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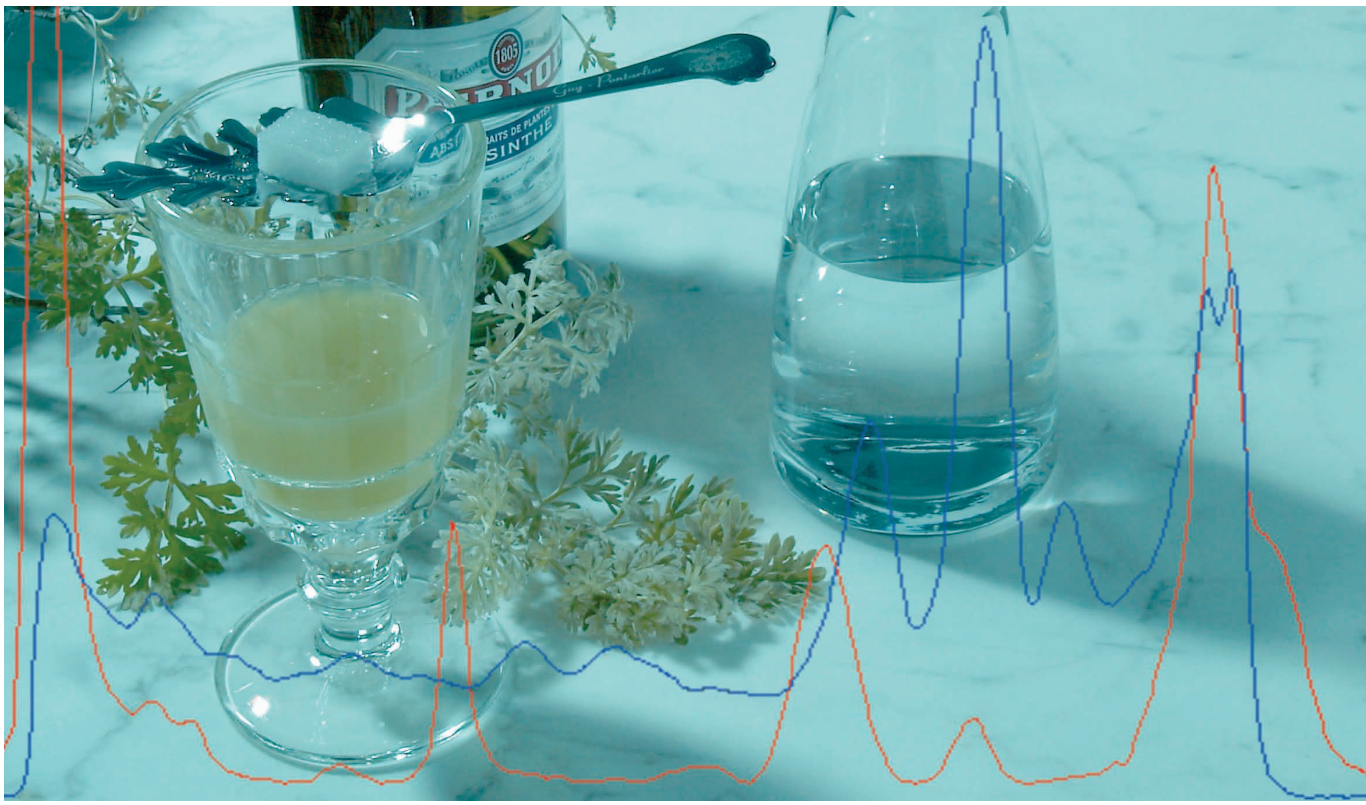

Planar-Chromatographie beantwortet Authentizitätsfragen

(Absinth Seite 6–7, Trigonellin Seite 9–11, Grüntee Seite 12–15)

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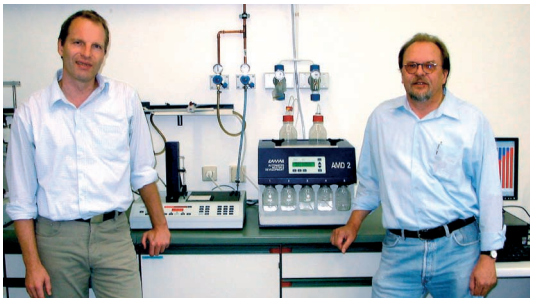
Strukturelle Charakterisierung von Gangliosiden mittels HPTLC/IR-MALDI-o-TOF



1 ◀ *Priv.-Doz. Dr. Klaus Dreisewerd und Prof. Dr. Johannes Müthing*

Die Arbeitsgruppen von Prof. Dr. Johannes Müthing* und PD Dr. Klaus Dreisewerd am Institut für Medizinische Physik und Biophysik der Westfälischen Wilhelms-Universität Münster entwickeln Verfahren zur direkten Kopplung von HPTLC und Matrix-unterstützter Laserdesorptions-/Ionisations-Massenspektrometrie (MALDI-MS). Dazu werden die zu analysierenden Probenstellen mit einem kleinen Tropfen einer flüssigen MALDI-Matrix (Glycerol) getränkt, die HPTLC-Platten bzw. Teile davon in das Massenspektrometer überführt und kleine Mengen im µm-Bereich mit dem Laser abgetragen. Ein Teil der desorbierten Moleküle ist elektrisch geladen, und diese Ionen werden in dem Massenspektrometer nach ihrer Masse aufgetrennt und nachgewiesen. Die Verwendung eines Infrarot(IR-)lasers besitzt im Gegensatz zu den für die MALDI standardmässig eingesetzten UV-Lasern eine grössere Eindringtiefe. Weil zudem nur Moleküle im Bereich des Laserfokus freigesetzt werden, können räumlich aufgelöste Mobilitätsprofile mit einer Auflösung im Bereich von 200 µm durch Abrastern der Probe gewonnen werden. Zur Verbesserung der Ionenausbeute wird die Analytzone mit Glycerol-Flüssigmatrix benetzt, was allerdings die Bildung von Adduktionensignalen begünstigt und die Spektren dadurch etwas kompliziert. Angewendet wird dieses Kopplungsverfahren in der Arbeitsgruppe von Prof. Müthing im folgenden bei der Charakterisierung von Glykosphingolipiden.

Einleitung

Glykosphingolipide (GSL) sind typische Lipide der Zellmembranen von tierischen Zellen. Sie besitzen eine wichtige biologische Funktion bei der Signaltransduktion und bei Zell-Zell-Erkennungsprozessen, fungieren aber auch bei verschiedenen Krankheiten als Rezeptoren für Influenzaviren, Bakterien und deren Toxine [1]. Das Ceramidmolekül, bestehend aus einem langkettigen zweiwertigen Aminoalkohol (Sphingosin, d18:1), der mit Fettsäuren verschiedener Kettenlängen (C16- bis C24-Fettsäuren) amidartig verknüpft ist, stellt den Lipidanker der GSL dar. Die terminale OH-Gruppe des Sphingosins ist mit einem Oligosaccharid glykosidisch verknüpft, das von der Zelloberfläche aus gesehen nach aussen ragt und so mit seiner Umwelt in Kontakt treten kann. Saure GSL, d.h. Ganglioside tra-

gen Sialinsäuren (hauptsächlich N-Acetylneuraminsäure, Neu5Ac), wodurch sie bei physiologischem pH-Wert negativ geladen sind. Das Gangliosid GM3 (Neu5Acα2-3Galβ1-4Glcβ1-1Cer) repräsentiert ein typisches Säugergangliosid.

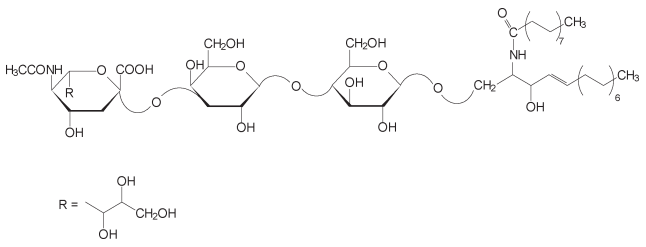
Die Planar-Chromatographie ist die am häufigsten verwendete Methode zur initialen Analyse von GSL-Präparationen [2]. Die Färbung der GSL nach der Chromatographie erfolgt entweder mit Orcin (Zuckerfärbung) oder mit dem Fluoreszenzfarbstoff Primulin (Lipidfärbung), wobei die zweitgenannte Färbung zerstörungsfrei arbeitet und somit zur präparativen DC von GSL eingesetzt werden kann. Weitere Detektionsarten sind ELISA-Verfahren direkt auf der HPTLC-Platte sowie die massenspektrometrische Analyse von Kieselgelextrakten aus immungefärbten GSL-Banden [3]. Die direkte Kopplung von IR-MALDI-o-TOF-MS mit HPTLC ist eine sehr sensitive Methode, da die bei alternativen MS-Verfahren verlustbehaftete Extraktion von Analyten entfällt. Grundsätzlich ist die HPTLC/MS-Kombinationstechnik für alle per HPTLC trennbaren Substanzklassen einsetzbar, wie es beispielhaft für native Oligosaccharide der Milch gezeigt wurde [4].

Probenvorbereitung

Chinese hamster ovary (CHO)-Zellen, die »Arbeitstiere« der molekularen Biotechnologen für die Herstellung von rekombinanten humanen Glykoproteinen, exprimieren hauptsächlich das Gangliosid GM3 (Neu5Ac). Eine GM3-Präparation wurde durch Aufreinigung über DEAE-Sepharose (Ionenaustauscher) und Kieselgel 60 (Adsorptions-Chromatographie) hergestellt.

Schicht

HPTLC-Platten Kieselgel 60 Merck, 10 x 10 cm (Art. 5633).



▲ *GM3(Neu5Ac, d18:1, C16:0)*

Probenauftragung

5 bis 10 µL der Probe werden strichförmig mit Linomat aufgetragen; Bandlänge 5 mm, Bahnabstand 10 mm

Chromatographie

In der Flachbodenkammer 20 x 20 cm mit 100 mL Chloroform – Methanol – Wasser 120:85:20 unter Zusatz von 2 mM $CaCl_2$ nach 3h Kammersättigung mit Filterpapier, Laufzeit 20 min, entspricht einer Laufstrecke von ca. 80 mm (vom unteren Plattenrand). Die Flachbodenkammer kann mehrfach für bis zu 10 Plattenentwicklungen benutzt werden. Nach der Chromatographie wird die Platte 5 min im Abzug bei Raumtemperatur getrocknet.

Anmerkung: Generell sollte die Kammergrösse der Plattengrösse angepasst sein. In diesem Fall benötigt eine Doppeltrogkammer 10 x 10 cm nur 10 mL Fliessmittel und 30 min Kammersättigung. (Hrsg.)

Derivatisierung

Die Platte wird mit einem Glasschneider, z.B. smartCUT, in 1 cm breite Streifen geschnitten. Ein Referenzstreifen wird nach dem Tauchen mittels Tauchvorrichtung in Orcinfärbelösung (0.3 % (w/v) in 3 M H_2SO_4) und 3 min Erhitzen bei 100 °C auf dem DC-Plattenheizer III angefärbt. Ein Parallelstreifen, welcher für die eigentliche IR-MALDI-o-TOF-Analyse eingesetzt werden soll, wird auf Höhe der GM3-Banden auf eine Länge von 3 cm gekürzt.

Alternativ können die GM3-Banden auch mit Primulin (0,02 % (w/v) in Aceton – Wasser 4:1) derivatisiert werden und dann direkt massenspektrometrisch vermessen werden. Denn im Gegensatz zur Orcinfärbung ist diese Detektionsart zerstörungsfrei, da nur eine Anlagerung an hydrophobe Lipide erfolgt.

IR-MALDI-o-TOF-Massenspektrometrie

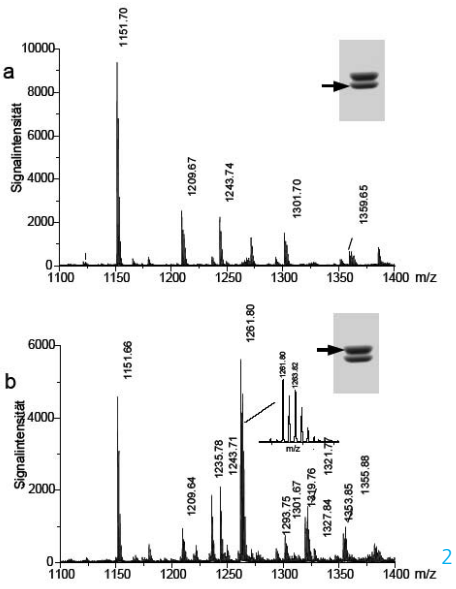
Die durch Laserbeschuss erzeugten Ionen werden orthogonal, d.h. senkrecht zur ihrer ursprünglichen Bewegungsrichtung, in das eigentliche Flugzeitmassenspektrometer beschleunigt. Bei dem für unsere HPTLC-Direktmessungen verwendeten Massenspektrometer handelt es sich um einen modifizierten Prototypen der Fa. Sciex (Ontario, Kanada), der mit einem ER:YAG-Laser (Erbium-dotierter Yttrium-Aluminium-Granat-Laser, Bioscope, BIOptics Laser Systems AG, Berlin) ausgestattet wurde. Dieser Infrarotlaser emittiert gepulste Strahlung (Impulsdauer

ca. 100 ns) mit einer Wellenlänge von 2940 nm. HPTLC-Plattenstücke von maximal 4,5 cm x 4,5 cm Grösse werden mit doppelseitigem Klebeband auf dem Probenhalter fixiert, in die Ionenquelle eingeführt und Analytionen dann mit Hilfe des Lasers desorbiert, ionisiert und anschliessend massenspektrometrisch analysiert. Als Flüssigmatrix dient Glycerol, das in Tropfen von ca. 0.3 µL auf die GM3-Bereiche gegeben wird. Massenspektren können von ungefärbten Platten oder auch von mit Primulin nichtkovalent fluorochrommarkierten Banden aufgenommen werden.

Ergebnisse und Diskussion

Direkte IR-MALDI-o-TOF-MS-Analyse von HPTLC-getrennten GM3-Spezies

Wie aus den Insets des Orcin-gefärbten Referenzlaufs ersichtlich, werden die GM3(Neu5Ac)-Gangliosid per HPTLC deutlich in zwei Hauptbanden aufgetrennt. Mittels MS werden in der unteren Bande (das zugehörige Massenspektrum wurde im negativen Ionenmodus aufgenommen) vorwiegend GM3-Spezies mit kurzkettigen Fettsäuren (hauptsächlich C16:0) und in der oberen Bande solche mit langkettigen Fettsäuren (hauptsächlich C24:1 und C24:0) detektiert.



▲ *Direkt erzeugte HPTLC-IR-MALDI-Massenspektren von GM3(Neu5Ac), gemessen im Negativionen-Modus. Gezeigt ist der m/z-Bereich zwischen 1100 und 1400 der unteren (a) und der oberen (b) GM3(Neu5Ac)-Bande. Das Inset zeigt den Orcin-gefärbten Referenzlauf. Die Massenspektren wurden von ungefärbten Banden erzeugt.*

Die ermittelten experimentellen *m/z*-Werte zeigen neben den deprotonierten GM3-Spezies $[M-H]^-$ auch Ionen, die durch Adduktbildungen mit der Glycerolmatrix und Salzmolekülen entstehen.

Vorgeschlagene Strukturen der dominanten Molekülionen, detektiert in HPTLC-getrennten GM3-Banden durch IR-MALDI-o-TOF-MS im negativen Ionenmodus: der Sphingoid-Teil von allen detektierten Gangliosiden hat die Zusammensetzung d18:1 (a) untere Bande, (b) obere Bande.

(a)

Vorgeschlagene GM3-Fettsäure	Typ des Molekülions	*m/z* (monoisotopisch)	
		Berechnet	Detektiert
C16:0	**$[M-H]^-$**	1151.71	**1151.70**
	$[M+NaCl-H]^-$	1209.66	1209.67
	$[M+G-H]^-$	1243.77	1243.74
	$[M+G+NaCl-H]^-$	1301.72	1301.70
	$[M+G+2NaCl-H]^-$	1359.67	1359.65

(b)

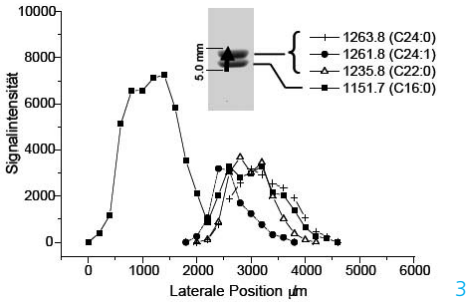
Vorgeschlagene GM3-Fettsäure	Typ des Molekülions	*m/z* (monoisotopisch)	
		Berechnet	Detektiert
C16:0	**$[M-H]^-$**	1151.71	**1151.66**
	$[M+NaCl-H]^-$	1209.66	1209.64
	$[M+G-H]^-$	1243.77	1243.71
	$[M+G+NaCl-H]^-$	1301.72	1301.67
C22:0	**$[M-H]^-$**	1235.80	**1235.78**
	$[M+NaCl-H]^-$	1293.74	1293.75*
	$[M+G-H]^-$	1327.86	1327.84*
C24:1	**$[M-H]^-$**	1261.82	**1261.80**
	$[M+NaCl-H]^-$	1319.77	1319.76
	$[M+G-H]^-$	1353.87	1353.85
C24:0	**$[M-H]^-$**	1263.83	**1263.82**
	$[M+NaCl-H]^-$	1321.77	1321.77
	$[M+G-H]^-$	1355.89	1355.88

GM3-Ionen mit hoher Signalintensität sind fett gedruckt
G: Glycerol
**Signal mit niedriger Signalintensität (S/N 2-3)*

Laterale Auflösung der direkten HPTLC-IR-MALDI-MS-Analyse

Mit Hilfe der Laserabtastung über die HPTLC-getrennten GM3-Banden kann ein Mobilitätsprofil der individuellen GM3-Spezies erstellt werden, wobei die laterale Auflösung durch den Laserspotdurchmesser von ca. 200 µm limitiert ist. Die Signal-intensitäten der vier dominanten GM3(Neu5Ac)-Spezies sind als Funktion der Laserfokuspositionen wiedergegeben. Jeder Datenpunkt repräsentiert die Aufsum-

mierung von ca. 50 Lasereinzelpulsen, wobei die GM3-Banden von dem Laser sukzessive in 200 µm-Schritten in Chromatographierichtung abgetastet werden.



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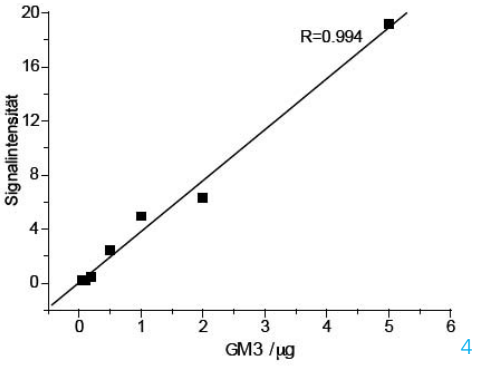
▲ *Mobilitätsprofil der [M-H]⁻-Ionensignalintensitäten der vier dominanten GM3(Neu5Ac)-Spezies mit C16:0-, C22:0-, C24:1- und C24:0-Fettsäuren, bestimmt durch direkte HPTLC-IR-MALDI-MS als Funktion der Laserspot-Position. Das Inset zeigt das Orcin-gefärbte Referenzchromatogramm. Die Massenspektren wurden von ungefärbten Banden erzeugt. Die Pfeile zeigen Richtung und Position des vom Laser abgetasteten Bereichs.*

Detektionslimit der direkten HPTLC-IR-MALDI-o-TOF-MS-Analyse

Das GM3-Detektionslimit wurde mittels Verdünnungsreihe von GM3-Mengen im Bereich von 50 ng/Bande bis 5 µg/Bande für die untere GM3(Neu5Ac)-Bande mit C16:0-Fettsäure (*m/z* 1151,7) ermittelt. Abbildung 4 zeigt, dass das Detektionslimit bei ca. 50 ng liegt. Dieser Wert entspricht in etwa der Nachweisgrenze von Immunfärbungen auf HPTLC-Platten.



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▲ *Korrelation zwischen IR-MALDI-Ionensignalintensität und der auf die HPTLC-Platte aufgetragenen GM3-Menge. Die Massenspektren wurden von der unteren HPTLC-getrennten GM3(Neu5Ac)-Bande mit C16:0-Fettsäure ([M-H]⁻-Ionen bei m/z 1151,7) aufgezeichnet (Korrelationskoeffizient R = 0,994).*

Ausblick

Weiterentwicklungen der direkten HPTLC/IR-MALDI-MS-Technik, an denen zur Zeit gearbeitet wird, sind die strukturelle Charakterisierung von mit Antikörpern detektierten (immungefärbten) GSL. Von besonderem Interesse sind dabei hochkomplexe Lewis^x-GSL, die nur mittels automatisierter Mehrfachentwicklung (AMD) befriedigend aufgetrennt werden können.

Danksagung

Wir danken der Firma Sequenom GmbH (Hamburg) für die Nutzung ihres o-TOF-Massenspektrometers. Weiterhin möchten wir uns bei Frau Prof. Dr. Jasna Peter-Katalinic und Herrn Prof. Dr. Franz Hillenkamp für ihre Unterstützung bedanken.

Referenzen

[1] J. Müthing. In Glycoscience: Chemistry and Chemical Biology, Vol. 3: Glycolipids; B. Fraser-Reid, K. Tatsuta, J. Thiem (Eds.) Springer: Heidelberg, Germany, 2001: pp. 2220-2252, 2001.

[2] J. Müthing, J. Chromatogr. A 72, 3-25, 1996.

[3] I. Meisen, J. Peter-Katalinic , J. Müthing, Anal. Chem. 76, 2248-2255, 2004.

[4] K. Dreisewerd, S. Kölbl, J. Peter-Katalinic, S. Berkenkamp, G. Pohlentz, J. Am. Soc. Mass Spectrom. 17, 139-150, 2006

[5] K. Dreisewerd, J. Müthing, A. Rohlfing, I. Meisen, Ž. Vukelic, J. Peter-Katalinic, F. Hillenkamp, S. Berkenkamp, Anal.Chem. 77, 4098-4107, 2005.

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Authentizitätsprüfung von Absinth

5

▲ *Mitglieder der Arbeitsgruppen HPTLC und Alkoholische Getränke des CVUA Karlsruhe (von links nach rechts): Silvia Gonzalez, Jürgen Geisser, Hannelore Heger und Dr. Dirk W. Lachenmeier**

Das Chemische und Veterinäruntersuchungsamt (CVUA) Karlsruhe ist Teil der amtlichen Lebensmittel- und Tiergesundheitsüberwachung in Baden-Württemberg, zu deren Aufgaben die Untersuchung eines grossen Produktspektrums (z.B. Lebensmittel, Kosmetika und Arzneimittel), tierärztliche Diagnostik wie auch Betriebskontrollen zählen. Hinsichtlich der Untersuchung und Beurteilung alkoholischer Getränke ist das CVUA Karlsruhe eines der renommiertesten Institute in Deutschland. Die HPTLC wird in Karlsruhe – ausser für die Analyse von alkoholischen Getränken – in einem weiten Anwendungsbereich eingesetzt, besonders zum Nachweis von Zusatzstoffen in allen Arten von Lebensmitteln und Kosmetika.

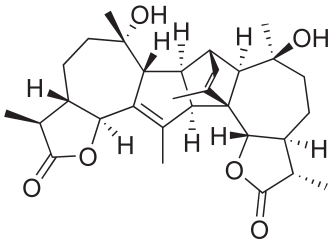
Einleitung

Das Modegetränk Absinth ist eine Spirituose von ausgesprochen bitterem Geschmack und grüner Farbe. Seinen bitteren Geschmack verdankt der Absinth den Inhaltsstoffen des Wermutkrauts (*Artemisia absinthium L.*, Asteraceae). Das alkoholische Getränk erlebt zurzeit eine Renaissance nach ca. siebzig Jahren Verkaufsverbot. In jüngster Zeit sind zahlreiche minderwertige Produkte auf dem Markt erhältlich, bei denen vor allem die sensorischen Eigenschaften des Wermuts fehlen. Folglich ist für die amtliche Lebensmittelüberwachung eine analytische Methode erforderlich, um den Wermutgehalt in Absinth zu prüfen. Aufgrund der in Deutschland und der EU fehlenden gesetzlichen Begriffsbestimmung für Absinth, stellt diese Authentizitätsprüfung eine Herausforderung für die Lebensmittelkontrolle dar.

Das bicyclische Monoterpen Thujon kann heute entgegen dem früheren Vorgehen (z.B. Angaben des Schweizer Lebensmittelbuchs) nicht als alleinige Markersubstanz für die Authentizität von Absinth betrachtet werden, weil Verfahren (z.B. überkritische Fluidextraktion) entwickelt wurden, um diese toxische Substanz im Wermutkraut zu entfernen. Darüber hinaus ist in Abhängigkeit vom Anbaugebiet auch thujonfreies Wermutkraut erhältlich, und es wurde festgestellt, dass traditionell hergestellter Absinth nur wenig Thujon enthält. Da die Getränkeindustrie mittlerweile thujonfreien Absinth produziert, kann als alternative Markersubstanz zur Authentizitätsprüfung von Absinth Absinthin, ein bitter schmeckendes dimeres Sesquiterpenlacton, das bis zu 0,28 % im Wermutkraut enthalten ist, herangezogen werden.

Die HPTLC ist eine oft angewendete Methode zur Analyse von Sesquiterpenlactonen, die nach Derivatisierung densitometrisch auswertbar sind. Sprühreagenzien wie Vanillin/o-Phosphorsäure, Anisaldehyd oder p-Dimethylaminobenzaldehyd/Schwefelsäure, Schwefelsäure, Resorcin/Schwefel- bzw. Phosphorsäure, Aluminiumchlorid oder Hydroxylamin werden dazu eingesetzt. Die vorgestellte Methode basiert auf der Wermutmethode (2005) des Europäischen Arzneibuchs, die auf alkoholische Getränke übertragen wurde.

Die Planar-Chromatographie ist derzeit die einzige effiziente Methode zur Bestimmung des Wermutanteils in Absinth. Aus den Validierungsdaten ist die hohe Empfindlichkeit, Selektivität und Reproduzierbarkeit der HPTLC-Methode ersichtlich. Die Eignung des Verfahrens wurde anhand der Untersuchung von 23 kommerziell erhältlichen Produkten gezeigt. Alle Ergebnisse entsprechen der Anforderung der amtlichen Lebensmittelüberwachung. Die niedrigen Kosten, der hohe Probendurchsatz und die minimalen Anforderungen an die Probenaufarbeitung sind klare Vorzüge dieser Screening-Methode.



▲ *Strukturformel von Absinthin*

Probenvorbereitung

25 mL Absinth wurden mit 50 mL Dichlormethan extrahiert. Die organische Phase wurde durch Zugabe von wasserfreiem Natriumsulfat getrocknet und nach anschliessender Filtration bis zur Trockene eingedampft. Der Rückstand wurde in 0,5 mL Ethanol (96 %) aufgenommen.

Standardlösung

100 g Wermutkraut von 3 verschiedenen Lieferanten wurden vermischt und mit einem Standardmixer zerkleinert. 2 g des erhaltenen Wermutpulvers wurden 5 min in 50 mL siedendem Wasser extrahiert. Nach dem Abkühlen wurden 5 mL 10 %-iger Bleiacetatlösung zur Klärung zugegeben. Das Filtrat wurde mit 50 mL Dichlormethan extrahiert und analog den Proben aufgearbeitet.

Schicht

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

Probenauftragung

Bandförmig mit DC-Probenautomat, Bandlänge 6 mm, 7 Bahnen, Auftragevolumen 10 µL, Bahnabstand 11,6 mm, seitlicher Randabstand 15 mm, unterer Randabstand 10 mm.

Chromatographie

In Doppeltrogkammer (10 x 10 cm) mit Aceton – Eisessig (98 %) – Toluen – Dichlormethan 1:1:3:5 (v/v/v/v); Laufstrecke 70 mm; die Platte wurde nach der Chromatographie im Luftstrom bei RT für 10 min getrocknet.

Derivatisierung

Mit der Tauchvorrichtung III wird die Platte in Essigsäureanhydrid – Schwefelsäure – Ethanol 1:1:10 (v/v/v) getaucht und anschliessend 5 min bei 104 °C erhitzt. Die Absinthin-Zone färbt sich bräunlich.

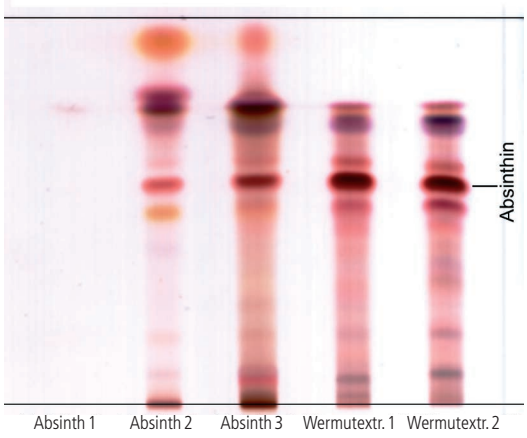
Densitometrie

TLC Scanner 3 mit winCATS Software, Absorptionsmessung bei 554 nm, polynome Kalibrierkurve (Peakfläche); Substanzidentifizierung anhand der Vis-Spektren (400–700 nm).

Ergebnisse und Diskussion

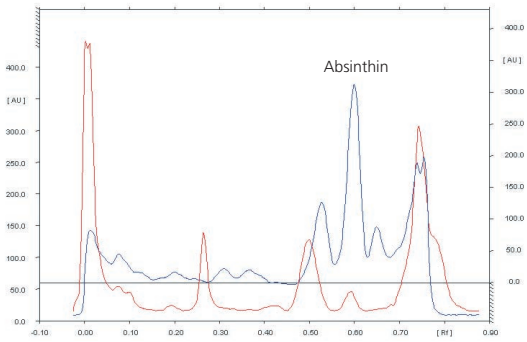
Die Identifizierung von Absinthin (hR_F 64) erfolgte mit Hilfe von authentischem Wermutextrakt und der Referenzsubstanz Resorcinol, die auf dieselbe Platte aufgetragen wurde. Gemäss des Europäischen Arzneibuchs besitzt Absinthin einen ähnlichen hR_F-Wert wie Resorcinol.

Die Kalibrationskurve für Absinthin zeigt im Bereich 0,1–10 g/L Wermutextrakt eine sehr gute Korrelation (r > 0,999). Die Bestimmungsgrenze für Absinthin in Absinth liegt bei 50 mg/L. Es wurden keine Störungen während der Analyse typischer Absinthbestandteile oder während der Routineanalytik von 23 Produktproben beobachtet. Die Präzision (RSD) war besser als 13,5 % (innerhalb eines Tages) bzw. 15,8 % (an unterschiedlichen Tagen). Bezüglich der Absinthin-Stabilität wurden weder eine signifikante Abnahme noch ein Verlust innerhalb 24 h vor und nach der Chromatographie festgestellt. Der Wermutextrakt-Gehalt der Proben lag im Bereich 0,1–7,8 g/L. In 10 Produktproben konnte kein Absinthin nachgewiesen werden (siehe Absinth 1 in nachfolgender Abbildung).



6

▲ *Trennung von 2 Wermutextrakten und 3 Absinthproben auf einer HPTLC-Platte Kieselgel 60; Absorptionsmessung bei 554 nm nach der Derivatisierung*



7

▲ *Analogkurven bei 554 nm von einem Wermutextrakt-Standard (blau) und einer authentischen Absinthprobe (rot)*

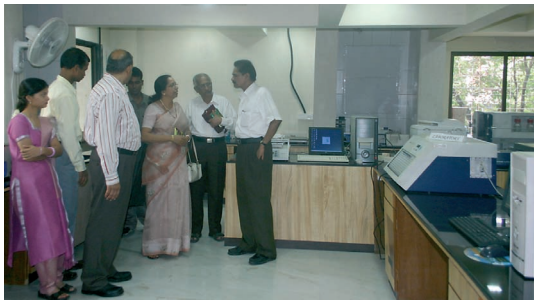
* Dr. W. Dirk Lachenmeier, Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weissenburger Str. 3, D-76187 Karlsruhe, Germany, Tel +49 -721-926-5434, E-mail: Lachenmeier@web.de

Einweihung in Indien: Anchrom investiert in die Zukunft!

8


9


10


11

Anchrom, Gewinner der CAMAG »Distributor of the Year« Auszeichnung 2005 (siehe CBS 95), konnte kürzlich neue Geschäftsräume in Mulund/Mumbai einweihen.

Dilip Charegaonkar, Gründer und Geschäftsführer von Anchrom, erklärte bei seiner Begrüssungsrede, dass seine Firma in den 28 Jahren der Zusammenarbeit mit CAMAG einen weiten Weg zurückgelegt hat. Anchrom ist heute der einzige Anbieter analytischer Geräte in Indien, der sich auf eine einzige Technik spezialisiert hat. Mit 8 Stützpunkten, verteilt über den gesamten Subkontinent, seinem auch international geschätzten Labor und ausgezeichneten Mitarbeitern fördert er den Einsatz der Planar-Chromatographie in Indien.

Anchrom unterstützt auch ständig Projekte, in denen Studenten und junge Wissenschaftler die Ressourcen der Firma gratis für ihre Forschung und Ausbildung benutzen können. Man ist stolz darauf, diesen kleinen Beitrag zur Entwicklung der Gesellschaft in Indien leisten zu können.

Ansprachen zur Einweihung hielten u.a.:

- Dr. P.D. Sethi, Direktor des Indian Pharmacopoeia Laboratory in Ghaziabad i. R. und Autor von fünf Büchern, drei davon zum Thema TLC/HPTLC. Er ist heute noch Quelle der Inspiration und guter Ratschläge für Dilip Charegaonkar.
- Joseph Koch, Direktor des Indian Swiss Business Forum und aktiver Promotor Schweizer Geschäftsinteressen in Indien.
- Peter Jänchen, CEO von CAMAG Muttenz.

Die traditionelle indische Einweihungsfeier mit Begrüssung, Festreden, Band zerschneiden, Kokosnuss zerbrechen und Öllampe anzünden wurde von der Anchrom Belegschaft sehr stilvoll organisiert. Im Anschluss an die Zeremonie wurden die Gäste durch die neuen Geschäftsräume geführt und ihnen dabei auch die Funktion der im Labor ausgestellten CAMAG-Geräte erläutert.

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CBS

Liebe Freunde

Nahrungsergänzungsmittel sowie Arzneimittel oder Kosmetika auf Basis pflanzlicher Wirkstoffe erleben seit einigen Jahren einen unglaublichen Boom. Wirkstoffe aus der Natur bergen aber auch eine gewisse Problematik. Die Authentizität (griech./lat. Echtheit, Zuverlässigkeit, Glaubwürdigkeit) von Naturstoffen ist eine wichtige Fragestellung, denn die Verfälschung von teuren pflanzlichen Drogen ist ein lukrativer Markt. Dabei können die zur Verfälschung eingesetzten Drogen oder Stoffe durchaus toxische Wirkungen aufweisen, zumindest aber die zugesprochene positive Wirkung beeinträchtigen. Die angestrebte Standardisierung von pflanzlichen Drogen und deren Wirkstoffen ist angesichts der Mannigfaltigkeit der Natur dabei zugegebenermaßen ein weites Feld...

Bei Authentizitätsfragen ist die bildliche Darstellungsweise der Planar-Chromatographie von Vorteil, denn ein Bild steigert den Informationswert. In dieser CBS-Ausgabe werden mit Hilfe der Planar-Chromatographie drei unterschiedliche Fragestellungen in diesem Kontext angegangen: Sei es der neu ausgewiesene Marker Absinthin für die Bestimmung des Wermutanteiles im Absinth (S. 6–7) oder der Marker Trigonellin, ein bedeutender Wirkstoff im Bockshornklee, der durch seine vielfältigen pharmakologischen Wirkungen in pflanzlichen Arzneimitteln eingesetzt wird (S. 9–11) oder das Polyphenolmuster bei der Unterscheidung von Teesorten (S. 12–15).

Herzlichst Ihre

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

For food supplements, medicinal products or cosmetics based on herbal plants and their active ingredients, a steady rise was observed in the last decade. However, the boom for natural active ingredients also causes concern. To begin with, quality assurance of herbals is often difficult, and, authenticity confirmation is essential as the practice of adulteration increases because these products are so profitable. Even more important is the fact that these adulterants can have adverse or even toxic health effects – not to mention the absence of intended positive health effects. The task of standardization of herbal drugs and their active ingredients would require an enormous effort, even if the only problem were the diversity of nature.

Regarding authenticity concerns, the planar chromatographic image is advantageous, since an image gives additional information and increases the information value. I mean, an image of a TLC/HPTLC plate with samples, reference material, standards when available, and known adulterants co-chromatographed side by side provides an overwhelming advantage over all other techniques. Read this CBS and you will see concrete evidence of this fact: Three different kind of authenticity concerns were solved by planar chromatography – see the new assigned marker absinthin for the determination of the wormwood content in absinthe (p. 6–7) or the marker trigonelline, an important active ingredient in fenugreek, which is used in herbals drugs due to its various pharmacological effects (p. 9–11), or the polyphenol pattern for the discrimination of different types of tea (p. 12–15).

Sincerely,

Gerda Morlock

Gerda Morlock
cbs@camag.com

CAMAG

SEPTEMBER
2006

97

THE CBS CLASSIFICATION SYSTEM

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

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

1. Reviews and books

97 001 S. R. DANDSTRA, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton PA, 18042, USA): Effects of diet and larval trematode parasitism on lipids in snails as determined by thin-layer chromatography. J. Planar Chromatogr. 19, 180-186 (2006). Review of the use of TLC for the analysis of neutral lipids and phospholipids in medically and economically important gastropod molluscs; discussion of methods for isolating lipids, the use of layers, mobile phases, and detection reagents for the TLC analysis including the review of quantitative densitometric studies, with particular emphasis on class separations of neutral lipids and phospholipids. Details can be found on sample preparation, extraction, TLC methods including stationary phases, standard and sample solution preparation and application, mobile phases and plate development, detection modes, identification and quantification methods, and statistical comparison of data.

review, biology 1, 11c

2. Fundamentals, theory and general

97 005 W. ZAPALA*, Monika WAKSMUNDZKA-HAJNOS (*Department of Chemical Engineering and Process Control, Chemical Faculty, Rzeszów University of Technology, Al. Powstanców Warszawy 6, 35-959, Rzeszów, Poland, e-mail: ichwz@prz.rzeszow.pl): Retention process in normal-phase TLC system. J. Liq. Chrom. & Rel. Technol. 27, 2127-2141 (2004). Study of the influence of mobile phase composition on the retention of selected test analytes in different normal-phase TLC systems and proposition of a new model for an accurate prediction of the analyte retention in the TLC with binary mobile phase. HPTLC of 15 analytes (phenol, 2-nitroaniline, 4-nitrophenol, quinoline, 4-aminophenol, hydroquinone, 1,2-phenylenediamine, 2-hydroxyquinoline, 4-nitroaniline, 2-iodoaniline, 8-methylquinoline, aniline,1-aminonaphthalene, 4-iodoaniline, 1,5-diaminonaphthalene) on cyano, diol and amino phase in horizontal chambers with binary mixtures of polar modifiers (2-propanol, ethyl acetate, ethyl methyl ketone, dioxane, or tetrahydrofuran with n-heptane). Detection under UV light at 254 nm.

HPTLC 2b

97 002 Z. ROZMER, P. PERJESI*, K. TAKACS-NOVAK (*Institute of Pharmaceutical Chemistry, University of Pécs, Rókus str. 2, H-7624 Pécs, Hungary): Use of RP-TLC for determination of log P of isomeric chalcones and cyclic chalcone analogues. J. Planar Chromatogr. 19, 124-128 (2006). Determination of log P values of 29 biologically active chalcone and cyclic chalcone analogues E-2-(X-benzylidene)-1-indanones and E-2-(X-benzylidene)-1-tetralones by an optimized and validated RP-TLC method. RP-TLC was performed on silanized silica gel with methanol - water 3:2. The experimentally determined log P(TLC) values were compared with the log P values predicted by use of the CLOGP program.

pharmaceutical, research, qualitative identification 2c

97 003 L. S. LITVINOVA (Institute of Macromolecular Compounds, Russian Academy of Siences, Bolshoi pr. 31, 199004 St. Petersburg, Russia): Method for correction of RF values in thin-layer chromatography. J. Planar Chromatogr. 19, 171-174 (2006). Correction of RF values to improve the reproducibility, repeatability, precision, and reliability of retention factors in TLC. HPTLC of phenacetin, acetanilide, meso-tetraphenylporphyrin, fullerene C60, anthracene, solvent green 3, Sudan I, and cytochrome C on silica gel (after heating at 120 °C for 40 min) in an S-type unsaturated and in saturated and unsaturated N-type chamber using toluene, diethyl ether, or tetrahydrofuran - cyclohexane. Detection of colorless spots under UV light at 254 nm. To improve

reproducibility retention factors are calculated based on an unretained compound analog to a void volume marker in column chromatography.

HPTLC, comparison of methods 2d

97 165 M. SAJEWICZ et al., see section 38

97 004 Monika WAKSMUNDZKA-HAJNOS*, A. HAWRYL (*Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University, Staszica 6, 20-081, Lublin, Poland; e-mail mwaks@panaceum.am.lublin.pl): Delta RM as the parameter characterizing chromatographic properties of polar bonded stationary phases in isoeluotropic systems. J. Liq. Chrom. & Rel. Technol. 27, 1467-1482 (2004). HPTLC of 8 phenol derivatives, 8 anilines and 5 quinolines on amine-, diol- and cyano-phases with mobile phases of different eluent strengths of the polar modifier. Detection under UV light at 254 nm. For the separation of phenols and aromatic amines the aminopropyl phase was most selective. The cyanopropyl phase was used for quinolines.

HPTLC, qualitative identification 2e

3. General techniques

97 009 R. SHAH*, B. SUHAGIA, I. RATHOD, S. SHAH, D. PATEL (*L.M.College of Pharmacy, Ahmedabad-380009, India): HPTLC method development for pharmacokinetic study of sparfloxacin in plasma. Indian J. Pharm Sciences 67 (6), 687-690 (2005). HPTLC of sparfloxacin extracted with dichloromethane from plasma. A standard solution was prepared in methanol - dichloromethane 1:1. HPTLC on silica gel with chloroform - toluene - methanol - diethylamine 44:15:2:1. Quantitative determination by absorbance measurement at 301 nm. The method had a linearity range of 80-200 ng/spot with an average recovery of 89.17 %.

pharmaceutical research, clinical routine analysis, HPTLC, densitometry, quantitative analysis 3a

97 007 V. G. BEREZKIN*, E. V. KORMISHKINA (*A. V. Topchiev Institute of Petro-Chemical Synthesis, Russian Academy of Sciences, Leninski Prospekt 29, Moscow 119991, Russia): Study of a new version of classical Thin-Layer Chromatography with a closed adsorbent layer. J. Planar Chromatogr. 19, 81-85 (2006). A simple device is proposed for chromatographic separation with a traditional plate under the condition of a closed adsorbent layer (TLC-CL). Compared with traditional TLC the new variant has certain advantages, it takes for example 20-30 % less time; the efficiency of TLC-CL was, however, usually less than that of traditional TLC.

qualitative identification, comparison of methods 3c

97 008 V. G. BEREZKIN*, G. A. NEKHOROSHEV (*A. V. Topchiev Institute of Petro-Chemical Synthesis, Russian Academy of Sciences, Leninski Prospekt 29, Moscow 119991, Russia): Use of an electroosmotic pump for organization of forced-flow TLC on a plate with an adsorbent layer closed with a polymer film. J. Planar Chromatogr. 19, 109-114 (2006). Investigation of a new version of TLC with a closed adsorbent layer and an electroosmotic pump which was placed on the front of the plate and used to induce rapid movement of the mobile phase. Experimental evaluation of the new version of forced-flow TLC suggests that further elaboration of this version of TLC is appropriate.

qualitative identification 3d

97 006 T. D. SAMANTA, S. LASKAR* (*Natural Products Laboratory, Chemistry Department, University of Burdwan, Burdwan-713104, W. Bengal, India): New reagent for detection of amino acids on TLC plates. J. Planar Chromatogr. 19, 252-254 (2006). TLC of 22 amino acids on silica gel with n-propanol - water 7:3. Detection by spraying with 0.25 % 2,3-dichloro-1,4-naphthoquinone in ethanol, followed by drying in the air at room temperature and heating in an oven at 110 °C for 10 min. After cooling spraying with 0.4 % isatin in ethanol. Visual detection of spot colors (varying from yellow, to orange, pink, purple, and gray). Detection limits were determined visually and ranged from 0.01 µg (cystine and arginine) to 0.30 µg (isoleucine, phenylalanine, methionine, aspartic acid, and glycine).

qualitative identification, postchromatographic derivatization 3e, 18a

97 010 R. ZAKREWSKI*, W. CIESELSKI (*Department of Instrumental Analysis, University of Lodz, Pomorska 163, 90-236 Lodz, Poland): Planar chromatography of heterocyclic thiols with detection by use of the iodine-azide reaction. J. Planar Chromatogr. 19, 4-9 (2006). TLC and HPTLC of 2-thioguanidine and 6-mercaptopurine on silica gel with methanol in a horizontal DS-chamber. Spots were visualized with a freshly prepared solution of sodium azide and starch, adjusted to a pH within the range 5.5-6.0, and then exposed to iodine vapor. The thiols became visible as white spots on a violet-gray background. The iodine-azide reagent enabled detection of quantities in the range 1-80 pmol per spot.

comparison of methods, HPTLC, quantitative analysis 3e

97 011 Gerda MORLOCK et al., see section 4e

4. Special techniques

97 011 Gerda MORLOCK*, W. SCHWACK (*Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, 70599 Stuttgart, Germany. gmorlock@uni-hohenheim.de): Quantification of ITX in food by HPTLC/FID coupled with ESI-MS and DART-MS. CBS 96, 11-13 (2006). HPTLC of isopropylthioxanthone (ITX) in food, on silica gel in horizontal developing chamber with toluene - n-hexane 4:1 over 50 mm. Quantitative determination by fluorescence measurement at UV 254/>400 nm. Polynomial calibration via peak height, working range was 20 - 200 µg/kg. LOD is 64 pg (n=3) and in spiked fatty matrix 1 µg/kg. Positive findings were confirmed by ESI-MS in selective ion monitoring mode at m/z 255 and 277 using a plunger-based extraction device. Further confirmation by DART directly coupled to TOF-MS.

food analysis, HPTLC, quantitative analysis, densitometry, online-coupling TLC-MS 4e, 3f, 24, 8

7. Phenols

97 012 J. KAC*, A. MLINARIC, A. UMEK (*Faculty of Pharmacy, Askerceva 7, SI-1000 Ljubljana, Slovenia) : HPTLC determination of xanthohumol in hops (Humulus lupulus L.) and hop products. J. Planar Chromatogr. 19, 58-61 (2006). HPTLC of xanthohumol and isoxanthohumol on silica gel with toluene - dioxane - acetic cacid 77:20:3 in an unsaturated flat-bottomed chamber. Quantification by scanning at 368 nm. The detection limit was 2 ng per spot. The method was validated for precision, accuracy, and repeatability. The method is specific; a linear relationship was obtained between response (peak area) and amount of xanthohumol in the range of 7.7-77 ng per spot; the correlation coefficient was 0.997. Recovery at the three levels was found to be 119.1 %, 95.7 %, and 96.7 %, respectively. Instrumental presision and repeatability were 0.38 and 1.5 %, respectively. Intra-day and Inter-day precision were 1.7 and 2.3 %, respectively.

food analysis, comparison of methods, HPTLC, densitometry, quantitative analysis 7

8.Substances containing heterocyclic oxygen

97 011 Gerda MORLOCK et al., see section 4e

97 013 M. A. HAWRYL, Monika WAKSMUNDZKA-HAJNOS* (Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University in Lublin, Staszica 6 St., 20-081 Lublin, Poland): Separation of phenolic compounds by NP and RP two-dimensional Thin Layer Chromatography on connected plates. J. Planar Chromatogr. 19, 92-97 (2006). Two-dimensional HPTLC of naringenin, acacetin, flavone, morin, hesperetin, quercetin, narcisin, kaempferol 3,7-dirhamnoside, naringin, rutin, astragalin, quercitrin, kaempferol 3-glyco-7-rhamnoside, naringenin 7-glucoside, ferulic acid, chlorogenic acid, elagic acid, caffeic acid, p-coumaric acid, m-coumaric acid, o-coumaric acid, and gallic acid by connecting diol or silica plates to RP18 plates, with e. g. acetone - water 2:3 or 1:1 in first direction of development and propan-2-ol - ethyl acetate 1:1 or methanol - ethyl acetate 1:9 in second direction of development. Derivatization by use of diphenylboric acid 2-aminoethylester (natural products reagent), followed by PEG 400 reagent; detection under 365 nm.

herbal, HPTLC, qualitative identification 8a

97 014 Anna BUDZIANOWSKA*, L. SKRYPCZAK, J. BUDZIANOWSKI (*Department of Pharmaceutical Botany, K. Marcinkowski University of Medical Sciences, 14 ul. Sw. Marii Magdaleny, 61-861 Poznan, Poland; e-mail: abudzian@amp.edu.pl): Phenylethanoid glucosides from in vitro propagated plants and callus cultures of Plantago lanceolata. Planta Med. 70, 834-840 (2004). Analytical and preparative TLC of flavonoids (lavandulifolioside, plantamajoside, acteoside, leucosceptoside, and martynoside) on silica gel, RP18, polyamide and cellulose. E. g. 2D-TLC on cellulose with n-butanol - acetic acid - water 4:1:5 in the first and acetic acid - water 15:85 in the second direction, preparatice TLC on polyamide with ethyl acetate - ethanol - water 20:3:2 (2 x), 50:3:10, and 25:3:3 (4 x). Detection under UV light at 366 nm before and after spraying with 0.1 % diphenylboric acid 2-aminoethylester (natural products reagent) or 1 % aluminium chloride solution in ethanol.

traditional medicine, herbal, preparative TLC, qualitative identification 8a

97 017 J. POTHIER*, J. RAGOT, N. GALAND (*University Francois Rabelais, Laboratory of Pharmacognosy, Faculty of Pharmacy, 31 Avenue Monge, F-37200 Tours, France): Planar chromatographic study of flavonoids and soyasaponins for validation of fingerprints of Desmodium adscendens of different origin. J. Planar Chromatogr. 19, 191-194 (2006). TLC of the flavonoid and triterpenoid soyasaponin content (rutin, vitexine, isovitexine, soyasaponin I and VI as standards) on silica gel in a twin-trough chamber with ethyl acetate - formic acid - acetic acid - water 100:11:11:26 for flavonoids. Detection with diphenylboric acid 2-aminoethylester (natural products reagent) followed by PEG reagent. For soyasaponins chloroform - methanol - water 6:4:1 was used. Detection by spraying with anisaldehyde - sulfuric acid reagent followed by heating at 115 °C.

herbal, traditional medicine, qualitative identification 8a, 14

97 015 P. LAUPATTARAKASEM, P. J. HOUGHTON*, J. R. S. HOULT (*Department of Pharmacy, King's College London, Franklin-Wilkins-Building, 150 Stamford Street, London SE1 9NN, U. K.; e-mail: peter.houghton@kcl.ac.uk): Anti-inflammatory isoflavonoids from the stems of Derris scandens. Planta Med. 70, 479-482 (2004). Analytical and preparative TLC of genistein and

7-O-alpha-rhamno(1-6)-beta-glucosylgenistein on silica gel with n-butanol - acetic acid - water 4:1:1, ethyl acetate - methanol - water 77:13:10, and ethyl acetate - methanol - acetic acid - water 13:3:4:3. Detection under UV at 254 nm and under 366 nm after spraying with 1 % methanolic diphenylboric acid beta-ethylamino ester (natural products reagent), followed by a 5 % ethanolic PEG 4000 solution.

traditional medicine, herbal, preparative TLC, qualitative identification 8a

97 016 C. MARUTOIU*, I. GOGOASA, I. OPREAN, O.-F. MARUTOIU, M.-I. MOISE, C. TIGAE, M. RADA (*Lucian Blaga University of Sibiu, Faculty of Agricultural Sciences, Food Industry, and Environmental Protection, 7-9, Ion Ratiu Street, 550012 Sibiu, Romania): Separation and identification of piperine and chavicine in black pepper by TLC and GC-MS. J. Planar Chromatogr. 19, 250-252 (2006). TLC of piperine and chavicine on silica gel with heptane - ethyl acetate 3:2 in unsaturated chambers. Detection under UV light at 254 nm.

food analysis, herbal, qualitative identification 8b

97 018 N. S. KAPADIA, N. S. ACHARYA, S. A. ACHARYA, M. B. SHAH* (*Department of Pharmacognosy, L. M. College of Pharmacy, Navarangpura, Ahmedabad (Gujarat)-380009, India): Use of HPTLC to establish a distinct chemical profile for Shankhpushpi and for quantification of scopoletin in Convolvulus pluricaulis Choisy and in commercial formulations of Shankhpushpi. J. Planar Chromatogr. 19, 195-199 (2006). HPTLC of scopoletin on silica gel with toluene - diethyl ether 1:1, saturated with 10 % glacial acetic acid, in a twin-trough chamber saturated with mobile phase for 45 min. Evaluation by densitometry at 366 nm. The method was validated for linearity, accuracy, interday and intraday precision, specificity, repeatability of measurement of peak area, and repeatability of sample application. Limit of detection was 50 ng/spot.

traditional medicine, herbal, HPTLC, quantitative analysis 8b

97 019 T. WENNBERG, I. VOVK, P. VUORELA, B. SIMONOWSKA, H. VUORELA* (*Division of Pharmaceutical Biology, Faculty of Pharmacy, P. O. Box 56, FIN-00014 University of Helsinki, Helsinki, Finland): Use of DryLab for simulation of TLC separation and method transfer from TLC to HPLC. J. Planar Chromatogr. 19, 118-123 (2006). The computer-assisted simulation program DryLab has been used to simulate TLC separations. The simulations were based on data from preliminary TLC separations. For DryLab data entry of Rf values from TLC were converted to retention times, the development distance on the plate was used as column length, and the thickness of the adsorbent was used as the column diameter. To achieve reasonably accurated simulations it was found necessary to run three preliminary runs in which differences between organic modifier concentration in two adjacent runs were more than 5 %. The possibility of predicting HPLC separation conditions on the basis of TLC separations was also studied. - TLC of gallic acid, rutin, (+)-catechin, naringenin, and quercitin on RP18 with mixtures of acetonitrile and 0.1 % aqueous formic acid. Scanning at 255 nm in reflectance mode.

densitometry 8b

10. Carbohydrates

97 020 È. SARDI, Eszter SZARKA*, G. CSILLERY, J. SZARKA (*Corvinus University of Budapest, Faculty of Horticulture Science, Department of Genetics and Plant Breeding, Ménesi ut 44, H-1118 Budapest, Hungary): Biochemical examination of the general defense system of plants by OPLC. J. Planar Chromatogr. 19, 233-237 (2006). OPLC-HPTLC and OPLC-TLC of xylose,

fructose, glucose, galactose, sucrose, maltose, and raffinose on silica gel with acetonitrile - water 17:3 (overrun, performed twice, successively). Detection by use of aniline - diphenylamine - phosphoric acid reagent. Densitometric quantitation at 540 nm.

HPTLC, densitometry, quantitative analysis, biology 10a

97 021 E. TAMBURINI*, T. BERNARDI, M. GRANINI, G. VACCARI (*Chemistry Department, University of Ferrara, Via Luigi Borsari 46, 44100 Ferrara, Italy): Separation and quantitative determination of aldoses and alditols by over-pressured layer chromatography (OPLC). J. Planar Chromatogr. 19, 10-14 (2006). OPLC of D-xylitol, L-arabitol, D-glucitol , D-xylose, L-arabinose, D-glucose, and L-rhamnose on silica gel with overrunning elution. Acetonitrile - acetic acid - water 63:33:5 was used as mobile phase. The upper limits of linearity were in the range 140-600 ng and detection limits were 15-50 ng per spot.

food analysis, densitometry 10a

11. Organic acids and lipids

97 022 A. BANERJEE*, R. T. SANE, K. MANGAONKAR, S. SHAILAJAN, A. DESHPANDE, G. GUNDI (*Analytical Chemistry Laboratory, S. P. Mandali's Ramnarain Ruia College, Matunga, Mumbai-400 019, India): Quantitation of oleanolic acid in Oldenlandia corymbosa L. whole-plant powder by High-Performance Thin-layer Chromatography. J. Planar Chromatogr. 19, 68-72 (2006). HPTLC of oleanolic acid on silica gel with dichloromethane - toluene - acetone - methanol 30:40:15:3. Detection by spraying with Liebermann-Burchard reagent; quantification by densitometry at 529 nm. Detection and quantitation limits were 0.1 µg and 0.5 µg, respectively. Oleanolic acid response was linear over the range 1 to 9 µg. The validated HPTLC method can be used for routine quality-control analysis of Oldenlandia corymbosa L. whole-plant powder and for quantitative determination of oleanolic acid.

pharmaceutical research, herbal, densitometry, HPTLC, quantitative analysis 11a

97 024 Anna NIESTROJ (Silesian University, Institute of Chemistry, 9 Szkolna Street, PL-40-006 Katowice, Poland): Use of RP-TLC to investigate the solubility in water of fatty acids, hydroxy fatty acids and their esters. J. Planar Chromatogr. 19, 208-211 (2006) . TLC of fatty acids and fatty acid esters (palmitic, stearic, alpha-hydroxypalmitic, 12-hydroxystearic, 9,10-dihydroxystearic acid, methyl alpha-hydroxypalmitate, methyl 12-hydroxystearate, and methyl 9,10-dihydroxystearate) on RP18 with methanol - water 19:1 and 9:1. Detection with iodine vapor. New methods for calculation of the solubility in water (log W) from experimental RM values and other physicochemical data have been proposed.

qualitative identification 11a

97 025 A. NIESTROJ, Alina PYKA*, J. KLUPSCH, J. SLIWIOK (*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska Street, PL-41-200 Sosnowiec, Poland; e-mail: alinapyka@wp.pl): Use of RP-TLC and structural descriptors to predict the log P values of higher fatty acids, hydroxy acids, and their esters. J. Liq. Chrom. & Rel. Technol. 27, 2449-2461 (2004). TLC of oleic, elaidic, ricinolic acid, methyl ricinoleate, alpha-hydroxypalmitic acid, methyl alpha-hydroxypalmitate, 12-hydroxystearic acid, methyl 12-hydroxystearate, 9,10-dihydroxystearic acid, and methyl 9,10-dihydroxystearate on RP18 with methanol; methanol - water 19:1 and methanol - water 9:1. Detection with iodine vapor.

qualitative identification 11a

97 026 Magdalena WOJCIAK-KOSIOR*, A. SKALSKA (*Department of Chemistry, Laboratory of Planar Chromatography, Medical Academy, Staszica 6, 20-081 Lublin, Poland): Thin-layer chromatography of phenolic acids on aminopropylsilica. J. Planar Chromatogr. 19, 200-203 (2006). TLC of 18 phenolic acids (salicylic, m-hydroxybenzoic, p-hydroxybenzoic, protocatechuic, alpha-resorcylic, beta-resorcylic, gallic, vanillic, syringic, gentisic, veratric, cinnamic, o-coumaric, m-coumaric, p-coumaric, caffeic, ferulic, and sinapic acid) an aminopropyl silica gel, prewashed with methanol and acetone, in a horizontal DS-chamber with mobile phases comprising mixtures of diisopropyl ether and acetic acid with toluene, petroleum ether, or heptane, partly with two developments. The best separations were obtained with heptane - diisopropyl ether - acetic acid 4:5:1, or petroleum ether - diisopropylether - acetic acid 6:3:1. Detection under UV light at 254 or 366 nm.

herbal, qualitative identification 11a

97 027 Magdalena WOJCIAK-KOSIOR*, G. MATYSIK, E. SOCZEWINSKI (*Department of Chemistry, Laboratory of Planar Chromatography, Medical Academy, Staszica 6, 20-081 Lublin, Poland): High-performance thin-layer chromatography combined with densitometry for quantitative analysis of phenolic acids in complex mixtures. J. Planar Chromatogr. 19, 21-26 (2006). HPTLC of protocatechuic and caffeic acid on silica gel using mixtures of heptane, diisopropyl ether, dichloromethane, and formic acid as mobile phases for multiple gradient development. Quantitation by scanning in absorbance/reflectance mode at 320 nm for caffeic acid and at 260 nm for protocatechuic acid.

pharmaceutical research, densitometry, quantitative analysis 11a

97 001 S. R. DANDSTRA et al., see section 1

97 023 D. L. MARTIN, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA, 18042, USA): High-performance thin-layer chromatographic analysis of neutral lipids and phospholipids in the medicinal leech Hirudo medicinalis maintained on different diets. J. Planar Chromatogr. 19, 167-170 (2006). HPTLC of standards (cholesterol, oleic acid, triolein, methyl oleate, and cholesteryl oleate as marker compounds for free sterols, free fatty acids, triacylglycerols, methyl esters, and steryl esters, respectively, as well as cholesterol, phosphatidylethanolamine, phosphatidylcholine, and lysophosphatidylcholine for the analysis of polar lipids) and prepared samples of leeches on silica gel (with preadsorbent zone and prescored lanes) in a presaturated twin-trough chamber with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 (for neutral lipids) and hexane - petroleum ether - diethyl ether - glacial acetic acid 50:25:5:1 (for steryl esters). Visualization by spraying with 5 % ethanolic phosphomolybdic acid solution and heating for 10 min at 110 °C. HPTLC of polar lipids on equal layer with chloroform - methanol - water 65:25:4. Visualization by spraying with 5 % aqueous cupric sulfate solution and heating for 10 min at 140 °C. Quantification of neutral lipids at 610 nm and of polar lipids at 370 nm.

HPTLC, quantitative analysis, densitometry, biology 11c

13. Steroids

97 028 M. FENSKE (Department of Animal Physiology, University of Bayreuth, 95440 Bayreuth, Germany): Thin-layer chromatographic competitive protein-binding assay for cortisol and cortisone, and its application to urine samples from healthy men undergoing water diuresis. Chromatogra-

phia 63 (7-8), 383-388 (2006). Specific and rapid determination of free cortisol and cortisone in human urine has been achieved by concentration of the urine samples, liquid-liquid extraction of the concentrated samples, TLC of ethanolic extracts on silica gel, location of the steroids under UV light, elution of cortisol and cortisone from sections of the plates with phosphate buffer, and measurement by competitive protein-binding assay. Chicken serum was used as the source of corticosteroid binding globulin, because it binds cortisol and cortisone with similar high affinity. The method combining TLC and competitive protein-binding assay is easy to perform, specific, sensitive, and quite rapid. Free cortisol and cortisone were measured in the urine of male individuals who abstained from water intake for 2 h or who ingested 1 L of water. The results show, for the first time, that short-term water diuresis markedly increases urinary free cortisone excretion, supporting our previous hypothesis that its excretion is positively correlated with urine volume, i.e. with the volume of 24-h urine samples.

clinical chemistry, research, HPTLC, quantitative analysis, qualitative identification 13

97 029 P. K. ZARZYCKI*, M. A. BARTOSUK, A. I. RADZIWON (Laboratory of Toxicology, Department of Environmental Biology, Technical University of Koszalin, Koszalin, Poland. pawel_k_z@hotmail.com): Optimization of TLC detection by phosphomolybdic acid staining for robust quantification of cholesterol and bile acids. J. Planar Chromatogr. 19, 52-57 (2006). TLC and HPTLC of cholesterol, cholic and lithocholic acid, and taurodeoxycholic acid sodium salt on silica gel with methanol - dichloromethane 1:4, and on RP18 with methanol - water 4:1 with chamber saturation. Detection by spraying with phosphomolybdic acid solution and placing the plates in a gravity convection oven. The plates were heated to different temperatures from 40 to 120 °C for different periods of time (from 2 to 40 min). Best conditions for sensitive and robust detection on silica gel and RP18 were low derivatization temperatures around 60 °C and long heating times of more than 15 min.

pharmaceutical research, postchromatographic derivatization, qualitative identification 13d

97 030 Alina PYKA*, M. DOLOWY (*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska St., PL-41-200 Sosnowiec, Poland; e-mail: alinapyka@wp.pl): Separation of selected bile acids by TLC. III. Separation on various stationary phases. J. Liq. Chrom. & Rel. Technol. 27, 2613-2623 (2004). TLC of cholic, glycocholic, glycolithocholic, deoxycholic, chenodeoxycholic, glycodeoxycholic, and lithocholic acid on different preparations of silica gel and a mixture of silica gel and Kieselguhr with n-hexane - ethyl acetate - acetic acid in various volume compositions; successful separation was achieved with the compositions 4:4:1 and 22:21:5. Detection by 10 % aqueous sulfuric acid followed by heating at 120 °C for 20 min. Densitometric measurement at 250 nm.

clinical routine analysis, densitometry, quantitative analysis, qualitative identification 13d

14. Steroid glycosides, saponins and other terpenoid glycosides

97 032 Erzsébet HAZNAGY-RADNAI*, S. CZIGLE, G. JANICSAK, I. MATHE (*Institute of Pharmacognosy, University of Szeged, Eötvös 6, H-6720 Szeged, Hungary): Iridoids of Stachys species growing in Hungary. J. Planar Chromatogr. 19, 187-190 (2006). Comparison of the iridoid composition of ten Stachys species by use of a TLC-densitometric method. TLC of harpagide, acetylharpagide, harpagoside, ajugoside, aucubin, and catalpol on silica gel with chloroform - methanol - water 25:10:1 and, occasionally, ethyl acetate - formic acid - water 9:2:1. Visualization of the spots by use of Ehrlich's spray reagent (1 % dimethylaminobenzaldehyde in concentrated hy-

drochloric acid) followed by heating at 105 °C for 5 min. Quantitation by densitometry at 540 nm after 3 h.

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

97 033 W.-Y. LIU (Wen-Yong Liu), W.-D. ZHANG* (Wei-Dong Zhang), H.-S. CHEN (Hai-Sheng Chen), Z.-B. GU (Zheng-Bing Gu), T.-Z. LI (Ting-Zhao Li), W.-S. CHEN (Wan-Sheng Chen) (*Department of Phytochemistry, College of Pharmacy, Second Military Medical University, 325 Guotte Road, Shanghai 200433, China; e-mail: wdzhangy@hotmail.com): New triterpenoid saponins from bulbs of Bolbastemma paniculatum. Planta Med. 70, 458-464 (2004). Preparative TLC of 6'-O-palmitoyltubeimoside I, a triterpenoid of the 8-formyldammarene type, the acetyl derivative of 7beta,20,26-trihydroxy-(2OS)-dammar-24E-en-3-O-alpha-L-arabinopyranosy-(1-2)-beta-D-glucopyranoside and the acetyl derivative of 7beta,18,20,26-tetrahydroxy-(2OS)-dammar-24E-en-3-O-alpha-L-arabinopyranosy-(1-2)-beta-D-glucopyranoside on silica gel with chloroform - methanol - water 13:7:2 and 13:4:1. Detection by spraying with 10 % sulfuric acid in ethanol, followed by heating.

herbal, traditional medicine, preparative TLC 14

97 017 J. POTHIER et al., see section 8a

97 034 C.-X. YANG (Cai-Xia Yang), S.-S. HUANG (Shuang-Shen Huang), X.-P. YANG (Xiu-Ping Yang), Z.-J. JIA* (Zhong-Jian Jia) (*College of Chemitry and Chemical Engineering, National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, Gansu, 730000, China; e-mail: jiazj@lzu.edu.cn): Nor-lignans and steroidal saponins from Asparagus gobicus. Planta Med. 70, 446-251 (2004). Preparative TLC of gobicusin B and 4-[5-(4-methoxy-phenoxy)-3-penten-1-ynyl]-phenol on cellulose with chloroform (60 mL x 3) and analytical TLC on silica gel. Detection under UV light at 254 nm or by spraying with 5 % sulfuric acid in ethanol, followed by heating.

traditional medicine, herbal, preparative TLC, qualitative identification 14

15. Terpenes and other volatile plant ingredients

97 035 Q. DU* (Qizhen Du), G. JERZ, P. CHEN (Ping Chen), P. WINTERHALTER (*Institute of Food and Biological Engineering, Hangzhou University of Commerce, Hangzhou, P. R. China; e-mail: qizdendu@mail.hzic.edu.cn): Preparation of ursane triterpenoids from Centella asiatica using high speed countercurrent chromatography with step-gradient elution. J. Liq. Chrom. & Rel. Technol. 27, 2201-2215 (2004). TLC of pentacyclic triterpene acids (asiatic acid, madecassic acid) and triterpene glycosides (asiaticoside, madecassoside) on silica gel with ethyl acetate - methanol - water 8:2:1. Detection of triterpenoids by spraying with 3 % sulfuric acid in ethanol, followed by heating to 110 °C for 5 min on a hot plate.

herbal, qualitative identification 15a

97 036 A. NAVARRETE*, B. AVULA, V. C. JOSHI, X. JI (Xiuhong Ji), P. HERSH, I. A. KHAN (*Universidad National Autónoma de México, Facultad de Química, Departamento de Farmacia, Ciudad Universitaria, Coyocan 04510, México D. F, México. anavarrt@servidor.unam.mx): Quantitative determination of triterpenes from Amphipterygium adstringens by Liquid Chromatogra-

phy and Thin-Layer Chromatography and morphological analysis of cuachalalate preparations. J. Assoc. Off. Anal. Chem. 89, 1-7 (2006). TLC of masticadienonic and 3-hydroxymasticadienonic acid on silica gel with hexane - acetone - formic acid - acetic acid 30:10:1:1. Quantitation by determination of the absorption at 200 nm. Detection by dipping into anisaldehyde - sulfuric acid reagent for 1 sec and heating at 100 °C for 5 min. Limit of detection was 0.1-0.2 µg/mL.

herbal, traditional medicine, quantitative analysis, densitometry 15a

97 037 R. TANAKA*, S.-I. WADA, Y. KINOUCHI, H. TOKUDA, S. MATSUNAGA (*Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan; e-mail: tanakar@gly.oups.ac.jp): A new seco-abietane-type diterpene from the stem bark of Picea glehni. Planta Med. 70, 877-880 (2004). Preparative TLC of a new seco-abietane-type diterpenoid 13S-hydroxy-9-oxo-9,10-seco-abiet-8(14)-en-18,10alpha-olide, pinoresinol and reduction products on silica gel with n-hexane - ethyl acetate - methanol 25:25:1, chloroform - methanol 9:1, and 19:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 15a

18. Amino acids and peptides, chemical structure of proteins

97 038 Jolanta FLIEGER*, M. TATARCZAK, H. SZUMILO (*Department of Inorganic and Analytical Chemistry, Medical University, 20-081 Lublin, Staszica 6, Poland): Multiple development HPTLC analysis of amino acids on cellulose layers. J. Planar Chromatogr. 19, 161-166 (2006). HPTLC of 16 amino acids on cellulose in a horizontal chamber with aqueous mixtures of 2-propanol, acetonitrile, and tetrahydrofuran in the range of 60-90 % (10 % increments). Acetic acid (at a concentration of 1%) was only added to the mobile phases containing 2-propanol. Best separation was achieved with tetrahydrofurane - water 17:3; acetonitrile - water 4:1; and 2-propanol - water - acetic acid 89.5 - 9.5 - 1 respectively. The adsorbed solvent was removed before repeating the development process. Visualization by spraying with ninhydrin solution and heating at 105 °C for 1.5-2 min. Densitometric evaluation by absorbance measurement at 415 nm.

HPTLC, densitometry, quantitative analysis, biochemical application 18a

97 006 T. D. SAMANTA et al., see section 3e

20. Enzymes

97 039 H. HIGASHID*, K.Z. HOSSAIN, H. TAKAHAGI, M. NODA (*Department of Biophysical Genetics, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan): Measurement of adenylyl cyclase by separating cyclic AMP on silica gel thin-layer chromatography. Anal. Biochem. 308 (1), 106-111 (2002). TLC of cyclic AMP (cAMP) on silica gel with water - ethanol - NH4HCO3 3:7:0.2 M. This procedure separated [32P]cAMP from other radioactive metabolites of [32P]ATP in up to 19 samples on one sheet (20×10 cm) over 40-60 min at room temperature (21 °C). This simple and rapid isolation method provides a novel and convenient technique for the assay of adenylyl cyclase.

review, AMD, adenylyl cyclase 20

22. Alkaloids

97 040 F. HANAWA*, N. FOKIALASIS, A.-L. SKALTSONNIS (*Department of Forest Chemistry and

Forest Products Research Institute (FFPRI), 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan; e-mail: fujinori@ffpri-affrc.go.jp): Photo-activated DNA binding and antimicrobial acitivities of furoquinoline and pyranoqtuinolone alkaoloids from Rutaceae. Planta Med. 70, 531-535 (2004). TLC overlay assays against a methicillin-resistant strain of Staphylococcus aureus and Candida albicans were employed to test antimicrobial properties. Skimmianine, kokusaginine, haplopine, flindersine, gentamycin, and 8-MOP were applied onto silica gel plates as spots of 9 mm diameter. Agar media was distributed over the plates; the plates were then irradiated with 31.7 kJ/sq.m. of UVA. After incubation at 37 °C in darkness, MTT solution (1 mg/mL in water) was sprayed.

traditional medicine, herbal 22

97 041 X. HUANG (Xueshi Huang), S. GAO (Song Gao), L. FAN (Lishua Fan), S. YU* (Shishan Yu), X. LIANG (Xiaotian Liang) (*Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1 Xiannongtan Street, Beijing 100050, China; e-mail: yushishan@imm.ac.cn): Cytotoxic alkaloids from the roots of Tylophora atrofolliculata. Planta Med. 70, 441-445 (2004). Preparative TLC of tylophoridicine E and F on silica gel with dichloromethane - methanol - ammonia 100:10:1 or 120:10:1. Detection with Dragendorff reagent.

traditional medicine, herbal, preparative TLC 22

97 042 Anna PETRUCZNYK*, Monika WAKSMUNDZKA-HAJNOS, M. L. HAJNOS (*Department of Chemistry, Medical University, Staszica 6, 20-081 Poland): The effect of chromatographic conditions on the separation of selected alkaloids in RP-HPTLC. J. Chromatogr. Sci. 43 (4), 183-194 (2005). HPTLC of selected alkaloid standards on RP18 W layer with various aqueous eluents containing an organic modifier and pH 3 buffer to suppress silanol ionization or an organic modifier and pH 8 buffer to suppress alkaloid ionization. Anionic ion pairs such as sodium dodecyl sulfate, octane-1-sulfonic acid sodium salt, pentane-1-sulfonic acid sodium salt, and bis(2-ethylhexyl)ortho-phosphoric acid are used to improve peak shape, efficiency, and selectivity. Amines (e.g., diethylamine, triethylamine, and tetrabutylamonium chloride) are incorporated into mobile phases to block surface silanols. The effect of chromatographic conditions on the separation of the investigated alkaloids is analyzed by the comparison of particular densitograms, asymmetry factor, or theoretical plate number. The best efficiency, peak symmetry, and separation selectivity of the investigated compounds is obtained through the addition of amine (especially diethylamine) to the mobile phases.

HPTLC 22

23. Other substances containing heterocyclic nitrogen

97 043 P. KUS*, K. WOJCIK, A. PASEWICZ (*Department of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): The chromatographic behavior of some meta- and para-alkyloxy derivatives of tetraphenylporphyrin. J. Planar Chromatogr. 19, 146-150 (2006). Comparison of the chromatographic behavior of twelve long-alkyl-chain derivatives of tetraphenylporphyrines on alumina and silica gel normal and reversed-phase TLC using several different organic mobile phases. TLC of 12 derivatives of tetraphenylporphyrins on alumina with dichloromethane - cyclohexane 3:7 and 7:13, on silica gel with chloroform - pentane 13:27, chloroform - hexane 9:11, dichloromethane - hexane 11:9, chloroform - cyclohexane 19:21, and dichloromethane - cyclohexane 11:9, and on RP18 with 7 mobile phases. Visual detection of spots.

qualitative identification 23a

97 044 Marzena PODGORNA*, J. DZIEGIELEWSKI (*Institute of Chemistry, Silesian University, 9 Szkolna St, 40-006 Kattowice, Poland): Effect of the structure of selected metalloporphyrins on their chromatographic properties. J. Planar Chromatogr. 19, 48-51 (2006). TLC of tetraphenylporphyrin and its Cu(II) and Ni(II) derivatives on silica gel with carbon tetrachloride - chloroform and on RP18 with methanol - chloroform. Visual detection of spots.

qualitative identification 23a

97 045 C. SULLIVAN, J. SHERMA* (*Department of Chemistry, Lafayette College, Easton, PA 18042, USA; e-mail: sherma@lafayette.edu): Development and validation of an HPTLC densitometry method for assay of caffeine and acetaminophen in multicomponent extra strength analgesic tablets. J. Liq. Chrom. & Rel. Technol. 26, 3453-3462 (2003). HPTLC of caffeine and acetaminophen on silica gel in a saturated twin-trough chamber with ethyl acetate - glacial acetic acid 19:1. Quantification at 254 nm. Diphenhydramine, pseudoephedrine, and acetaminophen were well separated from the caffeine zone. Precision (relative standard deviation) was 1.19 %; limit of detection was 0.2 µg for caffeine and 0.08 µg for acetaminophen; precision of duplicate samples (RSD) ranged from 0.95 to 7.56 %.

quality control, HPTLC, densitometry, quantitative analysis, postchromatographic derivatization 23a

24. Organic sulfur compounds

97 011 Gerda MORLOCK et al., see section 4e

27. Vitamins and various growth regulators

97 046 G. KATAY*, Z. I. NEMETH, E. KATAY, E. TYIHAK (*Plant Protection Institute, Hungarian Academy of Sciences, P. O. B. 102, H-1525 Budapest, Hungary): Identification of 1'-methylascorbigen in broccoli. J. Planar Chromatogr. 19, 139-145 (2006). Analytical OPLC-TLC and preparative TLC of ascorbigen and 1'-methylascorbigen on silica gel with chloroform - methanol 9:1. Visualization by use of Procházka's reagent (reaction with formaldehyde) as derivatization agent. Visual evaluation at 366 nm and by densitometry at 440 nm.

food analysis, densitometry, quantitative analysis 27

28. Antibiotics, Mycotoxins

97 047 Dorota KOWALCZUK*, H. HOPKALA (*Department of Medicinal Chemistry, Medical University, 4 Jaczewskiego Str., Lublin, Poland): Separation of fluoroquinolone antibiotics by TLC on silica gel, cellulose, and silanized layers. J. Planar Chromatogr. 19, 216-222 (2006). TLC of ciprofloxacin monohydrate hydrochloride, enoxacin sesquihydrate, fleroxacin, norfloxacin, pefloxacin dihydrate mesylate, sparfloxacin, and ofloxacin on silica gel, cellulose and RP18 with numerous mobile phases. Best separations were achieved on silica gel with methanol - acetone - 1mol/L citric acid - triethylamine 28:2:2:5, on cellulose with dichloromethane - isopropanol - tetrahydrofuran - 25% ammonia 4:6:3:3, and on RP18 with methanol - 0.07 mol/L phosphate buffer (pH 6) - 10 mmol/L benzyldimethyltetradecylammonium chloride 6:3:1. Detection under UV light at 254 nm was more sensitive than spraying with Dragendorff reagent, Forrest's reagent, 15 % $FeCl_3$ in 2 % HCl, iodic reagent (5 g $FeCl_3$ and 2 g I_2 in 100 mL acetone – 20 % tartaric acid 1:1), 20 % phosphomolybdic acid in 10 % sulfuric acid, and Folin-Ciocalteu reagent.

quality control, qualitative identification 28a

97 048 Irena Maria CHOMA (Department of Chromatographic Methods, M. Curie-Sklodowska University, M. Sklodowska Sq. 3, 20-031 Lublin, Poland): Screening of enrofloxacin and ciprofloxacin residues in milk by HPLC and TLC with direct bioautography. J. Planar Chromatogr. 19, 104-108 (2006). HPTLC and TLC of ciprofloxacin and enrofloxacin on silica gel with dichloromethane - methanol - 2-propanol - 25% aqueous ammonia 3:3:5:2. The plate was developed in a DS chamber to the top and the separation chamber was then uncovered for about 1 cm to enable the solvent to evaporate. In this way the plate was developed continuously for 2 h. Bioautography by immersion of the plate in a microorganism solution (Bacillus subtilis), incubation for 22 h at 37 °C. Visualization by spraying with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution and leaving for approximately 30 min at room temperature.

food analysis, qualitative identification, HPTLC, bioautography 28a

97 049 J. NOWAKOWSKA (Medical University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, Al. Gen. J. Hallera 107, 80-416, Gdansk, Poland): Effect of non-aqueous mobil phase composition on the retention of macrocyclic antibiotics in RP-TLC. J. Planar Chromatogr. 19, 62-67 (2006). TLC of erythromycin, troleandomycin, tylosin, rifamycin B, and rifampicin on RP18 with a wide range (from 0 to 100 %) of mixtures of alcohols with dimethyl sulfoxide or hexamethyldisiloxan in pre-saturated chambers. Visualization by spraying with a 1:4 mixture of concentrated sulfuric acid and methanol followed by heating in an incubator at 120 °C for 10 min.

pharmaceutical research, qualitative identification 28a

97 050 M. VEGA*, Maritza ALVARADO, M. ARANDA (*University of Concepcion, Faculty of Pharmacy, Department of Food Science, Nutrition and Dietetics, P.O. Box 237, Correo 3, Concepcion, Chile. mveha@udec.cl): Monitoring of oxytetracycline dose in medicated salmon feed. CBS 96, 6-7 (2006). HPTLC of oxytetracycline in salmon feed, on silica gel (pre-washed with methanol and dried at 120 °C for 30 min, followed by dipping into 5 % EDTA solution of pH 7.0 and drying at 120 °C for 1 h in an oven) with the organic layer of dichloromethane - methanol - 5 % EDTA 13:4:2 with chamber saturation for 30 min.Quantitative determination by fluorescence measurement at UV 366/>400 nm. Calibration (peak area) was performed via linear regression with r2 of 0.9925. Recovery rates for oxytetracycline at 500, 2500, and 5000 mg/kg were 73 ± 4.2 %, 101 ± 2.6 %, and 101 ± 4.0 %. Intermediate precisions at the same levels were 5.7, 2.6 and 4.0 %. At an application volume of 10 µL LOD was 14.8 mg/kg (n=3) and LOQ was 49.2 mg/kg (n=10). Quantification was achieved between 100 and 10000 mg/kg oxytetracycline in salmon feed due to the selectivity of fluorescence measurement.

food analysis, agricultural, HPTLC, densitometry, quantitative analysis 28a

29. Pesticides and other agrochemicals

97 053 R. S. MALI, B. D. DHONGADE, R. R. KuLKARNI, V. S. PANDAV* (Regional Forensic Science Laboratory, State of Maharashtra, Ganeshkhind, Pune-411007, India): Thin-Layer Chromatography for selective detection of methomyl in forensic casework. J. Planar Chromatogr. 19, 85-86 (2006). TLC of methomyl on silica gel with benzene - ethyl acetate 3:2 in a presaturated TLC chamber. After drying the plate was sprayed with 1 % phloroglucinol solution then with 50 % hydrochloric acid. The plate was heated at 100 °C for 5 min. Detection limit of the pink-violet spot was 5 µg.

toxicology, qualitative identification 29c

97 052 E. PLASS, A. KINAST (*Bayer Industry Services GmbH&Co. OHG, Bayer Chemistry Park, Building C 601, 41538 Dormagen, Germany. ernst.plass.ep@bayerindustry.de: Determination of amitrol in water by AMD. CBS 96, 2-5 (2006). AMD-HPTLC of amitrol in water samples, on LiChrospher silica gel pre-washed by immersion for 8 h in 1 % formic acid in methanol and drying over night in a desiccator. Development with a 18-step gradient from methanol (saturated with ammonia) to tert. butylmethyl ether over 50 mm. Detection by exposure to HCl vapor followed by dipping into a solution of 0.2 g Bratton - Marshall reagent (N-(1-naphtyl)ethylenediam inedihydrochloride) in 100 mL methanol - dichloromethane 1:4. Visual evaluation. Quantitative determination by absorbance measurement at 490 nm. LOD is 1 ng/spot. Linearity is given in the range of 1 - 10 ng amitrol.

environmental, AMD, quantitative analysis, HPTLC, densitometry 29d

97 051 M. MISZCZYK, Alina PYKA* (*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4, Jagiellonska Street, 41-200 Sosnowiec, Poland, alinapyka@wp.pl): Comparison of normal and reversed-phase TLC for separation of selected pesticides. J. Planar Chromatogr. 19, 15-20 (2006). TLC and HPTLC of fifteen urea pesticides (monolinuron, chlorotoluron, diuron, isoproturon, linuron, dimefuron, diflubenzuron, teflubenzuron, lufennuron, thifensulfuron methyl, triasulfuron, chlorsulfuron, rimsulfuron, amidosulfuron, tribenuron methyl) on RP18 with methanol - water and mixed organic (acetonitrile - methanol 1:1) - 0.1 % aqueous orthophosphoric acid mobile phases, and on silica gel with benzene - methanol and benzene - ethanol mobile phases. Relationships between Rf values and mobile phase composition were determined.

environmental, comparison of methods 29f

30. Synthetic and natural dyes

97 055 Nada U. PERISIC-JANJIC*, G. S. USCUMLIC, D. Z. MIJIN (*Department of Chemistry, Faculty of Sciences, Trg D. Obradovica 3, 21000 Novi Sad, Serbia and Montenegro): RP TLC of some newly synthesized azo-dye derivatives. J. Planar Chromatogr. 19, 98-103 (2006). TLC of 11 azo-dye derivatives on RP18 with water - methanol, water - acetone, water - dioxane, and water acetonitrile mobile phases. Detection under UV light at 254 nm. Investigation of the retention mechanism and determination of the retention constants.

qualitative identification 30a

97 054 T. HAYASHI, H. OKA*, Y. ITO, T. GOTO, N. OZEKI, Y. ITAKURA, H. MATSUMOTO, H. OHNO, K. YOSHIDA, T. MIYAZAWA, H. NAGASE (*Aichi Prefectural Institute of Public Health, 7-6 Nagare, Tsuji-machi, Kita-ku, Nagoya 462-8576, Japan; e-mail: hisao_oka@pref.aichi.lg.jp): An HPLC method for the analysis of orange color in food using beta-cryptoxanthin as an indicator. J. Liq. Chrom. & Rel. Technol. 27, 1579-1592 (2004). TLC of an orange color standard before and after saponification (as beta-cryptoxanthin) on RP18 with acetonitrile - acetone - n-hexane 11:7:2 and acetone - water 9:1. Measuring the visible absorption spectra in the range of 370-700 nm by scanning densitometry with its maximum absorption wavelength at 455 nm.

food analysis, qualitative identification, densitometry 30b

32. Pharmaceutical and biomedical applications

97 089 A. GHASSEMPOUR*, M. AHMADI, S. N. EBRAHIMI, D. H. Y. ABOUL-ENEIN (*Department

of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, 19835-389 Tehran, Iran) : Simultaneous determination of metformin and mlyburide in tablets by HPTLC. Chromatographia 64 (1-2), 101-104 (2006). TLC of metformin and glyburide in three different formulations of Glucovance®, on silica gel with water - methanol - ammonium sulfate 4:2:1. Rf value of metformin was 0.43 and of glyburide 0.64. Quantitative determination by absorbance measurement at 237 nm. The linear regression data for the calibration plot showed a good relationship with r = 0.99581 and 0.99982 for metformin and glyburide, respectively. The method was validated for precision and recovery. The limits of detection and quantification were 25 and 84 ng/spot for metformin and 12 and 41 ng/spot for glyburide, respectively. Stability study has been carried out for samples and standard solutions.

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

32

97 059 A. ABOURASHED, J. MOSSA (*Dept. of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia): HPTLC determination of caffeine in stimulant herbal products and power drinks. J. Pharm. Biomed. Anal 36, 617-620 (2004) HPTLC of caffeine in herbal products and energy drinks, on silica gel with ethyl acetate - methanol 17:3. Solid samples (capsules) were extracted with methanol, filtered and applied whereas liquid samples (coca cola) were applied after the effervescence has ceased. Quantitative determination by absorbance measurement at 275 nm. The developed method was validated for specificity, repeatability (CV < 5 %), recovery (98.90) and accurary (99.84). The levels of caffeine were 4.76-13.29 % and 0.011-0.032 % for the herbal products and the energy drinks resp.

pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods 32a

97 061 M. J. ANSARI*,S. AHMAD. K. KOHLI, J. ALI, R. K. KHAR (*Dept. of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard Univ., New Delhi 110062, India): Stability indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. J. Pharm. Biomed. Anal 39, 132-138 (2005). A simple, selective, precise and stability-indicating HPTLC method of analysis of curcumin both as a bulk drug and in formulations was developed and validated. HPTLC on silica gel with chloroform-methanol 37:3. This system was found to give compact spots of curcumin (Rf 0.48). Densitometric analysis of curcumin in the absorbance mode at 430 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r =0.996 and 0.994 via peak height and peak area, respectively, in the concentration range of 50-300 ng per spot. The method was validated for precision, recovery, robustness. The LOD and LOQ were 8 and 25 ng per spot, respectively. Curcumin was subjected to acid and alkali hydrolysis, oxidation and photodegradation.

quality control, traditional medicine, HPTLC densitometry, quantitative analysis 32a

97 063 A. AVACHAT, S. TAMBE, S. KALE* (*Mahatma Gandhi Vidyamandir Pharmacy College, Panchavati, Nasik 422003, MS, India): Characterization of tea-tree Melaleuca alternifolia oil HPTLC fingerprinting. Indian Drugs 42 (11), 731-734 (2005). Hydro-distilled volatile tea-tree oil of Melaleuca alternifolia was investigated by GC-MS, HPTLC, and X-Ray fluorescence. HPTLC on silica gel with toluene - ethyl acetate 93:7. Detection by spraying with anisaldehyde sulphuric acid reagent. Nine well-distinguished peaks were obtained.

herbal, HPTLC, comparison of methods, postchromatographic derivatization, qualitative identification 32a

97 065 V. B. BADGUJAR, P. S. JAIN, G. S. TALELE, S. J. SURANA (*R.C.Patel Coll of Pharmacy, Karvand Naka, Shirpur, Dhule-425405, India): HPTLC method for estimation of carvedilol from tablet formulation. Indian Drugs 42 (8), 511-515 (2005). HPTLC carvedilol in tablets on silica gel with toluene - methanol - ethyl acetate - ammonia 40:10:5:1. Quantitative determination by absorbance measurement at 254 nm. The method was linear in the range of 50-250 µg/µL with recovery of 98.5-100.2 %. Stability was studied during and after development. Accuracy, precision, and specificity of the method were established.

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

97 066 S. BAGADE, N. GOWEKAR*, A. TANKAR, K. KHANDELWAL, A. KASTURE (*Siddhant College of Pharmacy, Sadumbare, Pune, India): Chromatographic and spectrophotometric estimation of ambroxol hydrochloride and cetirizine hydrochloride from tablet dosage form. Abstract GP-55, IPC (2005). HPTLC of methanolic extracts of ambroxol and cetirizine combination tablets, on silica gel with methanol - ethyl acetate - toluene - ammonia 40:15:56:10 drops of ammonia. Quantitative determination by absorbance measurement at 231 nm. Rf values of cetirizine was 0.40 and of ambroxol 0.78, linearity range was 0.4-0.8 µg for cetirizine and 4-10 µg for ambroxol. Recovery was 99.3-100.4 % for both compounds. In comparison with a spectrophotometric method the HPTLC method had the advantage of higher throughput.

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

97 134 E. BODOKI*, R.OPREAN, I. VLASE, M. TAMAS, R. SANDULESCO (*University Med. and Pharm. 400023 Cluj-Napoca, Romania): Fast determination of colchicine by TLC densitometry from pharmaceutical and vegetal extracts. J. Pharm. Biomed. Anal 37 (5), 971-977 (2005). HPTLC of colchicine in Colchicum autumnale (meadow saffron) extracts, on silica gel with chloroform - acetone - diethyl amine 5:4:1. Quantification in absorbance mode at 350 nm. The peaks were optimized in area and shape by varying 4 scanning parameters (slit width and height, number of measurements and scanning speed). Calibration range of pure colchicine was 50-600 ng/mL. This method can be used in pharmaceutical industry for quick and accurate quantitative determination of colchicine because it eliminates the inteferences given by other bioactive or degradation compounds. The method was validated regarding linearity, accuracy, fidelity, and sensitivity.

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

97 163 R. BUSHAN et al., see section 38

97 071 K. BLASZCZAK-SWIATKIEWICZ, E. MIKICIUK-OLASIK*, A. CHOMKA (*Department of Pharmaceutical Chemistry and Drug Analysis, Medical University, Ul. Muszynskiego, Lodz, 90.151, Poland; e-mail: eolasik@farm.am.lodz.pl): Planar chromatography for new quinazoline derivatives. J. Liq. Chrom. & Rel. Technol. 27, 3121-3134 (2004). TLC of six quinazoline derivatives on silica gel with chloroform - ethyl acetate 19:1 and ethyl acetate - acetonitrile 7:13 in a horizontal chamber. Detection under UV light at 254 nm.

pharmaceutical research, qualitative identification 32a

97 085 J. FLIEGER*, M. TATARCZAK, M. WUJEC, M. PITUCHA, H. SZUMILO (*Department of Inorganic and Analytical Chemistry, Medical University of Lublin, Stascica 6, 20-081 Lublin, Poland): RP-TLC determination of the lipophilicity of some new derivatives of 1,2,4-triazole and thiosemicarbazide with potential antituberculosis activity. J. Planar Chromatogr. 19, 32-41 (2006). TLC of 11 new derivatives of 1,2,4-triazole and of 18 new derivatives of thiosemicarbazide on RP18 with mixtures of methanol, acetonitrile, and water. Amounts of organic modifiers were in the range of 20-70 % in 10 % increments. After development in horizontal DS chambers and drying, the plates were visualized under UV light at 254 nm.

pharmaceutical research, qualitative identification 32a

97 137 S. Y. GANDHE, S. V. PIMPLE*, M. A. JOSHI (*Emcure R & D Centre, T-184 MIDC Bhosari, Pune 411026, India): Determination of rutin in Ginkgo Biloba from a solid dosage form by High Performance Thin Layer Chromatography. Indian Drugs 42 (9), 592-596 (2005). HPTLC of rutin in Ginkgo biloba from a solid dosage form, on silica gel with n-butanol - n-propanol - chloroform - acetic acid-water 4:1:2:1:1 containing 1 % formic acid. Quantitative determination by absorbance measurement at 254 nm. Rf value of rutin was 0.24. The method was linear in the range of 30-70 ng/µL. No interference was observed from excipients present in solid dosage form.

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

97 090 C. GIAGINIS, D. DELLIS, Anna TSANTILI-KAKOULIDOU* (*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens 157 71, Greece): Effect of the aqueous component of the mobile phase on RP-TLC retention and its implication on the determination of lipophilicity for a series of structurally diverse drugs. J. Planar Chromatogr. 19, 151-156 (2006). Investigation of the reversed-phase TLC retention behavior of a series of structurally diverse drugs, mostly basic compounds, with different aqueous mobile phase components. Phosphate buffer, phosphate-buffered saline, and morpholinepropanesulfonic acid, with or without the addition of n-decylamine, at pH 7.4, and phosphate buffer at pH 11.0 were used with different portions of methanol as mobil phase. TLC of amitriptyline, chlorpromazine, diltiazem, fluoxetine, nifedipine, nimesulide, norfluoxetine, nortriptyline, phenazine, pindolol, promethazine, propanolol, protriptyline, tioconazole, and trimethoprim on RP18 with phosphate buffer pH 7.4, phosphate-buffered saline, 3-morpholinopropanesulfonic acid pH 7.4, 3-morpholinepropanesulfonic acid + 0.2 % n-decylamine pH 7.4, and phosphate buffer pH 11.0 in pre-saturated chambers. Detection under UV light at 254 nm.

pharmaceutical research, qualitative identification 32a

97 057 S. A. GOSAVI, A. A. SHIRKHEDKAR*, Y. S. JAISWAL, S. J. SURANA (*Department of Pharmaceutical Chemistry, R. C. Patel College of Pharmacy, Karwand Naka, Shirpur-Dhule, M. S.-(425405), India): A simple and sensitive HPTLC method for quantitative analysis of pantoprazole sodium sesquihydrate in tablets. J. Planar Chromatogr. 19, 228-232 (2006). HPTLC of pantoprazole sodium sesquihydrate (5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole) on silica gel after prewashing with methanol in a twin-trough chamber presaturated for 10 min with methanol - water - ammonium acetate 8:2:1. Quantitation by densitometric scanning at 290 nm. The method was validated for linearity, sensitivity, precision, accuracy, specificity, system suitability, ruggedness and robustness, and repeatability.

quality control, HPTLC, densitometry 32a

97 091 O. GROZDANOVIC, D. ANTIC, D. AGBABA* (*Faculty of Pharmacy, Institute of Pharm. Chem and Drug Anal. 11000 Belgrade, Serbia & Montenegro 11000): Development of a HPTLC Method for in-process purity testing of pentoxifylline. J. Sep. Sci. 28 (6), 575-580 (2005). HPTLC of pentoxifylline and related substances, impurities of reaction partners and side reaction products, on LiChrospher RP18 with acetone - chloroform - toluene - dioxane 2:2:1:1. Quantitative determination at 275 nm. Linearity (r2= 0.997), recovery (86.5-115.5 %) and determination limit (0.1-0.6 %) were evaluated and found to be satisfactory. This method enables monitoring of the synthesis as well as purity control of pentoxifylline-containing raw materials and pharmaceuticals.

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

97 093 Rajshree GUDE*, M. PAL, D. VERLEKAR (*Goa College of Pharmacy, Panaji, Goa, India): Development and validation of a new sensitive method for the estimation of tizanidine in tablets by using HPTLC. Abstract GP-41, IPC (2005). HPTLC of tizanidine in tablets on silica gel with ethyl acetate - methanol - acetic acid 60:4:1. Linearity range was 0.5-0.6 µg, LOQ 0.5 µg, and average recovery was 99.4-101.6 %. The method was validated according to ICH guidelines.

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

97 094 K. GUPTA*, S. WANKEDE, S. TAJNE, S. WADODKAR (*Dept. of Pharm Sc, Nagpur University, Nagpur-33) : A High Performance Thin Layer Chromatographic determination of indapamide in tablets. J. Pharmaceutical Research 4 (3), 55-57 (2005). HPTLC of indapamide on silica gel with toluene - methanol 7:3. Quantitative determination by absorbance measurement at 246 nm. The method was linear within the range of 1.4-3.7 µg/mL. Mean recovery values were 99.46-100.29 %. The method was validated for accuracy, precision, and specificity.

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

97 095 K. GUPTA*, S. WANKHEDE, M. TAJNE, S. WADODKAR (*Dept. of Pharm. Scinences, Nagpur University, Nagpur-440033, India): A validated HPTLC determination of fenofibrate. Indian J. Pharm Sciences 67 (6), 762-764 (2005). HPTLC of fenofibrate in methanolic capsule extracts on silica gel with toluene - chloroform 7:3 with chamber saturation for 10 min. Quantitative determination by absorbance measurement at 296 nm. Linearity range was 1.2-3.8 g with recovery of 101.43 %. The proposed validated method was stability indicating and useful for routine analysis.

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

97 109 A. JAMSHIDI*, H. MOBEDI, F. AHMAD-KHANBEIGI (*Department of Novel Drug Delivery Systems, Iran Polymer and Petrochemical Institute, P. O. Box 14185/456, Tehran, Iran): Stability-indicating HPTLC assay for leuprolide acetate. J. Planar Chromatogr. 19, 223-227 (2006). HPTLC of leuprolide acetate (a synthetic nonapeptide analog) on silica gel after prewashing with chloroform - methanol 1:1 using five-step isocratic incremental multiple development with ethyl acetate - methanol - 25 % ammonia. Detection under UV light at 254 and 280 nm. Quantitation by reflectance scanning at 280 nm.

quality control, AMD, HPTLC 32a

97 111 K. JINYVARGHESE, S.T. KARPE AND S.R. KULKARNI* (*Dept. of Pharmacognosy and Phytochemistry, The Bombay College of Pharmacy, Kalina, Mumbai 400098, India): Immuno-

stimulant activity of Adhatoda vasica, Lawsonia inermis and Alkanna tinctoria, TLC fingerprint profile for identification. Indian Drugs 42 (6), 345-352 (2005). TLC fingerprint identification of methanolic extracts of Adhatoda vasica, Lawsonia inermis and Alkanna tinctoria, on silica gel. For Lawsonia inermis and Alkanna tinctoria the developing solvent toluene - acetone - acetic acid 90:10:1 was used, and for Adhatoda vasica n-hexane - acetone - diethyl ether 3:1:1 for. Detection of alkannin by spraying with 10 % methanolic KOH.

pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods 32a

97 112 M. KAMIL*, F. AHMAD, M. A. NAJI (*Department of Pharmacognostic Sciences, Zaed Complex for Herbal Research & Traditional Medicine (ZCHRTM), General Authority for the Health Services for the Emirates of Abu Dhabi, P. O. Box 29300, Abu Dhabi, United Arab Emirates. drkamil2005@yahoo.co.in): Determination for glibenclamide adulteration in herbal drugs. CBS 96, 14-15 (2006). TLC of glibenclamide as adulterant in antidiabetic herbal drugs, on silica gel with toluene - ethyl formate - formic acid 5:4:1 in a saturated twin trough chamber over 150 mm. Detection under UV 365 nm, quantification of the image with VideoScan software. Rf of glibenclamide is 0.58. Results were compared with those obtained by UV spectrophotometry and HPLC and showed good correlation.

quality control, herbal, quantitative analysis, densitometry 32a

97 114 J. KOCHANA*, J. WILAMOWSKI, A. PARCZEWSKI (*Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Cracow, Poland; e-mail: kochana@chemia.uj.edu.pl): TLC profiling of impurities of 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate in MDMA synthesis. Influence of sample preparation methods and conditions. J. Liq. Chrom. & Rel. Technol. 27, 2463-2470 (2004). TLC of 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate product of MDMA (3,4-(methylenedioxy)metham phetamine, also known as ‚ecstasy', and impurities on silica gel in a horizontal chamber with chloroform - ethyl acetate 49:1. Detection of separated impurities under UV light at 254 and 366 nm.

toxicology, qualitative identification 32a

97 115 Dorota KOWALCZUK(Department of Medicinal Chemistry, Medical University, 4 Jaczewskiego st, 20-090 Lublin, Poland): Determination of nitrendipine in tablets by HPTLC-densitometry. J. Planar Chromatogr. 19, 135-138 (2006). HPTLC of nitrendipine on silica gel with n-hexane - ethyl acetate - acetone 6:3:2 in a horizontal chamber. Visualization under UV light. Densitometry was performed in absorbance mode at 335 nm. The calibration plot was constructed in the range 0.025 to 0.150 µg/µL (corresponding to 0.5-3.0 µg/spot) with good correlation (r2> 0.990). The method is also characterized by good precision and accuracy.

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

97 126 G. MAHESHWARI, G. SUBRAMANIAN, A. RANJITH KUMAR, Tara CHAND TAK* (*Dept. of Pharmacy, Manipal College of Pharm. Sci., Manipal, India): Stability indicating HPTLC determination of etoricoxib in formulations. Abstract GP-74 IPC (2005). HPTLC of etoricoxib in tablets on silica gel with toluene - 1,4 dioxane - methanol 17:2:1. Quantitative determination by absorbance measurement at 235 nm. Linearity range was 500-1500 ng/spot. For stability studies the sample was subjected to acid and alkali hydrolysis, thermal, oxidaline and photo degradation.

The peaks of all the degraded products were well resolved with significant different Rf values. The method was validated for different parameters and found suitable for routine quality control.

pharmaceutical research, densitometry, quantitative analysis, HPTLC 32a

97 130 S. MUNDHADA*, P. TATKE (*C.U.Shah College of Pharmacy, SNDT Women's University, Juhu Campus, Santacruz (W), Mumbai 400049, India): Preliminary phytochemical investigation and antimicrobial activity of fruits of Mimusops elengi Linn. TLC/HPTLC fingerprint profile. Indian Drugs 42 (7), 417-423 (2005). Unripe, powdered fruits of Mimusops elengi Linn. extracted individually and successively with acetone and methanol were evaluated for antimicrobial activity. HPTLC on silica gel with toluene - ethyl acetate - chloroform - acetic acid 35:35:28:2. Detection under UV 254 nm, 366 nm and after spraying with anisaldehyde sulphuric acid reagent for qualitative evaluation for different phyto constituents.

pharmaceutical research, quality control, densitometry, comparison of methods, qualitative identification, HPTLC 32a

97 131 M. NOWAK, K. PLUTA* (*Department of Organic Chemistry, The Medicl University of Silesia, Jagiellonska 4, 41-200 Sosnowiec, Poland): Study of the lipophilicity of novel diquinothiazines. J. Planar Chromatogr. 19, 157-160 (2006). Determination of the lipophilicity of twenty new diquinothiazines by reversed-phase thin-layer chromatography on RP18 with acetone-aqueous TRIS (tris(hydroxymethyl)aminomethane) buffer as mobile phase. TLC on RP18 with mixtures of acetone and aqueous TRIS buffer pH 7.4 in pre-saturated chromatographic chambers. Detection by UV 254 nm.

pharmaceutical research, qualitative identification 32a

97 096 B. H. PATEL*, K. M. PATEL, A. M. PRAJAPATI, M. PATEL, D. S. SANKHIA (*Dept. of Pharm. Chem, S. K. Patel College of Pharm. Edu. Research, Vidyanagar, Kherva, Gujarat, India): Development of stabililty indicating HPTLC method for determination of satranidazole in bulk, its formulations and to study degradation kinetics. Abstract GP-75, IPC (2005). HPTLC of satranidazole and its degradation products on silica gel with toluene - acetonitrile 3:2. For stability studies, the sample was treated with NaOH, HCl, H2O2 and photolysis. Degradation products were well resolved with significant different Rf values. The method had a linearity range of 100-500 ng. The proposed method suitable to investigate the kinetics of hydrolysis and photodegradation processes with first order in NaOH, and zero order for photolysis.

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

97 125 K. M. PATIL, S. L. BODHANKAR* (Dept. of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune-411038, India): Validated High Performance Thin Layer Chromatography method for simultaneous estimation of phenytoin sodium, phenobarbitone sodium and carbamazepine in tablet dosage forms. Indian J. Pharm. Sci. 67 (3), 351-355 (2005). HPTLC of carbamazepine, phenytoin, and phenobarbitone in tablets on silica gel with toluene - acetone 5:2. Quantification by absorbance measurement at 217 nm. This method was quantitatively evaluated in terms of linearity, accuracy, precision, repeatability and specificity proving its utility in estimation of drug content in tablet dosage form.

pharmaceutical research, quality control, densitometry, comparison of methods, qualitative identification 32a

97 136 K. M. PATIL, S. L. BODHANKAR* (*Dept.of Pharmacology, Bharati Vidypeeth Deemed University, Poona College of Pharmacy, Pune 411038, MS India): High Performance Thin Layer Chromatography method for therapeutic drug monitoring of anti-epileptic drugs in serum. Indian Drugs 42 (10), 665-670 (2005). HPTLC of carbamazepine, phenytoin, and phenobarbitone extracted with ethyl acetate from human serum, on silica gel with toluene - acetone 5:2. Quantification by absorbance measurement at 217 nm. Rf values were 0.20 for carbamazepine, 0.41 for phenytoin, and 0.49 for phenobarbitone. The linearity (r=0.998) was in the range of 100-2000 ng. LOQ was found to be 30 ng/spot for carbamazepine and 80 ng/spot for phenytoin and phenobarbitone. The accuracy was in the range of 88.5 to 98.1 % and the CV in range of 1.1 to 3.9 %. Intra day and inter day reproducibility was comparable and within the stated limits.

clinical chemistry, research, clinical routine analysis, HPTLC, densitometry, quantitative analysis 32a

97 133 Alina PYKA*, J. SLIWIOK (*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska St., PL-41-200 Sosnowiec, Poland; e-mail: alinapyka@wp.pl): Use of traditional structural descriptors in QSRR analysis of nicotinic acid esters. J. Liq. Chrom. & Rel. Technol. 27, 785-798 (2004). TLC of methyl, ethyl, isopropyl, butyl, hexyl, and benzyl nicotinate on silica gel and a mixture of silica gel and kieselguhr (heated at 120 °C for 20 min) with mixtures of n-hexane and acetone in the volume proportions 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4. Detection under UV light at 254 nm.

pharmaceutical research, qualitative identification 32a

97 138 M. SENTHIL*, G. SUBRAMANIAN, M. VASUDEVAN, S. RAVISANKAR (*Manipal College of Pharmaceutical Sciences, Manipal 576104, India): HPTLC estimation of tizanidine and diclofenac sodium in combination tablets. Indian Drugs 42 (7), 465-468 (2005). HPTLC of tizanidine and diclofenac in tablet formulations on silica gel with chloroform - methanol 4:1. Quantitative determination by absorbance measurement at 230 nm. Cetrizine was used as an internal standard. The solvent system was found to give compact spots for diclofenac sodium (Rf value 0.86), tizanidine (0.26) and cetrizine (0.52). The method was validated for linearity, accuracy and precision. Linearity for tizanidine was 0.6-1.4 µg/mL, and for diclofenac sodium 7.5-17.5 µg/mL . The mean recoveries obtained for tizanidine and diclofenac sodium were 98.73 % and 99.70 %, respectively. The proposed method was accurate, precise, selective and rapid for simultaneous estimation of tizanidine and diclofenac sodium in tablets.

pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods 32a

97 140 Sapna SHRIKUMAR*, M. ATHEM, M. SRIKUMAR, T. RAVI (*Dept. of Pharm Analysis, College of Pharmacy, SRIPS, Coimbatore-641044, India): A HPTLC method for standardization of curculigo orchioidesrhizomes and its marketed formulations using gallic acid as standard. Indian J. Pharm Sciences 67 (6), 721-726 (2005). HPTLC of gallic acid in ethanolic extracts of rhizomes from curculigo orchioides on silica gel with toluene - ethyl acetate - acetic acid 25:15:1. Quantitative determination by absorbance measurement at 260 nm. The method was validated according to ICH guidelines. Rhizomes and their marketed formulation were found to contain 2.5 % and 5.0 % of gallic acid.

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

97 139 S. D. SHANMUGAKUMARAN*, S. AMERJOTHY, K. BALAKRISHNA, M. S. VASANTH KUMAR (*Dept. of Botany, Presidency College, Chennai 600005, India): Antimycobacterial properties of leaf extracts of Pithecellobium dulce. Benth, identification by TLC fingerprint. Indian Drugs 42 (6), 392-395 (2005). Dried leaves of Pithecellobium Dulce. Benth were successively extracted with n-hexane, chloroform and alcohol. Each extract was evaluated for antimycobacterial activity. These extracts were subjected to TLC fingerprint profile for identification on silica gel with chloroform - methanol 9:1.

quality control, pharmaceutical research, qualitative identification, comparison of methods, densitometry 32a

97 143 R. SKIBINSKI, Genowefa MISZTAL*, L. KOMSTA, A. KOROLCZYK (*Department of Medicinal Chemistry, Medical University of Lublin, 4 Jaczewskiego Str., 20-090 Lublin, Poland): The retention behavior of some atypical antipsychotic drugs in normal-phase TLC. J. Planar Chromatogr. 19, 73-80 (2006). TLC of six atypical antipsychotic drugs (amisulpride, clozapine, olanzapine, quetiapine, risperidone, ziprasidone) on silica gel, amino, cyano, DIOL, and polyamide phases with mixtures of n-hexane and six polar modifiers (acetone, dioxane, diethylamine, ethanol, isopropanol, and tetrahydrofuran) in a horizontal DS chamber. After development the plates were inspected under UV light at 254 nm. Quantification by densitometry.

pharmaceutical research, qualitative identification, densitometry, quantitative analysis 32a

97 144 P. SOLAIRAJ, A. BHAT, Suvarna KINI, R. GOVINDARAJAN*, R. VENKATRAMAN (*Pharmacognosy & Ethnopharmacology Div., National Botanical Research Institute, Lucknow 226001, India): HPTLC method for the estimation of fexofenadine HCl in tablet dosage form. Indian Drugs 42 (7), 424-427 (2005) HPTLC of fexofenadine HCl from tablet dosage form on silica gel with dichloromethane - methanol 13:7. Quantitative determination by absorbance measurement at 260 nm. The linear detector response was observed between 0.2 and 1.0 µg. The method was validated to determine its accuracy and precision. The LOD was found to be 0.08 ng/µL, LOQ was 0.02 ng/µL. The recovery was carried out by standard addition method and was found to be 100.82 %.

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

97 087 S. G. TALELE, G. S .TALELE*, P.S .JAIN, V. B. BADGUJAR, S. J. SURANA (*R.C.Patel College of Pharmacy, Karvand Naka, Shirpur, Dt. Dhule- 425405, MS, India): Validated HPTLC Method for estimation of desloratadine from tablets formulations. Indian Drugs 42 (10), 671-674 (2005). HPTLC of desloratadine on silica gel with methanol - n-butanol - water - toluene - glacial acetic acid 20:30:10:20:1. Quantification in absorbance mode at 254 nm. The HPTLC system was quantitatively evaluated in terms of stability, precision, repeatability, specificity, accuracy and calibration, and was suitable for the analysis of desloratadine tablet dosage form. The linearity was in the range of 30-150 µg/mL with recovery of 98.8-102.0 %

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

97 097 M. H. VEGA*, E. T. JARA, M. B. ARANDA (*Department of Food Science, Nutrition and Dietetics, Faculty of Pharmacy, University of Concepcion, Barrio Universitario s/n Casilla 237, PO 403-0249 Concepcion, Chile): Monitoring the dose of florfenicol in medicated salmon feed by planar chromatography (HPTLC). J. Planar Chromatogr. 19, 204-207 (2006). HPTLC of florfe-

nicol on silica gel in a twin-trough chamber with ethyl acetate - n-hexane 4:1. Quantitative determination by absorbance measurement at 223 nm. Linearity range of calibration curve was 20 -80 ng with a correlation coefficient r2 of 0.9987. Limit of detection was 2.55 mg / kg and limit of quantification was 8.50 mg / kg. Recovery was 101.7 % at 50 mg /kg, 85.2 % at 500 mg / kg and 81.9 % at 1500 mg / kg. Precision was evaluated based on intra-laboratory dispersion or repeatability. RSD for 50, 500, and 1500 mg / kg was 2.30, 2.72, and 3.57 % respectively.

food analysis, quality control, HPTLC, quantitative analysis 32a

97 058 J. K. VERMA*, A. V. JOSHI (*Dept. of Chemistry, K.J.Somaiya College of Sc & Comm, Vidyavihar, Mumbai 400077, India): HPTLC method for determination of ursolic acid from Oscimum sanctum Linn (Tulsi) leaves and its formulations. Indian Drugs 42 (10), 650-653 (2005). A simple rapid, precise and cost-effective HPTLC method has been developed for the determination of ursolic acid in Oscimum sanctum (Tulsi) leaves and its formulations (Tulsi ghan tablets and Tulsi capsules). HPTLC on silica gel with toluene - ethyl acetate - acetic acid 30:3:1. Detection with anisaldehyde in sulphuric acid reagent followed by heating in an oven at 110 °C. Quantitative determination by absorbance measurement at 580 nm. Linearity of the detector response was given in the range of 40 - 280 ng. LOD was 8 ng. The correlation coefficient obtained from linearity was 0.9985. The standard error was 26.511. The mean assay values of ursolic acid wa found to be 3.485 mg/g, 0.553 mg/g and 3.221 mg/g in tulsi ghan tablets, tulsi capsule and tulsi leaves respectively.

traditional medicine, quality control, HPTLC, densitometry, quantitative analysis, postchromatographic derivatization 32a

97 148 C. VINODHINI, A. S.KALIDOSS*, V. VAIDHYALINGAM (*Dept. of Pharmaceutical Chemistry, Madras Medical College, Chennai 600003, India): Simultaneous estimation of cinnarizine and domperidone by High Performance Thin Layer Chromatography in tablets. Indian Drugs 42 (9), 600-603 (2005). HPTLC of cinnarizine and domperidone in tablets, on silica gel with toluene - ethyl acetate - methanol 14:1:5. Quantitative determination by absorbance measurement at 271 nm. R_f values of cinnarizine was 0.85 and of domperidone 0.4. Linearity was observed in the range of 0.1-0.4 for cinnarizine and 0.075-0.3 µg/µL for domperidone. The recoveries were in the range of 98.95-100.25 %. The tablet matrix did not interfere with the assay.

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

97 086 S. G. WALODE*, M. S. CHARDE, M. R. TAJNE, A. V. KASTURE (*Dept. of Pharm. Sciences, Nagpur University Campus, Amravati Road, Nagpur 440033, India): Development of HPTLC Method for simultaneous estimation of captopril and hydrochlorothiazide in combined dosage form. Indian Drugs 42 (6), 340-344 (2005). HPTLC of captopril and hydrochlorothiazide in tablets on silica gel with methanol - toluene - ethyl acetate - glacial acetic acid 2:12:6:1. Quantitative determination by absorbance measurement at 219 nm. The Rf value of hydrochlorothiazide was 0.38 and of captopril 0.57. The calibration curve response was 4-14 µg for both drugs. Recovery was determined by standard addition method. The percent recovery by area was found to be 100.25 for captopril and 99.98 for hydrochlorothiazide. The method was suitable for routine quality control of such formulations.

pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods 32a

97 154 Savita YADAV, Deepali MHASKE, A. KAKAD, B. PATIL, S. KADAM, S. DHANESHWAR* (*Dept. of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune 411038, India): A simple and sensitive HPTLC method for the determination of content uniformity of atorvastatin calcium tablets. Indian J. Pharm. Sci. 67 (2), 182-186 (2005). HPTLC of atorvastatin calcium in its commercial single component tablet formulations (10 mg/tablet), on silica gel with benzene-methanol 7:3. The Rf value was 0.46. Quantitative determination by absorbance measurement at 281 nm. The method was validated in terms of linearity (200-600 ng/spot), precision (intraday variation: 0.25 - 1.01 %, interday variation: 0.21 - 0.88 %), accuracy and specificity. The LOD for atorvastatin calcium was 40 ng/spot, the LOQ was 200 ng/spot. The proposed method was successfully applied to determine atorvastatin calcium content of 10 individual tablet units of two market formulations after extracting atorvastatin calcium with methanol. All formulations were compliant with USP specifications (RSD less than or equal to 6 %) of the content uniformity test. The proposed HPTLC method can analyse ten or more formulation units simultaneously on a single plate and provides a faster and cost effective quality control tool for routine analysis of atorvastatin calcium formulation.

pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, HPTLC 32a

97 102 I. HAZAI (Department of Pharmacokinetics, IVAX Drug Research Institute Ltd., P. O. Box 82, 1425 Budapest, Hungary): Thin-layer radiochromatographic investigation of denaverine metabolism in the rat. J. Planar Chromatogr. 19, 42-47 (2006). TLC of 14C metabolites of denaverin (2,2-diphenyl-2-(2-ethylbutoxy)acetic acid-2-(dimethylamino)-ethyl ester) on silica gel with chloroform - cyclohexane - methanol - ammonia 100:70:30:3. Detection with autoradiographic films after exposure for one week. Quantitation after scraping the adsorbent from the plate followed by determination of radioactivity by LSC.

pharmaceutical research, radioscanning, quantitative analysis 32b

97 135 A. RAMIC, Marica MEDIC-SARIC*, S. TURINA, I. JASPRICA (*Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, 10 000 Zagreb, Croatia): TLC detection of chemical interactions of vitamins A and D with drugs. J. Planar Chromatogr. 19, 27-31 (2006). Use of TLC to investigate possible chemical interactions of vitamins A and D with frequently used therapeutics (estrogens and progestins, corticosteroids, HMG CoA reductase inhibitors, vitamins, and non-steroidal anti-inflammatory drugs). Concentrations of vitamins and drugs applied to the plates were adjusted to mimic the doses usually prescribed in therapy. TLC on silica gel with cyclohexane - ether 1:1, 17:3, and ethyl acetate. The strength of interaction was measured as a surface below or above a distorted part of the sample band, visible under UV light at 254 nm or after exposing the plates to iodine vapor.

pharmaceutical research, qualitative identification 32b

97 060 H. AGRAWAL*, N. KAUL, A.R. PARADKAR, K.R. MAHADIK (*Department of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune 411038, India): Stability indicating HPTLC determination of clopidogrel bisulphate as bulk drug and in pharmaceutical dosage form. Talanta 61 (5), 581-589 (2003). TLC of clopidogrel bisulphate on silica gel with carbon tetrachloride - chloroform - acetone 12:8:3. Rf value of clopidogrel bisulphate was 0.30. Clopidogrel bisulphate was subjected to acid and alkali hydrolysis, oxidation, photodegradation and dry heat treatment. The drug was susceptible to acid - base hydrolysis, oxidation and dry heat degradation. Also the degraded products were well se-

parated from the pure drug with significantly different Rf values. Quantitative determination by absorbance measurement at 230 nm. The linear regression data for the calibration plots showed good linear relationship with r2=0.999 in the concentration range of 200-1000 ng. The mean value of correlation coefficient, slope and intercept were 0.999±0.001, 0.093±0.011 and 8.83±0.99, respectively. The method was validated for precision, accuracy, ruggedness and recovery. The limits of detection and quantitation were 40 and 120 ng per spot, respectively.

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

97 062 Sandra APERS*, Tania NAESSENS, L. PIETERS, A. VLIETINCK (*Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Antwerp, Belgium): Densitometric thin-layer chromatographic determination of aescin in a herbal medicinal product containing Aesculus and Vitis dry extracts. J. Chromatogr. A 1112 (1-2), 165-170 (2006). HPTLC of a herbal medicinal product containing 250 mg of Aesculus hippocastanum dry extract, 120 mg of Vitis vinifera dry extract and 50 mg of excipients. After purification with C18 SPE cartridges, HPTLC on silica gel with the upper layer of a mixture of acetic acid – water – butanol 1:4:5. Detection by spraying with anisaldehyde reagent followed by heating the plate for 5–10 min at 100–105 °C. Quantitative determination by measurement at 535 nm. The method was developed to analyze the total saponin content (also referred to as the aescin content) and is applicable for the quality control and stability investigation of both the Aesculus dry extract and HMP capsules thereof containing Vitis dry extract in combination with the Aesculus dry extract. The method was validated according to the International Conference on Harmonization (ICH) guidelines. The proposed assay method is specific for aescin in the presence of Vitis dry extract and formulation excipients. Analysis of stressed samples in forced degradation tests proves the method to be applicable for stability evaluation. The standard aescin curve is linear (r > 0.99) over a concentration range of 0.16-0.80 µg/spot. Recovery from the HMP capsules is statistically equal to 100 %. The precision of the method with respect to time and concentration is acceptable, with relative standard deviation values of 1.28 and 1.49 %, respectively.

pharmaceutical research, herbal, HPTLC, densitometry, quantitative analysis, qualitative identification, Aesculus hippocastanum 32c

97 069 L. I. BEBAWY (National Organization for Drug Control and Research, 6 Hussen Kamal el Deen, Ben-el-sariat, Dokki, Giza 12311, Egypt): Stability-indicating methods for the determination of linezolid in the presence of its alkaline-induced degradation products. Talanta 60 (5), 945-953 (2003). TLC of linezolid from its alkaline degradation product on silica gel with isobutanol - ammonia 9:1. Quantitative determination by densitometric measurement at 244 nm. The proposed method and two other methods (based on spectrophotometry) were successfully applied to the determination of the drug in bulk powder, in laboratory prepared mixtures with its degradation product and in commercial tablets.

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

97 072 Y. CHEN (Chen Yong)*, H. ZHEN (Zhen Hanshen), Y. LI (Li Yuehua), B. LIU (Liu Baocun), ZH. XIE (Xie Zhen), Q LIU (Liu Qing), H. XIN (Xin Hua) (*Coll. Pharm., Guangxi Acad. TCM, Guangxi, Nanning 530001 China): (Quality study of the products obtained from Hainan holly by different processing procedures) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (7), 786-790 (2005). TLC of Hainan holly on silica gel with 1) ethyl acetate - butanone - formic acid -

water 5:3:1:1; 2) ethyl acetate - formic acid - water 14:5:5; 3) benzene - acetone - methanol - formic acid 85:15:10:2. Detection 1) by spraying with 1 % $AlCl_3$ solution; 2) under UV 365 nm; 3) by spraying with 5 % phosphomolybdic acid in ethanol followed by heating at 105 ºC until the spots are visualized. Identification by fingerprint techniques. Quantification of rutin by HPLC. Discussion of the optimum processing procedure by comparison of TLC fingerprints and HPLC results of rutin content.

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

97 088 J. GAO (Gao Jianfeng)*, SH. SUN (Sun Shoujing), ZH. SUO (Suo Zheng) (*Shandong Provin. Ankang Hosp., Jining, Shandong, 272051, China): (Separation and simultaneous identification of the component drugs, liquorice and Albizia julibrissin Durazz flowers, in Anshen compound oral liquid by thin-layer chromatography) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (6), 740-742 (2005). TLC of liquorice and Albizia julibrissin Durazz flowers on silica gel with cyclo hexane - ethyl acetate - acetic acid 17:3:1. Detection by spraying with 5 % solution of vanillin - H_2SO_4 followed by heating until the spots are visualized. Identification by fingerprint technique.

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

97 110 Q. JIANG (Jiang Qing)*, R, YIN (Yin Rongli), Y. HU (Hu Youdan), L. ZHONG (Zhong Ling), (*Chengdu University TCM, Sichuan, Chengdu 611730, China): (Determination of chenodeoxycholic acid in Hedan tablets by thin-layer chromatography) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (7), 854-856 (2005). TLC of chenodeoxycholic acid in Hedan tablets on silica gel with n-hexane - ethyl acetate - acetic acid - methanol 20:25:2:3. Detection by spraying with 10 % H_2SO_4 in ethanol followed by heating at 105 ºC until the spots are visualized. Quantification of chenodeoxycholic acid by densitometry at 375 nm. Validation of the procedure by investigation of its linearity range (0.47 - 2.33 µg/spot, R = 0.9992); of its repeatability (3.3 %, n = 5); of its precision (3.9 %, n = 5 within plate and 4.7 % n= 5 plate-to-plate); and its standard addition recovery (98.4%, RSD = 2.5 %, n = 5).

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

97 104 SH. HU (Hu Shuang)*, H. DING (Ding Hong), Y. DU (Du Yan) (*Pharm. Coll., Shangxi Univ. Med., Shanxi, Taiyuan 030001, China): (Study of the quality control of Yixuning tablets) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (7), 769-772 (2005). TLC of the extracts of Yixuning tablets on silica gel with 1) ethyl acetate - chloroform - formic acid 15:30:1; 2) toluene - ethyl acetate - formic acid 90:5:2; 3) cyclo hexane - ethyl acetate 9:1. Detection under UV 365 nm. Identification by fingerprint techniques. Quantification by HPLC.

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

97 123 B. LIU (Liu Bonian)*, R. XU (Xu Ruilin) (*Shanghai Research Center of Sport Health, Shanghai 201100, China): (Study of the quality standard of Ganlu Xiaodu capsules) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (7), 859-861 (2005). TLC of Ganlu Xiaodu capsules on silica gel with chloroform - methanol - water 185:15:2. Detection under UV 254 nm. Identificati-

on by fingerprint techniques and by HPLC. Quantification of scutellarin by HPLC.

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

97 127 X. MAO (Mao Xiuhong)*, SH. JI (Ji Shen), X. BAI (Bai Xiaochun) (*Shanghai Municip. Inst. Drug Cont., Shanghai 200233, China): (Study of the quality standard for Yupingfeng oral liquid) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (6), 659-662 (2005). TLC of extracts of Yupingfeng oral liquid on silica gel with 1) cyclohexane - ethyl acetate 7:3. Detection by spraying with 5 % p-dimethylaminobenzaldehyde in H_2SO_4 - ethanol 1:9 and heating at 105 ºC until the spots are visualized. Identification by fingerprint techniques. Quantification of astragaloside by HPLC. The results for eight real life samples are given.

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

97 132 X. PENG (Peng Xia)*, C. CHEN (Chen Caiyi), Y. LIN (Lin Yanfang) (*Xishuangbanna Inst. Drug Cont., Yunnan, Xishuangbanna 666100, China): (Study of the quality standard for Qiwei Ketengzi pills) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (7), 780-782 (2005). TLC of Qiwei Ketengzi pills on silica gel with benzene - methanol - formic acid 180:30:2. Detection by spraying with 5 % vanillin - sulfuric acid solution followed by heating at 105 ºC until the spots are visualized. Identification by fingerprint techniques. Quantification of vitexicarpin by HPLC. The results for three batches of real life samples are given.

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

97 155 CH. YANG (Yang Chengxong)*, J. LU (Lu Jinqing), J. XIA (Xia Jiwei), X. YANG (Yang Xixong), L. FU (Fu Lianqun) (*Hubei Provin. Jingmen No.2 People's Hosp., Hubei, Jingmen 448000, China): (Determination of cinnamyl aldehyde in Chongcao Yangshen capsules by thin-layer chromatography) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27(5), suppl. 1-3 (2005). TLC of cinnamyl aldehyde in Chongcao Yangshen capsules on silica gel with petroleum ether (60 - 90 ºC) - ethyl acetate 6:1. Detection by spraying with 2,4-dinitrophenylhydrazine reagent. Quantification by densitometry at 514 nm. Validation of the method by investigation of its linearity range (0.0.3 µg - 2.5 µg, r = 0.99); precision (RSD = 1.1 %, n = 5); its reproducibility of five time assay towards the same sample (RSD = 1.2 %); standard addition recovery (99.9 %, RSD = 1.3 %, n = 5). The results for three batches of real life samples are given. Discussion of the application of the procedures for the quality control of the medicine.

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

97 156 R. ZHANG (Zhang Fengrui) (Changchun Coll. TCM, Changchun 130117, China): (Study of the quality standard for Suzi Jiangqi pills) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27(7), 775-777 (2005). TLC of extracts of Suzi Jiangqi pills on silica gel with 1) petroleum ether (60–90 ºC) - ethyl acetate 9:1; and 2) chloroform - ethyl acetate - methanol - water 15:40:22:10. Detection 1) under UV 365 nm; 2) 10 % H_2SO_4 in ethanol followed by heating until the spots are visualized. Identification by fingerprint techniques. Quantification of hespiridin by HPLC. The results for five batches of real life samples are given. Discussion of the application of the procedures for the quality control of the medicine.

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

97 159 SH. ZHAO (Zhao Shaohua)*, G. HAN (Han Guiru), H. XU (Xu Honghui), X. LI (Li Xiaoyan) (*Hebei Yiling Inst. Med., Hebei, Shijiazhuang 050035, China): (Determination of ecdultin in Bazi Bushen capsules by thin-layer chromatography) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (7), 783-785 (2005). TLC of ecdultin in Bazi Bushen capsules on silica gel with benzene - ethyl acetate 30:1. Detection under UV 365 nm. Quantitative determination of ecdultin by fluorescence measurement at 320 nm. Validation of the procedure by investigation of the optimum excitation wavelength; linearity range (0.022 - 0.13 µg/spot, R = 0.9998); repeatability (1.5 %, n = 6); precision (0.87 %, n = 6 within plate and 1.42 %, n= 6 plate-to-plate); and standard addition recovery (98.7 %, RSD = 1.83 %, n = 6). The results for six batches of real life samples are given.

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

97 056 M. A. ABBASI, V. U. AHMAD*, M. ZUBAIR, N. FATIMA, U. FAROOQ, S. HUSSAIN, M. A. LODHI, M. I. CHOUDHARY (*H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan; e-mail: vuahmad@cyber.net,pk) : Phosphodiesterase and thymidine phosphorylase-inhibiting salirepin derivatives from Symptocos racemosa. Planta Med. 70, 1189-1194 (2004). Preparative TLC of the new glycosides symploside and symploveroside on silica gel with methanol - acetone - chloroform 1:50:149 and 1:68:131. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 149 A. W. ANDAYI, A. YENESEW*, S. DERESE, J. O. MIDIWO, P. M. GITU, O. J. I. JONDIKO, H. AKALA, P. LIYALA, J. WANGUI, N. C. WATERS, M. HEYDENREICH, M. G. PETER (*Department of Chemistry, University of Nairobi, P. O. Box 30197, Nairobi, Kenya. ayenesew@nonbi.ac.ke): Antiplasmodial flavonoids from Erythrina sacleuxii. Planta Med. 72, 187-189 (2006). Preparative TLC of shinpherocarpin, 7,4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene, and 7-hydroxy-4'-methoxy-3'-prenylisoflavone (5-deoxy-3'-prenylbiochanin A) on silica gel with hexane - dichloromethane 3:7 (multiple development). Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 064 Chiara BACCELLI*, S. BLOCK, B. VAN HOLLE, A. SCHANK, D. CHAPON, B. TINANT, L. VAN MEERVELT, N. MOREL, J. QUETIN-LECLERCQ (*Laboratoire de Pharmacognosie, Unité d'Analyse Chimique et Physico-Chimique des Médícaments, Université Catholique de Louvain, UCL 72.30-CHAM, Av. E. Mounier 72, 1200 Bruxelles, Belgium; e-mail: chiara.baccelli@cham.ucl.ac.be): Diterpenes isolated from Croton zambesicus inhibit KCl-induced contractions. Planta Med. 71, 1036-1039 (2005). Preparative TLC of ent-18-hydroxytrachyloban-3beta-ol on silica gel with toluene - ethyl acetate - acetonitrile 5:2:3 and 40:9:1. Visualization by spraying with anisaldehyde - sulfuric acid reagent followed by heating at 105 °C for 5 min.

traditional medicine, herbal, preparative TLC 32e

97 067 C.H. BAGGIO, G. DE MARTINI OTOFUJI, W. M. DE SOUZA, C. A. DE MORAES SANTOS, L. M. B. TORRES, L. RIECK, M. DE ANDRADE MARQUES, Sonia MESIA-VELA* (*Depart-

ment of Pharmacology, Biological Science Sector, Universidade Federal of Paraná, UFPR, Centro Politecnico, Caixa Postal 19031, Jardim das Americas, CEP 81531-990, Curitiba-PR, Brazil; sm2418@columbia.edu): Gastroprotective mechanisms of indole alkaloids from Himatanthus lancifolius. Planta Med. 71, 733-738 (2005). Preparative TLC of (+)-ulein on silica gel with n-hexane - dichloromethane - methanol - diethylamine 25:20:4:1 and n-hexane - ethyl acetate - methanol - diethylamine 25:20:4:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 068 S.-P. BAI (Su-Ping Bai), Q.-Y. WEI (Qing-Yi Wei), X.-L. JIN (Xiao-Ling Jin), Q. -X. WU (Quan-Xiang Wu), L. YANG* (Li Yang) (*National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, Gansu 730 000, China; e-mail: yangl@zu.edu.cn): Two novel ent-kauranoid deterpeneoids from Isodon japonica leaves. Planta Med. 71, 764-769 (2005). Preparative TLC of 9 known diterpenoids and shikokianin and rabdoternin A on silica gel by two-fold development with chloroform - methanol 30:1, and of rabdosichuanin and lasiokaurin with chloroform - acetone 6:1. Detection under UV light at 254 nm.

traditional medicine, , herbal, preparative TLC 32e

97 070 P. BHANDARI, A. P. GUPTA, B. SINGH*, V. K. KAUL (*Natural Plant Products Division, Institute of Himalayan Bioresource Technology, Post Bag No. 06, Palampur-176 602, (HP), India): HPTLC determination of swertiamarin and amarogentin in Swertia species from the western Himalayas. J. Planar Chromatogr. 19, 212-215 (2006). HPTLC of swertiamarin and amarogentin on silica gel in a saturated twin-trough chamber with ethyl acetate - methanol - water 77:8:8. Detection under UV light. Quantitation in reflectance/absorbance mode at 235 nm.

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

97 073 J.-J. CHEN* (Jih-Jung Chen), C.-Y. DUH (Chang-Yih Duh), J.-F. CHEN (Jinn-Fen Chen) (*Department of Pharmacy, Tajen Institute of Technology, Pingtung, Taiwan 907, Republic of China; e-mail: jjchen@ccsun.tajen.edu.tw): New cytotoxic biflavonoids from Selaginella delicatula. Planta Med. 71, 659-665 (2005). Preparative TLC of robustaflavon 7,4',4'''-trimethyl ether on silica gel with chloroform - methanol 3:1, of robustaflavone 4',4'''-dimethyl ether on RP18 with methanol - water 6:1, of 2,3-dihydroamentoflavone 7,4',7'''-trimethyl ether on silica gel with ethyl acetate - methanol 4:1, of 2,3-dihydroamentoflavone-7,4'-dimethyl ether on RP18 with methanol - water 8:1, and of 2'',3''-dihydroisocryptomerin 7-methyl ether on silica gel with chloroform - methanol 3:1. Detection under UV light at 254 nm.

herbal, traditional medicine, preparative TLC 32e

97 074 J. CHEN* (Jih-Jung Chen), E. CHOU (En-Tzu Chou), C. DUH (Chang-Yih Duh), S. YANG (Sheng-Zehn Yang), I. CHEN (Ih-Sheng Chen) (*Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung, Taiwan 907, China. jjchen@mail.tajen.edu.tw): New cytotoxic tetrahydrofuran- and dihydrofuran-type lignans from the stem of Beilschmiedia tsangii. Planta Med. 72, 351-357 (2006). Analytical and preparative TLC of ergosta-4,6,8(14),22-tetraen-3-one, beta-sitosterone, 2,6,11-trimethyldodeca-2,6-10triene, and stigma-4-ene-3,6-dione on silica gel with n-hexane - ethyl acetate 6:1. Preparative TLC of tsangin A with dichloromethane - acetone 25:1; of tsangin B and beilschmien C with dichloromethane - acetone 20:1; of beilschmien A and B with n-hexane - ethylacetate 2:1. Detection under UV light at 254 nm.

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

97 075 J.-J. CHEN* (Jih-Jung Chen), H.-Y. FANG (Hui-Yu Fang),C. Y. DUH (Chang-Yih Duh), I.-S. CHEN (Ih-Sheng Chen) (*Department of Pharmacy, Tajen Institute of Technology, Pingtung, Taiwan 907, China; e-mail: jjchen@ccsun.tajen.edu.tw): New indolopyridoquinazoline, benzo(e)phenanthridines and cytotoxic constituents from Zanthoxylum integrifolium. Planta Med. 71, 470-475 (2005). Analytical and preparative TLC of three new alkaloids, 7,8-dehydro-1-methoxyrutaecarpine, isodecerine and 8-demethyloxychelerythrine together with 16 known compounds on silica gel with n-hexane - ethyl acetate 5:3, chloroform - ethyl acetate 25:1, and chloroform - methanol 25:1. Detection under UV light at 254 nm.

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

97 076 J.-J. CHEN* (Jih-Jung Chen), I.-S. CHEN (Ih-Sheng Chen), C.-Y. DUH (Chang-Yih Duh) (*Department of Pharmacy, Tajen Institute of Technology, Pingtung, Taiwan 907, China; e-mail: jjchen@ccsun.tajen.edu.tw): Cytotoxic xanthones and biphenyls from the root of Garcinia linii. Planta Med. 70, 1195-1200 (2004). Analytical and preparative TLC of the new xanthones linixanthone A, B, C, garcibiphenyl A and B and garcibenzopyran on silica gel with n-hexane - ethyl acetate 5:1 and 10:3, and chloroform - methanol 10:1 and 5:1. Detection under UV light at 254 nm.

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

97 077 H.-C. CHOU (Hsueh-Chun Chou), J.-J. CHEN (Jih-Jung Chen), C.-Y. DUH (Chang-Yih Duh), T.-F. HUANG (Tur-Fu Huang), I.-S. CHEN* (Ih-Sheng Chen) (*Graduate Institute oF Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan 807, China; e-mail: m635013@kmu.edu.tw) : Cytotoxic and anti-platelet aggregation constituents from the root wood of Melicope semecarpifolia. Planta Med. 71, 1078-1081 (2005). Preparative TLC of melicopone acetophenone derivative [1,2-bis(4-hydroxy-3-methoxyphenyl)ethanone] and 29 known compounds on silica gel with dichloromethane - ethyl acetate 5:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 078 M. CURINI, F. MALTESE, Maria Carla MARCOTULLIO*, L. MENGHINI, R. PAGIOTTI, O. ROSATI, G. ALTINIER, A. TUBARO (*Dipartimento di Chimica e Tecnologia di Farmaco, Sez. Chimica Organica, Università degli Studi, Via del Liceo 1, 06123 Perugia, Italy; e.mail: marcotu@unipg.it): Glaucopines A and B, new cyathane diterpenes from the fruiting body of Sarcodon glaucopus. Planta Med. 71, 194-196 (2005). Analytical TLC of glaucopines A and B on silica gel with dichloromethane - methanol 19:1. Detection by spraying with 50 % sulfuric acid.

traditional medicine, herbal, qualitative identification 32e

97 079 S.-J. DAI (Sheng-Jun Dai), Z.-M. MI (Zhong-Mao Mi), Z.-B. MA (Zhi-Bo Ma), S. LI (Shuai Li), R.-Y. CHEN (Ruo-Yun Chen) D.-Q. YU* (De-Quan Yu) (*Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, 1 Xian Nong Tan Street, Beijing 100050, China; e-mail: dqyu@imm.ac.cn): Bioactive Diels-Alder type adducts from the stem bark of Morus macroura. Planta Med. 70, 758-763 (2004). Preparative TLC of two new compounds, guangsangon A and guangsangon B, together with the known products kuwanon X, P, and Y on silica gel and RP18 with chloroform - methanol 7:3. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 080 Y. DENG (Yanshen Deng), R. A. NICHOLSON (Department of Biological Sciences, Simon Fra-

ser University, 8888ZUniversity Drive, Burnaby, British Columbia, V5A 1S6, Canada; e-mail: nicholso.@sfu.ca): Antifungal properties of surangin B, a coumarin from Mammea longifolia. Planta Med. 71, 364-365 (2005). Analytical TLC of surangin B on silica gel with chloroform - toluene 1:1 and toluene - acetone 9:1. Detection under UV light at 254 nm.

traditional medicine, herbal, qualitative identification 32e

97 081 K. DHALÖWAL, Y. S. BIRADAR, M. RAJANI* (*B. V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Thaltej-Gandhinagar Hwy, Thaltej, Ahmedabad 380 054, Gujarat, India. rajanivenkat@hotmail.com) : High-Performance Thin-Layer Chromatography densitometric method for simultaneous quantitation of phyllanthin, hypophyllanthin, gallic acid, and ellagic acid in Phyllanthus amarus. J. Assoc. Off. Anal. Chem. 89, 619-623 (2006). HPTLC of phyllanthin, hypophyllanthin, gallic acid, and ellagic acid on silica gel with toluene - ethyl acetate - formic acid 6:2:1 at 25 +/- 2 °C and 40 % relative humidity. Quantitation by densitometry at 280 nm. The method was validated for precision (0.54, 0.93, 0.08, and 1.06 %, respectively), repeatability (1.01, 0.79, 0.98, and 1.06 %, respectively), and accuracy, determined by a recovery study at 3 different levels (99.09 %, 99.27 %, 98.69 %, and 100.49 %, respectively).

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

97 082 N. DUARTE, N. GYEMANT, P. M. ABREU, J. A. MOLNAR, Maria-José U. FERREIRA* (*CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forcas Armadas, 1600-083 Lisbon, Portugal. mjuferreira@ff.ul.pt): New macrocyclic lathyrane diterpenes, from Euphorbia lagascae, as inhibitors of multidrug resistance of tumour cells. Planta Med. 72, 162-168 (2006). Preparative TLC of isofraxidin, latilagascene A, ent-16alpha,17-dihydroxykauran-3-one on silica gel with chloroform - methanol 9:1 (2 x). Visual detection under UV light at 254 nm or by spraying with sulfuric acid - acetic acid - water 1:20:4 or sulfuric acid - water 1:1 followed by heating.

traditional medicine, herbal, preparative TLC 32e

97 083 J. E. S. A. DE MENEZES, T. L. G. LEMOS, Otília DEUSDENIA, L. PESSOA*, R. BRAZ-FILHO, R. C. MONTENEGRO, D. V. WILKE, L. V. COSTA-LOTUFO, C. PESSOA, M. O. DE MORAES, E. R. SILVEIRA (*Departamento de Química Organica e Inorganica, Centro de Ciencias, UniveRsidade Federal de Ceará, Caixa Postal 12 200, CEP 60021-970 Fortaleza CE, Brazil): A cytotoxic meroterpenoid benzoquinone from roots of Cordia globosa. Planta Med. 71, 54-58 (2005). Preparative TLC of microphyllaquinone and (1aS*,1bS*,7aS*,8aS*)-4,5-dimethoxy-1a,7a-dimethyl-1,1a,1b,2,7,7a,8,8a-octahydrocyclopropa[3,4]cyclopenta[1,2-b]naphthalene-3,6-dione on silica gel with hexane - ethyl acetate 7:3 and dichloromethane - chloroform 7:3. Detection under UV light at 250 nm and by spraying with a solution of vanillin - perchloric acid - ethanol, followed by heating at 100 °C for 5 min.

traditional medicine, herbal, preparative TLC 32e

97 116 B. L. FIEBICH, M. GROZDEVA, S. HESS, M. HÜLL, U. DANESCH, A. BODENSIECK, R. BAUER* (*Institut für Pharmazeutische Wissenschaften, Pharmakognosie, Karl-Franzens-Universität Graz, Universitätsplatz 4, A 8010 Grau, Austria; e-mail: rudolf.bauer@uni-graz.at): Petasites hybridus extracts in vitro inhibit COX-2 and PGE2 release by direct interaction with the enzyme and by preventing p42/44 MAP kinase activation in rat primary microglial cells. Planta Med. 71, 12-19 (2005). TLC of petasin and isopetasin on silica gel without chamber saturation with

toluene - ethyl acetate 93:7. Detection with anisaldehyde - sulfuric acid reagent (anisaldehyde - 100 % acetic acid - methanol - sulfuric acid 1:20:170:10) followed by heating at 160 °C for 1.5 min. Observation under visible and UV light at 365 nm.

herbal, traditional medicine, preparative TLC 32e

97 092 J.-Q. GU (Jian-Qiao Gu), Y. WANG (Yuehong Wang), S. G. FRANZBLAU, G. Montenegro, D. Yang (Danzhou Yang), Barbara N. TIMMERMANN* (*Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, P. O. Box 210207, 1703 E. Mabel Street, Tucson, AZ 85721-0207, USA; e.mail: btimmer@pharmacy.arizona.edu): Antitubercular constituents of Valeriana laxifloria. Planta Med. 70, 509-514 (2004). Analytical and preparative TLC of a new iridolactone (4R,5R,7S,8S,9S)-7-hydroxy-8-hxdroxymethyl-4-methyl perhydrocyclop enta[c]pyran-1-one) on silica gel and RP18 with acetonitrile - water 1:1. Detection by dipping in phosphomolybdic acid or vanillin - sulfuric acid reagent followed by heating at 110 °C for 5 min.

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

97 098 M. HALABALAKI, X. ALEXI, N. ALIGIANNIS, G. LAMBRINIDIS, H. PRATSINIS, J. FLORENTIN, S. MITAKOU, E. MIKROS, A.-L. SKALTSONNIS, M. N. ALEXIS* (*Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 11635 Athens, Greece; e-mail: mnalexis@eie.gr): Estrogenic activity of isoflavonoids from Onobrychis ebenoides. Planta Med. 72, 488-493 (2006). Analytical and preparative TLC of ebenosin (8-(1,1-dime thylallyl)formomonetin) and 3 known isoflavonoids on silica gel with dichloromethane - hexane 3:2. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 099 A.-R. HAN (Ah-Reum Han), H.-Y. MIN (Hye-Young Min), T. WINDONO, G.-H. JEOHN (Gwang-Ho Jeohn), D. S. JANG (Dae Sik Jang), S. K. LEE (Sang Kook Lee), E.-K. SEO* (*Eun-Kyoung Seo) (National Products Chemical Laboratory, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea; e-mail: Yuny@ewha.ac.kr): A new cytotoxic phenylbutenoid dimer from the rhizomes of Zingiber cassumunar. Planta Med. 70, 1095-1097 (2004). Preparative TLC of (+/-)-trans-3-(4-hydroxy-3-methoxyphenyl)-4-[(E)--3,4-dimethoxystyryl]cyclohex-1-ene and related compounds on silica gel with n-hexane - ethyl acetate 2:1 and n-hexane - acetone 3:2. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 101 Q. HAN (Quanbin Han), J. ZHANG (Jixia Zhang), Y. LU (Yang Lu), Y. WU (Yunshan Wu), Q. ZHENG (Qitai Zheng), H. SUN* (Handong Sun) (*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming 650204, China; e-mail: hdsun@mail.kib.ac.cn): A novel cytotoxic oxetane ent-kauranoid from Isodon japonicus. Planta Med. 70, 581-584 (2004). Preparative TLC of mayoecrystal I, a new 11,20:1,20-diepoxy-ent-kaurane diterpenoid, and rubescensin on silica gel by three-fold development with petroleum ether - acetone 4:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 103 A. HAZEKAMP, R. SIMONS, A. PELTENBURG-LOOMAN, M. SENGERS, R. VAN ZWEDEN,

R. VERPOORTE (*Division of Pharmacognosy, Institut of Biology, Leiden University, Einsteinweg 55, 2300 RA, Leiden, The Netherlands; e-mail: ahazekamp@rocketmail.com): Preparative isolation of cannabinoids from Cannabis sativa by centrifugal partition chromatography. J. Liq. Chrom. & Rel. Technol. 27, 2421-2439 (2004). TLC of e. g. delta8-tetrahydrocannabinol, cannabigerol, cannabigerolic acid, cannabidiolic acid, and (-)-delta9-(trans)-tetrahydrocannabinolic acid on RP18 with methanol - 5 % acetic acid 19:1. Detection under UV light at 254 nm and by spraying with modified anisaldehyde - sulfuric acid spray reagent. For selective detection of cannabinoids, the plate was sprayed with 0.5 % fast blue B salt in water, followed by 0.1 M NaOH.

herbal, toxicology, qualitative identification 32e

97 105 CH. ITO, M. ITOIGAWA*, N. KOJIMA, H. T. TAN, J. TAKAYASU, H. TOKUDA, H. NISHINO, H. FURUKAWA (*Tokai Gakuen University, Ukigai, Miyoshi-cho, Nishikama-gun, Aichi 470-0207, Japan; e-mail: itoigawa@tokaigakuen-u.ac.jp) : Cancer chemopreventive activity of rotenoids from Derris trifolia (Corrected version of the paper first published in Planta Medica 70, 8-11 (2004). Planta Med. 70, 585-588 (2004). Preparative TLC of rotenon and 6a-alpha,12a-alpha-12a-hydroxyelliptone on silica gel with dichloromethane, benzene - methanol 24:1 and hexane - ethyl acetate 4:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 106 C. ITO, T. MURATA, M. ITOIGAWA*, K. NAKAO, M. KUMAGAI, N. KANEDA, H. FURUKAWA (*Tokai Gakuen University, 2-901 Nakahira, Tempaku-ku, Nagoya 468-8514, Japan. itoigawa@tokaigakuen-u.ac.jp): Induction of apoptosis by isoflavonoids from the leaves of Millettia taiwaiana in human leukemia HL-60 cells. Planta Med. 72, 424-429 (2006). Preparative TLC of furowanin A, millewanin F, isocrysenegalensein E, 8-gamma,gamma-di-gamma,gamma-dimethylallylwighteone, enchressone b10, 6,8-di-gamma,gamma-dimethylallylorobol on silica gel with n-hexane - acetone 3:1, chloroform - acetone 24:1 and 9:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 107 H. J. KIM (Hyoung Ja Kim), Y. S. LEE* (Yong Sup Lee) (*Department of Pharmaceutical Sciences,College of Pharmacy, Kyung Hee University, Hoegi-Dong, Dongdaemoon-Ku, Seoul 130-701, Korea; e-mail: kyslee@khn.ac.kr): Identification of new dicaffeoylquinic acids from chrysanthemum morifolium and their antioxidant activities. Planta Med. 71, 871-876 (2005). Preparative TLC of 3,5-dicaffeoylquinic acid and 1,3-dicaffeoyl-epi-quinic acid and 6 known dicaffeoylquinic acid derivatives on RP18 with 40 % aqueous methanol. Detection under UV at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 113 Christa KLETTER*, S. GLASL, A. PRESSER, J. WERNER, G. REZNICEK, S. NARANTUYA, S. CELLEK, E. HASLINGER, J. JURENITSCH (*Institute of Pharmacognosy, PharmaCenter-Vienna, University of Vienna, Althanstr. 14, 1090 Vienna, Austria; e-mail: Christa.Klett@univie.ac.at): Morphological, chemical, and functional analysis of Catuaba preparations. Planta Med. 70, 993-1000 (2004). Preparative TLC of catuabine and its hydroxymethyl derivative 7-exo-hydroxy-N-methyl-catuabine on silica gel with dichloromethane - acetone 97:3 and toluene - acetone - methanol - ammonia 45:45:7:3. Detection under UV light at 254 and 366 nm and by spraying with potassium iodoplatinate reagent (0.25 mL of 5 % hexachloroplatinic acid solution mixed

with 2.25 mL of 10 % potassium iodide solution and dissolved with 5 mL water).

traditional medicine, herbal, preparative TLC 32e

97 118 F. LARRONDE, T. RICHARD, J.-C. DELAUNAY, A. DECENDIT,. J.-P. MONTI, S. KRISA, J.-M. MERILLOU* (*Groupe d' Etude des Substances Végétales à Activité Biologique, EA 3675, Université de Bordeaux 2, 146 rue Leó Saignat, 33076 Bordeaux Cedex, France; e-mail: jean-michel.merillo@phyto.u-bordeaux2.fr): New stilbenoid glucosides isolated from Vitis vinifera cell suspension cultures (cv. Cabernet Sauvignon). Planta Med. 71, 888-890 (2005). TLC of (Z)-resveratrol 3,5-O-beta-diglucoside, (E)-resveratrol 3,5-O-beta-diglucoside, (Z)-resveratrol 3,5,4'-O-beta-triglucoside on silica gel with chloroform - methanol - formic acid 70:30:3. Visualization by spraying with anisaldehyde reagent.

food analysis, agricultural, preparative TLC 32e

97 119 Z.-L. LI (Zhan-Liu Li), X. LI* (Xian Li), L.-H. LI (Lin-Hao Li), N. LI (Ning Li), M. YU (Ming Yu), D. L. MENG (Da-Li Meng) (*Shenyang Pharmaceutical University, Box 49, Shenyang 110016, China; e-mail: Proflixian@163.com): Two new triterpenes from the husks of Xanthoceras sorbifolia. Planta Med. 71, 1068-1070 (2005). Preparative TLC of 21,22-diangeloyl-24-hydroxy-R1-barrigenol on silica gel with chloroform - methanol 15:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 120 J.-X. LI* (Jian-Xin Li), T. HAREYAMA, Y. TEZUKA, Y. ZHANG (Yuan Zhang), T. Miyahara, S, Kadota (*Key Lab of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China; e-mail: lijxnju@nju.edu.cn): Five new oleanolic acid glycosides from Achyranthes bidentata with inhibitory activity on osteoclast formation. Planta Med. 71, 673-679 (2005). Analytical and preparative TLC of 18-(beta-D-glucopyranosyloxy)-28-oxoolean-12-en-3beta-yl 3-O-(beta-D-glucopyranosyl)-beta-D-glucopyranosiduronic acid methyl ester, achyranthoside C dimethyl ester, achyranthoside C butyl dimethyl ester, achyranthoside E dimethyl ester, achyranthoside E butyl methyl ester (and 10 known compounds) on silica gel and RP18 with chloroform - methanol - water 8:5:2 or methanol - water 1:1, respectively. Detection under UV light at 254 nm or by spraying with cerium sulfate - 10 % sulfuric acid 1:99.

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

97 121 J. LIN (Jun-Xi Lin), X. WEI (Xiao-Ning Wei), Y. SHI* (Yan-Ping Shi) (*Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730 000, China. shiyp@lzb.ac.cn): Eremophilane sesquiterpenes from Ligularia myriocephala. Planta Med. 72, 175-179 (2006). Preparative TLC of 1beta,6beta-diangeloyloxy-8beta,10beta-dihydroxyeremophil-(11)-en-8alpha,12-olide, 1beta,6betadiangeloyloxy-8alpha,10alpha-dihydoxyeremophil-7(11)-en-8beta,12olide,1beta-angeloyloxy-8-oxoeremophil-6,9-diene-12-oic acid methylester on silica gel with petroleum ether (60-90°) - acetone 3:1. Detection under UV light at 254 nm or by spraying with 98 % sulfuric acid - ethanol 1:19 followed by heating.

traditional medicine, herbal, preparative TLC 32e

97 122 W. LIN (Wen-Yu Lin), C. PENG (Chien-Fang Peng), J. TSAI (Jan-Lin Tsai), J. CHEN (Jih-Jung

Chen), M. CHENG (Ming-Jen Cheng), I. CHEN* (Ih-Sheng Chen) (*Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, China; e-mail: m635013@kmu.edu.tw): Antitubercular constituents from the roots of Engelhardia roxburghiana. Planta Med. 71, 171-175 (2005). Preparative TLC of engelhardione on silica gel with dichloromethane - ethyl acetate 10:1 and of (-)-5-hydroxy-4-methoxy-1-tetralone on RP 18 with acetonitrile - water 1:1 and of 3-methoxycarbonyl-1,5-dihydroxyanthraquinone with n-hexane - dichloromethane 1:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 084 A. F. MAGALHAES*, A. M. G. A. TOZZI, E. G. MAGALHAES, L. C. SOUZA-NETA (*Departamento de Química Organica, IQ, UNICAMP, C. P. 6154, Campinas 13084-971, Brazil. aderbal@iqm.unicamp.br): New prenylated metabolites of Deguelia longeracemosa and evaluation of their antimicrobial potential. Planta Med. 72, 358-363 (2006). Preparative TLC of isorobustin, robustin, robustic acid, 4-hydroxy-3-(3',4'-methylenedioxyphenyl)-5-methoxy-6-(3,3-dimethylallyl-2",2"-dimethylpyrano-(5",6":8,7)coumarin, 4-hydroxy-3-(3'-hydroxy-4'-methoxyphenyl)-5-methoxy-6-(3,3-dimethylallyl-2",2"-dimethylpyrano-(5",6":8,7)coumarin, 4-hydroxy-3-(3'-hydroxy-4'-methoxyphenyl)-5-methoxy-6-(3,3-dimethylallyl-2",2"-dimethylpyrano-(5",6":6,7)coumarin, 4-hydroxy-3-[4'-O-(3'-hydroxy-4'-methoxyphenyl)-5-methoxy-6-(3,3-dimethylallylphenyl]-5-methoxy-2",2"-dimethylpyrano-(5",6":6,7)coumarin on silica gel with n-hexane - ethyl acetate 7:3 and 3:1, n-hexane - dichloromethane - ethyl acetate 3:1:1 and 9:1:4. Detection under UV light at 254 or 366 nm, and by derivatization with an ethanolic solution of anisaldehyde - sulfuric acid - acetic acid 90:5:1, followed by heating.

traditional medicine, herbal, preparative TLC 32e

97 124 A. M. MADUREIRA, A. MOLNAR, P. M. ABREU, J. MOLNAR, Maria-José U. FERREIRA* (*Centro de Estudos de Ciencias Farmaceuticas, Faculdade de Farmácia da Universidade de Lisboa, Av. das Forcas Armadas, 1600-083 Lisboa, Potugal; e-mail: mjuferreira@ff.ul.pt): A new sesquiterpene-coumarin ether and a new abietane diterpene and their effects as inhibitors of P-glycoprotein. Planta Med. 70, 828-833 (2004). Preparative TLC of driportlandin, portlanquinol as well as formonetin and davidigenin on silica gel (by x-fold development) with dichloromethane - methanol 49:1 (2 x), dichloromethane - diethyl ether 19:1, dichloromethane - ethyl acetate 19:1 to 47:3; chloroform - ethyl acetate 9:1 (4 x), and dichloromethane - methanol 19:1 (2 x). Detection under UV light at 254 nm and by spraying with sulfuric acid - acetic acid - water 1:20:4, followed by heating.

traditional medicine, herbal, preparative TLC 32e

97 128 W. MARKOWSKI*, A. LUDWICZUK, T. WOLSKI (*Department of Physical Chemistry, Medical University, Lublin, Poland): Analysis of ginsenosides from Panax quinquefolium L. by automated multiple development. J. Planar Chromatogr. 19, 115-117 (2006). HPTLC of eight ginsenosides on silica gel after cleaning with isopropanol for 1 h with methanol - chloroform. Two gradient programs and two different values of increment in the development distance were compared. Visualization by spraying with A) Godin's reagent (5% solution of sulfuric acid in ethanol), and B) 1% solution of vanillin in ethanol, followed by heating at 105°C for 10 min. Evaluation by scanning at 540 nm.

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

97 129 H. MATSUDA, T. MORIKAWA, H. XIE (Haihue Xie), M. YOSHIKAWA* (*Kyoto Pharmaceutical University, misasagi, Yamashina-ku, Kyoto 607 8412, Japan; e-mail: shoyaku@mb.kyoto-phu.ac.jp): Antiallergic phenanthrenes and stilbenes from the tubers of Gymnadenia conopsea. Planta Med. 70, 847-855 (2004). HPTLC and TLC of gymconopin A on silica gel and RP18 with the lower phase of chloroform - methanol - water 15:3:1.Visualization by spraying with 1 % cerium sulfate - 10 % aqueous sulfuric acid, followed by heating.

traditional medicine, herbal, qualitative identification, HPTLC 32e

97 145 M. T. T. NGUYEN, S. AWALE, Y. TEZUKA, J.-Y. UEDA, Q. L. TRAN, S. KADOTA* (*Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan. kadota@ms.toyama-mpu.ac.jp): Xanthine oxidase inhibitors from the flowers of Chrysanthemum chinense. Planta Med. 72, 46-51 (2006). Preparative TLC of acacetin, jaceidin, tricetin 3',4',5'-trimethylester, diosmetin, apigenin, eupafolin, chrysoeriol, (+-)eriodictyol, 3,4-dihydroxybenzaldehyde, p-coumaric acid, 5-O-caffeoylquinic acid methyl ester, 4,5-O-dicaffeoylquinic acid on RP18 with acetonitrile - methanol - water 1:1:3. Detection under UV at 254 nm.

herbal, traditional medicine, preparative TLC 32e

97 146 V. U. M. SARMA, P. V. SRINIVAS, V. ANURADHA, J. M. RAO* (*Natural Products Laboratory, Organic Division I, Indian Institute of Chemical Technology, Hyderabad-500 007, India): A simple and convenient method of standardization of Piper longum - an ayurvedic medicinal plant. J. Planar Chromatogr. 19, 238-240 (2006). HPTLC of plant extracts, using pellitorine and dihydropiperlongumine as markers, on silica gel in a twin-trough chamber saturated with the mobile phase hexane - ethyl acetate 3:1. Quantitation by densitometry in absorbance/reflectance mode at 260 nm.

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

97 141 E. SIMIONATTO, C. PORTO, I. I. DALCOL, U. F. DA SILVA, A. F. MOREL* (*Departamento de Química, Núcleo de Pesquisa de Produtos Naturais, Universidade Federal de Santo Maria, Campus Camobi, CEP 97105-900, Santa Maria RS, Brazil) : Essential oil from Zanthoxylum hyemale. Planta Med. 71, 759-763 (2005). Preparative TLC of hyemalol, nerolidol, and cadinol on silica gel with hexane - ethyl acetate 9:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 142 N. SINGH*, A. P. GUPTA, B. SINGH, V. K. KAUL (*Department of Natural Plant Products, Institute of Himalayan Bioresource Technology, P. O. Box No. 6, Palampur, 176061 (HP), India): Quantification of valerenic acid in Valeriana jatamansi and Valeriana officinalis by HPTLC. Chromatographia 63 (3-4), 209-213 (2006). HPTLC of valerenic acid in Valeriana jatamansi and Valeriana officinalis on silica gel with hexane - ethyl acetate - acetic acid 160:40:1. Detection with anisaldehyde-sulphuric acid reagent. Quantitative determination by absorbance measurement at 700 nm. The calibration curves were linear in the range of 500 ng - 2.5 µg/zone.

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

97 108 S. J. N. TATSIMO, P. TANE, J. MELISSA, B. L. SONDENGAM, C. O. OKUNJI, B. M. SCHUSTER, M. M. IWU, I. A. KHAN* (*National Center for Natural Products Research, The University

of Mississippi, University, MS 38677-1848, USA. ikhan@olemiss.edu): Antimicrobial principles from Aframomum longifolius. Planta Med. 72, 132-135 (2006). Analytical and preparative TLC of aframolin B (8beta(17)-epoxy-15,15-dimethoxylabd-12(E)-en-16-al), and aframodial on silica gel with hexane - ethyl acetate 2:3. Detection under UV light at 254 nm.

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

97 147 A. URBAIN, A. MARSTON, E. F. QUEIROZ, K. NDJOKO, K. HOSTETTMANN* (*Laboratory of Pharmacognosy and Phytochemistry, Geneva-Lausanne School of Pharmacy, University of Lausanne, BEP, 1015 Lausanne, Switzerland; e-mail: K. Hostettmann@pharm.unige.ch): Xanthones from Gentiana campestris as new acetylcholinesterase inhibitors. Planta Med. 70, 1011-1014 (2004). TLC bioautography of bellidin, bellidifolin and the respective glucosides on silica gel with chloroform - methanol - water 50:10:1 with huperzine A, galanthamin HBr, and physostigmine as reference substances.

traditional medicine, herbal, qualitative identification, bioautography 32e

97 117 M. L. VERAS, M. Z. B. BEZERRA, R. BRAZ-FILHO, O. D. L. PESSOA, R. C. MONTENEGRO, C. DO O PESSOA, M. O. DE MORAES, Letícia VERAS COSTA-LOTUFO* (*Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará, Rua Cel Nunes de Meló 1127, Caixa Postal-3157, 60430-270 Fortaleza, Ceará, Brazil; e-mail: lvcosta@secrel.com.br) e: Cytotoxic epimeric withaphysalins from leaves of Acnistus arborescens. Planta Med. 70, 551-555 (2004). Analytical TLC of withaphysalin F and two new epimeric withaphysalins ((17S,20R,22R)-5beta,6beta:18,20-diepoxy-4beta,18-dihydroxy-1-oxo-witha-24-enolide (18R and 18S)) on silica gel with chloroform - ethyl acetate 3:7. Detection by spraying with 10 % sulfuric acid in ethanol, followed by heating at 120 °C for 5 min (orange spots).

traditional medicine, herbal, qualitative identification 32e

97 150 H. WANGENSTEEN*, M. ALANGIR, S. RAJIA, A. B. SAMUELSEN, K. E. MALTERUD (*Department of Pharmacognosy, School of Pharmacy, University of Oslo, P. O. Box 1068, Blindern, 0316 Oslo, Norway; e-mail: helle.wangensteen@farmasi.uio.no): Rotenoids and isoflavones from Sarcolobus globosus. Planta Med. 71, 754-758 (2005). Analytical and preparative TLC of sarcolobin, sarcolobone, 6,7-dimrethoxy-2,3-dihydrochromone and 10 known compounds on silica gel with chloroform - petroleum ether - ethyl acetate 20:11:10. Visualization under UV light at 254 and 366 nm and/or by spraying with cerium sulfate (1 % in 10 % aqueous sulfuric acid) followed by heating at 105 °C for 5 min. Also centrifugally accelerated TLC on silica gel with an Chromatotron instrument in a nitrogen atmosphere using petroleum ether - ethyl acetate 2:1.

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

97 151 R. WILAIRAT, J. MANOSROI, A. MANOSROI, A. KIJJOA, M. S. J. NASCINMENTO, M. PINTO, A. M. S. SILVA, G. EATON, W. HERZ* (*Department of Chemistry and Biochemistry, Florida State University, Tallahassee FL 32306-4390, USA; e-mail: jdulin@chem.fsu.edu): Cytotoxicities of xanthones and cinnanate esters from Hypericum hookerianum. Planta Med. 71, 680-682 (2005). Analytical and preparative TLC of 5-hydroxy-2-methoxyxanthone, 2-hydroxy-3-methoxyxanthone, trans-kielcarin, 4-hydroxy-3-methoxyphenyl ferulate, and 3beta-O-caffeoylbetulinic acid on silica gel with chloroform - petroleum ether - formic acid 950:50:1, chloroform - acetone - formic acid 950:50:1, 900:100:1, and 850:150:1. Detection under UV light at 254 nm.

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

97 152 Q.-X. WU (Quan-Xiang Wu), Y.-P. SHI* (Yan-Ping Shi), L. YANG (Li Yang) (*Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China; e-mail: shiyp@lzu.edu.cn) : Eremophilane sesquiterpene lactones from Ligularia virgaurea ssp. oligocephala. Planta Med. 70, 479-482 (2004). Preparative TLC of 10alpha-hydroxy-1-oxoeremophila-7(11),8(9)-dien-12,8-olide, and toluccanolides A and C on silica gel with petroleum ether - diethyl ether 1:1, petroleum ether - ethyl actate 2:1, and petroleum ether - acetone 2:1. Detection under UV light or by spraying with 98 % sulfuric acid - ethanol 5:93 followed by heating at 110 °C.

traditional medicine, herbal, preparative TLC 32e

97 153 G. XU (Gang Xu), L. PENG (Li-Yan Peng), L. LU (Lei Lu), Z. WENG (Zi-Ying Weng), Y. ZHAO (Yu Zhao), X. LI (Xiao-Li Li), Q. ZHAO* (Qin-Shi Zhao), H. SUN (Han-Dong Sun) (*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan Kunming 650 204, China. qinshizhaosp@yahoo.com): Two new abietane diterpenoids from Salvia yunnanensis. Planta Med. 72, 84-86 (2006). Preparative TLC of yunnannin, danshenol C and A, dihydrotanshinone on silica gel with petroleum ether - ethyl acetate 4:1. Detection under UV light at 254 nm.

traditional medicine, herbal, preparative TLC 32e

97 157 Z. ZHANG (Zhizhen Zhang), S. LI* (Shigou Li), S. ZHANG (Shanmin Zhang) C. LIANG (Chun Liang), D. Gorenstein, R. S. Beasley (*Center for Medicinal Plant Research, Arthur Temple College of Forestry, Stephen F. Austin State University, Nacogdoches, Texas 75962-6109, USA; e-mail: lis@sfasu.edu): New camptothecin and ellagic acid analogues from the root bark of Camptotheca acuminata. Planta Med. 70, 1216-1221 (2004). TLC of 20-formylbenz[6,7]indolizino[1,2-b]quinolin-11(13H)one, 10-methoxy-20-O-acetylcamptothecin, 20-O-beta-glucopyranosyl-18-hydroxycamptothecin, 3,4-methylenedioxy-3'-O-methyl-5'hydroxy ellagic acid and 18 known compounds on silica gel with chloroform - methanol 4:1, chloroform - ethyl acetate 6:1, ethyl acetate - hexane 4:1, and ethyl acetate - acetone 1:4. Detection under UV light.

traditional medicine, herbal, qualitative identification 32e

97 158 R. ZHAO (Zhao Ruizhi), (TCM Lab., No.2 Clinical Hosp., Guangzhou Univ. Trad. Chinese Med. & Pharm., Guangzhou 510120, China): (Determination of resveratrol in Polygonum cuspidatum Sieb. et Zucc by thin-layer chromatography) (Chinese). J. Chinese Trad. Patent Med. (Zhongchengyao) 27 (5), 605-607 (2005). TLC of resveratrol on silica gel with petroleum ether (30 ºC-90 ºC) - ethyl acetate - methanol - glacial acetic acid 200:50:35:1. Detection under UV light. Identification by comparison with the standard. Quantification by densitometry at 293 nm. Validation of the method by investigation of its linearity range (0.5 µg - 5.0 µg, r = 0.999); precision (RSD = 0.98 %, n = 5); its reproducibility of five time assay towards the same sample (RSD = 2.52 %); standard addition recovery (101.6 %, RSD = 0.26 %, n = 5). The results for three real life samples are given. Discussion of the application of the procedures for the quality control of the medicine.

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

33. Inorganic substances

97 160 V. GHOULIPOUR, S. W. HUSAIN* (*Department of Applied Chemistry, Faculty of Chemistry, University of Tarbiat Moallem, 49 Mofatteh Avenue, Tehran-15614, Iran): Quantitative TLC of

toxic elements on inorganic ion-exchangers. VI. Separation and determination of cadmium. J. Planar Chromatogr. 19, 246-250 (2006). TLC of cadmium on titanium silicate ion-exchange plates in a twin-trough chamber (without chamber saturation) with ammonium buffer of pH 10 (5.354 g ammonium chloride and 42.5 mL ammonia solution in 100 mL water). Quantitation by scanning in absorbance mode at 390 nm after deivatization with a saturated solution of sodium sulfide and at 530 nm after deivatization with a mixed solution of 2,2'-bipyridine and iron(II) sulfate.

toxicology, HPTLC, densitometry, quantitative analysis 33a

35. Other technical products and complex mixtures

97 161 H. CHEN (Chen Hui)*, Y. WANG (Wang Yuan), R. ZHU (Zhu Ruohua) (*Chem. Dep., Beijing Norm. Univ., Beijing 100001, China): (Analysis of phthalates in plastic food-packaging bags by Thin Layer Chromatography) (Chinese). Chinese J. Chromatogr. (Sepu) 24 (1), 69-72 (2006). TLC of dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP) and di (2-ethylhexyl) phthalate (DEHP) in plastic food-packaging bags. Extraction with ethanol by ultrasonication, followed by filtration through a membrane of 0.45 ?m. Development with ethyl acetate - anhydrous ether - isooctane 1:4:15 on silica gel. Quantitative determination by densitometry at 275 nm (reference wavelength 340 nm) by use of an external standard. Good linearities were obtained for DMP, DEP, DBP and DEHP. The detection limits were 2.1 ng for DMP, 2.4 ng for DEP, 3.4 ng for DBP and 4.0 ng for DEHP. The relative standard deviations of the four phthalates were 2.8-3.5 %. The recoveries of the four phthalate standards in real sample were 79-111 %. The method presented has the advantages of high precision, high sensitivity, small sample size, and simple pretreatment. The contents in real samples were close to the results by gas chromatography.

qualitative identification, autoradiography, postchromatographic derivatization, quantitative analysis, phthalate 35

37. Environmental analysis

97 162 Danijela ASPERGER*, D. MUTAVDZIC, S. BABIC, A. J. M. HORVAT, M. KASTELAN-MACAN (*Faculty of Chemical Engineering and Technology, Laboratory of Analytical Chemistry, Marulicev Trg 19, 10000 Zagreb, Croatia): Solid-phase extraction and TLC quantification of enrofloxacin, oxytetracycline, and trimethoprim in wastewater. J. Planar Chromatogr. 19, 129-134 (2006). HPTLC of enrofloxacin, oxytetracycline, and trimethoprim on cyano phases with 0.5 M oxalic acid - methanol (5:5; 6:4; 7:3; 8:2). Detection under UV light at 254 nm. Quantitation by videodensitometry at 254 nm.

environmental, densitometry, quantitative analysis, HPTLC 37c

38. Chiral separation

97 163 R. BUSHAN*, D. GUPTA (*Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee-247 667, India) : Ligand-exchange TLC resolution of some racemic beta-adrenergic blocking agents. J. Planar Chromatogr. 19, 241-245 (2006). TLC of the enantiomers of the beta-blockers (+/-)-propanolol, (+/-)-metoprolol, and (+/-)-atenolol on silica gel impregnated with a Cu(II)-L-arginine complex in a glass chamber saturated for 20-25 min using different mixtures of acetonitrile, methanol, and water as mobile phases. Impregnated TLC plates were prepared by spreading a slurry of 50 g silica gel in a solution of 100 mL of the Cu(II)-L-arginine complex and activating the plates overnight at 60 °C. The Cu(II)-L-arginine complex was prepared by mixing 1 mM copper(II) acetate and 2 mM L-arginine in water - methanol 9:1 and adjusting the final pH

to 6-7 with aqueous ammonia. Detection with iodine vapor. Successful separation of all three racemic drugs was achieved with acetonitrile - methanol - water 15:2:2 and 15:2:1.

pharmaceutical research, quality control, qualitative identification 38, 32a

97 164 J. KRZEK*, M. STAREK, D. JELONKIEWICZ (*Department of Inorganic and Analytical Chemistry, Collegium Medicum, Jagiellonian University, 9 Medyczna Str, 30-688 Kraków, Poland): RP-TLC determination of S(+) and R(-) ibuprofen in drugs with the application of chiral mobile phase and UV densitometric detection. Chromatographia 62 (11-12), 653-657 (2005). TLC of S(+) and R(-) ibuprofen on RP phase with beta-cyclodextrin - methanol 15:1. The UV densitometric detection was carried out at 222 nm. Limit of detection for S(+) and R(-) ibuprofen is 1 µg/mg. Precision and repeatability are good, the obtained results are within the range x mean ± 2. Recovery for both isomers is approximately 99 % and linearity was found to be in the range of 0.01-0.3 %. The presence of both isomers S(+) and R(-) ibuprofen was observed in all preparations at comparable concentrations from 56-66% for S(+) isomer and from 34-44 % for R(-) isomer.

qualitative identification, densitometry, quantitative analysis, ibuprofen 38

97 165 M. SAJEWICZ*, R. PIETKA, A. PIENAK, T. KOWALSKA (*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): Application of Thin-Layer Chromatography to investigate oscillatory instability of the selected profen enantiomers in dichloromethane. J. Chromatogr. Sci. 43 (10), 542-548 (2005). The usefulness of TLC as an efficient measuring technique in the studies of oscillatory trans-enantiomerization of profens from the S to the R configuration (and vice versa) during their storage as 70 % ethanol solutions is demonstrated in the literature. S-(+)-ibuprofen, S-(+)-naproxen, and S,R-(±)-2-phenylpropionic acid are utilized as the test profens. It is proven possible to show oscillatory instability with the racemic S,R-(±)-2-phenylpropionic acid also. Correctness of the TLC assessment is successfully confirmed by means of polarimetry. Upon these preliminary results, it is concluded that the most probable mechanism might embrace the keto-enol tautomerism because of a convenient migration of the proton from one moiety of the profen molecule to another in an aqueous medium. To indirectly verify this hypothesis, profens are stored in dichloromethane, deliberately hampering their ability to dissociate and to re-structure. It is shown that the non-aqueous solvent considerably suppresses, although they do not completely eradicate, the oscillatory trans-enantiomerization of profens. In view of these findings, the reports which claim a predominant therapeutic potential of the respective S-profens become less convincing and certainly need reconsideration.

pharmaceutical research, oscillatory instability, profen enantiomers 38, 2d

Elke Hahn-Deinstrop

Applied Thin-Layer Chromatography – Best Practice and Avoidance of Mistakes

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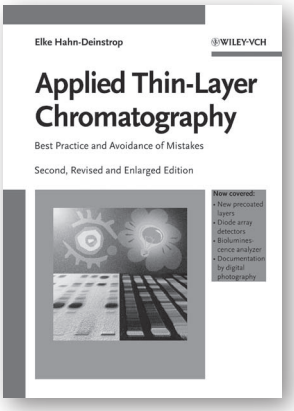

Thin-Layer Chromatography (TLC/HPTLC) is a mature analytical method found since more than 40 years in many laboratories across the globe. Even though pronounced dead once in a while, Planar Chromatography today is gaining ground concerning quality and importance thanks to new pre-coated layers, new instrumentation and a favorable cost – value ratio. In 1998 Elke Hahn-Deinstrop published her TLC textbook in German. The book focused on the practical aspects of the method and on how to avoid mistakes while applying it. For theoretical aspects of TLC/HPTLC reference was made to other texts in the literature section. In the beginning of the year 2000 Wiley-VCH published an English edition of the book which was very positively reviewed by Prof. C. Poole (see CBS 84). Since both books are out of print the author has now submitted a revised English edition.

What is new in the 2nd edition?

Considerable progress can be seen with pre-coated layers, instruments and methods. The book considers the state of the art. Elke Hahn-Deinstrop has performed own experiments with LUX and UTLC plates and shows corresponding figures and images of highest quality in a color print section at the end of the book. Actually new are chromatograms on ProteoChrom® pre-coated layers recently launched by Merck, which will make work in the life sciences much easier. All chapters have been revised and updated. Here are the most important topics: TLC/HPTLC hyphenated methods have been extended and literature references are given; radio-TLC was re-written and work with the J&M-Diode-Array-Scanner and the CAMAG BioLuminizer™ was included. The old fashioned documentation with Polaroid cameras was omitted; a detailed description of how to utilize digital cameras is given instead. The already existing 98 Figures have been supplemented by 11 additional ones.

The chapter concerning GxP-related issues shows that Elke Hahn-Deinstrop is rooted in the pharmaceutical industry: be it the fraud and fool proof TLC/HPTLC work on Merck silica gel GLP coded plates or the almost meticulous accuracy filling out most different forms or reports. The pharmacopoeias of this world prepare much headache to the author because the chromatographic systems described therein do rarely represent the developments in modern technology.

The book at hand has been completed by a recent market review. This service is one of the nice and convenient gifts for TLC/HPTLC users and those who want to become one. All steps of TLC/HPTLC are described in a very detailed way and assistance is given in order to avoid mistakes. With the second edition Elke Hahn-Deinstrop was able to carry on in elegant way with her book which already can be called standard work. It belongs into each analytic laboratory which uses (thin–layer) chromatography

Dr. Angelika Koch
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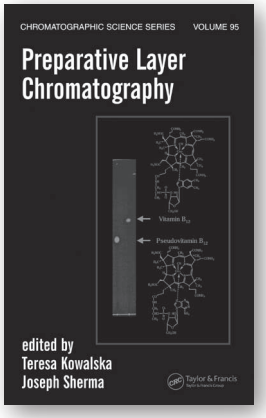


The editors claim that this book has been designed as a practical, comprehensive source of information on the field of classical preparative layer chromatography (PLC[1]), both designed for scientists with a high degree of experience and the relatively inexperienced chromatographer. This is, indeed, the first book dedicated specifically to this subject and it is well edited and does not show too much of the abundance we are often facing in these types of opus. The excellent introduction is followed by two sections, the first of which (encompassing Chapter 1 through 8) covers theory while the second (Chapter 9 through 16) deals with the application of PLC in selected substance classes and sample types.

The book does not include information on forced-flow PLC ("OPLC"), but only on procedures based upon capillary flow, nor on rotation planar chromatography (RPC) for which the transport of the solvent occurs due to centrifugal force. The reasons the editors give by for this exclusion are that a) both of these techniques require quite expensive and rather complex instrumentation and b) capillary PLC is intuitively closer to well-familiar "analytical" TLC. There may be others.

The well-known fundamentals of TLC which are equally relevant for most PLC operations are well presented throughout, though not overly correct in all their ramifications. For instance, throughout the book the term "mobile phase" is used as a full synonym for "solvent", which does not hold for multi-component solvents and unsaturated chambers where – because of gradient formations during development – several different mobile and stationary phases form along the layer. The "theoretical" chapter "Adsorption Planar Chromatography in the Nonlinear Range: Selected Drawbacks and Selected Guidelines" centers around the Fowler-Guggenheim model and covers aspects of separations on overloaded layers, but is perhaps a touch too theoretical to become mainstream application. This shortcoming of the book is well compensated by extensive practical Chapters on sorbents and precoated layers, location of separation zones and detection methods. Also the chapters on the selection and optimization of the solvent for PLC by Virginia Coman and sample application and chromatogram development by Gerda Morlock are closer to practical needs in the laboratory. The latter one excels by an extensive presentation of commercially available instrumentation and a "Roadmap to Your Own Procedure". Dzido and Polak plough deeply into the Methodological Possibilities of the Horizontal Chamber in PLC.

The described sectorial utilisations of PLC include a large array of medical applications, hydrophilic vitamins, various natural mixtures, lipids, natural pigments, inorganics and organometallics, geochemical samples and strategies for finding taxonomic marker substances in Olibanum resins. The scientific level and the presentation of these chapters are mostly high, partly outstanding.

In summary, this book can be recommended for purchase even to persons who are not in love with multi-author works, because the knowledge and expertise of 29 individual PLC specialists/authors collected in one volume is certainly better than no book at all. In the specialists' gardens one can graze abundantly.

FRIEDRICH GEISS
Author of the book 'Fundamentals of Thin-Layer Chromatography'
Ispra/Italy

[1]This acronym is also used for planar chromatography.

Validierte Bestimmung des Biomarkers Trigonellin

12

▲ *Shruti Chopra, Dr. Farhan J. Ahmad, Sanjay K. Motwani*

Shruti Chopra* und ihre Arbeitsgruppe unter der Leitung von Dr. Farhan J. Ahmad, von Jamia Hamdard, New Delhi, Indien, beschäftigen sich intensiv mit der Entwicklung und Validierung von HPTLC-Methoden zur Analytik von Biomarkern. Die Überwachung der Zusammensetzung und Qualität der pflanzlichen Arzneimittel bleibt stets eine Herausforderung im expandierenden Geschäft mit den pflanzlichen Medikamenten aus den Entwicklungsländern. Gehaltsschwankungen der pharmakologisch wirksamen Stoffe in diesen Arzneimitteln sowie nur begrenzt verfügbare Referenzsubstanzen zur Heilpflanzen-Analyse rücken diese Thematik bei allen Regierungen in Entwicklungs- und Schwellenländern an vordere Stelle. Die Erarbeitung von geeigneten, empfindlichen Methoden zur qualitativen und quantitativen Analytik der Biomarker kann die Qualitätskontrolle pflanzlicher Produkte aus unterschiedlichen Quellen erleichtern.

Einleitung

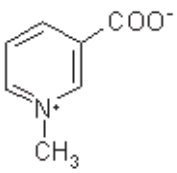
Trigonellin (TGF) ist ein bedeutender Wirkstoff im Bockshornklee (*Trigonella foenum-graecum*), der hypoglykämische, Cholesterin-senkende, antiseptische, antimigräne, antitumor, antimutagene und osmoregulatorische Eigenschaften besitzt [1]. Die Qualität pflanzlicher Produkte ist nur dann gewährleistet, wenn die Rohstoffe streng, einschließlich einer spezifischen botanischen Identifizierung des pflanzlichen Materials, kontrolliert werden. Es ist also wichtig, die geographische Herkunft und die Bedingungen zu kennen, unter denen das pflanzliche Arzneimittel gewonnen wurde [2]. Etliche analytische Methoden (UV-Photometrie [3], HPLC [4], HPTLC/TLC [5] und OPLC [6]) basieren auf der Bestimmung des Biomarkers Trigonellin, allerdings gibt es nur wenige analytische Untersuchungen zur Bestimmung von Trigonellin in Pflanzenextrakten und in deren pharmazeutischen Formulierungen. Aus diesen Gründen hat man eine einfache, empfindliche, selektive, präzise und robuste HPTLC-Methode entwickelt, die den ICH-Richtlinien zur Bestimmung von Trigonellin in Pflanzenextrakten und pharmazeutischen Erzeugnissen entspricht. Der Trigonellingehalt wurde in Pflanzenextrakten zweier verschiedener Lieferanten untersucht. Zudem wurden Referenzproben aus Placebogelen geprüft, um potentielle Einflüsse weiterer Extrakt-Inhaltstoffe auf die Trennqualität zu berücksichtigen.

Die HPTLC ist eine aufgrund ihrer Zuverlässigkeit, Einfachheit, Reproduzierbarkeit und Schnelligkeit effektive analytische Methode. Zusätzlich ist die Methode kostengünstig, da nur geringe Mengen an Lösungsmitteln verbraucht werden bei einem zugleich minimalen Anspruch an die Probenreinheit. Mehrere Proben können in kürzester Zeit gleichzeitig analysiert werden. Die HPTLC ist hinsichtlich der Wahl der mobilen Phase nicht limitiert und eröffnet zudem die Möglichkeit der direkten Auftragung von Suspensionen oder trüben Proben. Überdies erlaubt die Methode eine gleichzeitige Untersuchung mehrerer Bestandteile der medizinischen Multikomponenten-Produkte oder Pflanzenextrakte [7].

Probenaufarbeitung

(A) Bestimmung von TGF in pflanzlichen Extrakten

500 mg des Pflanzenextraktes wurden in einem 50 mL Messkolben mit 25 mL Methanol als Vorlage gegeben, für 30 min in ein Ultraschallbad gestellt und auf 50 mL mit Methanol aufgefüllt. Die erhaltene Lösung wurde 15 min bei 3000 U/min zentrifugiert.



▲ *Chemische Strukturformel von Trigonellin*

(B) Bestimmung von TGF in Arzneimittel-Zubereitungen

8,5 mg Gel (entspricht ca. 100 µg Trigonellin) wurden mit 25 mL Methanol 30 min im Ultraschallbad extrahiert, anschließend bei 12000 U/min 15 min bei 4 °C zentrifugiert. Die überstehende Flüssigkeit wurde filtriert und das Filtrat bei Raumtemperatur bis zu einem definierten Volumen getrocknet. Der Rückstand wurde in 5 mL Methanol aufgenommen. Die Referenzproben aus Placebogelen wurden analog behandelt.

Standardlösung

10 mg TGF wurden in 100 mL Methanol gelöst (100 µg/mL).

Schicht

DC-Alufolien Kieselgel 60 F_{254} (Merck) 20 x 10 cm

Probenauftragung

Bandförmig mit Linomat, Bandlänge 6 mm, Auftragevolumen 6 µL für Proben und 1–12 µL für die TGF-Standardlösung (100–1200 ng/6 mm), Bahnabstand 10 mm, unterer Randabstand 10 mm, seitlicher Randabstand 15 mm

Chromatographie

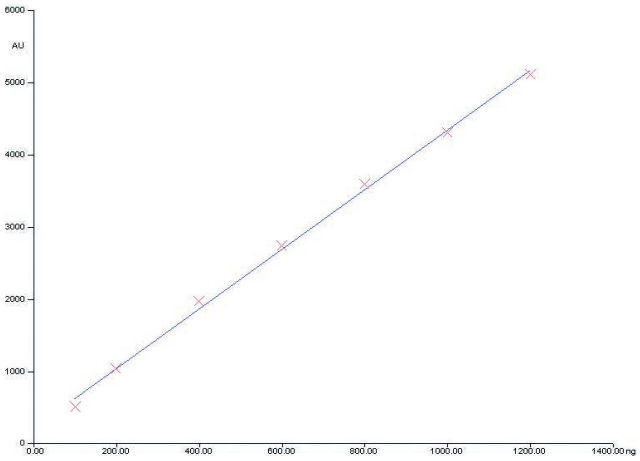
In der Doppeltrogkammer mit n-Propanol – Methanol – Wasser 4:1:4 (v/v/v) unter Kammersättigung, Laufstrecke 80 mm. Die Platten werden nach der Chromatographie 1 min im Warmluftstrom getrocknet.

Densitometrische Auswertung

TLC Scanner 3 mit winCATS Software, Absorptionsmessung bei 269 nm, lineare Kalibration über die Peakfläche.

Ergebnisse und Diskussion

Die HPTLC-Methode wurde hinsichtlich der Quantifizierung des Trigonellins in den Pflanzenextrakten optimiert. Anfangs wurde das Verhältnis der Fließmittel-Komponenten variiert, um die beste Trennung von der Matrix zu gewährleisten und dabei eine scharfe Peakform zu erhalten. Einen fokussierten Trigonellin-Peak (hR_F-Wert 46 ± 2) erhielt man schließlich mit n-Propanol – Methanol – Wasser im Verhältnis 4:1:4 (v/v/v). Aufgrund des signifikanten hR_F-Wert-Unterschiedes zu anderen Extraktbestandteilen waren keine Störeffekte festzustellen.



13

▲ *Kalibriergerade für Trigonellin*

Lineare Regressionsdaten zur Kalibrierkurve (n=3)	
Linearitätsbereich (ng)	100–1200
Korrelationskoeffizient (r ± SD)	0.9991 ± 0.0002
Steigung ± SD	4.1312 ± 0.0491
Konfidenzintervall der Steigung[a]	9.516 – 9.760
Schnittpunkt mit der y-Achse ± SD	208.2135 ± 4.5092
Konfidenzintervall des y-Achsen-Schnittpunktes[a]	190.93 – 219.87

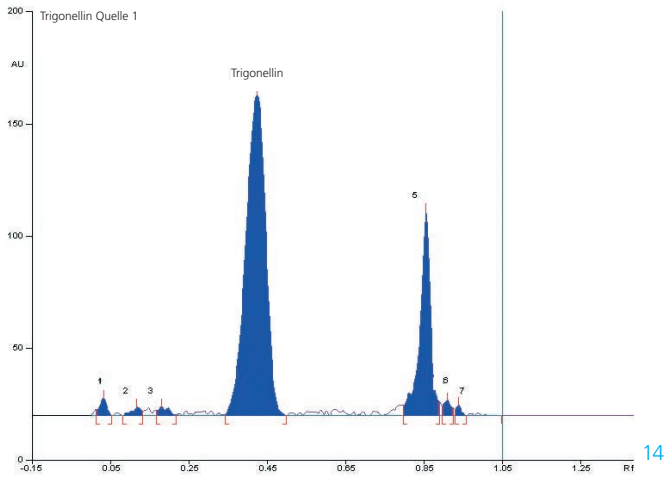
[a]Vertrauensbereich 95 %

Die Wiederholbarkeit der Probenauftragung (600 ng/Zone) und Messung der Peakflächen wurde zu jeweils ±0,09% und ±0,15% bestimmt. Die Messung von Peakflächen bei drei unterschiedlichen Konzentrationsniveaus (400, 600 und 800 ng/Zone) zeigte niedrige Werte für die Standardfehler (SE) und für die relative Standardabweichung (RSD). Die RSD lag unter 1% bei den Messungen an einem und an unterschiedlichen Tagen, was eine exzellente Präzision der Methode beweist. Die niedrigen RSD- und SE-Werte, die nach der Einführung geringfügiger Änderungen in den kritischen Parametern der entwickelten HPTLC-Methode wie Fliessmittel-Zusammensetzung und -volumen, Sättigungszeit und Aktivierungszeit der mit Methanol vorgewaschenen DC-Platten erhalten wurden, bestätigen die Robustheit des Verfahrens. Die Nachweis- und die Bestimmungsgrenze (S/N 3 und 10) wurden jeweils zu 2,3 ng und 7,6 ng bestimmt, was für eine gute Detektierbarkeit spricht.

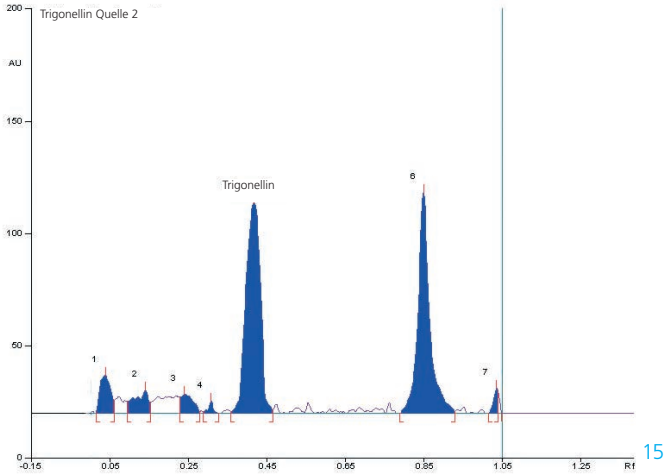
Bei der Anwendung der vorgeschlagenen Methode zur Extraktion und anschließenden Bestimmung des Trigonellin-Gehalts in den Arzneimittel-Zubereitungen wurden Wiederfindungsraten von

99–101% (Dotierniveau 50, 100 und 150% TGF) erreicht. Die Peakreinheit von Trigonellin wurde anhand des Spektrenvergleichs am Peakanfang, an der Peakspitze und am Peakende bewertet. Bei der Überprüfung der Spektrenidentität wurde eine gute Korrelation (r = 0,9992) zwischen den überlagerten Spektren des Trigonellin-Standards und der Proben erhalten. Hierbei waren keine Störungen durch weitere pharmakologisch wirksame oder sonstige Bestandteile der pflanzlichen Arzneimittel auf Gelbasis ersichtlich. Der Gesamtgehalt an Trigonellin in zwei unverarbeiteten Extrakten wurde jeweils zu 1,99% (w/w) and 2,10% (w/w) bestimmt.

Die für die Bestimmung von Trigonellin in pflanzlichen Extrakten und pharmazeutischen Zubereitungen entwickelte HPTLC-Methode ist genau, präzise, spezifisch und reproduzierbar. Mit der neu entwickelten mobilen Phase, die eine gute Abtrennung des Trigonellins von den anderen Inhaltsstoffen gewährleistet, kann das Verfahren sowohl zur qualitativen als auch zur quantitativen Analyse dieses Biomarkers angewendet werden. Weiterhin können die in den pflanzlichen Rohstoffen enthaltenen Verunreinigungen zur Gehaltsbestimmung der Alkaloid-Komponenten in Produkten aus regional unterschiedlichen klimatischen Bedingungen herangezogen werden. Außerdem kann die Untersuchung des Trigonellin-Abbaus unter verschiedenen Streßbedingungen gemäss der ICH-Richtlinie erfolgen.



14



15

▲ *Chromatogramme eines Trigonella foenum-graecum Extraktes (TGF 1200 ng/Zone) aus zwei Quellen: Rohstoff 1 (oben) und 2 (unten); Peaks 1–7 stammen von anderen Extrakt-Bestandteilen*

Anmerkung:
Die Autoren benutzen den Begriff »HPTLC«, um zu unterstreichen, dass sie instrumentelle Techniken einsetzen, obwohl sie konventionelle DC-Schichten benutzen. Die Methode kann aber ohne jegliche Änderung auf leistungsstärkere HPTLC-Trennmaterialien angewendet werden, was zudem die Detektierbarkeit verbessern würde. (Hrsg.)

Literatur

[1] S. Chopra, F.J. Ahmad, R.K. Khar, S.K. Motwani, S. Mehdi, Z. Iqbal, S. Talegaonkar, Anal. Chim. Acta, 577, 46–51, 2006.
[2] Note for guidance on quality of herbal medicinal product, The European Agency for the Evaluation of Medicinal Pro ducts, London, July, 26, 2006 (CPMP/QWP/2819/00, EMEA/CVMP/814/00)
[3] S.M.A. Wahab, M.A. Selim, Egypt. J. Pharm. Sci., 26 (1986) 335.
[4] H.Q. Zhao, Y. Qu, X.Y. Wang, H.J. Zhang, F.M. Li, H. Masao, Zhongguo Zhong Yao Za Zhi, 27 (2002) 194.
[5] S. Scinto, R. Chillemi, M. Piattelli, J. Nat. Prod., 51 (1988) 322.
[6] E. Mincsovics, E. Sardi, I. Velich, G. Katay, E. Tyihak, J. Planar Chromatogr., 15 (2002) 280.
[7] E.A. Abourashed, J.S. Mossa, J. Pharm. Biomed. Anal., 36, 617-620, 2004.

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HPTLC-Methoden für die Identifizierung von Grüntee und Grüntee-Extrakten

16 ▲ *R. Jorns*

17 ▲ *V. Widmer, E. Reich*

Seit mehreren Jahren werden im CAMAG – Labor qualitative HPTLC-Methoden zur Identifizierung von Heilpflanzen entwickelt. Der Schwerpunkt liegt dabei auf Drogen mit grosser wirtschaftlicher Bedeutung und gleichzeitig hohem Verwechslungs- oder Verfälschungspotential. Für die Gewährleistung von Qualität und Sicherheit eines Produktes auf pflanzlicher Basis ist die richtige Identifizierung von Rohmaterial eine Grundvoraussetzung. In diesem Zusammenhang hat sich die HPTLC als ein wichtiges Werkzeug in der Industrie etabliert. Die in diesem Beitrag beschriebenen Methoden wurden gemeinsam mit Frau Jorns von Frutarom Schweiz (Wädenswil) erarbeitet. Frau Jorns hat seit 14 Jahren unzählige Analysen-Methoden zur Identitätskontrolle für eine Vielzahl von Heilkräutern und standardisierten Extrakten, vorwiegend mittels HPTLC, entwickelt. Denn bei der Firma Frutarom bildet die HPTLC einen wichtigen Bestandteil in der Analytik und Entwicklung von neuen Produkten. Zu Beginn eines Projektes steht die Planar-Chromatographie für das Inhaltsstoff-Screening im Vordergrund. Ausgehend davon werden die Methoden zur Qualitätskontrolle entwickelt, wie die Prüfung auf Identität, auf Reinheit (entsprechende Pflanzenspezies bzw. Verfälschungen) oder auf relevante Inhaltsstoffe. Für diese Art der Analytik ist die HPTLC als schnelles und einfaches Verfahren bestens geeignet.

Einleitung

Tee aus Blättern von *Camellia sinensis* wird seit Jahrhunderten auf der ganzen Welt als Getränk genossen. Grüntee, die minimal fermentierte (oxidierte) Form der getrockneten Teeblätter, kann positive Wirkung auf die Gesundheit haben und erfreut sich deshalb wachsender Beliebtheit. Heute werden Teeblätter auch industriell verarbeitet. Die daraus gewonnenen Extrakte gehen in verschiedenste Produkte ein, u.a. Getränke (z.B. IceTea), Energie-Riegel, Eiscrem und sogar kosmetische Cremes. Strengere Vorschriften, die darauf ausgerichtet sind, Qualität und Sicherheit dieser Produkte zu gewährleisten, zwingen die Industrie, die Identität aller in ein Produkt eingegangenen Rohstoffe zu dokumentieren.

Für die quantitative Analyse einzelner Inhaltstoffe von Grüntee wurden in den letzten fünf Jahren verschiedene analytische Methoden publiziert. Solche Methoden allein können aber die Gesamtqualität eines Produktes nicht angemessen beschreiben. Deshalb wurde ein umfassenderer Ansatz bezüglich Identität und Produkteinheitlichkeit gewählt. Vier HPTLC-Methoden wurden als Fingerprint entwickelt, mit denen Polyphenole (Catechine), Flavonoide, Aminosäuren (Theanin) und Purinalkaloide (Coffein, Theobromin) untersucht werden können.

Probenvorbereitung

Teeproben (100 mg) oder Tee-Extrakte (40 mg) wurden mit 10 mL Ethanol – Wasser 4:1 für 5–10 min im Ultraschallbad extrahiert. Nach dem Zentrifugieren wurden die Überstände direkt als Probenlösung verwendet. Die chemischen Referenzsubstanzen wurden einzeln in Konzentrationen von 0,5 bis 5 mg/mL in Methanol gelöst.

Schicht

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

Probenauftragung

4–6 µL von Proben und Standards wurden strichförmig als 8 mm Banden mit dem DC Probenautomat 4 (ATS 4) aufgetragen. Bahnabstand mindestens 10 mm, unterer Randabstand 8 mm, linker Randabstand 20 mm.

Chromatographie

In der Doppeltrogkammer 20 x 10 cm für

- Flavonoide mit Ethylformiat – Toluen – Ameisensäure – Wasser 30:1.5:4:3 mit Kammersättigung über 70 mm
- Polyphenole mit Toluen – Aceton – Ameisensäure 9:9:2 ohne Kammersättigung über 60 mm
- Alkaloide mit Ethylacetat – Methanol – Wasser 20:2.7:2 ohne Kammersättigung über 50 mm
- Aminosäuren mit 1-Butanol – Aceton – Essigsäure – Wasser 7:7:2:4 ohne Kammersättigung über 50 mm.

Derivatisierung und Dokumentation

Durch Tauchen mit der Chromatogramm-Tauchvorrichtung III:

- Flavonoide in Naturstoffreagenz (0.5 % Diphenylborinsäureaminoethylester in Ethylacetat) gefolgt von Macrogolreagenz (5 % Polyethylenglykol 400 in Dichlormethan), Auswertung unter UV 366/>400 nm.
- Polyphenole in Echtblausalz B-Reagenz (0.07 % in Wasser – Methanol – Dichlormethan 1:14:5), Auswertung im Weisslicht.
- Alkaloide werden unter UV 254 nm direkt ausgewertet.
- Aminosäuren in Ninhydrinreagenz (0.2 % in Methanol), Auswertung im Weisslicht.

Digitale Bilder der Platten wurden mit dem DigiStore 2-System aufgenommen.

Ergebnisse und Diskussion

Insgesamt wurden 80 Teeproben aus China, Japan, Indien und anderer Herkunft untersucht. Neben Grüntees wurden Schwarztees, Weisser Tee, Oolong, Pu-Erh und einige andere Spezialtees in die Studie einbezogen.

Unterscheidung nach der geographischen Herkunft von Grüntee

Teeblätter ergeben ein charakteristisches Flavonoid-Fingerprint. Obwohl die einzelnen Proben in den relativen Intensitäten der getrennten Zonen erheblich variieren, scheinen drei unterschiedliche

18

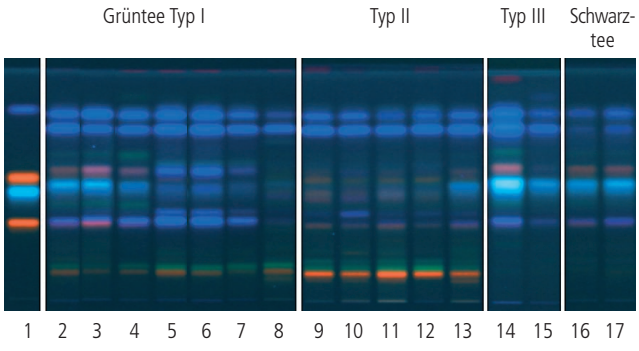
CAMAG DigiStore 2 Dokumentationssystem mit hochauflösender 12 bit CCD Kamera

Frutarom Schweiz setzt für die Dokumentation ihrer HPTLC-Platten das CAMAG DigiStore 2-System ein, denn es ermöglicht die professionelle Dokumentation von Planar-Chromatogrammen und anderen flachen Objekten.
Das schätzen unsere Kunden an ihrem DigiStore 2–System:

- Schnelle intuitive Bedienung aus winCATS mit hoher Reproduzierbarkeit
- Zugriff zu passenden Kamera-Parametern für alle Beleuchtungsarten
- Automatische Bildoptimierung
- Kontrastverstärkung ohne Veränderung des Original-Images
- Schneller Zugriff auf archivierte Bilder
- Objekte bis zu 4 cm Dicke können dokumentiert werden.
- Auf Wunsch kann das System IQ/OQ qualifiziert werden.
- Mit Zusatzoption ist es in einem 21 CFR Part 11-Umfeld einsetzbar.

Sollte der Wunsch nach einer digitalen quantitativen Bildauswertung bestehen, ist die Software mit CAMAG VideoScan kombinierbar.

Grundmuster aufzutreten (Typ I, II und III). Vor allem bei den Grüntees lassen sich diese mit der geographischen Herkunft der Proben korrelieren. Die meisten Proben vom Typ I kamen aus China, die meisten vom Typ II aus Japan und die vom Typ III aus Indien. Viele der untersuchten Schwarzteeproben gehören ebenfalls zum Typ III.

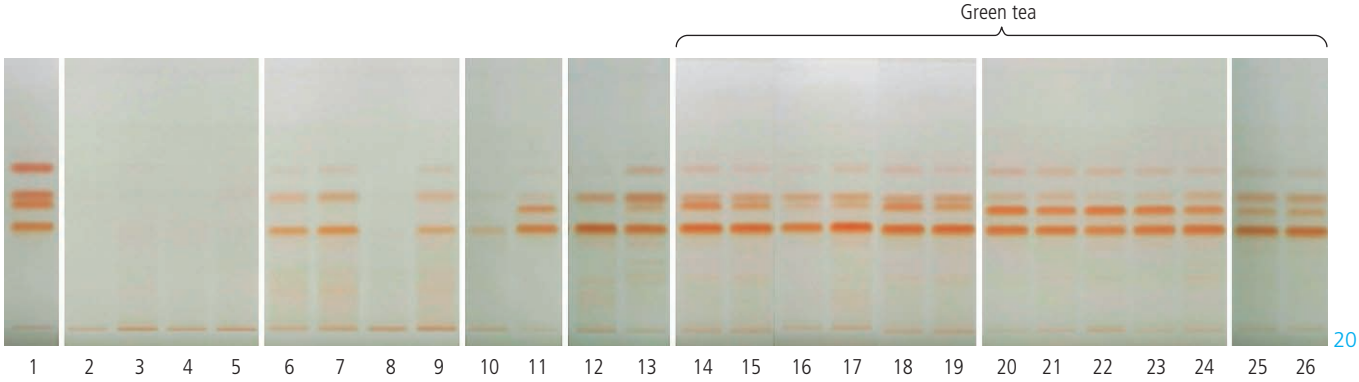


19

▲ *HPTLC-Fingerprints (Flavonoide) von Grünteeproben (auf verschiedenen Platten) unterschiedlicher geografischer Herkunft. Bahnbelegung: 1 Referenzsubstanzen mit steigendem R_F: Rutin, Chlorogensäure, Hyperosid, Gallussäure; 2–8 Proben aus China; 9–13 Proben aus Japan; 14–15 Proben aus Indien. Zum Vergleich: 16–17 Schwarztee aus Sri Lanka.*

Unterscheidung von Grüntee und anderen Teearten

Während der Verarbeitung der Teeblätter findet eine Fermentation (Oxidation) bis zu einem bestimmten Grad statt. Dabei wird das Polyphenolmuster (relativer Gehalt an Epigallocatechingallat, Epigallocatechin, Epicatechingallat und Epicatechin) beeinflusst. Während die Profile der untersuchten Proben von Weissem und Oolong Tee nicht konsistent und daher nicht repräsentativ waren, zeigten Schwarztees entweder gar keine Polyphenolzonen oder starke Zonen von Epigallocatechingallat und Epigallocatechin sowie eine schwache Zone für Epicatechin. Nur Grüntees zeigen vier Zonen, von denen jeweils die von Epigallocatechingallat die stärkste ist. Ein derartiges Muster konnte in allen Grünteeproben gefunden werden.



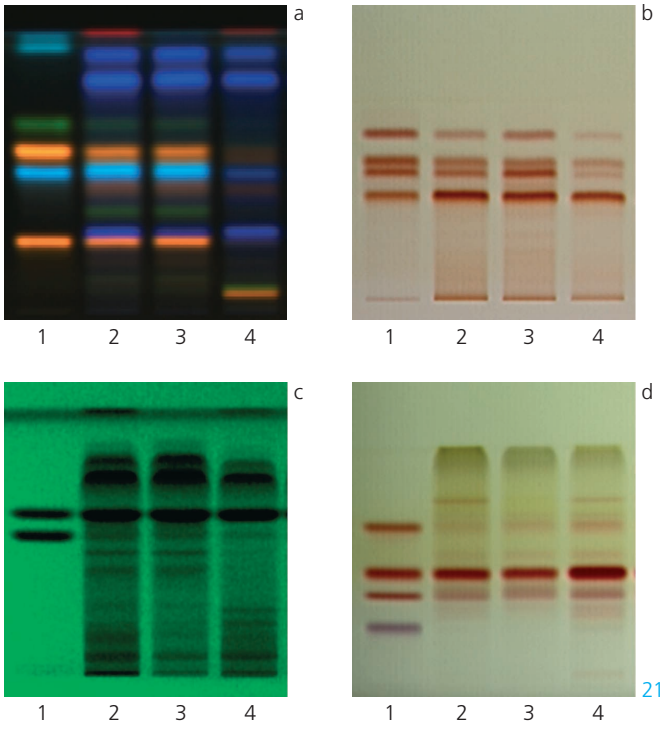
20

▲ *HPTLC-Fingerprints (Polyphenole) von Teeproben (auf verschiedenen Platten) unterschiedlichen Fermentationsgrades und unterschiedlicher geografischer Herkunft. Bahnbelegung: 1 Referenzsubstanzen mit steigendem R_F: Epigallocatechingallat, Epigallocatechin, Epicatechingallat und Epicatechin; 2–5 Schwarztee aus China; 6–9 Schwarztee aus Indien; 10–11 Oolongtee aus China (Probe 11 ist sehr wenig fermentiert); 12–13 Weisser Tee aus China; 14–19 Grüntee aus China; 20–24 Grüntee aus Japan; 25–26 Grüntee aus Indien.*

Hinweis: *Die Trennung von Epigallocatechin und Epicatechingallat (2 mittlere Zonen) wird von der relativen Luftfeuchte beeinflusst und ist daher auf den gezeigten Platten nicht genau gleich.*
Die ADC 2 ermöglicht die Einstellung einer reproduzierbaren Aktivität der Schicht und somit vergleichbare Chromatogramme. (Hrsg.)

Umfassende Charakterisierung eines Tee-Extrakts

Zusätzlich zu den oben beschriebenen Methoden kann man zwei weitere Methoden heranziehen, um Inhaltsstoffe von Grüntee zu untersuchen. Die resultierenden Fingerprints erlauben es dem Hersteller, nicht nur verschiedene Chargen von Rohmaterial mit einem botanischen Referenzmaterial (BRM) zu vergleichen, sondern auch zu belegen, dass während des Produktionsprozesses das gesamte Inhaltsstoffspektrum des Rohstoffes in das Produkt überführt wird.



21

▲ *HPTLC-Fingerprints von Grüntee und Grüntee-Extrakt. a) Flavonoide; b) Polyphenole; c) Alkaloide; d) Aminosäuren. Bahnbelegung: 1 Referenzsubstanzen mit steigendem R_F a) Rutin, Chlorogensäure, Isoquercitrin, Astragalin und Kaffeesäure; b) Epigallocatechingallat, Epigallocatechin, Epicatechingallat und Epicatechin; c) Theobromin und Coffein; d) Aspartamsäure, Glutaminsäure, Theanin und Tyrosin; 2 Grüntee BRM; 3 Grüntee-Extrakt; 4 Kommerzielles Grüntee-Produkt.*

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich.

[1] E. Reich, A. Schibli, V. Widmer, R. Jorns, E. Wolfram, A. DeBatt, J Liq Chromatogr & Rel Techn 14 (2006) 2141-2151

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22

CAMAG Automatische Entwicklungskammer ADC 2

Die Automatische Entwicklungskammer bietet Reproduzierbarkeit, Sicherheit und Komfort bei der Entwicklung von DC/HPTLC-Platten und -Folien im Format 20 x 10 und 10 x 10 cm. Bei den Arbeiten dieses CBS über Absinth (S. 5–6), Trigonellin (S. 9–11) und Grüntee (S. 12–15) könnte man die ADC2 mit Vorteil einsetzen.

Das schätzen die Kunden an der ADC 2:

- Der Benutzer wird von sämtlichen Überwachungsfunktionen entlastet, und der Ablauf bleibt in allen Einzelheiten nachvollziehbar.
- Betrieb im stand-alone Modus oder unter winCATS
- Die ADC 2 mit winCATS entspricht den Anforderungen von cGMP und kann IQ/OQ qualifiziert werden. Das Gerät ist in einem 21 CFR Part 11-Umfeld einsetzbar.
- Ganz besonders schätzen Kunden die Option »Feuchtekontrolle«, mit der reproduzierbare Chromatographie bei definierter Aktivität der Schicht ermöglicht wird.

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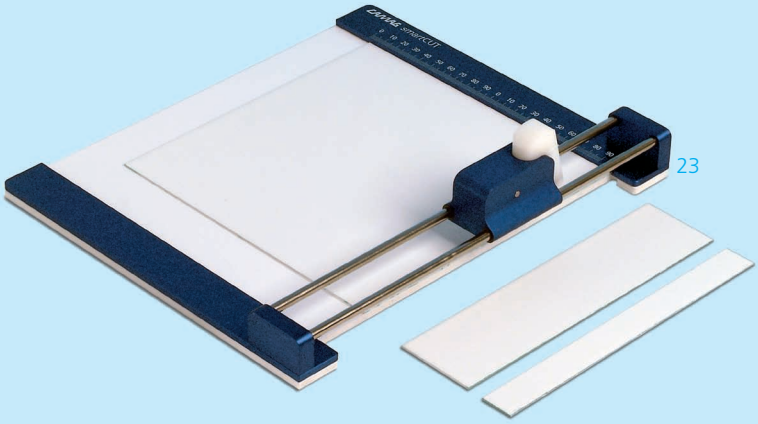

23

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