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Planar-Chromatographie
Herausgegeben von Gerda Morlock
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IN DIESER AUSGABE

Verfahren, Anwendungen

In dieser Ausgabe hervorgehobene Produkte und Dienstleistungen

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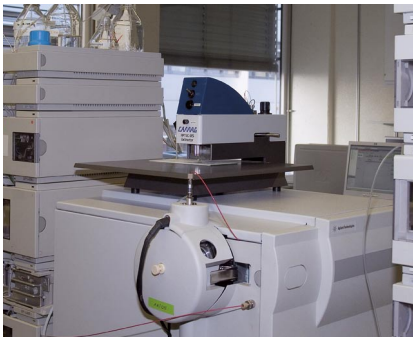
Titelseite:
Höckeriger Geweihschwamm (Axinella cannabina)
Foto: Andrej Jaklin, Rovinj, Kroatien

Das neue TLC-MS Interface


1


2

Links nach rechts: Dr. Matthias Loppacher, Leiter F & E CAMAG, Rolf Rolli, CEO CAMAG

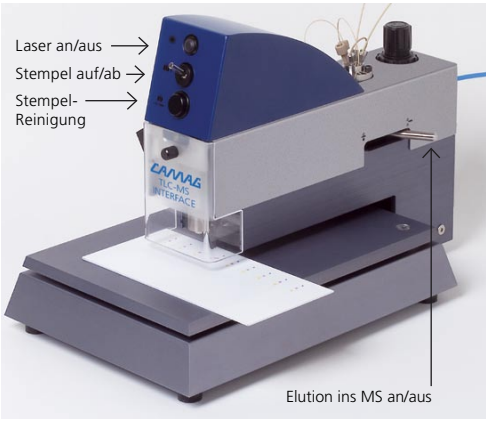
Seit mehr als 20 Jahren sind Bestrebungen im Gange, TLC mit Massenspektrometrie direkt zu verbinden, ähnlich der Kopplung HPLC-MS. Von Dr. Luftmann, Leiter der Abteilung Massenspektrometrie, Organisch-Chemisches Institut der Westfälischen Wilhelms-Universität in Münster, wurde ein Interface (ChromeXtractor) entwickelt, das eine echte online Kopplung TLC-MS ermöglicht [1, 2]. Dr. Morlock, Privatdozentin am Institut für Lebensmittelchemie der Universität Hohenheim in Stuttgart, modifizierte den ChromeXtractor und zeigte die Leistungsgüte dieses universellen Interfaces im Vergleich zu anderen Kopplungsarten auf [2–16].

Von CAMAG im Jahre 2007 angestellte Kundenumfragen ergaben, dass ein grosses Bedürfnis nach einer standardisierten Lösung besteht, und dass zunächst ein semi-automatischer Betrieb wünschenswert erscheint. Daher wurde ein entsprechendes kommerzielles Gerät entwickelt, das CAMAG jetzt unter der Bezeichnung TLC-MS Interface auf den Markt bringt. Funktion und Anwendung wurden bereits in Beiträgen der CBS-Ausgaben 93, 94, 96, 98, 100 und 101 vorgestellt. Der Vorteil dieses Interfaces liegt darin, dass es ohne Modifizierung in jedes beliebige HPLC/MS-System mit chemischer Ionisation bei Atmosphärendruck (APCI), Photoionisation bei Atmosphärendruck (APPI) oder Elektrospray-Ionisation (ESI) integriert werden kann. Das Interface wird in die Kapillarleitung zwischen der HPLC-Pumpe und dem Massenspektrometer geschaltet. Die interessierende Substanz wird direkt von der TLC/HPTLC-Platte eluiert und online in das Massenspektrometer überführt. Das Massenspektrum ist innerhalb einer Minute verfügbar.

Prinzip der Extraktion

Substanzgemische – auch solche mit hohem Matrixanteil – werden auf TLC/HPTLC-Platten oder -Alufolien kostengünstig aufgetrennt. Die zu untersuchende Zone kann, falls nicht sichtbar, unter UV 254 nm, UV 366 nm, durch Extrapolation der nach Derivatisierung sichtbaren benachbarten Zone oder mittels des durch den TLC Scanner 3 erhaltenen hR_F-Wertes markiert werden. Durch ein Laserkreuz kann die Zone im Interface genau unter dem Extraktionsstempel positioniert und extrahiert werden. Das TLC-MS Interface arbeitet semi-automatisch, d.h. nach manueller Positionierung der Zone wird per Knopfdruck der Stempel abgesenkt. Nach Umlegen eines Hebels fliesst Lösungsmittel durch die Schicht und extrahiert

die Zone. Zuvor wurde die Datenaufnahme gestartet, z.B. durch Fliess-Injektions-Analyse (FIA), Spritzeneinlass, Placeboinjektion oder einem direkten Aufnahmefenster. Anschliessend wird der Reinigungsmechanismus (ca. 5 s) gestartet, und das TLC-MS Interface steht für die nächste Analyse zur Verfügung.

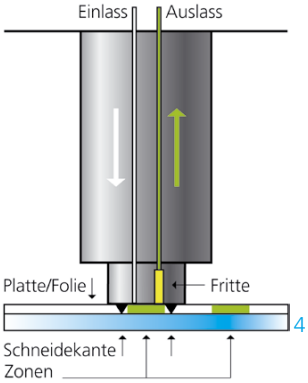


3

Interface-Funktionen und Positionierung der Zone im Laserkreuz

Extraktionsstempel

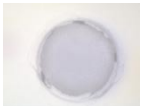
Der aktuelle Extraktionsstempel hat einen Durchmesser von 4 mm. Ovale Extraktionsstempel für bandförmige Zonen sind geplant. Bei der Absenkung des Extraktionsstempels auf die Schicht mit einer Kraft von ca. 20 kg wird die Extraktionszone komplett abgedichtet. Der Stempel hat eine Einlasskapillare, durch die ein geeignetes Lösungsmittel gepumpt wird, z. B. Methanol oder eine Mischung von Methanol und Ammoniumformiat-Puffer (10 mM, pH 4) 95:5 (v/v). Typische Flussraten der HPLC-Pumpe liegen im Bereich von 0.05 bis 0.5 mL/min, vorzugsweise bei 0.1 mL/min. Dabei durchströmt das Lösungsmittel die Schicht und löst die Substanz heraus. In der Auslasskapillare ist eine Fritte eingebaut, die die Verunreinigung des Massenspektrometers durch das Ausschwemmen von Kieselgelpartikeln verhindert.



4

Schema des Extraktionsstempels

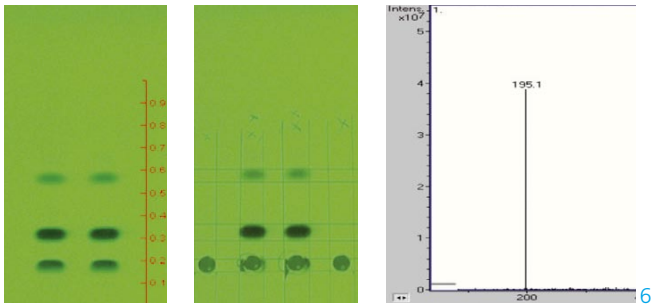

5a


5b

Plattenausschnitt mit blauer Farbstoffzone vor und nach der Extraktion

Anwendungsbeispiel

Zur Identifizierung der Zone bei hR_F15 aus einer Standardmischung von Coffein, Paracetamol und Acetylsalicylsäure wird das Massenspektrum der Zone aufgenommen. Auf der gleichen Höhe wird auch ein Hintergrund-Spektrum der Platte aufgenommen, welches vom Substanzzonen-Spektrum abgezogen werden kann. So erhält man ein von Systempeaks bereinigtes Massenspektrum, also vorrangig die Substanzsignale – hier das Massensignal *m/z* 195 $[M+H]^+$ für Coffein.



6

Links: Chromatogramm mit 4 mm Banden, Mitte: Chromatogramm nach Extraktion der Zone bei hR_F 15, rechts: Extrahierte Zone durch das Massensignal bei m/z 195 als Coffein identifiziert

Weiterführende Literatur zu dieser Kopplung

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Screening nach aktiven Naturstoffen aus Schwämmen

7 *Anne Klöppel und Andrea Kolm*

8 *Prof. Dr. Franz Brümmer*

Die Arbeitsgruppe von Prof. Dr. Franz Brümmer[1], Biologisches Institut der Universität Stuttgart, untersucht u.a. bioaktive Naturstoffe aus Schwämmen (Porifera). Dabei liegt der Fokus auf der chemischen Ökologie sowie der Aufklärung neuer interessanter Substanzen mit potentiell pharmakologischer Wirkung. Ein weiterer Aspekt stellt die Kultivierung ausgewählter Schwammarten in verschiedenen Systemen (*in situ, ex situ* und *in vitro*) und den damit verbundenen Änderungen im Metabolitspektrum dar.

In Zusammenarbeit mit Dr. Gerda Morlock[2], Institut für Lebensmittelchemie der Universität Hohenheim in Stuttgart, konnte durch den Einsatz der HPTLC und Kopplung mit der Bioaktivitätsdetektion und Massenspektrometrie zum ersten Mal eine hervorragende Methodik zur Naturstoffsuche aufgezeigt werden, um kulturbedingte Varianzen im Naturstoffmuster sowie in der Bioaktivität einzelner Substanzen zu erkennen.

Einleitung

Marine Invertebraten produzieren eine Vielzahl bioaktiver Substanzen. Sie sind eine der ergiebigsten Quellen von pharmakologisch wirksamen Naturstoffen, denn jedes Jahr werden bis zu 800 neue bioaktive Substanzen beschrieben, von denen etwa 45 % in Schwämmen gefunden werden. Diese nutzen die meist sessilen Filtrierer u. a. gegen Frassfeinde und Konkurrenzdruck, da sie über keine ausreichend wirksamen morphologischen Schutzmechanismen verfügen. Das Wirkspektrum der Stoffe reicht von antibakteriell und entzündungshemmend bis hin zu cytotoxisch und virostatisch. Trotz der Fülle an bioaktiven Metaboliten haben nur wenige schwammspezifische Substanzen die Produktreife erreicht, z. B. Ara A®, 9-β-D-arabino-furanosyl-adenin aus *Cryptotethya crypta,* das gegen das *Herpes simplex*-Virus wirkt.

Dem Einleiten der klinischen Testphasen bzw. dem endgültigen Einsatz in der Medizin geht eine aufwändige Kombination verschiedener Analysen voraus. Zunächst wird die generelle Bioaktivität der Rohextrakte mittels Standardbioassays ermittelt. Dazu gehören z. B. der Agardiffusionstest mit Standardbakterienstämmen wie *Escherichia coli* und *Bacillus subtilis* oder der Leuchtbakterientest mit *Vibrio fischeri*. Um jedoch eine klare Korrelation zwischen einem speziellen Metabolit und dessen Bioaktivität aufzuzeigen, ist eine Fraktionierung des Extraktes notwendig. Zur Isolierung und Reinigung aller möglichen bioaktiven Substanzen pro Extrakt und Spezies müssen verschiedene säulenchromatographische Systeme (z. B. Festphasenextraktion, Gelpermeationschromatographie, semipräparative HPLC) kombiniert werden. Danach folgt ein erneutes Bioaktivitätsscreening jeder einzelnen Substanz bzw. Fraktion. Gängige Kopplungen zur Strukturaufklärung bioaktiver Metabolite sind dann z. B. HPLC-MS und NMR.

Bei der aufwändigen Suche und Identifizierung neuer bioaktiver Substanzen stellt die HPTLC im Vergleich zur HPLC eine verkürzte, robustere (kaum Matrix-Effekte) und ökonomischere Analytik dar. Es ist möglich, bis zu 30 Schwammextrakte parallel chromatographisch aufzutrennen. Die Kombination mit einem Bioaktivitätstest basierend auf dem Leuchtbakterientest mit *Vibrio fischeri* nach DIN EN 11348 und anschliessender hochauflösender Massenspektrometrie zum Erhalt der Summenformel der neu gefundenen bioaktiven Substanzen

ermöglicht ein effektives Metabolit- und Bioaktivitätsscreening von Schwämmen [1]. Einzelne interessante Substanzen können dabei selektiv – ohne aufwändigen Isolierungs- und Reinigungsprozess – direkt von der HPTLC-Platte extrahiert und ins MS überführt werden. Innerhalb einer Minute erhält man das dazugehörige Massensignal und über die Hochauflösung die Summenformel. Die Detektierbarkeit ist vergleichbar empfindlich zur HPLC-MS, da das gesamte Substanzband von der Platte extrahiert wird.

Probenvorbereitung

Nach der Beprobung bzw. Kultivierung (*in situ* bzw. *in vitro*) wurden 1–5 cm³ je Schwamm in flüssigem Stickstoff eingefroren und gefriergetrocknet. Die Proben wurden pulverisiert und je 100 mg in sterile 50 mL Reaktionsgefässe eingewogen. Die Extraktion erfolgte mit 10 mL hochreinem Methanol auf dem Rollschüttler über 20 h, und nach Zentrifugieren wurde der Überstand eingesetzt.

Standardlösungen

Avarol und Avaron (isoliert aus *Dysidea avara,* Prof. Dr. Werner Müller, Universität Mainz) wurden jeweils in Methanol gelöst (0,1 mg/mL).

Schicht

HPTLC-Platten Kieselgel 60 F_{254} (Merck), 20 x 10 cm, vorgewaschen durch Entwicklung mit Methanol, anschliessend 15 min bei 100 °C auf dem DC-Plattenheizer getrocknet.

Probenauftragung

Bandförmig mit dem DC-Probenautomat 4, Bandlänge 4 mm, unterer Randabstand 8 mm, seitlicher Randabstand 10 mm, Bahnabstand 6 mm, Probenvolumina 20 µL pro Schwammextrakt, Standardvolumina 0,2–2 µL (20–200 ng/Band).

Chromatographie

Im AMD 2-System mit einem 15-Stufen-Gradient basierend auf Methanol, Dichlormethan und n–Hexan. Die Auftrennung erfolgte unter Stickstoff über 2,5 Stunden bis zu einer finalen Laufstrecke von 53 mm.

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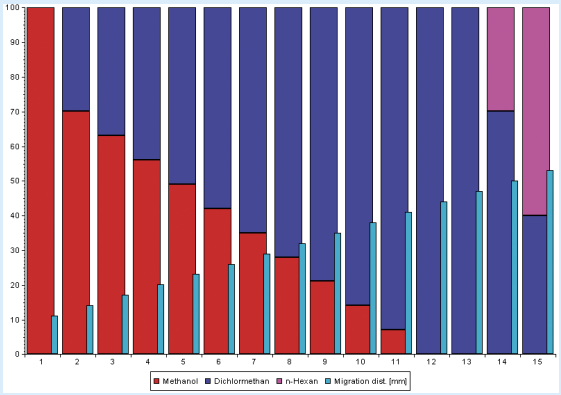
CAMAG AMD 2 System
(Automatisierte Mehrfachentwicklung)

Das AMD-Verfahren ermöglicht die Anwendung der Gradientenelution in der Planar-Chromatographie, vor allem in der Normalphase. Es wird angewendet zur Trennung komplexer, gegebenenfalls auch stark matrixbelasteter Proben mit einer Trennschärfe, die mit anderen Entwicklungstechniken nicht erreicht werden kann.

Hier wird das AMD-Verfahren bei der Suche nach bioaktiven Naturstoffen in Schwämmen eingesetzt. Dabei geht es weniger um die Nutzung der höchstmöglichen Trennleistung als vielmehr um den standardisierten Vergleich einer Vielzahl von Schwämmen hinsichtlich ihrer Inhaltsstoffe, weitgehend unabhängig von ihrer Matrixbelastung.

Dazu wurde der nachstehend wiedergegebene Gradient eingesetzt, der einen sehr grossen Polaritätsbereich abdeckt. Dass in manchen Fällen Überladungen unvermeidbar waren, ist für die hier gestellte analytische Aufgabe unerheblich.



10

Bioaktivitätsdetektion

Die Platten wurden unter Weisslicht sowie UV 254 nm und 366 nm mit dem DigiStore 2-System dokumentiert. Zusätzlich wurden sie für das Bioaktivitätsscreening in eine Suspension der *Vibrio fischeri*-Bakterien (BioLuminex-Schnelltest, ChromaDex, Boulder, USA) mit Hilfe der DC-Tauchvorrichtung III getaucht (Tauchgeschwindigkeit 3,5 cm, Eintauchzeit 1 s). Die Wirkung der einzelnen bioaktiven Substanzen auf das Photobakterium führte zu einer Abschwächung oder Verstärkung der Leuchtintensität, die mittels BioLuminizer dokumentiert wurde (Belichtungszeit 30 s). Das aufgenommene Bild kann zur digitalen Auswertung in die Software VideoScan importiert werden.

Kopplung mit ESI-MS

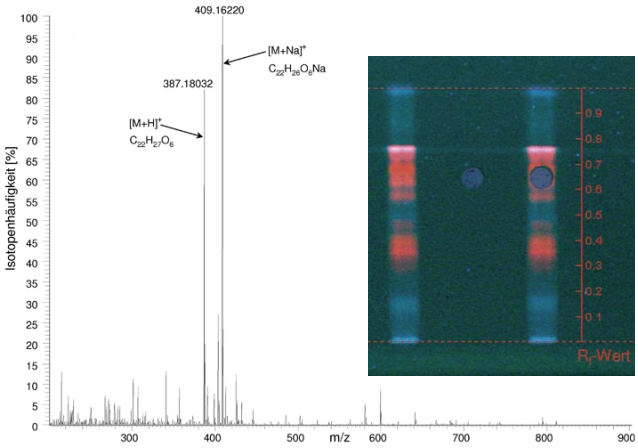
Interessante Banden wurden auf der HPTLC-Platte markiert und durch online Extraktion (ChromeXtraktor, ChromAn) bei einer Flussrate von 0,1 mL/min mit Methanol – Ammoniumformiat (10 mmol/L, pH 4) 95:5 ins ESI-MSD (Agilent) überführt (Kapillarspannung 4 kV, Desolvatisierungstemperatur 300 °C, Trocknungsgas 10 L/min, Vernebelungsgas 30 psig). Das Massenspektrum wurde im positiven full scan-Modus zwischen *m/z* 200 und 900 aufgezeichnet und mittels hochauflösender MS (LTQ Orbitrap XL hybrid FT-MS, Thermo Fisher Scientific) abgesichert.

Ergebnisse und Diskussion

Die *in vitro* Kultur von Schwammzellen (hier: Primmorphe, proliferierende 3D-Zellaggregate) stellt eine Möglichkeit dar, bioaktive Substanzen in einem grösseren Massstab zu produzieren.

Durch HPTLC-Bioaktivitätsscreening konnte nachgewiesen werden, dass auch in der Zellkultur die Synthese interessanter Sekundärmetabolite fortgeführt wird. Bei manchen Schwämmen (z.B. *Axinella polypoides*) traten jedoch unter *in vitro* Bedingungen Unterschiede in der qualitativen Metabolit-Zusammensetzung auf. Dies führte aber zu keinem Einfluss auf die Gesamttoxizität der Extrakte. Oftmals zeigten aus der Zellkultur gewonnene Schwammextrakte (z. B. *Axinella polypoides*, *Suberites domuncula*, *Petrosia ficiformis*) – bei gleicher Konzentration zum *in situ* Extrakt – eine intensivere Lumineszenzlöschung der gleichen Substanz. Dies könnte an einer stressbedingten Zunahme der Metabolit-Synthese liegen. Ausserdem kam es bei manchen Schwammarten zur Produktion weiterer bioaktiver, eventuell neuer noch unbekannter Substanzen, die im Metabolitmuster unter *in situ* Bedingungen fehlten (z. B. *Suberites domuncula*). So bildete der Schwamm *Dysidea avara* in der Zellkultur einen bioaktiven Stoff, der zu einer Lumineszenzverstärkung der Leuchtbakterien führte. Ein weiterer *Vibrio fischeri*-toxischer Metabolit ging hingegen verloren bzw. wurde kaum synthetisiert. Bei dem Vergleich, inwieweit assoziiert lebende Cyanobakterien in *Petrosia ficiformis* an der Metabolitsynthese beteiligt sind, stellte sich heraus, dass es durchaus Änderungen im Naturstoffspektrum gab, die hier aber keinen Einfluss auf die Gesamttoxizität hatten.

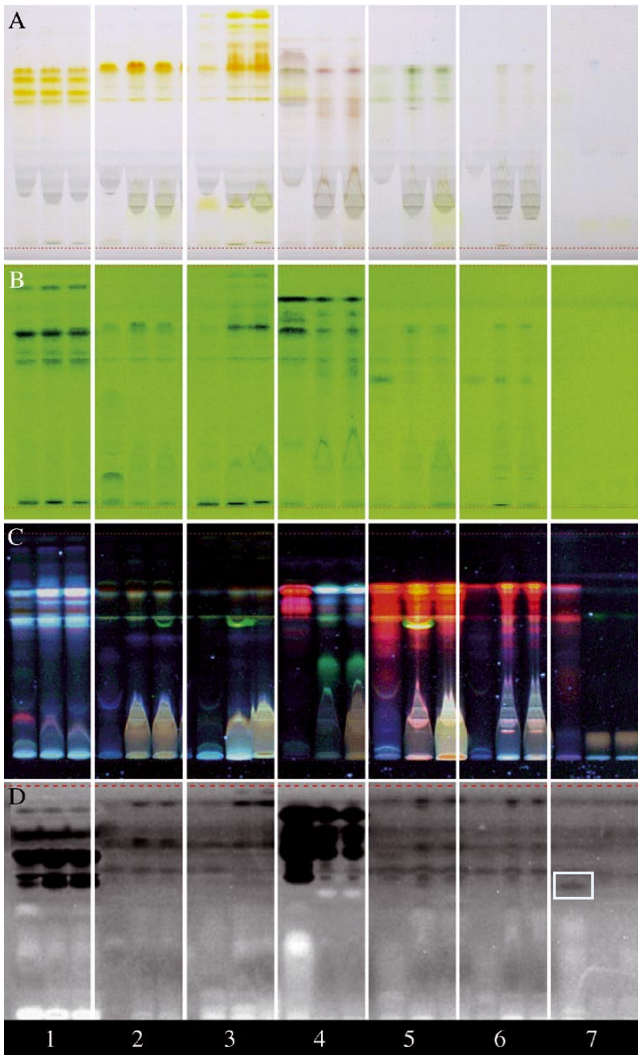
Die Kopplung der HPTLC mit dem BioLuminex-Schnelltest und der Massenspektrometrie ermöglichte es, 30 verschiedene Schwammextrakte sehr schnell und effektiv parallel zu trennen, deren Bioaktivität zu ermitteln und gezielt Strukturaufklärung interessanter Stoffe zu betreiben. Mit dieser Methodik (HPTLC-Bioaktivitätsdetektion-MS) konnte eine unbekannte bioaktive Substanz erstmalig in einem Süsswasserschwamm (*Ephydatia fluviatilis*) gefunden werden, die in der *in vitro* Kultur allerdings nicht mehr synthetisiert wurde. Durch die Kopplung mit hochauflösender Massenspektrometrie konnte die exakte monoisotope Masse mit *m/z* 387,18032 $[M+H]^+$ ermittelt werden sowie *m/z* 409,16220 für das Natrium-Addukt. Dies führte zu einer Summenformel von $C_{22}H_{27}O_6$.



11

Links: HPTLC-ESI-MS-Spektrum (positiver Modus) einer bioaktiven Zone des Süsswasserschwamms Ephydatia fluviatilis. Die Messungen führten zu einer monoisotopen Masse des protonierten Moleküls von m/z 387,18032 $[M+H]^+$ mit einer Massendivergenz von 0,7 ppm.

Rechts: Ausschnitt der entwickelten HPTLC-Platte dokumentiert unter UV 366 nm: Die bioaktive Substanz (hR_F 65) wurde per Lösungsmittelstempel (Ø 4 mm) extrahiert (linke Bahn: vor der Extraktion, Mitte: Blindwert, rechte Bahn: nach der Extraktion).

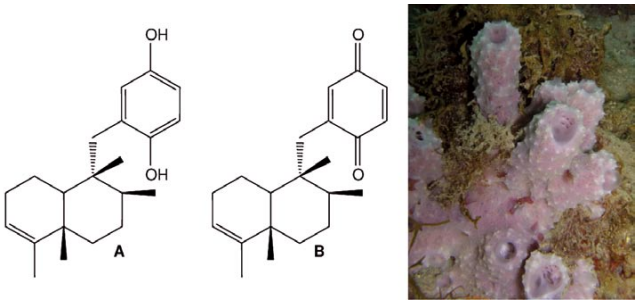


12

Detektion der Platte (A) unter Weisslicht, (B) bei 254 nm, (C) bei 366 nm und (D) mittels BioLuminizer. Im Teilbild 1–7 jeweils linke Bahn Schwammextrakte (in situ) sowie mittlere und rechte Bahn deren Primmorphen (in vitro, mittlere Bahn: Medium nach Le Pennec et al. (2003), rechte Bahn: Medium nach Zucht (2005)): Acanthella acuta (1), Axinella polypoides (2), Suberites domuncula (3), Dysidea avara (4), Petrosia ficiformis mit symbiotischen Cyanobakterien (5), Petrosia ficiformis ohne symbiotische Cyanobakterien (6), Ephydatia fluviatilis (7) hier: Medium nach Harsha et al. (1983).

Hemmung der Biolumineszenz (schwarze Zonen), Verstärkung der Biolumineszenz (weisse Zonen), unbekannter bioaktiver Stoff aus Ephydatia fluviatilis (weisse Box).

Bereits bekannte bioaktive Naturprodukte sind die antiviral und cytotoxisch wirkenden Metaboliten Avarol und Avaron. Die Detektionsgrenzen der Bioaktivität lagen bei 70 (Avarol) bzw. 60 ng/Band (Avaron) und waren für das Screening von Schwämmen sehr gut geeignet.



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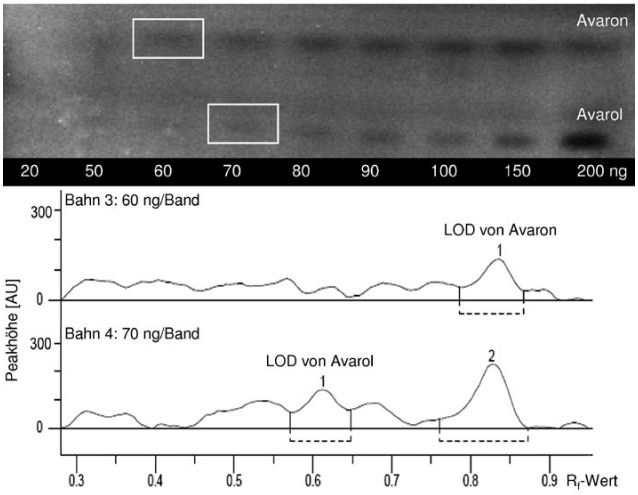
Strukturformeln von Avarol (A) und Avaron (B) aus dem Schwamm Dysidea avara (rechts). Avarol findet derzeit u.a. Anwendung in Salben gegen Psoriasis (International Patent Application DE 1991-4137093).



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Visuelle (weiße Box) und digitale bioaktive Detektionsgrenze (mittels VideoScan) von Avaron (60 ng/Band) und Avarol (70 ng/Band) nach HPTLC-Bioaktivitätsdetektion.

Durch die effektorientierte Detektion werden bioaktive Metabolite sichtbar, auch solche, die z. B. nicht UV-aktiv und so über gängige Detektoren wie HPLC-DAD nicht nachweisbar sind. Ein wesentlicher Vorteil gegenüber der HPLC-MS ist hierbei, dass zeitaufwändige Isolierungs- und Reinigungsprozesse komplett entfallen. Da alle Lösungsmittel nach der chromatographischen Trennung entfernt werden, können diese nicht mehr störend auf die Detektion einwirken, z. B. durch Inaktivierung von Enzymen oder lebenden Organismen wie den Leuchtbakterien.

[1] A. Klöppel, W. Grasse, F. Brümmer, G. Morlock, J. Planar Chromatogr. 21 (2008) 431-436

[1]Prof. Dr. Franz Brümmer, Biologisches Institut, Abteilung Zoologie, Universität Stuttgart, Pfaffenwaldring 56, 70569 Stuttgart, franz.bruemmer@bio.uni-stuttgart.de

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Kennen Sie CAMAG?

Die Online-Kopplung HPTLC-MS weiten Kreisen nahe zu bringen, haben wir uns für 2009 vorgenommen.

Die Kopplung DC/HPTLC-MS ist geeignet, die Planar-Chromatographie auch für solche Analytiker attraktiv zu machen, die ihr bisher reserviert gegenüber standen. Denn sie eröffnet der Planar-Chromatographie neue Dimensionen und wertet sie wissenschaftlich auf.

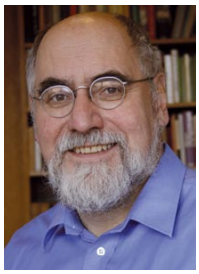
Das dazu benötigte Interface ist auf Seite 16 dieses CBS beschrieben, und einen Erfahrungsbericht finden Sie auf S. 2/3. Auch in den letzten CBS-Ausgaben wurde bereits wiederholt über diese Kopplung berichtet.

Jetzt veranstaltet CAMAG in Deutschland eintägige Seminare, in denen Sie eingehend über die Methode und deren Möglichkeiten informiert werden. Vormittags halten Wissenschaftler, die an der Entwicklung der Methode beteiligt waren oder bereits Erfahrungen damit sammeln konnten, Vorträge. Nachmittags finden praktische Demonstrationen im Labor statt, u.a. auch mit Proben (DC/HPTLC Platten), die von den Teilnehmern mitgebracht werden.

Veranstaltungstermine

26.03.2009	Zweckverband Landeswasserversorgung, D-89129 Langenau
30.04.2009	Fachhochschule D-77652 Offenburg
26.06.2009	Universität D-481490 Münster
01.10.2009	Technische Universität D-13355 Berlin

Bitte melden Sie Ihr Interesse bei Dr. Konstantinos Natsias (info@camag-berlin.de) an. Sie erhalten dann ausführliche Unterlagen. Die Teilnahme ist gebührenfrei.



15 *Dr. Heinrich Luftmann, Leiter der Abteilung Massenspektrometrie am Organisch-Chemischen Institut der Westfälischen Willhelms-Universität Münster*

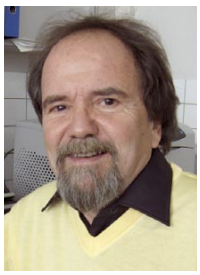
Arbeitsgebiet: Untersuchung von Proben unterschiedlichster Natur (>18000 pa) – Syntheseprodukte, metallorganische Verbindungen, Kohlenhydrate, Peptide, Polymere, etc. Spezielle Interessen: Entwicklung von Geräten und Zusatzeinrichtungen für die MS

Vortragsthema: ***TLC-MS in der organischen Synthese***

16 *Dr. Wolfgang Schulz, Laborleiter für den Bereich Sonderanalytik im Betriebs- und Forschungslabor, Zweckverband Landeswasserversorgung Langenau, langjähriger Dozent an der Hochschule Aalen*

Arbeitsgebiet: Non-Target Screening mit Planar-Chromatographie, HPLC, MS sowie wirkungsbezogene Analytik

Vortragsthema: ***Einsatz der HPTLC-MS Kopplung zur Identifizierung organischer Spurenstoffe in Roh- und Trinkwasser***



17 *Prof. Dr. Hans-Rudolf Schmutz, Fachhochschule für Life Sciences Nordwestschweiz, in Muttenz, Lehrtätigkeit in klassischer Analytik und Instrumentalanalytik Arbeitsgebiet: Pharma-Analytik unter Einsatz der verschiedensten Trenntechniken, speziell der gekoppelten Techniken GC-, HPLC-MS/MS, HPTLC-MS und MALDI-TOF.*

Vortragsthema: ***HPTLC-MS von pharmazeutischen Wirkstoffen und Pflanzeninhaltsstoffen***

18 *PD Dr. Gerda Morlock, Universität Hohenheim in Stuttgart. Lehrtätigkeit im Bereich Lebensmittelanalytik*

Arbeitsgebiet: Kopplung HPTLC-MS, wirkungsbezogene Analytik, digitale Auswerteverfahren für planare Chromatogramme, Aufdruckverfahren und nano-strukturierte Schichten in der Planar-Chromatographie

Vortragsthema: ***HPTLC-MS in der Lebensmittel- und pharmazeutischen Analytik***

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Liebe Freunde

In dieser Ausgabe liegt der Fokus auf Naturstoffanalytik und der Kopplung Planar-Chromatographie mit MS. Mithilfe des neuen TLC-MS-Interface (S. 2–8, 16) können nun Analytiker Fragen bezüglich der Identität nicht getrennter Substanzzonen oder möglicher Coelution von Substanzen innerhalb einer Minute beantworten. Für unbekannte Substanzzonen können die Summenformel und erste Hinweise zur Struktur erhalten werden, wenn das Interface z. B. mit einem hochauflösenden Massenspektrometer oder einem Tandem-MS verbunden ist.

Das Interface ermöglicht kontaminationsfreie, schnelle, kostensparende und vielseitige Transfermöglichkeiten. Zum Beispiel können Substanzzonen auch in je 100 µL eines Lösungsmittels aufgefangen werden. Die gelöste Zone kann dann weitergehend untersucht werden, z. B. mit NMR oder ATR-FTIR.

Die Kosteneinsparung wird deutlich am Beispiel vom Screening nach Naturstoffen (S. 4–7): Nur von interessierenden Zonen (z. B. bioaktiven Verbindungen) wird das Massenspektrum aufgenommen, jedoch nicht von Hintergrund und Matrix. Hingegen ist in der Säulenchromatographie bei unbekannten Proben die Aufnahme des gesamten Laufes nötig, weil man ja nicht im Voraus weiss, wann Analyten eluieren.

Gewinnen Sie einen Eindruck vom Potential des Interface auf einem der Fortbildungstage (S. 8) oder fragen Sie nach einer speziellen Vorführung in Ihrem Labor. Nutzen sie die Chance, sich zu informieren!

Mit freundlichen Grüssen

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

In this issue we focus on natural products analyses and the coupling of planar chromatography with MS. By means of the TLC-MS Interface (p. 2–8, 16) analysts can now answer questions regarding substance identity or potential co-elution of substances within a minute. For unknowns the sum formula and first hints on the structure can be obtained if the interface is connected e.g. to a high resolution mass spectrometer or tandem MS.

The interface offers contamination-free, rapid, cost-effective and versatile transfer options. For example it can also be used for collection of zones in 100 µL-portions of a solvent. The zones dissolved can be forwarded to further analysis, e.g. by NMR or ATR-FTIR.

Its cost-effective employment is clearly demonstrated for screening of natural products (p. 4–7): Just for zones of interest (e. g. bioactive compounds) the mass spectrum is recorded, and not for background and matrix. Whereas the recording of the whole run is status quo for unknown samples in column chromatography because one does not know when analyte(s) will elute.

You can get an impression of the interface´ potential attending the one-day seminar (p. 8) or asking for a special arrangement at your laboratory. Take the opportunity to get informed about it!

Sincerely,

Gerda Morlock

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cbs@camag.com

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MÄRZ
MARCH
2009

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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**

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

1. Reviews and books

102 001 L. CIESLA*, Monika WAKSMUNDZKA-HAJNOS (*Department of Inorganic Chemistry, Medical University, 20-081 Lublin, Poland): Two-dimensional thin-layer chromatography in the analysis of secondary plant metabolites. J. Chromatogr. A 1216 (7), 1035-1052 (2009). A review on two-dimensional TLC in the analysis of secondary plant metabolites. Plant extracts are usually very complex mixtures, therefore chromatographic methods are one of the most popular analysis techniques. The separation power of one-dimensional techniques is usually inadequate, therefore in this paper multidimensional planar chromatographic methods are reviewed. General aspects of multidimensionality are discussed. Attention is drawn to the potential of two-dimensional planar chromatography in the field of phytochemistry.

pharmaceutical research, qualitative identification, quantitative analysis, review 1

2. Fundamentals, theory and general

102 002 S. ERGÜL (Department of Science Education, Faculty of Education, Ondokuz Mayis University 55200, Atakum Yerleskesi-Samsun, Turkey): Linkage between separation of Cu2+, Co2+, and Ni2+ on TLC and crystal field theory. J. Chromatogr. Sci. 46 (10), 907-911 (2008). Examination of M(DEDTC)2 (M = Cu, Co, or Ni) and M(PyDTC)2 (M = Cu or Co) complexes prepared by reactions of sodium diethyldithiocarbamate (NaDEDTC) and ammonium pyrrolidinedithiocarbamate (NH4PyDTC) with metal (II) nitrates. Qualitative analysis and separation using TLC systems in the literature. Reexamination and discussion of the already known separation behaviour of the mentioned metal cations and their complexes in the context of relation to the crystal field theory (CFT) and TLC. Based on the chromatographic data it was found that CFT is closely related to the TLC separation of these metal cations and their complexes. This study is useful in understanding the linkage between the CFT of coordination chemistry and the chromatographic parameters, e.g., hRf value and theoretical plate numbers of the complexes.

quantitative analysis 2c, 33a

102 003 L. KOMSTA (Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland): A functional-based approach to the retention in thin layer chromatographic screening systems. Anal. Chim. Acta 629 (1-2), 66-72 (2008). Presentation of a QSRR approach for prediction of the retention in seven TLC screening systems. Optimization of the model by uninformative variable elimination-partial least squares (UVE-PLS) reduced the variables involved. Final equations with 7 - 32 variables were obtained which explain 32 - 60 % of overall explained variance. Their predictive ability (Q2) includes a range of 25 - 54 % (LOO crossvalidation) and 12 - 43 % (external validation).

prediction of retention, QSRR model 2c

3. General techniques

102 004 R. BHUSHAN*, C. AGARWAL (*Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee, 247667, India): Direct TLC resolution of (±)-ketamine and (±)-lisinopril by use of (+)-tartaric acid or (-)-mandelic acid as impregnating reagents or mobile phase additives. Isolation of the enantiomers. Chromatographia 68 (11-12), 1048-1051 (2008). TLC resolution of the enantiomers of the racemic drugs ketamine and lisinopril on silica gel with 1) ethyl acetate - methanol - water 3:1:1 and enantiomerically pure tartaric acid and (-)-mandelic acid as chiral impregnating reagents, for ketamine; and 2) acetonitrile - methanol - water - dichloromethane 14:2:2:1 with (+)-tartaric acid as the impregnating agent for lisinopril and using (+)-tartaric acid as mobile phase additive; and 3) acetonitrile - methanol - (+)-tartaric acid (0.5 % in water, pH 5) - acetic acid 70:10:11:7 which enabled successful resolution of the enantiomers of lisinopril. Investigation of the effects of temperature, pH, and the amount of chiral selector. Detection with iodine vapour. Isolation and identification of the separated enantiomers. The LOD was 0.25 and 0.27 µg for each enantiomer of ketamine with (+)-tartaric acid and (-)-mandelic acid, respectively, whereas 0.14 and 0.16 µg for each enantiomer of lisinopril with (+)-tartaric acid (both conditions) and (-)-mandelic acid, respectively.

quality control, quantitative analysis 3d, 38

102 088 P.K. ZARZYCKI et al., see section 30b

102 005 V.L. CEBOLLA*, Elena MATEOS, L. MEMBRADO, J. VELA (*CSIC, Instituto de Carboquimica, c/Miguel Luesma, 4, 50018 Zaragoza, Spain, vcebolla@icb.csic.es): A general detection technique for HPTLC based on changes in fluorescence. CBS 99, 9-11 (2007). HPTLC of petrochemical samples on silica gel pre- or post-chromatographically impregnated by dipping in methanolic berberine (60 mg/L) or coralyne (6 or 12 mg/L) solutions. Development in horizontal developing chamber with dichloromethane (saturated hydrocarbons), n-hexane (heavy gas oil), or petroleum ether - diethyl ether - acetic acid 80:20:1 (cholesterol). Quantitative determination by fluorescence measurement of berberine at 365/>450 nm and coralyne at 410/>450 nm. Linearity for alkenes was between 50 and 1500 ng and for naphtenes between 600 and 2400 ng.

HPTLC, postchromatographic derivatization, quantitative analysis 3e

102 006 Gertrud MORLOCK*, C. STIEFEL, W. SCHWACK (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany; gmorlock@uni-hohenheim.de): Efficacy of a modified printer for application of reagents in planar chromatography. J. Liq. Chromatogr. Relat. Technol. 30, 2171-2184 (2007). Modification of a commercially available printer for application of reagents in planar chromatography and investigation of optimal settings of the printer regarding utmost reagent transfer. Evaluation of the efficacy of printing. HPTLC of taurin in energy drinks on silica gel (prewashed with methanol) with ethanol - water 3:2 in a twin-trough chamber. For post-chromatographic derivatization the cartridge of a commercially available printer was filled with a modified ninhydrin solution which was printed onto the layer, followed by heating at 100 °C for 2 min. Quantitative determination by absorbance measurement at 525 nm. The results obtained for analysis of an energy drink were as good as such obtained by derivatization by dipping.

HPTLC, densitometry 3e

102 007 D. SZIKRA*, I.P. NAGY (*Department of Physical Chemistry, University of Debrecen, Hungary; deeezs@yahoo.com): Attenuated total reflectance as an alternative of diffuse reflectance infrared detection in the identification of compounds separated by thin layer chromatography. J. Liq. Chromatogr. Relat. Technol. 31, 161-168 (2008). TLC of 37 compounds, e.g. nitrotoluene, on silica gel, aluminium oxide, cellulose, and silanized silica gel after a short (few centimeters) elution with different eluents. After precise positioning, attenuated total reflectance (ATR) is used for measuring the spots against air background in the range of 650-4000/cm. Diffuse reflectance (DRIFT) and ATR are infrared sampling methods, based on the detection of infrared radiation reflected by the sample. They are widely used in surface analysis and are both capable of detecting small amounts of organic compounds. DRIFT is a well known on-layer method for the identification of analytes, separated on thin layers. In the present work, the possibility of using a diamond ATR unit to collect IR spectra of sample spots is examined.

comparison of methods 3g

4. Special techniques

102 010 U. SOTANAPHUN*, P. PHATTANAWASIN, L. SRIPHONG (*Faculty of Pharmacy, Silpakorn University, Nakhon-pathom, Thailand, h8773con@ella.hu) : Application of Scion Image software to the simultaneous determination of curcuminoids in turmeric (Curcuma longa). Phytochem. Anal. 20, 19-23 (2009). TLC of curcumin (1), demethoxycurcumin (2), and bisdemethoxycurcumin (3) from the rhizomes of Curcuma longa on silica gel with hexane - chloroform - methanol 10:10:1 as mobile phase. Quantitative determination by recording the chromatogram using a digital scanner and analyzing the density of the TLC spot with the Scion Image software. The hRf values of (1), (2), and (3) were 42, 25, and 18, respectively. Selectivity regarding matrix was given. Linearity was between 0.375 and 6 µg/spot for all curcuminoids. The intermediate precision of the method was satisfactory. Recovery was 101.9 % for (1), 104.8 % for (2), and 101.5 % for (3). The limits of detection and quantification were 43 and 143 ng/spot for (1), 69 and 230 ng/

spot for (2), and 73 and 242 ng/spot for (3). The method was compared with an official densitometric method, and the analytical results were not significantly different.

herbal, densitometry, comparison of methods 4c, 30b

102 008 A. CARMINATI*, G. GONCALVES, Muriel NIMOD (*Analytical Research and Development Department , Sanofi-Aventis, Chemin de Meteline, 04200 Sisteron, France, alain.carminati@sanofi-aventis.com): Fast identification of unknown impurities by HPTLC/MS. CBS 101, 9-11 (2008). Identification of an unknown impurity detected during an in-process control by HPTLC coupled to ESI-MS using the ChromeXtractor interface. HPTLC of two impurities (an aldehyde derivative and a chlorinated ketone derivative) on silica gel with n-butanol - water - acetic acid 3:1:1 in a twin-trough chamber. Detection by spraying with sulfuric acid reagent (10 % in methanol) followed by heating at 120 °C for 15 min.

HPTLC-MS online coupling 4e

102 009 Gertrud MORLOCK*, M. ARANDA, H. LUFTMANN (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany, gmorlock@uni-hohenheim.de): Automated HPTLC/ESI-MS coupling. CBS 100, 13-15 (2008). HPTLC of caffeine on silica gel with ethyl acetate - methanol - 25 % ammonia 90:15:1 (for samples of energy drinks) or chloroform - ethanol - 37 % acidic acid - acetone - water 54:27:10:2:2 (for samples of headache tablets). Detection under UV 254 nm. Quantitative determination by absorbance measurement at UV 274 nm. Automated online extraction with an HPTLC/MS interface connected to a ESI mass spectrometer. Without any internal standard the caffeine mass signal was recorded in the selected ion monitoring mode at m/z 195 [M+H]+. The method was validated. Repeatability was 5.6 % (%RSD, n=6) and reproducibility of the plate mean value was 1.5 % (%RSD, n=3).

food analysis, quality control, pharmaceutical research, HPTLC, comparison of methods, HPTLC/MS online coupling 4e

6. Alcohols

102 011 G. BHAVAR*, V. CHATPALLIWAR (*Dept. of Pharmaceutical Chemistry, R. C. Patel College of Pharmacy, Karvad Naka, Shirpur 425405, India): Quantiative analysis of propranolol hydrochloride by high performance thin layer chromatography. Ind. J. Pharm. Sci. 70 (3), 395 - 398 (2008). TLC of propranolol hydrochloride on silica gel with isopropanol - ethyl acetate - ammonia 2:17:1. Quantitative determination by absorbance measurement at 290 nm. The linearity of the method was between 200 and 2000 ng/spot. The method was successively applied for tablets, wherein, no interference from tablet excipients was observed.

pharmaceutical research, quality control, densitometry, quantitative analysis 6

8. Substances containing heterocyclic oxygen

102 013 M.A. HAWRYL, Monika WAKSMUNDSKA-HAJNOS*, J. MAKAR (*Faculty of Pharmacy, Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6, 20-081, Lublin, Poland; monica-hajnos@am.lublin.pl): Separation of selected flavonoids by use of RP-HPLC/NP-HPTLC coupled methods. J. Liq. Chromatogr. Relat. Technol. 30, 2253-2265 (2007). HPTLC of 17 flavonoids (caffeic acid, ferulic acid, flavone, naringenin, apigenin, acacetin, luteolin, hesperitin, catechin, epicatechin, hyperoside, hesperidin, quercitrin, narinin, rutin, resveratrol, kaempferol) on silica gel (prewashed with acetone) with 28 binary and ternary mobile phases with chamber saturation. Detection by derivatization with diphenylborinic acid 2-aminoethyl ester (natural products reagent) and evaluation under UV 366 nm. Also coupling of HPTLC and HPLC.

herbal, qualitative identification, HPTLC 8a

102 012 M.G. BOGDANOV, Y.N. MITREV, I.V. SVINYAROV, C.E. PALAMAREV, M.D. PALAMAREVA* (*Department of Chemistry, University of Sofia, 1, James Bouchier Avenue, Sofia 1164,

Bulgaria; mpalamareva@chem.uni-sofia.bg): Automatic selection of mobile phases. VII. Thin-layer chromatography on silica and alumina of 11,12-disubstituted trans/cis-11,12-dihydro-6H-dibenzo[c,h]chromen-6-ones. J. Liq. Chromatogr. Relat. Technol. 30, 2155-2169 (2007). TLC of fifteen 11,12-disubstituted trans/cis-11,12-dihydro-6H-dibenzo[c,h]chromen-6-ones on silica gel with sixteen mobile phases with close values of epsilon which were arbitrarily selected from lists prepared by complex calculations and used for TLC. The data obtained showed a good agreement between the theoretical and experimental data, thus providing a successful application of the approach.

qualitative identification 8b

9. Oxo compounds, ethers and epoxides

102 014 Malgorzata STAREK*, J. KRZEK, Monika TARSA, M. ZYLEWSKI (*Department of Inorganic and Analytical Chemistry, Collegium Medicum, Jagiellonian University, 9 Medyczna Str, 30-688 Kraków, Poland): Determination of piroxicam and degradation products in drugs by TLC. Chromatographia 69(3-4), 351-356 (2009). TLC of piroxicam and its degradation products on silica gel with ethyl acetate - toluene - butylamine 2:2:1. Quantification by absorbance measurement at 360 nm. It was found that piroxicam decomposes to produce pyridine-2-amine and 2-methyl-2,3-dihydro-4H-1λ6,2-benzotiazin-1,1,4-trione, based on 1H NMR and LC-MS-MS qualification data.

pharmaceutical research, quality control, densitometry, quantitative analysis, qualitative identification 9

10. Carbohydrates

102 015 S. ENDO, M. MORITA, M. UENO, T. MAEDA, T. TERABAYASHI* (*Department of Chemistry, School of Science, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa, Japan, terabaya@sci.kitasato-u.ac.jp): Fluorescent labeling of a carboxyl group of sialic acid for MALDI-MS analysis of sialyloligosaccharides and ganglioside. Biochem. Biophys. Res. Commun. 378, 890-894 (2009). TLC of 3‘-sialyllactose and 6‘-sialyllactose from bovine collostrum, before and after amidation with 2-(2-pyridilamino)ethylamine dihydrochloride (PAEA) on silica gel with tetrahydrofuran - acetonitrile - n-propanol - ammonium acetate 0.6 M - 28 % ammonia solution 5:10:50:35 with 1 drop of ammonia. TLC of ganglioside GM3 from the whole brain of a minke whale, before and after PAEA amidation on silica gel with chloroform - methanol - calcium chloride 0.2 % 6:4:1. Detection by spraying with resorcinol-hydrochloric acid.

pharmaceutical research, HPTLC, preparative TLC, qualitative identification 10a

102 033 T. HALKINA et al., see section 13c

11. Organic acids and lipids

102 019 E. JARYJ, K. LORENZ, B. SPANGENBERG* (*University of Offenburg, Institute of Process Engineering, Badstrasse 24, 77652 Offenburg, Germany; spangenberg@fh-offenburg.de): A simple method for the quantification of urethane in spirits. J. Liq. Chromatogr. Relat. Technol. 31, 1969-1976 (2008). TLC of urethane on spherical silica gel with methyl-t-butyl ether - methanol 7:3. Detection by immersion in a solution of 80 µL cinnamaldehyde in 40 mL acetone with 2.4 mL phosphoric acid followed by heating in an oven at 130 °C for 10 min. The fluorescence can be enhanced by the factor of 2 if the plate is dipped for 4 s into a solution of 10 % polyethylene glycol 600 in methanol. Quantitative determination by absorbance measurement in the range of 445 to 460 nm.

food analysis, toxicology, densitometry, quantitative analysis 11a

102 020 E. LÓPEZ-BOJÓRQUEZ, G. CASTANEDA-HERNÁNDEZ, M. GONZÁLEZ-DE LA PARRA, S. NAMUR* (*Fundación Liomont A. C. privada Jesús del Monte 77, Cuajimalpa, 05000, México D. F.; snamur@liomont.com.mx or snamur@gmail.com): Development and validation of

a high-performance thin-layer chromatographic method, with densitometry, for quantitative analysis of ketorolac tromethamine in human plasma. J. AOAC Int. 91, 1191-1195 (2008). HPTLC of ketorolac tromethamine ((+/-)-5-(benzoyl)-2,3-dihydro-1N-pyrrolizine-1-carboxylic acid tris hydroxymethylaminomethane) on silica gel prewashed with methanol with n-butanol - chloroform - acetic acid - ammonium hydroxide - water 9:3:5:1:2 in a horizontal developing chamber. Quantitative determination by absorbance measurement at 323 nm.

clinical routine analysis, HPTLC, densitometry, quantitative analysis 11a

102 023 D.R. MASSA, M.J. CHEJLAVA, B. FRIED, J. SHERMA* (*Department of Chemistry, Lafayette College, Easton, PA 18042, USA; shermaj@lafayette.edu): Thin layer and high performance column liquid chromatograpic analysis of selected carboxylic acids in standards and from Helisoma trivolvis (Colorado strain) snails. J. Liq. Chromatogr. Relat. Technol. 30, 2221-2229 (2007). HPTLC of acetic, fumaric, lactic, malic, pyruvic, and succinic acid on 1) cellulose with n-propanol - 2 M ammonium hydroxide 7:3 (triple development) in a twin-trough chamber with chamber saturation, detection with aniline-xylose reagent; 2) on silica gel with n-butyl formate - 90 % formic acid - water 7:2:1, detection with ethanolic bromocresol green reagent; 3) on cellulose with water-saturated isopropyl ether - formic acid 3:1 containing 2 - 3 mg/100 mL dichlorofluorescein, detection with pyridine vapor and evaluation under UV; 4) on silica gel with n-pentyl formate - chloroform - formic acid 14:3:3 or 2:7:1, detection with bromocresol green reagent; 5) on silica gel with diisopropyl ether - formic acid - water 16:3:1, detection with bromophenol blue reagent; 6) on silica gel with diisopropyl ether - formic acid - water 90:7:3, detection with aniline - glucose reagent, or bromocresol green, bromophenol blue, bromocresol purple, or potassium permanganate reagents. Best results were obtained with method 1 on cellulose.

HPTLC, qualitative identification, biological research 11a

102 026 D. NEDELCHEVA, D. ANTONOVA, S. TSVETKOVA, I. MAREKOV, S. MOMCHILOVA, Boryana NIKOLOVA-DAMYANOVA*, M. GYOSHEVA (*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; bmd@orgchm.bas.bg): TLC and GC-MS probes into the fatty acid composition of some Lycoperdaceae mushrooms. J. Liq. Chromatogr. Relat. Technol. 30, 2717-2727 (2007). Identification of lipid classes, e. g. fatty acid methyl esters, and polar lipids (presumably phospholipids), free fatty acids, sterols, triacylglycerols, fatty acid esters, by TLC on silica gel with n-hexane - acetone 25:4. Detection by spraying with 50 % ethanolic sulfuric acid and heating at 200 °C. The mushrooms contained a characteristic group of three isomeric hexadecenoic fatty acids (double bond in positions 6-, 9-, and 11-) which are resolved and determined separately for the first time.

food analysis, qualitative identification 11a

102 027 H. PANCHAL*, I. RATHOD, S. SHAH (*Dept. of Pharmaceutical Analysis, L. M. College of Pharmacy, Navaranpura, Ahmedabad 3890009, Gujarat, India, hir_143_2003@yahoo.com): Development of validated HPTLC method for quantitation of diclofenac in diclofenac gels. Indian Drugs 45(4), 301-306 (2008). HPTLC of diclofenac (extracted with 3N HCl and chloroform from single and multi-component diclofenac gel formulations) on silica gel with toluene - ethyl acetate - acetic acid 600:400:2. The hR_f value of diclofenac was 39, of salicylic acid 29, and of methyl salicylate 83. Quantitative determination by absorbance measurement at 283 nm. Linearity was between 200 and 600 ng/spot via peak area. In single component gel, recovery was 100.4 % whereas in multi-component gel it was 99.5 %. The method was found to be accurate and suitable for analysis in single and multi-component gel formulations.

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

102 028 P. PATEL, R. SHAH, S. PATEL, Unnati SHAH (* Pharmanza Herbal Pvt. Ltd., Anand, Gujarat, India): Development and validation of HPTLC method for determination of (-)hydroxy citric acid in fruits of Garcinia gummigutta D. 60th Indian Pharmaceutical Congress PA-206, (2008).

HPTLC of (-)hydroxy citric acid in fruits of Garcina Gummigutta on silica gel with n-propanol - water - acetic acid 50:50:1 in a twin-trough chamber saturated for 10 min. Quantitative determination by absorbance measurement at 210 nm. The method was linear in the range of 100-1000 ng/spot. Recovery was 99.8-100.9 %.

herbal, HPTLC, densitometry, quantitative analysis, qualitative identification 11a

102 029 Alina PYKA*, W. KLIMCZOK (*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska Street, 41-200 Sosnowiec, Poland; apyka@slam.katowice.pl): Application of densitometry for the evaluation of the separation effect of nicotinic acid derivatives. Part II. Nicotinic acid and its esters. J. Liq. Chromatogr. Relat. Technol. 30, 2419-2433 (2007). TLC and HPTLC of nicotinic acid and methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, and benzyl nicotinate on RP-18 (prewashed with methanol) with methanol - water and dioxane - water in 10 % volume ratio steps from 100:0 to 0:100 in a chamber saturated for 30 min. TLC on aluminium oxide (prewashed with methanol) with acetone - n-hexane 1:4 provided the optimum conditions for complete separation. Quantitative determination by absorbance measurement at 254 nm.

densitometry, HPTLC, quantitative analysis 11a

102 030 Alina PYKA*, W. KLIMCZOK (*Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, 4, Jagiellonska Str., 41-200 Sosnowiec, Poland; apyka@slam.katowice.pl): Analytical and densitometric evaluation of visualizing reagents of selected aliphatic compounds on thin layer. J. Liq. Chromatogr. Relat. Technol. 31, 1492-1510 (2008). TLC of stearic acid, stearyl alcohol, and methyl stearate on silica gel (prewashed with methanol) with methanol - chloroform 1:1 followed by drying for 24 h at room temperature. Six new derivatization reagents were evaluated: gentian violet, methylene violet, methylene blue, methyl green, malachite green, and Janus blue. Detection by dipping for 5 s, followed by drying for 24 h at room temperature. Quantitative determination by absorbance measurement. The results obtained indicate that all of the new derivatization reagents give better results than the universally applied Rhodamine B. The best reagents for quantitative determination of stearic acid are methylene blue and Janus blue, for stearyl alcohol malachite green and Janus blue, and for methyl stearate methylene blue, Janus blue, and malachite green.

densitometry, quantitative analysis, radioscanning, qualitative identification, postchromatographic derivatization 11a

102 016 U. BHANDARI*, M. ANSARI (*Hamdard University, Dept. of Pharmacology, Faculty of Pharmacy, New Delhi 110062, India, uma_bora@hotmail.com): High performance thin layer chromatographic method for quantification of embelin from Embelia ribes Burm. fruits. Indian Drugs 45(11), 908-910 (2008). HPTLC of embelin in ethanolic extract of dried fruits of Embelia ribes Burm. on silica gel with ethyl acetate - methanol 9:1. Quantitative determination by absorbance measurement at 365 nm. The total content in Embelia ribes fruit was 0.034 %. The proposed HPTLC method provides a good resolution of embelin from other constituents present in the ethanolic extract of dried fruits of Embelia ribes Burm.

pharmaceutical research, traditional medicine, densitometry, HPTLC, quantitative analysis 11c

102 017 B. FUCHS, A. NIMPTSCH, R. SÜSZ, J. SCHILLER* (*University of Leipzig, Institute of Medical Physics and Biophysics, Faculty of Medicine, Härtelstr. 16-18, 04107 Leipzig, Germany; juergen.schiller@medizin.uni-leipzig.de): Analysis of brain lipids by direct coupled matrix-assisted laser desorption ionization time-of-flight mass spectrometry and high-performance thin-layer chromatography. J. AOAC Int. 91, 1227-1236 (2008). HPTLC on silica gel using chloroform - ethanol - water - triethylamine 5:5:1:5 for separation of phospholipids and chloroform - acetone - methanol - acetic acid - water 46:17:15:14:8 and chloroform - methanol - acetic acid 13:5:2 for separation of glycolipids. Visualization by spraying with primuline reagent (Direct Yellow) and

observation under UV light at 366 nm. Also MALDI-TOF-MS analysis.

clinical chemistry research, HPTLC, qualitative identification 11c

102 018 D. HANDLOSER, Valeria WIDMER, E. REICH* (*CAMAG Laboratory, Sonnenmattstrasse 11, 4132 Muttenz, Switzerland; eike.reich@camag.com): Separation of phospholipids by HPTLC - An investigation of important parameters. J. Liq. Chromatogr. Relat. Technol. 31, 1857-1870 (2008). HPTLC of phospholipids (phosphatidic acid, phosphatidylcholin, phosphatidylethanolamine, phosphatidylinositiol, lysophosphatidic acid, lysophosphatidylcholine, lysophosphatidylethanolamine, and lysophosphatidylinositol) on silica gel with chloroform - methanol - water - 25 % ammonia 60:34:4:2 in a twin-trough chamber saturated for 20 min. Plates were conditioned to 47 % relative humidity. Detection by dipping for 6 s in modified copper sulfate reagent (20 g copper(II)sulfate pentahydrate were dissolved in 200 mL of cooled methanol, then under cooling 8 mL of sulfuric acid 98 % and 8 mL of ortho-phosphoric acid 85 % were added) followed by drying in cold air for 30 s and heating at 140 °C for 30 min. Quantitative determination by absorbance measurement at 360 nm, 420 or 720 nm, or by video densitometry. Investigation of several parameters of the chromatographic process, including chamber saturation, derivatization, plate activity, and batch to batch consistency of the plates. For reproducible results, the employed methodology must be strictly standardized.

pharmaceutical research, cosmetics, HPTLC, densitometry, quantitative analysis 11c

102 021 Ilko MAREKOV*, R. TARANDJIISKA, S. MOMCHILOVA, B. NIKOLOVA-DAMYANOVA (*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria): Quantitative silver ion thin layer chromatography of triacylglycerols from sunflower oils differing in the level of linoleic acid. J. Liq. Chromatogr. Relat. Technol. 31, 1959-1968 (2008). Quantitative Ag-TLC of eight samples of sunflower oil with different linoleic acid content on silica gel (impregnated by dipping into a 0.5 % methanolic solution of silver nitrate) with petroleum ether - acetone 25:1, and petroleum ether - acetone - ethyl acetate 100:5:2, and 50:3:2. Detection by consecutive treatment with bromine and sulfurylchloride vapors (30 min each) followed by heating at 180-200 °C. Quantitative determination by absorbance measurement at 450 nm. Evaluation of authenticity and possible adulteration of edible oils.

food analysis, quantitative analysis, densitometry 11c

102 022 D.R. MASSA, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA 18042, USA; friedb@lafayette.edu): Further studies on the neutral lipid content in the feces of BALB/c mice infected with Echinostoma caproni as determined by silica gel HPTLC-densitometry. J. Liq. Chromatogr. Relat. Technol. 31, 1871-1880 (2008). HPTLC for the determination of neutral lipid profils using a standard mixture (containing cholesterol, oleic acid, triolein, methyl oleate, cholesteryl oleate) on silica gel (plates with 19 scored lanes and a preadsorbent application area, prewashed by development with dichloromethane - methanol 1:1) with petroleum ether - diethyl ether - acetic acid 80:20:1 in a saturated twin-trough chamber. Detection by spraying with 5 % ethanolic phosphomolybdic acid and heating for 10 min at 115 °C. Quantitative determination by absorbance measurement at 610 nm.

HPTLC, densitometry, quantitative analysis 11c

102 025 S. MOMCHILOVA, Boryana NIKOLOVA-DAMYANOVA* (*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; bmd@orgchm.bas.bg): Quantitative TLC and gas chromatography determination of the lipid composition of raw and microwave roasted walnuts, hazelnuts, and almonds. J. Liq. Chromatogr. Relat. Technol. 30, 2267-2285 (2007). Analytical and preparative TLC of lipid classes, their fatty acid profiles, and the triacylglycerol and sterol composition on silica gel and modified silica gel (impregnated with silver nitrate for Ag-TLC or dimethyldichlorosilane for RP-TLC). TLC of lipid reference mixture on silica gel with hexane - acetone 25:4. Detection by spraying with 50% ethanolic sulfuric acid and heating at 200 °C. Preparative TLC for isolation and quantification,

followed by detection under UV light, spraying the edges with 2‘,7‘-dichlorofluorescein, scraping off, elution with diethyl ether and weighting. Quantitative Ag-TLC (impregnated by dipping into 0.5 % or 2 % methanolic solution of silver nitrate) followed by detection with bromine and sulfuryl chloride vapor for 30 min each, followed by heating at 180-200 °C. Preparative Ag-TLC with 4 different mobile phases. Quantitative RP-TLC on Kieselguhr treated for 6 h with vapors of dimethyldichlorosilane and washed with methanol using acetone - acetonitrile - water. Quantitative determination by absorbance measurement at 450 nm.

food analysis, preparative TLC, qualitative identification, densitometry, quantitative analysis 11c

102 031 P.A. ZANI*, J.L. COUNIHAN, J.D. VASTA, B. FRIED, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA 18042, USA; zanip@lafayette.edu): Characterization and quantification of the neutral lipids in the lizard Uta stansburiana stansburiana by HPTLC-densitometry. J. Liq. Chromatogr. Relat. Technol. 31, 1881-1891 (2008). HPTLC of various neutral lipid classes on silica gel (prewashed with dichloromethane - methanol 1:1) with petroleum ether - diethyl ether - acetic acid 80:20:1 in a twin trough chamber saturated for 20 min. Detection by spraying with 5 % ethanolic phosphomolybdic acid solution and heating for 10 min at 110 °C. Quantitative determination by absorbance measurement at 610 nm.

HPTLC, densitometry, quantitative analysis 11c

102 024 M. MIYAZAKI, A. YONESIGE, J. MATSUDA, Y. KURODA, N. KOJIMA, A, SUZUKI* (*Tokai University, Institute of Glycoscience, Hiratsuka, Kanagawa 259-1292, Japan; akmszk@tokai-u.jp): High-performance thin-layer chromatography/mass spectrometry for rapid analysis of neutral glycosphingolipids. J. AOAC Int. 91, 1218-1226 (2008). HPTLC of glycosphingolipids on silica gel with chloroform - methanol - water 60:35:8 or 65:25:4. Detection by spraying with orcinol reagent followed by heating at 110 °C. Detection with HPTLC/MS by direct coupling of HPTLC to matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry showed to be a reliable and reproducible method to obtain structural information and fundamental properties of glycosphingolipids.

clinical chemistry research, HPTLC 11e

12. Organic peroxides

102 032 S. AGARWAL*, A. ALI, S. AHUJA (*Dept. of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India): HPTLC determination of artesunate as bulk drug and in pharmaceutical formulations. Ind. J. Pharm. Sci. 69 (6) 841 - 844 (2007). HPTLC of artesunate on silica gel with toluene - ethyl acetate - acetic acid 20:80:2. Detection by treatment with vanillin reagent (1 % vanillin in 5 % ethanolic sulphuric acid) leads to pink zones which are stable for more than a day. Quantitative determination by absorbance measurement at 520 nm. The hR_f value for artesunate was 44. Linearity was between 100 and 600 ng per spot. Recovery (by standard addition method) was 98.9 - 99.9 % for tablets and injections.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 12

13. Steroids

102 034 S. KAMAT*, V. VELE, V. CHOUDHARI, S. PRABHUNE (*Ramnarian Ruia College, Dept. of Chem.,Matunga, Mumbai 400019, India, swarup_80@rediffmail.com): Determination of dutasteride from its bulk drug and pharmaceutical preparations by HPTLC. Asian J. Chem. 20(7), 5514-5518 (2008). HPTLC of dutasteride on silica gel with toluene - ethyl acetate - acetic acid 14:6:1. Absorbance measurement at 210 nm. The method was linear in the range of 50-500 µg/µL. Recovery was 99.3-99.5 %. The method was suitable for routine quality control.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 13a

102 035 M. KRISHNA*, V. MURUGAN, P. MUSMADE, S. VENKATARAM (*Dayanand Sagar College of Pharmacy, Bangalore, Karnataka, India): Stability indicating HPTLC method for determination of medroxyprogestrone acetate in bulk drug and pharmaceutical dosage forms. 60th Indian Pharmaceutical Congress PA-197, (2008). HPTLC of medroxyprogestrone acetate in bulk and injectable dosage form on silica gel with toluene - ethyl acetate - ammonia 800:200:1. Quantitative determination by absorbance measurement at 240 nm. The linearity was in the range of 50-1800 ng/spot. Recovery was 100.1 %. In the stability test (acid, base, peroxide, thermal, photodegradation) the compound was well separated from degradation products. The method was suitable for routine quality control and for monitoring stability.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 13a

102 038 M. SCHULZ*, Susanne MINARIK (*Merck KGaA, PC-RLP-SIL, Frankfurter Str. 250, 64293 Darmstadt, Germany, michael.schulz@merck.de): Use of reversed-phase (RP)-modified pre-coated plates. CBS 101, 5-7 (2008). HPTLC of steroids on RP-18W and RP-18 with methanol - water 3:2 in a flat bottom chamber. Detection by spraying with perchloric acid (20 % in ethanol) followed by heating at 100 °C for 5 min. Migration time on the hydrophobic RP-18 layer was 130 min whereas on the water-wettable RP-18W layer it was 39 min. The maximal water content of the mobile phase is 40 % for RP-18 layers and up to 100 % for RP-18W. Separation of steroids was better on RP-18W. The hR_f value of stanozolol was 4, of methyl testosterone 12, of Reichstein's S 26, of hydrocortisone 37 and cholesterol remained at the application position.

comparison of methods, HPTLC, qualitative identification 13a

102 037 Alina PYKA*, W. KLIMCZOK, D. GURAK (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, 41-200, Sosnowiec, Poland; apyka@slam.katowice.pl): Evaluation of visualizing reagents for estradiol on thin layer by densitometric method. J. Liq. Chromatogr. Relat. Technol. 31, 555-566 (2008). Five new derivatization reagents (gentian violet, methylene violet, methylene blue, malachite green, and Janus blue) were used to detect estradiol on aluminium oxide. Barton's reagent, rhodamine B, and sulfuric acid were used as the comparative derivatization reagents. Limit of detection, detection index, modified broadening index, modified contrast index, and linearity range were determined for estradiol after derivatization with these reagents. Quantitative determination by absorbance measurement between 200 and 600 nm.

densitometry, quantitative analysis 13b

102 033 T. HALKINA, J. SHERMA* (*Department of Chemistry, Lafayette College, Easton, PA 18042, USA; shermaj@lafayette.edu): Determination of sterols and fatty acids in prostata health dietary supplements by silica gel high performance thin layer chromatography with visible mode densitometry. J. Liq. Chromatogr. Relat. Technol. 30, 2329-2335 (2007). HPTLC of sterols and fatty acids on silica gel (prewashed with methanol) with petroleum ether (36-60 °C) - diethyl ether - acetic acid 80:20:1 in a twin-trough chamber with chamber saturation. Detection by spraying with a 5 % ethanolic phosphomolybdic acid solution followed by heating at 110 °C for 10 min. Quantitative determination by absorbance measurement in the visible range.

food analysis, quantitative analysis, densitometry, HPTLC 13c, 11a

102 039 P.K. ZARZYCKI*, M. BARAN, E. WLODARCZYK, M. A. BARTOSZUK (*Laboratory of Toxicology, Department of Environmental Biology, Koszalin University of Technology, Sniadeckich 2, 75-453 Koszalin, Poland; pkzarz@wp.pl or pawel_k_z@hotmail.com): Improved detection of ergosterol, stigmasterol, and selected steroids on silica coated TLC plates using phosphomolybdic acid staining. J. Liq. Chromatogr. Relat. Technol. 30, 2629-2634 (2007). TLC of ergosterol, stigmasterol, dihydrocholesterol, 4-cholesten-3-one, cholecalciferol, and cholesterol acetate on silica gel using methanol - dichloromethane 1:19 in a saturated horizontal chamber. Detection by spraying twice with 10 % phosphomolybdic acid in methanol and heating. Contrary to the data reported in literature, the experiments revealed that the best conditions for robust detection of

these analytes are achieved if the TLC plates are heated at relatively low temperatures (between 40 and 80 °C) and for longer times (more than 20 min).

densitometry 13c

102 036 Alina PYKA (Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, 4, Jagiellonska Str., 41-200 Sosnowiec, Poland; apyka@slam.katowice.pl): TLC of selected bile acids: Detection and separation. J. Liq. Chromatogr. Relat. Technol. 31, 1373-1385 (2008). TLC of cholic acid, glycocholic acid, glycolithocholic acid, deoxycholic acid, chenodeoxycholic acid, glycodeoxycholic acid, and lithocholic acid on silica gel with concentration zone, prewashed with methanol and dried for 24 h at room temperature, in a saturated chamber. Best separation of the bile acids was achieved with n-hexane - ethyl acetate - methanol - acetic acid 20:20:5:2. Detection by dipping for 15 s in sulfuric acid - methanol 1:19, followed by heating at 90 °C for 20 min provided better results than derivatization by spraying with 10 % phosphomolybdic acid in ethanol. Quantitative determination by absorbance measurement between 190 and 800 nm.

pharmaceutical research, densitometry, quantitative analysis 13d

14. Steroid glycosides, saponins and other terpenoid glycosides

102 040 Silvia CORAN*, G. BARTOLUCCI, M. BAMBAGIOTTI-ALBERTI (*Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy): Validation of a reversed phase high performance thin layer chromatographic-densitometric method for secoisolariciresinol diglucoside determination in flaxseed. J. Chromatogr. A 1207 (1-2), 155-159 (2008). HPTLC of secoisolariciresinol diglucoside in flaxseed on RP-18W with methanol - 0.1 % formic acid 2:3, using the alkaline hydrolysis in aqueous medium of undefatted samples. Quantitative determination by absorbance measurement at 282 nm. Validation of the method following the protocol proposed by the Société Francaise des Sciences et Techniques Pharmaceutiques lead to a dependable and high throughput procedure well suited for routine application. Linearity was between 321-1071 ng/zone and the RSD of repeatability and intermediate precision did not exceed 3.6 %.

quality control, pharmaceutical research, herbal, HPTLC, quantitative analysis, qualitative identification, densitometry 14

102 041 V. DIXIT*, A. SHARMA (*Dr. Hari Singh Gour Vishwavidyalaya, Dept of Pharma. Sc., Sagar 470003 (M.P.), India, vdixit111@rediffmail.com): Hypolipidemic activity of Murraya Koenill L. in rats. Indian Drugs 45(5), 401-406 (2008). HPTLC of sapogenins in ethanolic extracts of leaves and seeds of Murraya Koenill L. on silica gel with chloroform - ethyl acetate 1:1. Detection by spraying with antimony trichloride reagent.

herbal, HPTLC, comparison of methods, qualitative identification 14

102 042 B.G. KIPRE, A.A. COFFI, A.A. ADIMA, T. GOKOU, Y. ITO*, B. K. GOSSE (*Center for Biochemistry and Biophysics, National Heart, Lung, and Blood Institute, National Institute of Health, Bethesda, Maryland, USA; itoy@nhlbi.nch.gov): Total chemical analysis of the seed of Tieghemella heckelii by diverse chromatography techniques. J. Liq. Chromatogr. Relat. Technol. 31, 250-262 (2008). Analytical and preparative TLC of saponins (arganine A, C, D, and tieghemelin in the seed of Tieghemella heckelii) on silica gel with chloroform - methanol - 0.5 % TFA 12:8:1. Detection by spraying with a solution of 2 % cerium sulfate in 5.6 % sulfuric acid.

herbal, preparative TLC, qualitative identification, comparison of methods 14

102 043 C. RUMALLA, B. AVULA, Y. SHUKLA, Y WANG (Wang Yanhong), R. PAWAR, T. SMILLIE, I. KHAN* (*National Center for Natural Products Research, University of Mississippi, University, USA, ikhan@olemiss.edu): Chemical fingerprint of Hoodia species, dietary supplements, and related genera by using HPTLC. J. Sep. Sci. 31, 3959-3964 (2008). HPTLC of steroidal glycosides

of Hoodia species and dietary supplements that claim to contain Hoodia gordonii, on silica gel with dichloromethane - methanol - water 375:85:11. Detection by dipping in anisaldehyde reagent (0.5 mL p-anisaldehyde in a mixture of 85 mL methanol, 10 mL acetic acid, and 5 mL sulfuric acid), followed by heating at 100 °C for 5 min. The hRf values were 8 for Hoodigoside M, 18 for Hoodigoside L, 20 for Hoodigoside P, 25 for Hoodigoside U, 31 for Hoodigoside O, 41 for Hoodigoside E, 42 for Hoodigoside F, 46 for Hoodigoside J, 53 for Hoodigoside N, 62 for P57, and 68 for Hoodigoside C. LC-UV-MS confirmation was performed for the samples analyzed.
herbal, food analysis, HPTLC,

quantitative analysis, densitometry, comparison of methods 14

15. Terpenes and other volatile plant ingredients

102 044 Magdalena LIGOR*, B. BUSZEWSKI (*Faculty of Chemistry, Chair of Environmental Chemistry and Bioanalytics, Nicolaus Copernicus University, 7 Gagarin St., 87-100 Torun, Poland; mada@chem.uni.torun.pl): Thin layer chromatographic techniques (TLC, OPTLC) for determination of biological activated compounds from herb extracts. J. Liq. Chromatogr. Relat. Technol. 30, 2617-2628 (2007). TLC and HPTLC of monoterpenes, e. g. menthol and menthone from peppermint, on silica gel with toluene - ethyl acetate 3:7, 4:6, ... , 9:1, and 10:0 in a horizontal chamber. Detection with methanolic vanillin - sulfuric acid reagent and under UV 254 nm. Flavonoids from hawthorn, Passiflora incarnata, hop, cacao, as well as tea were also determined by TLC and OP TLC.

herbal, HPTLC, qualitative identification, quantitative analysis 15a

102 045 K. VIKANI*, R. DANGAR, N. KAPADIA, M. SHAH (*L. M. College of Pharmacy, Dept. of Pharmacognosy, Ahmedabad 380009, India, mamta_b_shah@yahoo.com): A pharmacognostic study on Sphaeranthus indicus. Journal of Natural Remedies 8(1), 61-67 (2008). During pharmacognostic studies on Sphaeranthus indicus (Asteraceae) the sesquiterpenoid 7-OH-eudesmanolide was isolated. HPTLC of the ethyl acetate soluble fraction of aqueous alcoholic Sphaeranthus indicus extracts, on silica gel with n-hexane - diethyl ether 3:7. The plant extract and the marker (7-OH-eudesmanolide) were chromatographed simultaneously. Quantitative determination by absorbance measurement at 213 nm.

traditional medicine, quality control, HPTLC, densitometry 15a

102 046 Valeria WIDMER, D. HANDLOSER, E. REICH* (*CAMAG Laboratory, Sonnenmattstr. 11, 4132 Muttenz, Switzerland; eike.reich@camag.com): Quantitative HPTLC analysis of artemisinin in dried Artemisia annua L.: A practical approach. J. Liq. Chromatogr. Relat. Technol. 30, 2209-2219 (2007). HPTLC of artemisinin in Artemisia annua on silica gel with cyclohexane - ethyl acetate - acetic acid 20:10:1 in a twin-trough chamber saturated for 20 min. Detection by immersion in modified anisaldehyde reagent (20 mL acetic acid, 4 mL sulfuric acid, 2 mL of anisaldehyde in a mixture of 100 mL ethanol and 80 mL water) for 1 s. After 1 min the plate was heated at 100 °C for 12 min. Quantitative determination by fluorescence measurement at 520 nm with cut-off filter at 540 nm.

herbal, quality control, HPTLC, densitometry, quantitative analysis 15a, 32e

17. Amines, amides and related nitrogen compounds

102 047 E.A. ABOURASHED (ElSohly Laboratories Inc., Oxford, MS 38655, USA, eabourashed@elsohly.com): Sildenafil determination in pharmaceutical products and aphrodisiac herbal preparations. CBS 99, 6-7 (2007). HPTLC of sildenafil in pharmaceutical products and herbal preparations (extracted with methanol) on silica gel with chloroform - methanol - diethylamine 90:10:1 in a twin-trough chamber saturated for 30 min. Quantitative determination by absorbance measurement at 305 nm. The hRf value of sildenafil was 48 and selectivity regarding matrix was given. The 4-level calibration (n=3) from 150 to 1200 ng/band showed a polynomial regression. Inter-day precision was <1% (n=6). Mean recovery (by standard addition) was 98.2 %

for three different concentration levels.

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

102 049 Suneela DHANESHWAR*, P. DESHPANDE, M. PATIL, G. VADNEKAR, S. DHANESHWAR (*Poona College of Pharmacy, Dept of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Erandwane, Pune 411038, India, suneeladhaneshwar@rediffmail.com): Development and validation of a HPTLC method for estimation of duloxetine hydrochloride in bulk drug and in tablet dosage form. Ind. J. Pharm. Sci. 70(2), 233-236 (2008). HPTLC of duloxetine hydrochloride (in bulk drug and in tablet dosage form) on silica gel with chloroform - methanol 8:1. Quantitative determination by absorbance measurement at 235 nm. The method was linear in the range of 40-200 ng/spot. The method was suitable for routine quality control.

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

102 051 S. KAMAT*, V. VELE, V. CHOUDHARI, S. PRABHUNE (*Therapeutic Drug Monitoring Lab., 194, Scheme No.6, Road No. 15, Sion (E), Mumbai 400022, India, swarup_80@rediffmail.com,swarup.prabhune@gmail.com): HPTLC determination of atomoxetine hydrochloride from its bulk drug and pharmaceutical preparations. Asian J. Chem. 20(7), 5409 - 5413 (2008). HPTLC of atomoxetine HCl on silica gel with acetonitrile - acetic acid 9:1. Quantitative determination by absorbance measurement at 269 nm. The method was linear in the range of 100-1000 µg/mL. The recovery was 99.8 %. The method was suitable for routine analysis of atomoxetine HCl in its pharmaceutical preparations.

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

102 052 Jolanta KOCHANA*, W. TOMASZEWSKI, T. MOSZCZYNSKI, A. ZAKRZWESKA, A. PARCZEWSKI (*Faculty of Chemistry, Department of Analytical Chemistry, Jagiellonian University, Ingardena 3, 30-060 Cracow, Poland; kochana@chemia.uj.edu.pl): Application of carbon adsorbents for extraction of MDMA impurities in TLC drug profiling. J. Liq. Chromatogr. Relat. Technol. 31, 819-827 (2008). TLC of MDMA (3,4-methylenedioxymethamphetamine, ‚ecstasy') and impurities on silica gel with chloroform - methanol - acetonitrile 5:2:3. Detection under UV 254 and 366 nm after drying the plates at 110 °C for 15 min.

quality control, pharmaceutical research, qualitative identification 17a

102 056 S. SATHE*, S. BARI, S. SURANA (*R.C. Patel College of Pharmacy, Dept. of Pharmaceutical Chemistry, Shirpur 425405, Dist.-Dhule (M.S.), India, sbbari@rediffmail.com): Development of HPTLC method for the estimation of metoprolol succinate in bulk and in tablet dosage form. Indian J. Pharma Educ. Res. 42(1), 32-35 (2008). HPTLC of metoprolol succinate on silica gel with toluene - methanol - triethylamine 30:50:3. Quantitative determination by absorbance measurement at 274 nm. The hR_f value was 40. The method was linear in the range of 5-10 ng/spot. The limit of detection and quantification was 430 ng/spot and 1310 ng/spot respectively. The method was suitable for routine quality control.

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

102 057 S. WAKODE*, V. SINGH, H. SINGH (*Delhi Institute of Pharmaceutical Science & Research New Delhi, India): Development and validation of analytical method on UV-VIS spectrophotometer and HPTLC for determination of sibutramine hydrochloride monohydrate in capsules. 60th Indian Pharmaceutical Congress PA-177, (2008). HPTLC of sibutramine hydrochloride on silica gel with benzene - methanol 9:1. Quantitative determination by absorbance measurement at 223 nm. The method was linear in the range of 2-22 µg/mL (UV-Visible) and 100-700 ng/spot (HPTLC). The recovery was 99.5-101.6 % for both methods. The proposed methods could be

used for routine analysis of the drug in capsule dosage-form

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

102 048 S. AGARWAL*, H. GONSALVES, R. KHAR (*Dept. of PharmaceuticalScinece, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India, agarwal_sp@yahoo.com): HPTLC method for the analysis of melatonin in bulk and pharmaceutical formulations. Asian J. Chem. 20(4), 2531-2538 (2008). TLC of melatonin on silica gel with toluene - ethyl acetate - formic acid 10:9:1. Quantitative determination by absorbance measurement at 290 nm. The method was linear in the concentration range of 100 to 600 ng/spot. The recovery of the drug from tablets (by standard addition method) was 99.7%. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of the drug. Forced degradation studies showed the effect of variations in pH, UV light and high temperature on the stability of melatonin. As the proposed method could effectively separate the drug from its degradation products, it can be employed as a stability indicating method.

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

102 050 U. HUBICKA, J. KRZEK*, J. LUKA (*Collegium Medicum of Jagiellonian University, Department of Inorganic and Analytical Chemistry, Medyczna 9, 30-688 Kraków, Poland; jankrzek@cm-uj.krakow.pl): Thin-layer chromatography-densitometric measurements for determination of N-(hydroxymethyl)nicotinamide in tablets and stability evaluation in solutions. J. AOAC Int. 91, 1186-1190 (2008). TLC of N-(hydroxymethyl)nicotinamide on silica gel with chloroform - ethanol 2:3. Quantitative determination by absorbance measurement at 260 nm.

quality control, quantitative analysis, densitometry 17c

102 053 A. MEHTA*, M. PATEL, P. PARMAR, V. MANDOWARA (* K. B. Raval College of Pharmacy, Gandhinagar, Gujarat, India): Stability-indicating HPTLC determination of alfuzosin hydrochloride in bulk drug and pharmaceutical formulations. 60th Indian Pharmaceutical Congress PA-49, (2008). HPTLC of alfuzosin hydrochloride on silica gel with toluene - methanol - triethyl amine 15:5:1. Densitometric evaluation at 245 nm. Linearity was in the range of 5-400 ng/spot. The method was stability indicating (acid, alkali, oxidation, dry and wet heat and photodegradation). The compound was stable to oxidation and alkaline degradation. The hR_f value of the main zone was 63, and of additional zones 10 and 33. The method could effectively separate the main drug from the degradation products.

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

102 054 S.S. PATEL*, R.S. KESHALKAR, M.B. PATEL (*Department of Pharmaceutical Chemistry, Shri S. K. Patel College of Pharmaceutical Education and Research Centre, Ganpat University, Mehsana, Gujarat, 411038, India): Stability-indicating HPTLC method for analysis of moclobemide, and use of the method to study degradation kinetics. Chromatographia 68 (9-10), 855-859 (2008). HPTLC of moclobemide on silica gel with benzene - methanol - 40 % ammonia 70:30:1. Quantification by absorbance measurement at 238 nm. The degradation products reached under acidic, basic, and oxidising conditions were well resolved from the pure drug. Linearity was in the range of 50-600 ng/band, with a determination coefficient r2 of 0.9967 ± 0.51. LOD and LOQ, determined experimentally, were 10 and 30 ng/band, respectively. The method was used to investigate the kinetics of alkaline degradation, the Arrhenius plot was constructed and the activation energy calculated.

quality control, qualitative identification, HPTLC, densitometry, quantitative analysis 17c

102 055 Alina PYKA*, W. KLIMCZOK (*Department of Analytical Chemistry, Faculty of Pharmacy,

Silesian Academy of Medicine, 4 Jagiellonska Street, 41-200 Sosnowiec, Poland; apyka@slam.katowice.pl): Application of densitometry for the evaluation of the separation effect of nicotinic acid derivatives. Part I. Nicotinic acid and its amides. J. Liq. Chromatogr. Relat. Technol. 30, 2317-2327 (2007). TLC and HPTLC of nicotinic acid, nicotinamide, N-methylnicotinamide, and N,N-diethylnicotinamide on RP-18 with methanol - water 3:7, and dioxane - water 1:4 and 1:9. The best separation was achieved on alumina with acetone - n-hexane 1:1. Detection under UV light at 254 nm. Quantitative determination by absorbance measurement at 254 nm.

pharmaceutical research, densitometry, HPTLC, quantitative analysis, qualitative identification 17c

18. Amino acids and peptides, chemical structure of proteins

102 058 C.S. BABU, A.G. SUNIL, H.R. VASANTHI, V.S.MUTHUSAMY, M. RAMANATHAN* (*Department of Pharmacology, PSG College of Pharmacy, Post Box No 1674, Peelamedu, Coimbatore, TN 641 004, India; muthiahramanathanin@yahoo.co.in): Development and validation of an HPTLC method for simultaneous estimation of excitatory neurotransmitters in rat brain. J. Liq. Chromatogr. Relat. Technol. 30, 2891-2902 (2007). HPTLC of L-glutamate and L-aspartate on silica gel by double elution with n-butanol - acetic acid - water 13:3:5 in a chamber saturated for 3 h. Detection by spraying with 0.2 % ninhydrin solution in acetone. Quantitative determination by absorbance measurement at 486 nm.

clinical chemistry research, HPTLC, densitometry, quantitative analysis 18a

102 060 M. SAJEWICZ, D. KRONENBACH, D. STASZEK, M. WRÓBEL, G. GRYGIERCZYK, Teresa KOWALSKA* (*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland; kowalska@us.edu.pl): Experimental investigation of the oscillatory transenantiomerization of L-tyrosine. J. Liq. Chromatogr. Relat. Technol. 31, 2006-2018 (2008). TLC of L-tyrosine on silica gel (prewashed by development with methanol - water 9:1) impregnated by dipping in 30 mMol/L L-prolin in water - methanol 9:1, with n-butanol - acetonitrile - water 6:2:3 at 22 °C. Quantitative determination by absorbance measurement at 200 nm. The results of the investigation confirm clearly the ability of L-tyrosine to undergo oscillatory transenantiomerization, similar to that of the previously studied profens and L-alpha-phenylalanine.

qualitative identification 18a, 38

102 061 M. SAJEWICZ, M. GONTARSKA, L. WOJTAL, D. KRONENBACH, M. LEDA, I. R. EPSTEIN, Teresa KOWALSKA* (*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland; kowalska@us.edu.pl): Experimental and model investigation of the oscillatory transenantiomerization of L-alpha-phenylalanine. J. Liq. Chromatogr. Relat. Technol. 31, 1986-2005 (2008). TLC of L-alpha-phenylalanine on silica gel (prewashed by development with methanol - water 9:1) impregnated by dipping for 2 s in a 24 mMol/L aqueous solution of copper sulfate, followed by drying for 10 min at 110 °C and dipping in 30 mMol/L proline in water - methanol 9:1. The mobile phase was n-butanol - acetonitrile - water 6:2:3. Quantitative determination by absorbance measurement at 200 nm. The ability of L-alpha-phenylalanine to undergo oscillatory transenantiomerization was demonstrated. A skeleton molecular mechanism for the same process was proposed.

qualitative identification 18a, 38

102 062 Roseline SBAFFO-POASEVARA* (*API Analytical Development, IPSEN, 5 avenue du Canada, 91966 Les Ulis, France, roseline.poasevara@ipsen.com): Identification and quantification of amino acids in peptides. CBS 101, 2-4 (2008). HPTLC of amino acids (from hydrolysis of peptides) on silica gel, Diol phase, and cellulose with either 2-butanol - acetic acid - pyridine - water 15:3:10:12 or 2-butanol - 25 % ammonia - pyridine - water 39:10:34:26 in a twin-trough chamber or horizontal chamber. Detection by dipping in ninhydrin solution (0.5 % in 2-propanol) followed by heating at 110 °C for 5 min. Better results are achieved by adding ninhydrin directly to the mobile phase at a 0.5 %-level. Quantitative determination by absorbance measurement at 440 nm.

Selectivity was better on the cellulose and silica gel plate. Selection of the chromatographic system depended on which amino acids had to be separated and no general recommendation could be given. HPTLC analysis of a hydrolyzed peptide sample (containing Phe, Trp, D-Bal, Apc and Inp) on cellulose with the acidic mobile phase. All five amino acids were quantified between 70 and 130 % of the theoretical value for non-stable amino acids (degradation 5 to 30 %).

pharmaceutical research, clinical chemistry research, HPTLC, densitometry, quantitative analysis 18a

102 063 D. TIAN (Tian Dating)*, H.-Q. XIE (Xie Hong-Quan) (*School of Chemical and Environmental Engineering, Hubei Institute for Nationalities, Enshi 44500, China; tiandating@163.com): Influence of microemulsion conditions on the thin layer chromatographic behavior of amino acids. J. Liq. Chromatogr. Relat. Technol. 31, 763-771 (2008). TLC of 23 amino acids on silica gel with cetyltrimethylammonium bromide - n-butanol - n-octane - water microemulsion. Detection by spraying with ninhydrin reagent. Investigation of the effects of the hydrous content of microemulsion and structures of amino acids on the hR_f values. Several amino acid mixtures were separated and determined using a microemulsion with 40 % hydrous content, which was compared with the traditional mobile phase ethanol - water - acetic acid.

pharmaceutical research, qualitative identification 18a

102 059 D. KAZMIERCZAK, W. CIESIELSKI, K. DYNSKA, R. ZAKRZEWSKI* (*Department of Instrumental Analysis, University of Lodz, Pomorska 163, 90-236 Lodz, Poland; robzak@chemul.uni.lodz.pl): Iodine-azide detection system for dipeptides in thin-layer chromatography. J. Liq. Chromatogr. Relat. Technol. 31, 752-762 (2008). TLC and HPTLC of nine dipeptides (gly-gly, ala-gly, pro-leu, pro-asp, pro-gly, leu-pro, ala-pro, phe-pro, val-pro) on silica gel with ethanol - dichloromethane 2:1 and methanol - dichloromethane 1:1 in a horizontal chamber saturated for 20 min. Detection by spraying with sodium azide and starch solution (25 mL aqueous starch solution, containing 2.5 g starch, was added to 20 mL aqueous sodium azide solution containing 2 g sodium azide, the mixture was adjusted to pH 5.5 with 0.1 mol/L hydrochloric acid and diluted to 50 mL with water to obtain 4 % and 5 % solution for sodium azide and starch, respectively). All solutions were prepared fresh daily. The limit of detection was 2-200 pmol/spot for the iodine azide procedure, 1-100 pmol/spot for iodine, 20-2000 pmol/spot for UV 254 nm, and 40-1000 pmol/spot for spraying with ninhydrine and drying at 110 °C.

HPTLC, quantitative analysis, qualitative identification 18b

20. Enzymes

102 064 J. CHOI (Choi JiHye), H. LEE, Y. KIM (Kim Youngwan), J. PARK (Park Jongtae), E. WOO (Woo Euijeon), M. KIM (Kim Myojeong), B. LEE (Lee Byonghoon), K. PARK (Park Kwanhwa)* (*Center for Agricultural Biomaterials and Department of Food Science and Biotechnology, Seoul National University, Seoul, Republic of Korea, parkkh@snu.ac.kr): Characterization of a novel debranching enzyme from Nostoc punctiforme possessing a high specificity for long branched chains. Biochem. Biophys. Res. Commun. 378, 224-229 (2009). TLC of the hydrolytic action patterns of the purified Nostoc punctiforme debranching enzyme on the following substrates: pullulan, amylopectin, soluble starch, amylose, cyclodextrins, and maltooligosaccharides (form glucose to maltoheptaose), on silica gel with 1-propyl alcohol - ethyl acetate - water 6:2:3. Detection by dipping into 0.3 % N-(1-naphthyl)-ethylenediamine and 5 % sulfuric acid in methanol, followed by heating for 10 min at 110 °C. Quantitative determination by radioactivity measurement of the 14C-labeled maltooligosaccharides.

pharmaceutical research, quantitative analysis, radioscanning 20

21. Purines, pyrimidines, nucleic acids and their constituents

102 066 A.K. KUMAR*, K. MANNINDER (*Kulkarni S K Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India): Estimation of adenosine and its major metabolites in brain tissues of rats using high-performance thin-layer chroma-

tography - densitometry. J. Chromatogr. A 1209 (1-2), 230-237 (2008). TLC of purines (adenosine and its major metabolites, inosine, and hypoxanthine) in rat brain tissue preparations, on silica gel with a two-step gradient mobile phase consisting of (1) n-butanol - water - acetonitrile - 10 % ammonia - acetic acid 10:4:8:2:1 and (2) n-butanol - chloroform - acetonitrile - 10 % ammonia - acetic acid 10:4:8:2:1. Quantitative determination by absorbance measurement at 258 nm (via peak area). Application of the method to estimate the endogenous purines in discrete regions of rat brain. Development of a novel protocol for tissue preparation using 0.1 M HCl and 0.15 M NaOH solutions in 60 % methanol, which provided well-resolved peaks and high recoveries.

clinical chemistry research, quality control, densitometry, qualitative identification, quantitative analysis 21a

102 067 S. RAVETTI, M. S. GUALDESI, Margarita Cristina BRINÓN* (*Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitária, 5000 Córdoba, Argentina; macribri@fcg.unc.edu.ar): Lipophilicity of 5‘-carbonates of lamivudine with antiretroviral activity. Correlation between different methods. J. Liq. Chromatogr. Relat. Technol. 31, 1014-1032 (2008). TLC of cytidine, lamivudine (2‘,3‘-dideoxy-3‘-thiacytidine) and seven new 5‘-carbonates of lamivudine on RP-18 with acetone - buffer pH 7.4 and methanol - buffer 7.4 mixtures with modifier contents between 40 and 80 % in 10 % increments. Detection under UV light. RP-TLC is a reliable and accurate technique to describe the lipophilic character of this nucleoside family of compounds.

pharmaceutical research, quantitative analysis 21a

102 065 R. FUKUNAGA, Y. HARADA, I. HIRAO, S. YOKOYAMA* (*Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan, yokoyama@biochem.s.u-tokyo.ac.jp): Phosphoserine aminoacylation of tRNA bearing an unnatural base anticodon. Biochem. Biophys. Res. Commun. 372, 480-485 (2008). 2D-TLC of alpha-32P ATP or alpha-32P UTP labeled nucleotides after RNase T2 treatment of tRNA transcripts synthesized by T7 RNA polymerase, on silica gel with isobutyric acid - ammonia - water - 66:1:33 for the first dimension and isopropyl alcohol - hydrocloric acid - water 14:3:3 for the second dimension. Quantitative determination by radioactivity measurement of the labeled nucleotides.

pharmaceutical research, HPTLC, quantitative analysis, radioscanning 21b

22. Alkaloids

102 068 R. PATEL*, A. PRAJAPATI, M. PATEL (*S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherava 382711, Mehsana, Gujarat, India, leomanrk@yahoo.co.in): High performance thin layer chromatographic method for quantification of atisine from Aconitum heterophyllum Roth. Indian Drugs 45(3), 222-225 (2008). HPTLC of atisine in Aconitum heterophyllum (Ranunculaceae) on silica gel with toluene - ethyl acetate - diethylamine 7:2:1. Absorbance measurement at 274 nm prior to derivatization. Detection by dragendorff‘s reagent followed by treatment with 10 % sodium nitrite. Quantitative determination by absorbance measurement at 520 nm. Linearity was between 10-60 ng/spot.

pharmaceutical research, herbal, HPTLC, densitometry, quantitative analysis 22

102 132 H. PULPATI et al., see section 32e

102 069 K.K. ROUT, S. PRADHAN, S. K. MISHRA* (*Utkal University, Pharmacognosy and Phytochemistry Division, University Department of Pharmaceutical Sciences, Vani Vihar, Bhubaneswar 751004, Orissa, India; skmishraudps@gmail.com): Estimation of berberine in Ayurvedic formulations containing Berberis aristata. J. AOAC Int. 91, 1149-1153 (2008). HPTLC of berberine on silica gel prewashed with methanol using n-butanol - acetic acid - water 8:1:1 in a twin-trough chamber with chamber saturation for 5 min at 33 °C at 57 % relative humidity. Quantitative determination by absorbance measurement at 350 nm.

traditional medicine, quality control, herbal, densitometry, HPTLC, quantitative analysis 22, 32g

102 070 A. SINGH*, S. JAYARAMAN, K. JAYARAM, A. RANI, R. NEMA (*Dept. of Pharmaceutical Chemistry, S. D. College of Pharmacy and Vacational Studies, Muzaffarnagar 251001, India, nema_pharmacy@yahoo.co.in): Stability-indicating HPTLC determination of piperine in bulk drug and pharmaceutical formulations. Asian J. Chem. 20(8), 6007- 6010 (2008). HPTLC of piperine as bulk drug and in formulations on silica gel with toluene - ethyl acetate 93:9. Quantitative determination by absorbance measurement at 254 nm. The method was linear in the range of 100-400 ng/µL. The sample was then subjected to degradation studies (acid, alkali, oxidation, photodegradation). The method could effectively separate the drug from degradation products thus being stability indicating.

pharmaceutical research, quality control, HPTLC 22

23. Other substances containing heterocyclic nitrogen

102 071 Marzena PODGÓRNA (Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): Application of topological index and the Rf parameter to the estimation of lipophilic properties of selected porphyrins. J. Liq. Chromatogr. Relat. Technol. 31, 1458-1464 (2008). TLC of porphyrins and four alkoxy porphyrin derivatives on RP-18 with dichloromethane - methanol 3:2 saturated for 30 min. Detection by visual evaluation under white light.

pharmaceutical research, qualitative identification 23a

27. Vitamins and various growth regulators

102 072 M. NISHIOKA, Y. TANIOKA, E. MIYAMOTO, T. ENOMOTO, F. WATANABE* (*Faculty of Agricultur, School of Agricultural, Biological and Environmental Sciences, Tottori University, Tottori 680-8553, Japan; watanabe@muses.tottori-u.ac.jp): TLC analysis of a corrinoid compound from dark muscle of the yellowfish tuna (Thunnus albacares). J. Liq. Chromatogr. Relat. Technol. 30, 2245-2252 (2007). TLC of authentic vitamin B12 and extract on silica gel with 2-propanol - 28 % ammonia - water 7:1:2 and 1-butanol - 2-propanol - water 10:7:10 in the dark at room temperature. After drying agar containing basal medium and pre-cultured E. coli 215 was overlaid and then incubated at 30 °C for 20 h. After spraying with a methanolic solution of 2,3,5-triphenyltetrazolium salt corrinoid compounds were detected as red zones under white light.

food analysis, qualitative identification 27

102 073 Y. TANIOKA, Y. YABUTA, E. MIYAMOTO, H. INUI, F. WATANABE* (*School of Agricultural, Biological, and Environmental Sciences, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan; watanabe@muses.tottori-u.ac.jp): Analysis of vitamin B 12 in food by silica gel 60 TLC and bioautography with vitamin B 12-dependent Escherichia coli 215. J. Liq. Chromatogr. Relat. Technol. 31, 1977-1985 (2008). TLC of vitamin B 12 and dicyanocobinamide on silica gel with 2-propanol - 28 % ammonia - water 7:1:2 in the dark at room temperature. After drying the plate was overlaid with 1.5 % agar containing a basal medium and a small volume of E. coli 215 culture, and then incubated at 30 °C for about 20 h. After spraying with a methanolic solution of 4 % 2,3,5-triphenyltetrazolium salt the plate was heated at 30 °C for 1 h. The method was applied to detect vitamin B12 or inactive corrinoids in foods.

food analysis, qualitative identification, bioautography 27

28. Antibiotics, Mycotoxins

102 074 Irena CHOMA (Department of Chromatographic Methods, University of M. Curie-Sklodowska, Lublin, Poland; ichoma@hermes.umcs.lublin.pl): TLC separation of cephalosporins: Searching for better selectivity. J. Liq. Chromatogr. Relat. Technol. 30, 2231-2244 (2007). HPTLC of eight cephalosporins (cefaclor, cefoperazone, cefazolin, cefotaxime, cefoxitin, cefuroxime, cephalotin, and p-chlorophenacyl cephalothin) on silica gel with diisopropyl ether - toluene - ethyl acetate -

80 % formic acid 1:4:13:2 in a sandwich chamber. Also HPTLC on diol-, amino-, and cyano-modified silica gel. Detection under UV light at 254 nm.

pharmaceutical research, HPTLC, qualitative identification 28a

102 075 U. HUBICKA, J. KRZEK* (*Jagiellonian University, Collegium Medicum, Department of Inorganic and Analytical Chemistry, Medyczna 9, 30-688 Krakow, Poland; jankrzek@cm-uj.krakow.pl): Effect of selected metal ions on the photodegradation of ciprofloxacin in the solid phase. J. AOAC Int. 91, 1331-1338 (2008). HPTLC of ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid hydrochloride) and degradation products (7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid and 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid) on silica gel with chloroform - methanol - 25 % ammonia 43:43:14. Detection with 0.3 % methanolic ninhydrin solution and dimethylaminobenzaldehyde and quantitative determination by absorbance measurement at 277 nm.

quality control, densitometry, quantitative analysis, HPTLC 28a

102 076 A. KONYA*, Z. SZABO, I. LANG, I. BARTA, J. SALAT (*IVAX Drug Research Institute Ltd., Budapest, Hungary, h8773con@ella.hu) : Production of FK520 by Streptomyces tubercidicus. Microbiol. Res. 163, 624-632 (2008). TLC of FK506 and FK520 from the fermentation broths of Streptomyces species, on silica gel with isopropyl alcohol - benzene 3:17 or methylene chloride - acetone 2:1. Detection by bioautography with the A. IDR 721 test organism, and also by spraying the plate with cesium sulphate 1 % in sulfuric acid 10 % followed by heating at 120 °C. The hR_f of the immunosupressant compounds were 50 or 60, depending on the developing solvent

pharmaceutical research, qualitative identification 28a

102 077 Irena M. CHOMA*, C. KOWALSKI, R. LODKOWSKI, A. BURMANCZUK, I. KOMANIEKKA (*Department of Chromatographic Methods, University of M. Curie-Sklodowska, M. Sklodowska Sq. 3, 20-031 Lublin, Poland; irena.choma@umcs.lublin.pl): TLC-DB as an alternative to the HPLC method in the determination of cefacetril residues in cow‘s milk. J. Liq. Chromatogr. Relat. Technol. 31, 1903-1912 (2008). TLC of cefacetril on silica gel with methanol - acetonitrile in a sandwich chamber. The developed TLC plates were dried, then immersed briefly in the microorganism solution, and incubated for 20 h at 37 °C. After incubation the plates were sprayed with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide solution and left at room temperature for 30 min. White inhibition zones were observed against a purple background (Chrom Biodip Antibiotic Test kit). The described procedure is suitable for screening and semi-quantitative determination of antibiotic residues in milk.

food analysis, qualitative identification, TLC-direct bioautography 28a

102 078 S.K. MOTWANI*, R.K. KHAR, F.J. AHMAD, S. CHOPRA, K. KOHLI, S. TALEGAONKAR (*Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India): Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on moxifloxacin. Anal. Chim. Acta 582(1), 75 - 82 (2007). TLC of moxifloxacin both as a bulk drug and from pharmaceutical formulation on silica gel with n-propanol - ethanol - 6M ammonia 4:1:2. Quantification by absorbance measurement at 298 nm. The hRf value of moxifloxacin was 58. Linearity was in the range of 100-800 ng/spot. LOD and LOQ were 3.9 and 11.8 ng/spot, respectively. In stability studies (acid and alkali hydrolysis, oxidation, dry heat, wet heat, photodegradation) degradation products were well resolved from the standard drug. The method was suitable to investigate the kinetics of the acidic and alkaline degradation processes at different temperatures.

pharmaceutical research, densitometry, quantitative analysis,
qualitative identification 28a

29. Pesticides and other agrochemicals

102 083 Y. YUE (Yue Yongde)*, R. ZHANG (Zhang Rong), W. FAN (Fan Wei), F. TANG (Tang Feng) (*International Center for Bamboo and Rattan, 100102 Beijing, China; yueyd@icbr.ac.cn): High-performance thin-layer chromatographic analysis of selected organophosphorous pesticide residues in tea. J. AOAC Int. 91, 1210-1217 (2008). HPTLC of monocrotophos, quinalphos, triazophos, parathion-methyl, isophenphos-methyl, temephos, parathion, phoxim, and chlorpyrifos on silica gel with automated multiple development. HPTLC of phoxim and chlorpyrifos on silica gel with dichloromethane - hexane 1:1 in a twin-trough chamber. Quantitative determination by absorbance measurement at 254 nm.

food analysis, toxicology, HPTLC, AMD, densitometry, quantitative analysis 29b

102 081 T. TUZIMSKI (Faculty of Pharmacy, Department of Physical Chemistry, Medical University, Lublin, Poland, tomasz.tuzimski@am.lublin.pl): Application of SPE-HPLC-DAD and SPE-TLC-DAD to the determination of pesticides in real water samples. J. Sep. Sci. 31, 3537-3542 (2008). After solid phase extraction of water samples HPTLC of clofentezine (1), neburon (2), chlorfenvinphos (3), lenacyl (4), trifluralin (5), thiram (6), procymidone (7), flufenoxuron (8), tralkoxydim (9), propaquizafop (10), and dinoseb (11) on silica gel with ethyl acetate - n-heptane 2:8, 3:7, 4:6, or 7:3 as mobile phase. Quantitative determination by absorbance measurement between 200 and 600 nm. Selectivity regarding matrix was given. Linearity was 0.1-1.5 µg/spot for (1), 0.2-1.0 µg/spot for (2), 0.5-1.0 µg/spot for (3), 0.2-1.0 µg/spot for (4), 0.3-9.0 µg/spot for (5), 0.2-1.0 µg/spot for (6), 2.0-11.0 µg/spot for (7), 0.1-2.0 µg/spot for (8), 0.3-1.0 µg/spot for (9), 0.1-1.0 µg/spot for (10), and 0.2-1.0 µg/spot for (11). The limits of detection and quantification were 0.23 and 0.70 µg/spot for (1), 0.06 and 0.18 µg/spot for (2), 0.16 and 0.49 µg/spot for (3), 0.04 and 0.12 µg/spot for (4), 0.06 and 0.18 µg/spot for (5), 0.16 and 0.49 µg/spot for (6), 0.65 and 1.92 µg/spot for (7), 0.10 and 0.31 µg/spot for (8), 0.07 and 0.22 µg/spot for (9), 0.06 and 0.17 µg/spot for (10), and 0.08 and 0.24 µg/spot for (11). The optimal wavelength for quantification was 278 nm for (1), 249 nm for (2), 247 nm for (3), 273 nm for (4), 277 nm for (5), 281 nm for (6), 208 nm for (7), 268 nm for (8), 284 nm for (9), 245 nm for (10), and 366 nm for (11). Advantages of the technique over the HPLC method are highlighted.

environmental, HPTLC, quantitative analysis, densitometry, comparison of methods 29d

102 082 T. TUZIMSKI (Medical University, Department of Physical Chemistry, Faculty of Pharmacy, 4 Staszica St, 20-081 Lublin, Poland; tomasz.tuzimski@am.lublin.pl): Determination of pesticides in water samples from the Wieprz-Krzna canal in the Leczynsko-Wlodawskie lake district of southeastern Poland by thin-layer chromatography with diode array scanning and high-performance column liquid chromatography with diode array detection. J. AOAC Int. 91, 1203-1209 (2008). HPTLC of atrazine, clofentezine, chlorfenvinphos, hexaflumuron, terbuthylazine, lenacyl, neburon, bitertanol, and metamitron on silica gel with ethyl acetate - n-heptane 1:4, 3:7, 2:3, or 7:3 in a horizontal chamber. Detection by scanning in the range of 200 to 600 nm with a TLC-DAD scanner.

environmental, qualitative identification, HPTLC 29d, 37c

102 079 D. MESHRAM*, S. BAGADE, M. TAJNE (*Dept. of Pharmaceutical Sciences, R.T.M. Nagpur University, Nagpur 40033 (M.S.) India): High performance thin layer chromatographic estimation of itraconazole in capsules. J. Pharm. Res. 6(4), 205-207 (2007). HPTLC of itraconazole on silica gel with toluene - acetone - triethylamine 30:30:1. Quantitative determination by absorbance measurement at 270 nm. The hR_f value of itraconazole was 62. Linearity was between 200 and 600 ng. The percent drug estimated from the market formulation was found to be 99.6 and 100.2 by peak height and peak area respectively. The percent recovery of the drug (by standard addition method) was between 99.4 and 100.3.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 29e

102 080 Alina PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, 41-200 Sosnowiec, Poland; apyka@slam.katowice.pl): The application of densitometry to evaluate the visualizing effects of salicylanilide using brilliant green. J. Liq. Chromatogr. Relat. Technol. 31, 1943-1958 (2008). TLC of salicylanilide on silica gel and silica gel mixed with Kieselguhr (with and without brilliant green as a detection reagent) with chloroform in a chamber saturated for 30 min. Detection without a reagent and by treatment with a 50 mg/100mL aqueous solution of brilliant green. Quantitative determination by absorbance measurement at 597, 305, and 268 nm. Proposition of a new index to evaluate objectively the visualizing effects of detected substances on thin layer using a densitometric method. The limit of detection, detection index, broadening index, modified contrast index, densitometric visualizing index, and linearity range were used to evaluate the visualizing effects of salicylanilide.

qualitative identification, densitometry, quantitative analysis 29e

30. Synthetic and natural dyes

102 084 S. DIXIT, S. K. KHANNA, M. DAS* (*Indian Institute of Toxicology Research (Council of Scientific and Industrial Research), Food Toxicology Division, Mahatma Gandhi Marg, P.O. Box 80, Lucknow 226001, U. P., India; mditrc.@rediffmail. com): A simple 2-directional high-performance thin-layer chromatographic method for the simultaneous determination of curcumin, metanil yellow, and sudan dyes in turmeric, chili, and curry powders. J. AOAC Int. 91, 1387-1396 (2008). HPTLC on silica gel with chloroform - methanol 9:1 in the first direction for curcumin, demethoxycurcumin, bis(demethoxy)curcumin, and the synthetic dye metanil yellow, and with toluene - hexane - acetic acid 50:50:1 for sudan I and sudan IV in the second direction. Quantitative determination by absorbance measurement at 420 nm for curcumin and metanil yellow, at 491 nm for sudan I, and at 520 nm for sudan IV.

food analysis, quantitative analysis, densitometry, HPTLC 30a

102 085 H. OKA*, N. OZEKI, T. HAYASHI, Y. ITAKURA (*School of Pharmacy, Kinjogakuin University, Omori, Morigama-ku, Nagoya 463-8521, Japan; oka@kinjo-u.ac.jp): Analysis of natural colorings in foods by thin layer chromatography. J. Liq. Chromatogr. Relat. Technol. 30, 2021-2036 (2007). TLC of carotenoid colorings of 95 commercial foods (33 for tomato color [lycopene], 38 for orange color [e.g. fatty acid ester of beta-cryptoxanthin], and 24 for marigold colorings [fatty acid ester of lutein]) on RP-18 with acetonitrile - acetone - n-hexane 11:7:2 and acetone - water 9:1. TLC of beta-carotene and paprika colorings of 77 commercial foods (e.g. capsanthin and its esters) on RP-18 with n-hexane - acetone - acetonitrile 2:7:1. TLC of quinone colorings (lac and cochineal colors) on RP-18 with methanol - 0.5 mol/L oxalic acid 11:9. TLC of anthocyanin colorings of 45 commercial foods (red cabbage color [derivatives of cyanidin acylglycoside]) on RP-18 with acetonitrile - 0.2 mol/L trifluoroacetic acid 1:2. Identification by recording of visible absorption spectra.

food analysis, quality control, densitometry, review, qualitative identification 30b

102 086 M. PARAMASIVAM*, R. POI, H. BANERJEE, A. BANDYOPADHYAY (*Department of Agricultural Chemicals, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, India, sivam25@gmail.com): High performance thin layer chromatographic method for quantitative determination of curcuminoids in Curcuma longa germplasm. Food Chemistry 113, 640-644 (2009). HPTLC of curcumin (1), demethoxycurcumin (2), and bisdemethoxycurcumin (3) from the rhizomes of Curcuma longa on silica gel with chloroform - methanol 24:1. Quantitative determination by absorbance measurement at 425 nm. The hR_f values of (1), (2), and (3) were 66, 48, and 30, respectively. Selectivity regarding matrix was given. Recovery was 98.7 % for (1), 96.3 % for (2), and 97.2 % for (3). The limit of detection for (1), (2), and (3) was 0.1 µg/spot. The linear regression equation was y=4447.26 + 61.993X for (1), y=1089.881 + 70.003X for (2), and y=2611.84 + 51.565X for (3).

herbal, quality control, HPTLC, quantitative analysis, densitometry 30b

102 010 U. SOTANAPHUN et al., see section 4c

102 087 K.K. ROUT, S. PARIDA, S. K. MISHRA (*Utkal University, Pharmacognosy and Phytochemistry Division, University Department of Pharmaceutical Sciences, Vani Vihar, Bhubaneswar 751004, Orissa, India; skmishraudps@gmail.com): Standardization of the Ayurvedic formulation Haridra Khanda using high-performance thin-layer chromatography/densitometry. J. AOAC Int. 91, 1162-1167 (2008). HPTLC of curcumin, demethoxycurcumin, and bisdemethoxycurcumin as standards on silica gel prewashed with methanol using chloroform - methanol 97:3 in a twin-trough chamber at 23 °C and 31 % relative humidity. Quantitative determination by absorbance measurement at 430 nm.

traditional medicine, quality control, herbal, HPTLC, densitometry, quantitative analysis 30b

102 088 P.K. ZARZYCKI*, M.B. ZARZYCKA (*Koszalin University of Technology, Section of Toxicology and Bioanalytics, Department of Environmental Biology, Koszalin Sniadeckich 2, 75-453 Koszalin, Poland; pkzarz@wp.pl or pawel_k_z@hotmail.com): Evaluation of the water and organic liquids extraction efficiency of Spirulina maxima dyes using thermostated micro thin-layer chromatography. J. AOAC Int. 91, 1196-1202 (2008). Thermostated micro HPTLC of Spirulina maxima dyes on RP-18 with acetone - n-hexane 3:7 in a homemade temperature-controlled removable horizontal micro TLC chamber at 40 °C with chamber saturation. Detection by direct digital scan under visible light conditions. Experimental data indicated that under such conditions, with an office scanner used for chromatogram digitalization, spot quantification could be accurately performed within the analyte mass range of two orders of magnitide. quality control,

quantitative analysis, HPTLC 30b, 3d

32. Pharmaceutical and biomedical applications

102 089 R. AHMED*, Z. ZAHEER, S. DHANESHWAR, M. FAROOQUI (*Y. B. Chavan College of Phamacy, Aurangabad, Maharashtra, India): Stability-indicating HPTLC determination of clozapine in tablet dosage form. 60th Indian Pharmaceutical Congress PA-201, (2008). HPTLC of clozapine on silica gel with toluene - acetonitrile - ethyl acetate - ammonia 80:20:1. The hR_f value was 35. Quantitative determination by absorbance measurement at 290 nm. The drug when subjected to forced degradation (acid, alkali, thermal, photodegradation) was well separated from degraded products. The compound was found to be stable to oxidative conditions. The method was suitable for routine quality control of formulation.

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

102 090 Y. ANJANEYULU*, R. MARAYYA, D. LINGA RAO, P. KRISHNA RAO (*Natco Pharma Ltd. QC / QA Chemical Div., Road No.2, Banjara Hills, Hyderabad 500033, India, mekaguda@natcopharma.co.in): High performance thin layer chromatographic determination of the related substances in alprazolam drug. Asian J. Chem. 19(5), 3375-3381 (2007). The TLC method described in USP 28 does not separate all the related substances of alprazolam. An alternative HPTLC method is described for separation and estimation of starting material and synthesis related intermediates in alprazolam: 2-chloro acetamide-5-chloro benzophenone (impurity 1) i.e. starting material, nordiazepam (impurity 2), thionordiazepam (impurity 3), 2-(2-aceto hydrazinyl)-7-chloro-5-phenyl-3H-1, 4-benzodiazapine (impurity 4). The hRf values of alprazolam, impurity 4, impurity 3, impurity 2, and impurity 1 were 25, 16, 77, 45, and 83 respectively. The HPTLC method developed is capable of detecting impurities at a level of 0.05 %.

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

102 092 S. BAGADE*, N. GOWEKAR, A. KASTURE (*University Dept. of Pharmaceutical Sciences,

R. T. M. Nagpur University Campus, Nagpur 440033, India, sbbagade@rediffmail.com): Simultaneous HPTLC estimation of ambroxol HCl and cetirizine HCl in their combined dose tablet. Asian J. Chem. 19(2), 1487-1493 (2007). TLC of ambroxol HCl and cetirizine HCl on silica gel with methanol - ethyl acetate - toluene - ammonia 3:10:12:1. Quantitative determination by absorbance measurement at 231 nm. The hR_f value of ambrocol HCl was 78 and of cetirizine HCl 40. The calibration curve response was observed between 4 and 10 µg for ambroxol HCl and 0.4 and 0.8 µg for cetirizine HCl via peak height and area. The percent drug estimated for ambroxol HCl and cetirizine HCl from marketed formulation was 99.9, 100.2 by height and 100.8, 99.1 by area respectively. Recovery of ambroxol HCl via peak height and via peak area was 102.7 and 100.0 % respectively, and for cetirizine HCl 99.3 and 98.1 %.

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

102 093 S. BHALERAO*, S. TAMBE, V. KASTURE, V. PAREEK (*M. G. V. Pharmacy College, Nashik, Maharashtra, India): Application of HPTLC for the determination of diacerein in pharmaceutical solid dosage form. 60th Indian Pharmaceutical Congress PA-205, (2008). HPTLC of diacerein on silica gel with ethyl acetate - n-hexane - acetic acid 120:19:1. The hRf value was 32. Quantitative determination by absorbance measurement at 258 nm. The method was linear in the range of 50-250 ng/spot. The recovery was 96-103 %.

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

102 094 L. BHAT*, K. BOTHARA, M. DAMLE (*AISSMS College of Pharmacy, Dept. of Pharmaceutical Analysis, Kennedy road, Pune 411001, India, mrunal.damle@rediffmail.com): Validated HPTLC method for simultaneous determination of nebivolol hydrochloride and hydrochlorothiazide from tablets. Indian Drugs 45(12), 948-951 (2008). HPTLC of nebivolol hydrochloride and hydrochlorothiazide on silica gel with ethyl acetate - methanol - ammonia 17:2:1. Absorbance measurement at 280 nm. The method was linear in the range of 500-1500 µg/mL and 100-500 µg/mL for nebivolol hydrochloride and hydrochlorothiazide respectively. Recovery was 99.3-101.9 % for both compounds. The method is suitable for routine quality control.

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

102 096 S. CHITLANGE*, D. SAKARKAR, S. WANKHEDE, S. WADODKAR (*Pad. Dr. D. Y. Patil Institute of Pharmaceutical Science and Research, S. T. Nagar, Pimpri, Pune 411018, India): High performance thin layer chromatographic method for simultaneous estimation of ibuprofen and pseudoephedrine hydrochloride. Ind. J. Pharm. Sci. 70(3), 398 - 400 (2008). HPTLC of ibuprofen and pseudoephedrine HCl on silica gel with tert-butanol - ethyl acetate - acetic acid - water 7:4:2:2. Quantitative determination by absorbance measurement at 254 nm. The hR_f value of pseudoephedrine was 68 and of ibuprofen 91. The method was linear in the concentration range of 45.6 - 75.6 µg/mL for ibuprofen and 6.8 - 11.3 µg/mL for pseudoephedrine. The recovery was between 100.7 and 101.0 % for both compounds. The method was suitable for routine quality control.

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

102 097 T. CSERMELY, H. KALÁSZ*, K. DEÁK, M. Y. HASAN, F. DARVAS, G. PETROIANU (*Department of Pharmacology and Therapeutics, United Arab Emirates University, Al Ain, P.O. Box 17666, United Arab Emirates; huba.kalasz@gmail.com): Lipophilicity determination of some ACE inhibitors by TLC. J. Liq. Chromatogr. Relat. Technol. 31, 2019-2034 (2008). TLC of captopril, delapril, enalapril, lisinopril, amd moexipril on silica gel (impregnated by continous development with 10 % paraffin oil in n-hexane for 18 h) and RP-18 with various mobile phases. Detection under UV 254 nm. TLC is a fast and economical method for the determination of lipophilicity.

pharmaceutical research, qualitative identification 32a

102 098 S.R. DHANESHWAR*,NARENDRA G. PATRE, M.V. MAHADIK (Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, 411038, India): Validated TLC method for simultaneous quantitation of amlodipine besylate and valsartan in bulk drug and formulation. Chromatographia 69 (1-2), 157-161 (2009). TLC of amlodipine besylate (AML) and valsartan (VAL) on silica gel with toluene - methanol - acetic acid 70:30:1. Quantification by absorbance measurement at 244 nm. Linearity was between 100 - 600 ng/spot for AML and 1600 - 9600 ng/spot for VAL. For AML the RSD of intra-day precision was 1.5 - 1.8 % and of inter-day precision 1.2 - 2.0 %. For VAL the RSD of intra-day precision was 0.1 - 0.4 % and of inter-day precision 0.2 - 0.5 %. Accuracy was 98.3 % for AML and 98.7 % for VAL.

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

102 101 M. GANDHIMATHI*, T.K. RAVI (*Sri Ramkrishna Institute of Paramedical Sciences, Dept. of Pharmaceutical Analysis, College of Pharmacy, Coimbatore 641044, India, gands72@yahoo.co.in): RP-HPTLC and HPTLC estimation of tramadol hydrochloride and paracetamol in combination. Asian J. Chem. 20(6), 4940-4942 (2008). HPTLC of paracetamol and tramadol hydrochloride on silica gel with ethyl acetate - toluene - ammonia 60:40:1. Absorbance measurement at 254 nm. The method was linear in the range of 0.1-0.5 µg/mL and 0.9-4.5 µg/mL for tramadol and paracetamol respectively. The recovery was 98.4-99.9 % for both compounds. The method was suitable for routine analysis.

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

102 102 R. GODGE*, Leena BHAT, A. VORA, M. DAMLE (*AISSMS College of Pharmacy Dept. of Pharmaceutical Chemistry, Kennedy road, near RTO, Pune 411001, Mha., India, mrunal.damale@rediffmail.com): A validated high performance thin layer chromatographic method for simultaneous estimation of ofloxacin and satranidazole in pharmaceutical dosage form. J. Pharm. Res. 6(4), 233-235 (2007). HPTLC of ofloxacin and satranidazole on silica gel with n-butanol - ethanol - ammonia 5:5:4. Quantitative determination by absorbance measurement at 320 nm. The method was linear in the range of 200-1000 ng/spot (ofloxacin) and 300-1500 ng/spot (satranidazole) with a recovery of 99.1 - 99.9 % for both component. The hR_f value was 54 for ofloxacin and 83 for satranidazole. The method is suitable for routine quality control of raw material and formulations.

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

102 104 L. GUPTA*, S. BHALERAO, S. TAMBE, V. KASTURE (*M. G. V.'s Pharmacy College, Nashik, Maharashtra, India): High performance thin layer chromatographic determination of carvedilol. 60th Indian Pharmaceutical Congress PA-204, (2008). HPTLC of carvedilol on silica gel with methanol - ethyl acetate 13:7. The hR_f was 49. Quantitative determination by absorbance measurement at 242 nm. Linearity was between 200-1000 ng/spot. The recovery was 98-102 %. The method was suitable for routine quality control of the drug in formulation.

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

102 105 K. GUPTA*, S. WANKHEDE, M. TAJNE, S. WADODKAR (*Dept. of Pharmaceutical Science, S.K.B. College of Pharmacy, Near Dragon Palace Temple, New Kamptee, Nagpur 441002, India, krishnargupta@rediffmail.com): High performance thin layer chromatographic estimation of atenolol and indapamide from pharmaceutical dosage form. Asian J. Chem. 19(6), 4183-4187 (2007). HPTLC of atenolol and indapamide in tablet formulation on silica gel with toluene - ethanol - acetone - acetic acid 70:25:30:3. Quantitative determination by absorbance measurement at 266 nm. The hR_f value of atenolol and indapamide was 21 and 74, respectively. The linearity

range was 3.8-10.9 ng/spot and 0.2-0.6 ng/spot for atenolol and indapamide respectively. The recovery was in the range of 98.7-100.1 % for both compounds.

pharmaceutical research, quality control, HPTLC, densitometry,

102 108 N. HARIKRISHNAN*, V. GUNASEKARAN, A. SATHISBABU, G. RAO (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Old Pallavaram, Chennai 600117, India, harry74velscollege@yahoo.co.in): Simultaneous estimation of aceclofenac and paracetamol by HPTLC in pure and pharmaceutical dosage form. Asian J. Chem. 19(5), 3918-3922 (2007). TLC of paracetamol and aceclofenac on silica gel with toluene - isopropyl alcohol - ammonia 20:20:3. Quantitative determination by absorbance measurement at 254 nm. Linearity was between 60 and 140 µg/mL for aceclofenac and 460 and 540 µg/mL for paracetamol. Recovery (by standard addition method) of paracetamol and aceclofenac was between 98.6 and 99.3 %. The proposed method was found to be accurate, precise, simple and rapid could be used for routine analysis.

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

102 110 R. KAKDE*, V. KOTAK, D. KALE (*R.T.M. University, Dept. of Pharmaceutical Sciences, Nagpur (Ms), India, drkakde@yahoo.com): High performance thin layer chromatographic method for simultaneous estimation of amlodipine besilate and bisoprolol fumarate in pharmaceutical preparations. Pharma Review 7(3), 168 - 170 (2008). HPTLC of amlodipine besilate and bisoprolol fumarate on silica gel with methanol - ethyl acetate - ammonia 50:60:5. Quantitative determination by absorbance measurement at 229 nm. The hR_f values were 39 and 52 for amlodipine and bisoprolol respectively. The linear range was 500-1000 ng/spot for both components. The method was suitable for routine quality control of combine dosage form.

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

102 112 M. KUMAR*, A. KHEDKAR, J. RAO, S. YADAV (*Poona College of Pharmacy, Bharti Vidyapeeth, Pune, India): Development of stability-indicating HPTLC method for bumetanide in bulk drug and pharmaceutical dosage form. 60th Indian Pharmaceutical Congress PA-194, (2008). HPTLC of bumetanide on silica gel with toluene - ethyl acetate - formic acid 14:7:1. Quantitative determination by absorbance measurement at 335 nm. In the stability test (stress conditions: acid, alkali, oxidation, dry heat, wet heat, photodegradation) the compound was well separated from degradation products. Linearity was in the range of 100 - 800 ng/spot.

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

102 114 R. LAUFER, M. BÁTHORI, T. CSERMELY, G. PETROIANU, K. KUCA, N. TÓTH, H. KÁLÁSZ* (*Department of Pharmacology and Pharmacotherapy, Semmelweis University, 1089 Budapest, Hungary; huba@kalasz.com): TLC determination of hydrophilicity parameter of some pyridinium aldoximes. J. Liq. Chromatogr. Relat. Technol. 30, 2337-2344 (2007). TLC of pralidoxime and obidoxime on silica gel impregnated by an 18 hours continous development with 10 % paraffin oil in n-hexane in an unsaturated chamber. Lipophilicity was determined on paraffin coated TLC plates using 30 %, 40 %, 50 %, 60 %, 70 %, and 80 % aqueous methanol, each mixture containing 1 % ammonium hydroxide). Hydrophilicity was determined by using plain silica gel with 70 %, 80 %, and 90 % aqueous methanol and 100 % methanol, each mixture containing 1 % ammonium hydroxide. Detection under white light and under UV 254 nm.

pharmaceutical research, qualitative identification 32a

102 115 S. MAGESWARI*, K. SURENDRA, R. MAHESWARI, N. KRISHNAN, C. ROOSEWELT, V. GUNASEKARAN (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Pallavaram, Chennai 600117, India, kalavaivgs30@rediffmail.com): HPTLC method for simultaneous estimation of rabeprazole sodium and itopride hydrochloride in capsule and bulk drug. Asian J.

Chem. 19(7), 5634-5638 (2007). TLC of rabeprazole sodium and itopride hydrochloride on silica gel prewashed with methanol, with toluene - chloroform - methanol - 25 % ammonia 5:6:2. Quantitative determination by absorbance measurement at 225 nm. Linearity was between 120 and 280 µg/mL for rabeprazole sodium and 900 and 1900 µg/mL for itopride hydrochloride. The recovery (by standard addition method) was between 98.1 and 99.5 % for both drugs. The proposed method is precise and accurate and can be used for routine analysis of rabeprazole sodium and itopride hydrochloride in capsule formulation.

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

102 116 R. MAHESWARI*, S. MAGESWARI, K. SURENDRA, V. GUNASEKARAN, P. SHANMUGASUNDARAM (*Vel's College of Pharmacy, Dept. of Pharmaceutical Analysis, Chennai 600117, India, samsimahe@yahoo.com): Simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form by HPTLC method. Asian J. Chem. 19(7), 5582-5586 (2007). HPTLC of telmisartan and hydrochlorothiazide on silica gel with ethyl acetate - chloroform - methanol 10:3:1. Absorbance measurement at 270 nm. The method was linear in the range of 500-750 ng/µL and 1600-2400 µg/µL for hydrochlorothiazide and telmisartan respectively. The recovery was 99.4-99.6 % for both compounds.

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

102 117 A. MALIYE*, S. WALODE, A. KASTURE, S. WADODKAR (*Dept. of Pharmaceutical Science, Nagpur University Campus, Amravati Rd., Nagpur 440033, India, amitmaliye@indiatimes.com): Simultaneous estimation of mefenamic acid and drotaverine hydrochloride in tablets by high performance thin layer chromatography Asian J. Chem. 18(1), 667-672 (2006). HPTLC of mefenamic acid and drotaverine HCl in tablets on silica gel with methanol - toluene - triethylamine 10:75:2. Quantitative determination by absorbance measurement at 241 nm. The hRf values were 31 and 47 for mefenamic acid and drotaverine HCl, respectively. The method was validated in terms of accuracy, precision, specificity, ruggedness. Linearity was between 3800 and 8400 ng for mefenamic acid and 1200 and 2700 ng for drotaverine HCl. The recovery (by standard addition method) was in the range of 99.1-100.9 % for both compounds. The method could be used for routine analysis of these compounds and their combined dosage form.

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

102 118 S. MATHUR*, R. SINGH, D. SHARMA, P. SAINI, G. SINGH, S. TUTEJA (*Central Indian Pharmacopoeia Laboratory, R & D Div. Indian Pharmacopoeia Commission, Gaziabad, U.P., India): Simultaneous estimation of lamivudine and zidovudine by HPTLC in pharmaceutical dosage form. 60th Indian Phamaceutical Congress PA-132, (2008). HPTLC of lamivudine and zidovudine on silica gel with acetone - methanol - toluene 2:1:2. Quantitative determination by absorbance measurement at 273 nm. The method was suitable for routine quality control of both drugs in combined dosage form.

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

102 119 Clare MCKINLAY (Analytical Sciences, GlaxoSmithKline, Medicines Research Park, Gunnels Wood Road, Stevenage, Hertfordshire, UK, SG 1 1WZ, clare@mckinlay@gsk.com): Use of HPTLC as a problem solving technique in pharmaceutical analysis. CBS 101, 12-13 (2008). During a drug substance stability study a mass imbalance was discovered in light degraded samples. HPTLC on silica gel first with ethyl acetate - heptane, then, after drying, with tetrahydrofuran in a horizontal developing chamber. Detection under UV 254 nm and by densitometry at 240 nm. During another project differences in color between batches of a drug substance were observed. HPTLC on amino phase with methanol in a horizontal developing chamber. Detection under white light and under UV 366/>400 nm.

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

102 121 S. MOGRE*, B. MEHTA (*Dept. of Chemistry, University of Mumbai, Vidyanagari, Santacruz (E) Mumbai 400098, India): Validated HPTLC method and content uniformity test for analysis of telmisartan and hydrochlorothiazide in tablet dosage forms. J. Pharm. Res. 7(2), 126-128 (2008). HPTLC on silica gel with acetone - chloroform - ethyl acetate - methanol 6:6:6:1. Quantitative determination by absorbance measurement at 280 nm. The hRf value for telmisartan was 27 and for hydrochlorothiazide 45. The regression curve shows good linear relationship in the concentration range of 25.5 - 128.0 µg for hydrochlorothiazide and 81.6 - 408.0 µg for telmisartan. The content uniformity test was carried out as per the USP specification of the content uniformity test. The percent drug estimated from the marketed formulations were found to be in the range 99.3 and 100.5 for both drugs. The percent recoveries of drug carried out by the standard addition method was found to be 100.3 and 99.4 for hydrochlorothiazide and telmisartan respectively. The proposed method was found suitable for routine quality control and content uniformity tests.

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

102 122 B. MORAK-MLODAWSKA, K. PLUTA* (*Department of Organic Chemistry, The Medical University of Silesia, Sosnowiec, Poland; pluta@slam.katowice.pl): RP TLC determination of the lipophilicity of new 10-substituted 2,7-diazaphenothiazines. J. Liq. Chromatogr. Relat. Technol. 31, 611-618 (2008). TLC of eleven new bioactive 10-substituted 2,7-diazaphenothiazines on RP-18 with acetone and aqueous TRIS (tris-(hydroxymethyl)aminomethane) buffer pH 7.4 in a saturated chamber. The concentration of acetone in the mobile phase ranged from 50-85 % in 5 % increments. Evaluation under UV 254 nm and 366 nm. The method was used for the experimental determination of lipophilicity.

pharmaceutical research, qualitative identification 32a

102 124 Izabela MUSZALSKA*, P. G. GÓRSKI, H. SLADOWSKA, D. SZKATULA, A. SABINIARZ (*Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland; imuszals@amp.edu.pl): Chromatographic separation of derivatives of 4-alkoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione by TLC and HPLC. J. Liq. Chromatogr. Relat. Technol. 30, 2103-2115 (2007). TLC of five derivatives of 4-alkoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione on silica gel with n-butanol - 2-propanol - cyclohexane - acetic acid 6:3:2:1 in the first dimension and chloroform - 2-propanol - acetic acid 40:15:6 or chloroform - cyclohexane - 2-propanol - acetic acid 20:20:15:6 in the second dimension. Quantitative determination by absorbance measurement at 254 nm.

quality control, qualitative identification 32a

102 125 M. PAI*, Vibhuti KARPE, Rajashree GUDE, S. KUDCHAKAR, (*Goa College of Pharmacy, Panaji, Goa, India): A new validated HPTLC method for the quantitative estimation of oflxacin and ornidazole in tablets. 60th Indian Pharmaceutical Congress PA-08, (2008). HPTLC of ofloxacin and ornidazole on silica gel with chloroform - methanol - toluene - diethyl amine - water 20:15:25:10:1 in a saturated (20 min) twin trough chamber. Densitometric evaluation at 304 nm. The method was linear in the range of 20-250 ng/µL for both compounds. Recovery was between 100.6 and 101.2 %. The method is suitable for routine quality control.

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

102 126 M. PAL*, Rajashree GUDE, Swati BHENDE (*PES‘s College of Pharmacy Education and Research, Ponda, Goa, India): Development and validation of sensitive method for the quantitative analysis of glibenclamide, rosiglitazone maleate and metformin hydrochloride in an anti-diabetic combination by HPTLC. 60th Indian Pharmaceutical Congress PA-207, (2008). HPTLC of gli-

benclamide, rosiglitazone and metformin in combined dosage form on silica gel with methanol - tetrahydrofuran - water - acetate acid 40:9:10:1 with chamber saturation for 10 min. The hRf values were 56, 60, and 80 for glibenclamide, rosiglitazone, and metformin respectively. Quantitative determination by absorbance measurement at 245 nm. The method was linear in the range of 200-1000 ng/zone (glibenclamide, rosiglitazone) and 120-600 ng/zone (metformin). Recovery was 0.97-99.3 % for all the three compounds. The method was suitable for simultaneous estimation of all three compounds in dosage form.

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

102 127 M.B. PATEL*, K.M. PATEL, G.S. PATEL, B.N. SUHAGIA, A.M. PRAJAPATI (*Nootan Pharmacy College , 17, Kadam Tenament, Opp. Nutan School Ghatlodia, Ahmedabad, Gujarat, India; mandev68@yahoo.com): Development and validation of a stability indicating HPTLC-densitometric method for satranidazole. J. Liq. Chromatogr. Relat. Technol. 30, 2755-2767 (2007). HPTLC of satranidazole (3-(1-methyl-5-nitroimidazol-2-yl)-1-(methylsulfonyl)imidazolidin-2-one) on silica gel (prewashed with methanol) with toluene - acetonitrile 3:2 in a twin-trough chamber saturated for 30 min at 25 °C. Quantitative determination by absorbance measurement at 314 nm. The method can effectively separate the drug from its degradation products and is therefore suitable for stability studies.

quality control, densitometry, quantitative analysis, HPTLC 32a

102 128 R.B. PATEL*, M.B. SHANKAR, M.R. PATEL, K.K. BHATT (*Sardar Patel University, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, PO Box No. 19, Vallabh Vidyanagar 388120, Gujarat, India; rashmru@gmail.com): Simultaneous estimation of acetylsalicylic acid and clopidogrel bisulfate in pure powder and tablet formulations by high-performance column liquid chromatofgraphy and high-performance thin-layer chromatography. J. AOAC Int. 91, 750-755 (2008). HPTLC of acetylsalicylic acid and clopidogrel bisulfate ((alphaS)-alpha-(chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5-(4H)-acetic acid methyl ester) on silica gel using ethyl acetate - methanol - toluene - acetic acid 50:10:40:1. Quantitative determination by absorbance measurement at 235 nm.

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

102 129 T. PATEL*, S. PATEL, N. PATEL, S. PATEL, S. SHELADIA (* Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mehesan, Gujarat, India): Simultaneous estimation of fluoxetine HCl and olanzapine by HPTLC method in pharmaceutical formulations. 60th Indian Pharmaceutical Congress PA-200, (2008). HPTLC of fluoxetine HCl and olanzapine on silica gel with acetone - methanol - triethylamine 10:6:1. Quantitative determination by absorbance measurement at 235 nm. The method was linear in the range of 300-1000 ng/spot for fluoxetine HCl and 50-500 ng/spot for olanzapine respectively. The method was suitable for quality control of combined dosage form.

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

102 130 A. PETRUCZYNIK, M. BRONCZYK, T. TUZIMSKI, Monika WAKSMUNDZKA-HAJNOS* (*Department of Inorganic Chemistry, Medical University of Lublin, 20-081, Lublin, Poland; monika.hajnos@am.lublin.pl): Analysis of selected anti-depressive drugs by high performance thin-layer chromatography. J. Liq. Chromatogr. Relat. Technol. 31, 1913-1924 (2008). TLC of eleven anti-depressive drugs (amitriptyline, doxepin, amizepin, chlorpromazine, clomipramine, flupentixol, haloperidole, moclobemide, perazine, risperidone, venlafaxine) on silica gel, RP-18 and cyano phased in a horizontal chamber with non-aqueous mobile phases containing of polar modifier - methanol, medium polar diluent - diisopropyl ether and aqueous ammonia or diethylamine. The best results were obtained with addition of ammonia. Detection under UV light and by videodensitometry.

HPTLC, quantitative analysis 32a

102 133 S. RANHER*, V. RAJMANE, S. GANDHI, K. BOTHARA (*A.I.S.S.M.S. College of Phamacy, Pune, Maharashtra, India): Simultaneous HPTLC determination of nabumetone and paracetamol in combined dosage form. 60th Indian Pharmaceutical Congress PA-203, (2008). HPTLC of paracetamol and nabumetone in combined tablet dosage form on silica gel with toluene - isopropyl alcohol - acetic acid 80:20:1. Quantitative determination by absorbance measurement at 236 nm. The method was linear in the range of 50-250 ng/spot for both compounds. The method was suitable for routine quality control of tablet dosage form.

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

102 134 L. RAO (Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, A.P., India): HPTLC method for the estimation of simvastatin in bulk and tablet dosage form. 60th Indian Pharmaceutical Congress PA-198, (2008). HPTLC of simvastatin on silica gel with toluene - ethyl acetate - formic acid 16:3:1. Quantitative determination by absorbance measurement at 242 nm. The method was linear in the range of 200-1000 ng/spot and suitable for routine quality control of bulk drug and its dosage forms.

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

102 136 P. RAVAL*, Manisha PURANIK, S. WADHERA, P. YEOLE (*P.G. Dept. of Q.A., Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha 42001, India): A validated HPTLC method for determination of ondansetron in combination with omeprazole or rabeprazole in solid dosage form. Ind. J. Pharm. Sci. 70(3), 386-390 (2008). HPTLC for the simultaneous estimation of ondansetron combinations in solid dosage form with omeprazole and rabeprazole, on silica gel with dichloromethane - methanol 9:1. Quantitative determination by absorbance measurement at 309 nm for combinations of ondansetron with omeprazole and at 294 nm for ondansetron with rabeprazole. The hR_f value of ondansetron and omeprazole was 42 and 54, respectively, while for ondansetron and rabeprazole hR_f values were 41 and 51 respectively. Linearity was between 100 and 500 ng/spot for three drugs. The method can be employed for routine quality control of such formulation.

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

102 137 T.S. REDDY, P.S. DEVI* (*Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka Hyderabad 500007, India; sitadevi@iictuet.org): Simultaneous determination of mirtazapine and its three main impurities by a high performance thin layer chromatography/densitometry method. J. Liq. Chromatogr. Relat. Technol. 31, 1204-1212 (2008). HPTLC of mirtazapine (1,2,3,4,10,14b-hexahydro-2-methyl-pyrazino[2,3-c][2-benzazepine]), and three impurities (2-(4-methyl-2-phenyl-piperazin-1-yl)nicotinic acid, [2-(4-methyl-2-phenyl-piperazinyl)-pyridin-3-yl]methanol, and 2-chloronicotinic acid on silica gel with toluene - acetone - methanol 6:2:2 with chamber saturation. Quantitative determination by absorbance measurement at 285 nm. The limit of detection and quantification for mirtazapine was 22 and 75 ng/spot, respectively.quality

control, quantitative analysis, densitometry, HPTLC 32a

102 138 C. ROOSEWELT*, A. MAGESH, A. Sheela REKHA, P. PANDIAN, V. GUNASEKARAN (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Old Pallavaram, Chennai 600117, India, mpharmroosewelt@yahoo.co.in): Simultaneous estimation and validation of esomeprazole and domperidone by HPTLC in pure and pharmaceutnal dosage forms. Asian J. Chem. 19(4), 2955-2960 (2007). TLC of esomeprazole and domperidone on silica gel with chloroform - acetonitrile - ammonia 20:40:1. Quantitative determination by absorbance measurement at 222 nm. The hR_f value was 76 for esomeprazole and 89 for domperidone. Linearity was between 600 and

1400 µg/mL for domperidone and 1200 and 2800 µg/mL for esomeprazole. Recovery (by standard addition method) was 99.6 %. The proposed method is precise, accurate and can be used for routine analysis of esomeprazole and domperidone in tablets.

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

102 139 C. ROOSEWELT*, N. HARIKRISHNAN, P. MUTHUPRASANNA, P. SHANMUGAPANDIYAN, V. GUNASEKARAN (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Pallavaram, Chennai 600117, India, kalavaivgs30@rediffmail.com): Validated high performance thin layer chromatography method for simultaneous estimation of rofecoxib and tizanidine hydrochloride in pure and tablet dosage forms. Asian J. Chem. 19(6), 4286 - 4290 (2008). HPTLC of rofecoxib and tizanidine hydrochloride on silica gel with acetone - methanol 1:1. The method was linear in the range of 2200-3300 ng/spot and 180-260 ng/spot for rofecoxib and tizanidine respectively. The recovery was between 99.7 and 102.6 % for both compounds. The method was useful for the simultaneous estimation of the drug content in pure and tablet dosage form.

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

102 140 M. SAJEWICZ, M. GONTARSKA, A. DABROWA, Teresa KOWALSKA* (*Institute of Chemistry, Silesian University, 9, Szkolna Street, 40-006 Katowice, Poland; kowalska@us.edu.pl): Use of video densitometry and scanning densitometry to study an impact of silica gel and L-arginine on the retention of ibuprofen and naproxen in TLC systems. J. Liq. Chromatogr. Relat. Technol. 30, 2369-2383 (2007). TLC of 2-arylpropionic acids, namely ibuprofen and naproxen, on silica gel and silica gel impregnated with a 0.03 mol/L solution of L-arginine in methanol by dipping for 2 s, with ethanol and ethanol containing several drops of acetic acid. Quantitative determination by absorbance measurement at 254 nm. It was demonstrated that the crystalline chirality of the silica gel adsorbent is most probably responsible for the horizontal enantioseparation, whereas the molecular chirality of L-arginine deposited on the silica gel layer is responsible for the vertical enantioseparation.

densitometry, qualitative identification 32a

102 142 V.L. SATHIYANARAYANAN*, A. KHEDKAR, J. RAO, S. YADAV (*Poona College of Pharmacy, Bharati Vidyapeeth, Pune, India): Development of stability-indicating HPTLC method for the determination of trandolapril in bulk drug and pharmaceutical dosage form. 60th Indian Pharmaceutical Congress PA-195, (2008). HPTLC of trandolapril on silica gel with toluene - ethyl acetate - methanol - formic acid 5:16:2:1. The hR_f value of trandolapril was 51. Trandolapril was subjected to different stress conditions like acidic and alkaline hydrolysis, oxidation, dry heat, wet heat, neutral condition and photodegradation. The degradation products were well resolved from the pure drug. Quantitative determination by absorbance measurement at 220 nm. Linearity was between 300-1800 ng/spot. The method was suitable for routine analysis of the drug in bulk and pharmaceutical dosage form.

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

102 143 C. SHAH*, B. SUHAGIA, N. SHAH, D. PATEL, & N. PATEL (*Shri B. M. Shah College Pharma. Edu. & Res., Dept. of Q.A., College Campus, Modasa-383315, India, crshah681@yahoo.com): Stability-indicating simultaneous HPTLC method for olanzapine and fluoxetine in combined tablet dosage form. Ind. J. Pharm. Sci. 70(2), 251-255 (2008). HPTLC of olanzapine and fluoxetine on silica gel with methanol - toluene 2:1. Quantitative determination by absorbance measurement at 233 nm. The method was linear in the range of 100-800 ng/spot for olanzapine and 1000-1200 ng/spot for fluoxetine. Recovery was 99.4-100.4 % for both compounds. Forced degradation studies (acid, base, oxidation, photolyses and thermal) revealed that all the degradation products were well resolved from the principal compound. The method was suitable for routine quality control.

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

102 144 N. SHAH*, B. SUHAGIA, R. SHAH, N. PATEL (*Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa 383315, India): Development and validation of a simultaneous HPTLC method for the estimation of omesartan medoxomil and hydrochlorothiazide in tablet dosage form. Ind. J. Pharm. Sci. 69 (6), 834-836 (2007). HPTLC of olmesartan medoxomil and hydrochlorothiazide on silica gel with acetonitrile - chloroform - acetic acid 14:4:1. Quantitative determination by absorbance measurement at 254 nm. The calibration curve was linear between 500 and 750 ng/spot for olmesartan medoxomil and between 100 and 600 ng/spot for hydrochlorothiazide. The limit of detection and quantification for olmesartan medoxomil was 170 and 500 ng/spot, and for hydrochlorothiazide 30 and 100 ng/spot. The proposed method can be successfully used to determine the drug content of marketed tablet formulation.

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

102 145 F. SHAH*, Sanjula BABOOTA, J. ALI, Alka AHUJA (*Jamia Hamdard, Faculty of Pharmacy, New Delhi, India): Stability indicating HPTLC determination of carvedilol as bulk drug and from solid nanoparticles. 60th Indian Pharmaceutical Congress PA-87, (2008). HPTLC of carvedilol on silica gel with toluene - chloroform - methanol - acetic acid 20:20:10:1. Densitometric evaluation at 240 nm. The method was linear in the range of 50-1000 ng/spot. The method could effectively separate the drug from its degradation products (acid, base, oxidaline, photodegradation). The kinetic studies confirmed that the drug is more degraded in alkaline medium than in acidic medium.

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

102 147 S. SHELADIA*, S. PATEL, N. PATEL, S. PATEL, T. PATEL (*Shree S. K. Patel College of Pharmaceutical Education and Research, Ganapat University, Mehsana, Gujarat, India): Simultaneous estimation of trifluoperazine HCl and chlordiazepoxide by HPTLC method in pharmaceutical formulation. 60th Indian Pharmaceutical Congress PA-199, (2008). HPTLC of trifluoperazine and hydrochloride and chlordiazepoxide on silica gel with carbon tetrachloride - acetone - triethylamine 12:6:1. Quantitative determination by absorbance measurement at 262 nm. The method was linear in the range of 20-280 ng/spot and 50-700 ng/spot for trifluoperazine HCI and chlordiazepoxide. The method was suitable for routine quality control of combined dosage form.

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

102 148 K. SHIVSHANKER*, N. SREEKANTH, N. HARIKRISHNAN, C. ROOSEWELT, P. PANDIYAN, G. RAO, V. GUNASEKARAN (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Old Pallavaram, Chennai 600117, India, kalavaivgs30@rediffmail.com): Simultaneous estimation and validation of simvastatin and ezetimibe by HPTLC in pure and pharmaceutical dosage form. Asian J. Chem. 19(5), 3627-3632 (2007). TLC of simvastatin and ezetimibe on silica gel with ethyl acetate - chloroform 4:1. Quantitative determination by absorbance measurement at 220 nm. The hRf value of simvastatin was 76 and of ezetimibe 89. Linearity was between 600 and 1400 µg/mL for simvastatin and ezetimibe. The recovery (by standard addition method) was in the range of 99.7 and 99.6 % for both drugs. The proposed method is precise, accurate and can be used for routine analysis of simvastatin and ezetimibe in tablets.

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

102 149 A. SINGH*, P. OM, R. SINGH, G. SINGH, B. GANESH (*Central Indian Pharmacopoeia Laboratory, Research & Development Div., Indian Pharmacopoeia Commission, Govt. of India, Mi-

nistry of Health & Family Welfare, Sector-23, Rajnagar, Ghaziabad, India): Development and validation of a HPTLC method for the estimation of trandolapril in tablet dosage form. 60th Indian Phamaceutical Congress PA-196, (2008). HPTLC of trandolapril on silica gel with chloroform - methanol - acetic acid 16:3:1 in a saturated chamber. Quantitative determination by absorbance measurement at 210 nm. Linearity was in the range of 200-1000 ng/spot. The method was suitable for routine quality control of drug in tablet dosage form.

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

102 150 S. SONAWANE*, A. SHIRKHEDKAR, S. SURANA (*Dept. of Pharmaceutical Chemistry, R.C. Patel College of Pharmacy Karwad Naka, Shirpur 425405, India, atul_shirkhedkar@yahoo.com): HPTLC method for determination of ezetimibe in tablets. Asian J. Chem. 19(6), 4925 - 4927 (2007). TLC of ezetimibe on silica gel with toluene - acetone 3:2. Quantitative determination by absorbance measurement at 233 nm. The hRf value was 52 for ezetimibe. Linearity was in the range of 300 to 2100 ng/spot. Recovery (by standard addition method) was 99-101 %.

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

102 152 N. SREEKANTH*, K. SHIVSHANKER, P. PANDIYAN, C. ROOSEWELT, G. RAO, V. GUNASAEKARAN (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Old Pallavaram, Chennai 600117, India, kalavaivgs30@rediffmail.com): Simultaneous determination and validation of ornidazole and cefixime by HPTLC in pure and pharmaceutical dosage forms. Asian J. Chem. 19(5), 3621-3636 (2007). TLC of ornidazole and cefixime on silica gel with methanol - water 3:2. Quantitative determination by absorbance measurement at 254 nm. Linearity was between 250 and 2500 µg/mL for ornidazole and 100 and 900 µg/mL for cefixime. The recoveries of drugs by standard addition method were found in the range of 98.0 and 89.4 % for both drugs. The method is suitable for routine quality control.

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

102 154 M. STOLARCZYK, M. ANNA, J. KRZEK* (*Department of Inorganic and Analytical Chemistry, Jagiellonian University, Collegium Medicum, 9 Medyczna Street, 30-688 Cracow, Poland; jankrzek@cm-uj.krakow.pl): Chromatographic and densitometric analysis of hydrochlorothiazide, walsartan, kandesartan, and enalapril in selected complex hypotensive drugs. J. Liq. Chromatogr. Relat. Technol. 31, 1892-1902 (2008). HPTLC of hydrochlorothiazide, walsartan, kandesartan, and enalapril on silica gel after chamber saturation using ethyl acetate - tetrahydrofuran - acetic acid 16:4:1 (for kandesartan and walsartan present together with hydrochlorothiazide) and 1-butanol - acetic acid - water 12:3:5 (for enalapril and hydrochlorothiazide). Quantitative determination by absorbance measurement at 252 nm for walsartan and kandesartan, at 274 nm for hydrochlorothiazide and at 208 nm for enalapril. The method was of high sensitivity and specific to analyte constituents.

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

102 155 A. SUGANTHI*, Sofiya JOHN, T. RAVI (*Dept. of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641044, India): Simultaneous HPTLC determination of rabeprazole and itopride hydrochloride from their combined dosage form. Ind. J. Pharm. Sci. 70(3), 366-368 (2008). TLC of rabeprazole and itopride hydrochloride in tablets on silica gel with n-butanol - toluene - ammonia 17:1:2. Quantitative determination by absorbance measurement at 288 nm. The hR_f value of rabeprazole was 23 and of itopride hydrochloride 75. Linearity was found to be in the range of 40 - 200 ng/spot for rabeprazole and 300-1500 ng/spot for itopride hydrochloride. The limit of detection and quantification for rabeprazole was 10 and 20 ng/spot and for itopride hydrochloride 50 and 100 ng/spot, respectively. The method is suitable for simultaneous anlysis of both the drugs in dosage form.

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

102 156 K. SURENDRA*, S. MAGESWARI, R. MAHESWARI, N. HARIKRISHNAN, C. ROOSEWELT, V. GUNASEKARAN (*Dept. of Pharmaceutical Analysis, Vel's College of Pharmacy, Chennai 6000117, India, kalavaivgs30@rediffmail.com): Simultaneous estimation of levofloxacin hemihydrate and ornidazole in tablet dosage form by HPTLC. Asian J. Chem. 19(7), 5647-5651 (2007). HPTLC of levofloxacin hemihydrate and ornidazole on silica gel with n-butanol - water - acetic acid 3:1:1. Quantitative determination by absorbance measurement at 366 nm. The method was linear in the range of 1050-1400 µg/mL and 2600-2900 µg/mL for levofloxacin and ornidazole respectively. The recovery was between 97.3 and 98.0 % for both drugs. The method was suitable for simultaneous estimation of both drugs in combined tablet dosage form.

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

102 157 J. SUSHEEL*, M. LEKHA, T. RAVI (*Dept. of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641044, India): High performance thin layer chromatographic estimation of lansoprazole and domperidone in tablets. Ind. J. Pharm. Sci. 69 (5), 689 - 686 (2007). A simple, fast precise and accurate HPTLC method has been developed for the simultaneous estimation of lansoprazole and domperidone in tablet formulations. This method allows the determination of 100-500 ng/spot of lansoprazole and 100-500 ng/spot of domperidone. HPTLC on silica gel with n-butanol - acetic acid - water 36:12:1:2. Quantitative determination by absorbance measurement at 288 nm. The hRf value of lansoprazole was 78 and of domperidone 21. The limit of detection and quantification for lansoprazole was 10 ng/spot and 40 ng/spot, and for domperidone 30 ng/spot and 65 ng/spot, respectivey. The method was suitable for routine quality control.

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

102 158 A. VORA*, R. DAREKAR, R. MAHENDRE, Mrinalini DAMALE (*AISSMS College of Pharmacy, Dept. of Pharmaceutical Chemistry, Kennedy Road, Pune 411001, India, mrunal.damale@rediffmail.com): Validated spectrodensitometric method for simultaneous determination of lumefantrine and artemether. J. Pharm. Res. (7)4, 229-232 (2008). HPTLC of lumefantrine and artemether on silica gel with toluene - ethyl acetate - formic acid 60:60:7. Quantitative determination by absorbance measurement of lumefantrine at 267 nm and of artemether at 561 nm. The hR_f value for lumefantrine was 54 and of artemether 89. Linearity was in the range of 1200-6000 ng/spot for lumefantrine and 200-1000 ng/spot for artemether. The method was successfully applied to the analysis of commercial formulations.

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

102 159 S. WALODE*, A. MALIYE, K. GUPTA, A. KASTURE, S. WADODKAR, M. TAJNE (*Dept. of Pharmaceutical Science, Nagpur University Campus, Amravati Road, Nagpur 440033, India, sanjuwalode@rediffmail.com): HPTLC method for simultaneous estimation of nimesulide and diclofenac sodium in capsule. Asian J. Chem. 18(2), 1078-1084 (2006). TLC of nimesulide and diclofenac sodium on silica gel with n-hexane - ethyl acetate - chloroform - acetic acid 16:2:3:1. Quantitative determination by absorbance measurement at 256 nm. The hR_f value of nimesulide was 3 and of diclofenac sodium 57. Linearity was between 1.0 and 3.2 µg/zone for nimesulide and 0.5 and 1.6 µg/zone for diclofenac sodium. The recoveries (by standard addition method) were in the range of 99.1 and 101.0 for both drugs. The proposed method is precise and accurate and can be used for routine analysis of nimesulide and diclofenac sodium capsule formulation.

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

102 160 S. WANKHEDE*, K. GUPTA, M. TAJNE, S. WADODKAR (*Dept. of Pharmaceutical Science, Nagpur Univeristy Campus, Amravati Rd., Nagpur 440033, India, sagar_277@rediffmail.com): A validated HPTLC method for simultaneous estimation of lamivudine and zidovudine in tablets. Asian J. Chem. 18(4), 2669-2672 (2006). TLC of lamivudine and zidovudine on silica gel with toluene - methanol - n-hexane 14:3:2. Quantitative determination by absorbance measurement at 275 nm. Linearity for lamivudine and zidovudine was in the range of 0.8-2.0 and 1.5-4.0 µg/zone, respectively. The average recovery was 99.4 - 100.3 % for both drugs. The method can be applied for routine simultaneous estimation in combined dosage form.

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

102 103 A. GOEL*, F. AHMAD, R. SINGH, R. GOEL, G. SINGH (*Indian Pharmacopeia Commission, Central Indian Pharmacopoeia Laboratory, Raj Nagar, Ghaziabad, U.P., India): Simultaneous determination of 3-acetyl-11-keto-beta-boswellic acid and 6-gingerol by HPTLC. 60th Indian Pharmaceutical Congress PA-202 (2008). HPTLC of 6-gingerol and 3-acetyl-11-keto-beta-boswellic acid on silica gel with n-hexane - ethyl actate 7:3 in a chamber saturated at ambient temperature. Quantitative determination by absorbance measurement at 254 nm. The hR_f values were 48 and 58 for 3-acetyl-11-keto-beta-boswellic acid and 6-gingerol respectively. The recovery was 98.7-100.8 % for both compounds. The chromatographic conditions were suitable for routine analysis.

pharmaceutical research, HPTLC, densitometry, quantitative analysis 32c

102 107 P. HAMRAPURKAR*, M.CHACHAD, H. MENGHANI, K. KAMAT (*Prin. K. M. Kundnani College of Pharmacy, Mumbai, India): Extraction and quantification of Eclipta alba from raw material using hi-tech sophisticated instruments. 60th Indian Pharmaceutical Congress PA-82, (2008). HPTLC of wedelolactone in supercritical fluid extrats of Eclipta alba Hassk. on silica gel with toluene - ethyl acetate - formic acid 50:50:1. Densitometric evaluation at 240 nm. The method is linear in the range of 100-1000 ng/spot. The method was used for estimation of wedelolactone in the raw material obtain from different regions of the country (India).

herbal, HPTLC, densitometry, quantitative analysis 32c

102 141 A. SARASWATHY*, R. SHAKILA (*CSM Drug Research Institute for Ayurveda, Anna Hospital Campus, Arumbakkam, Chennai 600106, India, saraswathy20042000@yahoo.co.in): Quantitative estimation of geranial and luteolin from Cymbopogon citratus (DC.) Stapf leaf using HPTLC. Indian Drugs 45(8), 663-666 (2008). HPTLC of geranial and luteolin from leaves of Cymbopogon citratus on silica gel with toluene - ethyl acetate 9:1 for geranial and toluene - ethyl acetate - formic acid 10:7:1 for luteolin. Densitometric evaluation at 200 nm (geranial) and 254 nm (luteolin). Alcoholic extracts of the plant leaves were found to contain 1.34 % and 1.49 % of geranial and luteolin respectively.

herbal, HPTLC, densitometry 32c

102 146 D. SHANBHAG, Sunita JAYARAMAN (*D. G. Ruparal College, Dept. of Chemistry, Mahim, Mumbai 400016, India, sunita_75in@yahoo.com): Application of HPTLC in standardization of homoeopathic mother tincture Andrographis paniculata and its comparison with market products. Asian J. Chem. 1, 509-513 (2008). HPTLC of andrographolide in Andrographis paniculata homoeopathic mother tincture on silica gel with chloroform - methanol 7:1. Quantitative determination by absorbance measurement at 225 nm. The standard mother tincture was found to contain 21.9 mg/100 mL andrographolide, where as 6 commercial samples contained varying amounts of andrographolide ranging from 4.89-54.62 mg/100 mL.

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

102 151 F. SOPONAR*, A. CATALIN MOT, C. SARBU (*Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Arany Janos Str. no. 11, 400028 Cluj-Napoca, Romania): Quantitative evaluation of paracetamol and caffeine from pharmaceutical preparations using image

analysis and RP-TLC. Chromatographia 69 (1-2), 151-155 (2009). HPTLC of paracetamol and caffeine on RP18W with methanol - acetic acid - water 250:43:707. Quantification by absorbance measurement at 254 nm. The limit of detection and limit of quantitation for paracetamol was 100 and 191 ng/spot and 40 and 76 ng/spot for caffeine. Recovery was in the range of 99.6 - 106.8 % and the repeatability of the method was RSD < 1.9 %.

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

102 091 Anita ANKLI*, E. REICH, M. STEINER (*CAMAG Laboratory, Sonnenmattstr. 11, 4132 Muttenz, Switzerland; anita.ankli@camag.com): Rapid high-performance thin-layer chromatographic method for detection of 5% adulteration of black cohosh with Cimicifuga foetida, C. heracleifolia, C. dahurica, or C. americana. J. AOAC Int. 91, 1257-1264 (2008). HPTLC of black cohosh extracts and references cimifugin, 23-epi-26-deoxyactein, actein on silica gel with toluene - ethyl formate - formic acid 5:3:2 in twin-trough chamber saturated for 20 min. HPTLC plates were conditioned to 5 % relative humidity or less. For identification of species derivatization by dipping for 1 s in sulfuric acid reagent (10 % in methanol) followed by heating at 100 °C for 5 min and evaluation under white light and UV 366 nm. For detection of adulteration with Cimicifuga foetida derivatization with boric acid/oxalic acid reagent, for detection of adulteration with C. heracleifolia and C. dahurica derivatization with antimony(III) chloride reagent. Evaluation under UV 254 nm and after derivatization under UV 366 nm and white light. The method allows visual detection of 5 % of these adulterants in Cimicifuga racemosa.

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

102 095 S. CHATTERJEE, A. ANANTHAKUMAR, P. VARIYAR*, A. SHARMA (*Food Technology Division Bhabha Atomic Research Center, Trombay, Mumbai, India, prasadpsv@rediffmail.com): Identification and estimation of a novel fluorescent compound in nutmeg. J. Food Comp. Anal. 21, 577-581 (2008). HPTLC of a novel fluorescent compound of nutmeg (Myristica fragrans) on silica gel with hexane - diethyl eter - acetic acid 50:50:1. Detection under UV at 265 nm or by spraying with sulphuric acid 50 % followed by heating at 180 °C. The major fluorescent band at hR_f 63 was further purified on silica gel using dioxane - acetonitrile - acetic acid 70:30:1. The hR_f value of the novel compound was 61. Quantitative determination by absorbance measurement at 376 nm. Linearity was between 1 to 50 µg/spot. The compound was identified as 2-methyl-1,4,4a,8a-tetrahydro-endo-1,4-methanonaphthalene-5,8-dione.

food analysis, herbal, HPTLC, quantitative analysis, densitometry 32e

102 099 S. DHUMAL*, S. KULKARNI (*The Bombay College of Pharmacy, Dept. of Pharmacognosy and Phytochemistry, Sunder Nagar, Kalina, Santacruz (E), Mumbai 400098, India, svt_kulkarni@yahoo.co.in): Antibacterial and wound healing activity of roots of Sesamum indicum. Indian Drugs 44(12), 937-944 (2007). HPTLC of the methanolic extract of Sesamum indicum root (Pedaliaceae) and its ethyl acetate fraction on silica gel with ethyl acetate - n-hexane 1:9. Absorbance measurement at 549 nm. The red zone was isolated by preparative TLC and identified by IR to be a 1,4-naphthoquinone derivative.

herbal, HPTLC, densitometry, comparison of methods 32e

102 100 Francesca GALLO*, G. MULTARI, M. GIAMBENEDETTI, E. FEDERICI (*Dipartimento del Farmaco, Istituto Superiore di Sanita, Roma, Italy, Francesca.gallo@iss.it): Chemical fingerprinting of Lawsonia inermis L. using HPLC, HPTLC and densitometry. Phytochem. Anal. 19, 550-559 (2008). HPTLC of the leaves of Lawsonia inermis L., on silica gel with ethyl acetate - formic acid - water 82:9:9 followed by drying at 110 °C for 15 min. Detection by spraying with diphenylborinic acid aminoethylester 0.5 % in ethyl acetate, followed by drying and dipping into macrogol reagent (1 g polyethylene glycol 400 in 20 mL dichloromethane). Quantitative determination by absorbance measurement at 337 nm. Chemical fingerprint was used for quality evaluation of herbal products and detection of adulteration. Comparison with an HPLC method gave comparable results.

herbal, quality control, HPTLC, quantitative analysis, densitometry, comparison of methods 32e

102 111 J. KALOLA, S. ANANDJIWALA, H. SRINAVASA, M. RAJANI* (*B. V. Patel Pharmaceutical Education and Research Development Center, Pharmacognosy and Phytochemistry Department, Thaltej, Ahemdabad 380 054, Gujarat, India; rajanivenkat@hotmail.com): Effect of hydrolysis on the yield of hederagenin and high-performance thin-layer chromatography densitometric quantification of hederagenin in fruit pericarp of Sapindus spp. J. AOAC Int. 91, 1174-1178 (2008). HPTLC of hederagenin on silica gel with toluene - ethyl acetate - formic acid 7:3:5 in a twin-trough chamber. Detection with anisaldehyde - sulfuric acid reagent by dipping for 1 s and heating for 7 min at 100 °C. Quantitative determination by absorbance measurement at 595 nm.

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

102 120 F. MELIANITA, S. CHOLIFAH, E. SUMARLIK, W. F. KARTINASARI, G. INDRAYANTO* (*Faculty of Pharmacy, Assessment Service Unit, Airlangga University, Surabaya, Jl. Dharmawangsa dalam, Surabaya 60286, Indonesia; gunawanindrayanto@yahoo.com): Simultaneous densitometric determination of 6-gingerol and 6-shogaol in some commercial gingers (Zingiber officinale Roscoe). J. Liq. Chromatogr. Relat. Technol. 30, 2941-2951 (2007). TLC of 6-gingerol and 6-shogaol in commercial Ginger on silica gel with n-hexane - diethyl ether 2:3. Detection by spraying with anisaldehyde - sulfuric acid reagent. Evaluation under white light and quantitative determination by absorbance measurement at 577 nm.

herbal, food analysis, quantitative analysis, densitometry 32e

102 123 K. MURTHY*, S. MISHRA (*Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, The M. S. University of Baroda, Vadodara, 390 001, Gujarat, India): TLC determination of betulinic acid from Nymphodies macrospermum: a new botanical source for tagara. Chromatographia 68 (9-10), 877-880 (2008). TLC of betulinic acid in Nymphoides macrospermum on silica gel with hexane - ethyl acetate - acetic acid 700:300:3. Detection by spraying with anisaldehyde-sulphuric acid reagent. Quantification by absorbance measurement at 540 nm. Linearity was in the concentration range of 100-600 ng/spot. The method is suitable for the routine quality control of Granthika Tagara.

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

102 131 W. POTHITIRAT, W. GRITSANAPAN* (*Mahidol University, Department of Pharmacognosy, Faculty of Pharmacy, 447 Sri-Ayudhaya Rd, Ratchatewi, Bangkok 10400, Thailand; pywgs@mahidol.ac.th): Thin-layer chromatography-densitometric analysis of alpha-mangostin content in Garcina mangostana fruit rind extracts. J. AOAC Int. 91, 1145-1148 (2008). TLC of alpha-mangostin on silica gel with dichloromethane - methanol 24:1 in a saturated twin-trough chamber. Quantitative determination by absorbance measurement at 320 nm.

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

102 132 H. PULPATI, Y.S. BIRADAR, M. RAJANI* (*B. V. Patel Pharmaceutical Education and Research Development Center, Pharmacognosy and Phytochemistry Department, Thaltej-Gandhinagar Highway, Thaltej, Ahemdabad 380 054, Gujarat, India; rajanivenkat@hotmail.com): High-performance thin-layer chromatography densitometric method for the quantification of harmine, harmaline, vasicine, and vasicinone in Peganum harmale. J. AOAC Int. 91, 1179-1185 (2008). HPTLC of harmine, harmaline, vasicine, and vasicinone on silica gel with ethyl acetate - methanol - ammonia 70:10:3 in a twin-trough chamber at 25 °C and 40 % relative humidity. Quantitative determination by absorbance measurement at 366 nm for harmine and harmaline, at 292 nm for vasicine, and at 233 nm for vasicinone.

traditional medicine, herbal, quality control, HPTLC, densitometry, quantitative analysis 32e, 22

102 135 S. RASTOGI*, M.M. PANDEY, A.K.S. RAWAT (*National Botanical Research Institute, Pharmacognosy and Ethnopharmacology Division, Lucknow, 226 001, India; subharastogil@rediffmail.com): High-performance thin-layer chromatography densitometric method for the simultaneous determination of three phenolic acids in Syzygium aromaticum (L.) Merr. & Perry. J. AOAC Int. 91, 1169-1173 (2008). HPTLC of gallic acid, caffeic acid, and syringic acid (in clove) on silica gel with toluene - ethyl acetate - formic acid 8:2:1 in a twin-trough chamber. Quantitative determination by absorbance measurement at 280 nm.

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

102 153 P. SRIVASTAVA, N. TIWARI, A.K. YADAV, V. KUMAR, K. SHANKER, R.K. VERMA, M. M. GUPTA*, A.K. GUPTA, S.P.S. KHANUJA (*Central Institute of Medicinal and Aromatic Plants, Analytical Chemistry Division, Lucknow 226015, India; guptammg@rediffmail.com): Simultaneous quantification of withanolides in Withania somnifera by a validated high-performance thin-layer method. J. AOAC Int. 91, 1154-1161 (2008). HPTLC of 3 key whithanolides, namely withaferin-A, 12-deoxywithastramonolide, and withanolide-A on silica gel with dichloromethane - methanol - acetone - diethylether 15:1:1:1 in a saturated twin-trough chamber at 25 °C and relative humidity of 35-40 %. Quantitative determination by absorbance measurement at 230 nm. Detection by immersion in freshly prepared vanillin-sulfuric acid reagent for 2 s followed by heating at 110 °C for 10 min.

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

102 046 Valeria WIDMER et al., see section 15a

102 109 N. JEGANATHAN*, K. KANNAN (*Annamalai University, Dept. of Pharmacy, Nagar 608002 (T.N.), India): Simultaneous estimation of piperine and sennoside A in Nilavakai Curanam by HPTLC method. Ind. J. Pharma Educ. Res. 42(1), 59-64 (2008). Curanams are an important group of formulations in ayurvedic and siddha medicine. HPTLC of sennoside A and piperine in methanolic and ethyl acetate extracts of Nilavakai Curanam on silica gel with n-hexane - ethyl acetate - formic acid - acetic acid 15:5:1:1. Quantitative determination by absorbance measurement at 254 nm. The methanolic extract of the laboratory formulation contained 0.61 % and 1.4 % piperine and sennoside A respectively, whereas the ethyl acetate extract contained 1.0 % and 4.2 %. The methanolic extract of the commercial formulation contained 0.27 % and 0.42 % and the ethyl acetate extract contained 0.27 and 0.53 % respectively. The method was linear in the range of 15-105 ng/spot and 5-30 ng/spot for piperine and sennoside A respectively. The recovery was 98.5 % for both compounds.

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

102 113 K. LADHA*, R. KASAR, J. CHAUDHARY, A.SHUKLA (*Medical Natural Products Research Lab., Pharmaceutical Div., A-282, University Institute of Chemistry Technology, University of Mumbai, Mantunga Mumbai 400019, India, ksladha@udct.org): A HPTLC densitometric determination of antioxidant consituents from chyawanprash. Indian Drugs 45 (7), 536 - 541 (2008). Chyawanprash contains many phytoconstituents, out of which gallic acid, catechin, epicatechin are considered to be responsible for the antioxidant activity and piperine is responsible for the bioavailability enhancing effect. HPTLC with toluene - ethyl acetate - formic acid - ethanol 60:40:3:4. Quantitative determination by absorbance measurement at 254 nm. The hRf values of epicatechin, catechin, gallic acid, and piperine were 13, 32, 44, and 82 respectively. Recovery was 99.0 % for epicatechin, 96.1 % for catechin, 102.5 % for gallic acid and 100.6 % for piperine in Chyawanprash. The results obtained were compared with similar formulations available in the

market employing tests for identification and purity determination. The developed method may be considered as an additional tool for quality control of Chyawanprash.

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

102 069 K.K. ROUT et al., see section 22

33. Inorganic substances

102 002 S. ERGÜL, see section 2c

34. Radioactive and other isotopic compounds

102 161 T. KIM, W. LEITNER, R. ADOCHIO, B. DRAZNIN* (*Department of Medicine, University of Colorado Denver School of Medicine, Mail Stop 8106, Colorado, USA, Boris.Draznin@ucdenver.edu) : Knockdown of JNK rescues 3T3-L1 adipocytes from insulin resistance induced by mitochondrial dysfunction. Biochem. Biophys. Res. Commun. 378, 772-776 (2009). TLC of lipids from adipocytes on silica gel pre-treated with a solution containing methanol - water - potassium oxide - EDTA 60:40:1%:1mM and activated at 100 °C for 1 hour. The plate was developed with n-propanol - water - glacial acetic acid 65:34:1. Detection and quantitative determination by autoradiography using storage phosphor technology.

pharmaceutical research, quantitative analysis, radioscanning 34

35. Other technical products and complex mixtures

102 162 E. DYTKIEWITZ, Gertrud MORLOCK* (*University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany; gmorlock@uni-hohenheim.de): Analytical strategy for rapid identification and quantification of lubricant additives in mineral oil by high-performance thin-layer chromatography with UV absorption and fluorescence detection combined with mass spectrometry and infrared spectroscopy. J. AOAC Int. 91, 1237-1243 (2008). HPTLC of zinc bis(O,O‘-diisobutyl dithiophosphate), zinc bis(O,O‘-didodecyl dithiophosphate), and Aglamol 99 on RP-2 by automated multiple development with methanol - water - acetic acid 6:3:2 for 25 mm, then acetonitrile - water 11:9 for 60 mm, and again acetonitrile - water for 80 mm, or on silica gel with a 14-step gradient based on toluene. For derivatization, the plate was dipped in a solution of 0.05 % primuline in acetone - water 4:1 for 1 s and immediately dried in warm air. Quantitative determination by fluorescence measurement at 366/>400 nm and by absorbance measurement at 220 nm. HPTLC-ATR-IR and HPTLC-FTIR, as well as HPTLC/DART-MS and HPTLC/ESI-MS were applied for identification.

quality control, AMD, HPTLC, densitometry, quantitative analysis 35c

37. Environmental analysis

102 163 S. GHOSAL (Natreon-Inc. CL-18A, Sector II, Salt lake City, Kolkata 700091, India, vishnu20024@rediffmail.com): The signatures of energy-transducing organic molecules in meteorites. Science & Culture Jan.-Feb., 22-30 (2008). Isolation and identification of some unique chemical compounds is reported using chemical, chromatographic and spectroscopic methods such as GC-MS, HPTLC and HPLC. The presence of bio-organic molecules such as oxygenated dibenzo-a-pyrones (DBPs), their amino acyl conjugates (DCPs) and polyphenyl benzoquinones (PBQs) was observed in all the four samples of meteorites. HPTLC on silica gel with 1) n-butanol - acetone - acetic acid - water 7:7:2:4 for amino acids, detection with ninhydrine reagent and absorbance measurement at 610 nm; 2) n-butanol - acetic acid - water 3:1:2 for sugars, detection with p-anisidine reagent and absorbance measurement at 380 nm; 3) chloroform - methanol 9:1 for DBPs and absorbance measurement at 240 nm and 360 nm.

environmental, HPTLC, densitometry, postchromatographic derivatization, qualitative identification 37a

102 164 Gertrud MORLOCK*, Stephanie KOPACZ (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany; gmorlock@uni-hohenheim.de): Fast and precise SBSE-HPTLC/FLD method for quantification of six polycyclic aromatic hydrocarbons frequently found in water. J. Liq. Chromatogr. Relat. Technol. 31, 1925-1942 (2008). HPTLC of benzo[a]pyrene, benzo[b]fluoroanthene, benzo[k]fluoroanthene, benzo[ghi]perylene, fluoroanthene, 2-methylanthracene, and indeno[1,2,3-cd]pyrene on silica gel impregnated with caffeine-solution (by dipping in a solution of 2 g caffeine in 120 mL acetonitrile for 20 min, followed by drying for 15 min at 120 °C) with isopropyl acetate - acetonitrile 7:3 in a twin trough chamber at -20 °C. For fluorescence enhancement the plates were dipped in a solution of paraffin - n-hexane 1:1 and dried for 1 min in cold air. Quantitative determination by fluorescence measurement at 366/>400 nm. The method can be applied for control of the limit levels of the six polycyclic aromatoc hydrocarbons in water. The limits of quantitation were 0.08-0.44 ng/band depending on the substance. Linearity showed coefficients of correlation > 0.9920. The recoveries by stir bar sorptive extraction (n = 3) were between 87-100 % depending on the substance. The whole procedure was optimized to reach a sample throughput of 30 water samples, inclusive sample preparation by stir-bar sorptive extraction (SBSE), per 8-hour day.

environmental, toxicology, HPTLC, quantitative analysis 37c

102 082 T. TUZIMSKI. see section 29d

38. Chiral separation

102 004 R. BHUSHAN et al., see section 3d

102 165 M. SAJEWICZ, G. GRYGIERCZYK, M. GONTARSKA, Teresa KOWALSKA* (*Institut of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland; kowalska@us.edu.pl): Enantioseparation of S,R-(+/-)-ketoprofen on plain silica gel layers with achiral mobile phase. J. Liq. Chromatogr. Relat. Technol. 30, 2185-2192 (2007). TLC of S,R-(+/-)-ketoprofen on silica gel (prewashed with methanol - water 9:1) with acetonitrile - water 5:1 containing several drops of acetic acid. Quantitative determination by absorbance measurement at 252 nm. Three different components of the investigated mixture were found which were again separated two-dimensionally in the one dimensional development mode, i. e., their positions differed in terms of the hR_f values and the respective migration tracks of these three species all deviated to the right. Earlier tests showed that silica gel employed in the planar chromatographic mode enables two-dimensional enantioseparation of the racemic mixtures in the one-dimensional development mode, without using chiral mobile phases. In the present study this effect was investigated with another racemic mixture from the group of profens. It was concluded that the two-dimensional enantioseparation in the one-dimensional planar chromatographic mode on the microcrystalline silica gel layers is a promising option, enhancing the enantioseparative potential of planar chromatography that cannot be challenged by the column liquid chromatography.

quantitative analysis, densitometry 38

102 060 M. SAJEWICZ et al., see section 18a

102 166 M. SAJEWICZ, M. GONTARSKA, M. WRÓBEL, Teresa KOWALSKA* (*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland; kowalska@us.edu.pl): Enantioseparation and oscillatory transenantiomerization of S,R-(+/-)-ketoprofen, as investigated by means of thin layer chromatography with densitometric detection. J. Liq. Chromatogr. Relat. Technol. 30, 2193-2208 (2007). TLC of S,R-(+/-)-ketoprofen on silica gel (prewashed with methanol - water 9:1 and impregnated by dipping in a 0.03 mol/L solution of L-arginine in methanol for 2 s at 22 °C) with acetonitrile - water 5:1 containing several drops of acetic acid (to fix the pH < 4.8) in one-dimensional and two-dimensional development. Quantitative determination by absorbance measurement at 252 nm. The results of the investigations demonstrated the ability of S,R-(+/-)-ketoprofen to undergo oscillatory transenantiomerization.

densitometry, qualitative identification, quantitative analysis 38

10-jähriges Jubiläum des französischen HPTLC-Clubs

19

10-jähriges Jubiläum im Oktober 2008 bei Sanofi-Aventis in Neuville-sur-Saône (Frankreich)

20

Louise Vicard erhält eine besondere HPTLC-Platte mit einer von Vibrio Fischeri-Leuchtbakterien geschriebenen Inschrift.

1998 wurde der französische Club de CCM (CCCM) von einer Gruppe von HPTLC-Fans gegründet. Die Vereinigung organisierte bisher 20 Konferenzen (jährlich zwei Tage) und drei internationale Symposia: Lyon im Jahr 2003, Berlin 2006 und Helsinki 2008 (www.hptlc.com). Das nächste International Symposium for HPTLC findet vom 6.-8. Juli 2011 in Basel (Schweiz) statt.

Anlässlich des letzten Treffens (Bild oben) wurde nicht nur das Clubjubiläum, sondern auch die Pensionierung von Louise Vicard gefeiert, welche über die Jahre regelmässig Beiträge für den CBS verfasst hat und heute als Kassiererin im Vorstand des CCCM mitwirkt.

Das nächste Treffen am 11. Juni befasst sich mit der Kopplung mit präparativer HPLC und mit dem grundlegenden Thema der Platten. Für weitere Informationen steht Pierre Bernard Savary, Präsident des CCCM, zur Verfügung (info@hptlc.com).

Seminare in Indien

21

Seminar in Ayush,New Delhi (Indien)

22

Podiumsdiskussion während des Indian Pharmaceutical Congress im Dezember 2008 in Neu Dehli

Seit 30 Jahren ist Anchrom CAMAG's Partner in Indien, eines der weltweit führenden Länder in der Anwendung von HPTLC. Anchrom pflegt aktiv den Kontakt mit zuständigen Regierungsbehörden und Wissenschaftlern im Bereich moderner und pflanzlicher Arzneimittel. So entschloss man sich, eine Reihe von HPTLC-Seminaren abzuhalten, welche die wichtigsten Städte Indiens abdecken sollen.

Im Dezember 2008 führte Dr. Eike Reich, Leiter des CAMAG Labors, eine dritte Seminarrunde in Kolkata (Jadhavpur Univ.), Bangalore (PES College) und Neu Delhi (AYUSH Committee Room) durch.

Dr. Reich wurde auch zu einem Vortrag auf dem Indian Pharmaceutical Congress eingeladen, bei dem 65 Publikationen hauptsächlich aus dem Bereich der quantitativen HPTLC vorgestellt wurden.

HPTLC-Bestimmung der Ginkgolide A, B und C und Bilobalid in *Ginkgo biloba*

23

Untere Reihe von links: Dmitry Demchenko, Dr. Svetlana Ivanova, Dr. Vera Kosman Obere Reihe: Dr. Irina Urakova, Prof. Dr. Alexander Shikov, Dr. Marina Karlina, Dr. Olga Pozharitskaya*

In der Abteilung für Standardisierung und Neue Technologien am Pharmazeutischen Institut Sankt Petersburg werden verschiedene chromatographische Trenntechniken verwendet. Die Planar-Chromatographie wird vor allem zur Entwicklung von qualitativen und quantitativen Analysenmethoden eingesetzt, die der Untersuchung von Arzneipflanzen und Naturprodukten sowie der Bestimmung von Arzneistoffen, Terpenen, Polyphenolen, Vitaminen und anderen biologisch aktiven Substanzen dienen.

Einleitung

Ginkgo biloba L. ist die älteste Baumart der Welt und repräsentiert den einzigen noch lebenden Vertreter aus der Familie der Ginkgoaceae. Charakteristische Inhaltsstoffe dieser Pflanze sind Diterpenlactone (Ginkgolide und Bilobalid) und Flavonoide. Ginkgolide sind Diterpene mit käfigartiger Struktur, die nur in *G. biloba* gefunden wurden. Fünf Ginkgolide sind bekannt, nämlich Ginkgolid A, B, C, J und M, sowie Bilobalid, ein Sesquiterpen mit ähnlicher Struktur (wird meist zusammen mit den Diterpenen erfasst). Die Ginkgolide A und B zeigen eine regulierende Wirkung auf die Glucocorticoid-Synthese, und Ginkgolid B sowie Bilobalid wirken dem programmierten Zelltod (Apoptose) entgegen. *G. biloba* erregte grosses Interesse durch seine vermeintliche Wirkung bei der Behandlung von nachlassender Gedächtnisleistung.

Verschiedene Analysentechniken wie HPLC, TLC, GC und MPLC wurden zur Isolierung und Bestimmung von Ginkgoliden und Bilobalid in *G. biloba*-Blättern, -Extrakten und seinen phytopharmazeutischen Zubereitungen eingesetzt. Die vorliegende Arbeit beschreibt eine einfache und kostengünstige HPTLC-Methode zur Bestimmung von wichtigen Diterpenlactonen (Ginkgolid A, B, C und Bilobalid) in *G. biloba*-Blättern und deren Produkten. Die Methode einschliesslich der Studie zu den Freisetzungsraten *in vitro* eignet sich zur routinemässigen Laboranalytik.

Standardlösungen

Methanolische Lösungen (1.0 mg/mL) von Ginkgolid A (Ga), B (Gb), C (Gc) und Bilobalid (B).

Probenvorbereitung

G. biloba-Blätter wurden im Juli 2007 im Botanischen Garten (Sankt Petersburg, Russland) gesammelt und bei 40 °C getrocknet. 30 g pulverisierte *G. biloba*-Blätter wurden 20 min mit 400 mL siedendem Wasser extrahiert, anschliessend wurde filtriert. Dann wurden 10 g Aktivkohle zum Filtrat gegeben, und die Mischung wurde 12 h bei Raumtemperatur gerührt und danach zentrifugiert (15 min, 1000 g). Der Überstand wurde verworfen und die Aktivkohle in 20 mL Aceton resuspendiert. Nach Filtration wurde das Lösungsmittel zur Trockne abgedampft und der Rückstand direkt vor der HPTLC-Analyse in 3 mL Methanol aufgenommen.

Freisetzungsuntersuchungen

Feste Gelatinekapseln, die Ginkgo-Wasserextrakt enthielten, wurden einer Freisetzungsprüfung bei 37 °C nach der »Basket«-Methode (Erweka, Deutschland) unterzogen. Als Lösungsmedien wurden Wasser, künstlicher Magensaft (pH 1,2) und künstlicher Darmsaft (pH 6,8) verwendet. In vorgegebenen Zeitintervallen (15, 30, 45 und 60 min) wurden Proben gezogen und diese filtriert. Ga, Gb, Gc und B wurden direkt in diesen Freisetzungsmedien mittels HPTLC bestimmt.

Schicht

HPTLC-Platten Kieselgel 60 F_{254} (Merck), 10 × 10 cm, imprägniert durch Tauchen in eine 4 %igen Lösung von Natriumacetat in Methanol-Wasser 3:2 für 5 s, danach 1h bei RT getrocknet [1].

Probenauftragung

Bandförmig mit Linomat V, Bandlänge 6 mm, 9 Bahnen, Bahnabstand 10 mm, Abstand zur Unterkante 10 mm, Abstand vom seitlichen Rand 10 mm, Auftragevolumen 1–20 µL für Probe und Standardlösungen.

Chromatographie

In der Doppeltrogkammer mit Toluen – Aceton 7:3 nach Kammersättigung mit dem Fliessmittel für 20 min. Zwei Entwicklungen erfolgten über 60 mm mit Zwischentrocknung (eine Entwicklung dauerte ca. 6 min). Nach dem zweiten Lauf wurden die Platten getrocknet und zur Detektion der aktiven Bestandteile 1 h auf 150 °C erhitzt [2].

Anmerkung: Unter Umständen genügt auch eine Einfachentwicklung mit Toluen – Ethylacetat – Aceton – Methanol 20:10:10:1.2 (CAMAG Applikation F-16A).

Densitometrie

Absorptionsmessung bei 254 und 400 nm mit dem TLC-Scanner 3 und winCATS Software

Ergebnisse und Diskussion

Die Kalibrierfunktionen für Ga, Gb, Gc und B mit Korrelationskoeffizienten ≥0,9971 und relativen Reststandardabweichungen (*%RSD*) ≤ 2,0 % waren sehr gut geeignet.

Substanz	hR_F-Wert	Regressionsgleichung	r	*%RSD*	Bereich (ng/Band)
Ginkgolid A	44	y= -65.6 + 23.9 x - 0.035 x^2	0.9998	1.7	20–200
Ginkgolid B	40	y= -30.5 + 20.4 x - 0.012 x^2	0.9998	1.6	17–175
Ginkgolid C	17	y= -81.6 + 14.4 x - 0.024 x^2	0.9971	2.0	14–135
Bilobalid	57	y= -3.7 + 30.8 x - 0.058 x^2	0.9999	0.3	10–130

Die Ergebnisse für die Präzision (*%RSD*, n = 3, bei 50 ng/Band) innerhalb eines Tages (1,1–1,2 %) und zwischen den Tagen (1,1–1,3 %) sowie die Wiederfindungsraten (98,5–104,6 % ± 1,2–2,2 %) bestätigten die hohe Effizienz der gewählten HPTLC-Bedingungen. Um die Richtigkeit der Bestimmung der Wiederfindungsrate zu ermitteln, wurde eine Dreifach-Analyse der Pflanzenproben durchgeführt, die mit verschiedenen Konzentrationen (0,05, 0,1 und 0,5 %, entspricht den in Blättern gefundenen Gehalten) der Stammlösungen von Ga, Gb, Gc und B aufgestockt wurden.

Die quantitative Bestimmung von Ga, Gb, Gc und B

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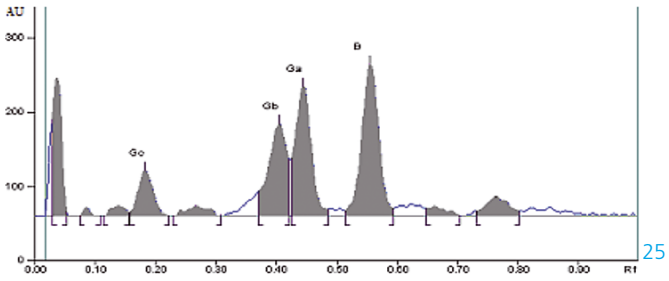
CAMAG Linomat 5

In diesem Beitrag ist der Linomat 5 unentbehrlich für die gute Auflösung zwischen Ginkgolide A (Ga) and Ginkgolid B (Gb), da kompakte, schmale Startbanden die bestmögliche Trennleistung mit dem gegebenen planarchromatographischen System gewährleisten.

Mit dem CAMAG Linomat 5 werden die Proben strichförmig auf die Schicht aufgesprüht. Dieses Verfahren ermöglicht es, grössere Probenvolumina als bei der Kontaktauftragung aufzutragen, da bei dem Sprühvorgang das Lösungsmittel praktisch vollständig verdampft. Selbst wenn stark polare Lösungsmittel verwendet werden, z.B. bei dieser Anwendung methanol- oder wasserhaltige Lösungsmittel, bleiben die aufgetragenen Zonen kompakt und schmal.

Ein weiterer Vorteil des Linomat 5 ist die selbstjustierende Objektauflage. Sie erlaubt den Wechsel zwischen Schichten unterschiedlicher Dicke ohne Nachjustieren der Sprühdüse. Gerade bei Anwendungen, bei denen oft mehrere Benutzer mit unterschiedlichen Schichtdicken auf dasselbe Auftragegerät zugreifen möchten, ist diese Eigenschaft sehr hilfreich.

in *G. biloba*-Blättern wurde dreimal durchgeführt. Für den durchschnittlichen Gehalt von Ga, Gb, Gc und B in den Blättern (in % des Trockengewichts) ergaben sich folgende Werte: Ginkgolid A 0,078 ± 0,008, Ginkgolid B 0,072 ± 0,007, Ginkgolid C 0,076 ± 0,008, Bilobalid 0,062 ± 0,006. Der Gesamtgehalt an Diterpenlactonen in *G. biloba*-Blättern lag bei 0,29 %



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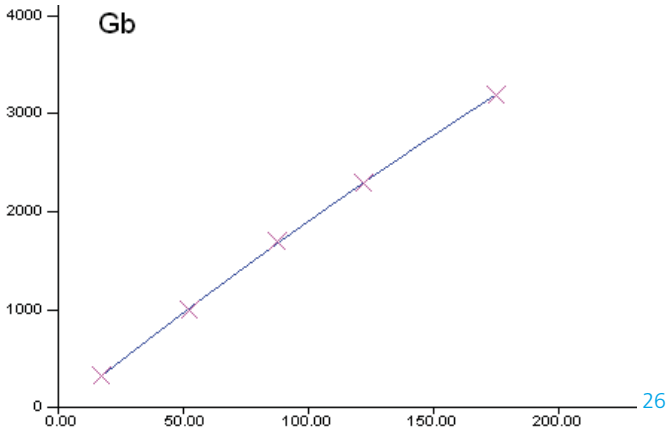
Absorptionsscan bei 254 nm: Auftrennung der Ginkgolide A (Ga), B (Gb), C (Gc) und Bilobalid (B) im Extrakt von Ginkgo biloba-Blättern. (Die sechs weiteren gezeigten Peaks wurden in dieser Studie nicht identifiziert.)



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Polynome Kalibrierkurve für Ginkgolid Gb

Ginkgolide sind in sehr kleinen Mengen in Ginkgoblättern enthalten. Zudem können andere Bestandteile wie Flavonoide, welche in höheren Konzentrationen vorliegen, die Bestimmung der Ginkgolide stören [3]. Bei der hier vorgestellten Vorgehensweise wurden die Diterpenlactone mit Hilfe von Aktivkohle extrahiert und aufgereinigt. Durch die Zugabe von Aktivkohle zur wässrigen Kolloidlösung wurden flavonoide Bestandteile zwar daran gebunden, die relativ unpolaren Verbindungen lagerten sich jedoch zu Aggregaten zusammen und waren nicht mehr in Lösung. Somit wurden diese relativ unpolaren Verbindungen im letzten Filtrationsschritt mit verworfen und beeinträchtigten die Analyse nicht.

Weiterhin wurde die Freisetzung der Ginkgolide und Bilobalid aus *G. biloba*-Blättern in vitro untersucht. Die Konstanten der Freisetzungsraten der Diterpenlactone, bestimmt über die Regressionsanalyse, betrugen 1,67 min-1, 0,87 min-1 and 0,42 min-1, jeweils in Wasser, künstlichem Magensaft (pH 1,2) und künstlichem Darmsaft (pH 4,5).

Zusammengefasst ist die hier vorgestellte HPTLC-Methode zur gleichzeitigen Analyse von Ginkgolid A, Ginkgolid B, Ginkgolid C und Bilobalid aus *G. biloba*-Blätter einfach, empfindlich, ökonomisch und zum schnellen Screening sowie zur Routineanalyse geeignet, bei der eine grosse Anzahl Pflanzenproben untersucht wird. Die Freisetzungsstudie von Ginkgoliden in verschiedenen Modelllösungen zeigte, dass die maximale Freisetzung in Wasser erzielt wurde, gefolgt von künstlichem Magensaft.

[1] Van Beek TA , Lelyveld GP. Phytochem Anal 4 (1993) 109.
[2] Tallevi SG, Kurz WGW. J Nat Prod 54 (1991) 624.
[3] Van Beek T.A. et al., J Chromatogr 543 (1991) 376.

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich.

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HPTLC-Identifizierung von *Hoodia gordonii*, einem populären Inhaltsstoff von pflanzlichen Schlankheitsmitteln

27

Valeria Widmer und Dr. Eike Reich

Im CAMAG Labor in Muttenz, Schweiz, arbeitet ein Team von Wissenschaftlern und Applikations-Spezialisten unter Leitung von Dr. Reich* daran, die Akzeptanz der HPTLC als standardisierte Analysenmethode zu erhöhen. Ein grosses Einsatzgebiet der Planar-Chromatographie ist die Analytik von Pflanzeninhaltsstoffen. Durch weltweite Kontakte mit führenden Vertretern der Nahrungsergänzungsmittel-Industrie, aus Forschungsinstituten, Universitäten und Arzneibuch-Kommissionen ist das CAMAG Labor am Puls der Zeit und kann gezielt HPTLC-Lösungen für aktuelle analytische Probleme bereitstellen.

Einleitung

Hoodia gordonii sieht aus wie ein Kaktus und ist eine sukkulente Pflanze aus der Unterfamilie der Seidenpflanzengewächse (Asclepiadoideae). Heimisch in den Wüstengebieten von Südafrika und Namibia, wurde die Pflanze in der lokalen Volksmedizin schon seit langer Zeit als natürlicher Appetitzügler verwendet. Seit die Schlankheitsindustrie Hoodia »entdeckt« hat, werden im Internet zahlreiche Abnehmpillen mit Zusätzen von *Hoodia gordonii* angeboten – die Nachfrage ist so gross, dass die langsam wachsende Pflanze heute nicht mehr in genügender Menge zur Verfügung steht und auf dem Markt gefälschte oder gestreckte Produkte auftauchen.

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Hoodia gordoni

Herstellerfirmen, die die Qualität ihrer Produkte garantieren wollen, sind angewiesen auf eine einfache Methode zur sicheren Identifizierung von *Hoodia gordonii* als Rohmaterial und in Fertigprodukten. In Zusammenarbeit mit der American Herbal Products Association (AHPA) und der American Herbal Pharmacopoeia (AHP) hat das CAMAG Labor eine schnelle und zuverlässige HPTLC-Methode zur Identifizierung von *Hoodia gordonii* entwickelt und bezüglich Spezifität, Reproduzierbarkeit und Robustheit validiert [1]. Basierend auf authentischem botanischen Referenzmaterial ermöglicht die Methode die sichere Unterscheidung zwischen *Hoodia gordonii*, den verwandten Spezies *Hoodia currorii* und *Hoodia parviflora* sowie den bekannten Verfälschungen Feigenkaktus (*Opuntia ficus-indica*) und Caralluma (*Caralluma fimbriata*). Dies wurde anhand von diversen in lokalen Apotheken, Drogerien und über das Internet gekauften Hoodia-Produkte gezeigt [2].

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Untersuchte Hoodia-Schlankheitsprodukte

Probenvorbereitung

Getrocknetes Pflanzenmaterial wurde fein gemahlen, 500 mg mit 5 mL Methanol – Wasser 4:1 versetzt und im Ultraschall über 10 min bei RT (23 °C) extrahiert. Nach dem Zentrifugieren wurde der Überstand verwendet.

Für quantitative Bestimmungen wurde eine erschöpfende Extraktion mittels Accelerated Solvent Extraction (ASE) durchgeführt: 500 mg gemahlenes Pflanzenmaterial wurden in der ASE 100 (Dionex) mit total 50 mL Methanol in 4 Teilen bei 40 °C extrahiert. Nach Abdampfen des Lösungsmittels wurden 25 mg des Rückstandes in 10 mL Methanol gelöst (die erzielbare Menge an Totalextrakt wurde mit 17 % bezogen auf das Trockengewicht der Droge ermittelt).

Standardlösungen

7 mg Fruktose wurden in 5 mL Methanol – Wasser 4:1 gelöst. 2 mg β-Sitosterol wurden in 5 mL Methanol gelöst. Wahlweise (wenn vorhanden) kann 1 mg des Steroidglykosids P57 (Wirkstoff aus *Hoodia gordonii*) in 5 mL Methanol gelöst werden.

Schicht

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

Probenauftragung

Bandförmig mit dem DC-Probenautomat 4, Bandlänge 8 mm, Auftragevolumen 1–10 µL, Bahnabstand mind. 10 mm, unterer Randabstand 8 mm, linker Randabstand mind. 15 mm.

Chromatographie

In der Automatischen Entwicklungskammer ADC 2 mit Chloroform – Methanol – Wasser 70:30:3 nach 20 min Kammersättigung, Laufstrecke 70 mm vom unteren Plattenrand. Da das chromatographische Ergebnis dieser Trennung von der Plattenaktivität beeinflusst wird, wurde diese mit einer gesättigten $MgCl_2$-Lösung bei 33 % rel. Luftfeuchte konstant gehalten.

Derivatisierung

Mit der Chromatogramm-Tauchvorrichtung III für 1 s in Anisaldehyd-Reagenz (1 mL Anisaldehyd in 170 mL Methanol mit 20 mL Essigsäure und 10 mL Schwefelsäure) tauchen. Nach 1 min wurde die Platte auf dem DC-Plattenheizer für 3 min bei 100 °C erhitzt.

Dokumentation

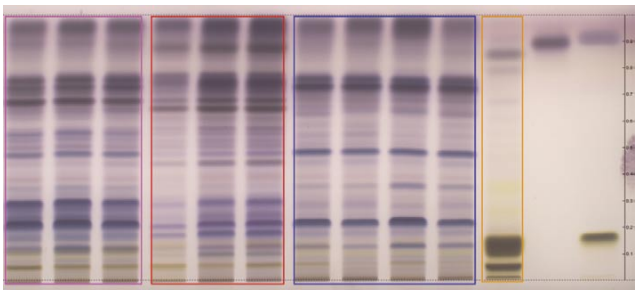
Mit dem DigiStore 2-System unter UV 366 nm und unter Weisslichtbeleuchtung

Auswertung

Durch Videodensitometrie mit der Software VideoScan

Ergebnisse und Diskussion

Die vergleichende Untersuchung verschiedener kommerzieller Schlankheitsprodukte führte zu erstaunlichen Ergebnissen. Obwohl auf allen Produkten als Hauptinhaltsstoff Hoodia deklariert war, konnte Pflanzenmaterial von *Hoodia gordonii* nur in 3 von 7 untersuchten Produkten nachgewiesen werden. Der gefundene Gehalt lag dabei zwischen 30 und 100 %. Mehrere Präparate wurden mit anderen Pflanzen verfälscht oder gestreckt, wobei als Verfälschung *Caralluma fimbriata* mit Sicherheit ausgeschlossen werden konnte.



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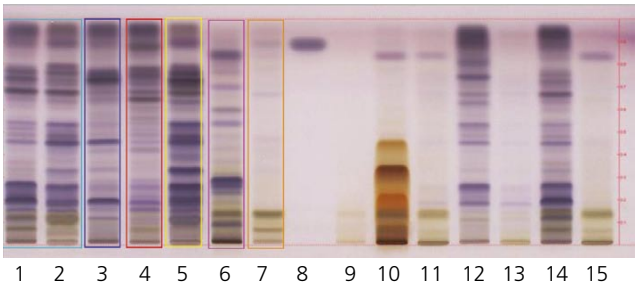
Unterscheidung von Hoodia-Arten und Feigenkaktus: Bahn 1–3 Hoodia gordonii, 4–6 Hoodia currorii, 7–10 Hoodia parviflora, 11 Feigenkaktus (Opuntia ficus-indica), 12 P57, 13 Fruktose, β-Sitosterol (mit steigendem R_F-Wert).



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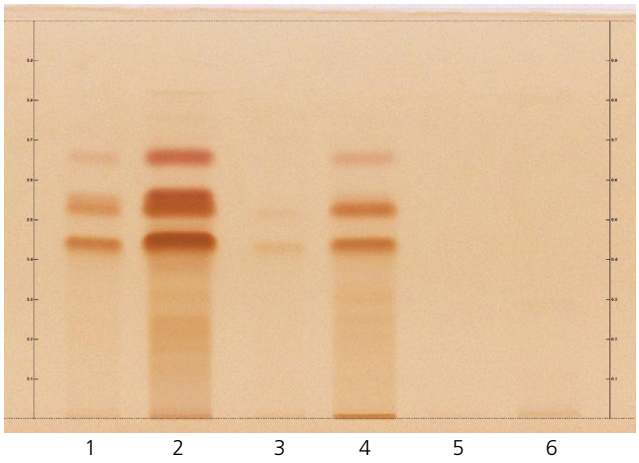
Untersuchung verschiedener kommerzieller Produkte: Bahn 1–2 Hoodia gordonii, 3 Hoodia parviflora, 4 Hoodia curorii, 5 Hoodia ruschii, 6 Caralluma fimbriata, 7 Opuntia ficus-indica, 8 β-Sitosterol, 9 Produkt S2627, 10 Produkt S3917, 11 Produkt S3918, 12 Produkt S3919, 13 Produkt S3920, 14 Produkt S3990, 15 Produkt S4062

Das HPTLC-Chromatogramm zeigt deutlich die grossen Unterschiede der untersuchten Produkte. Während die Produkte auf Bahnen 9, 11, 13, und 15 wenige oder keine typischen Zonen aufweisen, fallen bei Produkt S3917 (Bahn 10) intensive rotbraune Zonen in der unteren Hälfte des Chromatogramms auf. Diese Zonen konnten mit Grüntee korreliert werden, einem weiteren, auf der Verpakkung deklarierten Bestandteil des Produkts. Für den Nachweis von Grüntee wurde eine vom CAMAG Labor entwickelte und validierte Methode [3] eingesetzt. Dabei wurde festgestellt, dass Grüntee den Fingerprint von *Hoodia gordonii* zwar teilweise überdeckt, dessen chromatographisches Verhalten aber nicht verändert. Die Methode zur Identifizierung von Hoodia kann also auch auf das mit Grüntee kombinierte Produkt angewendet werden.



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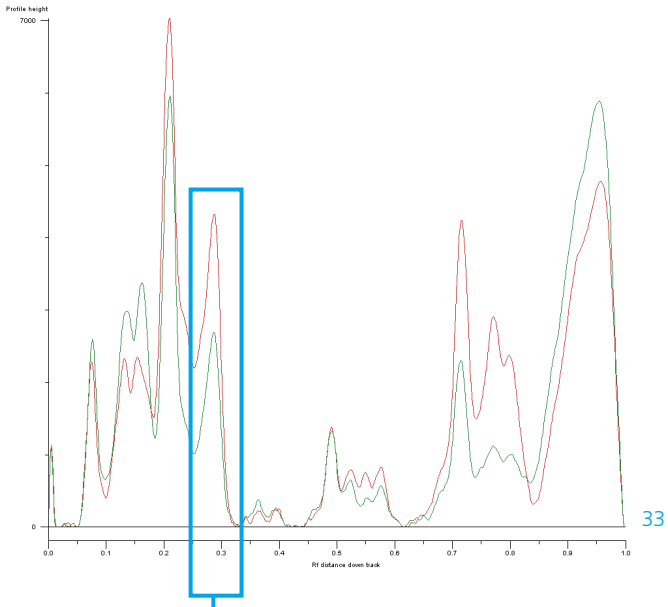
Nachweis von Grüntee in Produkt S3917: Bahn 1–2 Grüntee (0.1 und 1 µL), 3–4 Hoodia-Produkt S3917 (0.1 und 1 µL), 5–6 Hoodia gordonii (1 und 5 µL) [3]

Zwei Produkte (S3919 und S3990) weisen einen mit *Hoodia gordonii* vergleichbaren Fingerprint auf (Bahnen 12, 14 und 1–2). Diese Produkte und *Hoodia gordonii*-Rohmaterial (Referenzprobe) wurden mit ASE extrahiert. Zur VideoScan-Quantifizierung wurden unterschiedliche Volumina der Referenzprobe aufgetragen. Die quantitativen Untersuchungen bestätigten, was schon visuell aus dem Chromatogramm abgeschätzt werden konnte. Produkt S3919 (Bahn 12) wies im Vergleich mit *Hoodia gordonii* Rohmaterial schwächere Zonen auf und ergab in der Gehaltsbestimmung deutlich unter dem Sollwert (100 %) liegende Werte. In Produkt S3990 konnte mit 80–100 % (je nach ausgewerteter Substanzzone) der höchste Gehalt an *Hoodia gordonii* ermittelt werden.



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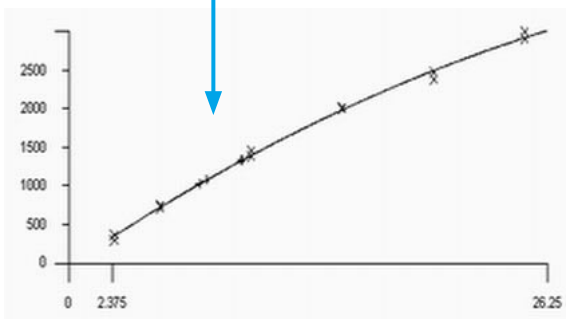

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Auswertung mit Videodensitometrie: Vergleich der HPTLC-Profile von Hoodia gordonii Standardextrakt (rot) mit Produkt S3990 (grün). Die blau markierte Zone wurde mittels polynomer Kalibration (6 Konzentrationen (je zweifach), relative Standardabweichung 3.4 %) quantifiziert.

Die hier vorgestellte Methode erwies sich als sehr geeignet für einen schnellen und sicheren Nachweis von *Hoodia gordonii* in den unterschiedlichsten Fertigpräparaten.

[1] V. Widmer, E. Reich, A. DeBatt, J. Planar Chromatogr. 21 (2008) 1, 21–26

[2] D. Arnold, Masterarbeit, Institut für pharmazeutische Wissenschaften, Universität Basel, 2008

[3] E. Reich, A. Schibli, A. DeBatt, J. AOAC Int. 91 (2008), 13–20

Weitere Informationen erhalten Sie auf Anfrage von den Autoren.

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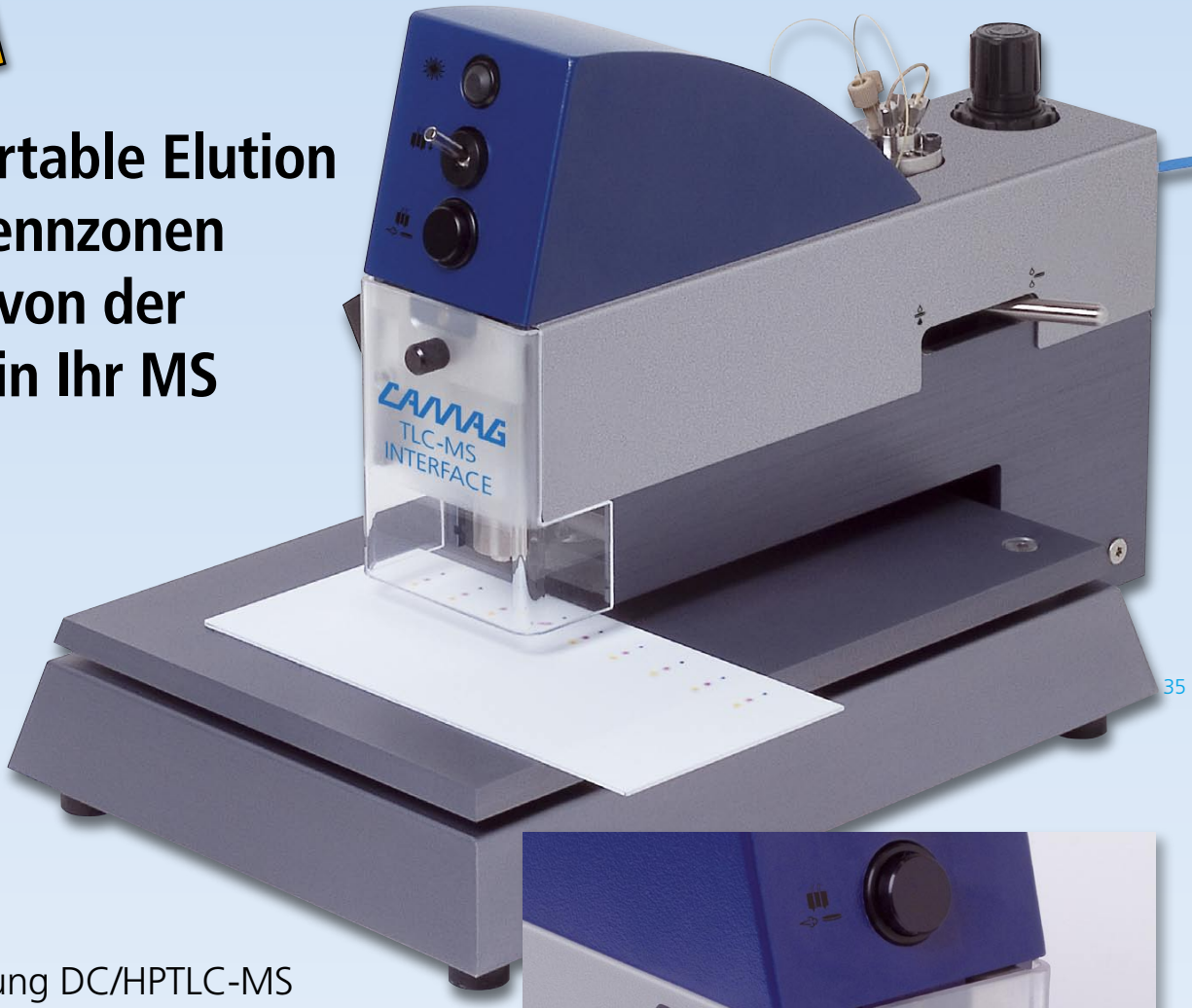

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