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In this issue:
Cleaning validation of industrial plants, sample preparation by planar chromatography, testing of honey for freshness… and more

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IN THIS ISSUE

Procedures, applications

Products featured in this issue

Column: Know CAMAG

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Cleaning validation using HPTLC

Bayer Weimar GmbH & Co. KG, produces and confections pharmaceutical preparations – contraceptives, hormone substitutes, etc. – for the Bayer AG.

To ensure product quality, each step in the procedure must be regulated and monitored which is effected by in-process control procedures. Cleaning validation is one of the steps. Ms Dipl-Chem Birgit Böckel is responsible for these critical analyses. It is necessary to ascertain that of all reaction vessels which come in contact with the products are effectively cleaned. CIP as well as manual rinsing must ensure that no cross contamination can occur.

The Weimar plant has employed HPTLC procedures for cleaning validation since 1998.

Considerations for this choice were:

- **Comparable results between HPTLC and HPLC procedures**
- **Evaluation of HPTLC chromatograms by densitometry, derivatization and R_F**
- **Low detection limits of sub components**
- **Side by side analysis of many samples**
- **Significant time and cost savings as compared with HPLC**

A swabbing procedure is predominantly used, whereby a defined surface area of the vessel is swiped in a prescribed way, usually an area of 200 cm², with a 2–3 cm pad of wool, pre-cleaned by Soxhlet extraction.

Sample preparation

The swabs are extracted three times with 50 mL chloroform (spectrograde) by sonication The combined extracts are evaporated to dryness in a rotary evaporator at 60 °C. The residue is taken up with chloroform and sonicated for a few minutes and, if not clear, it is filtered (0.45 µm) or centrifuged. A blank solution is prepared in the same way.

Standards

Calibration standards of three concentrations between determination level and its 20-fold are prepared.

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 x 10 cm

Sample application

With Automatic TLC Sampler (ATS4), application of 4 x 3 mm rectangles, 100 µL of sample solution and blank, standard solution sufficient to yield 3 calibration levels (see above); delivery speed 250 nL/s.

Chromatography

Automatic Developing Chamber (ADC) with toluene – ethyl acetate 3:2 after 10 min chamber saturation

Densitometry

TLC Scanner 3 with winCATS software. Identification of substances by spectra recording 200–350 nm, quantification with 3-level calibration

Post-chromatographic derivatization

By spraying with or immersion in methanol – sulfuric acid 9:1 and subsequent heating for 5 min at 105 °C. Immersion is recommended for quantitative evaluation.

Results and discussion

It was shown that in cleaning validation by HPTLC analysis correct results were obtained. R_F values, UV absorption spectra as well as colour/fluorescence after derivatization are suitable for identification. By checking selectivity and specificity it could be shown that the active ingredients as well as by-products are well resolved.

Depending on the concentration range, the function concentration/signal can be linear or polynomial; only functions with a correlation coefficient >0.990 are accepted

In a risk analysis defined sampling areas containing some residues were re-cleaned with released swabs and analyzed. Via calibration and extrapolation over the whole vessel area exposed to the product, the residue was found at <0.5 % of the allowed limit.

1

CAMAG Automatic Developing Chamber (ADC 2)

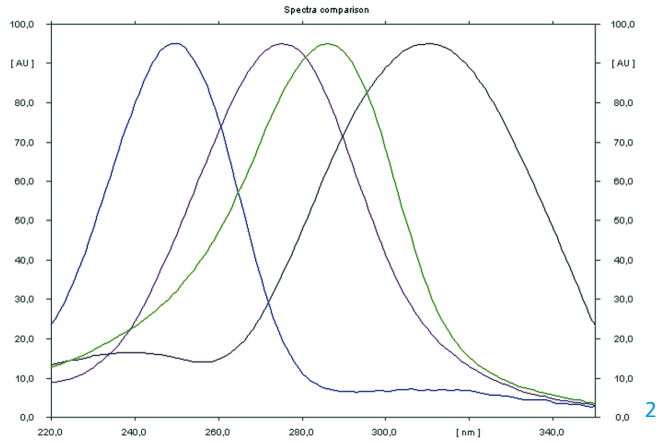
With the ADC 2 the development of 20 x 10 cm HPTLC plates is performed fully automatically, reproducibly, and independent of environmental effects. Pre-conditioning of the layer, control of relative humidity and chamber saturation, as well as developing distance and final drying can be pre-set and are automatically monitored. Two modes of operation are possible: stand-alone with input of parameters via keypad, or remote operation from a PC with winCATS

Note: If large sample volumes are to be applied as in the procedure described here, these can be sprayed on as rectangles and focused to narrow bands, prior to the separation run. This step including intermediate drying can be conveniently performed by the ADC 2.

Complete information can be found in the special brochure "Automatic Developing Chamber ADC 2" or under **www.camag.com/adc2**

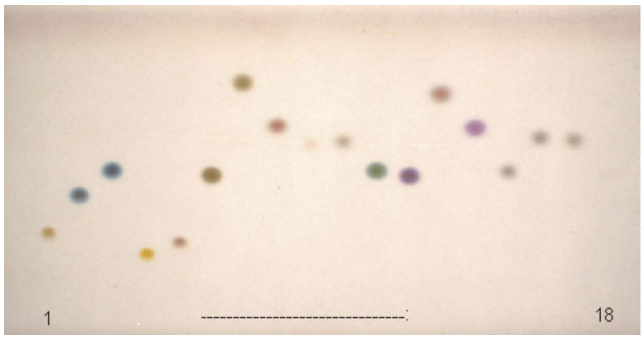
Conclusion

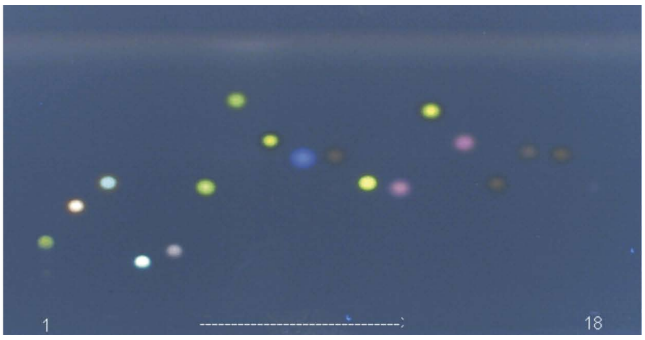
Cleaning validation by HPTLC analysis, i.e. determination of rest residues (of hormonal ingredients) can be achieved with the required accuracy. The method is simple and quick. Results obtained by quantitative densitometric evaluation are precise.



2

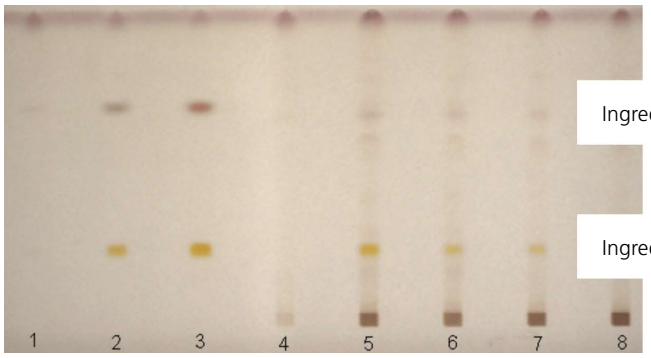
Absorption spectra of active ingredients processed in the Bayer Weimar plant

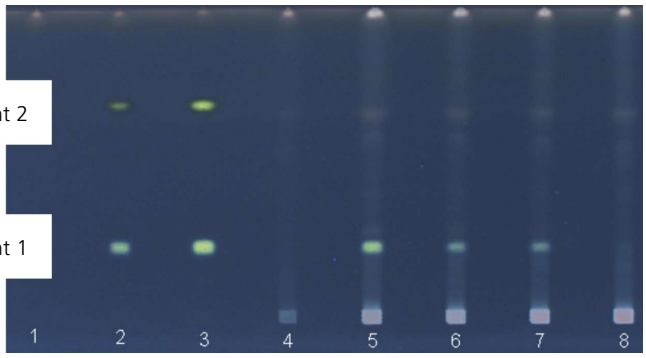




3

HPTLC plate after derivatization under visible light and under UV 366 nm for checking selectivity





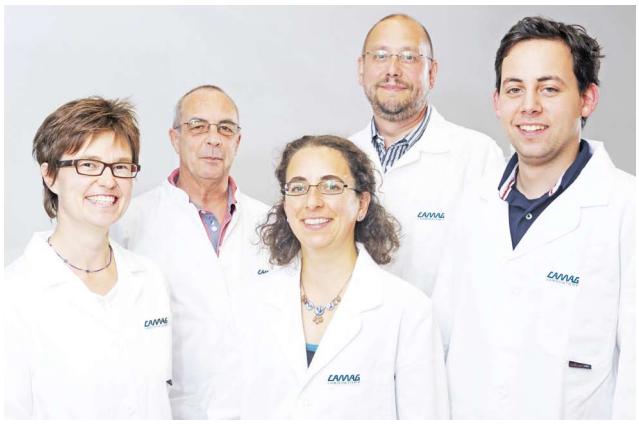
4

HPTLC plate after derivatization: Tracks 1–3 mixture of ingredients 1 & 2 for establishing the calibration function, track 4 blank, tracks 5–8 samples taken from 4 areas of a production vessel

Further information is available from the author upon request .

Contact: Ms Dipl.-Chem. Birgit Böckel, Bayer Weimar GmbH & Co. KG, Product Supply Pharma , QC Raw Materials, Döbereiner Str. 20, 99427 Weimar, birgit.boeckel@bayer.com

Rapid test for content uniformity of Coenzyme Q10 in soft gel capsules by HPTLC



5

The CAMAG laboratory team, Dr. Anita Ankli, Daniel Handloser, Valeria Widmer, Dr. Eike Reich, Eliezer Ceniviva

We are a team of highly qualified professionals including chemists, pharmacists and laboratory technicians with many years of HPTLC experience. Our goal is to share with you the advantages of HPTLC, especially flexibility, reproducibility and reliability. Moreover we assist our customers to resolve their analytical problems with HPTLC. Let us work together!

Introduction

Coenzyme Q10, found naturally in the body, is involved in the production of body energy. Thus heart, lung and liver have the highest concentration of Q10. The substance is active in many ways but primarily assumed to enhance the immune system and to work as an antioxidant, protecting against free radicals that damage cells. As a dietary supplement it is mostly sold in soft gel capsules. Most pharmacopoeas prescribe tests for content uniformity for pharmaceuticals that are administered in single doses. The procedures are laid down in the internal quality control of the manufacturers, in the official registration documents and in international trading certifications.

Our aim was to develop a rapid HPTLC method to test content uniformity of a large number of Coenzyme Q10 capsules. The key targets were simple sample preparation and rapid chromatographic development. The evaluation of the results was to be straight-forward and reliable.

Sample preparation

One soft gel capsule was placed into a flask and 50.0 mL of toluene were added. While submerged in the liquid the capsule was cut with a blade. The flask was placed on a shaker for 15 min. Based on the target content of the capsule an aliquot of the extract was diluted to a concentration of about 15 µg/mL. The samples were protected from bright light.

Standard solutions

A standard solution containing 1 mg/mL of Coenzyme Q10 in toluene was prepared. The stock solution was diluted with toluene to 10, 15 and 20 µg/mL of Coenzyme Q10. The standards were protected from bright light. The solutions are stable for about one week at 4 °C.

Layer

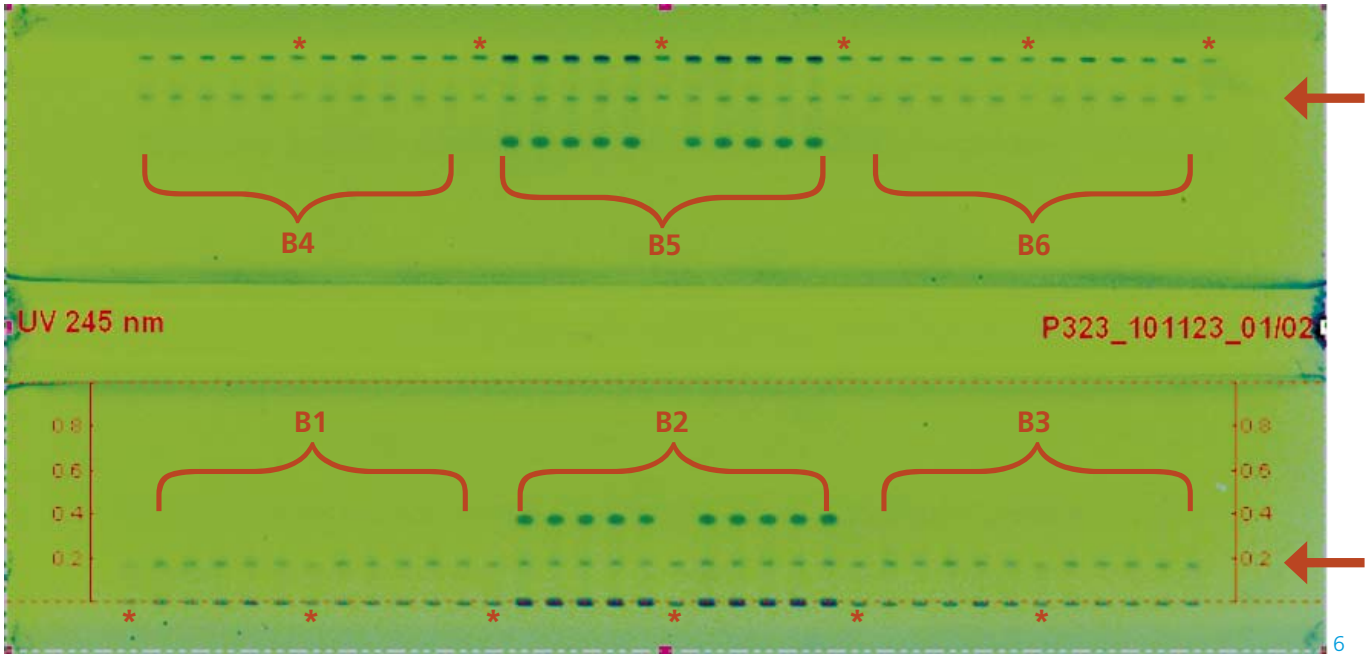
HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm, pre-washed by developing from both sides with methanol in an Horizontal Developing Chamber followed by drying in an oven at 120 °C for 30 minutes.

Sample application

Bandwise with ATS 4, band length 2 mm, track distance 5 mm, distance from lower edge 8 mm, distance from left edge 20 mm, application volumes 2 µL of samples and standard solutions.

Chromatography

In the Horizontal Developing Chamber (HDC) 20 × 10 cm or Twin Trough Chamber (TTC) or in the ADC 2 without saturation. For HDC 8 mL developing solvent for each side (36/72 samples), 10 mL of developing solvent for the Twin Trough Chamber in the front trough.



6

60 samples (6 batches, B1–B6) and 12 standards were applied onto one HPTLC plate; Coenzyme Q10 is seen at $R_f \approx 0.20$. In the Q10-batches on the center tracks vitamin B2 and vitamin E are also present ($R_f \approx 0$ and 0.4); (*NOTE: For better visualization in this figure the application volume was increased to 6 µL).*

Densitometry

TLC Scanner 4 with winCATS software, absorption measurement at 282 nm; slit dimension: 3.00 x 0.20 mm; evaluation via peak height, linear regression (20–50 ng); alternatively (20–150 ng) polynomial regression.

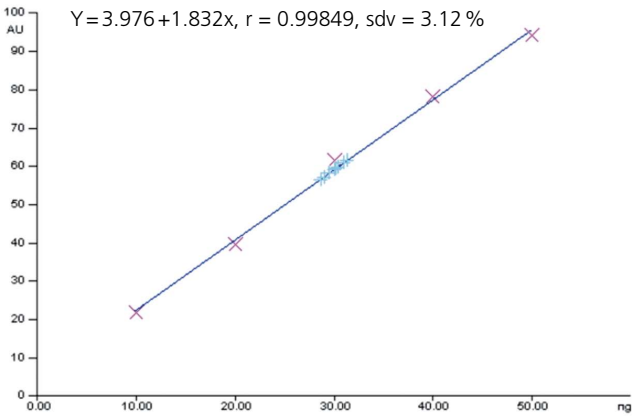
Results and discussion

As an example, 10 samples each from 6 different batches and 12 standards of various concentrations were applied on a 20 x 10 cm HPTLC plate and developed in a Horizontal Developing Chamber from both sides. The handling of the analysis was very easy due to the use of toluene as solvent for the sample and as mobile phase for chromatography. Only 86 min in total were required for the entire analysis of 72 samples. Sample application was the most time consuming step. Development and evaluation of the chromatogram were very fast.

The time requirement for a content uniformity test of 10 samples from 6 different batches and 12 standards is shown in the table.

Steps	[min]
Application of 72 tracks with Automatic TLC Sampler (ATS 4)	64
Chromatography in Horizontal Developing Chamber (HDC)	6
Drying of the plate	10
Evaluation with TLC Scanner 4	6
Total time required	**86 min**

In the working range of 20–50 ng the calibration curve was linear as can be seen from the diagram below.



7

Calibration function for Coenzyme Q10 at UV 282 nm. Measurement of 10 samples and 5 standards using linear calibration (P323_101011_03).

Summary

The test for content uniformity was performed with 10 samples according to the European Pharmacopoeia [1] and the USP 34 [2], respectively. The target content of the soft gel capsules according to the label claim was 30 mg. The average of the measured samples was 30.08 mg. The mean of individual contents expressed as a percentage of the label claim was calculated and resulted in $\bar{x}$ = 100.25 with a standard deviation of s = 2.76. According to [1, 2] the acceptability constant k for 10 samples is defined as k = 2.4. For the final calculation of the acceptance value (AV) the reference value M must be known; here for case 1 M = $\bar{x}$ [1, 2]. The calculated AV = 6.6 was smaller than the maximal allowed AV of 15. Therefore the content of the soft gel capsules was determined as uniform.

Calculation of Content Uniformity Test for dosage forms according to PhEur [1] and USP 34 [2], respectively. Sample size n = 10

X(Average)	X(Average)	Target content T (label claim)	30 mg
29.53 ng	29.53 ng	Average (calculated)	30.08 mg
X(Average)	X(Average)	Mean of individual contents expressed as a percentage of the label claim $\bar{x}$ (calculated)	$\bar{x}$ = 100.25
31.28 ng	31.28 ng		
X(Average)	X(Average)	Sample standard deviation s (calculated)	s = 2.76
30.17 ng	30.17 ng	Acceptability constant k (n = 10)	k = 2.4
X(Average)	X(Average)	M (case 1), if 98.5 % ≤ $\bar{x}$ 101.5 %	M = $\bar{x}$
30.05 ng	30.05 ng	Acceptance value AV	AV = |M – $\bar{x}$ | + ks
X(Average)	X(Average)	AV (calculated)	**AV = 6.6**
30.43 ng	30.43 ng	Max. allowed AV, L1 = 15.0	15.0

Further information is available on request from the authors (Lab@camag.com).

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

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

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CAMAG Automatic TLC Sampler 4 (ATS 4)

Automatic sample application is the key for precision and high sample throughput in routine HPTLC analysis. With the ATS 4, samples are either applied as spots through contact transfer (01–5 µL) or as bands or rectangles (0.5 – >50 µL) using the spray on technique. Starting zones in the form of narrow bands offer the best resolution attainable with a given chromatographic system. Application in the form of rectangles that are focused into narrow bands prior to chromatographic separation, allows precise application of large volumes without damaging the layer.

In the procedure described here, the samples were applied quasi spot wise in order to accommodate as many samples as possible on the plate. The spray on mode was chosen to keep the starting zones compact. Therefore 2 mm band length was selected, the shortest the ATS 4 program accepts in spraying mode.

In the cleaning validation example (page 2–4), application in the form of rectangles was chosen, however, without a pre-focusing step.

New Chairman of the Board

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Dr. Konstantinos Natsias has been elected Chairman of the CAMAG Board of Directors. This action was taken at the recommendation of the CAMAG Foundation, following the resignation of Mr. Christian Gfeller. The General Assembly unanimously elected Dr. Natsias at its annual meeting in June.

Dr. Natsias has been with CAMAG for more than 20 years. He joined CAMAG Berlin in August 1989 as its Sales & Marketing Manager and was promoted to General Manager in 2001. In June of 2010 he was elected a Member of the Board of the CAMAG mother company where he will now serve as its Chairman. All members of the "CAMAG Family" are convinced that we have found a competent and diligent person to fill this position. He stands for progress as well as for CAMAG tradition. All in the company value his mature and amicable way of getting along with people.

We are sincerely indebted to Christian Gfeller for 45 years of trustworthy and successful work for CAMAG, and we are very happy that he will continue as a Board Member.

Dr. Dieter Jänchen
President CAMAG Foundation

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Liebe Freunde

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

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

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

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

Herzlichst

Gerda Morlock

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Dear friends

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

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

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

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

Sincerely,

Gerda Morlock

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THE CBS CLASSIFICATION SYSTEM

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

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

1. Reviews and books

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

quantitative analysis, qualitative identification, review 1, 4e

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

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

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

review 1, 3d

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

tendency in current pharmacopoeias for favouring HPLC.

pharmaceutical research, comparison of methods, review, HPTLC 1

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

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

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

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

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

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

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

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

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

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

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

review, HPTLC 1, 3

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

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

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

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

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

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

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

2. Fundamentals, theory and general

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

pharmaceutical research 2

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

pharmaceutical research 2

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

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

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

pharmaceutical research 2

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

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

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

HPTLC 2f

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

pharmaceutical research, quantitative analysis 2c

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

pharmaceutical research 2

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

pharmaceutical research 2

3. General techniques

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

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

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

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

HPTLC, comparison of methods, biodetection 3c

107 002 Irena CHOMA et al., see section 1

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

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

quantitative analysis, qualitative identification 3f

107 008 A. MARSTON, see section 1

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

quantitative analysis, qualitative identification 3

107 010 Salwa POOLE et al., see section 1

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

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

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

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

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

4. Special techniques

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

pharmaceutical research, HPTLC, quantitative analysis 4e

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

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

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

5. Hydrocarbons and halogen derivatives

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

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

7. Phenols

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

food analysis, quality control, HPTLC, qualitative identification 7

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

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

8. Substances containing heterocyclic oxygen

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

quality control, herbal, densitometry, quantitative analysis 8b

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

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

qualitative identification, quantitative analysis 8a

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

quality control, herbal, densitometry, quantitative analysis 8b

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

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

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

herbal, densitometry, quantitative analysis 8b

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

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

10. Carbohydrates

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

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

HPTLC 10

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

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

11. Organic acids and lipids

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

quality control, herbal, densitometry, quantitative analysis 11a

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

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

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

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

traditional medicine, herbal, densitometry, quantitative analysis 11a

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

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

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

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

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

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

107 006 Beate FUCHS et al., see section 1

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

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

herbal, densitometry, quantitative analysis 11a

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

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

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

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

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

traditional medicine, quality control, herbal, densitometry 11a

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

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

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

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

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

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

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

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

pharmaceutical research 11a

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

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

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

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

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

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

food analysis, toxicology, qualitative identification 11d

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

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

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

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

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

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

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

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

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

17. Amines, amides and related nitrogen compounds

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

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

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

the respective compounds were scraped off and quantified by spectrophotometry.

pharmaceutical research, quality control, qualitative identification 17a

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

pharmaceutical research, quality control, quantitative analysis 17a

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

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

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

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

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

be used for stability studies.

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

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

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

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

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

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

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

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

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

18. Amino acids and peptides, chemical structure of proteins

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

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

pharmaceutical research, HPTLC, qualitative identification 18

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

pharmaceutical research, HPTLC, densitometry, qualitative identification 18a

21. Purines, pyrimidines, nucleic acids and their constituents

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

herbal, HPTLC, densitometry, quantitative analysis 21a

22. Alkaloids

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

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

pharmaceutical research 22

23. Other substances containing heterocyclic nitrogen

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

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

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

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

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

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

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

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

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

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

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

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

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

traditional medicine, herbal, densitometry, quantitative analysis 23e

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

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

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

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

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

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

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

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

quality control, HPTLC, densitometry, quantitative analysis 23e

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

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

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

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

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

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

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

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

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

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

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

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

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

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

herbal, densitometry, quantitative analysis 23e

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

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

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

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

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

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

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

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

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

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

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

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

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

24. Organic sulfur compounds

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

HPTLC 24

27. Vitamins and various growth regulators

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

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

28. Antibiotics, Mycotoxins

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

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

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

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

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

29. Pesticides and other agrochemicals

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

30. Synthetic and natural dyes

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

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

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

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

qualitative identification 30b

32. Pharmaceutical and biomedical applications

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

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

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

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

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

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

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

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

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

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

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

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

pharmaceutical research 32a

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

herbal, HPTLC, densitometry, quantitative analysis 32e

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

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

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

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

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

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

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

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

2) for *Cuscuta chinensis*, on polyamide phase with methanol - glacial acetic acid - water 4:1:5, detection by spraying with $AlCl_3$ solution and evaluation under UV 366 nm; 3) for Malaytea scurfpea fruit on silica gel with *n*-hexane - ethyl acetate 4:1, detection by spraying with 10 % NaOH in methanol and evaluation under UV 366 nm. Identification by fingerprint comparison with the individual component drug of the preparation.

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

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

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

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

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

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

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

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

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

herbal, densitometry, quantitative analysis 32g

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

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

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

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

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

herbal, HPTLC, quantitative analysis, densitometry 32e

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

pharmaceutical research 32a

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

herbal, densitometry, quantitative analysis 32e

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

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

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

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

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

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

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

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

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

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

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

herbal, qualitative identification 32e

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

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

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

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

herbal, HPTLC, quantitative analysis, densitometry 32e

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

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

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

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

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

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

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

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

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

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

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

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

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

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

35. Other technical products and complex mixtures

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

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

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

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

quantitative analysis, comparison of methods 35

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

37. Environmental analysis

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

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

38. Chiral separation

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

densitometry, qualitative identification, quantitative analysis 38

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

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

qualitative identification, quantitative analysis, densitometry 38

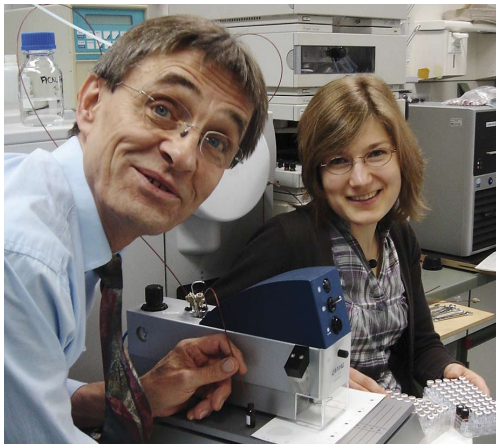
107 158 M. SAJEWICZ*, E. JOHN, D. KRONENBACH, M. GONTARSKA, T. KOWALSKA (*Silesian University, Institute of Chemistry, 9 Szkolna Street, 40-006 Katowice, Poland): TLC study of the separation of the enantiomers of lactic acid. Acta Chromatographica 20(3), 367-382 (2008). Investigation of the separation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

pharmaceutical research 38

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

pharmaceutical research, quality control, HPTLC, quantitative analysis 38

Planar solid phase extraction – a new clean-up concept in residue analysis of pesticides



10

Prof. Dr. W. Schwack and Claudia Oellig

One of the main research topics of Professor Schwack, University of Hohenheim, is method development in multi-residue analysis of pesticides in fruits and vegetables. The focus is on the extraction process as related to automation as well as to new clean-up methods for sample extracts. In addition there are intense studies on the special class of dithiocarbamate fungicides.

Introduction

In the European Union, maximum residue limits are regulated for over 500 pesticides in food and feed. Therefore, sensitive, selective and robust analytical techniques for pesticide residues analysis are required. Fruit and vegetables contain a huge amount of co-extracted matrix compounds, which cause signal suppression in GC-MS and LC-MS . An efficient clean-up of fruit and vegetable extracts is a reliable way preventing matrix effects in residue analysis of pesticides. Therefore, the present study is focused on the development of a feasible, easy and rapid planar chromatographic clean-up method for the separation of pesticides from matrix compounds followed by LC-MS.

In contrast to common clean-up techniques like dispersive solid phase extraction (dSPE) or cartridge SPE for multi-residue analysis of pesticides in food, the new high-throughput planar solid phase extraction (HTpSPE) method is a cost-effective, reliable and rapid alternative. It allows an efficient clean-up at low running costs and a solvent consumption of only 1 mL per sample. A sample clean-up of 20 extracts is possible in parallel in 20 minutes. [1]

Sample extracts

Organic fruit and vegetable samples (10 g) were extracted according to the QuEChERS method [2].

Pesticide standard solutions

Sample raw extracts were spiked with a mixture of seven representative pesticides (acetamiprid, azoxystrobin, chlorpyriofos, fenarimol, mepanipyrim, penconazole and pirimicarb) at levels of 0.1 and 0.5 mg/kg. Two internal standards were used, tris(1,3-dichloroisopropyl)phosphate (TDCPP) for quantification and sudan II as visible marker for the target analyte zone.

Chromatogram layer

TLC aluminum foils silica gel 60 NH_2 F_{254s} (Merck), 20 x 20 cm, prewashed with acetonitrile (15 cm) and then cut for 20 x 10 cm use.

Sample application

Area application with Automatic TLC Sampler 4 (ATS4), length 3.0 mm, height 4.0 mm, track distance 8.5 mm, distance from the side 16.5 mm, distance from lower edge 13 mm, application volume 50 µL

Planar solid phase clean-up

In Automatic Developing Chamber ADC2 with 10 mL acetonitrile, migration distance 75 mm, migration time 10 min, drying 5 min; 2^{nd} development in the backwards direction with acetone, migration distance 45 mm, migration time 3 min, drying 5 min

Documentation of the clean-up

With TLC Visualizer under UV 254 nm, UV 366 nm and white light illumination, and under UV 366 nm after dipping in primuline solution

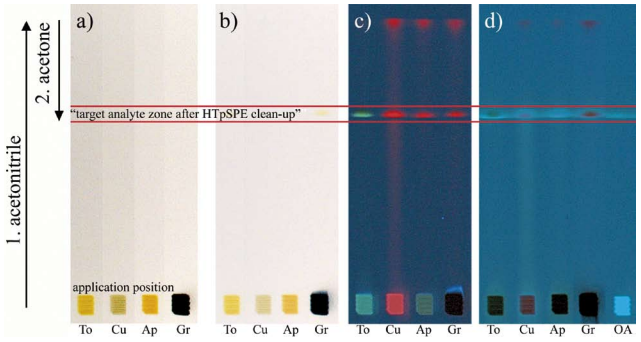
TLC-LC-(ESI)-MS

Extraction of the target analyte zone with TLC-MS Interface in autosampler vials with acetonitrile – 10 mM ammonium formate 1:1 (v/v), flow rate 0.2 mL/min, extraction time 1 min; separation of

pesticides on a Chromolith Performance RP-18e (100 mm x 3.0 mm, Merck) with gradient elution.

Results and discussion

Planar chromatography was used to separate pesticides from co-extracted matrix compounds and to focus the target compounds into a sharp zone. By variation of sorbent and solvent system this separation can easily be optimized. The best planar chromatographic clean-up of QuEChERS extracts of different fruit and vegetable samples was performed with a twofold development on amino phases. The multiple options of detection in planar chromatography make the success distinctly apparent.



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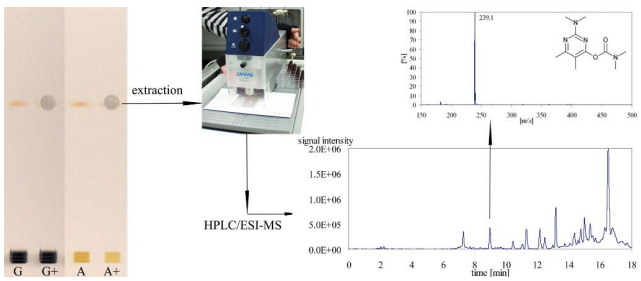
Separation of matrix and pesticides on TLC aluminum foils silica gel 60 NH_2 F_{254s} (tomatoes (To), cucumbers (Cu), apples (Ap) and red grapes (Gr); oleic acid (OA) was applied as a fatty acid example): before development under white light (a), after development under white light (b), UV 366 nm (c) and UV 366 nm after derivatization with primuline (d) (reprinted from [1] with permission).

Following this HTpSPE clean-up, the target zone (pesticides) can be extracted by the TLC-MS Interface into autosampler vials or directly online into the LC-MS system.



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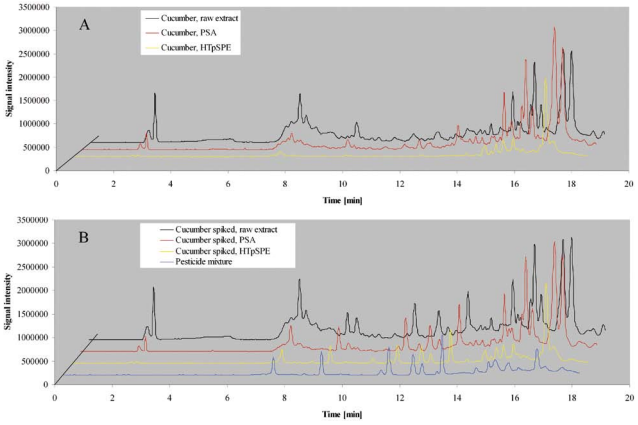
Extraction of a target zone with the TLC-MS Interface followed by LC-MS, total ion current chromatogram (TIC) of an apple extract spiked with a mixture of pesticides of various substance classes (A+) and mass spectrum of the peak with Rt=9 min (pirimicarb, m/z 239.1, $[M+H]^+$).

On a 20 cm-plate, HTpSPE offers a sample clean-up of 20 extracts, simultaneously within 20 minutes developing time. Including the automatic sample application, the total clean-up took about 70 min. With an overall clean-up time of 3.5 min per sample and a solvent consumption of only 1 mL per sample, HTpSPE is a cost-effective, reliable and rapid alternative to commonly used clean-up techniques. The new approach was successfully proven with pesticides from various substance classes in different fruit and vegetable matrices. Compared to common dispersive SPE methods, sample extracts are much cleaner and matrix effects are almost completely eliminated.



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Comparison of LC-MS total ion current chromatograms of cucumber blank extracts (A) and extracts spiked with a pesticide mixture at 0.5 mg/kg (B): QuEChERS raw extract, after dSPE (PSA), and after HTpSPE clean-up (reprinted from [1] with permission from Elsevier).

Recoveries of the applied pesticides were determined at two spiking levels from four different fruit and vegetable matrices. Average recoveries of the seven representative pesticides of 90–104 % with excellent relative standard deviations of 0.3–4.1 % ($n = 5$) confirm the powerful HTpSPE clean-up as reproducible and with excellent recoveries.

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

[2] www.quechers.com

Further information is available on request from the authors.

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Quantification and side component analysis of the cosmetic active tiliroside using planar chromatography


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

Planar chromatography is being used in both analytical and development departments for various applications at Merck KGaA Darmstadt and is especially important in the research and development of cosmetics.

Introduction

Planar chromatography provides many advantages to a broad field of applications. One example is the cosmetic active, RonaCare® Tiliroside, from Merck, which is obtained by extraction from a plant of the family Sterculiaceae. HPTLC covers several tasks, such as the quantification of the active ingredient in a complex plant matrix for determining the quality of the raw material, in-process control for monitoring the impurity profile during the manufacturing process and the quantification of the active ingredient in the final product. The method also has been proven to be suitable for stability tests. In this article further versatility of the method is demonstrated by the quantification and side component analysis based on visual evalution of the cosmetic active ingredient tiliroside.

The advantages of a high sample throughput and a simple sample preparation make planar chromatography a rapid and efficient method in the field of cosmetics. In comparison with column chromatography the fact that in HPTLC every plate is used only once, the risk of contamination is excluded, whereas in HPLC, contamination of the stationary phase by plant matrix and cosmetic oils must be considered.

Reference solutions

1. Quantification

Tiliroside-standard in methanol, 0.5 mg/mL

2. Side component analysis

Tiliroside-standard in methanol, 2.6 mg/mL, tiliroside-samples 1 and 2, kaempferol-3-glucoside, kaempferol-3-rutinoside, kaempferol, coumaric acid, each 1 mg/mL in methanol, glucose 1 mg/mL in water

Sample preparation

1. Quantification

25 mg tiliroside was dissolved in 50 mL methanol. For each plant sample, a certain amount of plant material was extracted four times with 80 mL of methanol under reflux. The extracts were filtered over filter paper and diluted with methanol to a volume of 500 mL after cooling to room temperature. The solutions were filtered again through a 0.45 micron membrane filter prior to analysis.

2. Secondary component analysis

The standards were weighed out and dissolved using an ultrasonic bath. The yellow or colourless solutions were filtered through a 0.45 micron membrane filter.

Layer

HPTLC-Plates Silica gel 60 F_{254s} Merck, 20 x 10 cm

Sample application

Band wise with Automatic TLC Sampler 4, band length 5 mm, track distance 10 mm, distance from the lower edge 10 mm, application volume 2 µl

Chromatography

In the twin trough chamber 20 x 10 cm with ethyl acetate – formic acid – acetic acid – water (100:11:11:27) + 1 % heptane

Post chromatographic derivatization

Using the TLC Sprayer, the respective reagent was homogenously applied:

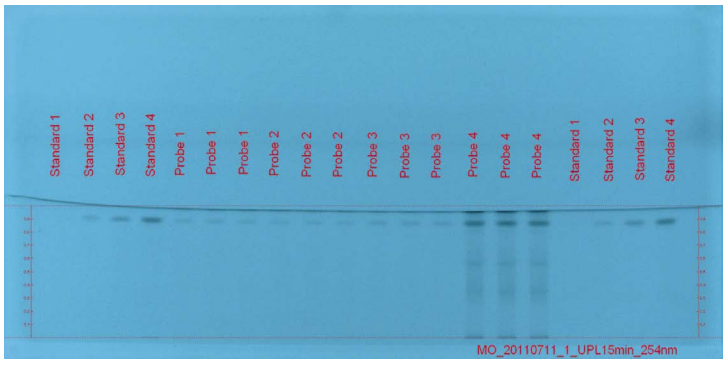
- Natural product reagent according to Neu (NSR): 1 % amino ethyl diphenylborate in methanol → UV 366 nm
- Anise aldehyde sulphuric acid reagent (AAR): 0.5 mL anise aldehyde in 85 mL methanol, 10 mL acetic acid and 8 mL conc. sulphuric acid (added ice-cooled) → Heating of the plate 90–125 °C for max. 15 min → white light

Densitometry

CAMAG TLC Scanner 3, absorbance measurement at 315 nm, slit dimension 4 × 0.3 mm, scanning speed 20 mm/s

Results and discussion

1. Quantification



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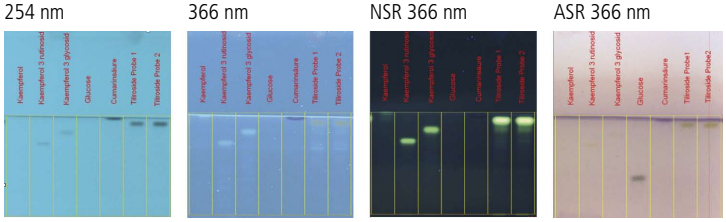
Chromatogram under UV 254 nm

The plate was scanned at 315 nm, which is the absorption maximum of tiliroside. For quantification and setting up a 4-point calibration curve, the respective peak areas of tliroside were used and the four extract samples were analyzed three times each. The tiliroside contents in the sample extracts were determined as: #1 = 1.09 µg (*RSD* = 0.40 %), #2 = 0.93 µg (*RSD* = 0.82 %), #3 = 1.19 µg (*RSD* = 1.18 %) and #4 = 3.32 µg (*RSD* = 0.31 %).



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Track 1: kaempferol, track 2: kaempferol-3-rutinoside, track 3: kaempferol-3-glucoside, track 4: glucose, track 5: coumaric acid, track 6: tiliroside sample , track 7: tiliroside sample 2

2. Side components analysis

The band of coumaric acid becomes visible under UV 254 nm at R_f 1, but it was not present in the tiliroside samples. Kaempferol (at R_f = 1) had been detected using Neu's reagent, but was not present in the tiliroside samples. Therefore the presence of coumaric acid and kaempferol could be excluded. Also the presence of glucose could be clearly excluded by staining with AAR. After staining with Neu's reagent, kaempferol-3-rutinoside and kaempferol-3-glucoside showed two fluorescent bands below the cosmetic active ingredient tliroside. The comparison with the tiliroside samples showed the typical, very small amounts of kaempferol-3-rutinoside and kaempferol-3-glucoside in the plant extract.

Note: In this work no optimization of the HPTLC conditions lege artis was performed. The R_f value of tiliroside was relatively high, and the reference compounds coumaric acid and kaempferol migrated with the solvent front. The data shown represent the results of a first "hands-on" approach, which was taken to get a quick but meaningful indication about Tiliroside content and impurities. All results were verified using HPLC and very good agreement was found.

Summary

The method is well suited for exclusion of relevant side components. By using appropriate derivatisation reagents, UV-inactive substances can be determined and the whole profile of side components can be shown. High sample throughput and precise quantification make HPTLC a powerful tool for the analysis of cosmetic active ingredients.

Further information is available from the authors on request.

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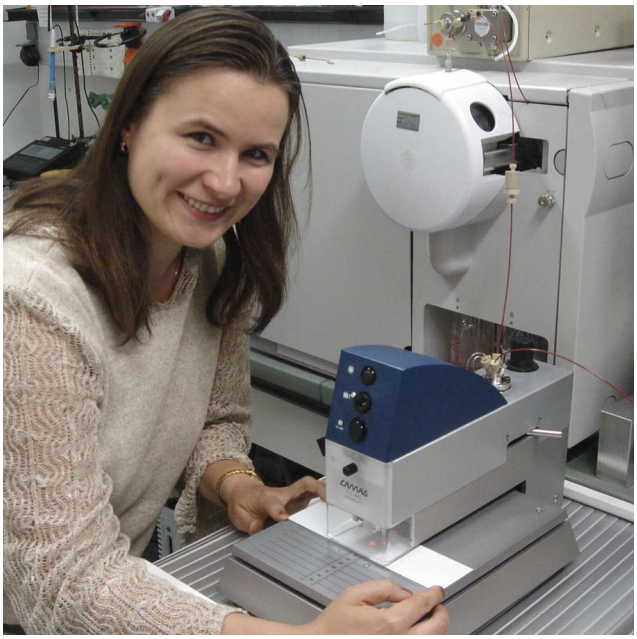
Fast quantification of 5-hydroxymethylfurfural in honey



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Dr. Elena Chernetsova

In the working group of Prof. Dr. Gertrud Morlock, Institute of Food Chemistry, University of Hohenheim in Stuttgart, Dr. Elena Chemestova, guest researcher from Russia, employs planar chromatography and mass spectroscopy in her research work.

Introduction

An analytical method for quality control is expected to provide high throughput and reliability while being cost-effective. 5-Hydroxymethylfurfural (HMF) is formed by decomposition of fructose of glucose by extended storing or by heat exposure of honey. Thus the HMF content in honey is an indicator of its freshness Quantification HMF in honey is usually performed by HPLC or by spectrophotometric methods according to White or Winkler [1–4]. However, both have shortcomings. The Winkler method is comparatively imprecise, the White method uses carcinogenic reagents and is not very reliable. Thus, HPLC is usually employed, although it is fairly demanding. Samples are dissolved in water, treated with Carrez reagent to suppress decomposition of HMF, and filtrated. Then they are chromatographed one by one which takes 10–15 minutes per sample.

Planar chromatography has proven to be an efficient, fast and cost-effective alternative [5]. After minimal sample preparation, 24 samples are chromatographed side by side under identical condition, within 5 minutes and at low solvent consumption. If chromatographed from both sides in the Horizontal Developing Chamber, even 48 samples can be separated simultaneously. Reliability of the new method has been verified by TLC/MS online coupling and also by selective derivatization.

Chromatogram layer

HPTLC plates silica gel 60 or silica gel 60 F_{254}, prewashed with methanol – water 6:1 and dried 20 min at 110 °C.

Standard solutions

Aqueous solutions of HMF of 0.1, 1.0, 2.5, 5 and 10 µg/mL

Sample preparation

Honey samples are homogenized and approx. 1 g is weighted in a measuring flask and water is added at 10 mL.

Sample application

Bandwise with TLC Sampler 4 (ATS 4), track distance 7.5 mm, distance from lower edge 8 mm, application volume 1–12 µL, 24 tracks

Chromatography

Automatic Dveloping chamber (ADC2) with 10 mL ethyl acetate, migration distance 50 mm, drying time 5 min

Densitometry

TLC Scanner 3 with winCATS software, spectra recording from 200–800 nm, quantification at 290 nm, slit dimension 5 x 0.45 mm, scanning speed 20 mm/s and; depending on the working range, evaluation by polynomial or Michaelis Menten 2 regression

HPTLC-MS (optional)

The positions of the HMF zones were marked with a soft pencil. Elution with the TLC-MS Interface with circular elution head with methanol 0.2 mL/min with an inline filter (0.5 µm frit Upchurch) fitted in the outlet capillary. Mass spectra were recorded using an electrospray ionization single quadrupole mass spectrometer (ESI-MS) and the LC/MSD Chemstation (Agilent).

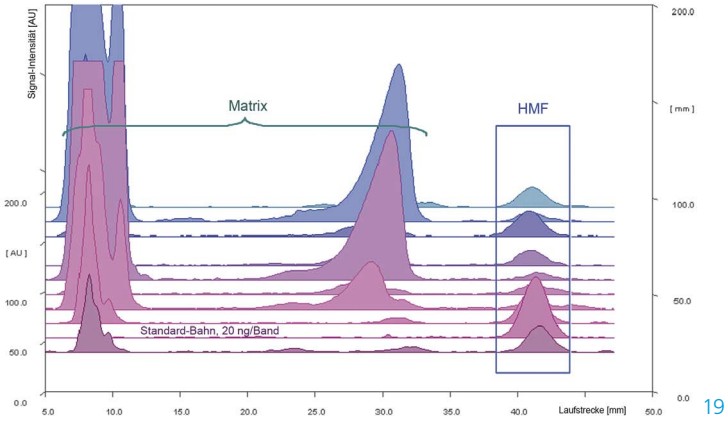
Post-chromatographic derivatization (optional)

The HPTLC plate was immersed with the TLC Immersion Device (speed 5.0 mm/s, immersion time 0 s) in *p*-aminobenzoic acid reagent (1 g dissolved in 36 mL acetic acid, then added 40 mL water, 2 mL phosphoric acid 86 % and 120 mL acetone). The plate was heated at 110 °C for 5–10 min with TLC Plate Heater.

Results and discussion

In the densitogram the HMF zones (hR_f 80) were clearly separated from the various matrix compounds of the honey samples. The identity of HMF was confirmed by UV spectra. The optimal wavelength of 290 nm for quantification was established by spectra recording.

The detection limit (LOD, S/N of 3, peak height) in the honey samples was comparable with that without matrix and corresponded to 0.75 mg/kg when 12 µL aqueous solution was applied. The limit of quantification (LOQ, S/N of 10, peak height) was 2.4 mg/kg. This proves that the method complies with the most stringent requirements worldwide, which are 15 mg/kg of HMF in honey. LOD and LOQ could be even lowered by increasing the sample volume applied.



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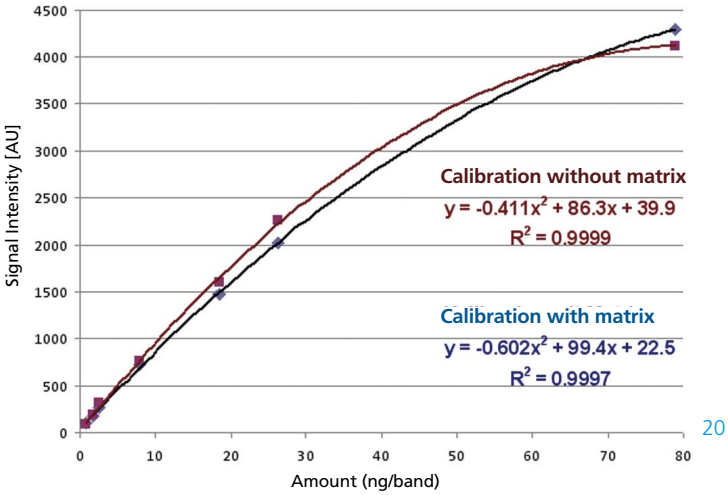
Densitogram of honey samples and HMF standard (track 2) absorbance at 290 nm

The calibration function was polynomial in a working range of 1:100, whilst Michaelis Menten 2 was suitable for higher HMF concentrations

Regression	Calibration range	Correlation coefficient r	Relative standard deviation sdv
Polynomial	1:100 (0.8–80 ng/band)	≥ 0.9998 (A) ≥ 0.9999 (H)	≤ 2.5 % (A) ≤ 1.4 % (H)
Michelis Menten 2	1:1000 (11–1100 ng/band)	n.d. n.d.	≤ 1.5 % (A) ≤ 2.3 % (H)

A peak area H peak height n.d. not defined

As matrix components obviously cause no interference, calibration is usually performed by external standards.



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Calibration function of HMF with and without honey matrix

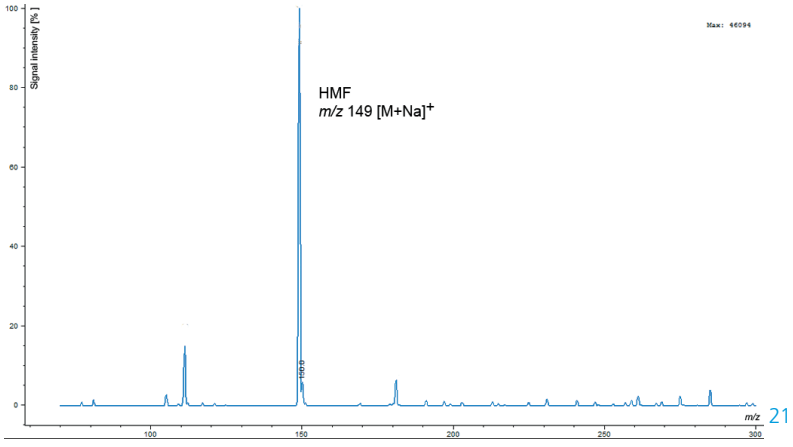
For 10 honey samples received from the Apicultural State Institute (Stuttgart) and from the Institute of Apiculture (Celle), the HMF results obtained with the Winkler method were compared with those obtained with HPLC-UV

and with the new HPTLC-UV methods. It is apparent that the differences between the two orthogonal chromatographic methods are minor (3.3 % 0.9 mg/kg), confirming the validity of the new method.

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

The repeatability in matrix (*%RSD*, *n* = 6, peak height) was 2.9 % for a 10 ng HMF band and 06 % for a 100 ng band. The mean reproducibility (*%RSD*, *n* = 2, peak height) of the whole procedure for 4 honey samples was 3.0 % (i.e. between 1.9 and 4.4 %).

HPTLC-MS online coupling proved suitable as complementary confirmation of the HMF results. HMF zones identified by UV scanning were eluted and subjected to ESI-MS full-scan modus. The mean deviation of the HMF results between HPTLC-UV and HPTLC-MA was found at 11 % (5.1 mg/kg).



Full-scan MS of an HMF zone of a 80 ng/band honey sample

Derivatization of the HMF zones to a blue fluorescing derivative followed by fluorescence scanning at 366/>400 nm served as additional verification.

Conclusion

The verification of the results of HMF quantification in honey obtained by the new HPTLC method was successfully demonstrated by comparison with the established methods. High sample throughput and cost-efficiency combined with reliability made it apparently superior for quality control analysis.

Further information is available from the authors.

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

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

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CAMAG AMD 2 System

Automated Multiple Development of thin-layer chromatograms

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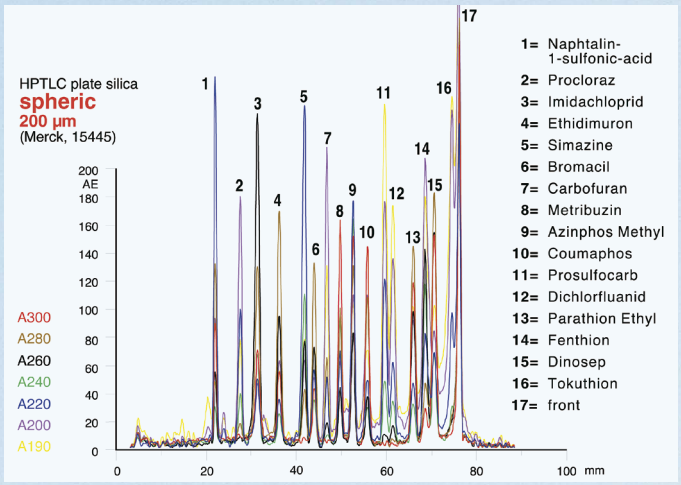

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Densitogram (MWL scan) of an AMD chromatogram of pesticides HPTLC plate Lichrosphere silica gel 60 F_{254} Merck

Principle

- The HPTLC plate is developed repeatedly in the same direction.
- Each successive run extends over a longer solvent migration distance than the one before.
- Each successive run uses a solvent of lower elution strength than the one used before.
- Between runs the solvent is removed and the plate is completely dried.

Result

- Due to the stepwise elution gradient, combined with the focussing effect of the subsequent runs, extremely narrow bands are formed with typical peak widths of about 1 mm.
- Over the available separation distance of 80 mm more than 40 components can be baseline separated.
- This ensures the highest resolution that can be attained with a planar chromatography system.

Applications of the AMD method most often used

Environmental protection – Impurities in water, contaminations in soil

CBS 105, p. 7–9, W. Weber et al: 1H-Benzotriazole and tolyltriazole in the aquatic environment (AMD – MS)

Using AMD in the monitoring program of raw and drinking water offers a number of advantages; for example in non-target screening analysis, not yet considered contaminants can be identified by online coupling with MS or by bioactive detection, so that their significance can be considered.

Lipids, phospholipids

CBS 105, p. 10–12, I. Schellenberg, K. Kabrodt: Optimization of an AMD 2 method for determination of stratum corneum lipids (AMD–bioluminescence)

AMD is the key for the employment of planar chromatography in lipid analysis. Its gradient elution potential provides the required resolution power. The planar medium facilitates post-chromatographic derivatization of lipid compounds that otherwise are difficult to detect.

Ingredients of plants and other natural products

CBS 102, p. 4–7, G. Morlock *et al.*: Screening for bioactive natural products in sponges (AMD-UV/Vis/FLD-bioluminescence-MS)

The cost and time intensive isolation and purification procedures required for HPLC-MS analyses are completely eliminated. Since solvents are completely removed after chromatography these cannot interfere in the detection of bioactive metabolites.

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CBS 107 E 996.2270-10