CBS 106 • March 2011

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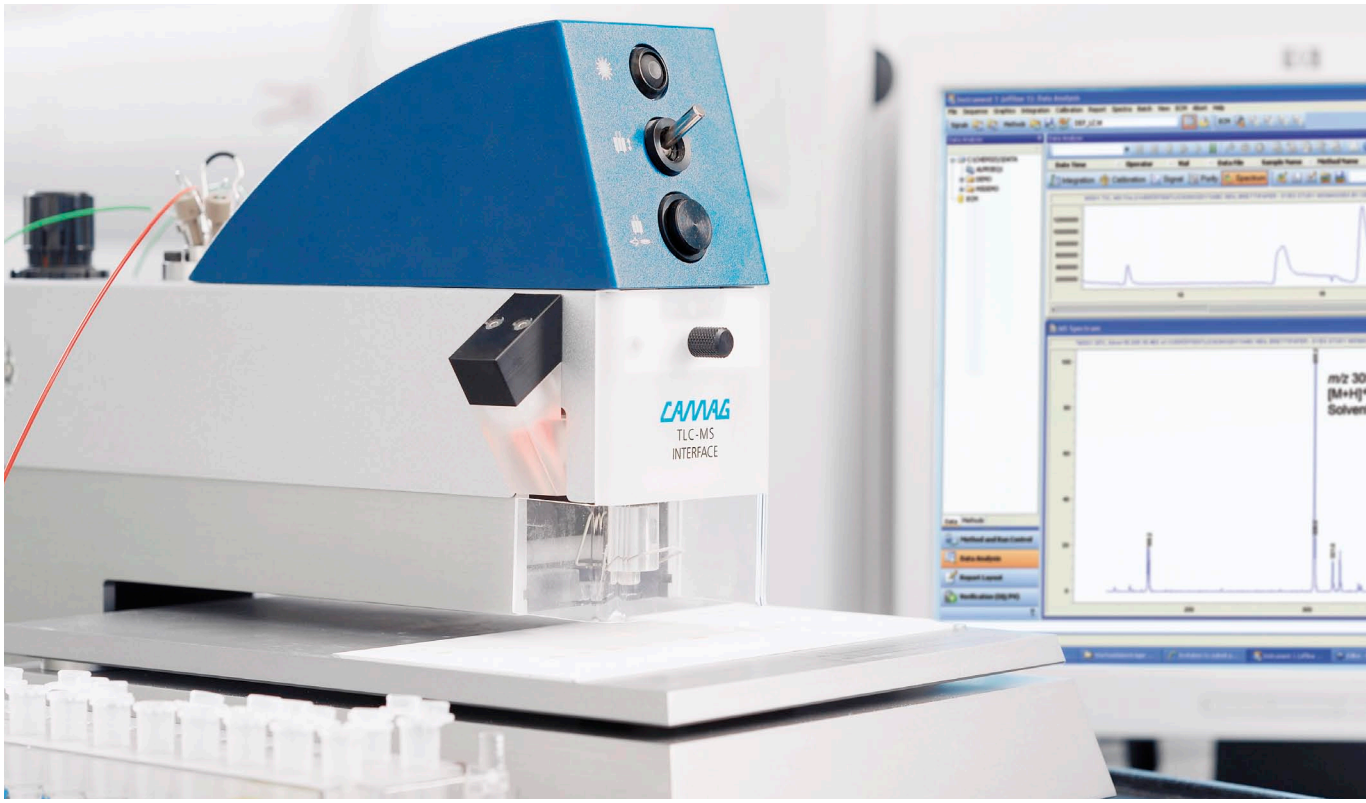

Hyphenation of planar chromatography with mass spectrometry is appreciated by many analysts

INTERNATIONAL SYMPOSIUM FOR HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY
Basel, Switzerland, 06–08 July 2011

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Planar Chromatography
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IN THIS ISSUE

Procedures, applications

Products featured in this issue

Column: Know CAMAG

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Determination of enrofloxacin and ciprofloxacin in milk by direct bioautography detection

1 *From left: M.Sc. Wioleta Bąk, Dr. hab. Irena Choma, M.Sc. Edyta Grzelak, Dr. Karol Pilorz and Dr. hab. Barbara Majer-Dziedzic.*

The research of Dr. hab. Irena Choma*, associate professor at the Chromatographic Methods Department, Maria Curie-Skłodowska University, Lublin, Poland, focuses on all kinds of chromatography. Presently, she employs mainly planar chromatography to develop new and improved analytical methods for determination of antibiotic residues in food matrices such as milk or meat. She uses the hyphenation with microbiological tests, so-called TLC/HPTLC-direct bioautography (TLC/HPTLC-DB), which gives information on the antibacterial activity of a given substance zone. This study was performed in cooperation with Dr. hab. Barbara Majer-Dziedzic, University of Life Science in Lublin.

Introduction

Fluoroquinolones are widely used in the treatment of both human and veterinary diseases. Ciprofloxacin, one of the most popular human antibiotics, is a main metabolite of enrofloxacin. In veterinary medicine it is mainly used to cure mastitis in cows. Hence, both of these drugs are potential residues in the milk of treated cows. Analytical methods should be sensitive and selective enough to distinguish between these antibiotics at their maximum residue level (MRL), which is 100 µg/kg for the sum of these drugs.

HPLC, most frequently used in antibiotic analysis, can often be replaced advantageously by HPTLC/TLC with microbiological detection. HPTLC/TLC-DB enables the separation of antibiotics, and at the same time, detection of their antibacterial properties. The developed plate is dipped directly in a bacterial suspension. After incubation and visualization, an inhibition of bacterial growth around the antibiotic zones can be observed. The method is simple, very sensi-

tive, accurate and precise. Its great advantage over HPLC is not only the analysis of many samples at the same time but also the additional information about their antibacterial properties. The detection of any further antibacterial compounds, which are not known (e.g. breakdown products) or not in the focus of the regular target analysis, is treasured as well.

Chromatogram layer

Pre-coated TLC plates silica gel 60 F_{254} (Merck), 10 × 20 cm

Standard solutions

The stock solution of a mixture of ciprofloxacin and enrofloxacin, 1 mg/mL of each, was prepared in methanol. The standard solutions were prepared by appropriate dilution with methanol.

Sample preparation

Milk samples are prepared by spiking of 2 % fat milk with an appropriate volume of stock solution. The analytes are extracted using a variant of matrix solid-phase dispersion (MSPD). For defatting the adsorbent is packed in a syringe and percolated (flushed) with hexane. The antibiotics are eluted with dichloromethane, the solution evaporated to dryness and the residue taken up with methanol.

Sample application

With the Linomat 5 the standard solution and milk extracts were applied bandwise; band length 3 or 5 mm, application volume 10–50 µL

Chromatography

In the horizontal DS chamber (Chromdes) with dichloromethane – methanol – isopropanol – 25 % aqueous ammonia 3:3:5:2.

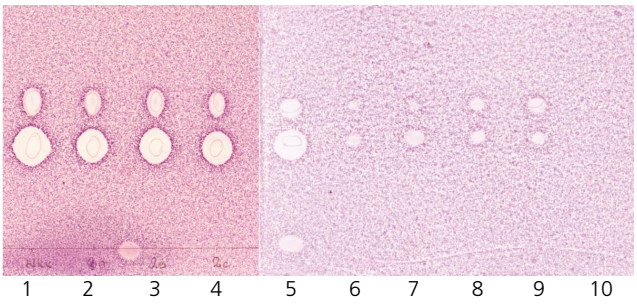
Direct bioautography

Bioautography detection with *Bacillus subtilis* strain was performed using the Chrom Biodip Antibiotic Kit (Merck) according to the recipe [1]. The assay with *Escherichia coli* strain was developed in our research group [2]. After drying, the plate was immersed in a broth inoculated with *E. coli* for 5 s using the CAMAG TLC Immersion Device. Then, the plate was incubated at 37 °C for 5 h in a moist chamber. Visualization was performed by spraying with an aqueous tetrazolium salt (MTT) solution (0.2 %). The cream-white inhibition zones were observed against a purple background, indicating the presence of ciprofloxacin and enrofloxacin. The inhibition zones were measured with a planimeter.

Results and discussion

For the *Bacillus subtilis* bioautogram, milk samples were spiked at 50 and 1000 µg/kg, and the application of 50 µL of the milk extracts was necessary to enable the detection at the lower trace level [3].

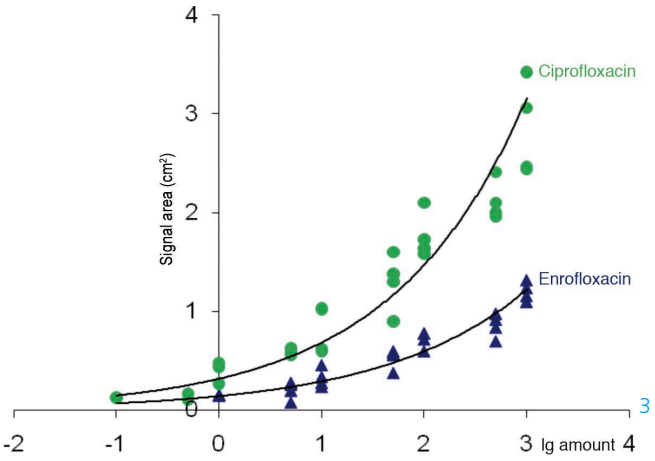
Note: The inhibition zones can also be measured with the TLC Scanner 4. As the background is not white, but colored, negative peaks would be obtained. Hence, special software settings for the absorption measurement are needed: maximal wavelength of the background color as measurement wavelength and fluorescence as measurement mode. This unusual measurement mode allows the regular (positive) peak display.



2

TLC-DB (B. subtilis assay) of milk extracts spiked with enrofloxacin (hR_F = 58) and ciprofloxacin (hR_F = 42) at 50 and 1000 µg/kg; track assignment: 1: standard solution (10 mg/L), 2–4: 10 µL of the milk extracts spiked at 1000 µg/kg, 5, 6: standard solution (1000 and 500 µg/kg), 7–9: 50 µL of the milk extracts spiked at 50 µg/kg, 10: blank sample

Bioautographic analysis is usually done by regression analysis of the inhibition zone areas. For a narrow range of concentration (one or two orders of magnitude), a linear correlation was observed between the inhibition area and the logarithm of the antibiotics' concentration. For a wider concentration range an exponential relation fits better [4].



Areas of inhibition zones (cm^2) versus logarithm of the antibiotics' amounts (ng); data were obtained from four plates

Presently, our research group works on two new bioautographic tests based on *Bacillus subtilis* and *Escherichia coli* [2], which shall be used for testing real milk samples of treated cows. The limit of detection for ciprofloxacin in *E. coli* test (25 µg/kg) is lower than that obtained using the Chrom Biodip test, while for enrofloxacin it is slightly higher (75 µg/kg).

Further information is available on request from the authors.

[1] R. Eymann *et al*. in: Planar Chromatography, Proceedings, Lillafüred, Hungary, 2000, 67.

[2] E.M. Grzelak *et al*. J. AOAC Int., in press.

[3] I.M. Choma, J. Planar Chromatogr. 19 (2006) 104.

[4] I.M. Choma *et al*. J. Liq. Chromatogr. Relat. Technol. 27 (2004) 2071.

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CAMAG BioLuminizer

Combining the separation power of planar chromatography with bioluminescence detection enables the identification of single biologically active compounds. All substances which are generating this distinct effect are detected in complex mixtures like degradation products, unknown metabolites or unauthorized ingredients.

In contrast to the bioassay used in this contribution that requires incubation and detection with the TLC Scanner 4, the luminescent bacteria assay is an instant bioassay (see CBS 105). Bioactive compounds are directly visible as dark or enhanced zones on a luminescent background (viable bacteria) after immersion into the bacteria suspension.

The BioLuminizer is a compact, user-friendly detection system for bioluminescence imaging, which shows an exceptional image quality and a high resolution for a short exposure time. All the consumables necessary for this detection are supplied by CAMAG as BioLuminex standard kit (No. 022.9765).

Use of Planar Chromatography for the analysis of peptides from tryptic protein digest

5 *Michael Schulz and Susanne Minarik*

6 *Dr. Gary van Berkel*

Merck has been producing pre-coated TLC plates since 1966. To the wide variety of plates with different layer materials and surface modifications, in 2006 ProteoChrom® HPTLC plates have been added. These are particularly suited for one and two-dimensional separations of peptides from tryptic digest of proteins and can be used for subsequent identification by mass spectrometry. These plates having a layer thickness of 100 µm and their binder system has been optimized for increased water stability, making them suitable for the application of relatively large volumes of aqueous samples. ProteoChrom® Silica gel plates are used for one-dimensional separations whilst ProteoChrom® Cellulose layers are particularly suited for two-dimensional chromatography. This contribution was created in collaboration between Michael Schulz and Susanne Minarik (Merck) with Gary van Berkel, Oak Rigde National Laboratory.

Introduction

Planar chromatography is a widely used and versatile analytical method. With the use of precise instruments for sample application and chromatogram evaluation, planar chromatography in the form of HPTLC has become well established for many years. Current developments indicate an increasing number of applications employing planar chromatography coupled with mass spectrometry (MS). This very promising trend and commercially available instruments for coupling planar chromatography with MS open up new possibilities in bio-analytical applications.

In this study, peptides from the tryptic digest of proteins like myoglobin, cytochrome C, ß-casein and bovine serum albumin (BSA) were separated on HPTLC plates and then read out by coupling with ESI-MS. Sequence coverage is determined and compared with HPLC [1, 2].

Sample preparation

The proteins were digested with the enzyme trypsin. The trypsin to protein ratio was 1:100 mg at a protein concentration of 2 mg/mL in 25 mM of ammonium carbonate buffer.

Stationary phase

HPTLC plate ProteoChrom® Silica gel 60 F_{254}s, 20 x 10 cm
HPTLC aluminum foil ProteoChrom® Cellulose, 10 x 10 cm

Sample application

Bandwise with Automatic TLC Sampler 4, band length 5 mm, track distance 10 mm, distance from lower edge 10 mm, application volume 7 µL

Chromatography

In the flat bottom chamber for 20 x 10 cm and 10 x 10 cm: One-dimensional on the silica gel layer with 2-butanol – pyridin-ammonia (25 %) – water 39:34:10:26; two-dimensional on the cellulose layer with 2-butanol – pyridine – acetic acid – water 15:10:3:12 and the same mobile phase as for silica gel layers (migration distance 50 mm each).

Post-chromatographic derivatization

Using the TLC sprayer, one of the following reagents was homogenously applied:

- Ninhydrin reagent (0.5 % ninhydrin in 2-propanol): the plate was sprayed with ninhydrin solution → 110 °C for 2 min → white light illumination
- Fluorescamine reagent (0.02 % fluorescamine in acetone and 10 % triethylamine in acetone) → the plate was sprayed with fluorescamine reagent → 10 min drying at RT → the plate was sprayed with triethylamine reagent → 10 min drying at RT → UV 366 nm
- Merck ProteoChrom® COLOR Peptide Staining Kit (ProteoChrom® COLOR Reagent and Ninhydrin Spray solution): the plate was sprayed with ProteoChrom® COLOR Reagent → 5 min drying at RT →

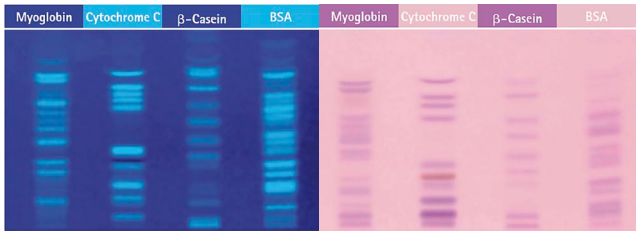
the plate was sprayed with ninhydrin solution → 110 °C for 2 min → white light illumination

Mass spectrometry

The developed, not derivatized plate was positioned on an x-y-z table. A track was scanned with a self-modified desorption electrospray beam (impact angle 55°, water as spray solvent) in a distance of 2–3 mm. The desorbed, ionized molecules were transferred via a prolonged transfer capillary, in a distance of 2 mm over the impact region, into the DESI-MS (LCQ Deca ion trap mass spectrometer, ThermoFinnigan). For locating the peptides, a track was recorded in the fullscan mode with a velocity of 100 µm/s over 55 mm (9 min/track). Thereafter MS or MS/MS spectra of the localised peptides were recorded.

Results and discussion

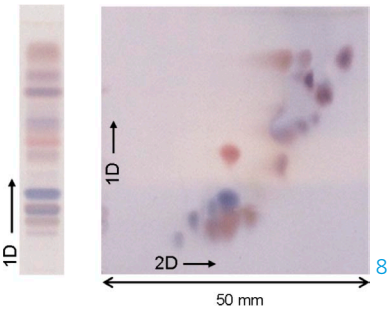
Up to 20 bands can be separated using one-dimensional HPTLC. That is about the number of peptides obtained by tryptic digest of small proteins (e.g. cytochrome C consists of 104 amino acids and 17 peptides are formed after tryptic digest). Very compact chromatogram zones are obtained by a one-dimensional separation on silica gel.



7

One-dimensional separation of peptides from tryptic digest of myoglobin, cytochrome C, ß-casein and BSA on silica gel layers stained with fluorescamine (left) and ninhydrin (right)

By two-dimensional separation, the number of the separated substances can be increased. Cellulose layers are particularly suited for this.



8

Two-dimensional separation of peptides from tryptic digest cytochrome C on a cellulose layer stained with ProteoChrom® COLOR Staining Kit.

The following table shows the sequence coverage obtained for cytochrome C and myoglobin after HPTLC/DESI-MS analysis. The sequence coverage varies depending on the layer material and separation quality. For two-dimensional separation on cellulose layers the sequence coverage is almost comparable with the sequence coverage achieved by HPLC/ESI-MS.

		HPTLC/ESI-MS	1D-HPTLC/DESI-MS	2D-HPTLC/DESI-MS
ProteoChrom® HPTLC Silica gel 60 F_{254s}	Cytochrome C		58%	
ProteoChrom® HPTLC Cellulose	Cytochrome C		72%	81%
Acclaim PepMap 100 C18	Cytochrome C	92%		
ProteoChrom® HPTLC Silica gel 60 F_{254s}	Myoglobin		62%	
ProteoChrom® HPTLC Cellulose	Myoglobin		68%	74%

This makes the method well suited to analyze simple and medium complex peptide mixtures. One special feature of HPTLC/MS coupling is the fact that detection can be done independent of the separation. The separated zones are stored on the HPTLC plate and can be read with mass spectrometry as needed. The general advantages of HPTLC to analyze many samples in parallel and the high flexibility in terms of detection can also be used for analysis of peptides. Simple and most rapid staining is achieved with the ninhydrin reagent, higher sensitivity with the fluorescamine reagent and the most characteristic staining with the ProteoChrom® COLOR-Peptide Staining Kit.

Further information is available from the authors on request.

[1] S. P. Pasilis *et al.* Anal. Bioanal. Chem. 391 (2008) 317
[2] S. P. Pasilis *et al.* J. Mass Spectrom. 43 (2008) 1627

Contact: Michael Schulz, Merck KGaA, Abt. MM-LER-C, Frankfurter Str. 250, 64293 Darmstadt; michael.schulz@merckgroup.com

HPLC-MS or simply HPTLC for analysis of sucralose in water?

Leonard Schuele (left) and Sebastian Grashorn

At the Institute of Food Chemistry, University of Hohenheim in Stuttgart all relevant analytical methods for samples in complex matrices are evaluated to find the most rational method with regard to efficacy. For the analysis of sucralose in food matrices, HPTLC was chosen [1, 2, CBS 94]. The method was applied to sewage effluent, surface water, and drinking water in the bachelor theses of Leonard Schuele and Sebastian Grashorn, supervised by Associate Prof. Dr. Gerda Morlock.

Introduction

Sucralose, a persistent chlorinated substance used as sweetener in Europe since 2005, has already been found in waste water. Therefore, various countries focused on the release of sucralose into the aquatic environment. HPLC-MS(/MS), HPLC-TOF and GC-MS were preferably used as analytical methods in the ultra-trace range after solid phase extraction.

A quantitative HPTLC method, which was orthogonal to the given analytical methods with regard to separation principle and detection, was highly suited for screening. Up to 17 samples were separated in parallel on an HPTLC plate within 15 min. The availability of post-chromatographic derivatization of sucralose was another benefit, which allowed its selective detection in sewage effluent and surface water. The sucralose content determined in four water samples of an interlaboratory trial was in good agreement to the mean laboratory values of that trial, analyzed by HPLC-MS/MS or HPLC-TOF-MS with the use of mostly isotopically labeled standards. The good accuracy, cost-efficiency and high sample throughput capacity proved HPTLC as the most preferable method.

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm, prewashed by pre-development with methanol, followed by drying at 100 °C for 15 min.

Standard solution

5 mg of sucralose were dissolved in 50 mL methanol and diluted 1:10 with methanol (10 ng/µL).

Sample preparation

The water sample (0.5 L, adjusted to pH 8) was extracted by solid phase extraction using a (styrene-) divinylbenzene copolymer adsorbent (500 mg/3 mL, Bond Elut PPL, Varian) at a flow rate of 10 mL/min. The adsorbent was dried by suction of air for 30 min. Elution by gravity followed with 1 mL methanol after 5 min residence time, which was repeated twice. The combined eluate was purified through an amino propyl phase (200 mg/3 mL Bond Elut NH_2, Varian), which was additionally eluted with 2 mL methanol (combined with the purified extract). After concentration to 2 mL, the extract was transferred to a sample vial.

Sample application

With ATS 4 by area application 8 × 5 mm, 17 tracks, track distance 10 mm, distance from the left side 20 mm, distance from lower edge 8 mm, application volume 100 or 300 µL for the sample solution and 1, 10, 20 and 30 µL for the standard solution, through heating the ATS4 spray nozzle to 60 °C (upscale of the dosing speed of 150 nL/s to 1000 nL/s, reduction by 70 % of the regular application time for large volumes), 100 and 300 µL volumes were applied in 1.7 and 5 min, respectively.

Chromatography

In the ADC2 with a mixture of isopropyl acetate, methanol and water 15:3:1, migration distance 60 mm,

continuation on page 9

New faces in the Sales & Marketing Department

We have extended this department, some new coworkers were hired and responsibilities have been newly assigned.


10

Jürg Leuenberger (49) works for CAMAG already since 2003. After his education as a chemical lab technician at Ciba-Geigy he worked in synthesis and quality control labs. Then he had an assignment with the Red Cross for 6 years during which he was active in Africa, Asia and Latin America. When he joined CAMAG he first attended to our customers in Switzerland. In view of his extensive international experience he was appointed area sales manager for Latin America in 2010. He travels frequently to these countries to support our distributors. At our home base, he takes care of customer projects and is active in distributor training courses.


11

Raphael Vizzini (36) joined CAMAG in August 2008. He received his federal diploma of higher education and senior laboratory technician in pharmaceutical research at Roche. Then he worked in process development at Roche, DSM and Synphabase. At CAMAG he is responsible for Europe, Middle East and Africa as area sales manager. This requires extensive traveling for the support of our distributors, to organize customer seminars and take part in exhibitions. He also plays an active role in distributors training at our home base.


12

Tobias Hohler (30) belongs to the team since July 2010. Before, he worked for an export company. He is bachelor of commerce and holds a trade diploma. His activities in our sales & marketing department include quotations in the export business, organization of customer and distributor training in collaboration with our lab and handling our export business with Latin America.


13

Lukas Frommenwiler (24) joined in August 2010. He had received his education as a chemical lab technician with the Laboratory Spiez and attended further training, e.g. marketing. He worked for EMS Chemie in a production lab and then at LONZA where he handled customer projects and took part in the training of apprentices. At CAMAG he attends to our homepage, assists in the organization of customers training and is gaining experience with sales literature and advertising.

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PLANAR CHROMATOGRAPHY

CBS

Liebe Freunde

Die Kopplung der Planar-Chromatographie mit der Massenspektrometrie wird von Analytikern sehr positiv aufgenommen. Sie unterstützt ihre Entscheidungen, sichert sie ab oder lässt neue Erkenntnisse gewinnen. Dabei ist die HPTLC-MS-Kopplung nur eine von vielen Möglichkeiten, Informationen aus einem chromatographischen Lauf zu gewinnen. Ein neuer GDCh-Kurs, der auf der letzten gelben Seite detailliert beschrieben ist, zeigt die Vielfältigkeit der aktuellen Kopplungsmöglichkeiten in der HPTLC auf. 2012 wird dieser Kurs in Englisch angeboten.

Das vorläufige Programm für das HPTLC-Symposium in Basel vom 6.–8. Juli 2011 ist fertig. Dieses sowie aktuelle Informationen entnehmen Sie der homepage www.hptlc.com. 179 Abstracts von Wissenschaftlern aus 23 Ländern wurden als Vorträge oder Poster mit aktuellen Themen aus den unterschiedlichsten Anwendungsbereichen angenommen. Die Registrierung ist noch offen (Anmeldeschluss: 30. Mai). Bisher sind schon über 120 Teilnehmer angemeldet. Erfahren Sie in Basel, ob HPTLC für ihre Fragestellungen geeignet ist. Alle Kopplungstechniken und Anwendungsbereiche sind vertreten. Sprechen Sie mit den Experten vor Ort.

Sie sind herzlich willkommen – auch das Rahmenprogramm ist attraktiv. Sehen wir uns dort? Ich würde mich freuen!

Herzlichst

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

Coupling of planar chromatography with mass spectrometry is obviously appreciated by many analysts. It is suited to support decisions or gain knowledge otherwise. Thereby TLC/HPTLC-MS coupling is just one of many possibilities to gain information from a chromatographic run.

A new course organized by the German Chemical society (see last yellow page) shows the diversity of current coupling techniques. This course will be held in English in 2012.

The tentative program for the International HPTLC Symposium in Basel, 6th–8th July 2011 is ready. This and other current information is available on the homepage www.hptlc.com. 179 abstracts of scientists from 23 countries have been accepted as oral or poster presentations on current topics from a variety of application fields. Registration is still open (deadline 30th May). So far, already more than 120 participants have registered. Join us and experience how far TLC/HPTLC is suited for your tasks. All coupling techniques and application fields are represented. Come and exchange with the experts. The social program is attractive too.

Will I have the privilege to see you in Basel?
I would appreciate it!

Sincerely,

Gerda Morlock

Gerda Morlock
cbs@camag.com

CAMAG

MARCH 2011 106

THE CBS CLASSIFICATION SYSTEM

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

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

1. Reviews and books

106 001 Marta KUCHARSKA*, J. GRABKA (*Institute for Engineering of Polymer Materials and Dyes, Department of Dyes and Organic Products in Zgierz, 2/4 Chemików Street, 95-100 Zgierz, Poland): A review of chromatographic methods for determination of synthetic food dyes. Talanta, 80 (3), 1045-1051 (2010). Review on chromatographic methods for synthetic food dyes, including the following techniques: TLC, HPTLC, traditional column chromatography, HPLC, ion-pair chromatography, RP HPLC, and high performance ion chromatography, demonstrated by using examples in different conditions for each technique.

quality control, food analysis, HPTLC, review 1, 30

106 002 J. SHERMA (Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA, shermaj@lafayette.edu): Pesticides: TLC analysis. Encyclopedia of Chromatography Third Edition 1, 1749-1756 (2009). This review describes the current materials and techniques most widely used for the analysis of pesticides by TLC. In detail, information regarding sample preparation, stationary and mobile phases, detection and quantification is included. Specific examples of pesticides analysis in water and soil are also described.

environmental, HPTLC, densitometry, quantitative analysis, review 1b

2. Fundamentals, theory and general

106 010 V.G. BEREZKIN*, Svetlana KHREBTOVA (*Topchiev Inst., Petrochem. Syth., Russian Acad. Sci., Moscow 119991, Russia): Investigation of TLC in an N-Chamber. Chromatographia, 72(11-12), 1169-1176 (2010). Experimental and theoretical study of the adsorption kinetics of the gas phase of solvents most often used in TLC (methanol, acetic acid, ethyl acetate, toluene, chloroform, and acetone) in the N-chamber (the chamber most often used for analytical TLC, in 95 % of publications). To describe the kinetics an equation is proposed, which relates the solvent vapor adsorption to the time, the vapor pressure in saturated chambers and the diffusion coefficients of the solvents. It was found that as the characteristics of the adsorbent layer substantially improved by the manufacturers, the weight of mobile phase adsorbed by plates has increased several fold.

HPTLC 2c

106 011 M. KAMINSKA, Irena CHOMA* (*Department of Chromatographic Methods, University of M. Curie-Sklodowska, M. Sklodowska Sq. 3, 20-031 Lublin, Poland; irena.choma@poczta.umcs.lublin.pl): The influence of perchlorate ion concentration on the retention of fluoroquinolones in RP-TLC. J. Liq. Chromatogr. Relat. Technol. 33, 894-902 (2010). Five amphoteric piperazynyl fluoroquinolones and flumequine were analyzed in an RP system on C8 plates with acetonitrile/aqueous acidic mobile phases containing various concentrations of potassium perchlorate. Perchlorate as a so-called chaotropic ion caused the increase in the retention of basic fluoroquinolones. TLC of sarafloxacin, difloxacin, norfloxacin, enrofloxacin, ciprofloxacin, and flumequine on RP-8 with acetonitrile - water containing constant concentration of citric acid but various concentrations of potassium perchorate. After air-drying, fluoroquinolone zones were detected at 366 nm and 254 nm and flumequine only at 254 nm. The retention increased with the increasing concentrations of perchlorate ion in the mobile phase and achieved a plateau for the mobile phases containing 10-20 mM of chaotropic perchlorate.

qualitative identification 2d, 28a

106 012 C. SARBU*, R. D. BRICIU (*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos Str. No. 11, 400028, Cluj Napoca, Romania; csarbu@chem.ubbcluj.ro):

Lipophilicity of natural sweeteners estimated on various oils and fats impregnated thin-layer chromatography plates. J. Liq. Chromatogr. Relat. Technol. 33, 903-921 (2010). A variety of TLC-plates impregnated with oils (paraffin, olive, sunflower, corn, castor, cod liver) and fats (margarine butter, pig, sheep, pullet, human) were indirectly evaluated regarding lipophilicity by employing a series of experimental parameters estimated for a representative group of natural sweeteners from retention data. TLC of 13 sugars on oil- and fat-impregnated silica gel layers using five organic solvents as organic modifiers. Best results were obtained with mixtures of acetonitrile and water with chamber saturation for 10 min. Detection of the sugars by treatment with silver nitrate and sodium hydroxide followed by heating at 105 °C for 5 min.

food analysis, qualitative identification 2d, 11

106 013 J. TRIFKOVIC, F. ANDRIC, P. RISTIVOJEVIC, D. ANDRIC, Z. TESIC, Dusanka MILOJKOVIC* (*Faculty Chemistry, University of Belgrade, 11158 Belgrade, Serbia, dusankam@chem.bg.ac.rs): Structure-retention relationship study of arylpiperazines by linear multivariate modeling. J. Sep. Sci. 33, 2619-2628 (2010). HPTLC of 33 newly synthesized arylpiperazines on RP-18 with 1) methanol - water (with increasing concentrations of methanol from 80-100 %), 2) dioxane - water (with increasing concentrations of dioxane from 60-80 %), and 3) dimethyl sulfoxide - water (with increasing concentrations of dimethyl sulfoxide from 80-100 %). In all cases the organic modifier was increased in steps of 5 %. Quantitative determination by absorbance measurement at 254 nm. A quantitative structure-retention relationship study allowed to understand the chromatographic behavior of similar compounds.

pharmaceutical research, HPTLC, quantitative analysis 2c

3. General techniques

106 014 Virginia COMAN*, S. KREIBIK, M. VLASSA, M. FILIP (*Babes-Bolyai University, Raluca Ripan Institute for Research in Chemistry, 40 Fântânele Street, 400294 Cluj-Napoca, Romania; coman_virginia@yahoo.com, mvcoman@chem.ubbcluj.ro): Study of electric field geometry using the vertical planar dielectrochromatographic chamber. J. Planar Chromatogr. 23, 434-439 (2010). TLC of indophenol blue, Sudan red G, and 4-dimethylaminoazobenzene on aluminium oxide with toluene at 20 +/- 2°C with chamber saturation for 20 min. Experiments were done with a vertical PDEC chamber changing the geometry of the elctric field to obtain the most favorable chromatographic results. The tests showed that different electric field geometries which do not cross the glass support of the TLC plates can be used. This results in enhancement of the intensity of the electric field in the chromatographic layer, especially for the spherical and conical armatures. With these two armatures the best resolution of the solutes were achieved.

planar electrochromatography 3d

106 015 Y.Q. CUI (Cui Yongquan), Z.H. GE (Ge Zhaohui), D.Y. WANG* (Wang Dongyuan), B. YUAN (Yuan Bo) (*Department of Analytical Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016 CHINA; wdylxsy@hotmail.com): The behavior of an improved RP-18 sintered plate in planar electrochromatography. J. Planar Chromatogr. 23, 426-433 (2010). Mobile phases without addition of buffer salts could be successfully used in planar electrochromatography with a new type of RP-18 sintered plate, prepared from a 1:4 mixture of silica gel for TLC and powdered glass which was suspended in a solution of 0.3 % CMC-Na. The sintering step was the same as already described in J. Planar Chromatogr. 19, 313-318 (2006) except for the temperature which was 651 °C. A suitable degree of bonding of C18 was from 0.03 to 0.06, and good end-capping was achieved by placing the bonded sintered plates in a solution of dimethyldichlorosilane in toluene (ratio 1:100 to 1:1000) at room temperature for 20 min.

planar electrochromatography 3b

106 016 Ágnes M. MÓRICZ*, H. KALÁSZ (*Plant Protection Institute, Hungarian Academy of Sciences, Herman O. Str. 15, 1022 Budapest, Hungary; moricz_am@nki.hu): Centrifugal layer chromatography - rotation planar chromatography. J. Planar Chromatogr. 23, 415-419 (2010). The paper summarizes the classification and applicability of centrifugal layer chromatography and rotation planar chromatography (RPC). The combination of TLC with centrifugal force resulted in the introduction of centrifugal layer chromatography which was renamed rotation planar chromatography because the abbreviation CLC had been used for column liquid chromatography. In analytical RPC three development modes are possible - circular, anticircular, and linear. On-line and off-line sample application, separation, and detection can be combined. RPC is suitable for analytical, micro-preparative, and preparative separations. The term RPC covers five basic techniques: normal chamber, micro-chamber, ultra-micro chamber, column and sequential RPC. Most RPC methods are applicable to preparative separation of a single sample. Analytical RPC is rarely used.

comparison of methods 3d

106 017 E. TYIHÁK*, E. MINCSOVICS (*Plant Protection Institute, Hungarian Academy of Sciences, Herman O. Str. 15, P. O. Box 102, 1525 Budapest, Hungary; etyih@nki.hu): Forced-flow planar liquid chromatographic techniques (after twenty-two years). J. Planar Chromatogr. 23, 382-395 (2010). The paper summarizes progress in the main forced-flow planar liquid chromatographic (FFPLC) techniques taking into account one group of FFPLC used in practice, OPLC and rotation planar chromatography and another group like e. g. electrochromatographic techniques and shear-driven chromatography which show interesting results at an experimental level. Progress in FFPLC deals with the diversity of further instrumental developments and its basis, the instrument developments, determination of the role of the adsorbent layer in OPLC, challenges in the OPLC instrument development, and analytical and preparative applications of OPLC. Centrifugal layer or rotation planar chromatography is mentioned briefly. Two electrochromatographic techniques have been developed to accelerate the mobile phase flow, planar electrochromatography and planar dielectrochromatography using direct and alternating currents, respectively. One of the newest techniques among FFPLC is shear-driven chromatography in which the mobile phase is between two plates and is forced to flow above and inside the very thin adsorbent layer with shear-driven force generated by a moving plate. For the study of the biological activity of natural and synthetic compounds the planar adsorbent layer is advantageous, namely for in-vitro and in-vivo studies.

review 3d

4. Special techniques

106 018 L. BLUMBERG*, M.S. KLEE (*Fast GC Consulting, P.O. Box 1243, Wilmington, DE 19801, USA): A critical look at the definition of multidimensional separations. J. Chromatogr. A 1217(1), 99-103 (2010). Discussion of the definition of multidimensional (MD) separations, especially some potentially powerful separation techniques such as comprehensive 2D LC (LC × LC), and comprehensive 2D GC (GC × GC). The definitions of MD separations have been extended by some researches beyond their intended scope. This disqualifies comprehensive 2D techniques as LC × LC, GC × GC and 2D TLC from being considered as 2D techniques. In other instances, extended treatment of the definition is used as a basis to justify design-parameters of comprehensive 2D separations despite the fact that these parameters lead to sub-optimal implementations. The review draws attention to the shortcomings in the definition, discusses the weaknesses in the currently used definitions, and proposes to define n-dimensional analysis as one that generates n-dimensional displacement information.

doping, quantitative analysis, qualitative identification, review 4

106 019 Gertrud MORLOCK*, W. SCHWACK (*University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany): Hyphenations in planar chromatography. J. Chromatogr. A 1217 (43), 6600-6609 (2010). A review on hyphenations of planar chromatography and its most important subcategory HPTLC. Examples from the field of natural product search, food, and lipid analysis point out the hyphenation with effect-directed analysis and mass spectrometry and illustrate the efficiency gain. Depending on the task at hand, hyphenations can readily be selected, for example with MS, bioassays etc. as required to reach the relevant information about the sample. At the same time, information is obtained for many samples in parallel. The flexibility and the unrivalled features through the planar format valuably assist separation scientists.

doping, HPTLC, qualitative identification, review 4e

106 020 O. OVCHINNIKOVA, G. VAN BERKEL* (*Organic and Biological Mass Spectrometry Group, Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6131, USA, vanberkelgj@ornl.gov): Thin-layer chromatography and mass spectrometry coupled using proximal probe thermal desorption with electrospray or atmospheric pressure chemical ionization. Rapid Commun. Mass Spectrom. 24, 1721-1729 (2010). Ambient proximal probe thermal desorption (TD) sampling of substances from a HPTLC plate and coupled with secondary ionization by atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). The method does not require a specialized ionization source. Different anaytical parameters and performance metrics are reported and the method covers a wide range of analyte types including explosives, dyestuffs, herbicides and pharmaceuticals.

pharmaceutical research, environmental, herbal, agricultural, HPTLC, quantitative analysis, comparison of methods 4e

5. Hydrocarbons and halogen derivatives

106 021 T. BOROLE*, R. MEHENDRE, M. DAMLE, K. BOTHARA (*Dept. of Pharmaceutical Chemistry, AISSMS College of Pharmacy, Pune, Maharashtra, India): Development and validation of stability indicating HPTLC method for determination of prasugrel. Journal of Chemical and Pharmaceutical Research 2(4), 907-913 (2010). TLC on silica gel with dichloromethane - methanol 99:1. The hR_f value of prasugrel was 58±3. Prasugrel was subjected to stress test conditions like acid, alkali, neutral hydrolysis, oxidation, dry heat, and photo degradation. The zones corresponding to degradation products were well resolved from the main drug. Densitometric evaluation in absorbance mode at 254 nm. Linearity was in the range of 300-1500 ng/band.
pharmaceutical research, densitometry

quantitative analysis, HPTLC 5c

106 022 R. PATEL*, Mrunali PATEL, K. BHATT, B. PATEL (*A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Sardar Patel University, Vallabh Vidyanagar 388120, India, rbp.arcp@gmail.com): HPTLC method development and validation quantification of paliperidone in formulations and in vitro release study. Analytical Methods 2, 521-531 (2010). An HPTLC method for determination for paliperidone in formulation as well as for in vitro release studies has been developed. HPTLC on silica gel with methanol - ethyl acetate 4:1. The hR_f value was 54. Quantification was performed by densitometric evaluation at 254 nm. The method was linear in the range of 100-600 ng/band. The method was suitable for estimation of the drug in mucoadhesive microemulsion formulations, as well as for solubility and diffusion studies.

pharmaceutical research, quality control, densitometry, quantitative, analysis, HPTLC 5c

106 023 W. SOLOMON*, M. MANU, R. SIVAKUMAR, P. ANAND, R. VENKATANARAYANAN (*Dept. of Pharmaceutical Analysis, RVS College of Pharmaceutical Sciences, Sulur, Coimba-

tore, T.N., India, samwd_2000@yahoo.com): Application of TLC-densitometry method for estimation of acebrophylline in pharmaceutical dosage forms. Journal of Pharmacy Research 3(11) (2010). TLC on silica gel (plates pre-washed with methanol) with chloroform - isopropanol - toluene 8:1:1. The hR_f value was 22. Densitometric evaluation at 254 nm. The method was linear in the range of 100-5000 ng/band. The recovery was in the range of 99.8-101.5 %.

pharmaceutical research, quality control, densitometry, quantitative, analysis 5c

6. Alcohols

106 024 S. ARIYANATHAN*, A. SARASWATHY, G. RAJAMANICKAM (*Centre for Advanced Research in Indian system of Medicine (CARISM) SASTRA University, Thanjavur 613402, India, saraswathy20042000@yahoo.co.in): Quality control standards for the roots of three plumbago species. Ind. J. Pharma. Sci. 72(1), 86-91 (2010). Three species of Plumbago (Plumbaginaceae), i.e. P. zeylanica, P. carpensis, and P. rosea were studied for different physico-chemical parameters in addition to the estimation of microbial contamination, aflatoxins and pesticide residues and heavy metal content. All three species are used as herbs. The fingerprint profile of each species was compared using plumbagin as marker. Chloroform extracts of each plant were subjected to chromatography on silica gel with toluene - ethyl acetate 4:1 in a saturated twin trough chamber. Detection under UV 254 nm and 366 nm. The hR_f value of plumbagin was 70. The identity of plumbagin in the samples was shown by overlay of the UV spectra. Linearity was between 200 and 1000 ng/zone. The amount of plumbagin in the three species was between 0.01 and 0.17 %.

quality control, herbal, traditional medicine, densitometry, quantitative analysis 6

7. Phenols

106 025 V. DIGHE*, G. CHAREGAONKAR (*Dept. of Chemistry, Ramanarain Ruia College, Mantunga (E), Mumbai 400019, India): HPTLC quantification of sinigrin from seed powder of Brassica juncea (L.) czern and Brassica nigra (L.). Asian J. Chem. 22(7), 5349-5354 (2010). An HPTLC method is reported for estimation of sinigrin from seeds of Brassica juncea and Brassica nigra (Cruciferae). HPTLC of methanolic seed extracts on silica gel with ethyl acetate - methanol - water 6:2:1. Densitometric evaluation at 230 nm. The method was linear in the range of 400-1200 ng/band. Amounts of 0.43 and 1.55 % sinigrin were found in Brassica juncea and B. nigra, respectively. The method is suitable for routine quality control of herbal raw material.

traditional medicine, quality control, herbal, densitometry, quantitative analysis, HPTLC 7

106 026 Vidya DIGHE*, G. CHAREGAONKAR (*S. P. Mandal's Ramnarain Ruia College, Mantunga, Mumbai 400019, India): HPTLC quantitation of eugenol from leaf and berry powder of Pimenta dioica (L) merr. Analytical Chemistry - An Indian Journal 8(1), 29-33 (2009). A sensitive and accurate HPTLC method has been developed for the determination of eugenol from the leaf and berry powder of Pimenta dioica (L) Merr. The leaf and berry powders were extracted with methanol. HPTLC on silica gel with ethyl acetate - methanol - water 6:2:1. Detection and quantification by densitometry at 280 nm. Linear response to eugenol was found to be in the concentration range of 200-600 ng/band. The developed method can be used for routine quantitative monitoring of eugenol from the dried leaf and berry powder of Pimenta dioica (L) Merr.

quality control, herbal, densitometry, quantitative analysis, HPTLC 7

106 027 M. FAIYAZUDDIN*, S. AHMAD, Zeenat IQBAL, Sushma TALEGAONKAR, F. AHMAD, Aseem BHATNAGAR, R. KHAR (*Dept. of Pharmaceutical Faculty of Pharmacy, Hamdard University (Jamia Hamdard), Hamdard Nagar, New Delhi 110062, India): Stability indicating TLC method for determination of terbutaline sulphate in bulk and from submicronized dry pow-

der inhalers. Analytical Sciences 26 (4), 467-472 (2010). TLC of terbutaline sulphate on silica with chloroform - methanol 9:1. The hR_f value was 34. Densitometric evaluation at 366 nm. The method was linear in the range of 100–1000 ng/band. The recovery was being 99.6 %. When subjected to different stress conditions (acid, base, oxidative, and UV) the drug was not stable under acidic condition but stable under all other conditions. All degradation products are well resolved from the main compound. The proposed method is stability-indicating and suitable for quality control.

pharmaceutical research, quality control, densitometry, quantitative analysis 7

8. Substances containing heterocyclic oxygen

106 028 V. BORHADE*, Hema NAIR, D. HEGDE, C. BARHADE (*Dept. of Pharmacognosy & Phytochemistry, Bombay College of Pharmacy, University of Mumbai, Kalina, Santacruz (East), Mumbai, India): Development and validation of HPTLC method for estimation of tacrolimus in formulations. Drug Development and Industrial Pharmacy 35, 440-448 (2009). TLC of tacrolimus on silica gel with toluene - acetonitrile - glacial acetic acid 60:40:1. For quantitative evaluation the plate was derivatized with anisaldehyde sulfuric acid reagent and scanned at 675 nm. Using this method a compact spot was obtained at an hR_f value of 40. The linearity range was 100-800 ng/band. The method is suitable for routine analysis of formulations.

pharmaceutical research, quality control, densitometry, postchromatographic derivatization, quantitative analysis, HPTLC 8b

106 029 G. CHAKRABORTHY (SVKM'S, NMIMS University, School of Pharmacy and Technology Management, Shirpur Campus Shirpur, Maharashtra 425405, India): Quantitative estimation of ascorbic acid by HPTLC in different varieties of Amla. J Young Pharm 1(5), 82-85 (2010). HPTLC of ascorbic acid in different varieties of amla fruit (collected from different geographical regions) on silica gel with ethanol - acetic acid 19:1. The hR_f value of ascorbic acid was 76. Densitometric evaluation at 254 nm. The method was linear in the range of 1-5 µg/band. Bigger fruits were found to contain higher concentrations of ascorbic acid. The reported method was applied for estimation of ascorbic acid in crude drug as well as in herbal and pharmaceutical dosage form with reproducible results.

pharmaceutical research, herbal, food analysis, HPTLC 8b

106 030 P. HAMRAPURKAR*, M. PHALE, N. SHAH (*Dept. of Pharmaceutical Analysis, Prin. K. N. Kundanani College of Pharmacy, Jote Joy Bldg., R. S. Rd., Cuffe Parade, Coloba, Mumbai 400005, India): Quantitative estimation of efavirenz by high-performance thin-layer chromatography. J Young Pharm 1(4), 359-363 (2010). TLC of efavirenz on silica gel with acetonitrile - water - glacial acetic acid 12:8:1 in a twin-trough chamber saturated for 10 min. Densitometric evaluation at 247 nm. The method was linear in the range of 10-400 ng/band. The average recovery was 99.7 %.

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

106 031 P. KHUSHBOO*, V. JADHAV, V. KADAM (*Dept. of Q. A., Bharati Vidyapeeth's College of Pharmacy, Sector 8, CBD Belapur, Navi-Mumbai 400614, India, drvmjadhav_bvcop@rediffmail.com): Development and validation of a HPTLC method for determination of psoralen in Psoralea corylifolia (Bavachi). International Journal of ChemTech Research 1(4), 1122-1128 (2009). TLC on silica gel with toluene - ethyl acetate 3:1. The hR_f value of the furanocoumarin psoralen was 47. Densitometric evaluation at 299 nm. The method was linear in the range of 10-100 ng/band. The average recovery was 99.7 %.

herbal, densitometry, quantitative analysis 8b

106 032 U. PAWAR*, A. SULEBHAVIKAR, A. NAIK, S. PINGALE, K. Mangaonkar (*Dept. of Chemistry, Mithibai College of Arts, Chauhan Institute of Science & Amrutben Jivanlal College of Commerce & Economics, Vile Parle, Mumbai 400056, India): Simultaneous determination of rofecoxib and tizanidine by HPTLC. E-Journal of Chemistry 6(1), 295-302 (2009). HPTLC of rofecoxib and tizanidine on silica gel (plates pre-washed with methanol and dried at 110 °C) with toluene - ethyl acetate - methanol - triethyl amine 60:30:5:1 with chamber saturation for 15 min. The hR_f value of tizanidine was 49 and of rofecoxib 68. Rosiglitazone was used as internal standard. Densitometric evaluation at 235 nm. The method was linear in the range of 3.75-11.35 µg/band for rofecoxib and 0.30-0.70 µg/band for tizanidine. The recovery was 99.6-101.0 %.

pharmaceutical research, quality control, densitometry, quantitative analysis, HPTLC 8b,17a

106 033 S. RAKESH*, P. PATIL, V. SALUNKHE, P. DHABALE, K. BURADE (*Govt. College of Pharmacy, Vidyanagar, Tal. Karad 415124, Dist. Satara, M.S., India, sachinrakesh@rediffmail.com): HPTLC method for quantitative determination of quercetin in hydroalcoholic extract of dried flower of Nymphaea stellata willd. International Journal of ChemTech Research 1(4), 931-936, (2009). TLC of quercetin in hydroalcoholic extracts of dried flower of Nymphaea stellata (Nymphaceae). Separation on silica gel with toluene - ethyl acetate - formic acid 25:20:1. The hR_f value was 26. Densitometric evaluation at 380 nm. The method was linear in the range of 20-200 ng/band. The average recovery was 99.3 %.

quality control, herbal, densitometry, quantitative analysis 8b

106 034 A. RAO*, D. VARMA (*Dept of Pharmaceutical Ana, Shri vishnu college of Pharmacy, Vishnupur, Bhimavaram 534 202 (A.P) India): Development and validation of HPTLC method for the simultaneous estimation of rofecoxib and tizanidine hydrochloride in tablet dosage form. Int. J. Chem Sci 7(2), 986-992 (2009). A validated HPTLC method is reported for estimation of rofecoxib and tizanidine hydrochloride in combined dosage form. HPTLC on silica gel with methanol - ethyl acetate 1:1. The hR_f value of tizanidine was 45 and of rofecoxib 67. Densitometric evaluation at 254 nm. The method was linear in the range of 180-260 ng/band for tizanidine and 2000-3000 ng/band for rofecoxib. Recovery was in the range of 99.5–102.5 % for both compounds. The method was suitable for routine quality control of combine dosage form.

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

106 035 R. THORAT*, V. JADHAV, V. KADAM, S. KAMBLE & K. SALASKAR (*Dept. of Q. A., Bharati Vidyapeeth's College of Pharmacy, Sector 8, CBD Belapur, Navi-Mumbai 400614, India, drvmjadhav_bvcop@rediffmail.com): Development of HPTLC method for estimation of wedelolactone, quercetin and jatamansone in polyherbal formulation. International Journal of ChemTech Research 1(4), 1079-1086 (2009). TLC of wedelolactone, quercetin, and jatamansone in a polyherbal formulation. The formulation (oil) was extracted with methanol, the supernatant was concentrated under vacuum and used for chromatographic analysis. TLC on silica gel with toluene - acetone - formic acid 11:6:1 for wedelolactone, toluene - ethyl acetate - methanol 44:50:6 for quercetin and petroleum ether - acetone 3:1 for jatamansone. The hR_f value of jatamansone was 34, of wedelolactone 56, and of quercetin 47. Densitometric evaluation at 254 nm, 285 nm and 366 nm for quercetin, jatamansone, and wedelolactone respecticely. The method was linear in the range of 500-2500 ng/band for wedelolactone, 3000-8000 ng/band for quercetin and 2000-6000 ng/band for jatamansone. The polyherbal oil formulation contained 1.62 %, 0.24 % and 0.11 % of wedelolactone, quercetin, and jatamansone respectively.

herbal, clinical routine analysis, densitometry, quantitative analysis, HPTLC 8b

106 036 K. WANG (Wang Keqin)*, J. LUO (Luo Junwu), J. CHEN (Chen Jingping), L. CHEN (Chen Liang) (*Key Lab. of Tea Science, Ministry of Education, Hunan Agr. Univ., Changsha 410128, China): (Quantitative analysis of apigenin in celery by high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC)) (Chinese). Chinese J. Food Sci. 29 (4), 291-295 (2008). HPTLC of celery extracts on silica gel with chloroform - methanol - water 360:46:7. Detection and identification of the flavonoids by the characteristic chemical reaction colors and the UV spectrum of the prepared samples. Quantitative determination of apigenin by absorbance measurement at 345 nm. The linearity was between 330 and 990 ng/zone. Precision (*% RSD*) was 1.33 % ($n = 5$) and the recovery was 97.8 % ($n = 5$, *RSD* = 2.3 %).

food analysis, HPTLC, densitometry, quantitative analysis, qualitative identification, comparison of methods 8a

10. Carbohydrates

106 037 J. ANTOSCH, N. HADZIFEJZOVIC*, M. HUBBERT, L.-N. PRENNER, B. DONNER, J. SCHRAMM (*Rottapharm/Madaus, Madaus GmbH, Cologne, Germany; hadnih@hotmail.de): Establishment of xylose in Plantago ovata forssk. as a leading compound for quantification in raw material and finished product. J. Liq. Chromatogr. Relat. Technol. 33, 996-1004 (2010). TLC of xylose with acetonitrile - water 9:1 with chamber saturation at ambient temperature. Detection by dipping into 4-aminobenzoic acid reagent for 1 to 2 s followed by heating at 110 °C for 10 min. The hR_f of xylose was 56. Quantitative determination by densitometric evaluation at 366 nm. The linearity determination coefficient was $r^2 = 0.9999$. The recovery of xylose was between 102 and 106 %. The precision of a six-time preparation was 1.6 %.

herbal, quality control, densitometry, quantitative analysis 10a

106 038 S. ARORA*, P. SINGH, V. SHARMA, B. WADHWA, V. GEORGE, A. SINGH, G. SHARMA (*Dairy Chemistry Div. National Dairy Research Institute, Karnal 132001, India, sumitak123@yahoo.com): Analysis of sucralose and its storage stability in burfi. J Food Sci Technol 46(2), 114-117 (2009). A method for isolation and analysis of the artificial sweetener sucralose from the Indian sweet Burfi has been developed. For the isolation samples were suspended in water, treated with $K_3Fe(CN)_6$ solution and zinc sulfate, and filtrated. Semi-quantitative HPTLC analysis on amino layer with acetonitrile - water 4:1. The developed plate was heated at 190 °C for 20 min and spots were detected under UV 365 nm. Treating the plate with 5 % methanol CTMA increased the sensitivity (25 ng/zone). Quantitative HPTLC on silica gel with dichloromethane - methanol 4:1. Detection by spraying with 15 % methanolic sulfuric acid and heating at 100 °C for 10 min. The spot density was evaluated with Bio-rad fluorescence imaging software and confirmed by comparison with the standard. The method was linear in the range of 0.5-1.25 µg/band.

food analysis, HPTLC, quantitative analysis, qualitative identification 10a

106 039 M. VLASSA, Virginia COMAN*, M. FILIP, F. ONITA (*Babes-Bolyai University - Raluca Ripan Institute for Research in Chemistry, 30 Fântânele Street, 400294 Cluj-Napoca, Romania; coman_virginia@yahoo.com): OPLC of carbohydrate content for quality control of commercial Romanian wines. J. Planar Chromatogr. 23, 400-405 (2010). OPLC of fructose, glucose, and sucrose in 31 different types of wine (white and red; dry, semi-dry, semi-sweet, and sweet) on silica gel with acetontrile - water 13:2 (as the best of 6 mobile phases tested) under isocratic conditions in overrunning operation mode. Detection by spraying with a mixture of aniline and diphenylamine. Quantitative determination by absorbance measurement at 420 nm.

food analysis, quality control, quantitative analysis, densitometry 10a

11. Organic acids and lipids

106 040 S. AHMAD*, Y. KAMAL, M. SINGH, R. PARVEEN, M. MUSTHABA (*Dept. of Pharmacognosy & Phytochem., Faculty of Pharmacy, Jamia Hamdard, New Delhi 110062, India, sahmad_jh@yahoo.co.in): HPTLC determination of gallic acid in crude drugs and herbal formulations. Asian Journal of Chemistry 23(1), 469-470 (2011). Several herbal formulations were analyzed for gallic acid contents. Tablets were powdered, subjected to hydrolysis by refluxing with 10 % HCl, filtrated and extracted with chloroform. Acidic aqueous extracts were concentrated and the residue was taken up in methanol. TLC on silica gel with ethyl acetate - formic acid 85:11. Gallic acid was observed at an hR_f value of 89. Densitometric quantification of gallic acid at 272 nm. The method was linear in the range of 100-3000 ng/band. The method was suitable for analysis of formulations without interference from excipients. Gallic acid contents of different tablet samples varied from 0.06-0.15 % w/w.

traditional medicine, herbal, densitometry, quantitative analysis 11a

106 041 S. AZHLWAR*, T. RAVI (*Dept. of Pharmaceutical Analysis, College of Pharmacy, Sri Ramkrishna Institute of Paramedical Science, Coimbatore, India): Simultaneous densitometric analysis of Drotaverine and aceclofenac by HPTLC method. Scholars Research Library 2(2), 328-332 (2010). A validated densitometric method has been development for simultaneous estimation of drotaverine and aceclofenac in combined dosage form. TLC on silica gel with methanol - ethyl acetate - glacial acetic acid 100:90:1. The hR_f value of drotaverine was 18 and of aceclofenac 51. Densitometric evaluation at 300 nm. The method was linear in the range of 80-360 ng/band for drotaverine and 100-700 ng/band for aceclofenac. The average recovery was 99.8-100.2 %.

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

106 042 Girija BHAVAR, V. CHATPALLIWAR*, D.PATIL, S. SURANA (*Dept. of Pharmaceutical Chemistry, R. C. Patel College of Pharmacy Karvad Naka, Shirpur, Dist. Dhule 425405, vchatpalliwar@yahoo.co.in): Validated HPTLC method for simultaneous determination of quinapril hydrochloride and hydrochlorothiazide in a tablet dosage form. Indian J. Pharma. Sci. 70(4), 529-531 (2008). HPTLC on silica gel with ethyl acetate - acetone - acetic acid 13:6:1 with chamber saturation for 15 min. Densitometric evaluation at 208 nm. The hR_f value was 51 for quinapril hydrochloride and 76 for hydrochlorothiazide. The method was linear in the concentration range of 400-2800 ng/zone for quinapril and 500-3500 ng/zone for hydrochlorothiazide. The recovery was in the range of 98.3-100.8 %.

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

106 043 J. BOLSTRIDGE, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA 18042, USA, fried@lafayette.edu): Effects of temperature on the neutral lipid content of Biomphalaria glabrata as determined by high-performance thin-layer chromatography-densitometry and observations on snail fecundity. J. Liq. Chromatogr. Relat. Technol. 33, 1005-1012 (2010). HPTLC of lipids (free sterols, free fatty acids, triacylglycerols, methyl esters, and steryl esters) on silica gel (plates with concentration zone) with petroleum ether - diethylether - glacial acetic acid 80:20:1. Detection by spraying with 5 % ethanolic phosphomolybdic acid reagent and heating for 10 min at 110 °C. Quantitative densitometric analysis was performed at 610 nm.

HPTLC, densitometry, quantitative analysis 11c

106 044 S. CHITALANGE*, N. KUMAR, S. WANKHEDE (*Dept. of Pharmaceutical Chem., Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Sant Tukaram Nagar, Pimpri 411018, India, sohanchitlange@rediffmail.com): Stability-indicating HPTLC method for estimation

of dexibuprofen in pharmaceutical dosage form. Journal Pharmacy Research 2(9), 1542-1546 (2009) TLC on silica gel with *n*-hexane - ethyl acetate - glacial acetic acid 75:25:2. The hR_f value was 38. Densitometric evaluation at 225 nm. The method was linear over a concentration range of 100-350 ng/band. The average recovery was 100.2 %. The samples were subjected to different stress conditions (acid, base, oxidative, heat) and all the degradation products were well resolved from the main compound. The method is suitable for stability studies and routine quality control.

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

106 045 S. CHITLANGE*, G. PAWBAKE, A. MULLA, S. WANKHEDE (*Padm. DR. D. Y. Patil Institute of P'ceutical Sciences & Research, Pimpri, Pune 411018, M.S., India, sohanchitlange@rediffmail.com): Stability-indicating HPTLC method for estimation of diacerein in pharmaceutical dosage form. Research Journal of Pharmaceutical, Biological and Chemical Sciences 1(2), 226-234 (2010). TLC on silica gel with toluene - isopropanol - 25 % ammonia 23:23:4. The hR_f value was 30. Densitometric evaluation at 258 nm. The linearity range was 100-350 ng/band. For stability studies diacerein was subjected to different stress conditions (acid, alcohol, oxidative, thermal, photolytic). Degradation products were well resolved from the main zone of diacerein with significantly different hR_f values.

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

106 046 Fatma HELMY*, S. WHITE, S. AMIRI, R. AMIRI, A. SALIU (*Department of Biological Sciences, Delaware State University, 1200 North Dupont Highway, Dover, DE 19901, USA; fhelmy@desu.edu): On the different lipolytic capability of diverse organs from young adult guinea pigs. A chromatographic study. J. Planar Chromatogr. 23, 277-281 (2010). TLC of cardiolipin, monolysocardiolipin, and phosphatidylethanolamin plasmalogen on silica gel and alumina prewashed sequentially with chloroform - methanol 2:1 and acetone. For one-dimensional chromatography the mobile phase was 1-propanol - chloroform - ethyl acetate - methanol - water 50:50:50:21:18. Detection of phospholipids with thionine reagent. Two-dimensional chromatography in the first direction with the mobile phase mentioned before and in the second dimension with hexane - diethyl ether 4:1 after hydrolysis with 1 % hydrochloric acid to reveal alkenyl phospholipids. Detection by spraying with leucofuchsin reagent. TLC of choline lipids, phosphatidylcholine, and sphingomyelin on alumina with chloroform - methanol - water 65:30:4. Detection by treatment with Biebrich scarlet. Densitometric evaluation at 600 nm for thionine reagent and at 560 nm for leucofuchsin reagent.

densitometry, quantitative analysis, biochemical research 11

106 047 K. HUSSAIN*, Z. ISMAIL, A. SADIKUN (*Dept. of Pharmaceutical Chem. School of Pharmaceutical Science, University Sains Malaysis, Pulau Pinang 11800, Malaysia, hussain_761@yahoo.com): High-performance thin-layer chromatographic method for quantification of betulinic acid in extracts of leaves of Orthosiphon stamineus benth. Asian Journal of Chemistry 23(2), 977-979 (2011). Part of the aqueous extract of Orthosiphon stamineus (Lamiaceae) was chromatographed on a silica gel column and eluted with *n*-hexane - ethyl acetate 3:2. The eluate was concentrated in order to obtain betulinic acid for use as standard solution. TLC on silica gel with *n*-hexane - ethyl acetate - formic acid 150:100:1. For derivatization the plate was sprayed with anisaldehyde reagent followed by heating at 100 °C for 10 min. Quantification of betulinic acid by densitometric measurement in fluorescence mode at 366 nm. The method was linear in the range of 0.2-500 µg/band. The recovery was 96.1-98.4 %.

traditional medicine, herbal, densitometry, quantitative analysis, preparative TLC 11a

106 048 Snehal INGALE*, Dipali TAJANE, V. MODAK, S. GITE, V. CHOUDHARI, B. KUCHEKAR (*Maharashtra Institute of Pharmacy, MIT Campus, Paud Raod, Kothrud, Pune, MS, India): Development and validation of a HPTLC method for simultaneous estimation of drotaverine hydrochloride and diclofenac potassium in combined dosage form. Der Pharma Chemica 2(5), 126-132 (2010). HPTLC of drotaverine hydrochloride (DRO) and diclofenac potassium (DFK) in bulk and pharmaceutical dosage form on silica gel with toluene - ethyl acetate - methanol 1:4:1. Densitometric evaluation at at 298 nm. The hR_f value was 28 ± 5 for DRO and 51 ± 5 for DFK. The linearity was in the range of 160-1280 ng/band and 100-800 ng/band. The recovery was in the range of 99.8-101.2 % for DRO and 98.3-101.4 % for DFK.

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

106 049 Varsha JADHAV*, U. KEDAR, S. GHOLVE, V. KADAM (*Dept. of Q. A., Bharati Vidyapeeth's College of Pharmacy, Sector 8, CBD Belapur, Navi-Mumbai 400614, India, drvmjadhav_bvcop@rediffmail.com): Development and validation of HPTLC method for determination of glycyrrhizin in herbal extract and in herbal gel. International Journal of ChemTech Research 1(4), 826-831 (2009). A herbal extract and herbal gel were extracted with 70 % ethanol, filtered and used for application. TLC on silica gel (plates pre-washed with methanol) with ethyl acetate - methanol - water - formic acid 15:2:1:1. The hR_f value was 34. Densitometric evaluation at 252 nm. Glycyrrhizin monoammonium salt was used as standard. The method was linear in the range of 200-1000 ng/band, the recovery was 95.9-98.2 % . The glycyrrhizin contents in the extract and gel were in the range of 17.9-18.5 %.

quality control, herbal, densitometry, quantitative analysis 11a

106 050 R. JESWANI*, P. SINHA, K. TOPAGI, M. DAMLE (* Dept. of Pharmaceutical Chem., A.I.S.S.M.S. College of Pharmacy, Pune 411001, India, mcdamle@rediffmail.com): A validated stability indicating HPTLC method for determination of cephalexin in bulk and Pharmaceutical formulation. International Journal of PharmaTech Research 03, 527-538 (2009). A stability indicating HPTLC method has been developed for analysis of cephalexin in bulk and dosage formulation. HPTLC on silica gel with ethyl acetate - methanol - 25 % ammonia 6:4:1. The hR_f value was 56. Densitometric quantification at 260 nm. The method was linear in range of 500-1500 ng/band. Cephalexin was subjected to forced degradation (acid, alkali, oxidation, thermal, photolytic). All degradation products were well separated from the drug, indicating specificity of the method.

pharmaceutical research, densitometry, quantitative analysis, HPTLC 11a

106 051 R. KUMAR*, N. SRISUTHERSON, P. NALLASIVAN, P. ARULRAJ, R. VENKATNARAYANAM (*Dept. of Pharmaceutical Analysis, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore 641402, Tamil Nadu, India, andrilan@rediffmail.com): HPTLC method for the simultaneous estimation of aceclofenac and diacerein in tablets dosage forms. Research J. Pharm. and Tech. 3(3), 825-827 (2010). TLC on silica gel (plates pre-washed with methanol) with chloroform - methanol 4:1. The hR_f value of aceclofenac was 38 and of diacerein 66. Densitometric evaluation at 256 nm. The linearity range was 10-50 µg/band for aceclofenac and 5-25 µg/band for diacerein. The recovery was in the range of 99.3-101.8 % for both compounds. The method was suitable for routine quality control of combined dosage forms without any interference from the excipients.

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

106 052 S. MANIMARAN*, M.V.N.L. CHAITANYA, S. DHANABAL, B. SURESH, R. CHANDRASEKAR, M. NANJAN (*Dept. for Phytopharm. & Phytomedicine TIFAC CORE in Herbal Drugs

J.S.S. College of Pharmacy, Ootacamund, Tamilnadu, India): Estimation of n-valeric acid content in Valeriana officinalis by HPTLC technique. Indian Drugs 47(3), 55-56 (2010). An HPTLC method is described for estimation of n-valeric acid in Valeriana officinalis (Valerianaceae). Methanolic extracts of the plant were subjected to chromatographic analysis on silica gel with toluene - ethyl acetate 3:1. Densitometric evaluation at 254 nm. The method was linear in the range of 500-1500 ng/band with a recovery between 98.4-104.1 %. The plant extract was found to contain 1.13 % of *n*-valeric acid.

herbal, densitometry, quantitative analysis, HPTLC 11a

106 053 I. MAREKOV*, S. PANAYOTOVA, R. TARANDJIISKA (*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., blok 9, 1113 Sofia, Bulgaria; ilko@orgchm.bas.bg): Silver ion TLC of minor triacylglycerol components for unambiguous detection of adulteration of olive oil with vegetable oil. J. Liq. Chromatogr. Relat. Technol. 33, 1013-1027 (2010). Preparative TLC of triacylglyerol (TAG) fractions on silica gel with hexane - acetone 25:4. Analytical TLC of TAG classes from sunflower, corn, soybean, cotton, and olive oil (differing in saturation) on silica gel, impregnated by dipping into a 5 % methanolic solution of silver nitrate, with petroleum ether - acetone - ethyl acetate 100:3:2 and 50:3:2. Detection by exposure to bromine and sulfuryl chloride vapor and heating at 180-200 °C . Quantitative determination by absorbance measurement at 450 nm.

food analysis, quality control, quantitative analysis, densitometry 11c

106 054 A. MOHAMMAD*, S. SHARMA, S. BHAWANI (*Analytical Research Lab., Dept. of Applied Chemistry, Faculty of Engineering & Technology, Aligarh Muslim Univ., Aligarh 202002, India, alimohammad08@gmail.com): Identification of ketoprofen in drug formulation and spiked urine samples by micellar thin-layer chromatography and its quantitative estimation by high performance liquid chromatography. International Journal of ChemTech Research 2(1), 89-96 (2010). A micellar thin layer chromatographic method has been reported for identification of ketoprofen in drug formulation and spiked urine samples, followed by quantification of the drug by HPLC both in formulation and spiked urine samples. TLC on amino layer with a micellar mobile phase of 0.5 % aqueous solution of sodium dodecyl sulphate -Triton X100 - acetone 16:10:3. The drug was extracted from spiked urine samples with ethylene dichloride containing 10 % each of isomyl alcohol and diethyl ether, then the organic phase was evaporated and the residue was taken up in acetonitrile. Derivatization by exposure to iodine vapor and by spraying with Dragendorffs reagent. The hR_f value of ketoprofen was 50.

pharmaceutical research, comparison of methods, qualitative identification, postchromatographic derivatization 11a

106 055 Preeti NAYAK*, S. UPADHYAYA, Anubha UPADHYAYA (*Dept. of Crop & Herbal Physiology, College of Agriculture, JNKV, Jabalpur, M.P., India): A HPTLC densitometer determination of sinapic acid in Chandrasur (Lepidium sativum). J. Sci. Res. 1(1), 121-127 (2009). An HPTLC method is described for qualitative and quantitative estimation of sinapic acid in Lepidium sativum (Brassicaceae). Methanolic extracts of the plant material were subjected to chromatographic separation on HPTLC silica gel with *n*-butanol - acetic acid - water 4:1:5. Derivatization with anisaldehyde sulfuric acid reagent, followed by heating at 110 °C for 10 min. Denstiometric quantification at 326 nm. Identification was confirmed by comparison of hR_f values of sample and standard.

quality control, herbal, HPTLC, densitometry, qualitative identification, quantitative analysis, postchromatographic derivatization 11a

106 056 K. PAGI*, S. LAHIRI, G. YADAV, Mamta SHAH (*Dept. of Phytochem. and Pharmacognosy, L.M. College of Pharmacy, Navarangpura, Ahmedabad, Gujarat 380009, India, mbshah2007@rediffmail.com): Development and validation of HPTLC method for determination of betulinic acid in Helicteres isora root extract. International Journal of ChemTech Research 2(2), 851-855 (2010). Defatted powdered roots of Helicteres isora (Sterculiaceae) were extracted with ethyl acetate. TLC on silica gel with toluene - acetone - formic acid 25:5:2. The hR_f value was 43. Densitometric evaluation at 540 nm. The method was linear in the range of 100-500 ng/band. Recovery was 98.4-99.9 %.

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

106 057 Marta PALUSINSKA-SZYSZ*, M. JANCZAREK, R. KALITYNSKI, A. DAWIDOWICZ, R. RUSSA (*Department of Genetics and Microbiology, Institute of Biotechnology and Microbiology, Maria Curie-Sklodowska University, Akademicka St. 19, Lublin, Poland, marta.szysz@poczta.umcs.lublin.pl): Legionella bozemanae synthesizes phosphatidylcholine from exogenous choline. Microbiol. Res.166, 87-98 (2011). Two-dimensional TLC of phospholipids from Legionella bozemanae on chloroform - methanol - water 14:6:1 in the first dimension and chloroform - methanol - glacial acetic acid 13:5:2 in the second dimension. Lipids were detected by spraying with concentrated sulfuric acid or by exposure to iodine vapor. The following phospholipids were identified: phosphatidylcholine, phosphatidyl-N,N-dimethylethanolamine, phosphatidylethanolamine, phosphatidyl-N-monomethylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.

pharmaceutical research, HPTLC, quantitative analysis, densitometry 11c

106 058 A. PAPRIKAR, K. PAtil, S. RANAFE, V. PANE, S. RAO, M. DAMLE* (*Dept. of Pharmaceutical Chemistry, AI.S.S.M.S. College of Pharmacy, Kennedy Rd., Near R.T.O. Pune 411001, mcdamle@rediffmail.com): Stress degradation monitoring of ofloxacin by HPTLC and bioautography. Research J. Pharm. and Tech. 3(4), 1275-1278 (2010). A stability indicating HPTLC method has been developed for ofloxacin. The results were confirmed by bioautography. HPTLC on silica gel with *n*-butanol - ethanol - 25 % ammonia 5:5:4. The hR_f value was 53. Densitometric evaluation at 299 nm. The method was linear in the range of 500-2500 ng/band. The sample was subjected to forced degradation (acid, base, oxidation, thermal, photolytic) and both degraded and un-degraded sample were analyzed by HPTLC and bioautography. The result obtained by bioautography confirmed that the antimicrobial activity of ofloxacin was directly related to its degree of degradation under different stress conditions. Further no inhibition zone was observed at another hR_f than that of ofloxacin, which indicates that degradation products do not exhibit antibacterial activity. Bioautography studies were carried out in petri plates (10 cm diameter) using as media Mueller Hinton agar and as organism Escherichia coli NCIM 2066.

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

106 059 N. PATEL*, V. JAIN, A. BHARGAV, D. SHAH (*C. K. Pithawala Institute of Pharmaceutical Science & Research Surat 395007, Gujarat, India): High-performance thin-layer chromatographic method for quantification of gallic acid in bark powder of Terminalia crenulata Roth. Indian Drugs 47(6), 59-61 (2010). HPTLC of gallic acid in bark powder of Terminalia crenulata (Combretaceae) on silica gel with toluene - ethyl acetate - formic acid - methanol 60:60:16:4 with chamber saturation for 30 min. Densitometric evaluation at 254 nm. The method was linear in the range of 200-1200 ng/band. Samples extracted with different solvents were compared. Both alcoholic and water extracts were found to contain gallic acid where as it was absent in the chloroform extract.

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

106 060 L. POTALE, Mrinalini DAMLE*, A. KHODKE, K. BOTHARA (*AISSIMS College of Pharmacy, Kennedy Rd., Near RTO Pune 411001, M.S., India, mcdamle@rediffmail.com): A validated stability indicating HPTLC method for simultaneous estimation of ramipril and telmisartan. International Journal of Pharmaceutical Sciences Review and Research 2(2), 35-39 (2010). TLC on silica gel with methanol - chloroform 1:6. The hR_f value was 38 and 68 for ramipril and telmisartan respectively. Densitometric evaluation at 210 nm. The method was linear in the range of 300-3000 ng/band and 500-4000 ng/band for ramipril and telmisartan respectively. The recovery was 98-102 %. The sample was subjected to different stress conditions (acid, base, oxidative, heat, photolytic) and all the degradation products were well separated from drug.

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

106 061 Manisha PURANIK*, D. BHAWSAR, Prachi RATHI, P. YEOLE (*Institute of Pharmaceutical Education and Research, P. G. Dept. of Q. A., Borgaon (Meghe), Wardha 442001, India, manisha68_12@yahoo.com): Simultaneous determiniation of ofloxacin and ornidazole in solid dosage form by RP-HPLC and HPTLC techniques. Ind. J. Pharma. Sc. 72(4), 513-517 (2010). TLC on silica gel with dichloromethane - methanol - 25 % ammonia 95:10:3. Ofloxacin and ornidazole were well separated. Linearity was in the range of 20-100 ng/band for ofloxacin and 50-250 ng/band for ornidazole. Recovery was in the range of 99.3-100.5 %.

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

106 012 C. SARBU et al., see section 2

106 062 A. RAO* D. SUNEETHA (*Dept. of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupuri, Bhimavaram 5342012, India): High-performance thin-layer chromatographic estimation of simvastatin in bulk and tablet dosage form. Asian J. Chemistry 22(1), 27-30 (2010). HPTLC of simvastatin (in bulk and tablet dosage form) on silica gel with toluene - ethyl acetate - formic acid 10:3:1. The hR_f value of simvastatin was 26. Densitometric evaluation at 242 nm. The method was linear in the range of 200-1000 ng/band.

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

106 063 A. RAO*, N. PRUDHVI, K. BASAVARAJ, Y. MANOHARA (*Dept. of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram 534202, AP, India): Development and validation of HPTLC method for the estimation of cefetamet. Rasayan J. Chem. 2(3), 720-723 (2009). HPTLC of cefetamet on silica gel with toluene - chloroform - methanol 3:6:1. The hR_f value of cefetamet was 35. Densitometric evaluation at 236 nm. The method was linear in the range of 1-5 µg/band. The recovery was 99.3-101.5 %. The method was found suitable for routine quality control.

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

106 064 P. RATHEE*, Sushila RATHEE, Deepti AHUJA (no address given): Simultaneous quantification of glycyrrhetinic acid and apigenin using HPTLC from Glycyrrhiza glabra linn. Eurasian J. Anal. Chem 5(1), 95-103 (2010). An HPTLC method is reported for quantification of glycyrrhetinic acid and apigenin in Glycyrrhiza glabra. Methanolic and acidic-methanolic extracts are subjected to chromatographic separation on silica gel with ethyl acetate - ethanol - water - 25 % ammonia

63:20:4:1. The method was linear in the range of 160-960 ng/band for glycyrrhetinic acid and 32-96 ng/band for apigenin. Glycyrrhiza glabra was found to contain 0.65 % of glycyrrhetinic acid and 0.0004 % of apigenin.

quality control, herbal, densitometry, quantitative analysis, HPTLC 11a

106 065 R. RELE*, S. SAWANT (*Dept. for Chemistry, D.G. Ruparel College, Mahim, Mumbai 400016, India): Simultaneous determination of paracetamol and Ibuprofen from combined dosage formulation by HPTLC method. Analytical Chemistry - An Indian Journal 9(1) (2009). An HPTLC method has been developed for simultaneous estimation of paracetamol and ibuprofen. HPTLC on silica gel with ethyl acetate - acetone - *n*-butanol - 25 % ammonia 3:4:3:1. Densitometric quantification at 254 nm. The hR_f value of ibuprofen was 32 and of paracetamol 86. The method was linear in the range of 120-600 ng/band for paracetamol and 130-650 ng/band for ibuprofen.

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

106 066 C. ROOSEWELT*, N. HARIHRISHNAN, V. GUNASEKARAN, S CHANDRASEKARAN, V. HARIBASKAR, B. PRATHAP (*Dept. of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Vel's University, Pallavaram, Chennai 600117, India): Simultaneous estimation and validation of tramadol and paracetamol by HPTLC in pure and pharmaceutical dosage form. Asian Journal of Chemistry 22(2), 850-854 (2010). HPTLC of tramadol and paracetamol in pharmaceutical dosage form on silica gel with chloroform - methanol - glacial acetic acid 90:20:1. The hR_f value of tramadol was 48 and of paracetamol 68. Densitometric quantification at 270 nm. The method was linear in the range of 500-2000 ng/band for both compounds. Recovery was 98.9-99.7 %.

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

106 067 P. ROVINA, C. GRAF, F. BORNANCIN* (*Novartis Institutes for Biomedical Research, Brunnerstrasse 59, Vienna, Austria, frederic.bornancin@novartis.com): Modulation of ceramide metabolism in mouse primary macrophages. Biochem. Biophys. Res. Commun. 399, 150-154 (2010). HPTLC of the lipids from mouse primary macrophages on silica gel with butanol - acetic acid - water 3:1:1. Detection by imaging using Fujifilm intelligent dark box in SYBR green fluorescent light. Quantitative determination with a TLC plate imaging software.

pharmaceutical research, HPTLC, quantitative analysis, densitometry 11c

106 068 M. SAJEWICZ, D. KRONENBACH, M. GONTARSKA, Teresa KOWALSKA* (*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland, teresa-kowalska@us.edu.pl): TLC and polarimetric investigation of the oscillatory in vitro chiral conversion of R-beta-hydroxybutyric acid. J. Liq. Chromatogr. Relat. Technol. 33, 1047-1057 (2010). TLC of R-beta-hydroxybutyric acid on silica gel pre-washed with methanol - water 9:1 and impregnated by dipping for 2 s in a 0.05 mol/L water - methanol solution of copper acetate. TLC was carried out at 22 +/- 1 °C with dioxane - water 9:1. Detection by spraying with 5 % sulfuric acid in ethanol and heating at 100-110 °C for 10 min. Quantitative determination by absorbance measurement at 326 nm. It was experimentally established that R-beta-hydroxybutyric acid can undergo oscillatory chiral conversion.

quantitative analysis, densitometry 11a

106 069 Smita SHARMA*, M. SHARMA, D. KOHLI, A. SHARMA (*Dept. of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar (M.P.), India): Development and validation of TLC-densitometry method for simultaneous quantification of montelukast sodium and levocetirizine

dihydrochloride pharmaceutical solid dosage form. Scholars Research Library 2(1), 489-494 (2010). HPTLC of montelukast sodium and levocetirizine dihydrochloride on silica gel with toluene - chloroform - methanol - glacial acetic acid 6:20:10:1 in a twin trough chamber with chamber saturation for 25 min. The hR_f value of levocetirizine was 64 and of montelukast 89. Densitometric evaluation at 302 nm. The method was linear in the range of 200-3200 ng/band for montelukast and 400-1200 ng/band for levocetirizine. The recovery was 99.9-100.0 %.

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

106 070 M. SHARMILA, S. SHARMA* (*Devi Ahilya Vishwavidyalaya Takshshila Campus, Khandwa Rd., Indore 452001, M.P., India, mukeshcsharma@yahoo.com): Development and validation of an HPTLC method for determination of oseltamivir phosphate in pharmaceutical dosage form. Indian Drugs 47(11), 68-72 (2010). TLC on silica gel with ethyl acetate - acetic acid - water 15:3:2. The hR_f value was 57. Densitometric evaluation at 265 nm. The method was linear in the range of 200-2400 ng/band. The method was suitable for routine quality control of the drug in formulation as there is no interference from excipients.

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

106 071 P. SINHA*, Munalini DAMLE, K. BOTHARA (*AISSMS College of Pharmacy,Kennedy Rd., Pune 411001, India): A validated stability indicating HPTLC method for determination of aspirin and clopidogrel bisulphate in combined dosage form. Eurasian J. Anal Chem 4(2), 152-160 (2009). A stability indicating HPTLC method has been reported for estimation of aspirin and clopidogrel bisulphate in combined dosage form. HPTLC on silica gel with carbon tetrachloride - acetone 5:2. The hR_f value of aspirin was 13 and of clopidogrel 78. Densitometric evaluation at 220 nm. Linearity was in the range of 200-600 ng/spot for aspirin and 300-600 ng/spot for clopidogrel. Degradation products (acid, base oxidative, dry heat, photodegradation) did not interfer with the analysis.

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

106 072 Malgorzata STAREK*, J. KRZEK (*Department of Inorganic and Analytical Chemistry, Jagiellonian University, Collegium Medium, 9 Medyczna Str., 30-688 Cracow, Poland): TLC chromatographic-densitometric assay of ibuprofen and its impurities. J. Chromatogr. Sci. 48(10), 825-829 (2010). TLC of ibuprofen and its impurities in pharmaceutical preparations on silica gel with toluene - ethyl acetate - glacial acetic acid 17:13:1. Quantification by densitometry. The limit of detection and quantification ranges from 0.13-0.27 µ g/zone. The recovery is 96.8-99.0 % for the individual constituents. The method is suitable for routine quality-control analysis of pharmaceuticals containing ibuprofen.

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

106 073 M. TARES*, L. BEBAWY, S. MOHAMMED (*Analytical Dept. Faculty of Pharmacy, Cairo University, Egypt): Determination of lacidipine by thin-layer chromatographic densitometric, spectrophotometric method and modified vierordt‘s method in presence of its photodegradates. Analytical Chemistry - An Indian Journal 8(3) (2009) (without page number). A TLC method has been developed for determination of lacidipine in presence of its photodegradates. TLC on silica gel with toluene - acetone - methanol - 25 % ammonia 30:10:3:1. Densitometric evaluation at 284 nm. The chromatographic method was compared with a derivative spectrophotometric method and vierordt‘s method and was found suitable for analysis of lacidipine in bulk drug as well as in dosage form.

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

106 074 T. TOGAWA, I. KAWASHIMA, T. KODAMA, T. TSUKIMURA, T. SUZUKI, T. FUKUSHIGE, T. KANENURA, H. SAKURABA* (*Department of Analytical Biochemistry, Meiji Pharmaceutical University, Tokyo, Japan,sakuraba@my-pharm.ac.jp): Tissue and plasma globotriaosylsphingosine could be a biomarker for assessing enzyme replacement therapy for Fabry disease. Biochem. Biophys. Res. Commun. 399, 716-720 (2010). HPTLC of globotriaosylceramide (Gb3) in mouse tissues on silica gel with chloroform - methanol - water 65:25:4. Detection by HPTLC-immunostaining with an anti-Gb3 monoclonal antibody. Quantification by densitometry with a luminescent image analyzer. The limit of detection of Gb3 was 50 ng/zone.

pharmaceutical research, HPTLC, quantitative analysis, densitometry 11e

106 075 N. VEKARIYA*, G. PATEL, H. BHATT, M. PATEL, R. DHOLAKIYA, G. RAMANI (*Shree Dhanvantary Pharmacy College, Kim, Dist Surat, Gujarat, India, Smt B.N.B. Swaminarayan Pharmacy College, Salvav Dist. Valsad, Gujarat, India): Application of TLC-densitometry method for simultaneous estimation of telmisartan and amlodipine besylate in pharmceutical dosage form. International Journal of PharmTech Research 1(4), 1644-1649 (2009). TLC of telmisartan and amlodipine besylate on silica gel with tetrahydrofuran - dichloromethane - methanol - 25 % ammonia 30:10:5:1. The hR_f value for telmisartan was 22 and for amlodipine 45. Densitometric evaluation in absorbance mode at 326 nm. The method was linear in the range of 1200-7200 ng/band for telmisartan and 400-1400 ng/band for amlodipine. The average recovery was 100.7-101.5 %.

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

106 076 N. VEKARIYA*, M. PATEL, G. PATEL, R. DHOLAKIYA (*Shree Dhanvantary Pharmacy College, KIM, Smt. B.N.B. Swaminarayan Pharmacy College, Salvav, India, nitin.vekariya@gmail.com): Development and validation of TLC-densitometry method for simultaneous determination of telmisartan and amlodipine besylate in bulk and tablets. J Young Pharm 1(3), 259-263 (2009). A validated HPTLC method is described for simultaneous estimation of amlodipine besylate and telmisartan in dosage form. HPTLC on silica gel with tetrahydrofuran - dichloroethane - methanol - 25 % ammonia solution 60:20:10:4. The hR_f value of amlodipine besylate was 45 and of telmisartan 22. Densitometric evaluation at 326 nm. Linearity was in the range of 1200-7200 ng/band for telmisartan and 400-1400 ng/band for amlodipine besylate. The limit of detection was 149 ng/zone and 53 ng/zone for telmisartan and amlodipine besylate, respectively. The limit of quantification was 453 ng/zone for telmisartan and 161 ng/zone for amlodipine besylate. The recovery was between 100.4 and 100.8 %.

pharmaceutical research, quality control, HPTLCdensitometry, quantitative analysis 11a, 23e

13. Steroids

106 077 C. ONISOR, M. POSA, S. KEVRESAN, K. KUHAJDA, C. SARBU* (*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos 11, 400028 Cluj-Napoca, Romania, csarbu@chem.ubbcluj.ro): Estimation of chromatographic lipophilicity of bile acids and their derivatives by reversed-phase thin layer chromatography. J. Sep. Sci. 33, 3110-3118 (2010). HPTLC of bile acids and their derivatives on 1) RP-18, 2) RP-18W and 3) cyano phase with methanol - water mixtures. The volume fraction of organic solvent in the mobile phase ranged from 70-90 % for (1), 50-70 % for (2) and 45-65 % for (3). Detection by spraying with a solution of manganese chloride in sulfuric acid, followed by heating at 100-120 °C for 10 min. Quantifica-

tion by absorbance measurement at 254 nm and 365 nm.

pharmaceutical research, HPTLC, quantitative analysis, densitometry 13d

15. Terpenes and other volatile plant ingredients

106 078 Rani BHAGAT, D. KULKARNI* (*Botany Group, Agharkar Research Institute, Pune 411004, India, dilipkkulkarni@gmail.com): Quantification of beta-sitosterol from three Jatropha species by high-performance thin-layer chromatography. Asian Journal of Chemistry 22(10), 8117-8120 (2010). HPTLC of beta-sitosterol in leaves, roots and seed oil of 3 species of Jatropha. Chromatographic separation on silica gel with toluene - methanol 9:1. The hR_f value of beta-sitosterol was 54. Derivatization with anisaldehyde-sulfuric acid reagent, followed by heating at 120 °C. After derivatzation densitometric evaluation at 525 nm. The method was linear in the range of 100-500 ng/band. The proposed method was used for the estimation of beta-sitosterol in extracts of different species of plants. The presence of beta-sitosterol in samples was confirmed by multi-wave length scanning.

traditional medicine, quality control, herbal, HPTLC, densitometry, quantitative analysis 15a

106 079 Ágnes M. MÓRICZ*, G. HORVÁTH, P. MOLNÁR, B. KOCSIS, A. BÖSZÖRMÉNYI, É. LEMBERKOVICS, P. G. OTT (*Plant Protection Institute, Hungarian Academy of Sciences, Herman O. Str. 15, 1022 Budapest, Hungary; moricz_am@nki.hu): Investigation of thyme (Thymus vulgaris L.) essential oil by use of the BioArena system. J. Planar Chromatogr. 23, 406-410 (2010). TLC and OPLC of essential oil of thyme (thymol, carvacrol, and linalool as standards) on silica gel with chloroform (previously extracted with a 0.1 % aqueous solution of sodium hydrogen carbonate and dried on sodium sulfate to eliminate the stabilizer amylene, free hydrochloric acid, and chlorine) in an unsaturated chamber. Detection at 254 nm, by spraying with vanillin - sulfuric acid reagent (50 mg vanillin with 12 mL ethanol and 200 mL 98 % sulfuric acid) and heating to 70 °C for 10 min, and by use of the BioArena system. Quantitative determination by densitometry at 275 and 600 nm (dual-wavelength measurement).

herbal, traditional medicine, densitometry, quantitative analysis, qualitative identification 15b

106 080 F.A. MEHTA*, B.G. PATEL*, S.S. PANDYA, K.B. AHIR (*Indukaka Ipcowala College of Pharmacy, P. O. Box 53, PO Vithal Udyognagar, Beyond GIDC Phase IV, New Vallabh Vidyanagar, Gujarat 388 121, India; fm999@ymail.com): Densitometric HPTLC method for analysis of oleanolic acid in Achyranthes aspera L. J. Planar Chromatogr. 23, 289-292 (2010). HPTLC of oleanolic acid on silica gel pre-washed with methanol and acetone using toluene - ethyl acetate - formic acid 9:1:2 in a twin-trough chamber saturated for 30 min. Detection by spraying with 10 % sulfuric acid in ethanol, followed by heating at 130 °C for 3 min. Quantitative determination by densitometry at 490 nm. Linearity was between 600 and 1000 ng/band; the correlation coefficient was 0.994. The limit of detection and quantification was 27 and 82 ng/band. The repeatability (*%RSD, n* = 6) was 0.61 %, and the recovery was between 97.8 and 99.2 %. The inter-day and intra-day precision (*RSD, n* = 3) was 1.25 and 0.83 % (600 ng/band), 1.00 and 1.03 % (800 ng/band), and 1.11 and 0.81 % (1000 ng/band).

herbal, traditional medicine, densitometry, quantitative analysis, qualitative identification 15a

106 081 S. MUSTHABA*, M. ATHAR, S. BABOOTA & S. AHMAD (*Dept. of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110062, India, sahmad_jh@yahoo.co.in): HPTLC analysis of neem oil in herbal dosage form. Asian Journal of Chemistry 23(1), 385-387 (2011). HPTLC on silica gel with chloroform - ethyl acetate containing 1 % acetic acid. Two well resol-

ved zones with hR_f values of 33 and 55 were obtained by illumination at 254 nm. The zones were labelled as substance I and II and used for standardization of the oil. Densitometric evaluation at 265 nm. The linearity range for both substances was 4-100 µg/band. The recovery was in the range of 97.4-98.7 %. Several commercially available tablets and capsules were analyzed for the content of Neem oil using substances I and II as marker (in the absence of chemical markers).

herbal, traditional medicine, densitometry, quantitative analysis, qualitative identification, radioscanning 15b

106 082 A. PAREKH, V. JADHAV* (*Dept. of Pharmacology Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, India, drvmjadhav_bvcop@rediffmail.com): Development of validated HPTLC method for quantification of jatamansone in jatamansi oil. Journal of Pharmacy Research 2(5), 975-977 (2009). Quantitative analysis of jatamansone in jatamansi oil obtained by steam distillation of rhizome extracts of Nardostachys jatamansi (Valerianaceae). HPTLC on silica gel with petroleum ether - acetone 3:1 in a saturated twin trough chamber. The hR_f value of jatamansone was 43. Densitometric evaluation at 285 nm. The method was linear in the range of 250-1500 ng/band. The recovery was 98.8-102.2 %. The oil was found to contain 20.3 % of jatamansone.

herbal, HPTLC, quantitative analysis, densitometry 15b

106 083 K.C. PATRA*, K.J. KUMAR (*Institute of Pharmaceutical Sciences, Guru Ghasidas Viswavidyalaya (Central University), Bilaspur 495009, India: herbalkartik@gmail.com): A validated HPTLC method for simultaneous analysis of eugenol and piperine in a Siddha formulation. J. Planar Chromatogr. 23, 293-297 (2010). TLC of eugenol and piperine on silica gel with toluene - ethyl acetate 9:3 with chamber saturation for 15 min at room temperature. The hR_f value of eugenol was 73 and of piperine 28. Linearity was between 1 and 12 µg/band for both eugenol and piperine. A good linear relationship with $r^2 = 0.9979$ and 0.9980 for eugenol and piperine was obtained. The limit of detection was 0.1 ng/band for eugenol and 10 ng/band for piperine. Recovery of eugenol and piperine was above 97 %. The recovery (measured at different concentration levels) of eugenol was 95.9- 97.5 % and of piperine 91.8-96.6 %. For eugenol the robustness of the method (*%RSD*, $n = 3$) was 0.74 % (12 ng/band) and 0.69 % (25 ng/band) for toluene - ethyl acetete 9:3 and 0.79 % (12 ng/band) and 0.88 % (25 ng/band) for toluene - ethyl acetate 9:2. For piperine the robustness of the method (*%RSD*, $n = 3$) was 0.74 % (5 ng/band) and 0.89 % (10 ng/band) for toluene - ethyl acetate 9:3 and 0.72 % (5 ng/band) and 0.73 % (10 ng/band) for toluene - ethyl acetate 9:2.

herbal, traditional medicine, quality control, HPTLC, densitometry, quantitative analysis 15

106 084 M. SAJEWICZ, L. WOJTAL, D. STASZEK, M. HAJNOS, Monika WAKSMUNDZKA-HAJNOS, Teresa KOWALSKA* (*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland; teresa.kowalska@us.edu.pl): Low temperature planar chromatography - densitometry and gas chromatography of essential oils from different sage (Salvia) species. J. Liq. Chromatogr. Relat. Technol. 33, 936-947 (2010). TLC of essential oils (multicomponent mixtures of mono-, di, tri-, and sesquiterpenes) from Salvia species at 22 +/- 1 °C and at -10 +/- 0.5 °C on silica gel with toluene - ethyl acetate 19:1 in sandwich chambers saturated with mobile phase for 15 min. Quantitative absorbance measurement at 340 nm. Also preparative TLC after bandwise application at -10 +/-2 °C in saturated sandwich chambers. After extraction of the separated bands GC with mass spectrometric detection was performed. Low temperature TLC densitometry can successfully be used for fingerprinting of essential oils contained in the different sage species.

pharmaceutical research, herbal, traditional medicine, preparative TLC, densitometry, qualitative identification, quantitative analysis 15b

106 085 M. SAJEWICZ, L. WOJTAL, M. HAJNOS, Monika WAKSMUNDZKA-HAJNOS, Teresa KOWALSKA* (*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland; teresa.kowalska@us.edu.pl): Low-temperature TLC-MS of essential oils from five different sage (Salvia) species. J. Planar Chromatogr. 23, 270-276 (2010). TLC of essential oils (obtained from different sage species by vapor destillation) on silica gel at -10 +/- 0.5 °C with toluene - ethyl acetate 19:1 in sandwich chamber saturated with mobile phase for 15 min. Quantitative absorbance measurement at 340 nm and 254 nm. After densitometry mass spectrometric analysis was performed using a TLC-MS Interface for direct elution of a given band from the plate.

herbal, qualitative identification, densitometry 15b

106 086 S. SAPANA, V. JADHAV*, V. KADAM (*Dept. of Q. A., Bharati Vidyapeeth's College of Pharmacy, Sector 8, CBD Belapur, Navi-Mumbai 400614, India, drvmjadhav_bvcop@rediffmail.com): Development and validation of HPTLC method for determination of 3-hydroxy androstane [16,17-C] (6'methyl, 2'-1-hydroxy-isopropene-1-yl) 4,5,6 H pyran in jambul seed (Syzygium cumini). International Journal of ChemTech Research 1(4), 1129-1135 (2009) The marker compound was first isolated by column chromatography over silica gel by elution with toluene - ethyl acetate 17:3. TLC of ethanolic extracts of Syzgium cumini seed on silica gel with toluene - ethyl acetate 17:3. The hR_f value of the marker compound was 50. Densitometric evaluation in fluorescence mode at 366 nm. The method was linear in the range of 1-5 µg/band. The extract of the powdered sample contained 7.4 % of the marker compound.

quality control, herbal, environmental, densitometry, quantitative analysis 15

106 087 S. SARFARE*, S. MENON, S. SHAILAJAN (*Herbal Research Lab, Ramnarain Ruia College, Mumbai 400019, India): Cornsilk as a bioavailable source of beta-sitosterol: a pharmacokinetic study using HPTLC. Asian Journal of Plant Sciences 9(1), 44-50 (2010). Beta-sitosterol is one of the major phytoconstituents in cornsilk (style and stigma of Zea mays Linn.). An HPTLC method for the standardization of cornsilk as a bioavailable source of beta-sitosterol is reported. HPTLC of methanolic extracts of dried cornsilk and plasma of rabbits (1 h after injection of plant slurry) on silica gel with toluene - ethyl acetate - methanol - glacial acetic acid 80:10:5:3 with chamber saturation for 30 min. Detection by treatment with Liebermann-Burchard reagent. Beta-sitosterol was well resolved with an hR_f value of 48. Quantitative determination by fluorescence measurement at 366 nm. The method was suitable for the pharmacokinetic study (absorption - elimination). The study confirmed the bioavailability of beta-sitosterol from cornsilk, which is therefore a potential natural source of beta-sitosterol.

herbal, clinical routine analysis, clinical chemistry research, HPTLC 15a

106 088 Willy SHAH*, S. PEDNEKAR, Sunita SHAILAJAN, V. VAIDYA (*Ramnarain Ruia College, Matunga, Mumbai, India): A rapid densitometric method for simultaneous quantification of two biologically active compounds in Vernonia cinerea whole plant powder using HPTLC. Analytical Chemistry - An Indian Journal 8(4), 608-612 (2009). An HPTLC method is reported for estimation of lupeol and beta-sitosterol from the whole plant of Vernonia cinerea (Acanthaceae). Methanolic extracts of the plant were subjected to chromatographic analysis on silica gel with toluene - methanol 220:3. Derivatization with Liebermann-Burchard reagent. Densitometric evaluation at 366 nm. The plant was found to contain 0.49 mg/g and 1.4 mg/g of lupeol and beta-sitosterol respectively. The method was suitable for quality control of herbal raw material.

quality control, herbal, densitometry, HPTLC, postchromatographic derivatization, quantitative analysis 15a

106 089 Smruti SHAH*, Sunita SHAILAJAN (*Herbal Research Lab, Ramnarain Ruia College, Matunga,

Mumbai, India): Quantitation of beta-sitosterol from Woodfordia fruticosa (Linn.) Kurz and a polyherbal formulation used for treating female reproductive disorders. Analytical Chemistry - An Indian Journal 8(1), 82-86 (2009). An HPTLC method is reported for estimation of beta-sitosterol in flowers of Woodfordia fruticosa (Lythraceae) and its polyherbal formulation. HPTLC on silica gel with toluene - ethyl acetate - methanol - glacial acetic acid 80:10:5:3. Methanolic extracts of the sample were used for chromatographic separation. Derivatization with Liebermann-Burchard reagent, densitometric quantification at 366 nm. The linearity range was 100-700 ng/band. The flowers of the plant contained 0.18 µg/mg beta-sitosterol and the tablet herbal formulation contained 0.15 µg/mg.

traditional medicine, quality control, herbal, densitometry, quantitative analysis, postchromatographic derivatization, HPTLC 15a

106 090 S. SHAIKH*, K. MANGAONKAR (*Analytical Chemistry lab., Mithibai College of Arts, Chauhan Institute of Science & Amrutben Jivanial College of Commerce & Economics, Vile Parle (W) Mumbai 400056, India): High-performance thin-layer chromatographic method for quantification of beta-sitosterol from Tridax procumbens. Analytical Chemistry - An Indian Journal 9(2) (2010) (without page number). An HPTLC method has been reported for estimation of beta-sitosterol in whole plant powder of Tridax procumbens. HPTLC on silica gel with toluene - ethyl acetate - glacial acetic acid 40:10:1. Methanolic extracts of the plant material were used. For detection the plate was sprayed with anisaldehyde - sulfuric acid reagent. Densitometric quantification at 550 nm. The method was linear in the range of 10.0-70.0 µg/band. The plant material was found to contain 0.04 % of beta-sitosterol. The proposed method was suitable for routine quality control of plant as well as quantification of beta-sitosterol .

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

106 091 Sunita SHAILAJAN*, S. MENON (*Herbal Research Lab, Ramnarain Ruia College, Matunga, Mumbai, India): Simultaneous quantitation of lupeol and beta-sitosterol from the whole plant powder of Asteracantha longifolia Nees. Analytical Chemistry - An Indian Journal 8(1), 77-81 (2009). An HPTLC method is reported for estimation of lupeol and beta-sitosterol in the whole plant powder of Asteracantha longifolia (Acanthaceae). The proposed method was also employed for estimation of beta-sitosterol in herbal formulation containing Asteracantha longifolia. Methanolic extracts of the sample were used for chromatography. HPTLC on silica gel with toluene - ethyl acetate - methanol 75:15:7. Derivatization with Liebermann-Burchard reagent. Densitometric quantification at 366 nm. The concentration of lupeol and beta-sitosterol was 0.162 mg/g and 0.045 mg/g, respectively in the whole plant, while the concentration of beta-sitosterol was 0.039-0.048 mg/g in the formulation. The method was suitable for the quality control of the herbal formulation.

herbal, HPTLC, densitometry, postchromatographic derivatization, quantitative analysis 15a

106 092 Maria-Loredana SORAN*, I. LUNG (*National Institute of Research and Developmen for Isotope anf Molecular Technology, 65-103 Donath Street, 400293 Cluj-Napoca, Romania; loredana_soran@yahoo.com)t: HPTLC analysis of thymol in extracts of Satureja hortensis L. obtained by different techniques. J. Planar Chromatogr. 23, 320-322 (2010). HPTLC of thymol in Satureja hortensis L. extracts on silica gel with toluene - ethyl acetate 93:7. Detection by spraying with anisaldehyde reagent. Quantitative determination by absorbance measurement at 513 nm. The quantity of thymol extracted depends on the composition of the extraction solvent and the extraction technique. The best results were obtained by maceration with ethanol.

traditional medicine, herbal, quality control, HPTLC, densitometry, quantitative analysis 15

17. Amines, amides and related nitrogen compounds

106 093 Anindita BEHERA*, D. SANKAR, S. CHANDRA (*Dept. of Pharmaceutical Science Andhra University waltair, Visakapatnam 530003, AP, India): Development and validation of an HPTLC - densitometric method for determination of levodopa in seeds of Mucuna pruriens and its dosage form. Eurasian J. Anal. Chem. 5(1), 25-27 (2010). An HPTLC method is reported for estimation of levodopa in seeds of Mucuna pruriens and its herbal formulation. HPTLC on silica gel with *n*-butanol - glacial acetic acid - water 5:1:4. Densitometric evaluation at 280 nm. The hR_f value of levodopa was 39. The method was linear in the range of 100-1000 ng/band. The method is suitable for estimation of levodopa in herbal formulation.

quality control, herbal, densitometry, quantitative analysis, HPTLC 17c

106 094 R. CHAKRABORTY*, K. PAL, M. SHAIBA, N. SANGEPU, P. SRIDEVI (*CM College of Pharmacy, Maisammaguda, Hakimpet Post, Komapally, Secunderabad 500014, A.P., India): High-performance thin-layer chromatographic estimation of ranolazine. Research Journal of Pharmaceutical, Biological and Chemical Sciences 1(4), 152-157 (2010). TLC on silica gel with methanol - 10 mM ammonium acetate 3:2 . The hR_f value of ranolazine was 49. Densitometric evaluation at 271 nm. The method was linear in the range of 1-6 µg/band with a mean recovery of 100.0 %.

pharmaceutical research, quality control, densitometry, quantitative analysis 17

106 095 P. DESHPANDE, S. GANDHI*, Vandana BHAVNANI, R. BANDEWAR, A. DHIWARE, Vrushali DIWALE (*Dept. of Q.A. AISSMS College of Pharmacy, Kennedy Rd., near R.T.O. Pune 411001, M.S., India, santoshgandhi@rediffmail.com): High-performance thin-layer chromatographic determination of famotidine and domperidone in combined tablet dosage form. Research Journal of Pharmaceutical, Biological and Chemical Sciences 1(4), 354-359 (2010). TLC on silica gel with toluene - methanol - triethylamine 12:6:1 with chamber saturation for 15 min. Densitometric evaluation at 290 nm. The hR_f value was 23 and 67 for famotidine and domperidone, respectively. The method was found to be linear in the range of 100-500 ng/band. The recovery was 98.8 %.

pharmaceutical research, densitometry, quantitative analysis 17c

106 096 M. FAISAL*, N. ZRINE, S. FAIYAZ, A. SAYEED, K. KOHLI, R. KHAR (*Dept. of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard Nagar, New Delhi 110062, India): A new TLC densitometric method for stability assessment of modafinil. Chem. Anal. (Warsaw) 54, 1-12 (2009). An HPTLC method has been developed for estimation of modafinil in tablet formulation and stability assessment. HPTLC on silica gel with toluene - chloroform - methanol 2:2:1. The hR_f value of modafinil was 46. Densitometric evaluation at 220 nm. The method was linear in the range of 100-500 ng/band. The sample compound was well separated from all degradation products. The method was suitable for analysis and also for stability studies.

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

106 097 N. JAIN,* G. JAIN, Zeenat IQBAL, Sushma TALEGAONKAR, F. AHMAD, R. KHAR (*Dept. of Pharmaceutical, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110062, India): Retraction: an HPTLC method for the determination of minocycline in human plasma, saliva and gingival fluid after single step liquid extraction. Analytical Science 25(1), 57-62 (2009). HPTLC of minocycline on silica gel plates previously treated with 10 % EDTA solution of pH 9.0 with methanol - acetonitrile - isopropyl alcohol - water 10:8:1:1. The hR_f value was 32. Densitometric evaluation at 345 nm. The method was linear in the range of 100-1200 ng/band for all biological samples. The average recovery was 95.1 %. The method was found suitable for estimation of the drug in

different biological fluids (human plasma, saliva, gingival fluid) and can be used for pharmacokinetic studies.

clinical routine analysis, densitometry, quantitative analysis, HPTLC 17c

106 098 D. PATEL*, N. PATEL (*Dept. of Pharmaceutical Chemistry, S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva, Mehsana 382711, Gujarat, India, diptibpatel_24980@yahoo.co.in): Validated stability indicating HPTLC method for the determination of tamsulosin hydrochloride in pharmaceutical dosage forms. International Journal of ChemTech Research 2(1), 646-652 (2010). TLC on silica gel with toluene - methanol - triethylamine 9:3:1 with chamber saturation for 30 min. The hR_f value was 71. Densitometric evaluation at 282 nm. The method was linear in the range of 100-2000 ng/band with $r^2 = 0.9973$. The repeatability of sample application and measurement of peak area (*%RSD*, $n = 6$) was 0.461 % and 0.363 %. The *%RSD* for intra-day and inter-day variation was 0.752-0.961 % and 0.848-1.082 % respectively. The LOD and LOQ was 10 and 50 ng/zone, respectively. The average recovery was 99.5 %. The samples were subjected to stress conditions (acid, base, oxidative, thermal) and all degradation products were well resolved from the main compound. The method was found suitable for stability studies and for routine analysis of the drug in biological fluids.

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

106 099 A. PATIL*, Darshana PATIL, A. SHARMA, N. CHANDRA (*Dept. of Botany-Herbal Sciences, Birla College, Kalyan 421304,India, dravinashpatil@rediffmail.com): Quantification of beta-carotene from Diplocyclos palmatus jeff. fruits rind by using high-performance thin-layer chromatography. Asian Journal of Chemistry 23(2), 788-790 (2011). A simple HPTLC method has been developed for estimation of beta-carotene in fruit rind of Diplocyclos palmatus (Cucurbitaceae). The rind of fruits was extract with acetone. HPTLC on silica gel with petroleum ether as mobile phase. The hR_f value of beta-carotene was 30. Densitometric evaluation at 450 nm. The method was linear in the range of 6-60 ng/band. The recovery was 99.4 % for beta-carotene.

traditional medicine, herbal, HPTLC, densitometry, quantitative analysis 17a

106 032 U. PAWAR et al., see section 8

106 100 B. PRATHAP*, G. NAGARAJAN, C. ROOSEWELT, V. GOPAL (*Faculty of Pharmacy, PRIST Univeristy, Thanjavur 614904, Tamil Nadu, India): Simultaneous estimation of gatifloxacin and ambroxol HCl in tablet formulation by HPTLC method. Scholars Research Library 2(3), 163-167 (20100. TLC on silica gel with *n*-butanol - water - methanol - ammonia 8:1:1:2 with chamber saturation for 10 min. The hR_f value was 33 for gatifloxacin and 89 ambroxol HCl. The method was linear in the range of 100-600 ng/band for gatifloxacin and 12.5-62.5 ng/band for ambroxol HCI. The recovery was 99.3-100.3 %. Densitometric evaluation at 310 nm.

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

106 101 A. RAMADAN*, A. BODAKJI, I. MAHMOUD (*Dept. of Chemistry, Faculty of science, University of Aleppo, Syria, dramadan@scs-net.org): TLC-densitometric determination of vitamins B1, B6 and B12 in puree and pharmaceutical formulations using treated aleppo bentonite. Asian Journal of Chemistry 22(4), 3283-3291 (2010). TLC of vitamins B1, B6, and B12 in formulations on bentonite-coated TLC plates (0.3 mm) with iso-butylalcohol - methanol - chloroform - acetic acid - ammonia 75:10:35:4:5. The hR_f value of vitamin B1 was 26, of B6 37 and of B12 68. The method was linear in the range of 500-4000 ng/band for all three vitamins. Densitometric quantification at 275 nm for B1, B6, and B12 and at 525 nm for B12.

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

106 102 Madhuri RAMTEKE*, A. KASTURE, N. DIGHADE (*V.R.M. Institute of Diploma in Medical Lab. Technology, R-2, Electronic Zone, MIDC, Hingna Rd., Nagpur 441110, India, shende_madhuri@rediffmail.com): Development of high-performance thin-layer chromatographic method for simultaneous estimation of atenolol and nifedipine in combined dosage form. Asian Journal of Chemistry 22(8), 5951-5955 (2010). TLC on silica gel with cyclohexane - methanol - ethyl acetate - 25 % ammonia 10:3:6:1. The hR_f value was 7 for atenolol and 69 for nifedipine. Densitometric evaluation at 230 nm. The linearity was in the range of 3-21 µg/band for atenolol and 1.2-8.4 µg/band for nefidipine. The recovery was 100.7 % for both drugs. The method was found suitable for analysis of the combined dosage form of atenolol and nefidipine without interference of excipients.

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

106 103 N.M. RAO*, K.R. KUMAR, J. BHAGYALAKSHMI, T.K. RAVI (*Dept. of Pharmaceutical Analysis, College of Pharmacy Institute of Paramedical Sciences, Coimbatore TN, India, mallimpharmmba@gmail.com): Development and validation of a stability indicating TLC method for the estimation of voglibose in bulk and tablet dosage forms. International Journal of Pharma World Research 1(2) (2010). A validated stability indicating assay method is described for estimation of voglibose in bulk and dosage formulation. TLC on pre-washed silica gel plates with acetonitrile - methanol - 25 % ammonia 150:40:1. The hR_f value of voglibose was 66. Densitometric evaluation at 284 nm. The method was linear in the range of 100-450 ng/band. The average recovery was 101.4 %.

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

106 104 W. SOLOMON*, P. ANAND, R. SHUKLA, R. SIVAKUMAR, R. VENKATNARAYANAN (*Dept. of Pharmaceutical Analysis, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore 641402, Tamil Nadu, India, samwd_2000@yahoo.com): Application of TLC-densitometric method for simultaneous estimation of tramadol HCl and paracetamol in pharmaceutical dosage forms. International Journal of ChemTech Research 2(2), 1188-1193 (2010) TLC on silica gel with chloroform - ethanol 7:3. The hR_f value of tramadol HCl was 48 and of paracetamol 85. Densitometric evaluation at 254 nm. The method was linear over a concentration range of 2.5-32.5 µg/band for tramadol and 10-50 µg/band for paracetamol. The recovery was 100.7-101.8 %. There was no intereference with excipients from the dosage form.

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

18. Amino acids and peptides, chemical structure of proteins

106 105 D. DAS*, A. SAHANA, R. SAHA, P. KUNDU, S. LASKAR (*Department of Chemistry, The University of Burdwan, Burdwan 713104, India; ddas100in@yahoo.com): Anthracene-anchored derivatized methionine: A new ligand for detection of amino acids, and estimation of binding constants. J. Planar Chromatogr. 23, 255-259 (2010). Synthesis of a new ligand by anchoring anthracene to L-methionine. The ligand allows identification of amino acids on TLC plates based on distinguishable colors. A theoretical calculation (Hartree-Fock) was performed to investigate interaction of the ligand with the amino acids. TLC of 22 amino acids on silica gel with *n*-propanol - water 7:3. Detection by spraying with 0.01 % anthracenylmethyl L-methionine in acetone, followed by drying and spraying with 0.25 % ninhydrin in acetone. After drying and heating at 110 °C for 10 min the zones were evaluated visually. Detection limits were between 1 and 200 ng/zone.

qualitative identification 18a

106 106 A. MOHAMMAD*, N. HAQ (*Analytical Research Laboratory, Department od Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh 202 002, India; alimohammad08@gmail.com): TLC separation of amino acids with a green mobile phase. J. Planar Chromatogr. 23, 260-264 (2010). TLC of 15 amino acids on silica gel and alumina (with or without impregnation) with micellar solutions of cetrimide and cetylpyridiniun chloride and aqueous solutions of dextrose with chamber saturation for 10 min. A TLC system comprising of silica gel impregnated with micellar solution of cetrimide (5.0 mM) as stationary phase and 40 % aqueous solution of dextrose as mobile phase was best suitable for the separation of amino acids. Detection by spraying with 0.3 % ninhydrin solution in acetone and heating for 15-20 min at 60 °C.

qualitative identification 18a

106 107 J.D. VASTA, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA, 18042, USA; fried@lafayette.edu): Determination of estivation-induced changes in the amino acid content of Biomphalaria glabrata snails by high-performance thin-layer chromatography-densitometry. J. Liq. Chromatogr. Relat. Technol. 33, 1028-1037 (2010). HPTLC of amino acids (e. g. alanine, arginine, glycine, leucine/isoleucine, lysine, serine, and valine) on silica gel or cellulose pre-washed with dichloromethane - methanol 1:1 using either 2-butanol - pyridine - glacial acetic acid - water 39:34:10:26 or 2-butanol - pyridine - 25 % ammonia - water 39:34:10:26 in a saturated twin-trough chamber. Detection by treatment with ninhydrin reagent (0.3 g ninhydrin in 100 mL of *n*-butanol with 3 mL of glacial acetic acid) and heating at 110 °C for 10 min. Quantitative determination by densitometric absorbance measurement at 610 nm.

densitometry, quantitative analysis, HPTLC 18a

21. Purines, pyrimidines, nucleic acids and their constituents

106 108 Claudia CIMPOIU*, A. HOSU, L. SESERMAN, M. SANDRU, V. MICLAUS (*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos, 400028 Cluj-Napoca, Romania, ccimpoiu@chem.ubbcluj.ro): Simultaneous determination of methylxanthines in different types of tea by a newly developed and validated TLC method. J. Sep. Sci. 33, 3794-3799 (2010). HPTLC of caffeine (1), theobromine (2) and theophylline (3) in different types of tea on silica gel with chloroform - dichloromethane - isopropanol 4:2:1. Quantitative determination by absorbance measurement at 254 nm. The hR_f of (1), (2) and (3) was 65, 45 and 56, respectively. Limits of detection and quantification were 22 and 45 ng for (1), 23 and 46 ng for (2) and 22 and 43 ng for (3), respectively. The intra-day and inter-day precisions had a *%RSD* lower than 2.55 % ($n = 6$) for all substances. Recoveries (by standard addition) were between 95.1-101.5 % for all the three methylxanthine derivatives. The values of LOD and LOQ obtained are similar with those obtained by HPLC.

food analysis, quality control, HPTLC, quantitative analysis, comparison of methods, densitometry 21a

106 109 CH. JENDRESEN*, M. KILSTRUP, J. MARTINUSSEN (*Center for Systems Microbiology, Department of Systems Biology, Technical University of Denmark, 2800 Lyngby, Denmark): A simplified method for rapid quantification of intracellular nucleoside triphosphates by one-dimensional thin-layer chromatography. Anal. Biochem. 409 (2), 249-259 (2011). Presentation of a less time-consuming, more sensitive, and more precise method for the quantitative determination of nucleoside triphosphates (NTPs), 5-ribosyl-1-pyrophosphate (PRPP), and inorganic pyrophosphate (PPi) in cell extracts by TLC: Separation of an acid extract of L. lactis by charcoal filtration into a filtrate and an eluate, which then was separated by TLC either in the Cashel solvent (0.85 M potassium phosphate, pH 3.4) or in the AFC solvent (3 M ammonium formate [pH 2.4] and 0.7 M ammonium chloride). Two-dimensional separation of 18 µL of the eluate sample using the

AFC solvent in the first dimension and using 0.75 M LiCl in 7.5 % lithium borate (pH 6.8, borate solvent) in the second dimension.

food analysis, environmental, agricultural, clinical chemistry research, HPTLC, qualitative identification, quantitative analysis, comparison of methods, radioscanning 21

106 110 P. SAINI*, R. SINGH, S. MATHUR, G. SINGH, S. TUTEJA, U. SINGH (*Indian Pharmacopoeia Commission Ministry of Health & Family Welfare, Government of India, Sector 23 Rajnagar, Ghaziabad 201002, UP, India): Simultaneous HPTLC method for estimation of lamivudine and zidovudine in bulk and in tablet dosage forms. Indian Drugs 47(5), 42-45 (2010). HPTLC of lamivudine and zidovudine on silica gel with acetone - toluene - methanol 2:1:2. The hR_f of lamivudine was 34 and of zidovudine 74. Densitometric quantification at 267 nm for zidovudine and 272 nm for lamivudine. The method was linear in the range of 10-210 ng/band for lamivudine and 30-420 ng/band for zidovudine. The recovery was between 99.5-100.4 % for the compounds. The method is suitable for estimation of compounds in combined formulations.

pharmaceutical research, HPTLC, densitometry, quantitative analysis 21a

106 111 V. VENKATESH*, A. PRABHAKAR, P. SURESH, U. MAHESHWARI, N. RAO (*Dept. of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Vhalapathi Nagar, Lam, Guntur 522034, India, vedullapalli.pharma@gmail.com): HPTLC method for the simultaneous estimation of etophylline and theophylline in tablet dosage form. Asian Journal of Chemistry 23(1), 309-311 (2011). TLC on silica gel with toluene - isopropyl alcohol - acetic acid 12:12:1. The hR_f values was 63 for etophylline and 75 for theophylline. Densitometric evaluation at 261 nm. The method was linear in the range of 200-400 ng/band for etophylline and 60-80 ng/band for theophylline.

pharmaceutical research, quality control, densitometry, quantitative analysis 21a

22. Alkaloids

106 112 H. MISRA*, D. MEHTA, B. MEHTA, M. SONI, D. JAIN (*School of studies in Chemistry & Biochemistry, Vikram University, Ujjain 456010, India): Study of extraction and HPTLC-UV method for estimation of caffeine in marketed tea (Camellia sinensis) granules. International Journal of Green Pharmacy 3 (1), 47-17 (2009). HPTLC of caffeine on silica gel with ethyl acetate - methanol 9:1. Densitometric quantification at 274 nm. The method was linear in the range of 2-14 µg/band. The sample extracted with 5 % diethyl amine in water gave the maximum yield of caffeine (2.1 %). The proposed chromatography gave the best resolution with caffeine at an hR_f value of 40. The method can be used for quality control of tea samples in respect of caffeine contents.

quality control, herbal, densitometry, HPTLC, quantitative analysis, qualitative identification 22

106 113 A. SUTHAR*, K. KATKAR, P. PATIL, P. HAMARAPURKAR, G. MRUDULA, V. NAIK, G. MUNDADA, V. CHAUHAN (*Pharmacognosy Dept., Manipal College of Pharmaceutical Science, Manipal University, Karanataka, India, ashish.suthar@piramal.com): Quantitative estimation of vasicine and vasicinone in Adhatoda vasica by HPTLC. Journal of Pharmacy Research 2(12), 1893-1899 (2009). HPTLC of vasicine and vasicinone in methanolic aqueous extracts of the dried herb and in cough syrup on silica gel with chloroform - methanol 9:1. The hR_f value of vasicine was 11 and of vasicinone 45. Densitometric quantification at 280 nm. The method was linear in the range of 2-100 ng/band for vasicine and 25-1000 ng/band for vasicinone. The recovery was 95-102 %. The limit of detection of vasicine was 1 ng and of vasicinone 25 ng. Vasicine and vasicinone were well separated from other constituents of Adhatoda vasica.

quality control, herbal, HPTLC densitometry, quantitative analysis 22

23. Other substances containing heterocyclic nitrogen

106 114 L.S. ABDEL-FATTAH, Z. EL-SHERIF, K.M. KILANI, Dalia A. EL-HADDAD* (*National Organization for Drug Control & Research, 6&7 AboHazem St, Pyramids, P. O. Box 29, Giza, Egypt; daliaelhaddad@hotmail.com): HPLC, TLC, and first-derivative spectrophotometry stability-indicating methods for the determination of tropisetron in the presence of its acid degradates. J. AOAC Int. 93, 1180-1191 (2010). TLC of tropisetron and its acid-induced degradation products on silica gel with methanol - glacial acetic acid 22:3 with chamber saturation for 45 min. Detection under UV light at 254 nm. Quantitative determination by scanning at 285 nm. The hR_f values were 44, 84, and 92 for tropisetron and degradates I and II, respectively. Linearity was between 1 and 10 µg/zone. Mean accuracy was 100.2 %. The precision was 0.64 %.The limit of detection and quantification for tropisetron was 0.26 and 0.80 µg/zone, respectively. The intraday and interday precisions were evaluated by assaying freshly prepared samples of tropisetron in triplicate concentrations, i. e. 3, 5, and and 9 µg/zone, resulting in 99.8 %, 100.3 %, and 100.4 %, respectively, for interday precision and 99.7 %, 100.2 %, and 99.7 %, respectively, for intraday precision (with a precision of 0.9 %, 0.6 %, 0.8 % and 0.6 %, 0.4 %, 0.6 %, respectively).

quality control, pharmaceutical research, densitometry, quantitative analysis 23

106 115 Mrinalini DAMLE*, M. DANGI, Darshana CHAUDHARI, M. SINKAR, Veena RACHA (*AISSMS College of Pharmacy, Kennedy Rd., Pune 411001, India): Stability indicating HPTLC method for estimation of nebivolol hydrochloride and amlodipine besylate in combination. Eurasian J. Anal.Chem. 5(1), 21-24 (2010). A stability-indicating HPTLC method has been developed for simultaneous estimation of amlodipine besylate and nebivolol hydrochloride. HPTLC on silica gel with ethyl acetate - methanol - 25% ammonia 17:2:2. The hR_f value of amlodipine besylate was 40 and of nebivolol hydrochloride 60. Densitometric quantification at 240 nm for amlodipine and 260 nm for nebivolol. The method was linear in the range of 500-2000 ng/band for both compounds. Degradation products formed under stress conditions (acid, alkali, oxidative, dry heat, photo degradation) were well separated from the main compound. The method was suitable for quality control and stability studies.

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

106 116 B. DHANDAPANI*, N. THIRUMOORTHY, P. JOSH (*Dept. of Pharmaceutical Analysis, A. M. Reddy Memorial College of Pharmacy, Petlurivaripalem, Narasaraopet, Guntur 522601, A.P., India, dhandapani@gmail.com): Development and validation for the simultaneous quantification of nebivolol hydrochloride and hydrochlorothiazide by UV spectroscopy, RP-HPLC and HPTLC in tablets. E-Journal of Chemistry 7(2), 341-348 (2010). HPTLC of nebivolol hydrochloride and hydrochlorothiazide on silica gel with ethyl acetate - methanol - 25 % ammonia 17:2:1 with chamber saturation. The hR_f value of hydrochlorothiazide was 21 and of nebivolol 41. Densitometric evaluation at 285 nm. The method was linear in the range of 200-1000 ng/band for nebivolol and 500-2500 ng/band for hydrochlorothiazide. The recovery was 98.9-102.4 %. Comparison of the HPTLC method with a RP-HPLC method and a UV spectroscopic method gave comparable results. The HPTLC method is suitable for routine quality control.

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

106 117 H. KALÁSZ*, E. MINGSOVICS, N. RAM, K. KUCA (*Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, 1089 Budapest, Nagyvárad tér 4, Hungary; drkalasz@gmail.com): Thin-layer chromatography of pyridinium aldoximes using dis-

tinct techniques for development. J. Liq. Chromatogr. Relat. Technol. 33, 922-935 (2010). TLC and HPTLC of 22 different pyridinium aldoximes on silica gel with water - acetonitrile - acetic acid 8:1:1, water - acetone - acetic acid 8:1:1, and acetone - 50 mM aqueous sodium acetate 1:4 using unsaturated and saturated vapor phase as well as OPLC. Detection under UV 254 nm.

toxicology, clinical chemistry research, HPTLC, qualitative identification, comparison of methods 23d

106 118 W. PARYS, Alina PYKA* (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, 41-200 Sosnowiec, Poland; apyka@sum.edu.pl): Use of TLC and densitometry to evaluate the chemical stability of nicotinic acid and its esters on silica gel. J. Liq. Chromatogr. Relat. Technol. 33, 1038-1046 (2010). TLC of nicotinic acid and methyl, ethyl, isopropyl, butyl, hexyl, and benzyl nicotinate (heated for 1 to 7 h at 120 °C) on silica gel pre-washed with methanol using methanol - benzene (for nicotinic acid) and acetone - hexane 2:3 for nicotinic acid esters. Quantitative determination by densitometric absorbance measurement at the respective absorption maximums (263 nm for nicotinic acid, 220-222 nm for nicotinic acid esters). The hR_f of nicotinic acid was 44, and of methyl, ethyl, isopropyl, butyl, hexyl, and benzyl nicotinate 48, 46, 51, 54, 56, and 44, respectively.)

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

106 119 L. POBLOCKA, Miroslawa KRAUZE*, D. GLOD, A. KAWIAK, E. LOJKOWSKA (*Department of Pharmacognosy, Medical University of Gdansk, Gen. J. Hallera 107 Street, Gdansk, Poland, krauze@amg.gda.pl): Chromatographic analysis of simple phenols in some species from the genus Salix. Phytochem. Anal. 21, 463-469 (2010). HPTLC of pyrocatechol in the barks of different species and clones from the genus Salix on diol phase by mutiple gradient development with chloroform - hexane 7:3 - ethyl acetate - formic acid in the following gradients: (I) 18:2:0.01, (II) 17:3, and (III) 16:4. Detection by spraying with thymol reagent. Quantitative determination by absorbance measurement at 254 nm.The hR_f of pyrocatechol was 25. Detection and quantification limits were 30 and 100 µg/mL, respectively. The intra-day and inter-day precisions had a *%RSD* lower than 2.5 %. Recovery (by standard addition) was 96.3 %. The correlation coefficient of pyrocatechol concentrations determined by HPTLC and HPLC was 0.9932.
herbal, toxicology, HPTLC,

quantitative analysis, densitometry 23

106 120 A. SHIKHEDKAR*, S. SURANA (*R. C. College of Pharmacy Dept. of Pharma. Chemistry, Shirpur, Dhule (MS), India): Application of stability-indicating RP-TLC densitometric determination of rabeprazole sodium in bulk pharmaceutical formulations. Eurasian J. Anal. Chem. 4(1), 87–97 (2009). HPTLC of rabeprazole sodium on silica gel with acetone - water 7:30. The hR_f value was 45. Densitometric quantification at 284 nm. Linearity was in the range of 400-2400 ng/band. The recovery was 99.6-101.4 %. The sample was subjected to forced degradation (acid, alkali, oxidative, thermal, photolytic). The degradation products were well separated from the main compound and the method is suitable for stability studies.

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

106 121 Shradhanjali SINGH*, Kirti TOPAGI, Mrinalini DAMLE (*Dep of Pharmaceutical Chem, AISSMS Collage of Pharmacy, Pune 411001, India, mcdamle@rediffmail.com): A validated high-performance thin-layer chromatographic method for simultaneous estimation of nebivolol hydrochloride and valsartan in pharmaceutical dosage form. Research Journal Pharm and Tech. 2(4), 746-748 (2009). A validated HPTLC method is reported for simultaneous estimation of nebivolol

hydrochloride and valsartan in combined dosage form. HPTLC on silica gel with ethyl acetate - methanol - 25 % ammonia. The hR_f value of valsartan was 27 and of nebivolol 75.The method was linear in the range of 800-2400 ng/band for nebivolol and 200-1000 ng/band for valsartan. Densitometric quantification at 280 nm. The method was found suitable for routine quality control of the combined dosage form.

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

106 122 L. SIVASUBRAMANIAN* DEVARAJAN, K. BELL (*Dept. of Pharmaceutical Analysis, SRM College of Pharmacy, Kattankulathur, Tamil Nadu, India): HPTLC for the simultaneous determination of tizanidine and valdecoxib in pharmaceutical dosage form. Journal of Pharmacy Research 2(1), 189-195 (2009). A validated HPTLC method has been developed for simultaneous estimation of valdecoxib and tizanidine in dosage form. HPTLC on silica gel with toluene - methanol - ethyl acetate 2:2:1. The compounds were well separated as compact bands. The method was linear in the range of 10-100 ng/band for tizanidine and 100-1000 ng/band for valdecoxib. The recovery was in the range of 99.3–101.2 %. Densitometric quantification at 254 nm. The method is suitable for quality control of combined dosage form.

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

106 076 N. VEKARIYA et al., see section 11

106 123 S. WALODE*, H. CHAUDHARI, M. SARASWAT, A. KASTURE, S. WADODKAR (*Sinhgad Institute of Pharmaceutical Science, Kusgaon (BK), Lonavala, Pune 410401, India): Validation high performance thin layer chromatographic determination and content uniformity test for rosiglitazone tablets. Indian J. Pharm. Sci. 72(2), 249-252 (2010). HPTLC of rosiglitazone on silica gel with methanol - toluene - chloroform - triethyl amine 2:161:1. Caffeine was used as internal standard. The hR_f value of rosiglitazone was 31 and of caffeine 52. Densitometric evaluation at 264 nm. The method was linear in the range of 5-50 µg/band with average recovery of 99.8-100.2 %. The method was suitable for content uniformity testing according to USP and could be used for stability studies as well.

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

24. Organic sulfur compounds

106 124 M. ARANDA, Gertrud E. MORLOCK* (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany; gmorlock@uni-hohenheim.de): Quantification of pyritinol in solid pharmaceutical formulation by high-performance thin-layer chromatography-ultraviolet detection and selective evaluation by mass spectrometry. J. Liq. Chromatogr. Relat. Technol. 33, 957-971 (2010). HPTLC of pyritinol on silica gel with dichloromethane - methanol - formic acid 9:1:1 in a twin trough chamber. Quantitative determination by absorbance measurement at 300 nm. Repeatability and intermediate precision in matrix were 0.4 % and 3.0 %, respectively. Recoveries of spiked samples at three levels ranged from 98.5 to 101.9 % with intermediate precisions of *RSD* 3.7 to 4.7 %. The limit of detection and quantification was 0.6 and 2.0 µg/mL (6 and 20 ng/band), respectively. Selectivity was evaluated determining the peak purity by UV-spectrophotometry and showed correlation coefficients (r) > 0.9997. Additionally the selectivity was proven by mass spectrometry. Mass spectra showed only the analyte signals which indicated a highly satisfying selectivity.

HPTLC, quantitative analysis, densitometry 24

26. Organometallic and related compounds

106 125 R. BAOSIC*, A. RADOJEVIC, Z. TESIC (*Faculty of Chemistry, University of Belgrade, Studentski trg 12, P.O. Box 158, 11000 Belgrade, Serbia): Prediction of the retention of beta-diketonato complexes in TLC systems on silica gel by quantitative structure-retention relationships. J. Serb. Chem. Soc. 75(4), 513-521 (2010). A TLC method has been reported for prediction of retention behavior of several beta-diketonato complexes of cobalt(III), chromium(III), and ruthenium(III). Aim of this study was to develop a model for accurate quantitative relationships between molecular structures and retention behavior of mixed beta-diketonato complexes. TLC of 36 complexes on silica gel with four mono- and five two-component mobile phases. Quantitative structure - retention relationships (QSSRS) shall allow the prediction of retention of new solutes and structurally similar compounds, as well as their molecular description.

pharmaceutical research, qualitative identification 26c

27. Vitamins and various growth regulators

106 126 K. UETA, M. NISHIOKA, Y. YABUTA, F. WATANABE* (*The United Graduate School of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan; watanabe@muses.tottori-u.ac.jp): TLC-bioautography analysis of vitamin B12 compound from the short-necked clam (Ruditapes philippinarum) extract used as a flavoring. J. Liq. Chromatogr. Relat. Technol. 33, 972-979 (2010). TLC of vitamin B12 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. Detection by spraying with a methanolic solution of 2,3,5-triphenyltetrazolium salt, B12 compounds appeared as red zones indicating E. coli growth.

food analysis, agricultural, qualitative identification 27

28. Antibiotics, Mycotoxins

106 127 A. AHMAD, M. MUJEEB, B. P. PANDA* (*Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India 110062; bibhu_panda31@rediffmail.com): An HPTLC method for the simultaneous analysis of compactin and citrinin in Penicillium citrinum fermentation broth. J. Planar Chromatogr. 23, 282-285 (2010). HPTLC of compactin and citrinin on silica gel with toluene - ethyl acetate - formic acid 3:2:1 in a twin-trough chamber saturated for 30 min at room temperature and a relative humidity of 60 +/- 5 %. Quantitative determination by absorbance measurement at 238 nm and 366 nm. The hR_f of compactin and citrinin was 47 and 62, respectively. Good correlation was obtained between peak area and concentration, with a determination coefficient r^2= 0.998 for compactin and 0.996 for citrinin.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis, mycotoxins 28b

106 128 Monika DABROWSKA*, J. KRZEK (*Jagiellonian University, Collegium Medicum, Department of Inorganic and Analytical Chemistry, Medyczna 9, Kraków, Poland; mtylka@cm-uj.krakow.pl) : Separation, identification, and quantitative analysis of the epimers of cefaclor by TLC-densitometry. J. Planar Chromatogr. 23, 265-269 (2010). TLC of the epimers of cefaclor on silica gel (impregnated with beta-cyclodextrin by development with 1:9 aqueous beta-cyclodextrin solution - methanol) with chloroform - ethyl acetate - glacial acetic acid - water 4:4:4:1 with chamber saturation. Chromatograms were developed at 5 °C and dried at room temperature. Quantitative determination by absorbance measurement at 274 nm. The limit of detection was 0.24 and 0.27 µg/band for the epimers at hR_f 26 and 33, respectively. The limit of quantification was 0.74 and 0.83 µg/band, respectively. The recovery was between 100.0 and 100.8 % and the precision

between 0.7 and 1.7 %.

quality control, pharmaceutical research, densitometry 28a

106 129 M. DHOKA*, V. GAWANDE, P. JOSHI (*Dept. of Q.A. AISSMS College of Pharmacy, Kennedy Rd., near R.T.O. Pune 411001, M.S., India): HPTLC determination of amoxicillin trihydrate and bromhexine hydrochloride in oral solid dosage forms. J. Pharma. Sci. & Res. 2(8), 477-483 (2010). TLC on silica gel with ethyl acetate - glacial acetic acid - methanol - water 10:5:3:2. The hR_f value was 51 and 74 for amoxicillin and bromhexine, respectively. Densitometric evaluation at 260 nm (bromhexine) and at 320 nm (amoxicillin). The method was linear in the range of 200-1000 ng/band and 10-30 µg/band for bromhexine and amoxicillin, respectively. The recovery was between 98.1-101.5 %.

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

106 130 Mária HEVESI*, A. M. MÓRICZ, Z. KIRÁLY-VÉGHELY, M. TÓTH, G. KÁTAY, E. TYIHÁK (*Corvinus University of Budapest, Faculty of Horticultural Sciences, Department of Pomology, Villányi Str. 35-44, 1118 Budapest, Hungary; maria.hevesi@uni-corvinus.hu): Effect of trans-resveratrol and ascorbigens on the fire blight pathogen Erwinia amylovora in the BioArena system. J. Planar Chromatogr. 23, 411-414 (2010). OPLC of ascorbigen and 1'-methylascorbigen on silica gel with *n*-hexane for the first step and chloroform - methanol 9:1 for the second step; OPLC of trans-resveratrol on silica gel with chloroform - methanol 10:1. Detection by bioautography (immersion in E. amylovora cell suspension, incubation for 2 h at 26 °C and 100 % relative humidity, and staining with a solution of 80 mg MTT and 100 mg Triton X-100 in 100 mL water. Quantitative determination by densitometry.

agricultural, quality control, HPTLC, densitometry 28

106 011 M. KAMINSKA et al., see section 2

29. Pesticides and other agrochemicals

106 131 R. AKKAD, W. SCHWACK* (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany): Multi-enzyme inhibition assay for the detection of insecticidal organophosphates and carbamates by high-performance thin-layer chromatography applied to determine enzyme inhibition factors and residues in juice and water samples. J. Chromatogr. B 878 (17-18), 1337-1345 (2010). Use of rabbit liver esterase, Bacillus subtilis esterase, and cutinase from Fusarium solani pisi for the detection of 21 organophosphorus and carbamate pesticides by HPTLC-enzyme inhibition assays (HPTLC-EI) on silica gel with *n*-hexane - ethyl acetate - dichloromethane 13:4:3. HPTLC-EI assay of three groups of organophosphate and carbamate insecticides with 1) *n*-hexane - ethyl acetate 63:37, 2) chloroform - ethyl acetate 9:1, 3) *n*-hexane - acetone - dichloromethane 15:2:3. Detection by treatment with Fast Blue Salt B and enzymatically coupling to alpha-naphthol released from the respective acetate used as substrate. Quantification by densitometry at 533 nm. Calculation of enzyme inhibition factors derived from HPTLC-EI using linear calibration curves. Comparison to published inhibition constants showed good correlation. The limits of detection ranged from a few pg/zone for organophosphates as strongest inhibitors to a few ng/zone for most carbamates and was around 60 ng/zone for chlorpyrifos and 14 ng/zone for parathion without oxidation. The CUT was able to detect insecticides of high and low inhibitory power in the range of ng to µg/zone. The HPTLC-EI with rabbit liver esterase was applied to the analysis of apple juice and drinking water samples spiked with paraoxon (0.001 mg/L), parathion (0.05 mg/L) and chlorpyrifos (0.5 mg/L). The mean recovery was 71-112 % with standard deviations of 2.0-18.3 %.

food analysis, environmental, agricultural, HPTLC, densitometry,
qualitative identification, quantitative analysis 29c

106 132 Vera BAUMGARTNER*, W. SCHWACK (*State Laboratory of the Canton Basel-City, Kannenfeldstrasse 2, 4056 Basel, Switzerland; vera.baumgartner@bs.ch): Enhanced quantitative evaluation of the HPTLC-bioluminescence detection. J. Liq. Chromatogr. Relat. Technol. 33, 980-995 (2010). HPTLC of bronopol and Kathon CG on silica gel (pre-washed with methanol) with methanol - dichloromethane - *n*-hexane 2:12:7. For detection, luminescent Vibrio fischeri bacteria were cultivated. Plates were dried for 30-40 min at 120 °C on a plate heater and then immersed for 1 s in the bacteria suspension using an immersion device and a glass dip tank. Excess bacteria suspension was removed. Images were taken using the BioLuminizer with an exposure time of 55 s. The effective and reasonably fast quantitative method is in routine use at several laboratories.

quantitative analysis, HPTLC 29f

106 133 M. BROSZAT, H. ERNST, B. SPANGENBERG* (*Institute of Process Engineering, University of Offenburg, Badstrasse 24, 77652 Offenburg, Germany; Spangenberg@FH-Offenburg.de): A simple method for quantifying triazine herbicides using thin-layer chromatography and a CCD camera. J. Liq. Chromatogr. Relat. Technol. 33, 948-956 (2010). HPTLC of triazine herbicides (atraton, terbumelon, simazine, and terbutylazine) on silica gel with methyl-t-butyl ether - cyclohexane 1:1 in a vertical developing chamber without chamber saturation. Detection by derivatization using chlorine and starch-iodine. Quantitation with a CCD camera; the range of linearity covers two magnitudes (for atrazine from 10 ng to 1000 ng).

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

30. Synthetic and natural dyes

106 001 Marta KUCHARSKA et al., see section 1

32. Pharmaceutical and biomedical applications

106 134 R. BAJWA*, R. SANE (*Guru Nanak Institute for Research & Development G. N. Khalsa College, Nathalal Parekh Rd., Matunga, Mumbai 40019, India): Studies in phytochemical investigation, anti bacterial activity and acute toxicity study of Prosopis cineraria pods. Indian Drugs 47(6), 31-38 (2010). Pods of Prosopis were extracted with different solvents and the extracts were subjected to phytochemical evaluation using 2D paper chromatography and HPTLC. Analysis of phenolic acids by two-directional paper chromatography with toluene - acetic acid - water 6:7:3 in the first direction and sodium formate - formic acid - water 10:1:200 in the second direction. For detection of phytoconstituents HPTLC on silica gel with different mobile phases and detection wavelengths: 1) beta-sitosterol with toluene - methanol 9:1, measured at 258 nm, 2) coumaric acid with toluene - ethanol 4:1, measured at 292 nm, 3) quercetin with toluene - methanol 4:1, measured at 271 nm, and 4) caffeine with toluene - acetone 7:3, measured at 272 nm.

pharmaceutical research, herbal, qualitative identification, HPTLC 32e

106 135 Z. CHUNFANG*, X. YIN, Y. LONGJIANG, L. SHUO, W. ZEQIANG (*Resource of Biology and Biotechnical Lab, College of Life Science and Technology, Huazhong University of Science and Technology,Wuhan City, Hubei Province, 430074 China): TLC and HPLC methods to follow the synthesis of vinorelbine. J. Chromatogr. Sci. 48(8), 685-689 (2010). TLC combined with HPLC for detection of the intermediates and the end-product from the synthesis process of vinorelbine. Specific TLC separation of vinblastine sulfate, anhydrovinblastine, and vinorelbine on silica gel with petroleum ether - chloroform - acetone - diethyl amine 47:24:4:5. Quantitative evaluation with HPLC.

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

106 136 L. CIESLA, D. STASZEK, M. HAJNOS, Teresa KOWALSKA, Monika HAJNOS* (*Department of Inorganic Chemistry, Medical University of Lublin, 6 Staszika Street, Lublin, Poland,monika.hajnos@am.lublin.pl): Development of chromatographic and free radical scavenging activity fingerprints by thin layer chromatography for selected Salvia species. Phytochem. Anal. 22, 59-65 (2011). TLC and free radical scavenging fingerprints of nineteen Salvia species on silica gel with toluene - ethyl acetate - formic acid 60:40:1 for the less polar constituents and ethyl acetate - water - formic acid - acetic acid 100:26:11:11 for the medium and highly polar substances. After drying at room temperature for 15 min derivatization with vanillin sulfuric acid reagent (1 g vanillin with 20 % sulfuric acid in methanol) followed by heating for 5 min at 105 °C. Quantitative determination by absorbance measurement at 254 nm. Free radical scavenging properties were investigated by spraying the plate with DPPH radical reagent (0.2 %) in methanol and left at ambient temperature for 30 min. The strongest free radical scavenging activity was observed for rosmarinic acid, with an hR_f of 70.

herbal, quantitative analysis, postchromatographic derivatization, qualitative identification 32e

106 137 M. DAMLE*, S. PHADTARE, H. RASKAR, K. GADGE, R. MEHENDRE, K. BOTHARA (*Dept. of Pharmaceutical Chemistry, AISSMS College of Pharmacy, Kennedy Rd., Near R.T.O. Pune 411001, M.S., India, mrunal.damle@rediffmail.com): A validated HPTLC method for determination of arbidol from pharmaceutical formulation. International Journal of ChemTech Research 2(2), 1042-1046 (2010) HPTLC on silica gel with dichloromethane - methanol 9:1 in a twin-trough chamber saturated for 30 min. The hR_f value was 70. Densitometric evaluation in absorbance mode at 254 nm. The method was linear in the range of 400-2000 ng/band. The average recovery was 101.2 %.

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

106 138 B. DHANDAPANI*, A. SOMASUNDARAM, S. RASEED, M. RAJA & K. DHANABAL (*Dept. of Pharmaceutical Analysis, K.M.C.H. College of Pharmacy, Kovai Medical Centre Research & Educational Trust, Kovai Estate, Kalapatti Rd., Coimbatore-641045, India, dhandapanirx@gmail.com): Development and validation of HPTLC method for estimation of quetiapine in bulk drug and in tablet dosage form. International Journal of ChemTech Research 1(2), 139-141 (2009). TLC on silica gel with toluene - methanol 3:4. The hR_f value was 41. Densitometric evaluation at 235 nm. The method was linear in the range of 100-500 ng/band. The average recovery was 98.9 %. The proposed method was suitable for estimation of quetiapine in bulk drug and dosage form.

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

106 139 B. DHANDAPANI*, N. ANJANEYULU, Y. VENKATESHWARLU & H. SHAIK (*K.M.C.H. College of Pharmacy, Kovai Estate, Lalappatti Rd., Coimbatore 641035, Tamil Nadu, Dept. of Phamraceutical Analysis, Donbosco College of Pharmacy, Guntur, AP, India, dhandapanirx@gmail.com): HPTLC method development and validation for the simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in tablet dosage form. Journal of Pharmacy Research 3(2), 332-334 (2010). HPTLC on silica gel with methylene chloride - methanol - ammonia 17:2:1. The hR_f value of amlodipine was 19 and of nebivolol 41. Densitometric evaluation at 285 nm. The method was linear in the range of 100-500 ng/band for both compounds. The average recovery was 99.9-102.1 for both compounds and there was no interference with additives present in tablet dosage forms.

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

106 140 P. GAIKWAD, S. BHOPE*, V. KUBER, M. PATIL (*Tulip Laboratories Pvt. Ltd., MIDC Ranjangaon, Pune 412220, India, bshrinivas16@gmail.com): Validated TLC method for simultaneous quantitation of kutkoside and picroside-I from kutki extract. Phytochem. Anal. 22, 36-41 (2011). TLC of kutkoside (1) and picroside-I (2) in the kutki extract (Picrorhiza kurroa) on silica gel with ethyl acetate - methanol - glacial acetic acid - formic acid 25:5:1:1. Quantitative determination by absorbance measurement at 265 nm.The hR_f of (1) was 42 and of (2) 61. The precision was 0.77 % and 1.01 % for (1) and (2), respectively. Linearity was between 80-480 ng/zone for both substances. Detection and quantification limits were 24 and 79 ng/zone for both. The intra-day and inter-day precisions were 0.4 % and 0.3 % (n = 3) respectively. The recovery for (1) was 96.5 % and for (2) 96.0 %, respectively. The results were comparable with those obtained by HPLC.

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

106 141 S. GANDHI*, P. DESHPANDE, U. PATIL, P. MUNDHE, P. R. DESHMUKH (*Dept. of Pharmaceutical Analysis, AISSMS College of Pharmacy, Kennedy Rd., Pune, India): HPTLC determination of cefpodoxime proxetil and potassium clavulanate in combined tablet dosage form. The Pharma Review 149-150 (2010). TLC on silica gel with chloroform - methanol - toluene 4:3. The hR_f value was 16 and 66 for potassium clavulanate and cefpodoxime proxetil respectively. Densitometric analysis was carried out at 215 nm. The method was linear in the range of 500-2500 and 2000–10000 ng/band for cefpodoxime proxetil and potassium clavulanate respectively. The recoveries are in the range of 99.1-100.7 %.

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

106 142 V. GANGADEVI *, J. MUTHUMARY (*Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India): A simple and rapid method for the determination of taxol produced by fungal endophytes from medicinal plants using high-performance thin-layer chromatography. Chinese J. Chromatogr. 26 (1), 50-55 (2008). TLC of some endophytic fungi isolated by column chromatography from selected medicinal plants on silica gel plates with 1) chloroform - methanol 7:1, 2) chloroform - acetonitrile 7:3, 3) ethyl acetate - 2-propanol 19:1, 4) methylene chloride - tetrahydrofuran 3:1, 5) methylene chloride - methanol - dimethylformamide 90:9:1. Detection by spraying with 1 % vanillin sulfuric acid reagent after gentle heating. Also HPTLC on silica gel with chloroform - methanol 9:1. Detection by densitometry at 254 nm and 366 nm. If taxol was present, derivatization by spraying with 1 % vanillin sulfuric acid reagent and heating for 2 min, then detection under UV 366 nm and white light. Only 13 fungal species produced taxol in the artificial culture medium of the 20 screened fungi. pharmaceutical research

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

106 143 A. GANGWAL*, S. PARMAL, N. SHETH (*Dept. of Pharmaceutical Sciences, Suarashtra University, Rajkot 360005, India): Phytochemical investigation and immunomodulatory activity of Lagenaria siceraria fruits. Journal of Natural Remedies 10(2), 170-174 (2010). The powdered plant material was defatted, extracted with methanol, concentrated and successively extracted with ethyl acetate and *n*-butanol. The resulting fractions were concentrated and subjected to chromatographic fingerprint analysis of the phytoconstituents, i.e. alkaloids, saponins, tannins, flavonoids, anthraquinones and sterols. TLC on silica gel with *n*-butanol - acetic acid - water 4:1:5 for the ethyl acetate fraction and with chloroform - methanol 9:1 for the *n*-butanol fraction. Densitometric evaluation at 254 nm and 366 nm for the *n*-butanol fraction and at 290 nm for the ethyl acetate fraction. Derivatization with vanillin-sulfuric acid reagent for the *n*-butanol fraction and

with $AlCl_3$ reagent for the ethyl acetate-fraction, evaluation at 600 nm. The *n*-butanol fraction contained sterols and saponins, the ethyl acetate fraction flavonoids.

quality control, herbal, densitometry, qualitative identification 32e

106 144 A. HALKA, P.W. PLOCHARZ, A. TORBICZ, T.H. DZIDO* (*Department of Physical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland; tadeusz.dzido@umlub.pl): Reversed-phase pressurized planar electrochromatography and planar chromatography of acetylsalicylic acid, caffeine, and acetaminophen. J. Planar Chromatogr. 23, 420-425 (2010). TLC and pressurized planar electrochromatography (PPEC) of acetylsalicylic acid, caffeine, and acetaminophen on RP-18 and RP-18 W (prewashed with methanol) using acetonitrile - buffer - water (e. g. 15 % acetonitrile in pH 3.8 buffer) in a horizontal chamber saturated for 15 min. Evaluation by densitometry. The results showed that PPEC of these drugs is characterized by faster separation, better performance, and different separation selectivity. The technique is still under development, however, rather slow because a variety of technical challenges must be overcome.

quality control, comparison of methods 32a

106 145 Maha HEGAZY*, Fadia METWALY, M. ABDELKAWY, NADA ABDELWAHAB (*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562 Cairo, Egypt): Validated chromatographic methods for determination of hydrochlorothiazide and spironolactone in pharmaceutical formulation in presence of impurities and degradants. J. Chromatogr. Sci. 49, 129-135 (2011). TLC of hydrochlorothiazide (HCT), spironolactone (SPR) and their impurities and degradation products on silica gel with ethyl acetate - chloroform - formic acid - triethyl amine 70:30:1:1. Quantification by densitometry. The different method parameters were optimized for maximum separation. The method was applied for determination of HCT and SPR in commercial tablets. Statistical comparison with the also performed HPLC method showed that there is no significant difference in the performance of the methods.

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

106 146 K. HULLATTI*, M. SHARADA (*Dept. for Pharmacognosy, National College of Pharmacy, Shimoga-577201, DOS in Botany, Univ. of Mysore 570006, India, kkhullatti@gmail.com): Comparative phytochemical investigation of the sources of Ayurvedic drug Patha: a chromatographic fingerprinting analysis. Ind. J. Pharma. Sci. 72(1), 39-45 (2010). The Ayurvedic Pharmacopoeia of India recognized roots of the three plants Cissampelos pareira, Cyclea peltata, and Stephania japonica (all Menispermaceae) as source for the marketed drug Patha. HPTLC fingerprint analysis of methanolic extracts of roots of all three plants on silica gel with *n*-butanol - ethyl acetate - formic acid - water 3:5:1:1. Detection under UV 365 nm (crude extract) and 295 nm (total alkaloids). Quantification of the marker berberine by HPLC. The three plants exhibit significantly different physico-chemical features and chromatographic fingerprints. Roots of C. pareira contained the highest concentration of berberine, S. Japonica contained very low amounts and C. peltata no berberine at all.

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

106 147 Malgorzata JANICKA*, D. PIETRAS-OZGA (*Faculty of Chemistry, Department of Physical Chemistry, Maria Curie-Sklodowska University, M. Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland; malgorzata.janicka@poczta.umcs.lublin.pl): Chromatographic evaluation of the lipophilicity of N-phenyltrichloroacetamide derivatives using micellar TLC and OPLC. J. Planar Chromatogr. 23, 396-399 (2010). TLC of 8 newly synthesized N-phenyltrichloroacetamides on RP-18 W with

micellar-organic solutions composed of 0.02, 0.04, or 0.06 M Brij 35 (2-dodecoxyethanol, a non-ionic surfactant) and tetrahydrofuran 4:1 in saturated sandwich chambers. Detection under UV 254 nm. According to the results obtained from the OPLC studies, especially log km values are excellent descriptors of the lipophilicity of the compounds.

pharmaceutical research, HPTLC, qualitative identification, biochemical research 32a

106 148 S. KAUSHIK*, P. SHARMA, A JAIN, M. SIKARWAR (*Nagaji Institute of Pharmaceutical Sciences,Gwalior (MP), India): Preliminary phytochemical screening and HPTLC fingerprinting of Nicotiana tabacum leaf. Journal of Pharmacy Research 3(5), 1144-1145 (2010). The plant material (Nicotiana tabacum leaves) was extracted with non-polar and polar solvents. The resulting extracts were subjected to screening for different phyto-constituents (such as terpenes, saponins, flavonoids, alkaloids, and steroids) on silica gel using *n*-hexane - ethyl acetate 5:1 for fingerprint profiling. Detection under UV 254 nm. For derivatization the plate was sprayed with anisaldehyde-sulfuric acid reagent followed by densitometric evaluation at 550 nm. It was observed that fingerprint profiling after derivatization was more suitable than under UV before derivatization. Methanolic extracts of the plant yielded a superior profile.

herbal, HPTLC, qualitative identification, densitometry 32e

106 149 A. KHODKE, L. POTALE, Mrinalini DAMLE, K. BOTHARA (Dept. of Q. A. A.I.S.S.M.S College of Pharmacy Kennedy Rd., Near R.T.O. Pune 411001, India, mcdamle@rediffmail.com): A validated stability-indicating HPTLC method for simultaneous estimation of irbesartan and hydrochlorothiazide. Pharmaceutical Methods 1(1), 39-43 (2010). TLC on silica gel with acetonitrile - chloroform 5:6. The hR_f value of irbesartan was 27 and of hydrochlorothiazide 45. Densitometric evaluation at 270 nm. The method was linear in the range of 200-1000 ng/band for irbesartan and 200-600 ng/band for hydrochlorothiazide. The drug was subjected to different stress conditions (acid, base, oxidation, thermal, photolytic). All the degradation product were well resolved from the main drug. The proposed method is stability-indicating and suitable for routine analysis of fixed dose formulation in presence of degradation products.

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

106 150 A. KRISHNAVENI*, S. THAAKUR (*Dept. of Pharmacognosy, College of Pharmacy, Madurai Medical College, Tamil Nadu, India, akrishmaveni72@rediffmail.com): Quantification of harmaline content in Passiflora foetida by HPTLC technique. Journal of Pharmacy Research 2(5), 789-791 (2009). TLC of harmaline in methanolic leaf extracts of Passiflora foetida on silica gel with chloroform - acetone - diethylamine 5:4:1. The hR_f value of harmaline was 76. Densitometric quantification at 351 nm. The plant was found to contain 0.75 % w/w of harmaline. The method was linear in the range of 1-10 µg/band.

herbal, densitometry, quantitative analysis 32e

106 151 K. LAKSHMI*, Lakshmi SIVASUBRAMANIAN, A. PANDEY (*Dept. of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur 603203, Tamil Nadu, India, lakshmiss@hotmail.com): A validated HPTLC method for simultaneous determination of losartan and perindopril in tablet. Research J. Pharm. and Tech. 3(2), 559-561 (2010). TLC on silica gel with toluene - acetonitrile - formic acid 50:50:3 with chamber saturation for 30 min. The hR_f value was 27 for perindopril and 55 for losartan. Densitometric evaluation at 212 nm. The method was linear in the range of 5-30 µg/band and 1-10 µg/band for losartan and perindopril respectively. The average recovery was 98.2-99.9 %.

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

106 152 M. LI* (Li Min), J. CHEN (Chen Jianrong), L. LI (Li Li) (*Ningxia Inst. Cont. of Med. For Animal, Yinchuan, Ningxia 750001, China): (Identification of liquorice and Rhizoma zingiberis in Siyi decoction for animal by thin-layer chromatography) (Chinese). J. Tech. & Sci. of Ningxia Agr. & Forest, 5, 38-39 (2010). For liquorice TLC on silica gel with ethyl acetate - glacial acetic acid - formic acid - water 15:1:1:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 ºC. For Rhizoma zingiberis TLC on silica gel with cyclohexane - diethyl ether 1:1, detection by spraying with 10 % vanillin in sulfuric acid and heating at 105 ºC, followed by evaluation in daylight.

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

106 153 S. MENNICKENT*, R. FIERRO, M. VEGA, M. DIEGO, C. GODOY (*Department of Pharmacy, Faculty of Pharmacy, University of Concepción, P.O. Box 237, Concepción, Chile, smennick@udec.cl): Quantitative determination of fluoxetine in human serum by high-performance thin-layer chromatography. J. Sep. Sci. 33, 2206-2210 (2010). HPTLC of fluoxetine on silica gel with toluene - glacial acetic acid 4:5. Derivatization of fluoxetine with 4-dimethylamino-azobenzene-4-sulfonyl chloride. Quantitative determination by absorbance measurement at 272 nm. The hR_f of fluoxetine was 45. Linearity was between 0.05-0.35 ng/µL. Detection and quantification limits were 230 and 700 ng/µL, respectively. The intra-day and inter-day precisions showed a *%RSD* lower than 3.9 %. Recovery (by standard addition) was 94.7 %. The method was designed to cover the usual serum concentration level of patients taking the drug.

clinical routine analysis, HPTLC, quantitative analysis, densitometry 32f

106 154 P. MIGAS*, M. SWITKA (*Department of Pharmacognosy, Medical University of Gdansk, al. Gen. J. Hallera 107, 80-416 Gdansk, Poland; pmig@amg.gda.pl): TLC with an adsorbent gradient for the analysis of taxol in Taxus baccata L. J. Planar Chromatogr. 23, 286-288 (2010). TLC of taxol with a stationary-phase gradient whereby parts of different TLC plates were connected by use of a MIGAS device. Separated taxol-containing zones were developed with methanol over 1 cm and thus moved to another plate. The lipophilic substance zone was cut out after separation of the sample on a silica gel plate with n-heptane - ethyl acetate 1:1. Further separation of taxol from accompanying hydrophilic substances was carried out on HPTLC RP-18W with methanol - water 4:1. The taxol-containing fraction was finally separated on silica gel with chloroform - acetone 3:1 in a horizontal chamber at constant temperature (30 +/- 1 °C) and humidity (35 +/- 1 %). Detection under UV 254 and 366 nm. Quantitative determination by densitometry at 220 nm. The precision ($n = 7$) was 0.63 % and the repeatability ($n = 7$) 3.35 %. The limit of detection and quantification was 0.50 and 1.00 µg/zone, respectively; the correlation coefficient from linear regression was >0.98, and the linear calibration range was 1–10 µg/zone.

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

106 155 F. MIRZAJANI, A. GHASSEMPOUR, M. JALALI*, M. HOSSEIN (*Department of Chemistry, Sharif University of Technology, P.O. Box 11155-9516, Tehran, Iran, jalali@sharif.edu): Optimisation of a microwave-assisted method for extracting withaferin A from Withania somnifera Dunal. using central composite design. Phytochem. Anal. 21, 544-549 (2010). HPTLC of withaferin A in the aerial part of Withania somnifera Dunal. on silica gel with ethyl acetate - toluene - formic acid - 2-propanol 14:4:1:1. Quantitative determination by absorbance measurement at 220 nm. The hR_f of withaferin A was 29. Detection and quantification limits were 18 and 60 ng/zone respectively. The intra-day and inter-day precisions were 4.24 % and 14.28 %, respectively. Recovery (by standard addition) was 88.2 %.The correlation coefficient of the withaferin A determination was 0.9963.

herbal, HPTLC, quantitative analysis, densitometry 32e

106 156 H. MISRA*, B. DWIVEDI, Darshana MEHTA, B. MEHTA, D. JAIN (*School of Studies in Chemistry & Biochem, Vikram University, Ujjain 456010, MP, India): Development and validation of high-performance thin-layer chromatographic method for determination of alpha-mangostin in fruit pericarp of mangosteen plant (Garcinia mangostana L.) using ultraviolet-visible detection. Rec. Nat. Prod. 3/4, 178-186 (2009). An HPTLC method has been reported for quantitative estimation of alpha-mangostin in fruit pericarp of Garcinia mangostana (Hypericaceae). HPTLC on silica gel with chloroform - methanol 9:1 in a saturated chamber (10 min). The plate was derivatized with anisaldehyde sulfuric acid reagent. Densitometric quantification at 382 nm. Alpha-mangostin was detected as a compact band with hR_f value of 46. The method was linear in the range of 1-5 µg/band. The maximum yield of alpha-mangostin was obtained from the plant by hot extraction with methanol.

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

106 157 A. MOHAMMAD*, S. SHARMA, S. BHAWANI, R. SINGH (*Analytical Research Lab., Dept. of Applied Chem., Faculty of Eng. & Tech., Aligarh Muslim University Aligarh 202002, India): Identification and separation of Cannabis sativa, Embleia ribes, Myristica fragrans and Piper longum from organic extract on silica gel surface with anionic micellar solvent system: application in ayurvedic medicine. The Open Nutraceuticals Journal 2, 2-6 (2009) A TLC method using a micellar solution of sodium dodecyl sulfate (SDS) as mobile phase has been developed for identification of four herbals present in Jatiphaladya, a powdered herbal formulation containing Cannabis sativa, Myristica fragrans, Piper longum, and Embleia ribes. The formulation was extracted with 80 % ethanol. TLC on laboratory made plates coated with silica gel and activated at 100 °C for 60 min, with a 5 % solution of SDS as mobile phase. The resolved spots were identified by spraying with a 2 % solution of vanillin in 5 % methanolic sulfuric acid. Spots corresponding to different herbals were well resolved. Different detection reagents were evaluated, i.e. iodine, vanillin sulfuric acid, and anisaldehyde-sulfuric acid. Vanillin sulfuric acid reagent was found to be the most sensitive. Of the different surfactants used, anionic, cationic and nonionic, SDS was found to be most suitable. The most suitable pH of the mobile phase was pH 4.2-5.7, it provided optimum resolution of zones.

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

106 158 D. MUKHERJEE, N. KUMAR, T. KHATUA, P. MUKHERJEE* (*School of Natural Products Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India, pulokm@gmail.com): Rapid validated HPTLC method for estimation of betulinic acid in Nelumbo nucifera (Nymphaeaceae) rhizome extract. Phytochem. Anal. 21, 556-560 (2010). HPTLC of betulinic acid in the rhizomes of Nelumbo nucifera on silica gel with chloroform - methanol - formic acid 49:1:1. Detection by spraying with anisaldehyde - acetic acid - methanol - sulfuric acid 5:100:850:50 and drying at 50 °C for 10 min. Quantitative determination by absorbance measurement at 420 nm. The hR_f of betulinic acid was 30. Linearity was between 2 and 10 µg/zone. Detection and quantification limits were 0.4 and 2.30 µg, respectively. The intra-day and inter-day precisions were 0.4 % and 0.3 % ($n = 3$) respectively. Recovery (by standard addition) was 98.4 %.

herbal, HPTLC, quantitative analysis, densitometry, postchromatographic derivatization 32e

106 159 S. MUNNA*, K. JAYAVEERA, C. CHETTY, K. GNANAPRAKASH, K. ADINARAYANA (*Annamacharya College of Pharmacy, Rajampet, A.P., India, sreenivasulu_munna@yahoo.com): Heavy metal analysis of various parts of Ficus mollis (vahl) by TLC. International Journal of ChemTech Research 2(2), 807-812 (2010). Chloroform and ethyl acetate extracts of leaves and bark of Ficus mollis (Moraceae) were subjected to TLC fingerprint profiling on silica gel with toluene - ethyl acetate - formic acid 16:2:1. Evaluation under UV 254 nm. Derivatization with vanillin-sulfuric acid reagent, followed by heating at 105 °C until colorization. In the bark 7 well-defined zones were observed, whereas in leaves 10 zones were observed. Heavy metal and mineral analysis was performed by atomic absorption spectroscopy.

pharmaceutical research, quality control, herbal, clinical routine analysis, qualitative identification, postchromatographic derivatization 32e

106 160 Smita NAGAGOUDA*, A. KARIGAR, V. JOSHI, M. SIKARWAR (*Sonia College of Pharmacy, Dharwad, Karnataka, India, smithasanglad@ymail.com): Validated HPTLC method for mangiferin in Salacia chinesis. Journal of Pharmacy Research 3(5), 1107-1109 (2010). HPTLC of mangiferin in Salacia chinesis (Hippocrateaceae) on silica gel with ethyl acetate - methanol 2:3 at 25 °C with chamber saturation for 30 min. Densitometric evaluation at 254 nm. Derivatization by spraying with acetic anhydride-sulfuric acid-ethanol reagent, followed by heating at 110 °C for 2 min. The method was linear in the range of 10–200 ng/band. The plant was found to contain 1.54 % of mangiferin.

herbal, quality control, HPTLC, quantitative analysis, postchromatographic derivatization 32e

106 161 I. NAGUIB*, M. ABDELKAKAWY (*Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt, inaguieb@bsu.edu.eg): Development and validation of stability indicating HPLC and HPTLC methods for determination of sulpiride and mebeverine hydrochloride in combination. Eur. J. Med. Chem. 45, 3719-3725 (2010). HPTLC of sulpiride and mebeverine on silica gel with ethanol - methylene chloride - triethyl amine 35:15:1. Quantitative determination by absorbance measurement at 221 nm. The hR_f of sulpiride and mebeverine was 42 and 62, respectively. Linearity was between 0.4-1.4 µg/band for sulpiride and 0.2-1.6 µg/band for mebeverine. Detection and quantification limits were 0.02 and 0.3 µg/band for sulpiride and 0.04 and 0.2 µg/band for mebeverine. The intra-day and inter-day precisions had a *%RSD* lower than 2.6 %.Recoveries (by standard addition) were 99.3 % and 100.7 % for sulpiride and mebeverine, respectively. The proposed method showed comparable statistical results with the standard HPLC method

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

106 162 R. NANDA*, S. POTAWALE, V. BHAGWAT, R. DESHMUKH, P. DESHPANDE (*Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pimpri, Pune 411018, India, sachin_potawale@yahoo.co.in, rabindrananda@rediffmail.com): Development and validation of a HPTLC method for simultaneous densitometric analysis of ranitidine hydrochloride and dicyclomine hydrochloride as the bulk drugs and in the tablet dosage form. Journal of Pharmacy Research 3(8), 1997–1999 (2010). TLC on silica gel (plates pre-washed with methanol) with methanol - water - acetic acid 80:20:1. The hR_f value of ranitidine HCl was 27 and of dicyclomine HCl was 67. Derivatization by exposure to iodine vapor. Densitometric evaluation at 410 nm. The method was linear in the range of 400–2400 ng/band for dicyclomine hydrochloride and 150–900 ng/band for ranitidine hydrochloride. The mean recovery was 98.8 ± 0.5 % for ranitidine HCl and 99.1 ± 0.8 % for dicyclomine HCl.

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

106 163 J. ODOVIC*, M. ALEKSIC, B. STOJIMIROVIC, D. MILOJKOVIC, Z. TESIC (*Faculty of Pharmacy, University of Belgrade, 11001 Belgrade, Serbia): Normal-phase thin-layer chromatography of some angiotensin converting enzyme (ACE) inhibitors and their metabolites. J. Serb. Chem. Soc. 74(6), 677-688 (2009). An NP-TLC method has been reported to study the lipophilicity of 5 ACE inhibitors (lisinopril, quinapril, fosinopril, enalapril, cilazapril and their metabolites). TLC on silica gel with several non-aqueous mono and binary solvent systems. Binary mobile phases demonstrated a decrease in hR_f values of all 5 ACE inhibitors, i.e. increased retention with increased amounts of the less polar component in the mobile phase. Metabolites usually exhibited stronger retention, i.e. lower hR_f values than the corresponding ACE inhibitor. This is probably due to a different interaction with silica gel because of two carboxylic groups in the structure of the metabolites, whereas ACE inhibitors contain only one carboxyclic group. Results obtained on NP-TLC were compared with those by RP-TLC and no significant difference was found regarding lipophilicity.

pharmaceutical research, qualitative identification 32a

106 164 P.N. OKUSA*, C. STÉVIGNY, M. DEVLEESCHOUWER, P. DUEZ (*Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, Université Libre de Bruxelles (ULB), CP 205/09, Bd du Triomphe, 1050 Brussels, Belgium; okusandj@ulb.ac.be): Optimization of the culture medium used for direct TLC-bioautography. Application to the detection of antimicrobial compounds from Cordia gilletii De Wild (Boraginaceae). J. Planar Chromatogr. 23, 245-249 (2010). TLC of methanol extracts of Cordia gilletii on silica gel with dichloromethane, dichloromethane - methanol 9:1 or 4:1, or petroleum ether - diethyl ether - acetic acid 90:10:1. Detection by spraying with 1 % vanillin in ethanol followed immediately by 10 % ethanolic sulfuric acid, and heating at 105 °C for 10 min. For bioautography, 1 mL of 0.5 McFarland microorganism suspension was diluted with 9 mL of the tested mixture of Mueller-Hilton broth and agar 9:1) at 37 °C. After distribution of the inoculated mixture over the plate and solidification of the medium at ambient temperature, the plate was incubated overnight at 37 °C. The bioautogram was subsequently sprayed with an aqueous solution of MTT 0.8 mg mL-1 and incubated at 37 °C for 4 h. Also application of the Bioluminex assay (a TLC detection method based on the natural bioluminescence of the non-pathogenic bacteria Vibrio fischerii). The bioluminescent bacterial coating was visualized by use of the Bioluminizer.

herbal, quality control, qualitative identification, bioautography 32 e

106 165 Mrunali PATEL*, Rashmin PATEL, J. PARIKH, B. PATEL (*Indukaka Ipcowala College of Pharmacy, Sardar Patel University, New Vallabh Vidyanagar 388121, India, mrunalipatel@gmail.com): HPTLC method for estimation of tazarotene in topical gel formulations and in vitro study. Analytical Methods 2, 275-281 (2010). HPTLC on silica gel with toluene - methanol 9:1. The hR_f value was 75. Densitometric evaluation at 372 nm. The method was linear in the range of 100-600 ng/band. The recovery was 99.2-100.4 %. The method was suitable for microemulsion based formulations without any interference from excipients.

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

106 166 P. PIKUL*, J. NOWAKOWSKA, P. ROGULSKI (*Medical University of Gdansk, Department of Physical Chemistry, Al. Gen. Hallera 107, 80-416 Gdansk, Poland; pikul.piotr@gmail.com): Simple thin-layer chromatographic method for analysis of ranitidine in its pharmaceutical formulations: Application to the analysis of photolytic degradation products. J. Planar Chromatogr. 23, 304–308 (2010). Chromatographic investigation of ranitidin and degradation products by TLC on silica gel, RP-18, alumina, cellulose, and amino phase and by HPTLC on silica gel and amino phase. HPTLC of ranitidin and degradation products in pharmaceutical preparations on silica gel

with pure solvents (methanol, ethyl acetate, acetonitrile, and DMSO) and binary mixtures (acetonitrile - DMSO, acetonitrile - methanol, and ethyl acetate - methanol) with chamber saturation. The best separation of ranitidine and its photolytic degradation products was obtained on RP-18 W with methanol - water mixtures in a wide range of concentrations as mobile phase. Detection by spraying with sulfuric acid - methanol 1:4 and heating for 10 min at 120 °C.

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

106 167 V.J. RAO*, L. SATHIYANARAYANAN, S. YADAV (*Dept. of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy Pune 411038, M.S., India, janhavirao@rediffmail.com): Stability indicating HPTLC method for trandolapril estimation in the bulk drug and tablet dosage form. Indian J. Pharma. Educ. Res. 44(4), 341–344 (2010). HPTLC of silica gel with toluene - ethyl acetate - methanol - formic acid 5:16:21. The hR_f value was 51. Densitometric evaluation at 220 nm. The method was linear in the range of 300–1800 ng/band. The sample was subjected to different stress conditions (acid, base, oxidative, thermal, and photolytic). Degradation products were well separated from trandolapril, therefore the method can be used for stability studies.

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

106 168 L. RUSU, C. MARUTOIU, M. RUSU, A. SIMIONESCU, M. RUSU, Z. MOLDOVAN, C. BARBU* (*Spiru Haret University, Brazda lui Novac Street, No.4, Craiova, Dolj, Romania, cristina_barbu2000@yahoo.co.uk): HPTLC and MS for separation and identification of some beta-blockers in urine. Asian Journal of Chemistry 22(6), 4209-4213 (2010). HPTLC of beta-blockers on silica gel with toluene - ethyl acetate - acetone - 25 % ammonia 10:20:20:1. The method was applied to biological samples (urine and blood). The drugs were extracted from biological samples by liquid-liquid extraction. Detection at 254 nm. The corresponding separated spots were scraped from the plate, extracted with methanol and analyzed by mass spectrometry. Finally the identity was confirmed by comparing the spectra with a data library. The proposed HPTLC method coupled with MS is very sensitive and highly suitable for biological samples.

pharmaceutical research, quantitative analysis, HPTLC 32a

106 169 G.A. SALEH, F.A. MOHAMED, S.R. EL-SHABOURY, A.H. RAGEH* (*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut Univeristy, 71526 Assiut, Egypt): Selective densitometric determination of four alpha-aminocephalosporins using ninhydrin reagent. J. Chromatogr. Sci. 48, 68-75 (2010). TLC of cefaclor, cefadroxil, cefalexin and cefradine on silica gel with ethyl acetate - methanol - water in different ratios. The studied drugs had hR_f values between 40 and 60. Detection of blue to violet colored zones after spraying with ninhydrin reagent. Quantification by densitometry. Linearity was in the range of 2-10 µg/zone with correlation coefficients ranging from 0.9990 to 0.9996 and determination coefficients from 0.9986 to 0.9992 for all studied drugs. The limits of detection and quantification for all studied drugs were between 90 to 230 ng/zone and 270 to 840 ng/zone, respectively.

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

106 170 A. SHARMA, B. PATEL, R. PATEL* (*Prof. Parul Institute of Pharmacy, Vaghodiya, Vadodara, Gujarat, India, drbhp@rediffmail.com): Simultaneous estimation of nebivolol hydrochloride and *s*-amlodipine besylate by high-performance thin-layer chromatography. International Journal of Pharma and Bio Sciences 1(4), 339-345 (2010). TLC on silica gel (plates pre-washed with methanol) with chloroform - toluene - methanol - glacial acetic acid 50:20:30:1. The hR_f value of *s*-amlodipine was 33 and of nebivolol 48. Densitometric evaluation at 271 nm. The linearity range

was 500-2500 ng/zone for nebivolol and 250-1250 ng/zone for s-amlodipine. The method was suitable for routine analysis of the formulation.

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

106 171 D. SHEWIYO*, E. KAALE, C. UGULLUM, M. SIGONDA, P. RISHA, B. DEJAEGHER, J. VERBEKE, Y. HEYDEN (Tanzania Food and Drugs Authority, P.O. Box 77150, Dares Salaam, Tanzania): Development and validation of a normal-phase HPTLC method for the simultaneous analysis of lamivudine, stavudine and nevirapine in fixed-dose combination tablets. J. Pharm. Biomed. Analysis 54, 445-450 (2011). An improved HPTLC method has been developed for simultaneous estimation of lamivudine (LVD), stavudine (STV), and nevirapine (NVP) in fixed dose formulation. HPTLC on silica gel with ethyl acetate - methanol - toluene - 25 % ammonia 12:6:12:1. The hR_f value is 24 for LVD, 38 for STV and 69 for NVP. Densitometric evaluation at 254 nm. The method was linear in the range of 42-209 ng/band (LVD), 38-192 ng/band (STV) and 42-208 ng/band (NVP). The recovery values were in the range of 98.4-102.2 % for all three compounds. The method was found suitable for quality control of LVD, STV and NVP in fixed dose formulation.

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

106 172 Malgorzata STAREK*, J. KRZEK, M. TARSA (*Department of Inorganic and Analytical Chemistry, Collegium Medicum, Jagiellonian University, 9 Medyczna Str., 30-688 Kraków, Poland; mstarek@interia.pl): TLC-densitometric method for quantification of oxaprozin and its degradation products in pharmaceutical preparations. J. Planar Chromatogr. 23, 298-303 (2010). TLC of oxaprozin on silica gel with *n*-hexane - chloroform - glacial acetic acid 4:1:1 with chamber saturation for 15 min at room temperature. The hR_f value of oxaprozin was 56. Quantitative determination by absorbance measurement at 286 nm. The limit of detection and quantification was 16 and 40 µg/mL, respectively. Linearity was between 33 and 433 µg/mL. The recovery was between 98.9 and 101.6 % at different spiking levels. Intra-day precision (*%RSD*) at different concentration levels was 0.86-1.03 % and inter-day precision 0.99-1.15 %. Oxaprozin was subjected to stress tests (acidic and alkaline hydrolysis) and the method was able to separate the degradation products from the main compound.

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

106 173 T. TUZIMSKI (Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland, tomasz.tuzimski@umlub.pl): Determination of clofentezine in medical herb extracts by chromatographic methods combined with diode array scanning densitometry. J. Sep. Sci. 33, 1954-1958 (2010). HPTLC of clofentezine in medical herb extracts on silica gel with tetrahydrofuran - *n*-heptane 3:7. After drying for 20 min, the plate was turned 90 ° and developed with ethyl acetate - *n*-heptane 1:4. Quantitative determination by absorbance measurement at 254 nm. Limits of detection and quantification were 230 and 700 ng/zone respectively. The average recovery (by standard addition) was 55.8 %.

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

106 174 S.J. VARGHESE*, T.K. RAVI (*Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Department of Pharmaceutical Analysis, Coimbatore 641 044, Tamil Nadu, India; susheeljv@yahoo.com): Determination of rosuvastatin and ezetimibe in a combined tablet dosage form using high-performance column liquid chromatography and high-performance thin-layer chromatography. J. AOAC Int. 93, 1222-1227 (2010). HPTLC of rosuvastatin (ROS) and ezetimibe (EZE) on silica gel with n-butyl-acetate - chloroform - glacial acetic acid 1:8:1 with chamber saturation for 30 min. Quantitative determination by densitometry at 245 nm. The hR_f value of

ROS was 30 and of EZE 58. The linearity for ROS and EZE was 0.1 to 0.9 µg/zone.The intraday and interday precision was 0.62 % and 0.73 % for ROS and 0.59 % and 0.69 % for EZE ($n = 3$). The LOD values for ROS and EZE were found to be 0.04 and 0.07 µg/spot, respectively, and their LOQ values were 0.07 and 0.1 µg/spot, respectively. The recovery study results ranged from 98 to 101 % for ROS and EZE.

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

106 175 N.E. WAGIEH*, Maha HEGAZY, M. ABDELKAWY, E.A. ABDELALEEM (*Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Egypt): Quantitative determination of oxybutynin hydrochloride by spectrophotometry, chemometry and HPTLC in presence of its degradation product and additives in different pharmaceutical dosage forms. Talanta, 80 (5), 2007-2015 (2010). Presentation of a method for determination of oxybutynin hydrochloride (OX) in presence of its degradation product and additives in its pharmaceutical formulations. UV spectrophotometry using the first derivative of ratio spectra and measurment at 216 nm. Chemometric analysis using principal component regression and partial least-squares. HPTLC of OX and its degradation products methylparaben and propylparaben on silica gel with chloroform - methanol - ammonia - triethylamine 500:15:2.5:1. Quantitative determination by densitometry at 220 nm. Comparison of the results obtained with all three methods showed no significant differences.
pharmaceutical research, quality control, qualitative identification, autoradiography,

comparison of methods, HPTLC, quantitative analysis 32c

106 176 S. WANKHEDE*, K. RAKA, S. WADKAR, S. CHITLANGE (Dept. of Pharmaceutical Chemistry, Padmashri Dr. D. Y. Patil Institute of Phaemaceutical Science and Research, Sant Tukaram Nagar, Pimpri, Pune 411018, India, drsagarwankhede@rediffmail.com): Development and validation of stability-indicating HPTLC method for analysis of amlodipine besilate, losartan potassium and hydrochlorothiazide in pharmaceutical dosage form. Journal of Pharmaceutical Research 9(2), 60-62 (2010). TLC on silica gel with chloroform - ethyl acetate - methanol - ammonia 20:20:10:1 with chamber saturation for 30 min. Densitometric evaluation at 232 nm. The method was linear in the range of 100-800 ng/band for amlodipine, 400-2400 ng/band for hydrochlorothiazide and 1000-8000 ng/band for amlodipine. The average recovery was 99.1-100.4 % for all three compounds. The method was suitable for stability studies (stress conditions: acid, base, thermal, UV).

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

106 177 H. ZUO (Zuo Hongxiang)*, Y. JIN (Jin Yong), CH. ZHANG (Zhang Chengyi),J. MA (Ma Jimei) (*Pharm. Coll. Beihua Univ.,Jilin, Jilin 132013, China): (On the quality standard of Kongzhenyizhi tablets) (Chinese). J. of Beihua Univ. (Natural Sci.), 11 (5), 420-423 (2010). TLC on silica gel with 1) petroleum ether (60-90 ºC) - ethyl acetate 4:1 and 2) chloroform - propanone - *n*-hexane - acetic acid 80:40:2:5. Detection under UV 365 nm. Derivatization by spraying with 10 % vanillin in sulfuric acid and heating at 105 ºC until the zones were visualized. Identification by comparison with the standards of the components in the individual composition drug.

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

33. Inorganic substances

106 178 A. MOHAMMAD*, S. BHAWANI (*Analytical Research Lab., Dept. of Applied Chemistry, Faculty of Eng. & Tech., Aligarh Muslim University, Aligarh, UP, India): On plate resolution of three-component mixture of cationic surfactants with mixed aqueous-organic eluents containing

formate ion. Separation Science and Technology 44, 1007-1021 (2009). A TLC method for mutual separation of three longchain aliphatic quaternary ammonium halides (cationic surfactants) is reported. TLC of dodecyltrimethyl ammonium bromide (DTAB), tetradecyltrimethyl ammonium bromide (TTAB) and hexadecyltrimethyl ammonium chloride (HTAC) on laboratory made TLC plates coated with Kieselguhr (0.25 mm), with methanol - 10 % sodium formate 3:7 as mobile phase. Detection with modified Dragendorff reagent comprised of solution A (containing bismuth subnitrate and KD) and solution B (containing barium chloride). Solutions A and B were mixed 2:1. The compounds appeared as orange-coloured spots. The effects of alcohols (ethanol, *n*-propanol), substitution of the formate ion by benzoate and acetate ion, different adsorbents (silica gel, aluminum), and the interference of metal ions (Cu^{2+}, Zn^{2+}, Hg^{2+}, Co^{2+}, Pb^{2+}) on the resolution of the three surfactants was studied. The limits of detection of DTAB, TTAB, and HTAC estimated were 3.3, 3.1, and 2.8 µg/zone respectively. The method was applied for separation of these compounds in water samples.

cosmetics, quality control, qualitative identification 33a

35. Other technical products and complex mixtures

106 179 V. GHOULIPOUR, S. AMINI, A. HAGHSHENAS, S. WAQIF-HUSAIN* (*Chemistry Department, Faculty of Science, Science and Research Branch, Islamic Azad University, P. O. Box 14515-775, Poonak-Hesarak, Teheran, Iran; syedwaqifhusain@yahoo.com): Chromatographic behavior of food additives on thin layers of titanium(IV) silicate ion-exchanger. J. Planar Chromatogr. 23, 250-254 (2010). TLC of 30 food additives (aldehydes, organic acids, esters and alcohols, and various sodium salts) on titanium(IV)silicate ion-exchanger with 10 different mobile phases: e. g. methanol, heptane - diethylether 4:1, aqueous ammonia - methanol - ethyl acetate 1:3:6, methanol - aqueous ammonia 9:1, methanol - ethanol 17:3, 0.5 M ammonium sulfate solution, 0.25 M, 0.5 M and 0.1 M potassium bromide solution, and phosphate buffer pH 2.5 in a twin-trough chamber without chamber saturation. Detection by spraying with 1 % iron chloride solution, 0.5 % potassium permanganate and 3 % barium chloride solution 1:1, 1 % ninhydrin in ethanol, 2 % phosphomolybdic acid in ethanol, and 5 % potassium dichromate in concentrated sulfuric acid. The study shows that the quality of separation depends to a large extent on the mobile phase, and selectivity is achieved by varying the composition of the mobile phase.

food analysis, qualitative identification 35b

106 180 M. IDRIS, S. SRIVASTAVA, T. R. BAGGI, S. K. SHUKLA* (*Central Forensic Science Laboratory, Directorate of Forensic Science, Ministry of Home Affairs, Government of India, Ramanthapur, Hyderabad 500 013 India; drskshukla@gmail.com): High-performance thin-layer chromatography analysis of saccharin in foods and beverages. J. Planar Chromatogr. 23, 339-342 (2010). HPTLC of saccharin in foodstuffs (e. g. cola drinks, lemon juices, betel nut powder, mouth fresheners, ice candy, and tabletop sweeteners) on silica gel with chloroform - methanol - acetic acid 64:35:1 or acetone - isopropanol - acetic acid 60:39:1. Quantitative determination by absorbance measurement at 230 nm. Linearity was between 250 - 1250 ng/µL. The limit of detection and quantification for saccharin were 40 and 130 ng, respectively. Mean recovery from spiked samples was 102.3 % for cola drinks and 98.8 % for lemon juices. Relative standard deviation (*% RSD*) for cola drinks, lemon juices, ice candy, mouth freshener, betel nut powders, and tabletop sweeteners were 2.1, 4.2, 3.4, 3.0, 4.9, and 4.1 %, respectively.

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

106 181 G. XIAO, G. LI, L. CHEN, Z. ZHANG, J.J YIN, T. WU*, Z. CHENG, X. WEI, Z. WANG (*The MOE Key Laboratory for Standardization of Chinese Medicines, the SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, and the Shanghai Key Laboratory for Compound Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of

Traditional Chinese Medicine, 1200 Cailun Road, Zhangjiang Hi-Tech Park, Shanghai 201210, China): Isolation of antioxidants from Psoralea corylifolia fruits using high-speed counter-current chromatography guided by thin layer chromatography-antioxidant autographic assay. J. Chromatogr. A 1217(34), 5470-5476 (2010). Description of a combination method using high-speed counter-current chromatography and TLC as an antioxidant assay to separate antioxidant components from the fruits of Psoralea corylifolia by dipping in a ethanolic DPPH radical solution (2.54 mM) for autography. Bands with the DPPH scavenging activity were observed as white yellow bands on a purple background. High-speed counter-current chromatography of five flavonoids and three coumarins from the fruits of P. corylifolia with *n*-hexane - ethyl acetate - methanol - water 10:11:13:1. Preparative TLC for clean-up improves the substance yield from the fruits.

herbal, pharmaceutical research, qualitative identification, autoradiography, preparative TLC 35

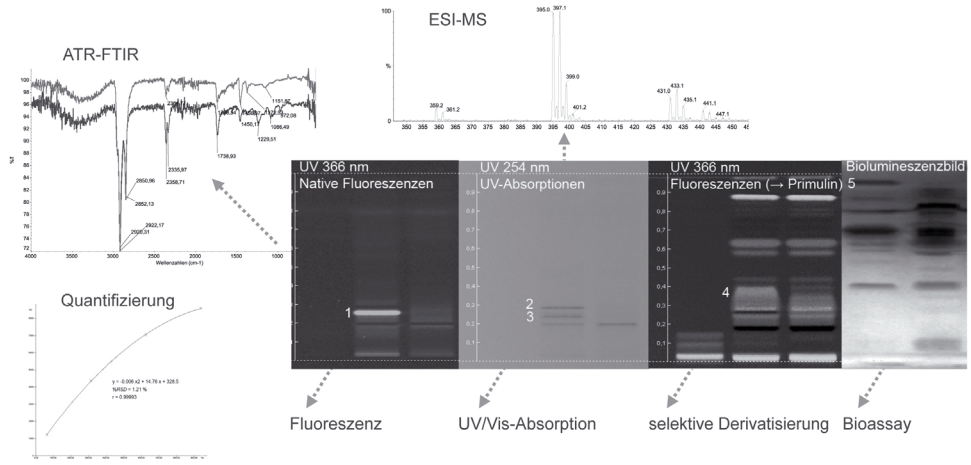
38. Chiral separation

106 182 R. BHUSHAN*, Shivani TANWAR (*Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, India): Different approaches of impregnation for resolution of enantiomers of atenolol, propranolol and salbutamol using Cu(II)-L-amino acid complexes for ligand exchange on commercial thin-layer chromatographic plates. J. Chromatogr. A 1217(8), 1395-1398 (2010). TLC of atenolol, propranolol and salbutamol and their enantiomers by using different modes of loading/impregnating the Cu(II) complexes of L-proline, L-phenylalanine, L-histidine, N,N-dimethyl-L-phenylalanine, and L-tryptophan on silica gel with 1) the Cu(II)-L-amino acid complex as chiral mobile phase additive, 2) ascending development of plain commercial plates in the solutions of Cu complex, and 3) using a solution of Cu(II)acetate as mobile phase additive for the commercial TLC plates impregnated by development with the amino acid solutions. Detection by exposure to iodine vapor. The detection limit was 0.18 µg for each enantiomer. For the best method performance the Cu(II)cation has to be involved.

quality control, pharmaceutical research, quantitative analysis, qualitative identification 38

GDCh-Kurs 335/11
Hochleistungs-Dünnschicht-Chromatographie-Massenspektrometrie (HPTLC-MS)

in Zusammenarbeit mit der Universität Hohenheim, Stuttgart



Ziele
- Potential der HPTLC erkennen
- Aktuelle Kopplungen der HPTLC kennenlernen
- Erkennen, wie Hyphenations (Kopplungen) in der HPTLC die Analytik effizient unterstützen

Zielgruppe
Analytiker, Lebensmittelchemiker, Pharmazeuten, Chemiker etc., die das Potential der HPTLC samt ihren Kopplungsmöglichkeiten für ihre Zwecke ausloten möchten

Veranstaltungsort
Universität Hohenheim, Institut für Lebensmittelchemie, Garbenstrasse 28, 70599 Stuttgart

Programm am Dienstag, 15.11.2011

9:30 Begrüßung und Einführung in die HPTLC (Morlock)
10:00 HPTLC erfahren – Experimente Gruppe 1 (Oellig), Gruppe 2 (Morlock)
11:00 Kaffeepause
11:15 Hyphenations in der Planar-Chromatographie (Morlock)
12:00 Gruppe 1 Experiment DC-HPLC/DAD-ESI/MS (Schwack, Oellig)
Gruppe 2 Experiment HPTLC-UV/Vis/FLD-MALDI-TOF MS/MS (Schürenberg)
12:45 Gruppe 1 Experiment HPTLC-UV/Vis/FLD-Bioaktivität-ESI/MS (Morlock)
Gruppe 2 Experiment HPTLC-UV/Vis/FLD-ATR FTIR (Dytkiewitz)
13:30 Mittagspause (Mensaessen)
14:00 Gruppe 1 Experiment HPTLC-UV/Vis/FLD-MALDI-TOF MS/MS (Schürenberg)
Gruppe 2 Experiment DC-HPLC/DAD-ESI/MS (Schwack, Oellig)
14:45 Gruppe 1 Experiment HPTLC-UV/Vis/FLD-ATR FTIR (Dytkiewitz)
Gruppe 2 Experiment HPTLC-UV/Vis/FLD-Bioaktivität-ESI/MS (Morlock)
15:30 Kaffeepause (Umbau ESI auf DART)
15:45 Experiment HPTLC-UV/Vis/FLD-DART-MS (Morlock)
16:15 Diskussion der Ergebnisse anhand der Vergleichsproben (Morlock)
16:45 Ende

Kursleitung: apl. Prof. Dr. G. Morlock, Universität Hohenheim, Stuttgart
Referenten:
· Prof. Dr. Wolfgang Schwack, Universität Hohenheim, Stuttgart
· Dr. Martin Schürenberg, Bruker Daltonik GmbH, Bremen
· Claudia Oellig, Universität Hohenheim, Stuttgart
· Elisabeth Dytkiewitz, Universität Hohenheim, Stuttgart
Teilnehmerzahl: max. 16 Personen
Gebühren: GDCh-Mitglied € 570.–, Nichtmitglied € 680.–
Anmeldeschluss: 18.10.2011
Anmeldung: siehe www.gdch.de/vas/fortbildung.htm oder per E-Mail an fb@gdch.de

High-performance thin-layer chromatography – mass spectrometry (HPTLC-MS)
This course organized by the German Chemical Society will be held November 2011 in German language. The same course in English is tentatively planned for November 2012.

Objectives
- Recognize the potential of HPTLC
- Learn more about current hyphenations in HPTLC
- Figure out how HPTLC hyphenations efficiently support analyses

Target group
Analysts, food chemists, pharmacists, chemists etc. who like to evaluate for their projects the benefits of hyphenating HPTLC with mass spectrometry etc.

Location
University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart

Program

9:30 Welcome and introduction in HPTLC
10:00 Realize the power of HPTLC – experiments
11:15 Hyphenations in planar chromatography
12:00 Group 1 Experiment TLC-HPLC/DAD-ESI/MS
Group 2 Experiment HPTLC-UV/Vis/FLD-MALDI-TOF MS/MS
12:45 Group 1 Experiment HPTLC-UV/Vis/FLD-bioassay-ESI/MS
Group 2 Experiment HPTLC-UV/Vis/FLD-ATR FTIR
13:30 Lunch at the cafeteria
14:00 Group 1 Experiment HPTLC-UV/Vis/FLD-MALDI-TOF MS/MS
Group 2 Experiment TLC-HPLC/DAD-ESI/MS
14:45 Group 1 Experiment HPTLC-UV/Vis/FLD-ATR FTIR
Group 2 Experiment HPTLC-UV/Vis/FLD-bioassay-ESI/MS
15:45 Experiment HPTLC-UV/Vis/FLD-DART-MS
16:15 Discussion of the results based on a common sample

Course leader: Assoc. Prof. Dr. G. Morlock, University of Hohenheim, Stuttgart
Further tutors:
· Prof. Dr. Wolfgang Schwack, University of Hohenheim, Stuttgart
· Dr. Martin Schürenberg, Bruker Daltonik GmbH, Bremen
· Claudia Oellig, University of Hohenheim, Stuttgart
· Elisabeth Dytkiewitz, University of Hohenheim, Stuttgart
Number of participants: max. 16
Fee: GDCh members € 570.–, non-members € 680.–
Interested? Please email fb@gdch.de

continuation from page 7

migration time 15 min; automatic drying for 1 min each after sample application and chromatography

Post-chromatographic derivatization

With the Chromatogram Immersion Device the plate was immersed (vertical speed 2.5 cm/s, immersion time 0 s) into the aniline diphenylamine *o*-phosphoric acid reagent, followed by heating the plate on the TLC Plate Heater at 120 °C for 20 min. The reagent (1.5 g of aniline and diphenylamine each were dissolved in 150 mL methanol and 15 mL *o*-phosphoric acid was added dropwise) stored in the refrigerator is stable for at least one month.

Documentation

With the DigiStore2 Documentation System, exposure time 7 ms (white light, reflectance and transmission combined) and 1300 ms (UV 366, reflectance mode), gain of 1

Densitometry

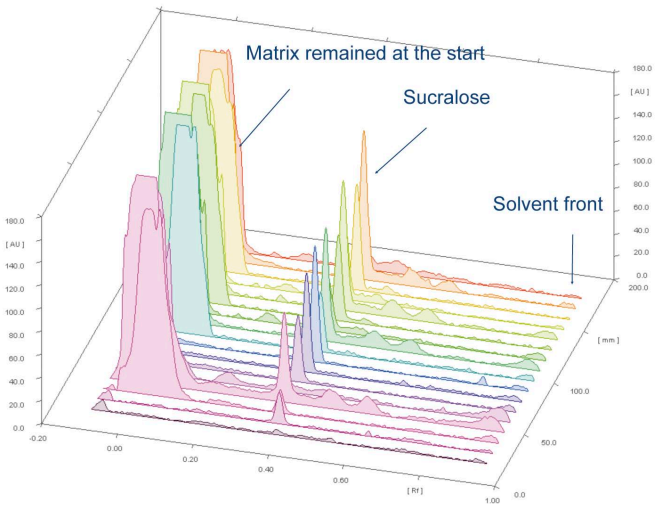
TLC Scanner 3 with winCATS software, absorption measurement at 400 nm, slit dimension 6 × 0.45 mm, scanning speed 20 mm/s

Mass spectrometry

Via the TLC-MS Interface, the respective zones were eluted using methanol – ammonium formiate buffer (10 mM, pH 4) 19:1 at a flow rate of 0.1 mL/min (HP 1100 pump, Agilent) and transferred to the single-quadrupole mass spectrometer (MSD, Agilent). The electrospray ionization (ESI) mass spectra were recorded in the full scan mode: capillary voltage + 4 kV and – 4 kV, nebulizer gas pressure 20 psig, drying gas temperature 300 °C, drying gas flow rate 10 L/min, fragmentator voltage 100 V, gain 1, threshold 10, and step size 0.25.

Results and discussion

For method validation, the mean recovery of sucralose in drinking water spiked at 1 µg/L was determined to be 84 ± 7 % (n = 3). If a 0.5 L sample being extracted at a supposed recovery rate of 80 % and thereof 300 µL being applied on the plate, the limit of detection was 100 ng/L (6 ng/band). The calibration curve (10–300 ng/band, coefficient of correlation r = 0.9999, residual standard deviation sdv = 1.3 %) was suited to analyze sucralose findings in water samples ranged between 0.1 and 5 µg/L. The calibration in matrix was performed by overspraying start zones of water extracts (multi-fold applied) with different volumes of sucralose standard solution. The slope of this curve was comparable to the external standard calibration curve, which was herewith proven to be suited for routine use. However, for low sucralose findings (< 200 ng/L), matrix calibration was preferred as it corrected any potential matrix influence.



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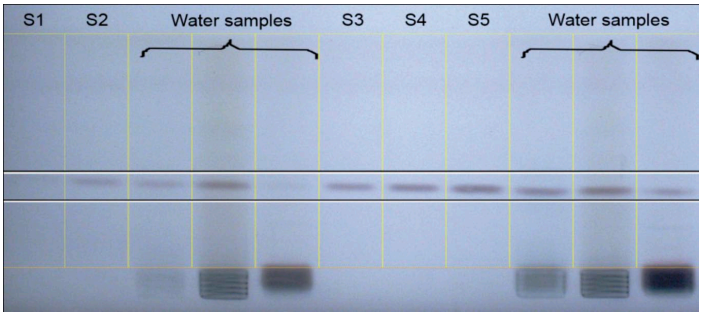
For proof of principle, samples obtained in an interlaboratory study were analyzed by HPTLC and the results were compared to the respective means of the interlaboratory study obtained by HPLC-MS, mostly corrected by isotopically labeled standards. The HPTLC results corrected by the recovery rate showed good accuracy and the bias (in %) to the specified value was determined to be between 0 and 33 %. According to the t-test, both mean values were statistically not different (calculated values < t-table value of 9.93, P = 99 %, two-sided, υ = 2).



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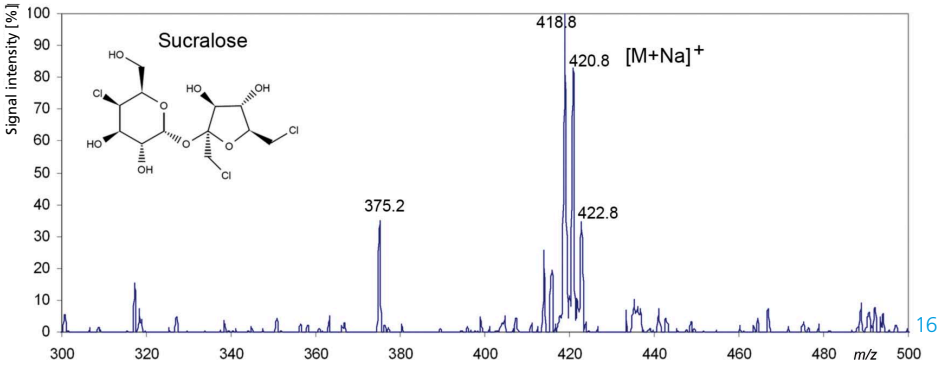
Sucralose in sample (ng/L)	Effluent water I	Effluent water II	Surface water I	Surface water II
Mean value of HPTLC-Vis (*%RSD*, $n = 2$)	5863 ± 5	7034 ± 1	247 ± 28	218 ± 13
Mean value of HPLC-MS/MS or HPLC-TOF MS (*%RSD*, $n = 6$ laboratories*)	5869 ± 17	7302 ± 24	186 ± 21	200 ± 23
Bias in %	0.1	3.7	32.8	9.0
Calculated t-value	0.04	5.42	1.25	0.90

*mostly corrected by expensive, isotopically labeled standards

For mass spectra, the formiate adduct was the most pronounced signal group in the negative ionization mode and the sodium adduct in the positive ionization mode, whereby the 3-fold chlorine isotope pattern was clearly visible. The capability of detection (S/N of 3) was about 100 ng/zone using standard settings.



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As the HPTLC method is intended to find key sources of sucralose input, a high sample throughput is required. Up to 17 samples can be analyzed at low running costs within 1.4 h (without SPE), which is very effective compared to the given analytical methods, also considering that HPTLC using a simple selective derivatization can yield comparable results to HPLC-TOF-MS or HPLC-MS/MS with isotopically-labelled standards.

Further information is available on request from the author.

[1] G. Morlock, S. Prabha J. Agric. Food Chem. 55 (2007) 7217

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[3] G. Morlock *et al.* J. Chromatogr. A, doi 10.1016/j.chroma.2010.11.063

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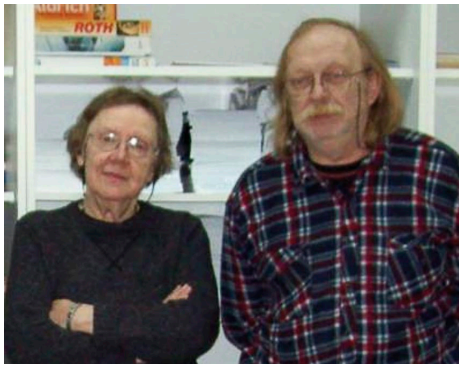
TLC/HPTLC fingerprinting of herbal essential oil followed by liquid chromatography hyphenated with the TLC-MS Interface



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Prof. Teresa Kowalska und Dr. Mieczysław Sajewicz

Planar chromatography has been the special focus of attention of Prof. Teresa Kowalska and Dr. Mieczysław Sajewicz, Department of General Chemistry and Chromatography, University of Silesia, Katowice, Poland. They use this technique on an everyday basis both for analytical and physicochemical purposes. The departmental laboratory is a well equipped laboratory, a modern facility for performing state-of-the-art analyses. In the course of their work, they have learned to compare the performance of planar chromatography with that of the other chromatographic techniques. They have discovered multiple application areas for which HPTLC is fully comparable with HPLC, even some where HPTLC outperforms HPLC. Currently, their research group is exploring the potential of the TLC-MS Interface, testing new and not yet fully recognized possibilities of this smart device.

Introduction

Among the most urgent demands in chemical analysis are certainly those raised by the biomedical and environmental analysis sectors, as well as by the food industry. Many analytical tasks consist of rapid screening of a large series of pharmaceutical and food samples for a possible forgery or contamination. In the chemical analysis of botanical samples, one difficulty is the unpredictability of the chemical composition of a given plant extract, plus the problem of the high costs of phytochemical standards. For rapid screening of herbs, traded mostly in a dried and crumbled or granulated form, and for chemotaxonomic studies by botanists who want to take into account chemical (not only morphological) similarities and dissimilarities among individual species, an HPTLC instrumental system can prove invaluable indeed. The system must feature efficient fingerprinting of herbal material with the detection system ensuring partial yet immediate identification. A remarkable feature of planar chromatography is that it can even be used for the separation of the volatile fractions (e.g., essential oils), when employed at the low temperatures (–20, or –10 °C) and using silica gel as a strong enough adsorbent [1, 2]. In our own studies, we have successfully tested this possibility upon the essential oils derived from several species belonging to the sage (*Salvia*) genus [3].

It seems highly possible that the well recognized simplicity and versatility of HPTLC in combination with the exactness of mass spectrometric detection can in a near future successfully compete with the fully instrumental liquid and gas chromatographic techniques as a particularly versatile and handy tool for rapid screening.

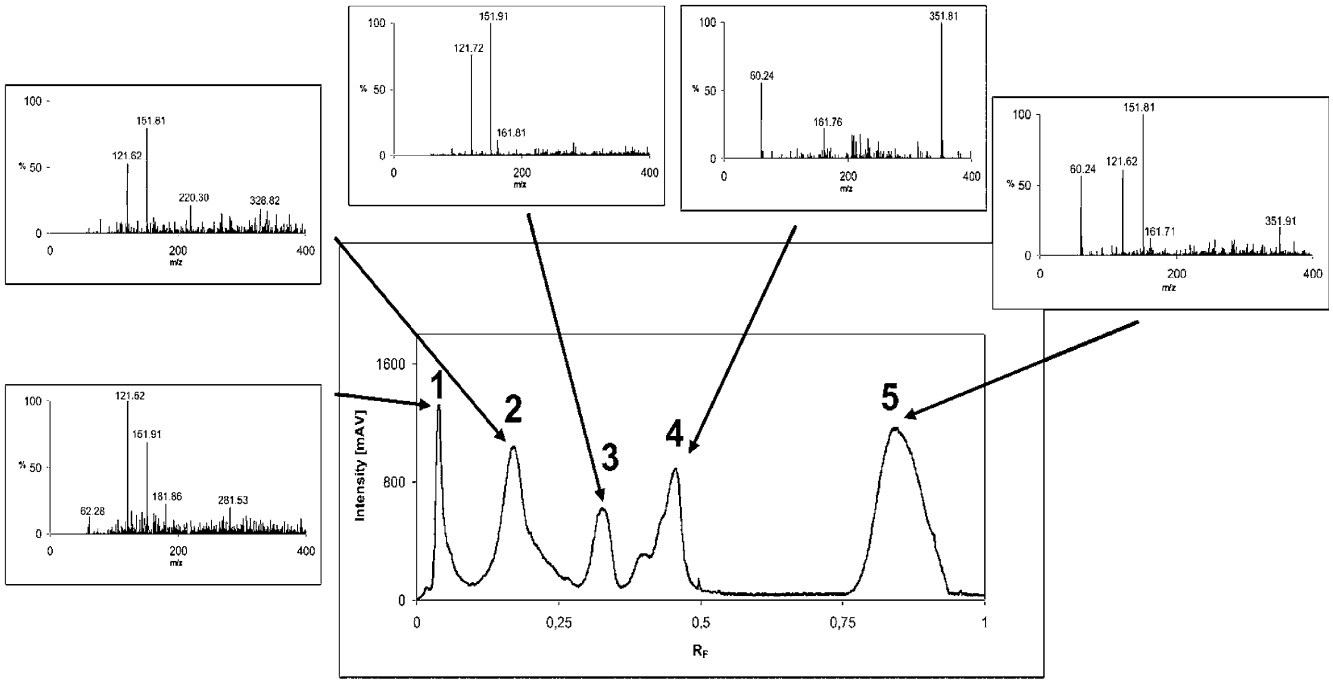
Note: In the following this is shown by the use of conventional TLC layers, however, the method could be easily and advantageously adapted for HPTLC separation material: improved separation, reduced migration times and mobile phase savings.

Sample preparation

Different groups of plant constituents (e.g., essential oils, phenolic acids, flavonoids, etc.) can be extracted from plant material in a more or less selective way, e.g., including hydrodistillation, classical solvent extraction, accelerated solvent extraction, etc.

Chromatogram layer

TLC plates silica gel 60 F_{254} (Merck), 20 × 20 cm



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Densitogram and mass spectra of the five separated chromatographic zones derived from the essential oil sample of Salvia lavandulifolia

Sample application

Spotwise application of 5 µL of the undiluted essential oil

Chromatography

In the twin-trough chamber with toluene – ethyl acetate 95:5 at -10 ± 0.5 oC up to a migration distance of 15 cm

Densitometry

Absorbance measurement at 340 nm

Mass spectrometry

Zones of interest were marked on the plate and directly eluted with the TLC-MS Interface into the Varian 500 mass spectrometer operating in the positive ESI mode using the MS Workstation v. 6.9.1 software: full scan, spray chamber temperature 45 °C, drying gas temperature 150 °C, drying gas pressure 25 psi, capillary voltage 70 V, needle voltage 5 kV.

Results and discussion

The densitogram for the essential oil derived from *Salvia lavandulifolia* by means of hydrodistillation showed five separated bands, of which the mass spectra were recorded [4].

In general, this approach can be extended to a wide variety of complex mixtures of compounds [5, 6]. Contrary to popular belief, fragmentation of compounds can occur even when employing the electrospray ionization mass spectrometry (ESI-MS) under the mild operational conditions. For example, the ions corresponding to certain truncated entities can be recognized in several mass spectra derived from the investigated *Salvia lavandulifolia* essential oil and one can point out to the *m/z* values equal to 60, 122 and 152. However, target compounds can be identified without much trouble in such a spectrum, which can be recognized as the reliable fingerprints of the analyzed fractions.

Existing hyphenations in TLC/HPTLC [7, 8] are expanded by the TLC-MS interface, which can be used for devising multidimensional liquid chromatography modes. This is due to its technical design which enables direct transferring of a fraction to the HPLC system, equipped with any given detector. One can benefit from rapid preliminary fractionation of a complex mixture by means of planar chromatography, followed by an enhanced separation with use of the HPLC system and a choice of different detection possibilities like:

- MS detector (see CBS 103, 2009, 13-15)
- UV/VIS detector

- Diode array detector (DAD)
- Evaporative light scattering detector (ELSD, see CBS 105, 2010, 2-5)
- Polarimetric detector
- Circular dichroism (CD) detector
- Any other detection system.

A comparison was made of the analysis of the same plant extracts in the one-dimensional (1D) TLC-MS and the two-dimensional (2D) TLC-LC-MS system [4–6]. A conclusion was drawn that the 2D system provides a more abundant analytical material (in form of the mass spectrometric fingerprints) than the 1D one, which is an evident advantage of the 2D approach. The remarkable flexibility of the TLC-HPLC system coupled off-line by the TLC-MS interface is its multidimensionality in a selective manner, as not all bands undergo the transfer. Besides, one can perform HPLC fractionation using for each separated TLC/HPTLC band a different chromatographic column, a different eluent composition and mode (isocratic or gradient), and also the most suitable detector.

Further information is available on request from the authors.

[1] C. Mathis, G. Ourisson Phytochemistry 3 (1964) 115
[2] A. Koch *et al.* in Thin Layer Chromatography in Phytochemistry, M. Waksmundzka-Hajnos, J. Sherma, T. Kowalska (Eds.) CRC Press, Boca Raton, USA, 2008, 451
[3] M. Sajewicz *et al.* J. Planar Chromatogr. 23 (2010) 270
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[6] M. Sajewicz *et al.* J. Chromatogr. Sci., in press
[7] G. Morlock, W. Schwack Trends Anal. Chem. 10 (2010) 1157
[8] G. Morlock, W. Schwack J. Chromatogr. A 1217 (2010) 6600

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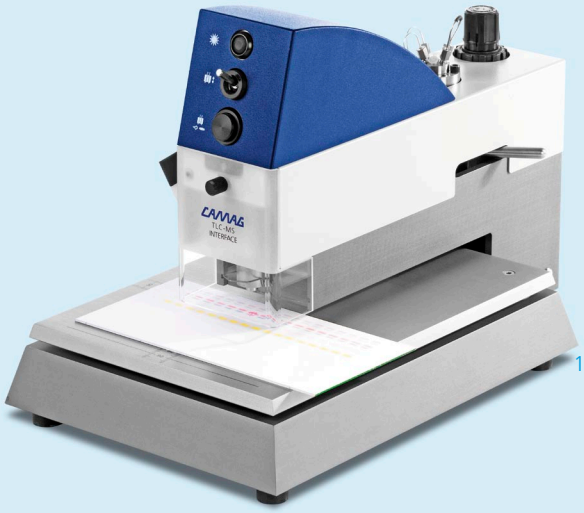

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CAMAG TLC-MS Interface

This interface allows rapid and contamination-free elution of TLC/HPTLC zones with online transfer to the respective mass spectrometer. The advantage of this universal interface is the plug & play integration into any given HPLC/MS system without modification.

Within a minute the substance can be identified via its mass spectrum, or for unknown substance zones the respective sum formula can be obtained depending on the mass spectrometer used. The capability of detection is comparable to HPLC-MS, as the whole substance zone inclusive its depth profile is eluted.

Also interesting zones can be eluted into a vial for further investigations with, *e. g.*, NMR, (ATR-)FTIR, static nanospray, direct inlet EI-MS and MALDI.

Quality control of Traditional Chinese Medicines by HPTLC

20

Professor Dr. Irmgard Merfort and her working group

For his diploma thesis in the group of Professor Irmgard Merfort* at the Department of Pharmaceutical Biology and Biotechnology, Institute for Pharmaceutical Sciences at the Albert-Ludwigs-University Freiburg (Germany) Mr. Zhi Li worked on the development and validation of an HPTLC method for the quality control of multi-herbal drugs at the CAMAG laboratory.

Introduction

Multicomponent herbal drugs, mixtures of medicinal plants or plant extracts, are very common to Traditional Chinese Medicine (TCM) and other traditional medicines in the world. Many of such drugs and the corresponding plants have been monographed in pharmacopoeias for a long time. However, because the manufacture of TCM is now regulated by current good manufacturing practice (cGMP) the existing monographs often neither meet the requirements for proper identification of each ingredient nor do they include suitable assays for the evaluation of the potency of the final product.

Using as an example the traditional Chinese veterinary combination medicine Cangzhu Xianglian San (CXS) a systematic approach to improving the quality control of multi-herbal drugs by the use of HPTLC fingerprints was elaborated [1]. HPTLC proved to be a rapid and powerful technique for proper identification, detection of adulteration as well as quantitative analysis of complex herbal mixtures.

Sample preparation

Commercial samples of CXS and samples of the ingredient herbal drugs Coptis rhizome, Aucklandia root, and Atractylodes rhizome were extracted with methanol (0.1 g/mL) by sonication for 15 min. For quantification of berberine 25.0 mg of CXS were extracted with 50 mL of methanol by sonication in a water bath at 60 °C for 30 min, after cooling the extract was made up to 50.0 mL and filtered.

Standard solutions

Methanolic solutions (1.0 mg/mL) of berberine HCl, palmatine HCl, jatrorrhizine HCl, dehydrocostuslactone and costunolide were prepared. For quantification a 3.3 µg/mL methanolic solution of berberine HCl was used.

Layer

HPTLC plates silica gel 60 F_{254} (Merck), 20×10 cm, pre-washed by developing with methanol followed by drying in an oven at 120°C for 20 min.

Sample application

Bandwise with ATS4, band length 8 mm, track distance min. 10 mm, distance from lower edge 8 mm, distance from left edge min. 15 mm, application volumes 2–10 µL of samples and 1–8 µL of standard solutions.

Chromatography

In the ADC2 with chamber saturation for 20 min to a migration distance of 70 mm using toluene, ethyl acetate, methanol, isopropanol, water (60:30:20:15:3 v/v/v/v/v) as mobile phase in the front trough and 10 mL of 25 % ammonia (filter paper) in the rear trough of the chamber. Separation was affected by the activity of the adsorbent, therefore the plate was conditioned prior to both developments at 33 % relative humidity for 10 min using a saturated solution of magnesium chloride.

Documentation

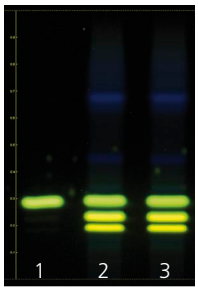
With TLC Visualizer documentation system under UV 366 nm.

Densitometry

TLC Scanner 3 with winCATS software, fluorescence measurement at 366/>400 nm.

Results and discussion

Two commercial CXS samples were analyzed using the TLC method of the official monograph of the Chinese Veterinary Pharmacopoeia 2005 (ChVPh). Both samples showed a yellow green zone at the position of berberine and were therefore in compliance with the monograph. However, this TLC method for identification seems meaningless because berberine is present in many other TCM drugs, and any plant powder adulterated with berberine would pass the test. Therefore, a more specific HPTLC identification of the drug was developed.



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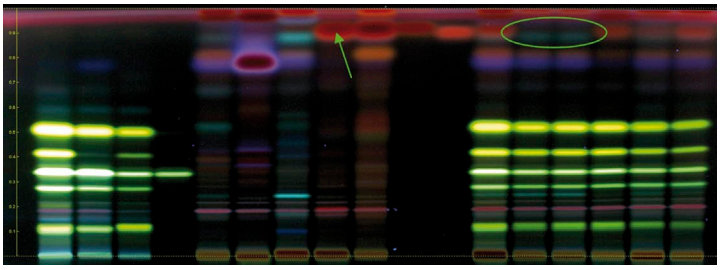
Identification of CXS according to ChVPh 2005. Track assignment: 1: Berberine HCl, 2: CXS-a1 (Company A, batch 1), 3: CXS-a2 (Company A, batch 2).

For this purpose, methods for identification of each individual herbal constituent were elaborated with the aim to properly distinguish a) all three acceptable Coptis species, b) Aucklandia radix from its adulterants Vladimiria radix and Inula radix as well as c) the two acceptable species of Atractylodes rhizome and discrimination from the prepared drug. In a second step it was determined whether those methods are also suitable for establishing the presence of all constituents in the multicomponent herbal drug. The proposed method met these requirements. The analysis of 5 commercial CXS samples from different sources revealed that 3 samples did not contain all specified ingredients. Both samples analyzed with the method of the ChVPh now failed the test (track 13 and 14) because they lack the zone specific for Aucklandia (arrow and oval). One sample contained prepared Atractylodes instead of unprepared. Two other commercial samples passed the test.

For the quantitative analysis of CXS, berberine was selected because it is a well detectable compound of the main CXS ingredient



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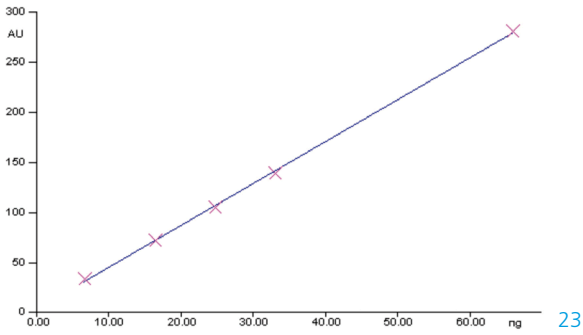
Analysis of commercial CXS samples. Track 1: Coptis chinensis; 2: Coptis teeta; 3: Coptis deltoidea; 4: berberine HCl; 5: Atractylodes chinensis; 6: Atractylodes lancea; 7: Atractylodis rhizome (prepared); 8: Aucklandia lappa; 9: Radix Vladimiriae; 10: dehydrocostuslactone; 11: costunolide; 12: mixture of reference drugs; 13: CXS-a1 (Company A, batch 1); 14: CXS-a2 (Company A, batch 2); 15: CXS-b (Company B); 16: CXS-c1 (Company C, batch 1); 17: CXS-c2 (Company C, batch 2).

Coptis rhizome. According to the Chinese Pharmacopoeia Coptis rhizome must contain not less than 3.6 % berberine expressed as berberine HCl, this corresponds to a minimum content of 0.98 % in CXS. During validation of the method for berberine HCl, the working range for fluorescence measurement at 366/>400 nm was established between 10–1500 ng with an sdv of 2.15 % using a Michaelis-Menten function I. In a smaller working range between 6 and 60 ng the calibration curve was linear (r = 0.99970 and sdv = 2.15 % via peak height and r = 0.99959 and sdv = 2.34 % via peak area).



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Linear calibration curve for berberine HCl (r = 0.9997, sdv 2.2 %).

The recovery of a spike added to the raw material was 95.4–97.3 % with *%RSD* (n = 3) of 3.9 % for repeatability. This precision includes the sample preparation. The berberine content of the 2 samples that passed the identification test was 2.4 % (CXS-b, track 15) and 1.4 % (CXS-c2, track 17), both samples therefore complied with the minimum content of 0.98 %.

Further information is available on request from the authors.

[1] Z. Li, I. Merfort, E. Reich, J AOAC 93 (2010) 1390

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