CBS 111 • SEPTEMBER 2013

CBS

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111

SEPTEMBER 2013

Focus of this issue:
HPTLC for the analysis of cosmetics

CAMAG BIBLIOGRAPHY SERVICE PLANAR CHROMATOGRAPHY

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Planar Chromatography
Edited by Gerda Morlock
cbs@camag.com
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IN THIS ISSUE

Procedures, applications

Products featured in this issue

Column: Know CAMAG

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

CAMAG Scientific Inc. (USA)
515 Cornelius Harnett Drive
Wilmington, NC 28401
Phone 800 334 3909 • Fax 910 343 1834
tlc@camag.com • www.camagusa.com

Quantification of alkaloids in *Sceletium tortuosum*

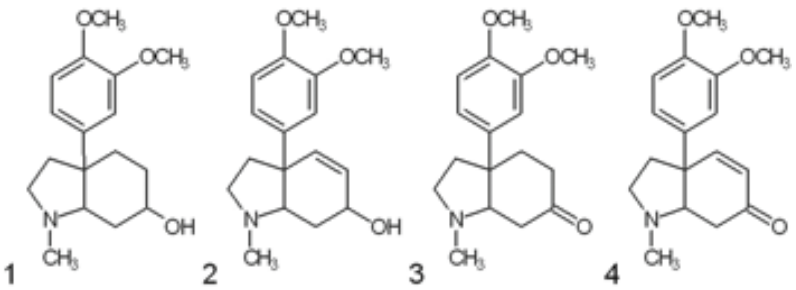
This is an excerpt of the paper of Emmanuel A. Shikanga, Ilze Vermaak, and Alvaro M. Viljoen in Journal of Planar Chromatography 25 (2012) 283 with kind permission of Akadèmiai Kiadó, Budapest.

Prof. Alvaro Viljoen with his research group

The research group under the leadership of Professor Alvaro Viljoen at Tshwane University of Technology, Pretoria, South Africa, is active in the area of quality control of African traditional medicines. A range of techniques are used in their laboratory such as vibrational spectroscopy, LC-MS and GC-MS but HPTLC remains a pivotal and indispensable tool in developing quality control protocols for routine analysis.

Introduction

Scientific investigations on alkaloids with structural similarities to neurotransmitters in the human central nervous system (CNS), such as dopamine, serotonin, and acetylcholine, have led to the development of drugs used as analgesics and immunomodulators, although some have resulted in addiction and/or caused severe adverse affects. Many alkaloid-producing plants have been reported to be used as mind-altering and mood enhancing substances. *Sceletium tortuosum* (L.) N.E.Br (Mesembryanthemaceae) is used traditionally as a treatment for CNS-related disorders. Its mesembrine-type alkaloids are reported to be responsible for the psychoactive properties. [1–3]



Chemical structures of the alkaloids mesembranol (1), mesembrenol (2), mesembrine (3), and mesembrenone (4)

In this study a simple, rapid, and precise HPTLC method for the simultaneous determination of mesembrenol, mesembranol, mesembrine, and mesebrenone in *S. tortuosum* raw materials and commercial preparations was developed for use in routine quality control.

Sample preparation

Aerial parts of wild *S. tortuosum* plants were collected from various localities in the south-western region of South Africa in October 2009. The aerial plant parts were dried at 30 °C for 2 weeks prior to extraction. Dry plant material was pulverized in a ball mill and sieved through a 500 µm mesh sieve. The alkaloids were extracted using an acid-base extraction with 0.5 M sulfuric acid (24 mL) was added to 2 g of each sample and centrifuged. Supernatants of the mixtures were filtered and then neutralized using 6 mL of 20 % aqueous ammonia solution. For liquid-liquid extraction, dichloromethane (14 mL) was added, and the organic phase was filtered into a clean glass vial. Organic extraction was repeated, and both filtrates were combined. They were dried in a vacuum oven at 40 °C and 0.2 bar. For commercial preparations, 2 g powder were removed from the capsules and prepared as described.

Standard solutions

Methanolic alkaloid solutions (0.6 mg/mL), except for mesembrenone (0.2 mg/mL)

Layer

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

Sample application

Bandwise with Automatic TLC Sampler 4 (ATS 4), band length 6 mm, track distance 9 mm, 18 tracks

Chromatography

Development in the Automatic Developing Chamber ADC2 with dichloromethane – methanol – 10 % NH_3 9:1:0.01 (v/v/v) after chamber saturation (with mobile phase for 20 min at 25 °C) and control of the plate activity (with potassium thiocyanate to 47 % relative humidity) up to a migration distance of 85 mm (measured from bottom plate edge)

1

CAMAG
TLC-MS Interface

This interface allows rapid and contamination free elution of TLC/HPTLC zones with online transfer to the mass spectrometer. Advantages are plug & play integration with any given HPTLC-MS system and very low solvent consumption.

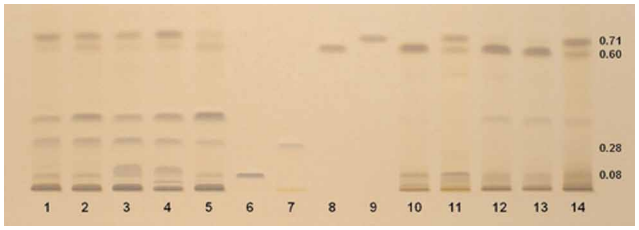
Since 2008 several applications using online coupling of HPTLC-MS have been reported in the CBS. This article describes for the first time the preparative collection of chromatogram zones using the interface. Layers up to 0.5 mm thickness can be eluted with a special elution head. Such use is of interest *e.g.* for collecting marker substances which are not commercially available.

Further information can be found under **www.camag.com/tlcms** or in the special brochure CAMAG TLC-MS Interface.

Also reference CBS 110, p. 10/11 for Merck's new special plates for TLC/HPTLC–MS coupling.

Post-chromatographic derivatization

For visualization only, the iodoplatinate reagent (0.3 % aqueous hydrogen hexachloroplatinate (IV) hydrate mixed with 100 mL 6 % potassium iodide solution) was used.

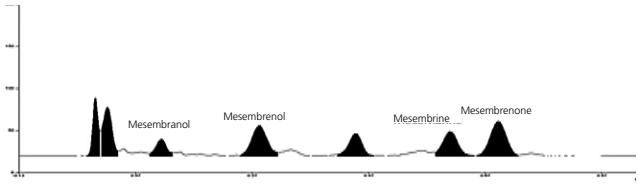


1

HPTLC chromatogram of sample extracts (tracks 1-5), alkaloid standards (track 6: mesembranol, 7: mesembrenol, 8: mesembrine, 9: mesembrenone) and commercial products (tracks 10-14)

Densitometry

TLC Scanner 3 with winCATS software, absorbance measurement at 280 nm, slit dimensions 5 x 0,45 mm, evaluation by linear regression

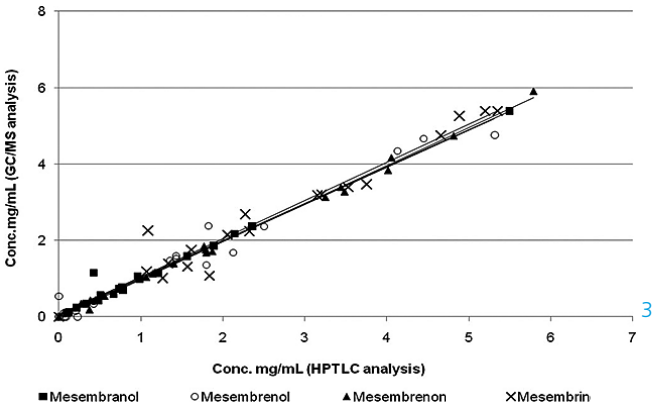


2

HPTLC densitogram of a sample (track 2) containing the four alkaloids

Results and discussion

The method was validated. With regard to linearity, the regression analyses of the calibration data showed a good linear relationships with mean determination coefficients (r^2) ranging from 0.996 to 0.999. Limits of detection and quantification were 18–32 ng/band and 44–95 ng/band, respectively, depending on the alkaloid. Repeatabilities were ≤ 1.6 % and interlaboratory precisions ≤ 1.9 %. Repeatabilities were further confirmed using ANOVA. Accuracy was expressed as recovery, which means ranged between 90.1 ± 3.1 % and 104.7 ± 2.2 %, depending on the alkaloid. The results were compared with those of GC/MS analysis and showed a good correlation with determination coefficients (r^2) of 0.969 for mesembranol, 0.914 for mesembrenol, 0.997 for mesembrenone and 0.953 for mesembrine.



3

Linear correlation between HPTLC and GC/MS data

The HPTLC densitometric method proved to be a simple, reliable, precise, and rapid method for identification and simultaneous quantification of mesembrine-type alkaloids in *S. tortuosum* raw materials and commercial preparations. This method was found to effectively separate psychoactive alkaloids from other components present in plant material. Results obtained showed linear correlation with those obtained from GC/MS analysis. Hence, this method shows potential for use in routine quality control of *S. tortuosum* raw material and commercial products.

[1] W.P. Armstrong, Mind-Altering Plant Alkaloids, http://waynesword.palomar.edu/ww0703.htm.

[2] M.T. Smith, N.R. Crouch, N. Gericke, M. Hirst, J Ethnopharmacol 50 (1996) 119.

[3] B-E. van Wyk, N. Gericke, People's Plants, Briza Publications, Pretoria, 2003.

Further information is available from the author

Contact: Prof. Dr. A.M. Viljoen, Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa, viljoenam@tut.ac.za

Detection and determination of caffeine, taurine and arginine in shampoos

Michaela Oberle

Michael Schulz

Over the last few years a new trend in hair care products, particularly shampoos, has become apparent. Now that the cleaning effect is no longer the main focus, the cosmetic industry has started to develop products with additional features. Anti-dandruff shampoos or shampoos with remedial functions are available in every supermarket. The use of semi-natural additives seems to be part of the trend, and as one might expect, there follows a need for analytical methods to identify and to quantify these substances.

Below the quantification of caffeine and taurine is demonstrated in two commercially available shampoo products, using a very simple sample preparation. HPTLC offers unique advantages, especially in this field of cosmetics analysis. Many samples can be analyzed quickly and simultaneously under exactly the same conditions. They can be compared side by side in the image. The ingredients can usually be separated directly from complex matrices and quantified. The separation of the typical cosmetics matrix components such as oils, fats and other components of the formulation is achieved by selecting the appropriate mobile phase or in some cases, stationary phases with concentration zone.

Sample and standard solutions

1 g shampoo sample was stirred with 10 mL isopropanol at room temperature and filtered with a 0.45 µm syringe filter; methanolic standard solutions of caffeine, taurine and arginine (1 mg/mL)

Layer

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

Sample application

Bandwise with Automatic TLC Sampler 4, band length 5 mm, track distance 10 mm, distance from the lower edge 10 mm, application volume 0.1–5 µL

Chromatography

In a twin trough chamber (10 x 10 cm) with isopropanol – *n*-heptane – water 7:3:1 (v/v/v) for caffeine, and isopropanol – water 4:1 (v/v) for arginine and taurine; migration distance 50 mm

Postchromatographic derivatization

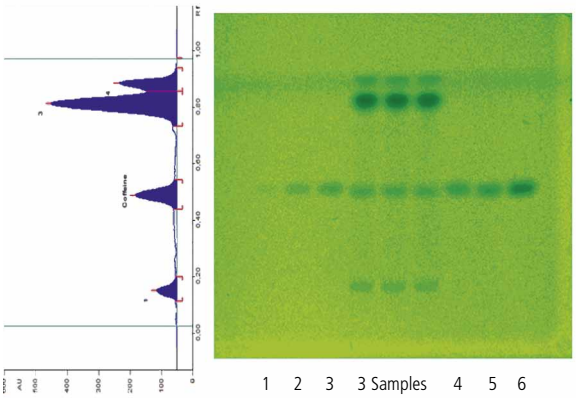
Arginine and taurine were detected by spraying with ninhydrin reagent (Merck # 1.06705.0100)

Documentation

With DigiStore 2 under UV 254 nm (caffeine) and under white light (arginine and taurine)

Densitometry

Absorbance measurement with TLC Scanner 3 at 254 nm (caffeine) and 600 nm (arginine and taurine), slit dimension 4 x 0.3 mm, scanning speed 20 mm/s, evaluation via peak area



1

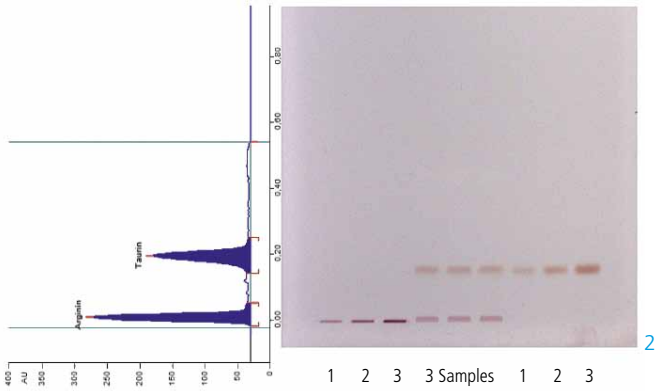
Absorption measurement (254 nm) of the middle shampoo sample in the chromatogram under UV 254 nm

Editor's note: Usually, absorbance measurements are performed at the absorption maximum of the substance to ensure the maximum sensitivity. This is at 275 nm for caffeine on silica gel plates.

Results and discussion

The caffeine mean (hR_F 54, n = 3) was determined to be 0.94 mg/g in shampoo sample 1 via polynomial calibration (r = 0.9991, sdv = 3.9 %).

For detection of taurine and arginine, the plate was derivatized with the ninhydrin spray reagent and documented under white light. Arginine was not moved under these chromatographic conditions and not evaluated. The taurine mean (hR_F 24, n = 3) was evaluated to be 0.75 mg/g in shampoo sample 2, also via polynomial calibration.



2

Absorption measurement (600 nm) of the last shampoo sample in the chromatogram under white light

The taurine and caffeine amounts found corresponded to the usual amounts of 0.1 % active ingredient in a formulation.

Further information is available from the authors on request.

Contact: Michaela Oberle, Merck KGaA, MM-LER-CP, Frankfurter Str. 250, 64293 Darmstadt, Germany, michaela.oberle@merck-group.com

3

CAMAG TLC Scanner 4

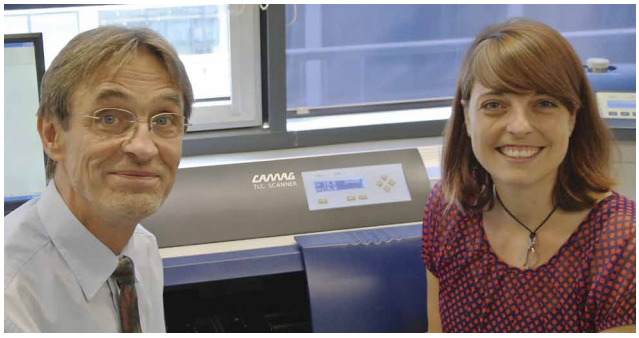
The CAMAG TLC Scanner 4 with winCATS Software is the most advanced workstation for densitometric evaluation of TLC/HPTLC chromatograms.

- Measurement by absorbance or fluorescence
- Spectral range from 190 to 900 nm with high spectral resolution
- Scanning speed 1–100 mm/s
- Data step resolution 25–200 µm
- Dual-wavelength scanning for background correction, *e.g.* after derivatization
- Multi-wavelength scanning for correct quantitation of fractions with different absorption maxima and for checking fractions for purity
- Automatic recording of absorption spectra and of fluorescence excitation spectra
- Scanner selftest as an indispensible tool for IQ/OQ qualification

Further informationen under:
www.camag.com/tlc-scanner

continuation on page 9

Screening method to study the reactivity of cosmetic UV filters on skin proteins



Professor Schwack, Constanze Stiefel

Besides method development for the residue analysis of special pesticide groups, the research of Professor Schwack is focused on photodegradation of pesticides on fruits and vegetables and of cosmetic UV filters on the skin. Planar chromatography plays an important role, such as in the development of planar solid phase extraction and for the screening of additives in plastics or of antibiotic residues in animal-derived food. A new approach is the use of amino plates to study the efficacy of cosmetic ingredients to form protein adducts.

Introduction

Most consumers today are aware of the correlation of excessive sun exposure and possible skin damage. In the past the use of UV filters was mainly limited to sun protection products. Today they are also found in many daily body care products. Most of the UV filters that are used contain reactive carbonyl groups allowing a reaction with peptides or free amino acids of the skin. For the safety assessment of cosmetics, the determination of the sensitizing potential of the ingredients plays an important role. Since the new Cosmetics Regulation (EC) No. 1223/2009 EU bans all animal testing, the development of non-animal alternative methods is gaining importance.

In comparison to existing *in vitro* methods, which are partly associated with great instrumental efforts or long incubation times, the developed HPTLC screening on an amino phase as protein model provides an easy and rapid way to estimate the reactivity of UV filters on skin proteins.

Standard solutions

Benzophenone-3 (BP-3), butyl methoxydibenzoylmethane (BM-DBM), 3-benzylidene camphor (3-BC), 4-methylbenzylidene camphor (4-MBC), octocrylene (OCR), ethylhexyl methoxycinnamate (EHMC), ethylhexyl salicylate (EHS), diethylhexyl butamido triazone (DEBT), and octyldimethyl *p*-aminobenzoic acid (ODPABA) were solved in acetonitrile, 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (HMBS) in methanol, ethylhexyl triazone (EHT) in ethanol – acetonitrile – toluene 2:2:1 (150 µg/mL each).

Layer

HPTLC plates NH_2 F_{254} (Merck), 20 × 10 cm, prewashed with methanol, sparingly cut to 6 plates of 6.7 × 5 cm

Sample application

Bandwise with ATS 4, band length 4 mm, track distance 8 mm, distance from left and right edge 10 mm, distance from lower edge 8 mm, 3 tracks (2 µL/band) of one UV filter and after reaction 4 more tracks (0.2–2.9 µL/band) for calibration

Reaction on the plate (up to 2h)

Storage in the dark at room temperature (RT), heating to 33 °C with a TLC plate heater, irradiation under natural sunlight or with a Suntest CPS+ (Atlas) at 350 W/m^2

Chromatography

In a twin trough chamber (10 × 10 cm) with 8 mL petroleum ether – *t*-butyl methyl ether – methanol 7:2:1 (v/v/v) and 1 % triethylamine (HMBS with methanol – acetic acid 9:1 v/v), migration distance max. 48 mm from the lower edge

Documentation

With DigiStore 2 under UV 254 nm

Sales & Marketing under New Leadership

This January I joined CAMAG as the new Head Sales & Marketing. I am very aware that this assignment will be a big challenge, a challenge which I expect to meet with success. My confidence stems from my background and experience and my natural inclination.

After I finished my studies in Biology I did my Ph.D. at the University of Basel, working in the laboratories of Ciba-Geigy Ltd., Department of Plant Protection. I then went to the USA for postdoctoral studies at the University of California in Berkeley. I returned to Switzerland where I performed metabolism studies for the registration of pesticides at Harlan Ltd. using *e.g.* HPLC, Thin-Layer Chromatography and Gel-Electrophoresis. With this valuable practical experience, I got the chance at LONZA/Basel to combine scientific work with commercial aspects which led to opportunities in sales and marketing. Afterwards I was given a challenging task involving three Swiss companies, which was to build and lead for each company their sales and marketing teams. In each case I was a member of the management team.

I do see potential for growth in CAMAG's major business area, the planar chromatography market, provided that we have efficient public relations and the ability to expand into additional markets. I have already visited in my first six months our two subsidiaries and the main distributors in Europe as well as those overseas. I was thereby able to gain some insight into measures that I think will be necessary to expand our markets.

I am sure that together with the highly motivated CAMAG team we will be able to further increase the acceptance of the planar chromatography worldwide.

Markus Wyss, Ph.D.

Head Sales & Marketing

CBS 111

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SEPTEMBER 2013

Liebe Freunde

Das vorherige *International Symposium for High-Performance Thin-Layer Chromatography* 2011 in Basel war ein grosser Erfolg. Über 310 Teilnehmer aus ca. 40 Ländern nahmen daran teil. Umso größer ist die Herausforderung für das bevorstehende *International Symposium for HPTLC* in Lyon, 2. bis 4. Juli 2014. Die Vorbereitungen dafür haben begonnen; den aktuellen Stand erfahren Sie unter **www.hptlc.com**. Abstracts für Poster oder Vorträge können bis zum 1. März 2014 eingereicht werden.

Themenkomplexe für Vorträge und Poster sind für alle Bereiche der HPTLC geplant. Neueste Informationen zu Miniaturisierung, zu innovativen Plattenmaterialien, Fortschritte in der Gerätetechnologie und Aktuelles von der wirkungsbezogenen Detektion werden mit Sicherheit dabei sein, da diese Bereiche stark gefragt sind. Es begeistert auch, das grosse Potenzial der bildhaften Planar-Chromatography zu erkennen. Informieren Sie sich über Trends und die neuesten Errungenschaften – noch vor ihrer Publikation!

International anerkannte Vortragende bringen die HPTLC forschend voran und sind eingeladen, ihr Wissen mit uns zu teilen und zu diskutieren. Wir freuen uns schon jetzt, von den Teilnehmern mit neuen Ideen angeregt zu werden- Auch Nachwuchswissenschaftler kommen zum Zuge, und die besten Vorträge und Poster werden mit Preisen ausgezeichnet. Das endgültige Programm wird im März unter **www.hptlc.com** online verfügbar sein. Nutzen Sie die Gelegenheit und aktualisieren Sie Ihr Wissen zur HPTLC – es ist an der Zeit, sich für dieses Symposium zu entscheiden!

Mit freundlichen Grüssen

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

The last *International Symposium on High-Performance Thin-Layer Chromatography*, held in Basel in 2011 was a great success and attracted more than 310 participants from about 40 countries. Now we are looking at an even greater challenge, which is to continue the success at the upcoming *International Symposium on HPTLC* in Lyon, 2nd–4th July 2014. Arrangements for HPTLC 2014 are already underway, viz. **www.hptlc.com**. The Call for Papers is open for poster and oral presentations until 1st of March 2014.

Sessions are planned in every discipline of HPTLC and you can be sure that the latest information about miniaturized planar chromatography, novel plate materials, progress in instrumentation, and effect-directed detection will be presented, since these are considered to be emerging fields. The possibilities of what might be on the horizon for planar chromatography with its unique features for image acquisition are very thrilling. The symposium will give you the opportunity to get informed about trends and latest achievements, even before they are published!

Internationally recognized speakers move HPTLC forward and they are invited to share their knowledge with us and discuss new ideas. We look forward to what they might have to show us. Particularly young researchers are encouraged to present their work. The best oral and poster presentations will be recognized with an award. The final symposium program will be published in March 2014 under **www.hptlc.com**. Advance your knowledge in HPTLC – now is the right time to register for this event!

Regards from Switzerland,

Gerda Morlock

Gerda Morlock
cbs@camag.com

CAMAG

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PLANAR CHROMATOGRAPHY

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

111 001 Clara O'SULLIVAN, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA, USA, friedb@lafayette.edu): Metabolic profiling of *Echinostoma caproni* and *Schistosoma mansoni* in their definitive and intermediate hosts. *Acta Parasitologica* 58/1, 1-5 (2013). Review focused on chromatographic methods, mainly TLC or HPTLC, for metabolic profiling of the trematodes *Echinostoma caproni* and *Schistosoma mansoni*. More than 12 references each for *Schistosoma* and for *Echinostoma* are reviewed.

research, HPTLC, densitometry, quantitative analysis, qualitative identification, comparison of methods 1

2. Fundamentals, theory and general

111 002 M. JANICKA*, K. STEPNIK, A. PACHUTA (*Maria Curie-Skodowska University, Department of Physical Chemistry, Faculty of Chemistry, Maria Curie-Skodowska Sq. 3, 20-031 Lublin, Poland, malgorzata.janicka@poczta.umcs.lublin.pl): A comparative study of the lipophilicity of 1,2,4-triazoles by reversed-phase and micellar TLC and OPLC. J. Planar Chromatogr. 26, 153-159 (2013). TLC lipophilicities of 21 newly synthesized 1,2,4-triazoles on RP-8 and RP-18 with buffered solutions of acetone, 1,4-dioxane, tetrahydrofuran and acetonitrile as well as on cyano phase with solutions of 0.03, 0.04, 0.06, 0.08 and 0.1 M sodium dodecyl sulfate modified with the constant (20 %) addition of organic modifier (acetone, 1,4-dioxane, tetrahydrofuran and acetonitrile). Partitioning lipophilicity indices showed mobile phase composed of sodium dodecyl sulfate and tetrahydrofuran as the system providing the best linear correlations between chromatographic and partitioning lipophilicities of tested 1,2,4-triazoles.

pharmaceutical research, qualitative identification, lipophilicity 2c

111 003 L. LITVINOVA*, N. BELNIKEVICH (*Institute of Macromolecular Compounds of the Russian Academy of Sciences, Bolshoi pr. 31, 199004 St.-Petersburg, Russia, larissa_litvinova@hotmail.com): The peculiarities of adsorption-exclusion transition of poly(methyl methacrylate) in binary mobile phases with acetonitrile as displacer. J. Planar Chromatogr. 26, 147-152 (2013). TLC of poly(methylmethacrylate) (PMMA) on silica gel with acetonitrile binary solvent mixtures with carbon tetrachloride, toluene, methylene chloride, chloroform and dichloroethane. Mark-Kuhn-Houwink parameters for PMMAs, Snyder's parameters and the measured intrinsic viscosity values for PMMA. The characteristics of adsorption-exclusion transition depends both on thermodynamic affinity of the solvent for the polymer and macromolecule-to-pore size ratio.

pharmaceutical research, qualitative identification 2b

111 004 J. POLANSKI, M. SAJEWICZ, M. KNAS, Monika GONTARSKA, T. KOWALSKA* (*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland, teresa.kowalska@us.edu.pl): Lateral relocation in thin-layer chromatography. J. Planar Chromatogr. 25, 208-213 (2012). The phenomenom of lateral relocation in the migration tracks of chiral analytes was reviewed as a retention fundamentals in TLC. By employing silica gel plates and others impregnated with L-arginine and DL-arginine, the non-linear motion of (±)-2-phenylpropionic acid was studied. TLC on impregnated or non-impregnated silica gel (pre-washed by development with methanol - water 9:1) with acetonitrile - methanol - water 20:4:3. For plates impregnated with arginine, the mobile phase additionally contained 0.5 % (v/v) glacial acetic acid to set the pH to < 5.

pharmaceutical research, HPTLC, review 2c

111 005 L. SNYDER (LC Resources, 26 Silverwood Ct., Orinda, CA 94563, USA, snyder0036@comcast.net): Localization in adsorption chromatography. J. Planar Chromatogr. 25, 184-189 (2012). The author reviewed the effects of the localization of molecules of solute or solvent on the adsorbent surface in adsorption chromatography, particularly on sample retention and resolution.

pharmaceutical research, HPTLC 2c

3. General techniques

111 006 J.Z. HALL, M.T. TASCHUK, M.J. BRETT* (*Dep. Electr. & Comp. Engin., Univ. of Alberta, 2nd Floor ECERF, Edmonton, Alberta T6G 2V4, Canada): Polarity-adjustable reversed phase ultrathin-layer chromatography. J. Chromatogr. A 1266, 168-174 (2012). RP-TLC plates modified with C18, C8 or C2 are available on the market, however, RP-plates with tunable polarity have not been reported. Because of the limited variety of RP-plates mobile phase optimization is needed which is often time consuming. The presented polarity-adjustable RP-UTLC plates simplify the mobile phase screening process and expand the selection of reversed phase plates. The plates were prepared using the glancing angle deposition (GLAD) technique to deposit SiO_2 nanopillars on glass plates and functionalized with octadecyltrichlorosilane for hydrophobicity. By O_2 plasma treatment the silane carbon chain was shortened and COOH groups were introduced to the surface, producing plates with finely tunable polarities. Confirmation of the tuning of surface polarities by measurement of retention behavior changes in the separation of a model dye mixture of Sudan blue and Sudan IV, the elution order of which reversed as a result of the change in surface polarity. Testing on commercial RP-plates showed no change in the separation behavior which proves that plasma treatment of GLAD structures with highly accessible surfaces improves control over interfacial properties, producing better reverse phase separations.

pharmaceutical research, quality control, qualitative identification, HPTLC, comparison of methods, glancing angle deposition, UTLC 3b, 30

111 007 R. KAISER (Institute f. Chromatography, Bad Duerkheim, Germany, rudolf.kaiser@t-online.de): Three-phase chromatography: planar chromatography using simultaneously flowing gas phase(s), liquid mobile phase(s), and one stationary phase. J. Planar Chromatogr. 26, 190-195 (2013). Enlarging of the two-phase microcircular planar liquid chromatography (PLC) into a three-phase chromatographic technique by adding gas flow containing specific chemicals like acids, bases, alcohols, esters, hydrocarbons. The flow speed of 2 L/min flushes the gas volume over the 100 mm × 100 mm PLC plate 200 times per minute at room temperature. Reproducibility was obtained in the ±0.1 % range in qualitative evaluations and ±0.5 % down to ±0.05 % range for quantitative analyses.

pharmaceutical research, quantitative analysis, three-phase chromatography, micro circular planar chromatography. 3d

111 008 S. KUSTRIN*, C. LOESCHER, R. SINGH (*School of Pharmacy and Applied Science, La Trobe University, Edwards Rd, Bendigo, Victoria 3550, Australia, s.kustrin@latrobe.edu.au): Quantification of phenylpropanoids in commercial Echinacea products using TLC with video densitometry as detection technique and ANN for data modelling. Phytochem. Anal. 24, 303-308 (2013). HPTLC of chicoric acid (1), chlorogenic acid (2) and echinacoside (3) in commercial Echinacea products on silica gel with ethyl acetate - formic acid - acetic acid - water 100:11:11:17. Qualitative identification under UV 366 nm. Images were quantified and trans-

formed into chromatograms, as signal intensities (peaks) versus hR_F (retention factor) for a artificial neural network (ANN) correlation. LOD were 19, 46 and 29 ng/band, whereas LOQ were 63, 154 and 98 ng/band for (1), (2) and (3), respectively.

herbal, quality control, HPTLC, qualitative identification 3f

111 009 W. MARKOWSKI*, Karol WROBLEWSKI, T. DZIDO (*Department of Physical Chemistry, Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland, wojtek@bg.umlub.pl): Stepwise gradient elution in RP-HPTLC with a new horizontal developing chamber. J. Planar Chromatogr. 25, 200-207 (2012). New horizontal developing chamber with advantages for stepwise gradient elution in RP HPTLC. It is shown that a previously developed computer program can be successfully applied to predictive calculations of solute retention in stepwise gradient elution.

HPTLC 3d

111 010 Olumuyiwa OGEGBO*, S. EYOB, S. PARMAR, Z.T. WANG, Annie BLIGH (*Plantalysis, Institute for Helath Research and Policy, London Metropolitan University, 166-220 Holloway Road, London, UK, o.ogegbo@londonmet.ac.uk): Metabolomics of four TCM herbal products: application of HPTLC analysis. Anal. Methods 4, 2522-2527 (2012). HPTLC of the petroleum ether extracts of the herbal TCM root drugs of *Aster tataricus*, *Atractylodes lancea, Gentiana rigescens*, and *Gentiana macrophylla* on silica gel with toluene - ethyl acetate 15:1 over 70 mm. Detection under UV 366 nm and white light after immersion in 1 % vanillin solution dissolved in 10 % conc. sulfuric acid in ethanol, heating at 105 °C for 5 min. Each sample track on the UV 366 nm image was digitalized. For statistical data exploration multivariate analysis based on the models principal component analysis (PCA), partial least-square-discriminant analysis (PLS-DA) and orthogonal PLS-DA was used. The model score plots showed for all three models good spatial distributions with clear cluster for grouping each sample and high reproducibility and predictive values (> 0.5).

herbal, HPTLC, qualitative identification 3g, 32e

111 011 M. SCHULZ*, B. SCHUBACH, Susanne MINARIK, H. GRIESINGER (*Merck KGaA, MMLER-CP. Frankfurterstr. 250, 64293 Darmstadt, Germany, michael.schulz@merckgroup.com): Introduction of special HPTLC and TLC plates for coupling with mass spectrometry. CBS 110, 10-11 (2013). New HPTLC silica gel plates specially suited for coupling with MS and TLC plates for coupling with NMR. These MS-grade HPTLC plates are low in impurities and show less background noise, the layer thickness is 100 µm. HPTLC of human insulin and its secondary components on MS-grade silica gel with 2-butanol - pyridine - ammonia 25 % - water 39:34:10:26, detection under UV 366 nm after treatment with fluorescamine and by elution with the TLC-MS Interface and Q-TOF MS analysis in ESI positive mode, the eluent was acetonitrile - water 19:1. Separation and identification of insulin and desamido insulin was achieved, even though there is only 1 Da mass difference.

HPTLC 3b, 19

111 012 W. SCHWACK, Claudia OELLIG* (*Institute of Food Chemistry, University of Hohenheim, 70599 Stuttgart, Germany, claudia.oellig@uni-hohenheim.de): Solid phase extraction as clean-up for pesticide residue analysis of tea samples using planar chromatographic developing techniques CBS 110, 12-15 (2013). Clean-up of matrix-rich samples using high-troughput planar

solid phase extraction (HTpSPE). Black and green tea samples were spiked with 7 pesticides (acetamiprid, azoxystrobin, chlorpyrifos, fenarimol, mepanipyrim, penconazole, and primicarb) at level 0.01, 0.1 and 1 mg/kg. Extraction with acetonitrile, pre-cleaning by dispersive SPE. TLC on silica gel (prewashed with acetonitrile) of samples applied as rectangles of 3 x 16 mm first with acetonitrile - water 19:1 over 85 mm and after drying for 5 min with acetone - water 7:1 in the opposite direction over 31 mm. Detection under UV 254 and 366 nm and by dipping in primuline reagent (0.2 % in acetone - water 4:1) and detection under UV 366 nm and white light. Elution of target zones into autosampler vials by TLC-MS Interface with acetonitrile - 10 mM ammonium formate buffer 1:1, flow rate 0.2 mL/min. After clean-up the samples are free of caffeine which interferes with pesticide detection.

food analysis, quality control 3a, 29f

111 013 A. SINHABABU*, B. KUMAR, H. DEY, S. LASKAR (*Natural Products Laboratory, Department of Chemistry (UGC-CAS), The University of Burdwan, Burdwan 713104, West Bengal, India, sinhababu04@yahoo.co.in): Identification of amino acids with modified ninhydrin reagents on thin-layer chromatography plates. J. Planar Chromatogr. 26, 26-30 (2013). Four new spray reagents for the detection of amino acids were introduced: (1) 0.25 % solution of benzoic acid in ethanol, (2) 0.1 % solution of *p*-fluorobenzoic acid in ethanol, (3) 0.1% solution of *p*-chlorobenzoic acid in ethanol and (4) 0.05 % solution of *p*-iodobenzoic acid in ethanol. Depending on the different amino acids tested the LOD on silica gel was in the range of 0.1-1.0 µg for all four reagents. For example for cysteine the LOD was 0.1 µg with reagents (1), (3), and (4), and 0.2 µg with reagent (2).

pharmaceutical research, HPTLC, qualitative identification, postchromatographic derivatization 3e, 18

4. Special techniques

111 014 E. MINCSOVICS*, P. OTT, A. ALBERTI, A. BOSZORMENYI, E. HETHELYI, E. SZOKE, A. KERY, E. LEMBERKOVICS, A. MORICZ (*Department of Genetics and Plant Breeding, Faculty of Horticultural Sciences, Corvinus University, Villányi Str. 29-45, 1118 Budapest, Hungary, emil.mincsovics@t-online.hu): *In-situ* clean-up and OPLC fractionation of chamomile flower extract to search active components by bioautography. J. Planar Chromatogr. 26, 172-179 (2013). OPLC with on-line detection and fractionation, *In-situ* sample clean-up in the planar layer adsorbent bed, direct bioautography (DB), OPLC-MS, solid phase microextraction (SPME)-GC-MS, and LC-MS/MS for the bioassay-guided isolation and characterization of bioactive compounds from chamomile flower extract. The bioassay-guided isolation of antibacterial chamomile components was based on OPLC separation with on-line detection and fractionation combined with previous sample clean-up *In-situ* in the adsorbent bed after sample application. First the adsorbent layer was partially pre-weted between the edge of the layer and the sample application zone with the goal to fill up the »dead« area behind the trough, which leads the components to leave the adsorbent layer during the clean-up step. With this process, the zone behind the trough can be protected from stucking of any components in it, otherwise the stucked compounds could be detected continuously during the separation/detection/fraction collection. During the *In-situ* sample clean-up the mobile phase flow was in the opposite direction, from outlet toward inlet of the chamber. In this step the load of the adsorbent can be decreased for the fractionation, which is done in the normal direction of the mobile phase.

herbal, pharmaceutical research, HPTLC, qualitative identification 4e

111 015 G. SCHLOTTERBECK*, S. GAUGLER, Uta SCHERER, A. GOESSI, S.WYSS, A.BUETTLER, T. HETTICH, A. BARON (*School of Life Sciences, Institute for Chemistry and Bioanalytics, Gruendenstrasse 40, 4132 Muttenz, Switzerland; goetz.schlotterbeck@fhnw.ch): Rapid structure confirmation and quantitation by HPTLC-NMR. CBS 110, 2-4 (2013). HPTLC of caffeic acid, chlorogenic acid, and rutin hydrate on silica gel (prewashed with methanol and dried under vacuum at 50 °C for 30 min) with formic acid - ethyl acetate - water - methyl ethyl ketone 5:30:6:18 with chamber saturation for 5 min over a developing distance of 5 cm. Detection under UV 254 nm. Elution of substance zones with TLC-MS Interface using methanol and a flow-rate of 0.3 mL/min for 6 min. Evaporation of methanol under nitrogen, residue taken up with methanol-d4. Subsequent off-line quantitative 1H-NMR spectroscopic analysis of the residue, acquisition time 30 min. Linearity for all substances was confirmed in the range of 10 - 80 µg/mL. Recoveries were in the range of 100.5 % for chlorogenic acid and up to 103.4 % for caffeic acid, with precisions under 3.9 % (%*RSD*, *n*=3).

pharmaceutical research, HPTLC, quantitative analysis, densitometry 4e

111 016 Q. ZHU (Zhu Qin), X. SU (Su Xiao), H. WU (Wu Haijun), Y. ZHAI (Zhai Yanjun), J. XIA (Xia Jinming), BUHEBATE, Y. XU (Xu Yizhuang)*, J. WU (Wu Jinguang) (*The State Key Lab. of Rare Earth Materials Chem. & Appl., Coll. of Chem. & Molec. Eng., Peking Univ., Beijing 100871, China): (Study of the technique of Fourier Transform Infrared Spectroscopy (FTIR) coupled with TLC based on silver iodide stationary phase) (Chinese). Chinese J. of Spectroscopy & Spectral Anal. 32 (7), 1790-1794 (2012). TLC is an efficient and economical alternative separation technique for those samples not suitable to be processed by HPLC due to no or low UV response of the compounds or their impurities. Fourier Transform Infrared Spectroscopy (FTIR) is a powerful technique of qualitative and semiquantitative analysis and of discrimination of functional groups towards pure organic compounds. FTIR coupled with TLC is a useful hyphenated technique for the analysis of complex organic mixture samples. In order to abate the interference from IR absorbance signal caused by conventional TLC stationary phases, plates coated with specially processed silver iodide particles are employed. Preparation of silver iodide by reaction of silver nitrate and potassium iodide and isolation of produced silver iodide particles (average size around 100 nm) by dialysis. The silver iodide plates are coated by precipitation method. TLC of bromophenol blue and rhodamine B on the prepared silver iodide plates, with methanol - acetone 1:3, detection in daylight and by in situ reflection FTIR.

qualitative identification 4e

5. Hydrocarbons and halogen derivatives

111 017 Sophie BEHRINGER, W. SCHWACK* (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany, wolfgang.schwack@uni-hohenheim.de): Determination of PAHs in toys by HPTLC. CBS 108, 12-15 (2012). HPTLC of anthracene (ANT), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF), pyrene (PYR), acenaphthene (ACE), benzo[a]anthracene (BAA), benzo[a]pyrene (BAP), benzo[ghi]perylene (BPE), chrysene (CHR), dibenzo[a,h]anthracene (DBA), indeno[1,2,3-c,d]pyrene (IND), fuorene(FLU), fuoranthene (FLA), and phenanthrene (PHE) in toys on RP-18 phase with acetonitrile - water 9:1 by three-fold development over 45, 55 and 65 mm using automated multiple development (AMD) under nitrogen. Detection at 254 and 366 nm. Quantitative fluorescence measurement at different excitation wavelengths with cut-off filters: 220 nm/>320 nm for ACE, 250/>320 for ANT, 366/>400 for BAA and BAP, 270/>400 for BBF, BPE, BKF, CHR, DBA, FLA, IDN (after dipping in nitromethane), 250/>320 for FLU and PHE and at 270/>320 for PYR. Polynomi-

al regression with high coefficients of correlation and low standard deviations. Coeffivients of variation for repeatability and reproducibility were below 10 %. This method allows the determination of 14 of the 16 PAHs. With LODs of 0.1-0.2 mg/kg the demands for the German GS mark (label for checked safety) are fulfilled. The results by HPTLC were comparable to results obtained by GC-MS.

comparison of methods, HPTLC, quantitative analysis, densitometry 5d

111 018 M. CHAVAN, A. VAIDYA* (*Unilever R & D India, 64 Main Road, Whitefield, Bangalore, India, ashish.vaidya@unilever.com): Development of high-performance thin-layer chromatography (HPTLC) technique for evaluation of sunscreen photostability. J. Planar Chromatogr. 25, 122-126 (2012). HPTLC of 4-tert-butyl, 4-methoxydibenzoylmethane along with a photostabilizer on silica gel, then exposed to solar simulated sunlight and developed with *n*-hexane - ethyl acetate 9:1. Detection under UV 366 nm. Quantitative determination of the amount of sunscreen left after solar exposure by absorbance measurement at 357 nm.

pharmaceutical research, HPTLC, quantitative analysis 5b

7. Phenols

111 019 I. SCHELLENBERG, Kathrin KABRODT* (*Hochschule Anhalt, Center of Life Sciences, AG Institut für Bioanalytische Wissenschaften, Strenzfelder Allee 28, 06406 Bernburg, Germany, k.kabrodt@loel.hs-anhalt.de): Identification of polyphenolic compounds in Rheum officinale Baill. by TLC-MS-coupling. CBS 109, 5-7 (2012). HPTLC of *Rheum* root extracts on silica gel (pre-washed with isopropanol, activated for 30 min at 120 °C) with toluene - ethyl acetate - formic acid 5:4:1 for fraction (1), 4:5:1 for fractions (2) to (6), 3:6:1 for fraction (7), 3:7:1 for fraction (8) and 2:7:1 for fractions (9) and (10). Detection under white light and UV 366 nm after dipping in 1 % ethanolic vanillin solution for 3 s, drying, heating at 63 °C for 5 min and exposure to 37 % HCl vapors. Elution of target zones by TLC-MS Interface, flow rate 0.1 mL/min, for ESI MS in negative mode.

herbal, HPTLC, postchromatographic derivatization, qualitative identification 7

8. Substances containing hetrocyclic oxygen

111 020 E. SHAWKY (Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt, Alexandria 21521, Egypt, shawkyeman@yahoo.com): Development and validation of an HPTLC method for the simultaneous determination of diosmin and hesperidin in different citrus fruit extracts and pharmaceutical formulations. J. Planar Chromatogr. 25, 138-144 (2012). HPTLC of diosmin (1) and hesperidin (2) on silica gel with ethyl acetate - methanol - water - acetic acid 25:2:2:1. Quantitative determination by absorbance measurement at 330 nm. Linearity was in the range of 100-3000 ng/zone for (1) and 250-7500 ng/zone for (2). The method did not show any statistically significant deviation when compared with a validated HPLC.

food analysis, quality control, comparison of methods, quantitative analysis, HPTLC 8a

111 021 K. SPEER*, Sandra BUCHMANN, Isabelle KOELLING-SPEER (*Professorship of Special Food Chemistry and Food Production, TU Dresden, Bergstrasse 66, 01062 Dresden, Germany, karl.speer@chemie.tu-dresden.de): TLC screening for the detection of Robusta admixtures to Arabica coffee. CBS 109, 2-4 (2012). Extraction of arabica coffee with 2 to 50 % robusta coffee by accelerated solvent extraction with tBME. A part was saponified with10 % ethanolic KOH solution for 2 h. HPTLC of coffee extracts and standards 16-O-methylcafestol (16-OMC) and 16-OMC esters on silica gel with toluene - ethyl acetate - acetic acid 93:7:1 for 16-OMC esters and

with tBME - chloroform 1:1 for 16-OMC. Detection by spraying with vanillin sulfuric acid (1 g in 250 mL ethanol and 2 mL of sulfuric acid, prepared freshly) and heating for 1 min at 80 °C. Quantitative absorption measurement at 530 nm. The LOD was 163 ng/band. After saponification the LOD of free 16-OMC was 43 ng/band. Thus 2 % robusta can be detected in a coffee blend.

quality control, food analysis, HPTLC, densitometry, quantitative analysis, postchromatographic derivatization 8b

10. Carbohydrates

111 022 N. POPOVIC, B. FRIED*, J. SHERMA (*Department of Chemistry, Lafayette College, Easton, PA 18042, USA friedb@lafayette.edu): Effects of increased salinity on glucose and maltose composition of *Biomphalaria glabrata* snails infected with *Schistosoma mansoni* as determined by high-performance thin-layer chromatography-densitometry. J. Planar Chromatogr. 26, 137-140 (2013). HPTLC of glucose (1) and maltose (2) in the digestive gland-gonad complex of *Biomphalaria glabrata* snails on silica gel with 1-butanol - glacial acetic acid - diethyl ether - water 27:18:5:3. Detection by spraying with alpha-naphthol-sulfuric acid detection reagent followed by heating at 110 °C for 10 min. The hR_F values of (1) and (2) were 41 and 28, respectively.

HPTLC, environmental, quantitative analysis, salinity 10a

111 023 Anitta PUSCAS, Anamaria HOSU, Claudia CIMPOIU* (*Babes-Bolyai Univ., Faculty of Chem. & Chem. Engin., 11 Arany Janos, 400028 Cluj-Napoca, Romania): Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration. J. Chromatogr. A 1272, 132-135 (2013). Honey is a saturated solution of sugars, used for a long time as a natural source of sugars and is an important ingredient in traditional medicine due to its antimicrobial, anti-inflammatory and antioxidant effects. For quality control and detection of adulteration, TLC of glucose, fructose and sucrose on silica gel twice with ethyl acetate - pyridine - water - acetic acid 12:6:2:1, followed by dipping in an immersion solution. Documentation of plates by using a TLC visualization device and processing the images of plates by using a digital processor. Validation of the method for its selectivity, linearity and range, LOD and LOQ, precision, robustness and accuracy. The method was proved to be simple and economical and then applied for quantitative determination of glucose, fructose and sucrose from different types of Romanian honeys, commercially available.

pharmaceutical research, quality control, food analysis, agricultural, HPTLC, quantitative analysis, qualitative identification 10

111 024 Dinah SCHICK, W. schwack, F. GAMLICH, Gertrud MORLOCK* (*Justus Liebig University of Giessen, Institute of Nutritional Science, IFZ, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, gertrud.morlock@ernaehrung.uni-giessen.de): The fingerprint of biopolymers (polysaccharides). CBS 108, 9-11 (2012). HPTLC of fructose, galacturonic acid, rhamnose, xylose, and galactose in samples of agar agar, arabic gum, carubin, guaran, traganth, xanthan, pectin, alginic acid, alginates and carrageen on silica gel with *i*-propyl acetate - ethyl acetate - methanol - water 50:40:10:1 to a migration distance of 60 mm. Densitometric absorption measurement at 370 and 630 nm and peak area evaluation by linear or polynomial regression. Detection by immersion in aniline diphenylamine o-phosphoric acid reagent (20 % *o*-phosphoric acid (85 %) added to 1:1 mixtures of 2 % solutions of diphenylamine and aniline in acetone) and heating at 110 °C for 5 min. Comparison of HPTLC with GC gave comparable results, however, the HPTLC method is more effective in terms of sample throughput, robustness, costs and analysis

time (8 times faster than GC).

food analysis, quantitative analysis, postchromatographic derivatization, HPTLC, densitometry 10b

11. Organic acids and lipids

111 025 R. NASCU, C. SARBU* (*Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Arany Janos Str. No. 11, Cluj Napoca, 400028, Romania, csarbu@chem.ubbcluj.ro): Lipophilicity of oils and fats estimated by TLC. J. Sep. Sci. 36, 1317-1326 (2013). HPTLC of 1. aflatoxine G1; 2. ochratoxine A; 3. zearalenone; 4. quinine; 5. cinconine, 6. hydroquinine; 7. *o*-(4-chlorobenzoyl) hydroquinine; 8. hydroquinine 4-methyl-2-quinolyl ether; 9. (DHQ)2 Phal; 10. (DHQ)2 AQN and 11. camptothecin on RP-18, RP-8, and RP-2 with 85 to 95 % methanol in steps of 2.5 %. In the case of the other stationary phases, the methanol fraction modification was of 5 %. The methanol fraction ranged from 75 to 95 % for RP-18 W, from 35 to 55 % for amino phase, from 50 to 70 % for diol phase, and finally from 65 to 85 % for cyano phase. Qualitative identification by absorbance measurement at 254 and 366 nm. The lipophilicity indices indicated that the mRM appears to be the most suited descriptor.

pharmaceutical research, qualitative identification, HPTLC, lipophilicity 11c

111 026 F. SAHLE, H. METZ, J. WOHLRAB, R. NEUBERT* (*Department of Pharmaceutical Technology and Biopharmaceutics Martin Luther University Halle-Wittenberg Wolfgang-Langenbeck Str. 4 06120 Halle (Saale), Germany, reinhard.neubert@pharmazie.uni-halle.de): Lecithin-based microemulsions for targeted delivery of ceramide AP into the *stratum corneum*: formulation, characterizations, and *in vitro* release and penetration studies. Pharm. Res. 30, 538-551 (2013). HPTLC of ceramide AP in an *in vitro* release and penetration into the *stratum corneum* on silica gel with chloroform - methanol - acetic acid 189:10:1. Detection by dipping into an aqueous 10 % copper sulphate solution in 8 % phosphoric acid and 5 % methanol for 20 s, followed by heating at 160 °C for 20 min. Quantitative determination by absorbance measurement at 358 nm.

pharmaceutical research, qualitative identification, HPTLC, microemulsions 11

111 027 A. SEIGEL, B. MILZ, B. SPANGENBERG* (*University of Offenburg, Institute of Process Engineering, Badstrasse 24, 77652 Offenburg, Germany, spangenberg@hs-offenburg.de): Quantification of parabens by diode-array thin-layer chromatography coupled with a Vibrio fischeri bioluminescence assay. J. Planar Chromatogr. 26, 119-124 (2013). HPTLC of methyl- (1), ethyl- (2), propyl- (3), and butylparaben (4) in cosmetics on cyanopropyl plates with water - acetonitrile - dioxane - ethanol 8:2:1:1+1 drop ammonia. Quantitative determination by absorbance measurement at 255 nm and by bioutographic analysis using *Vibrio fischeri* bacteria. LOD for (3) and (4) was 100 ng/zone, for (1) 80 ng/zone, and 69 ng/zone for (2). The LOQ for (3) and (4) was 120 ng/zone, for (1) 90 ng/zone, and 78 ng/zone for (2).

cosmetics, HPTLC, quantitative analysis, bioautography 11a

13. Steroids

111 028 K. BIELICKA*, A. VOELKEL, D. RUSINSKA, P. ZARZYCKI (*Institute of Technology and Chemical Engineering, Poznan University of Technology, Poznan, Poland, Katarzyna.Bielicka-Daszkiewicz@put.poznan.pl): Estimation of the breakthrough volume of selected steroids for C-18 solid-phase extraction sorbent using retention data from micro-thin layer chromatography.

J. Sep. Sci. 36, 1104-1111 (2013). Micro TLC of equilin (1), estetrol (2), estriol (3), 17alpha-estradiol (4), 17beta-estradiol (5), estrone (6), 17alpha-hydroxyprogesterone (7), 20alpha-hydroxyprogesterone (8) and progesterone (9) on RP-18 with 30 to 100 % methanol. Detection by dipping into PMA reagent (10 % phosphomolybdic acid in methanol) and heating at 100 °C for 10 min. The breakthrough volume was calculated to optimize the extraction process of nine steroids using solid-phase extraction retention data.

pharmaceutical research, HPTLC, qualitative identification 13

14. Steroid glycosides, saponins and other terpenoid glycosides

111 029 S. LONDHE*, S. NANAWARE (*Sinhgad College of Pharmacy, Department of Pharmaceutical Chemistry, Vadgaon, Pune- 411041, India, smitaks1@rediffmail.com): HPTLC method for simultaneous analysis of stevioside and rebaudioside-A in *Stevia rebaudiana*. J. AOAC Int. 96, 24-26 (2013). HPTLC of stevioside (1) and rebaudioside-A (2) in the leaves of *Stevia rebaudiana* on silica gel with ethyl acetate - ethanol - acetone - water 5:1:2:2. Detection by spraying with anisaldehyde - sulfuric acid reagent, followed by heating at 110 °C for 10-15 min. Quantitative determination by absorbance measurement at 580 nm. The hR_F values of (1) and (2) were 34 and 28, respectively. Linearity was 1-7 µg/zone for both. LOD and LOQ were 127 and 387 ng/zone for (1) and 393 and 1191 ng/zone for (2). The interday and intra-day precisions were below 1.2 % (n=3). Recovery (by standard addition) ranged 89.6-93.9 % for (1) and 86.7-90.6 % for (2).

herbal, densitometry, HPTLC, quantitative analysis 14

111 030 Stephanie MEYER, Gertrud MORLOCK* (*Justus Liebig University of Giessen, Institute of Nutritional Science, IFZ, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, gertrud.morlock@ernaehrung.uni-giessen.de): Quantitative determination of steviol glycosides (Stevia sweetener). CBS 109, 10-12 (2012). HPTLC of steviol glycosides (stevioside, rebaudioside, dulcoside A, steviolbioside) on silica gel (pre-washed with methanol and dried at 100 °C for 15 min) with ethyl acetate - methanol - acetic acid 3:1:1 over 60 mm. Detection under white light after immersion in ß-naphthol reagent (2 g in 180 mL ethanol with 12 mL 50 % sulfuric acid) and heating at 120 °C for 5 min. Quantitative absorption measurement at 500 nm after derivatization. LOD was 10 ng/band and LOQ 30 ng/zone. Using the calibration curve method the LOQ was reduced to 12 ng/band via peak height and 20 ng/band via peak area.The calculated expected tolerance range over the whole procedure inclusive sample preparation considered recovery rates at 3 different concentration levels (0.02, 0.13, and 0.20 %) in milk-based matrix. The accuracy (recovery tolerance limit of 92-120 %), repeatability (3.1-5.4 %) and intermediate precision (4.0-8.4 %) were highly satisfying, exemplarily shown for stevioside in milk-based matrix. ANOVA was successfully passed to prove the working range. With the newly developed and validated HPTLC method, steviol glycosides in Stevia leaves, Stevia formulations, and food products were investigated.

food analysis, clinical chemistry research, quality control, HPTLC, quantitative analysis, densitometry, postchromatographic derivatization 14

111 031 N. TIWARI, A. KUMAR, M. MOHAN* (*Analytical Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India, guptammg@rediffmail.com): Validated HPTLC method for the simultaneous quantification of diterpenoids in Vitex trifolia L. J. Sep. Sci. 36, 2373-2378 (2013). HPTLC of 6alpha,7alpha-diacetoxy-13-hydroxy-8(9),14-labdadien (1), 13-hydroxy-5(10),14-halimadien-6-one (2), and 9-hydroxy-13(14)-lab-

den-16,15-olide (3) in the leaves of *Vitex trifolia* L. on silica gel with chloroform - acetone 49:1. Detection by spraying with vanillin-sulfuric acid reagent. Quantitative determination by absorbance measurement at 610 nm. The hR_F of compounds (1), (2) and (3) were 74, 57 and 51, respectively. Linearity was 333-1000 ng/zone for (1) and (2) and 670-2000 ng/zone for (3). LOD were in the range of 73, 56, and 92 ng/zone and LOQ were found to be 242, 186, and 307 ng/zone for (1), (2) and (3), respectively. The intra- and inter-assay precisions were in the range of 1.0-1.2 % and 1.1-1.3 %, respectively for compounds (1) to (3). Recovery (by standard addition) ranged 99.1-102.7%.

herbal, quality control, quantitative analysis, HPTLC 14

18. Amino acids and peptides, chemical structure of proteins

111 032 A. MOHAMMAD*, A. SIDDIQ, A. MOHEMAN, G. EL-DESOKY (*Department of Applied Chemistry, Faculty of Engineering & Technology, Aligarh Muslim University, Aligarh-202 002, India, alimohammad08@gmail.com): Aqueous urea solution promoted resolution of five-component mixture of amino acids on silica TLC plates. J. Planar Chromatogr. 26, 31-36 (2013). TLC of a five-component mixture of the amino acids lysine (1), histidine (2), leucine (3), alanine (4) and glutamic acid (4) on silica gel with 1.0% aqueous urea solution pH 7.44. The hR_F values of amino acids (1) to (5) were 38, 59, 78, 87 and 98, respectively. LOD for (1), (3) and (4) was 1.5 µg/zone, whereas for (2) and (5) it was 1.2 and 3.0 µg/zone, respectively.

pharmaceutical research, quality control, qualitative identification 18a

111 033 B. POLAK*, K. BALASA, T. DZIDO (*Department of Physical Chemistry, Medical University of Lublin, Chodyki 4A, 20-093 Lublin, Poland, beata.polak@umlub.pl): Separation of amino acid 2,4-dinitrophenyl-5-L-valine amide diastereomeric derivatives with high-performance planar chromatography and pressurized planar electrochromatography. J. Planar Chromatogr. 26, 180-189 (2013). HPTLC of 2,4-dinitrophenyl-5-L-valine amide derivatives of some amino acids (leucine, isoleucine, valine, asparagine, cysteine, tryptophane) L and D-enantiomers on RP-18 with aqueous buffer pH 2.2 (1.47 mM citric acid, 0.06 mM disodium hydrogen phosphate) and acetonitrile 50 %. The statistic evaluation of the migration distance compared with pressurized planar electrochromatography (PPEC) shows similar RSD.

pharmaceutical research, HPTLC, comparison of methods 18a

111 034 M. SAJEWICZ, M. MATLENGIEWICZ, M. JUZIUK, M. PENKALA, M. WELOE, M. SCHULZ, T. KOWALSKA* (*University of Silesia, 9 Szkolna Street, 40-006, Katowice, Poland, teresa.kowalska@us.edu.pl): Thin-layer chromatographic evidence of proline peptidization in solution and its thin-layer chromatographic enantioseparation. J. Liq. Chromatogr. Relat. Technol. 36, 2497-2511 (2013). HPTLC evidence of rapid peptidization of L-proline and DL-proline on silica gel with 2-butanol - pyridine - ammonia (25 %) - water 39:34:10:26 and on silica gel plates impregnated with Mn(II) and Cu(II) acetate with dioxane - water 13:7+1 drop 2 % acetic acid. HPTLC enantioseparation of DL-proline was performed with 2-butanol - pyridine - glacial acetic acid - water 15:10:3:12. Detection by dipping into 0.5 % ninhydrin solution in 2-propanol, followed by heating for 2 min at 110 °C. Quantitative determination by absorbance measurement at 340 nm.

pharmaceutical research, HPTLC, qualitative identification 18a

111 013 A. SINHABABU *et al.*, see section 3e

111 035 M. VLASSA, Virginia COMAN*, M. FILIP, F. COPACIU, A. MOCANU, M. COTISEL (*Babe-Bolyai University, »Raluca Ripan« Institute for Research in Chemistry, 30 Fântânele Street, 400294 Cluj-Napoca, Romania, coman_virginia@yahoo.com): OPLC separation and identification of some amino acids from different proteins. J. Planar Chromatogr. 26, 165-171 (2013). OPLC of essential and non essential amino acids in proteins like hen egg yolk protein, bovine serum albumin (BSA), and Type I Collagen from bovine achilles tendon on silica gel with *n*-butanol - glacial acetic acid - water 40:3:12 and 50 bar external pressure, 300 µL rapid eluent flush, 100 µL/min eluent flow rate, 3200 µL development volume and 1950 s developing time. Detection by spraying first with 4-hydroxybenzaldehyde (1 % in acetone) and secondly, with ninhydrin (0.25 % in acetone), followed by heating at 110 °C for 10 min. Qualitative determination by absorbance measurement at 460 nm.

pharmaceutical research, qualitative identification, OPLC 18a

19. Proteins

111 036 M. SCHULZ*, Susanne MINARIK, B. SCHUBACH, I. FAHR (*Merck KGaA, MM-LER-CP. Frankfurterstr. 250, 64293 Darmstadt, Germany, michael.schulz@merckgroup.com): Analysis of insulin samples from different species by HPTLC-MS. CBS 108, 4-5 (2012). HPTLC of human insulin recombinant and porcine and bovine pancreas insulin on ProteoChrom silica gel with 2-butanol - pyridine - 25 % ammonia - water 39:34:10:26 to 50 mm migration distance. Detection under UV 366 nm after spraying with 0.02 % fluorescamine in acetone. Elution with the TLC-MS Interface and MS analysis in ESI positive mode, the eluent was acetonitrile - water 1:1. The hR_F value of human, porcine and bovine insulin was 50. All mass spectra could be clearly assigned to the different insulin samples.

HPTLC, postchromatographic derivatization, peptide analysis 19

111 011 M. SCHULZ *et al.*, see section 3b

20. Enzymes

111 037 Anita ANKLI, D. HANDLOSER, Valeria WIDMER, E. REICH*, E. CENIVIVA (*CAMAG Laboratory, Sonnenmattstr. 11, 4132 Muttenz, Switzerland, lab@camag.com): Rapid test for content uniformity of coenzyme Q10 in soft gel capsules by HPTLC. CBS 107, 5-7 (2011). HPTLC of coenzyme Q10 in toluene extracts of soft gel capsules on silica gel (pre-washed by development from both sides with methanol) with toluene in the horizontal developing chamber from both sides. Detection under UV 254 nm. The hR_F value of coenzyme Q10 is 20. Quantitative absorption measurement at 282 nm, evaluation via peak height. Linearity was in the range of 20-50 ng/band. Polynomial calibration ranged 20-150 ng/band. The parallel analysis of 60 samples (10 samples each from 6 batches) and 12 standards required only 86 min. The test for content uniformity was performed with 10 samples according to the European Pharmacopoeia and the USP 34 and the requirements were met.

quality control, cosmetics, HPTLC quantitative analysis, densitometry 20

22. Alkaloids

111 038 W. CHEN (Chen Weishen)*, Y. CHEN (Chen Yan), J. HE (He Jiang) (*The Xinjiang Uygur Autonomous Region Inst. of Pharm., Wulumuqi, Xinjiang 830004, China): (Study on the quality control of the fruit of *Capparis spinosa* L.) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22(1), 133-134 (2011). *Capparis spinosa* L. is a plant with an important ecological function which is mainly growed in the sand of the Gobi desert. It has a huge root system which

helps to maintain the humidity of the land. As a Uygur traditional medicinal herb, it is effective in curing diseases like rheumatism, toothache, and dysentery. However, hitherto only *Capparis spinosa* root has been used in the preparations. This wrecks seriously the resource of the herbal drug and its ecological function. Therefore, its fruits have recently been applied as replacement for the root in the preparations. Quality control of the fruits by TLC on silica gel with chloroform - methanol - formic acid - water 20:90:10:3. Detection by spraying with 5 % potassium iodobismuthate solution. The fingerprints were compared with the extracts from the root, taking one of the typical components, betaine, as the marker substance. In addition the fruits were identified based on their shape, size, surface colors, smell, and the sarcocarp; by microscopy and by determination of the moisture content.

pharmaceutical research, traditional medicine, quality control, environmental, herbal, qualitative identification 22

23. Other substances containing heterocyclic nitrogen

111 039 A. KUMAR Verma, S. BALI Prasad* (*Cell andTumor Biology Laboratory, Department of Zoology, School of Life Sciences, North-Eastern Hill University, Shillong 793022, India, sbpnehu@hotmail.com): Antitumor effect of blister beetles: An ethno-medicinal practice in Karbi community and its experimental evaluation against a murine malignant tumor model. J. Ethnopharmacol. 148, 869-879 (2013). HPTLC of cantharidin in the blister beetles *Epicauta hirticornis* and *Mylabris cichorii* on silica gel with ethyl acetate - chloroform. Identification by absorbance measurement at 254 nm. The hR_F of cantharidin was 78 and structurally defined as hexahydro-3a,7a-dimethyl-4,7-epoxyisobenzofuran-1,3-dione.

pharmaceutical research, traditional medicine, HPTLC, qualitative identification, zootherapy 23e

27. Vitamins and various growth regulators

111 040 Claudia CIMPOIU*, Anamaria HOSU, Anitta PUSCAS (*«Babes-Bolyai« Univ., Faculty of Chem. & Chem. Eng., 11 Arany Janos, 400082 Cluj-Napoca, Romania): Thin-layer chromatography with stationary phase gradient as a method for separation of water-soluble vitamins. J. Chromatogr. A 1223, 142-146 (2012). Hydrophilic vitamins play an important role in human health, and their lack or excess produces specific diseases. Development of a method for the analysis of these compounds for monitoring their content in pharmaceuticals and food. Application of TLC in the simultaneous analysis of hydrophilic vitamins with the optimized conditions towards different chemical characteristics of analytes. Due to the difficulty of separation of the structural analogues of these hydrophilic vitamins in one chromatographic run, taking the advantage of TLC to perform 2-D separations by using stationary phase gradient of silica gel and cellulose, achieving the highest resolution by combining two systems with different selectivity. The method has been used for identifying the water-soluble vitamins in alcoholic extracts of *Hippophae rhamnoides* and of *Ribes nigrum.*

quality control, pharmaceutical research, food analysis, HPTLC 27

28. Antibiotics, Mycotoxins

111 041 U. HUBICKA, B. ZUROMSKA, P. ZMUDZKI, B. MATWIEJ, J. KRZEK* (*Department of Inorganic and Analytical Chemistry, Medical College of Jagiellonian University, 9 Medyczna Str, 30-688 Krako´w, Poland, jankrzek@cm-uj.krakow.pl): Thin-layer chromatography with densitometry for the determination of difloxacin and its photodegradation products. Kinetic evaluation of the degradation process and identification of photoproducts by mass spectrometry. J. Liq. Chromatogr. Relat. Technol. 36, 2431-2445 (2013). HPTLC of difloxacin and its degra-

dation products on silica gel with methylene chloride - methanol - 2-propanol - ammonia 25 % 4:4:5:2. Quantitative determination by absorbance measurement at 294 nm. The hR_F values of difloxacin and its degradation products were 43, 25, 32, 39, respectively. Linearity was 1.2-2.4 µg/zone. LOD and LOQ were 0.01 and 0.03 µg/zone, respectively. Intermediate precision was below 2.0 %. Recovery (by standard addition) was in the range of 101.1-110.9 %.

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

111 042 J. KRZEK*, J. PIOTROWSKA, B. WITEK, U. HUBICKA, E. LYSON (*Department of Inorganic and Analytical Chemistry, Medical College of Jagiellonian University, Krakow, Poland, jankrzek@cm-uj.krakow.pl): Validation of the method of identification and quantitative determination of bacitracin in the form of bacitracin derivative with dabsyl chloride by thin-layer chromatography and densitometry. J. Planar Chromatogr. 26, 67-72 (2013). HPTLC of bacitracin derivative on silica gel with *n*-butanol - 2-butanone - 25 % ammonia - water 10:5:2:2. Quantitative determination by absorbance measurement at 460 nm. Linearity was in the range of 0.09-0.85 ng for bacitracine. LOD and LOQ were 9 ng/zone and 26 ng/zone, respectively. Intermediate precision was below 2.47 %. Comparable results with those obtained by spectrophotometric method.

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

111 043 R. RAN, C. WANG (Wang Canhua), Z. HAN (Han Zheng), A. WU, D. ZHANG (Zhang Dabing), J. SHI (Shi, Jianxin)* (*National Center for Molecular Characterization of Genetically Modified Organisms, School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China, sjianxin@gmail.com): Determination of deoxynivalenol (DON) and its derivatives: Current status of analytical methods. Food Control. 34, 138-148 (2013). TLC was reviewed as a fast screening methodology for the determination of deoxynivalenol (DON) showing LOD in rice of 100 mg/kg. 2D-TLC on silica gel with dichloromethane - ethyl acetate - propan-2-ol 18:1:1 and toluene - ethyl acetate - formic acid 6:3:1 for the first and second runs, respectively. Detection by spraying with chromotropic acid, followed by heating at 110 °C for 5 min. Quantitative determination by absorbance measurement at 366 nm. The hR_F values of DON were 13 and 20. Advantages and disadvantages of current methods as well as comparison with other techniques were also described for the effective monitoring and surveillance of DON and its derivatives.

toxicology, food analysis, review, HPTLC, comparison of methods 28b

29. Pesticides and other agrochemicals

111 044 A. MOHAMMAD*, A. AMIN, A. MOHEMAN (*Department of Applied Chemistry, Aligarh Muslim University, Aligarh, India, alimohammad08@gmail.com): Chromatographic behavior and separation of pesticides on thin silica gel layers impregnated with cationic micelles. J. Planar Chromatogr. 25, 101-107 (2012). HPTLC of mixtures of the pesticides glyphosate, acephate, chlorpyrifos, malathion/methyl parathion, and isoproturon on silica gel impregnated with 0.01 % CTAB (Ncetyl-N,N,N-trimethyl ammonium bromide) and developed with hexane - acetone 1:1. LOD of the pesticides was aproximately 20 µg/band. The method can also be applied for fast determination of pesticides in cereals, vegetables and fruit grains.

food analysis, quality control, HPTLC, quantitative analysis 29f

111 045 S. MUCHARRAF*, M. SHOAIB, D. KUMAR, M. NAJAM (*Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Kara-

chi-75270, Pakistan, musharraf1977@yahoo.com): Effective separation and simultaneous quantification of permethrin isomers in household products by validated TLC-densitometric method. J. Planar Chromatogr. 26, 14-20 (2013). HPTLC of permethrin on silica gel with hexane - diethylether - ethyl acetate 22:2:1. Quantitative determination by absorbance measurement at 227 nm. The hR_F values of cis- and trans-permethrin were 72 and 62, respectively. Linearity was in the range of 300-1800 ng/zone. LOD of cis- and trans-permethrin was 1.6 and 2.4 ng/zone, respectively. LOQ was 4.9 and 7.4 ng/zone for cis- and trans-permethrin, respectively. Intermediate precision was below 2 %.

toxicology, pharmaceutical research, HPTLC, quantitative analysis 29a

111 012 W. SCHWACK *et al.*, see section 3a

111 046 Y. SHI (Yanhong Shi), Y. YUE (Yongde Yue), H. CAO* (Haiqun Cao), F. TANG (Feng Tang), R. HUA (Rimao Hua), W. WU (Xiangwei Wu), J. TANG (Jun Tang) (*Key Laboratory of Agri-Food Safety, College of Resource and Environment, Anhui Agricultural University, 230036 Hefei, Anhui, China, caohq@vip.163.com, haiquncao@yahoo.com.cn): Photodegradation kinetics of octachlorodipropyl ether in organic solvents using an HPTLC method. J. Planar Chromatogr. 25, 117-121 (2012). HPTLC of octachlorodipropyl ether on silica gel with toluene - acetic acid - water 20:20:1. Detection by spraying with 2 N alcoholic potassium hydroxide, followed by heating at 120 °C for 30 min, overspraying with 1 % silver nitrate in 30 % nitric acid and then exposed to unfiltered UV illumination for approximately 15 min. Quantitative determination by absorbance measurement at 399 nm. The hR_F values of octachlorodipropyl ether and photodegradation products O_1 and O_2 were 93, 19 and 82, respectively.

quality control, environmental, HPTLC, quantitative analysis 29a

30. Synthetic and natural dyes

111 006 J.Z. HALL *et al.*, see section 3b

111 047 H. WANG (Wang Hongzhong)*, L. XIE (Xie Liping), Y. LI (Li Yuming), G. ZHANG (Zhang Guiyou), R. ZHANG (Zhang Rongqing) (*Coll. of Life Sci., Qinghua Univ., Beijing 100084, China): (A new approach for the analysis of chloroplast pigments by thin-layer chromatography and their preparation by column chromatography) (Chinese). Chinese Bulletin of Biol. 47 (7), 44-46 (2012). Chloroplast pigments play an important role in plant photosynthesis and become a kind of main raw material widely applied in the preparation of antitumor photodynamic drugs and functional foods. Phytoxanthin has outstanding function in vision protection, reducing the incidence of cataracts, anticancer therapy, and delaying the occurrence of arteriosclerosis. As a demonstration experiment for high school students analysis of chloroplast pigments by TLC and column chromatography. TLC of the extracts of fresh spinach leaves on silica gel with petroleum ether (30-60 °C) - benzene - acetone 7:1:5, detection in daylight, identification of beta-carotene (orange), chlorophyll a (dark green), chlorophyll b (light green) and phytoxanthin (bright yellow) by fingerprint comparison with the standards. Preparation by column chromatography on silica gel eluted with the same sovent system, collection of fractions, and the pure products were obtained by solvent evaporation. The results indicate that the TLC method is simple, sensitive, easy to operate, more intuitive, robust and more suitable for the purpose.

food analysis, qualitative identification 30b

31. Plastics and their intermediates

111 048 C. JARNE, V. CEBOLLA*, L. MEMBRADO, E. GALVEZ, J. VELA, R. GARRIGA (*Instituto de Carboquímica, ICB-CSIC, C/ Miguel Luesma, 4, 50018 Zaragoza, Spain, vcebolla@icb.csic.es): Revisiting molecular weight distribution of polystyrenes using adsorption high-performance thin-layer chromatography. J. Planar Chromatogr. 26, 5-13 (2013). HPTLC of polystyrenes with molecular weight from 1920 to 520000 Da on silica gel with cyclohexane - tetrahydrofuran 39:11. The method allowed different ranges of molecular weight to be separated as a function of mobile phase composition using slight variations of cyclohexane and tetrahydrofuran. Comparison with other techniques for molecular weight distribution analysis is also discussed.

pharmaceutical research, HPTLC, qualitative identification, comparison of methods 31

32. Pharmaceutical and biomedical applications

111 049 AMEEDUZAAFAR, A. ALI, A. ALI* (Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi-62, India, alipharm@gmail.com*): Stability-indicating HPTLC method of carteolol in bulk drug and in pharmaceutical dosage forms. J. Planar Chromatogr. 26, 86-92 (2013). HPTLC of carteolol on silica gel with chloroform - methanol 5:1. Quantitative determination by absorbance measurement at 254 nm. The hR_F of carteolol was 31. Linearity was in the range of 200-1200 ng/zone. LOD and LOQ were 34 ng/zone and 104 ng/zone, respectively. Intermediate precision was below 2 %. The method could effectively separate the drug from its degradation products.

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

111 050 E. ABDELALEEM, N. ABDELWAHAB* (*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Bani-Sueif University, Alshaheed Shehata Ahmad Hegazy St., 62514, Beni-Suef, Egypt, nadasayed2003@yahoo.com): Simultaneous determination of some antiprotozoal drugs in their binary and ternary mixtures with mebeverine hydrochloride in different dosage forms. J. Liq. Chromatogr. Relat. Technol. 36, 1528-1539 (2013). HPTLC of mebeverine hydrochloride (1), metronidazole benzoate (2), diloxanide furoate (3) and metronidazole (4) in pharmaceutical suspensions on silica gel with hexane - acetone - triethylamine 35:15:3. Quantitative determination by absorbance measurement at 254 nm. The hR_F values of compounds (1) to (4) were 10, 34, 49 and 64, respectively. Linearity was 0.4-2.4 µg/zone for (1), 0.3-2.0 µg/zone for (2) and 0.4-2.4 µg/zone for both (3) and (4). Intermediate precision was below 1.2 %. Recovery (by standard addition) was 99.9-100.1 % for compounds (1) to (4). Comparable results were obtained when compared with reported RP-HPLC methods.

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

111 051 H. ADHAMI*, U. SCHERER, H. KAEHLIG, T. HETTICH, G. SCHLOTTERBECK, E. REICH, L. KRENN (*Department of Pharmacognosy, University of Vienna, Vienna, Austria, adhami@univie.ac.at): Combination of bioautography with HPTLC-MS/NMR: a fast identification of acetylcholinesterase inhibitors from galbanum. Phytochem. Anal. 24, 395-400 (2013). HPTLC bioautography of the oleo gum-resin galbanum from *Ferula gummosa* Boiss on silica gel with chloroform - ethyl acetate - methanol 50:5:1 and detection under UV 366 nm. Bioautographic assay to detect the zones inhibiting acetylcholinesterase (AChE). The zones that showed activity in the assay on AChE inhibition were selected for HPTLC-MS. Auraptene and

farnesiferol A were identified as AChE Inhibitors.

pharmaceutical research, herbal, qualitative identification, HPTLC 32e

111 052 Huguette AGNANIET, Anita ANKLI* (*CAMAG Laboratory, Sonnenmattstr. 11, 4132 Muttenz, Switzerland, anita.ankli@camag.com): Bioautographic HPTLC assays for screening of Gabonese medicinal plants used against Diabetes mellitus. CBS 110, 5-7 (2013). HPTLC of extracts of (1) *Nauclea diderrichii*, (2) *Sarcocephalus pobeguinii*, (3) *Hua gabonii*, (4) *Morinda lucida*, and (5) *Momordica foetida* on silica gel with A) toluene - ethyl acetate 19:1; B) chloroform - methanol - water 35:15:2; C) ethyl acetate - acetic acid - formic acid - water 100:11:11:27, D) acetonitrilie - water - formic acid 15:4:1, and E) 1-butanol - acetic acid - water 7:1:2. For (1) mobile phases B and C were best suited, for (2) mobile phase B, for lipophilic compounds of the essential oil of (3) mobile phase A and for (4) and (5) mobile phase B. Bioautographic analysis using alpha- and beta-glucosidase enzym assays, acetylcholinesterase inhibition assay, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reagent for detecting radical scavenging activity.

herbal, HPTLC, qualitative identification, postchromatographic derivatization, bioactivity 32e

111 053 N. ALI*, M. HEGAZY, M. ABDELKAWY, E. ABDELALEEM (*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Alshaheed Shehata Ahmad Hegazy St., 62514 Beni-Suef, Egypt, dr.nourali@hotmail.com): Simultaneous determination of methocarbamol and its related substance (guaifenesin) in two ternary mixtures with ibuprofen and diclofenac potassium by HPTLC spectrodensitometric method. J. Planar Chromatogr. 25, 150-155 (2012). HPTLC of methocarbamol (1) and its related substance guaifenesin (2) in two ternary mixtures with ibuprofen (3) and diclofenac potassium (4) on silica gel with ethyl acetate - acetone - triethylamine 62:35:6 + 1 drop formic acid. Quantitative determination by absorbance measurement at 222 nm for the first mixture and 278 nm for the second mixture. The hR_F values for agents (1) to (4) were 78, 54, 14 and 12, respectively. Linearity was in the range of 2-12 µg/band for (1), 2-10 µg/band for (2), 4-20 µg/band for (3) and 0.2-2.2 µg/band for (4). The intermediate/inter-day/intra-day precision was below 1.5 %. Mean recovery for (1) to (4) was between 100.7 and 100.8 %.

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

111 054 I. ALI*, V. GUPTA, P. SINGH, U. NEGI (*Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi 110025, India, drimran_ali@yahoo.com): Monitoring of haloperidol and its metabolites in plasma by SPE-RP-TLC spectrometry. J. Planar Chromatogr. 25, 156-161 (2012). HPTLC of haloperidol (1) and metabolites I (2), II (3) and III (4) in plasma on RP-18 with methanol - 0.001 % diethylamine. Quantitative determination by absorbance measurement at 230 nm. The hR_F values of haloperidol and metabolites (2) to (4) were 22, 6, 16, and 87, respectively. Recoveries for (1) to (4) were 85, 88, 87, and 77 %, respectively. LOD for haloperidol was 1.0 mg/mL and for all the three metabolites (2) to (4) it was 0.8 mg/mL.

clinical routine analysis, HPTLC, quantitative analysis 32c

111 055 S. ALQASOUMI, P. ALAM*, M. ABDEL (*Department of Pharmacognosy, College of Pharmacy, Salman Bin AbdulAziz University, P.O. Box 173, Al-Kharj 11942, Kingdom of Saudi Arabia, prawez_pharma@yahoo.com): Stability-indicating densitometric HPTLC method for qualitative and quantitative analysis of arbutin in commercial whitening creams. J. Planar Chromatogr. 25,

168-173 (2012). HPTLC of arbutin in commercial whitening creams with methanol - chloroform - acetic acid 7:12:1. Quantitative determination by absorbance measurement at 254 nm. The hR_F value of arbutin was 40. LOD and LOQ were found to be 42 and 112 ng/zone, respectively.

quality control, cosmetics, HPTLC, quantitative analysis 32a

111 056 P. BHATTACHARYA, A. SAHA* (*Department of Chemical Technology, University of Calcutta 92, APC Road, Kolkata 700009, India, asct@caluniv.ac.in): Evaluation of reversible contraceptive potential of *Cordia dichotoma* leaves extract. Brazilian Journal of Pharmacognosy. 23, 342-350 (2013). HPTLC of apigenin (1) and luteolin (2) in the leaves of *Cordia dichotoma* on silica gel with toluene - ethyl acetate - methanol 10:9:1. Quantitative determination by absorbance measurement at 254 nm. The hR_F of compounds (1) and (2) were 80 and 65, respectively. Linearity was 50-200 ng/zone for both (1) and (2). LOD and LOQ for both compounds were found to be 10 and 40 ng/band, respectively. Intra-day precision was in the range of 1.0-1.2 % and 0.9-1.0 %, and inter-day precision ranged 1.1-1.3 % and 1.0-1.2 %, for 50 and 200 ng/band of (1) and (2), respectively (n=3). Average recoveries at two different levels of AP and LT were found to be 99.2 and 99.9 %, respectively.

herbal, quality control, quantitative analysis, HPTLC 32e

111 057 Birgit BOECKEL (Bayer Weimar GmbH & Co. KG, Product Supply Pharma, QC Raw Materials, Doebereiner Str. 20, 99427 Weimar, Germany, birgit.boeckel@bayer.com): Cleaning validation using HPTLC. CBS 107, 2-4 (2011). HPTLC of chloroform extracts from cleaning swabs on silica gel with toluene - ethyl acetate 3:2 after 10 min chamber saturation. Densitometric evaluation for identification of substances by spectra recording at 200-350 nm, quantification with 3-level calibration. Detection by immersion in methanol - sufuric acid 9:1 and heating at 105 °C for 5 min. Cleaning validation by HPTLC (i.e. determination of residues of hormonal ingredients) can be achieved with the required accuracy. The method is simple and quick and the quantitative results are precise.

pharmaceutical research, HPTLC, quantitative analysis, postchromatographic derivatization, densitometry 32a

111 058 L. BOUDESOCQUE, J. DORAT, J. POTHIER, A. GUEIFFIER, Cecile ENGUEHARD* (*Université de Tours François Rabelais, 31 Avenue Monge, 37200 Tours, France, cecile.enguehard-gueiffier@univ-tours.fr): High-performance thin-layer chromatography-densitometry: A step further for quality control of cranberry extracts. Food Chemistry. 139, 866-871 (2013). HPTLC of catechin (1), proanthocyanidin (PAC) A2 (2) and PAC-B1 (3) in American cranberry (*Vaccinium macrocarpon*) extracts on silica gel with dichloromethane - ethyl acetate - formic acid 6:10:1. Detection by dipping into a hydrochloric solution of 1 % (w/v) vanillin, followed by heating at 110 °C for 2 min. Quantitative determination by absorbance measurement at 254 and 366 nm. The hR_F values of (1), (2) and (3) were 63, 48 and 25, respectively. Linearity was in the range of 0.6-5 µg/band. Intermediate precision was below 2.1 %. Average recoveries (by standard addition) were 98.4 % for (1), 99.6 % for (2) and 98.5 % for (3).

herbal, food analysis, quality control, quantitative analysis, HPTLC 32e

111 059 L. CAI (Cai Lixing)*, Z. GE (Ge Zaochuan) (*School of Chem. & Chem. Eng., Shenzhen Univ., Shenzhen, Guangdong 518060, China): (Study on the fingerprints of *Fallopia multiflora* (Thunb.) Harald growed in different regions of China by micellar thin-layer chromatography and infrared spectrography) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (4),

860-862 (2011). *Fallopia multiflora* (Thunb.) Harald, a medicinal herbal drug widely applied in traditional Chinese medicines, is available from different provinces of China. Presentation of fingerprint methods of *Fallopia multiflora* from different regions by micellar thin-layer chromatography (MTLC) and IR techniques. MTLC of methanol extracts of *Fallopia multiflora* and emodin standard solution on polyamide layer with 0.4 % cetylpyridinium chloride in acetone - methanol - 1.0 mol/L NaOH 12:4:5 after chamber saturation for 15 min. Detection of the purple zones in daylight. The ratio of the hR_F values of the component zones and those of emodin was calculated. In addition, FTIR of the methanolic extracts and emodin standard solution mixed in KBr tablets. The results of the real life samples available from five different regions are given and proved that the method is suitable for the quality control of the drugs.

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

111 060 X.CAI (Cai Xiaocui), J. HE (HE Jinhua)*, W. LI (Li Weiqiang), Y. MAO (Mao Yan) (*Xinjiang Inst. of Med., Xinjiang, Urumqi 830004, China): (Study of the method for the Quality Control of Qinyu Shaoshang burn liquid) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 30 (11), 1499-1501 (2012). Qinyu Shaoshang burn liquid is a herbal TCM preparation for treatment of burns. For quality control TLC on silica gel plates 1) for *Borneolum Syntheticum*, with chloroform - methanol - ammonia 100:20:1, detection under UV 366 nm; 2) for Amur Cork-tree Bark, with petroleum ether (60-90 °C) ethyl acetate - chloroform 3:1:1, detection by spraying with 5 % phosphomolybdic acid in ethanol and viewing in daylight. Quantification of baicalin by HPLC.

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

111 061 X. CHE (Che Xiaoping)*, X. ZHAO (Zhao Xiaowei), Y. LI (Li Yang) (*Dep. of Pharm. Prep., Affil. Hosp. of Trad. Chinese Med., Capital Univ. of Med., Beijing 100010, China): (Study of the method for the quality control of Ekou powder) (Chinese). Beijing J. of Trad. Chinese Med. 31 (7), 542-545 (2012). Ekou powder is a herbal TCM preparation for treating mycotic and follicular stomatitis and mouth erosion. For quality control TLC on silica gel 1) for *Coptis chinensis*, with cyclohexane - ethyl acetate - isopropanol - methanol - water - triethylamine 6:7:2:3:1:2, detection under UV 366 nm; 2) for borneol, with *n*-hexane - ethyl acetate 17:3, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:200 and heating at 105 °C, viewing in daylight; 3) for Indigo naturalis, with toluene - chloroform - acetone 5:4:1, detection in daylight.

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

111 062 D. CHEN (Chen Duo), S. GUO (Guo Siyuan), J. QI (Qi Ji), M. GUO (Guo Ming), A. WANG (Wang Airong)* (*Inst. for Medicinal Plants, Beijing Xiehe Med. Coll., Chinese Acad. of Med. Sci., Beijing 100094, China): (Study of the method for the quality control of Niuhuang Jiedu Ruanjiaonang capsules) (Chinese). Modern J. of Integrated Trad. Chinese & Western Med. 22 (6), 659-661 (2013). Niuhuang Jiedu Ruanjiaonang capsules are a herbal TCM preparation for treating sore throat, gingival swelling and pain, aphthae, swollen red eyes, etc. For quality control, TLC on silica gel 1) for Artificial *Calculus Bovis* and the standards cholic acid and hyodesoxycholic acid, with *n*-hexane - ethyl acetate - acetic acid - methanol 20:25:2:3, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm;

2) for *Radix et Rhizoma Rhe*i, with the upper phase of petroleum ether (30-60 °C) - ethyl formate - formic acid 15:5:1, detection under UV 366 nm; 3) for *Radix Scutellariae* and the standard baicalin, with ethyl acetate - butanone - formic acid - water 5:3:1:1, detection by spraying with 1 % ferric chloride in ethanol and viewing in daylight; 4) for Borneol, with cyclohexane - ethyl acetate 4:1, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C, viewing in daylight. Quantification of borneol by GC.

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

111 063 S. CHEN (Chen Suying)*, J. HUANG (Huang Jian), X. ZHUANG (Zhuang Xuechao), Y. CHEN (Chen Yunzi) (*Prepar. Centre, Fushan Municip. Hosp. of Trad. Chinese Med., Guangdong, Fushan 528000, China): (Study of the method for the identification of Shangke Tiegao wound plaster by thin-layer chromatography) (Chinese). Yunan J. of Chinese Trad. Med. & Pharm. 34 (2), 44-46 (2013). As a herbal TCM preparation for treating sprain, contusion, blood stasis and innominate toxic swelling, Shangke Tiegao wound plaster is a new generation of trauma plaster preparations, based on the traditional trauma powder and a hot melt pressure sensitive adhesive which improves the release of the drug and reduces skin irritation. The new formulation not only retains the original dosage form and characteristics of the efficacy of transdermal drug delivery, but is also convenient to use. For quality control, TLC on silica gel 1) for *Coptis chinensis* Franch, with toluene - ethyl acetate - isopropanol - methanol - ammonia 12:6:3:3:1, detection under UV 366 nm; 2) for *Radix et Rhizoma Rhei*, with *n*-hexane - ethyl acetate - formic acid 60:20:1, detection at 254 nm; 3) for *Fructus Gardeniae*, with ethyl acetate - acetone - formic acid - water 5:5:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 110 °C, viewing in daylight; 4) for *Radix Sanguisorbae*, with toluene (saturated with water) - ethyl acetate - formic acid 6:3:1, detection by spraying with 1 % ferric chloride in ethanol and viewing in daylight.

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

111 064 X. CHEN (Chen Xingyu), L. HAN (Han Liping)*, J. LIANG (Liang Junle) (*General Hosp. of Guangzhou Military Region, The Chinese PLA, Guangdong, Guangzhou 510010, China): (Qualitative and quantitative determination of timosaponin B-II in *rhizoma anemarrhenae* total steroidal saponins) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 23 (2), 401-402 (2012). The dried root of *Anemarrhena asphodeloides* Bge. is a traditional Chinese medicinal crude drug specially effective for cleaning heat, purging the pathogenic fire, reinforcing body fluid and nourishing the blood, promoting the secretion of saliva or body fluids. Timosaponin B-II is a water soluble saponin extracted from *Anemarrhenae*. TLC of *rhizoma anemarrhenae* total steroidal saponins on silica gel with *n*-butanol - glacial acetic acid - water 4:1:5, detection by spraying with 10 % vanillin in ethanol and heating at 105 °C until the zones are visible, evaluation in daylight.

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

111 065 X. CHEN (Chen Xueting)*, S. LI (Li Sumei), Y. LI (Li Yangxue) (*Guangzhou Univ. of Trad. Chinese Med., Guangdong, Guangzhou, 510405, China): (Study on the method for the quality control of Qufengjiangu tablets) (Chinese). Chinese J. of Today‘s Pharm. 22 (11), 690-692 (2012). Qufengjiangu tablets are a herbal TCM for the treatment of waist and knee pain and rheumatic

arthralgia. For quality control, TLC on silica gel 1) for *Radix Angelicae Pubescentis*, with cyclohexane - ethyl acetate - formic acid - glacial acetic acid 70:20:2:3, detection under UV 366 nm; 2) for *Radix Notoginseng* and *Radix Dipsaci*, with *n*-butanol - ethyl acetate - 10 % diluted ammonia 4:1:5, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 3) *Herba Taxilli*, with toluene saturated with water - ethyl formate - formic acid 5:4:1, detection by spraying with 5 % aluminium chloride in ethanol and heating at 105 °C, viewing under UV 366 nm. Quantification of osthole by HPLC.

pharmaceutical research, traditional medicine, quality control, herbal, qualitative identificationn 32e

111 066 Y. CHEN (Chen Yinjuan) (Guangdong Provinc. Trad. Chinese Med. Hosp., Guangdong, Guangzhou 510120, China): (Study of the method for the identification of Shugan capsules) (Chinese). Chinese J. of Northern Pharmacy 9 (4), 8-9 (2012). Shugan capsules are a herbal TCM herbal preparation for nourishing the liver and tonifying spleen, used for women's climacteric symptoms. For quality control, TLC on silica gel 1) for *Radix Paeoniae Alba*, with chloroform - ethyl acetate - methanol - formic acid 200:25:50:1, detection by spraying with 5 % vanillin in ethanol - sulfuric acid 4:1 and heating mildly, viewing under daylight; 2) for *Rhizoma Cyperi*, with *n*-hexane - ethyl acetate 17:3, detection under UV 254 nm.

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

111 067 U. CHHALOTIYA*, K. BHATT, D. SHAH, S. NAGDA, J. PATEL (*Indukaka Ipcowala College of Pharmacy, Beyond GIDC, P.B. No. 53, Vitthal Udyognagar- 388 121, Gujarat, India, usmangani84@gmail.com): Development of HPTLC method for the estimation of antidepressant drugs melitracen and flupentixol in their combined dosage form. J. Liq. Chromatogr. Relat. Technol. 36, 1231-1242 (2013). HPTLC of melitracen (1) and flupentixol (2) in combined dosage form on silica gel with methanol - toluene 4:1+1 drop ammonia. Quantitative determination by absorbance measurement at 270 nm. The hR_F values of (1) and (2) were 79 and 88, respectively. Linearity was in the range of 1000-8000 ng/zone for (1) and 50-400 ng/zone for (2). LOD and LOQ were 225 and 683 ng/zone for (1) and 4.7 and 14.1 ng/zone for (2). Intermediate precision was below 3.6 %. Recovery (by standard addition) for (1) and (2) was in the range of 98.6-102.1 % and 97.8-101.8 %, respectively.

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

111 068 A. DOSHI*, B. PATEL, C. PATEL (*Department of Quality Assurance, Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India, doshi007.1989@gmail.com): Development and validation of HPTLC method for simultaneous estimation of propranolol hydrochloride and flunarizine dihydrochloride in combined tablet dosage form. J. Planar Chromatogr. 26, 62-66 (2013). HPTLC of propranolol hydrochloride (1) and flunarizine dihydrochloride (2) on silica gel with methanol - chloroform - toluene 6:14:14+1 drop glacial acetic acid. Quantitative determination by absorbance measurement at 260 nm. The hR_F values of (1) and (2) were 35 and 64, respectively. Linearity was in the range of 3000-15000 ng/zone and 750-3700 ng/zone for (1) and (2), respectively. Intermediate precision was below 2 %.

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

111 069 N. DUBEY*, D. KUMAR, S. JADHAWANI (*College of Pharmacy, IPS Academy, Indore 452015 Madhya Pradesh, India, nitindubeympharm@yahoo.com): Stability-indicating HPTLC

method for simultaneous estimation of famotidine, paracetamol, and ibuprofen in combined tablet dosage forms. J. Planar Chromatogr. 25, 162-167 (2012). HPTLC of famotidine (1), paracetamol (2), and ibuprofen (3) in combined dosage forms on silica gel with chloroform - methanol - ethyl acetate - acetic acid 215:161:591:32. Quantitative determination by absorbance measurement at 256 nm. The hR_F values of compounds (1) to (3) were 21, 80 and 89, respectively. Linearity was in the range of 160-960 ng/zone for (1), 400-2400 ng/zone for (2) and 600-3600 ng/zone for (3).

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

111 159 W. FANG (Fang Wenqing) (Fujian Vocational Coll. of Bioengineering, Fujian, Fuzhou 350002, China): (Study of the method for the quality control of Yangyin Qingfei compound oral liquid) (Chinese). J. Strait Pharm. 24 (11), 16-18 (2012). Yangyin Qingfei compound oral liquid is a herbal TCM preparation for treating cough, sore throat, tonsillitis, etc. For quality control, TLC on silica gel 1) for *Rehmannia*, with chloroform - methanol - water 14:6:1, detection by immersion into vanillin - sulfuric acid - acetic acid 1:10:1000 and heating at 105 °C, viewing in daylight; 2) for *Radix Ophiopogonis*, with toluene - methanol - glacial acetic acid 800:50:1, detection under UV 254 nm. Quantification of catalpol by HPLC.

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

111 070 H. GAO (Gao Hui)*, X. MA (Ma Xiaojun), Y. LIN (Lin Yongqiang), H. YOU (You Huilian), L XU (Xu Lihua) (*Shandong Inst. for Drug Contr., Shandong, Jinan 250101, China; 2. Inst. of Medicinal Plant Develop., Beijing Union Med. Univ., Chinese Acad. of Med. Sci., Beijing 100094, China): (Study of the method for the qualitative and quantitative analysis of Jiannao Bushen PiIls) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 23 (1), 176-178 (2012). Jiannao Bushen Pills are a traditional Chinese compound preparation effective for brain-strengthening, tonifying kidney, replenishing Qi to invigorate the spleen, relieve uneasiness of mind and body tranquilization. TLC on silica gel 1) for *Angelica sinensis*, with petroleum ether (60-90 ºC) - ethyl acetate 4:1, detection at UV 366 nm; 2) for *Fructus Forsythiae*, with petroleum ether (30-60 ºC) - methanol 20:1, detection by exposure to ammonia vapors for 15 min, then spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under daylight; 3) for *Cinnamomum cassia* and cassia twig, with petroleum ether (60-90 ºC) - ethyl acetate17:3, detection by spraying with 0.4 % 2,4-dinitrophenylhydrazine in 12N hydrochloric acid and viewing under daylight; 4) for bighead *atractylodes rhizome*, with petroleum ether (60-90 ºC) - ethyl acetate 50:1, detection by spraying with 0.5 % *p*-dimethylaminobenzaldehyde in ethanol - sulfuric acid 5:1 and viewing under daylight; 5) for *Radix Ginseng rubra*, with chloroform - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under daylight.

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

111 071 S. GIZAWY, L. BEBAWY, O. ABDELMAGEED, M. OMAR, S. DERYEA, A. ABDEL-MEGIED* (*October University for Modern Sciences and Arts (MSA), Pharmaceutical Analytical Chemistry, 26 July Mehwer Road Intersection with Wahat Road, 6th October City, Egypt, Gizah, 6th October, 61111 Egypt, dr_ahmed80@hotmail.com): High-performance liquid chromatography, TLC-densitometry, and first-derivative spectrophotometry for simultaneous determination of amlodipine and perindopril in bulk powder and its tablets. J. Liq. Chromatogr. Relat.

Technol. 36, 1323-1329 (2013). HPTLC of amlodipine (1) and perindopril (2) in bulk powder and tablets on silica gel with *n*-butanol - water - glacial acetic acid 4:5:1. Quantitative determination by absorbance measurement at 365 nm and 215 nm, for (1) and (2), respectively. The hR_F values for (1) and (2) were 72 and 48, respectively. Linearity was 1-6 µg/mL for (1) and 2-10 µg/mL for (2). LOD and LOQ were 0.28 and 0.86 µg/mL for (1) and 0.24 and 0.75 µg/mL for (2), respectively. The interday and intra-day precisions were below 1.3 % (n=3). Recovery (by standard addition) was 98.0-99.6 % for both (1) and (2). Comparable results were obtained when compared with validated HPLC and first-derivative spectrophotometry methods, resulting in short scan time, large sample capacity, and use of minimal volume of solvent.

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

111 072 J. GONG (Gong Ju) (Gaoan Municip Huangshagang health center, Jiangxi, Gaoan 330803, China): (Study of the method for the quality control of Genianan capsules by thin-layer chromatography) (Chinese). Chinese J. of Jiangxi Ind. (3), 68-70 (2012). Genianan capsules are a herbal TCM for treating some diseases during women's menopause such as tinnitus, palpitation, insomnia, blood pressure instability, etc. For quality control, TLC on silica gel 1) R*adix Polygoni Multiflori* and *Caulis Polygoni Multiflori* and standard emodin, with toluene - ethyl acetate - formic acid 15:2:1, detection under UV 366 nm; 2) for *Fructus Schisandrae Chinensis* and the standards deoxyschizandrin and schizandrin B, with petroleum ether (30-60 °C) - ethyl acetate - formic acid 15:5:1, detection under UV 254 nm; 3) for *Radix Ophiopogonis*, with chloroform - acetone 22:5, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 4) *Rhizoma Curculigins* and the standard curculigoside, with ethyl acetate - methanol - formic acid 100:10:1, detection by spraying with 0.1 mol potassium ferricyanide - 0.1 mol ferric chloride 1:1 and viewing in daylight.

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

111 073 Q. GUAN (Guan Qijia)*, R. ZHONG (Zhong Ruijian), G. ZHOU (Zhou Guoping), E. XIE (Xie Erlei), Y. QIN (Qin Yumei), L. XIAO (Xiao Lili) (Jiangxi Provinc. Inst. of Drug & Food Contr., Jiangxi, Nanchang 330046, China): (Study on the method for the quality control of Shenbao Heji compound oral liquid) (Chinese). J. of Jiangxi Univ. of TCM 24 (3), 54-57 (2012). Shenbao Heji compound oral liquid is a herbal TCM for the treatment of impotence, nocturnal emission, low back pain, lack of energy, aversion to cold, and women with menorrhagia. For quality control, TLC on silica gel 1) for *Epimedium davidii* Franch and the standard icariine, with ethyl acetate - methanol - water 100:17:13, detection by spraying with 1 % aluminium chloride in ethanol and heating at 105 °C, viewing at UV 254 nm; 2) for *Radix Polygoni Multiflori* and the standard emodin, with petroleum ether (60-90 °C) - ethyl formate - formic acid 15:10:1, detection by exposing to ammonia vapors and viewing in daylight; 3) for Leguminosae and the standard astragaloside A, with the lower phase of chloroform - methanol - water 13:6:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm; 4) for *Fructus Psoraleae* and the standard psoralen, with *n*-hexane - ethyl acetate 3:1, detection by spraying with 10 % KOH in methanol and viewing under UV 366 nm; 5) for *Radix Angelicae Sinensis* and *Rhizoma Ligustici* Chuanxiong, with *n*-hexane - ethyl acetate 9:1, detection under UV 366 nm. Quantification of icariine by HPLC.

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

111 074 L. GUO (Guo Lirong), H. LIU (Liu Hongwei), L. ZHOU (Zhou Liling)* (*Guangzhou Univ. of Traditional Chinese Med., Guangzhou 510006, China): (Study of the quality standard for Dieda Zhitong microemulsion spray) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (5), 1045-1048 (2011). Dieda Zhitong microemulsion spray is a traditional Chinese medicine effective in invigorate the circulation of blood, relieving blood stagnate, subsidence of a swelling, stop bleeding and relieving pain caused by injuries from falls, fractures, contusions, strains and traumatic injuries. For quality control, TLC of the extracts of the medicine 1) for *Cnidium monnieri* L. Cuss and Doubleteeth Pubescent Angelica root, on silica gel with petroleum ether (30-60 ºC) - ethyl acetate 9:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones were visible, viewing under UV 366 nm; 2) for *Ligusticum wallichii* and *Rheum officinale* on silica gel with petroleum ether (30-60 ºC) - ethyl acetate 9:1, detection under UV 254 nm; 3) for *Oleum Ocimi gratissimi* on silica gel with cyclohexane - ethyl acetate 4:1, detection by spraying with 10 % ferric chloride in ethanol and heating at 105 °C until the zones were visible; 4) for *Oleum Anisi stellati* on silica gel with petroleum ether (60-90 ºC) - ethyl acetate 19:1, detection by spraying with 5 % vanillin in ethanol and heating at 105 °C until the zones were visible; 5) for *Oleum Eucalypti* on silica gel with cyclohexane - diethyl ether - ethyl acetate 16:3:1, detection by spraying with 5 % vanillin in ethanol and heating at 105 °C until the zones were visible.

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

111 075 DEZHI*, H. WANG (Wang Hugejiletu) (*Coll. of Mongolian Med. & Pharm., Inner Mongolia Univ. for Nationalities, Inner Mongolia, Tongliao 028000, China): (Study on the method for the identification of Yuzan Qingyan Shiwuwei pills by thin-layer chromatography) (Chinese). J. of Inner Mongolia Univ. for Nationalities (Natural Sci. Edit.) 27 (1), 80-83 (2012). Yuzan Qingyan Shiwuwei pills are a traditional Mongolian herbal medicine for treating colds, sore throat, asthma, cough, etc. For quality control, TLC on silica gel 1) for *Fructus Gardeniae* and the standard gardenoside, with ethyl acetate - acetone - formic acid - water 5:5:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 2) for *Radix Aucklandiae* and the standards costunolide and dehydrocostus lactone, with cyclohexane - formic acetate - formic acid 15:5:1, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:200 and heating mildly, viewing in daylight; 3) for *Radix Glycyrrhizae* and the standard glycyrrhetinic acid, with benzene - petroleum ether (30-60 °C) - ethyl acetate - glacial acetic acid 50:25:20:3, detection under UV 366 nm.

pharmaceutical research, traditional medicine, quality control, herbal, HPTLC, densitometry 32e

111 076 X. HAN (Han Xu), CH. JIN (Jin Chaodong)* (*Anal. & Testing Center, Tianjin Inst. of Pharm. Res., Tianjin 300193, China): (Study of the method for the quality control of Wuzi Yanzong pills by thin-layer chromatography) (Chinese). J. of Anhui Coll. of Trad. Chinese Med. 31 (4), 77-79 (2012). Wuzi Yanzong pills are a herbal TCM preparation for treating male impotence, sterility, and premature ejaculation. For quality control, TLC on silica gel 1) for *Fructus Lycii* and China Dodder, with cyclohexane - acetone 3:2, detection under UV 366 nm; 2) for *Fructus Schisandrae Chinensis*, with chloroform - butanone 30:1, detection under UV 254 nm. Quantification of schisantherin A by absorbance measurement at 230 nm. Linearity was between 0.25-1.10 µg/zone (r= 0.999, n=5). Plate-to-plate %*RSD* was 2.1 % (n=5) and 0.98 % within plate (n=5). Recovery (by standard addition) was 100.9 % (%*RSD* = 0.51, n=6).

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

111 077 M. HAWRYL, M. NIEMIEC, Monika WAKSMUNDSKA-HAJNOS* (*Department of Inorganic Chemistry, Medical University of Lublin, 4a Chodzki Street, 20-093 Lublin, Poland, monika.hajnos@am.lublin.pl): Micro-two-dimensional TLC in search of selected Mentha sp. extracts for their composition and antioxidative activity. J. Planar Chromatogr. 26, 141-146 (2013). Micro 2D TLC fingerprint of *Mentha sp.* extracts on 5 x 5 cm cyano and diol phase with non-aqueous eluents (polar modifier dissolved in *n*-heptane) as the first direction eluents and aqueous eluents (organic modifier - methanol dissolved in water) as the second direction eluents. Detection by absorbance measurements at 254 and 366 nm. The method allowed the identification of 17 substances to differentiate varieties of *Mentha piperita.*

herbal, qualitative identification, HPTLC, Micro-thin-layer chromatography 32e

111 078 X. HE (He Xueqing)*, A. WANG (Wang Aiwu), N. WANG (Wang Nannan) (*Provincial Hosp. Affiliated to Shandong Univ., Shandong, Jinan 250021, China): (Study of the method for the limit test of aconitine in Hulisan capsules, Yaoxitong capsules and Panlongqi tablets by thin-layer chromatography) (Chinese). Chinese J. of Inform. on TCM 19 (9), 59-60 (2012). Some Aconitum medicinal herbs, such as Aconite root, *Radix Aconiti* Kusnezoffii, *Radix Aconiti Lateralis Preparata* etc. are used as the component drugs in TCM anti rheumatic preparations like Hulisan capsules, Yaoxitong capsules and Panlongqi tablets. The main active component in Aconitum is aconitine which has significant anti-inflammatory and analgesic activity, but is a potent toxic ingredient as well. For quality control of the formulations and ensuring their medication safety, a method for the limit test of aconitine in Hulisan capsules, Yaoxitong capsules and Panlongqi tablets has been presented. TLC on silica gel with cyclohexane - ethyl acetate - diethylamine 10:7:1, detection first by spraying with 5 % potassium iodobismuthate in water - hydrochloric acid 200:1 and then by exposing to iodine vapors, viewing in daylight. Semiquantification of aconitine by comparison of zones with the standard applied in concentrations meeting the safety limit.

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

111 079 M. HU (Hu Mingxia) (Xiantao Municip. Hosp. of Trad. Chinese Med., Hubei, Xiantao 433000, China): (Identification of the flavonoids, quercetin and kaempferol, in Polygonum orientale Linn. by thin-layer chromatography) (Chinese). J. of Trad. Chinese Med. & Pharm. Consult. 4 (5), 481-482 (2012). As a widely used TCM herbal drug, *Polygonum orientale* Linn. is prescribed for treatment of rheumatic arthritis, coronary heart disease, stomach pain, etc. Description of a method for identifying the counterfeit drug and for routine quality control of the crude drug by identification of the flavonoids quercetin and kaempferol in the drug samples obtained by extraction of the dry stem, leaves and fruits of the herb after acid hydrolysis. TLC on silica gel with toluene - ethyl acetate - formic acid 20:14:1, detection by spraying with 1 % aluminium chloride in ethanol and heating mildly, viewing under UV 366 nm.

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

111 080 W. HU (Hu Wangsheng)*, G. PENG (Peng Guihua), Y. HUANG (Huang Yihua), W. TAN (Tan Wansheng) (*Huangshi Municip. Inst. of Food & drug superv. & insp., Hubei, Huangshi 435000, China): (Study on the method for the identification of Dannangyan compound oral liquid by thin-layer chromatography) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 23 (7), 1822-1823 (2012). Dannangyan compound oral liquid is a herbal TCM preparation for tre-

ating cholecystitis and cholelithiasis. For identification, TLC on silica gel 1) for *Radix et Rhizoma Rhei*, with the upper phase of petroleum ether (30-60 ºC) - ethyl acetate - formic acid 15:5:1, detection at UV 366 nm and by exposure to iodine vapors and viewing in daylight; 2) for *Cortex Magnoliae officinalis*, with chloroform - benzene - ethyl acetate 10:4:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:200 followed by heating at 105 °C for 5 min and viewing in daylight.

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

111 081 B. HUANG (Huang Bo), P. WU (Wu Peie), J. ZHOU (Zhou Juan), Y. QING (Qing Yan), D. LU (Lu Daohui), W. ZHAO (Zhao Wenji), M. LI (Li Min)* (*Pharm. School, Chengdu Univ. of Trad. Chinese Med. Sichuan, Chengdu 611137, China): (Study of the method for the identification of *Rhizoma Curcumae Longae* and its three near-source species by thin-layer chromatography and high performance liquid chromatography fingerprints) (Chinese). J. of Modern Trad. Chinese Med. 14 (7), 7-10 (2012). Curcumins are phenolic compounds from the root and stem of *Zingiberaceae* plants with outstanding anti-inflammatory and antioxidative activity. Identification of *Rhizoma Curcumae Longae* and its three near-source species *Curcuma wenyujin* Y.H.Chen et C.Ling, *Curcuma phaeocaulis* Val., and *Curcuma kwangsiensis* S. G.Lee et C.F. Liang by TLC on silica gel with chlorofom - methanol - formic acid 960:40:7, detection under UV 366 nm. The standards were curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

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

111 082 A. HUSSAIN, M. RAHMAN*, M. HUSSAIN, M. MIRZA, Z. IQBAL, R. HARWANSH, L. RATNAKAR (*Faculty of Pharmacy, Integral University, Kursi Road, Lucknow-226026, India, rahmanpharma@gmail.com): HPTLC method for analysis of sertraline in pure bulk and lipidic nano delivery system: a stress degradation studies. J. Liq. Chromatogr. Relat. Technol. 36, 700-716 (2013). HPTLC of sertraline in bulk and formulation on silica gel with toulene - ethyl acetate 1:5+1 drop ammonia. Quantitative determination by absorbance measurement at 273 nm. The hR_F value of sertraline was 70. Linearity was in the range of 25-2000 ng/zone. LOD and LOQ were 15.3 and 46.8 ng/zone. Intermediate precision was below 0.83 %. Recovery range was in the range of 99.6-100.3 %. The hR_F values of acid and base-induced degradation products were 40, 52, 90 and 25.

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

111 083 W. JESIONEK, E. GRZELAK, B. MAJER, Irena CHOMA* (*Department of Chromatographic Methods, University of Maria Curie-Skodowska, M. Skodowska Sq. 3, 20-031 Lublin, Poland, irena.choma@umcs.lublin.pl): Thin-layer chromatography - direct bioautography for the screening of antimicrobial properties of plant extracts. J. Planar Chromatogr. 26, 109-113 (2013). Bioautographic fingerprinting of *Salvia officinalis*, *Thymus vulgaris*, and *Mentha piperita* on silica gel with toluene - ethyl acetate 93:7, followed by dipping into bacterial suspensions of *Escherichia coli* and *Bacillus subtilis* for 8 s. Detection by spraying with 0.2 % MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) aqueous solution, followed by re-incubation at 37 °C for 0.5 h. The hR_F values for thymol, menthol, and alpha-thujone were 51, 33 and 29 respectively.

herbal, pharmaceutical research, qualitative identification, bioautography 32e

111 084 J. JI (Ji Jia) (Pharm. Prepar. Dep., Zhengzhou Municip. People's Hosp., Henan, Zhengzhou 450000, China): (Determination of partition coefficient of alkaloids of *Coptis chinensis* in the solvent system of high speed countercurrent chromatography by thin-layer chromatography and fluorescence spectrophotometry) (Chinese). Chinese J. of Med. Guide 2 (9), 109-110 (2012). *Coptis chinensis* is a herbal TCM drug for relieving internal heat or fever. High speed countercurrent chromatography (HSCCC) is frequently applied to analyse the alkaloids of *Coptis chinensis*. To optimize the solvent system of HSCCC the method for determination of the partition coefficient of *Coptis chinensis* alkaloids was done by TLC and fluorescence spectrophotometry. The major alkaloids are coptisine, berberine, palmatine and epiberberine. The crude drug was extracted with ethanol and contained 9.2 % coptisine, 18.5 % berberine, 4.0 % palmatine and 3.5 % epiberberine. TLC on silica gel with cyclohexane - ethyl acetate - isopropanol - methanol - water - triethylamine 6:7:2:3:1 with chamber saturation for 20 min with ammonia vapors, detection at UV 366 nm. The zones were quantitatively scraped off the layer and eluted with ethanol for fluorescence spectrophotometry at UV 366 nm. Quantification by external standard calibration over the linearity range of 0.5-2.5 g/L for coptisine, berberine and epiberberine with standard addition recovery of 98.3 % (%*RSD* = 1.9 %, *n*=6), 95.3 % (%*RSD* = 3.4 %, *n*=6), 103.9 % (%*RSD* = 2.6 %, n=6), respectively; and 1.0-3.0 g/L for palmatine with standard addition recovery of 97.6 % (%*RSD* = 3.7 %, *n*=6). Calculation of the partition coefficient, *i.e.* the ratio of observed values of the analytes from the two phases, respectively, with the results for coptisine 2.20, berberine 0.29, palmatine 0.21 and epiberberine 0.61.

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

111 085 R. JIANG (Jiang Rongbin), X. ZHOU (Zhou Xin)* (*Res. Center for Quality Contr. of Natural Med., Guizhou Normal Univ., Guizhou, Guiyang 550001, China): (Study of the method for the quality control of *Kalimeris indica* (Linn.) Sch. by thin-layer chromatography) (Chinese). J. of Guizhou Normal Univ. (Natural Sci.) 30 (3), 1-3 (2012). *Kalimeris indica* (Linn.) Sch. is a perennial herb used in TCM preparations for treating cold fever, hepatitis, parotitis, pharyngitis, indigestion, etc. For quality control, TLC on silica gel with chloroform - methanol 9:1, detection by spraying with 5 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight. The standard was alpha-spinasteryl-3-O-beta-D-glucoside.

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

111 086 Y. JIANG (Jiang Yan)*, Y. LIU (Liu Yanli) (*Handan Health Hosp. for Women & Children, Hebei, Handan 056001, China): (Study on the method for the quality control of *Nauclea officinalis* decoction pieces and three formulations by thin-layer chromatography) (Chinese). J. of China Pharm. 26 (4), 368-369 (2012). *Nauclea officinalis* is a tree growing in tropical regions. In TCM its branch, trunk and bark are used for treatment of acute tonsillitis, acute pharyngitis, conjunctivitis, upper respiratory tract infection, etc. For quality control of its decoction pieces and the three formulations, Danmu injection, Danmu Jingao tablets, and Danmu Jingao syrup, TLC on silica gel with *n*-hexane - ethyl acetate - methanol 15:8:2, detection under UV 366 nm. As standards the alkaloids naucleficine, mauclefidine, and nauclefline were used.

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

111 087 ZH. JIANG (Jiang Zhenou)*, H. QIU (Qiu Hongcong), M.HUANG (Huang Minggui), Y. DENG (Deng Yuyin) (*Guangxi Acad. of Trad. Chinese Med. & Pharm., Nanning, Gu-

angxi 530022, China): (Development of the method for the quality control of Andrographis paniculata (Burm.f.) Wall. ex Nees. (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (6), 1411-1412 (2012). The extracts of *Andrographis paniculata*, a traditional Chinese medicinal herb, have been widely used as the core ingredient in a variety of traditional Chinese patent medicines, such as Chuanxinlian pills, Chuanxinlian capsules, Lianzhi pills, and some cosmetics and veterinary drugs. The quality of the extracts became significant for guaranteeing the validity, security, stability and homogeneity of the medicines. TLC of the herb extracts on silica gel with chloroform - ethyl acetate - methanol 20:15:2. Detection under UV 254 nm and by spraying with 2 % 3,5-dinitrobenzoic acid in ethanol - 2 mol/L KOH solution 1:1 followed by evaluation in daylight.

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

111 088 SH. JU (Ju Shanji)*, M. ZHANG (Zhang Mingzi) (*Yanbian Inst. for Food & Drug, Jilin, Yanbian 133001, China): (Study of the method for the identification of Weikang granules by thin-layer chromatography) (Chinese). J. of China Pharm. 21 (14), 57-58 (2012). Weikang granule is a herbal TCM preparation for curing chronic atrophic gastritis. For quality control, TLC on silica gel 1) for *Fructus Aurantii* with the upper phase of ethyl acetate - methanol - water 6:3: 1, detection by spraying with 1 % aluminium chloride in ethanol and viewing under UV 366 nm; 2) for *Rhizoma Corydalis* with cyclohexane - acetone 7:3, detection by spraying with aqueous potassium iodobismuthate 5 % - HCl 200:1 and viewing in daylight; 3) for *Coptis chinensis* with *n*-butanol - glacial acetic acid - water 7:1:2, detection under UV 366 nm.

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

111 089 T.S. KHEDKAR, Y.R. REDDI, B.D. MALI* (*Toxicology Division, Regional Forensic Science Laboratory State of Maharastra, Cantonment, Aurangabad 431005, India, malibdm@yahoo.co.in): Thin-layer chromatographic detection of some benzodiazepines. International Journal of Medical Toxicology & Legal Medicine 15 (1&2), 61-63 (2012). HPTLC for five benzodiazepines (alprazolam, clonazepam, diazepam, lorazepam and nitrazepam) on silica gel G with chloroform - acetic acid 9:1 in a presaturated TLC chamber. Detection by evaporisation with chlorine gas for 5 min, and after complete removal of chlorine, by spraying with *o*-tolidine reagent. Stabilisation of blue colored zones for a day by spraying the plate with 1 % phosphomolybdic acid. LOD for alprazolam, clonazepam, diazepan, lorazepam and nitrazepam was found to be 0.1, 0.5, 0.5, 1 and 0.5 µg/zone, respectively.

pharmaceutical research, postchromatographic derivatization, benzodiazepine drugs, HPTLC 32a

111 090 E. KILINC*, V. OKUMUS, M. DUZ, F. AYDIN (*Laboratory of Chemical Analysis, Department of Chemistry, Faculty of Science, University of Dicle, 21280 Diyarbakir, Turkey, ekilinc@dicle.edu.tr): Simultaneous high-performance thin-layer chromatographic determination of indole acetic acid, indole butyric acid, and absisic acid in in vitro seedling of watermelon exposed to heavy metals. J. Planar Chromatogr. 25, 108-111 (2012). HPTLC of indole acetic acid (1), indole butyric acid (2), and absisic acid (3) in watermelon seeds exposed to heavy metals on silica gel with cyclohexane - methanol - 2-propanol 4:2:1 +1 drop ammonia. Quantitative determination by absorbance measurement at 230 nm. Limit of quantification was 10 ng/zone

for (1) and (2) and 6 ng/zone for (3).

toxicology, quality control, food analysis, HPTLC, quantitative analysis 32d

111 091 M. KOBA*, M. MARSZALL, W. SROKA, M. TLUCHOWSKA, T. BACZEK (*Department of Medicinal Chemistry, Faculty of Pharmacy, Collegium Medicum of Nicolaus Copernicus University, Bydgoszcz, Poland, kobamar@cm.umk.pl): Determination of lamotrigine in tablets using HPTLC, HPLC, and derivative spectrophotometry methods. J. Liq. Chromatogr. Relat. Technol. 36, 537-548 (2013). HPTLC of lamotrigine in tablets on silica gel with toluene - acetone - ammonia 6:14:1. Quantitative determination by absorbance measurement at 312 nm. The hR_F value of lamotrigine was 55. LOD and LOQ were 0.57 and 1.73 µg/mL, respectively. Intermediate precision was below 1.9 %. Average recovery (by standard addition) was 101.5 %. Comparable results were obtained as compared with a validated HPLC method.

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

111 092 P. KOU (Kou Peiyan) (Baotou Municip. Test Center for Foods & Pharm., The Inner Mongolia Autonomous Region, Baotou 014030, China): (Study of the quality control of Chongderi pills) (Chinese) Chinese J. of Northern Pharmacy 9 (4), 4-5 (2012) Chongderi pills are a herbal traditional Mongolian medicine for treating diarrhea, invigorating the circulation of blood, stimulating the menstrual flow, and dissipating blood stasis, etc. For quality control, TLC on silica gel 1) for *Radix aucklandiae*, with benzene - methanol 27:1, detection by spraying with 1% vanillin in sulfuric acid - ethanol, detection under daylight; 2) for *Rhizoma acori tatarinowii*, with petroleum ether (60-90 º) - ethyl acetate 9:1, detection by exposure to iodine vapors, viewing under daylight; 3) for safflower carthamus, with ethyl acetate - formic acid - water - methanol 35:10:15:2, detection under daylight. Identification by comparison with the standards.

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

111 093 A. KUMAR, V. MANDAL, S. MANDAL (*Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India, subhashmandal@yahoo.com): Design of experiment approach for the process optimisation of microwave assisted extraction of lupeol from *Ficus racemosa* leaves using response surface methodology. Phytochem. Anal. 24, 230-247 (2013). HPTLC of lupeol in the leaves of *Ficus racemosa* on silica gel with toluene - chloroform - ethyl acetate 10:2:1 + 1 drop glacial acetic acid. Quantitative determination by absorbance measurement at 366 nm. The hR_F of lupeol was 31. Average recovery was in the range of 98.8-99.4 %. The intra- and inter-assay precision was below 0.2 %.

herbal, quality control, HPTLC, quantitative analysis 32e

111 094 Q. LI (Li Qiyan)*, J. YOU (You Jia), L. XU (Xu Lihua) (*Shandong Provinc. Inst. for Food & Drug Contr., Shandong, Jinan 250101, China): (Study on the method for the quality control of Yinxie Granules) (Chinese). J. of Qilu Med. & Pharm. 31 (6), 339-340 (2012). Yinxie Granules are a herbal TCM for treating difficult skin diseases, like psoriasis, etc. For quality control, identification of *Smilax china* L. by TLC on silica gel with chloroform - ethyl acetate - methanol - water 3:8:3:2, detection under UV 366 nm. Quantification of astilbin by HPLC.

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

111 095 SH. LI (Li Shenghua) (Bayan Nur Municip. Inst. for Drug Contr., Inner Mongolia, Bayan Nur, Linhe 015000, China): (Study on the method for the quality control of Luman-12 by thin-layer chromatography) (Chinese). Chinese J. Ethnopharm.(9), 36-38 (2012). Luman-12 is a traditional Mongolian herbal medicine preparation for treatment of cough, asthma, etc. For quality control of the main component herbal drugs TLC on silica gel 1) for *Radix Polygalae*, with toluene - ethyl acetate - formic acid 28:8:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 2) for *Radix Glycyrrhizae*, with toluene - ethyl acetate - formic acid 10:8:1, detection under UV 366 nm; 3) for *Piper nigrum*, with benzene - ethyl acetate - acetone 7:2:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight.

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

111 096 S. LI (Li Sumei)*, X. BI (Bi Xiaoli), Y. LI (Li Yangxue), W. LUO (Luo Wenhui), H. XIONG (Xiong Hong), ZH. TAN (Tan Zhican) (*Guangdong Provinc. Inst. of Trad. Chinese Med., Guangdong, Guangzhou 510095, China): (Study of the method for the quality control of Fufang Sanqidan capsules by thin-layer chromatography) (Chinese). Jiangxi J. of Trad. Chinese Med. 43 (350), 59-61 (2012). Fufang Sanqidan capsules are a herbal TCM preparation for treating frequent urination, cardiovascular and cerebrovascular diseases, nephropathy etc. For quality control TLC on silica gel for 1) *Radix Notoginseng* and *Radix Astragali*, with the upper phase of *n*-butanol - ethyl acetate - 10 % ammonia 4:1:5, 2) for *Salvia miltiorrhiz*a, with toluene - ethyl acetate 19:1, detection by viewing in daylight; and 3) for Mulberry Fruit, with petroleum ether (30-60 °C) - chloroform - ethyl acetate 3:8:5. All were detectied by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C for 5 min, viewing under UV 366 nm. Quantification of astragaloside A by HPLC.

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

111 097 T. LI (Li Tiegang), Y. LI (Li Yuting), X. WU (Wu Xiaozhui), X. WANG (Wang Xin), G. ZHONG (Zhong Guoyue), X. FAN (Fan Xinyue), X. DI (Di Xianyou), Y. ZHANG (Zhang Yi), Y. ZHANG (Zhang Yi), W. LUO (Luo Weizao)* (*Chongging Municip. Inst. of Trad. Chinese Med., Chongqing 400065, China): (Methodological study of assay of rhizome of Chinese goldthread and its processed products by thin-layer chromatography) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (3), 677-680 (2011). Chinese Goldthread is a traditional Chinese medicinal herbal drug, the dry rhizome of *Coptis chinensis* Franch, *Coptis dehoidea* C.Y. Cheng et Hsiao and Coptis teeta Wal. is widely used as key component in preparations effective for clearing heat, eliminating dampness, purging intense heat and relieve internal heat or fever. Presentation of a TLC method for simultaneous determination of five varieties of alkaloids in Chinese Goldthread rhizome and identification of the characteristic component of *Evodia rutaecarpa* (Juss.) in the preparation Yuhuanglian. TLC of the extracts of Chinese Goldthread rhizome on silica gel with cyclohexane - ethyl acetate - isopropanol - methanol - water - triethylamine 6:7:2:3:1:2 after saturation for 20 min, detection under UV 366 nm, identification of jatrorrhizine hydrochloride, palmatine hydrochloride, berberine hydrochloride, epiberberine hydrochloride, and coptisine hydrochloride by comparison with the standards. TLC of the

extracts of Yuhuanglian on silica gel with petroleum ether (60-90 ºC) - chloroform - acetone - methanol - diethylamine 25:10:10:5:1, detection by spraying with 2 % vanillin in ethanol - sulfuric acid 200:1 and heating at 105 °C until the zones were visible, identification of evodine by comparison with the standard.

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

111 098 ZH. LI (Li Zhiyong)*, D. SUN (Sun Dongmei), L. WANG (Wang Luolin) (*Guangdong Provinc. Inst. of Trad. Chinese Med., Guangdong, Guangzhou 510095, China): (Study of the method for the quality control of Yangning capsules) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (1), 100-101 (2011). Yangning capsules are a herbal TCM for treating skin pruritus caused by various reasons. For quality control, TLC on silica gel 1) for *Radix Saposhnikoviae* and *Radix Angelicae Sinensis*, with petroleum ether (60-90 °C) - ethyl acetate 4:1, detection under UV 366 nm; 2) for *Radix Glycyrrhizae*, with ethyl acetate - formic acid - glacial acetic acid 15:1:1:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm; 3) for *Cortex Dictamni*, with toluene ethyl acetate - formic acid 25:15:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C, viewing in daylight. Quantification of matrine and oxymatrine by HPLC.

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

111 099 K. LIANZA, J. SHERMA* (*Department of Chemistry, Lafayette College, Easton, PA, USA, shermaj@lafayette.edu): Application of an expanded model procedure for transfer of TLC screening for substandard and fake drugs designed for use in developing countries to quantitative HPTLC-densitometry methods. J. Liq. Chromatogr. Relat. Technol. 36, 2446-2462 (2013). HPTLC of amitriptyline in tablets on silica gel with acetone - ammonium hydroxide 99:1. Quantitative determination by absorbance measurement at 254 nm. The hR_F value of amitriptyline was 46. Linearity was 0.70-1.30 µg/zone. The earlier model TLC to HPTLC-densitometry transfer procedure was expanded by adding sample peak identification confirmation and peak purity (specificity) tests.

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

111 100 F. LIAO (Liao Fang)*, D. ZHANG (Zhang Dan), M. LI (Li Min), CH. LI (Li Chunlin), M. LAN (Lan Minghui), R. YIN (Yin Rongli) (*Chengdu Municip. Xindu District Hosp. of Trad Chinese Med., Sichuan, Chengdu 610500, China): (Study of the method for the quality control of Jiegu Xujin capsules by thin-layer chromatography) (Chinese). Jiangxi J. of Trad. Chinese Med. 43 (354), 62-64 (2012). Jiegu Xujin capsules are a herbal TCM for the treatment of fractures, bone dislocation and joint pain. For quality control, TLC on silica gel 1) for *Rhizoma Drynariae* and the standard naringin, with cyclohexane - ethyl acetate - formic acid - water 2:24:5:6, detection under UV 366 nm; 2) for *Radix Angelicae Sinensis* and *Rhizoma Ligustici* Chuanxiong, with *n*-hexane - ethyl acetate 9:1, detection under UV 366 nm; 3) for *Eupolyphaga seu steleophaga*, with toluene - dichloromethane - acetone 10:10:1, detection by spraying with 3 % vanillin in sulfuric acid - ethanol 1:200 and heat at 105 °C, viewing in daylight; 4) for *Radix Notoginseng* and the standards ginsenoside Rb1, Re, Rg1, R1, with the lower phase of chloroform - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm.

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

111 101 Y. LIAO (Liao Yong), X. ZHANG (Zhang Xiaorong)*, SH. WANG (Wang Shengmin), Y. WANG (Wang Yiping), M. TAO (Tao Min) (*Coll. of Life Sci. & Eng., Southernwest Jiaotong Univ., Chengdu, Sichuan 610031, China): (Study of the effect of superpressure jet flow processing technology on the contents of aconitine in traditional Chinese medicinal preparations containing *Aconitum carmichaeli* Debx (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (3), 620-621 (2011). Superpressure jet flow processing technology has been recently applied in puffing of some traditional Chinese medicinal herbs like *Aconitum carmichaeli* Debx. The effect of the technology on the traditional Chinese medicinal preparations containing *Aconitum carmichaeli* Debx was studied by determination of the content change of the active components. TLC of the extracts before and after superpressure jet flow processing on silica gel with chloroform - acetone - methanol 6:1:1. Detection by spraying with 5 % potassium iodobismuthate solution and evaluation under UV 366 nm. Identification of aconitine by comparison with the standards.

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

111 102 J. LIN (Lin Jinfeng)*, Y. ZHOU (Zhou Yingyi) (*Guangdong Provinc. Inst. for Drug Control, Guangdong, Guangzhou 510180, China): (Development of a method for the identification of *Radix Morindae officinalis* in Chuankezhi injection by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 8 (11), 134-135 (2012). Chuankezhi injection is a traditional Chinese medicinal herb preparation with *Radix Morindae officinalis* as the key active component. It is effective for tonifying kidney, relieving cough, regulation of immune function, and is prescribed clinically to cure asthma, and capillary bronchitis. Development of a method for the quality control of the preparation. TLC of the extracts of the preparations on silica gel with *n*-butanol - ethanol - water 5:4:1, detection by spraying with a solution of 1.5 g alpha-naphthol in ethanol - water - sulfuric acid 50:4:13 and heating at 105 °C.

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

111 103 L. LIU (Liu Lijun), L. ZHENG (Zheng Lin), Y. WANG (Wang Yonglin), A. WANG (Wang Aimin), Y. LI (Li Yongjun), X. HE (He Xun), Y. LAN (Lan Yanyu)* (*(The Key Lab. of Pharm. Preparation in Guizhou, School of Pharm., Guiyang Med. Coll., Guizhou, Guiyang 550004, China): (Study on the method for the quality control of Gongyanping capsules by thin-layer chromatography) (Chinese). Chinese J. of Guiyang Med. Coll. 37 (6), 605-607 (2012). Gongyanping capsules are a herbal TCM for treating acute and chronic pelvic inflammatory disease, irregular menstruation, etc. For quality control, TLC on silica gel 1) for *Melastoma dodecandrum* Lour. and the standard gallic acid, with chloroform - ethyl acetate - formic acid 25:20:4, detection by spraying with 2 % ferric chloride in ethanol and heating mildly, viewing in daylight; 2) for *Zanthoxylum nitidum*, with *n*-butanol - glacial acetic acid - water 7:1:2, detection by spraying with 5 % potassium iodobismuthate in water - hydrochloric acid 200:1 and viewing in daylight; 3) for *Radix Angelicae Sinensis* and the standard ferulic acid, with toluene - chloroform - glacial acetic acid 6:5:1, detection under UV 366 nm; 4) for *Ficus simplicissima* Lour. and the standard psoralen, with cyclohexane - toluene - ethyl acetate 10:10:3, detection by spraying with 10 % KOH in ethanol and viewing under UV 366 nm.

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

111 104 L. LIU (Liu Linna)*, ZH. CHI (Chi Zhihua), ZH. GUO (Guo Zhiwei), Y. ZHANG (Zhangyan), H. ZHANG (Zhang Haifeng), W. ZHANG (Zhang Wenjuan) (*Dep. Of Pharm., Tangdu Hosp., The 4th Milit. Med. Univ., Shanxi, Xian 710038, China): (Study of the method for the quality control of Xiaochaihutang dripping pills) (Chinese). J. of Practical Pharmacy & Clinic 15 (11), 742-744 (2012). Xiaochaihutang dripping pills are a herbal TCM for the treatment of chronic hepatitis and liver cirrhosis. For quality control, TLC on silica gel 1) for *Bupleurum chinense*, with ethyl acetate - ethanol - water 8:2:1, detection by spraying with 2 % *p*-dimethylaminobenzaldehyde in sulfuric acid - ethanol 2:3 and heating at 105 °C, viewing under UV 366 nm; 2) for *Radix Scutellariae*, with ethyl acetate - formic acid - water 14:5:5, detection by spraying with 2 % ferric chloride in ethanol, viewing in daylight. Quantification of baicalin by HPLC.

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

111 105 P. LIU (Liu Pinjian)*, J. CHEN (Chen Jian), Y. REN (Ren Yanan) (*Jiujiang Inst., School of Med., Jiangxi, Jiujiang 332000, China): (Separation and identification of chlorogenic acid in Cleavers by thin-layer chromatography) (Chinese). J. of Guangzhou Chem. Industry. 40 (24), 122-123 (2012). Cleavers (*Galium aparine*) is a herb used as a key component drug in various TCM formulations for the treatment of tumours, dysentery, mastitis, urinary tract infections, gallbladder diseases etc. For quality control and to guarantee the medication safety of relevant formulations, TLC of chlorogenic acid, the main active ingredient, on silica gel with ethyl acetate - formic acid - water 14:1:1, detection 1) by viewing under UV 366 nm; 2) by spraying with 2 % ferric chloride in ethanol and heating mildly then viewing in daylight; 3) by densitometry at 585 nm.

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

111 106 K. MANJULA, K. RAJENDRAN*, T. EEVERA, S. KUMARAN (*Department of Biotechnology, Periyar Maniammai University, Tamil Nadu-613 403, India, nkraj64@yahoo.co.uk): Quantitative estimation of lupeol and stigmasterol in *Costus igneus* by high-performance thin-layer chromatography. J. Liq. Chromatogr. Relat. Technol. 36, 197-212 (2013). HPTLC of lupeol (1) and stigmasterol (2) in the stems of *Costus igneus*on silica gel with *n*-hexane - ethyl acetate 4:1 for (1) and toluene - acetone - acetic acid 89:9:2 for (2). Detection by dipping in a solution of 0.5 mL *p*-anisaldehyde in 50 mL glacial acetic acid and 1 mL of 97 % sulfuric acid, followed by heating at 105 °C. Quantitative determination by absorbance measurement at 538 nm. The hR_F values of (1) and (2) were 55 and 58, respectively. Linearity was in the range of 5-10 µg/zone for (1) and 1-6 µg/zone for (2). LOD and LOQ were 131 and 430 ng/zone for (1) and 80 and 212 ng/zone for (2). Intermediate precision was below 2.9 %. Average recovery (by standard addition) for (1) and (2) was 100.2 % and 99.9 %, respectively.

herbal, quantitative analysis, HPTLC 32e

111 107 D. MODI*, B. PATEL (*Institute of Pharmaceutical Education and Research, Department of Pharmaceutical Chemistry, Kadi Sarva Vishwavidyalaya, Gandhinagar-382023, Gujarat, India, darshana_pharma@yahoo.co.in): Rapid and sensitive simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride in tablet formulation by HPTLC method. J. Liq. Chromatogr. Relat. Technol. 36, 618-627 (2013). HPTLC of metformin hydrochloride (1) and pioglitazone hydrochloride (2) in tablet on silica gel with butanol - 1,4-dioxane - glacial acetic acid 5:3:2. Quantitative determination by absorbance measurement at 226 nm. The hR_F values of (1) and (2) were 36 and 73, respectively. Linearity was in the range of 2-20 µg/zone for (1)

and 60-600 ng/zone for (2). LOD and LOQ were 630 and 1909 ng/zone for (1) and 9 and 26 ng/zone for (2). Intermediate precision was below 1.0 %. Average recovery (by standard addition) for (1) and (2) was 99.4 % and 98.5 %, respectively.

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

111 108 S. MORE, S. TANDULWADKAR, A. NIKAM, A. RATHORE, L. SATHIYANARAYANAN, K. MAHADIK* (*Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune 411038, Maharashtra, India, krmahadik@rediffmail.com): Separation and determination of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet dosage form by thin-layer chromatographic-densitometric method. J. Planar Chromatogr. 26, 78-85 (2013). HPTLC of lamivudine (1), tenofovir disoproxil fumarate (2) and efavirenz (3) on silica gel with chloroform - methanol - toluene 30:4:1. Quantitative determination by absorbance measurement at 260 nm. The hR_F values of (1), (2) and (3) were 20, 61 and 73, respectively. Linearity was in the range of 400-800 ng/zone for (1) and (2) and 800-1600 ng/zone for (3). Intermediate precision was below 2 %. LOD and LOQ were 180 and 300 ng/zone for (1), 150 and 210 ng/zone for (2) and 300 and 400 ng/zone for (3), respectively.

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

111 109 K. NAUMOSKA, B. SIMONOVSKA, A. ALBREHT, Irena VOVK* (*National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia, irena.vovk@ki.si): TLC and TLC-MS screening of ursolic, oleanolic and betulinic acids in plant extracts. J. Planar Chromatogr. 26, 125-131 (2013). HPTLC of positional isomers ursolic (1) and oleanolic (2) acids and their structural isomer betulinic acid (3) in the leaves or whole fruits of fresh vegetables on RP-18 phase with *n*-hexane - ethyl acetate 5:1. Qualitative identification by absorbance measurements at 366 nm. The hR_F of compounds (1) to (3) were 42, 48 and 62, respectively.

food analysis, quality control, qualitative identification, HPTLC 32e

111 010 Olumuyiwa OGEGBO *et al.* see section 3g

111 110 Y. OU (Ou Yanglu)*, W. LI (Li Wenqiang) (*Affilliated People's Hosp., Hubei Coll. of Med., Hubei, Shiyan 442000, China): (Study of the method for the identification of Bushen Huayu granules by thin-layer chromatography) (Chinese). Modern J. of Integrated Trad. Chinese & Western Med. 21 (36), 4087-4088 (2012). Bushen Huayu granules are a herbal TCM for treating osteoporosis. For quality control, TLC on silica gel 1) for *E. brevicornum Maxim* and the standard icraiin, with ethyl acetate - acetone - methanol - water 20:2:3:2, detection by spraying with 1 % aluminium chloride in ethanol and viewing under UV 366 nm; 2) for *Radix Rehmanniae* and the standard 5-hydroxymethylfurfural, with petroleum ether (60-90 °C) - ethyl acetate 1:1, detection under UV 254 nm.

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

111 111 L. PAILLAT, Christine PERICHET, S. LAVOINE, U. MEIERHENRICH, X. FERNANDEZ* (*Institut de Chimie de Nice, Université de Nice-Sophia Antipolis, Parc Valrose, 06108 Nice Cedex 2, France, xavier.fernandez@unice.fr): Validated high-performance thin-layer chromato-

graphy method for the determination of nicotine in tobacco (*Nicotiana tabacum* L.) extracts. J. Planar Chromatogr. 25, 23-29 (2012). HPTLC of nicotine in tobacco extracts on silica gel with ethyl acetate - methanol - ammonia 28 % 10:5:2. Quantitative determination by absorbance measurement at 263 nm. The hR_F of nicotine was 20. Linearity was in the range of 90-900 ng/zone. The intermediate/inter-day/intra-day precision was below 2 %.

herbal, quality control, quantitative analysis, HPTLC 32e

111 112 J. PENG (Peng Jiangli), L. WANG (Wang Lijing), T. LIU (Liu Tasi), W. DONG (Dong Weiwei), SH. LI (Li Shunxu), D. YANG (Yang Dajian)* (*The Key Lab. of Research on Pharmacy & Molecular Pharmacology of Trad. Chinese Med. at Shenzhen, Guangdong, Shenzhen 518057, China): (Study on the method for the differentiation of *Ganoderma lucidum* from its counterfeit, Ganoderma) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (7), 1703-1704 (2011). *Ganoderma lucidum* as a traditional Chinese medicinal herbal crude drug is the dried fruiting body of Ganoderma. It is used for mind and body tranquilization and to relieve cough and asthma, and is prescribed clinically to treat palpitation, lung deficiencies, and shortness of breath. TLC of the extracts of the crude drugs on silica gel with dichloromethane - ethanol - formic acid 16:10:1, detection under UV 254 nm. Identification of *Ganoderma lucidum* based on the characteristic marker compound ganodermic acid A which is not found in the counterfeits. Differentiation also by identifying the shape and properties by microscopy.

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

111 113 L. PENG (Peng Lala), ZH. LI (Li Zhiyong)*, D. SUN (Sun Dongmei), J. ZHANG (Zhang Jianjun), L. WANG (Wang Luolin) (*Guangzhou Hosp. of Trad. Chinese Med., Guangdong, Guangzhou 510130, China): (Study of the method for the quality control of Rulekang gel ointment) (Chinese). Chinese J. of Today's Pharm. 22 (11), 659-663 (2012). Rulekang gel ointment is a herbal TCM for treating diseases of the mammary glands. For quality control, TLC on silica gel 1) for *Nidus vespae*, with *n*-hexane - chloroform - ethyl acetate 16:3:4, detection under UV 366 nm; 2) for Olibanum and Myrrh, with toluene - ethyl acetate 19:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C, viewing in daylight; 3) for *Syzygium aromaticum*, with petroleum ether (60-90 °C) - ethyl acetate 8:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C, viewing in daylight; 4) *Rhizoma Curcumae Longae*, with chloroform - methanol - formic acid 96:4:1, detection by viewing in daylight; 5) for *Herba Asari*, with *n*-hexane - ethyl acetate 4 1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C, viewing under UV 366 nm; 6) for *Fructus Piperis Longi*, with *n*-hexane - ethyl acetate - formic acid 600:400:1, detection under UV 366 nm. Quantification of curcumin by HPLC.

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

111 114 Y. QING (Qing Yan), M LI (Li Min)*, J. ZHOU (Zhou Juan), ZH. YAO (Yao Zhiang), W. LIU (Liu Wengang) (*Chengdu Univ. of Trad. Chinese Med. & Pharm., Sichuan, Chengdu 611137, China): (Investigation of current situation of adulteration of processed monkshood and *radix aconiti agrestis* prepared in small pieces ready for decoction) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (8), 2001-2002 (2011). Monkshood and *Radix Aconiti agrestis*, as traditional Chinese herbal drugs, are usually processed into small pieces ready for decoction and are clinically effective for relieving rheumatic pains. However, in the market some processed

drugs were found to be mixed with counterfeits. Presentation of an assay method to investigate the situation of adulteration of these drugs. TLC of the extracts of the drugs 1) for alkaloids, on silica gel with *n*-hexane - ethyl acetate - methanol 32:18:5 with chamber saturation with ammonia vapors for 20 min, detection by spraying with 5 % potassium iodobismuthate solution. Identification a) by fingerprint comparison with the individual standard component drug; b) by microscopy distinguishing the cluster crystal of calcium oxalate consisted in the thin-wall cells of the drug; 2) for peoniflorin, on silica gel with chloroform - ethyl acetate - methanol - formic acid 40:5:10:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C.

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

111 115 M. QU (Qu Meilun) (Liuyang Municip. Hosp. of Trad. Chinese Med., Hunan, Liuyang 410300, China): (Study of the method for the quality control of Zhiheng tablets by thin-layer chromatography) (Chinese). Chinese J. Mod. Drug Appl. 7 (1), 118-119 (2013). Zhiheng tablets are a herbal TCM for treatment of hyperlipidemia. For quality control, TLC on silica gel 1) for *Radix Notoginseng*, with dichloromethane - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 2) for *Salvia miltiorrhiza* and the standard salvianolic acid A sodium, with dichloromethane - acetone - formic acid 25:10:4, detection by exposing to ammonia vapors for 10 min and viewing under UV 366 nm; 3) for *Pericarpium Citri Reticulatae* and the standard hesperidin, with ethyl acetate - methanol - water 100:17:13 to a distance of 3 cm and then with toluene - ethyl acetate - formic acid - water 20:10:1:1 to a distance of 8 cm, detection by spraying with 3 % aluminium chloride in ethanol and viewing under UV 366 nm; 4) for *Fructus Gardeniae* and the standard gardenoside, with ethyl acetate - acetone - formic acid - water 5:5:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight.

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

111 116 B. RAMESH, S. RAMAKRISHNA, R. REDDY, K. HARI, V. SARMA, P. DEVI* (*Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500007, India, sitadeviiict@gmail.com): HPTLC method for determination of darunavir in rat plasma and its application in pharmacokinetic studies. J. Liq. Chromatogr. Relat. Technol. 36, 167-179 (2013). HPTLC of darunavir in rat plasma on silica gel with toluene - acetone - methanol 3:1:1. Quantitative determination by mass spectrometry in the ESI+ mode and absorbance measurement at 254 nm. The hR_F value of darunavir was 53. Linearity was in the range of 5-150 ng/µL. LOD and LOQ were found to be 1.2 and 3.9 ng/µL, respectively. Intermediate precision was below 2.7 %. Recovery (by standard addition) was 94.8-98.4 %. The recovery of the drug is improved compared with the HPLC method (96.7 %).

clinical chemistry research, HPTLC, quantitative analysis, comparison of methods 32f

111 117 A. RAWAT, A. SRIVASTAVA, S. SHANKAR, S. SRIVASTAVA* (*National Botanical Research Institute, Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, UP, India, sharad_ks2003@yahoo.com): Quantification of protodioscin and prototribestin in fruits of *Tribulus terrestris* L. collected from different phyto-geographical zones of India. J. Liq. Chromatogr. Relat. Technol. 36, 1810-1821 (2013). HPTLC of protodioscin (1) and prototribestin (2) in fruits of *Tribulus terrestris* on silica gel with *n*-butanol - glacial acetic acid - water 40:3:10. Quantitative determinati-

on by absorbance measurement at 366 nm. The hR_F values of (1) and (2) were 41 and 39, respectively. Linearity was in the range of 100-1000 ng/zone for both (1) and (2). LOD and LOQ were 40 and 100 ng/zone for (1) and 35 and 100 ng/zone for (2). Intermediate precision was below 1.0 %. Recovery ranged 98.0-103.0 % for (1) and 99.1-101.9 % for (2).

herbal, quality control, quantitative analysis, HPTLC 32e

111 118 M. SCHULZ*, Susanne MINARIK, Michaela OBERLE, Sylvia EISENBERG (*Merck KGaA, MM-LER-CP. Frankfurterstr. 250, 64293 Darmstadt, Germany, michael.schulz@merckgroup.com): Quantification and side component analysis of the cosmetic active tiliroside using planar chromatography. CBS 107, 11-12 (2011) HPTLC of tiliroside (a cosmetic active obtained by extraction from a plant of the family *Sterculiaceae*) and side components kaempferol-3-glucoside, kaempferol-3-rutinoside, kaempferol, and coumaric acid on silica gel with ethyl acetate - formic acid - acetic acid- water 100:11:11:27 + 1% heptane. Detection under UV 366 nm after spraying with natural products reagent (1 % diphenylborinic acid 2-aminoethylester in methanol) and under white light after spraying with anisaldehyde sulfuric acid reagent (0.5 mL anisaldehyde in 85 mL methanol with 10 mL acetic acid and 8 mL conc. sulfuric acid) and heating at 90-125 °C for 15 min. Quantitative absorption measurement of tiliroside at 315 nm using a 4-point calibration via peak area. The tiliroside contents in the sample extracts were determined as: 1 = 1.09 µg (*RSD* = 0.4 %), 2 = 0.93 µg (*RSD* = 0.8 %), 3 = 1.19 µg (*RSD* = 1.2 %) and 4 = 3.32 µg (*RSD* = 0.3 %). During the side component analysis no kaempferol, coumaric acid, and glucose were detected in the tiliroside samples. Small amounts of kaempferol-3-glucoside and kaempferol-3-rutinoside were found. HPTLC covers several tasks: 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.

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

111 119 D. H. SHEWIYO, E. KAALE, P.G. RISHA, B. DEJAEGHER, J. SMEYERS-VERBEKE, Y. VANDER HEYDEN* (*Dep. Anal. Chem. & Pharm. Technol. (FABI), Center for Pharm. Res. (CePhaR), Vrije Univ. Brussel (VUB), Laarbeeklaan 103, 1090 Brussels, Belgium): Optimization of a reversed-phase-high-performance thin-layer chromatography method for the separation of isoniazid, ethambutol, rifampicin and pyrazinamide in fixed-dose combination antituberculosis tablets. J. Chromatogr. A 1260, 232-238 (2012). Presentation of a new RP-HPTLC method for the separation of pyrazinamide, isoniazid, rifampicin and ethambutol in a four fixed-dose combination tablet formulation by detection of pyrazinamide, isoniazid and rifampicin at UV 280 nm firstly, and then derivatization and detection of ethambutol at 450 nm after RP-plate development. Evaluation of methanol, ethanol and propan-1-ol as the modifiers to form alcohol-water mobile phases. Systematic optimization of the composition of each alcohol in the mobile phase by using the window diagramming concept to obtain the best separation. Examination of the R_F distribution of the separated compounds indicated that separation of the compounds with the mobile phase containing ethanol at the optimal fraction situated within the optimal hR_F-values region of 20-80, thus ethanol was selected as the organic modifier and the optimal mobile phase composition was found to be ethanol - water - glacial acetic acid - 37 % ammonia 70:30:5:1.

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

111 120 B. SHI (Shi Baoquan), L. XU (Xu Li)*, G. FU (Fu Gang) (*Dep. of Pharm., Hosp. of Hebei Provinc. Armed Police Force of PLA, Hebei, Shijiazhuang 050081, China): (Study of the method for the determination of indirubin and indigo in Banlian Chongji infusion by thin-layer chromatography) (Chinese). J. of Bethune Milit. Med. Coll. 10 (4), 282-283 (2012). Banlian Chongji infusion is a herbal TCM preparation for treating influenza and upper respiratory tract infections. For quality control, TLC of indirubin and indigo on silica gel with petroleum ether (60-90 °C) - ethyl acetate - chloroform 1:1:8, detection in daylight. Quantification by densitometry at 505 nm for indirubin and at 608 nm for indigo. Validation by investigation of the linearity range of the calibration curves (0.1-0.5 µg/zone, n=5, r = 0.999 for both), stability (%RSD = 1.2 %, n=5 for both), and precision (%RSD = 2.2 %, n=5 for both). The recovery (by standard addition) was 95.5 % (%RSD = 4.5 %, n=5) for indirubin and 96.0 % (%RSD = 2.6 %, n=5) for indigo.

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

111 121 R. SHI (Shi Rui) (Liaoning Univ. of Trad. Chinese Med., Liaoning, Shenyang 110032, China): (Study on *Citrus limon* (L.) Burm. F., *Rutaceae* - a class of traditional Chinese medicinal plants by thin-layer chromatography) (Chinese). Chinese J. of Jilin Med. Col l. 33 (1), 8-10 (2012). Citrus limon is a class of traditional Chinese medicinal plants used in various TCM preparations. To guarantee the safety and effectiveness it is significant to identify the authenticity of the crude drugs. TLC of 11 varieties of standard crude drug 1) for alkaloids with the standard synephrine, on 0.5 % NaOH containing silica gel with chloroform - ethanol - ammonia 5:5:1, detection by spraying with 1 % ninhydrin in ethanol and heating at 105 °C, viewing under UV 366 nm; 2) for flavonoids with the standard hesperidin and naringin, on silica gel with ethyl acetate - methanol - water 100:17:13, detection by spraying with 1 % aluminiumchloride in ethanol and viewing under UV 366 nm. The method demonstrated the merits of TLC, such as high throughput, fast analysis, no interferences, specificity, intuitive use and can be a base of establishing the operational standard method for the quality control of the crude drugs.

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

111 122 I. SIMA, D. CASONI, S. SARBU* (*Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Arany Janos Str., No 11, 400028, Cluj-Napoca, Romania, csarbu@chem.ubbcluj.ro): Simultaneous determination of carbidopa and levodopa using a new TLC method and a free radical as detection reagent. J. Liq. Chromatogr. Relat. Technol. 36, 2395-2404 (2013). HPTLC of carbidopa (1) and levodopa (2) in tablets on RP-18 with citrate buffer (pH 3.0) - methanol - formic acid 96:4:5. Detection by spraying with 0.02 % 2,2-diphenyl-1-picrylhydrazyl solution in ethanol (DPPH radical reagent). The hR_F values of compounds (1) and (2) were 39 and 63, respectively. Linearity was in the range of 50-300 ng/zone for both (1) and (2). Intermediate precision was below 3.7 %. LOD and LOQ were 20.5-60.7 ng/zone for (1) and 27.8-61.1 ng/zone for (2), respectively. Recovery (by standard addition) was 99.6-95.4 % for both.

pharmaceutical research, HPTLC, quantitative analysis, DPPH free radical 32a

111 123 S. SINGH, S. KHATOON*, H. SINGH, S. BEHERA, P. KHARE, A. RAWAT (*Pharmacognosy and Ethnopharmacology Division, CSIR National Botanical Research Institute, Lucknow, India, sayyadak@nbri.res.in): A report on pharmacognostical evaluation of four *Adiantum species*, *Pteridophyta*, for their authentication and quality control. Brazilian Journal of Pharmacognosy. 23, 207-216 (2013). HPTLC fingerprint of four similar looking Adiantum species: *A. capillus-*

veneris L. (1), *A. lunulatum* Burm. f. (2), *A. peruvianum* Klotzsch (3), and *A. venustum* D. Don. (4) on silica gel with toluene - ethylacetate 4:1. (1) could be clearly differentiated from other species by the presence of characteristic blue colored bands at hR_F 57 under UV 366 nm. (2) and (3) shared additional bands at hR_F 22 and 27 under UV 366 nm and at hR_F 74 under UV 254 nm. A characteristic band at hR_F 41 was observed only in (3) under UV 366 nm.

herbal, quality control, HPTLC, qualitative identification 32e

111 124 Y. SONG (Song Yue)*, H. SHEN (Shen Hongkuan), Y. ZHANG (Zhang Yajuan) (Jiamusi Municip. Inst. for Drug Contr., Heilongjiang, Jiamusi 154007, China): (Study on the method for the quality control of Liuwei Xiaohuang pills by thin-layer chromatography) (Chinese). Chinese J. of Prac. Med. 7/20, 245-247 (2012) . Liuwei Xiaohuang pills are a herbal TCM for treating abdominal distention and constipation. For quality control, TLC on silica gel 1) for *Radix et Rhizoma Rhei* and standard rhein, with petroleum ether (30-60 °C) - ethyl acetate - formic acid 15:5:1, detection under UV 366 nm and by exposing to ammonia vapors, viewing in daylight; 2) for *Fructus Aurantii Immaturus* and the standard synephrine, with the upper phase of *n*-butanol - glacial acetic acid - water 4:1:5, detection by spraying with 0.5 % ninhydrin in ethanol and heating at 105 °C, viewing in daylight.

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

111 125 L. SUN (Sun Lili)*, Y. NI (Ni Yana) (*Baicheng Municip. Inst. for Drug Contr., Jilin, Baicheng 137000, China): (Study of the method for the identification of Zhitongfengshi pills by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 10 (34), 113-114 (2012). Zhitongfengshi pills are a herbal TCM for treating numbness of limbs and rheumatic pain. For quality control TLC on silica gel 1) for *Fructus Gardeniae*, with ethyl acetate - acetone - formic acid - water 20:14:4:1, detection by spraying with 13 % sulfuric acid in ethanol and heating mildly, viewing in daylight; 2) for *Cortex Phellodendri Chinensis*,with *n*-butanol - formic acid - water 75:16:9, detection under UV 366 nm; 3) for R*adix Scutellariae*, with ethyl acetate - acetone - acetic acid - water 12:6:4:3, detection by spraying with 1.5 % ferric chloride in ethanol and viewing in daylight; 4) for *Radix Clematidis*, with toluene - ethyl acetate - glacial acetic acid 100:15:1, detection by spraying with 15 % sulfuric acid in ethanol and heating mildly, viewing in daylight.

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

111 126 P. SYAL, M. SAHOO, R. RAUT, A. HABLE, A. BATTEWAR, V. CHOUDHARI*, B. KUCHEKAR (*Maharashtra Institute of Pharmacy, MIT Campus, Paud Road, Kothrud, Pune 411038, MS, India, viraj1404@rediffmail.com): Development and validation of an HPTLC method for simultaneous estimation of thiocolchicoside and aceclofenac in combined dosage form. J. Planar Chromatogr. 25, 133-137 (2012). HPTLC of thiocolchicoside (1) and aceclofenac (2) in combined dosage form on silica gel with methanol - chloroform - water 48:1:1. Quantitative determination by absorbance measurement at 254 nm. The hR_F values of (1) and (2) were found to be 70 and 83, respectively. Linearity was in the range of 30-180 ng/band for (1) and 750-4500 ng/band for (2). Recovery was in the range of 98.7-101.2 % for both.

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

111 127 P. TANGYUENYONGWATANA, V. KEERATINIJAKAL, W. GRITSANAPAN* (*Mahidol University, Faculty of Pharmacy, Department of Pharmacognosy, 447 Sri-Ayudthaya Rd, Rat-

chatewi, Bangkok, 10400, Thailand, pywgs@mahidol.ac.th): Thin-layer chromatography-densitometry analysis of dimethoxyphenylbutadiene content in *Zingiber cassumunar* rhizomes. J. AOAC Int. 95, 1614-1617 (2012). HPTLC of (E)-4-(3,4-dimethoxyphenyl)butadiene (DMPBD) in the rhizome of *Zingiber cassumunar* on silica gel with hexane - dichloromethane 3:2. Quantitative determination by absorbance measurement at 289 nm. The hR_F value of DMPBD was 30. Linearity was between 130 and 703 ng/zone. LOD and LOQ were 40 and 10 ng/zone, respectively. The interday and intra-day precisions were 0.9 and 0.3 % (n=3). Average recovery (by standard addition) was 103.1 %.

herbal, HPTLC, densitometry, quantitative analysis 32e

111 128 V. TOPIC, S. FILIPIC, G. POPOVIC, K. NIKOLIC, D. AGBABA* (*Department of Pharmaceutical Chemistry, University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia, danica@pharmacy.bg.ac.rs): TLC determination of glimepiride and its main impurities in pharmaceuticals. J. Liq. Chromatogr. Relat. Technol. 36, 2422-2430 (2013). HPTLC of glimepiride (1) and its main degradation impurities, glimepiride-sulfonamide (2), and glimepiride-carbamate (3) in pharmaceuticals on silica gel with toluene - ethyl acetate - methanol 8:5:1. Quantitative determination by absorbance measurement at 230 nm. The migration distances of (1), (2) and (3) were 48, 43 and 35 mm, respectively. LOD for the impurities (2) and (3) were found to be 2.2 and 2.3 ng/zone, respectively. LOQ values for the impurities (1) and (2) were 7.2 and 7.7 ng/zone. Intermediate precision was below 4.2 % (n=6). Recovery (by standard addition) was in the range of 94.9-102.3 % for (1) to (3).

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

111 129 CH. WANG (Wang Chunyi), X. YE (Ye Xuelan), W. LI (Li Weimin), Y. GAO (Gao Ying)* (*Guangzhou Univ. Trad. Chinese Med. & Pharm., Guangdong, Guanzhou 510006, China): (Exploration of the quality standard for the extracts of general saponin in astragalus) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 23 (1), 94-96 (2012). Astragalus as a widely used medicinal raw drug is the dried root of *Astragalus membranaceus* (Fisch.) and *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao. Its pharmacological effects are nourishing the vitality, invigorating splenic yang, promoting tissue regeneration, inducing diuresis for removing edema, and invigorating the circulation of blood. The chemical constituents of the extracts of astragalus mainly are saponins, polysaccharides and flavones. TLC of the astragalus extracts on silica gel with the lower phase of methanol - chloroform - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones are visible, evaluation in daylight and under UV 366 nm. By comparison with standards the markers astragaloside I-Vlll, acetylastragaloside, isoastragaloside I, II, lV, and soyasaponin I were identified.

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

111 130 J. WANG (Wang Junxiang)*, J. ZHAO (Zhaojike) (*Shandong Univ. of Trad. Chinese Med., Shandong, Jinan 250014, China): (Study of the method for the quality control of Kexian capsules) (Chinese). Chinese J. of Food & Drug 14 (7), 284-286 (2012). Kexian capsules are a herbal TCM preparation for treating epilepsy and phlegm blockage syndrome. For quality control TLC, on silica gel 1) for Gambir plant, with toluene - chloroform - acetone - concentrated ammonia - methanol 20:25:15:4, detection under UV 366 nm; 2) for Borneol, with petroleum ether (60-90 °C) - ethyl acetate - chloroform 11:1:3, detection by spraying with 1 % vanillin in sulfuric acid -

ethanol 1:200 and heating at 105 °C, viewing in daylight. Quantification of cholic acid by UV spectrophotometry.

quality control, pharmaceutical research, traditional medicine, erbal, qualitative identification 32e

111 131 SH. WANG (Wang Shuping), CH. GUO (Guo Chunqiu), Q. QU (Qu Qianwen), H. QUAN (Quan Hong)*, CH. YUE (Yue Changli) (*Inst. of Trad. Chinese Med. & Pharm., Heilongjiang Univ. of Trad. Chinese Med. & Pharm., Heilongjiang, Haerbin 150040, China): (Study of the quality standard for Gongbaokang suppository) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (4), 829-830 (2011). Gongbaokang suppository is a traditional Chinese recipe for curing cervicitis, preventing pathological changes which may cause cervical carcinoma. Description of a method for the quality control. TLC of the extracts of the medicine 1) for *Sophora flavescens*, on silica gel with benzene - acetone - ethyl acetate - ammonia 10:15:20:1, detection by spraying with 5 % potassium iodobismuthate solution and viewing under daylight; 2) for *Fructus Cnidii*, on silica gel with toluene - ethyl acetate - *n*-hexane 3:3:2, detection under UV 366 nm; 3) for borneol, on silica gel with benzene - acetone 9:1, detection by spraying with 1 % vanillin in ethanol - sulfuric acid 4:1 and heating at 105 °C, viewing under UV 366 nm.

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

111 132 Y. WANG (Wang Yonghui), R. SUN (Sun Rongjin)*, F. YE (Ye Fang) (*School of Pharm., Hubei Univ. of Med. & Pharm., Hubei, Shiyan 442000, China): (Study on the method for the identification of chlorogenic acid and luteoloside in honeysuckle by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 9 (11), 136-137 (2012). Honeysuckle, as a traditional Chinese medicinal herbal crude drug, is the dried flower bud of *Lonicera japonica* Thunb. It is effective in clearing heat and removing toxicity. For quality control, a method for the identification of chlorogenic acid and luteoloside in honeysuckle has been developed and optimized. TLC of the extracts of the crude drug on silica gel with ethyl acetate - acetone - formic acid - water 35:15:5:6, detection by spraying with 5 % aluminium chloride in ethanol, followed by 1) evaluation under UV 366 nm for luteolosid; 2) by spraying again with the upper phase of 1 % ferric chloride - 1 % potassium ferricyanide 1:1 and viewing under daylight for chlorogenic acid.

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

111 133 S. WANKHEDE*, A. MAHAJAN, S. CHITLANDE (*Padmashree Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411018, Maharashtra, India, drsagarwankhede@rediffmail.com): A simple TLC-densitometric method for the estimation of labetalol hydrochloride in tablets. J. Planar Chromatogr. 25, 145-149 (2012). HPTLC of labetalol hydrochloride on silica gel with ethyl acetate - methanol 4:1 + 1 drop ammonia. Quantitative determination by absorbance measurement at 309 nm. The hR_F of labetalol was 69. Linearity was in the range of 400-2400 ng/band. The intermediate/interday/intra-day precision was below 2 %. Recovery was in the range of 98.6-100.5 %.

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

111 134 N. WEI (Wei Na)*, P. LI (Li Peipei), Y. WANG (Wang Yong) (*Coll. of Pharm., Hainan Univ. of Med., Nainan, Haikou 571101, China): (Study on the pharmacognostical identification method for the stem and leaf of Elaeagnus gonyanthes Benth.) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 23 (5), 1180-1181 (2012). *Elaeagnus gonyanthes* Benth. is a medicinal herb which grows in southern China. The crude drug is used in folk medicine for treating asthma, chronic bronchitis, arthritis, low back pain, traumatic swelling, etc. For identification, TLC of the extracts of different plant parts (leaf, stem) and the standards oleanic acid and ursolic acid on silica gel with cyclohexane - chloroform - ethyl acetate - glacial acetic acid 200:50:80:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight. Quantification of oleanic acid and ursolic acid by HPLC.

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

111 135 Y. WEN (Wen Yue), Y. LAI (Lai Ya), D. MENG (Meng Desheng)* (*The Third Milit. Med. Univ., The Third Affil. Hosp., Res. Inst. of field surgery, Pharm. Prepar. Section, Chongqing 400042, China): (Study on the method for the quality control of Yijingling oral liquid) (Chinese). J. of China Pharm. 21 (11), 22-24 (2012). Yijingling oral liquid is a herbal TCM preparation for mind and body tranquilization and nourishing the liver and kidney and is prescribed clinically to treat insomnia, amnesia, and the climacteric syndrome. For quality control, TLC on silica gel 1) for *Radix Polygoni Multiflori* with toluene - ethyl acetate - formic acid 20:2:1, detection by exposing to iodine vapor and viewing in daylight; 2) for *Fructus Psoraleae* with *n*-hexane - ethyl acetate 3:1, detection by spraying with 10 % KOH in ethanol and viewing under UV 366 nm.

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

111 136 D. WIDIRETANI, I. LUAILIA, G. INDRAYANTO* (*Section Analytical Development, Department R & D, Bernofarm Pharmaceutical Company, Sidoarjo, Indonesia; and G. Indrayanto, Faculty of Pharmacy, Airlangga University, Dharmawangsa dalam, Surabaya 60286, Indonesia, gunawanindrayanto@yahoo.com): Simultaneous densitometric determination of hydrocortisone acetate and 2-phenoxyethanol in creams. J. Planar Chromatogr. 26, 37-42 (2013). HPTLC of hydrocortisone acetate (1) and 2-phenoxyethanol (2) in cream on RP-18 with water-methanol 4:11. Quantitative determination by absorbance measurement at 270 nm. Linearity was in the range of 4.2-13.0 µg/zone for (1) and 0.8-4.3 µg/zone for (2). LOD and LOQ were 0.6 and 1.7 µg/zone for (1) and 0.2 and 0.5 µg/zone for (2), respectively. Intermediate precision was below 2 %.

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

111 137 L. WU (Wu Lizhong)*, L. WANG (Wang Luhong), H. NIU (Niu He) (*Jilin Oilfield General Hosp., Jilin 138000, China): (Study on the method for the quality control of Danbaijing capsules by thin-layer chromatography) (Chinese). Chinese J. Mod. Drug Appl. 6 (9), 124-125 (2012). Danbaijing capsules are a herbal TCM for treating intractable proteinuria. For quality control, TLC on silica gel 1) for *Radix Astragali* and the standard astragaloside A, with chloroform - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 2) for *Centella asiatica* and the standard asiaticoside, with chloroform - methanol - water 14:6:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at

105 °C, viewing in daylight.

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

111 138 J. XIE (Xie Jili), H. DING (Ding Hong)*, Y. DU (Du Yan), CH. SUN (Sun Chuan) (*Teaching & Research Section of Pharm., Coll. of Pharm., Shanxi Univ. of Med., Shanxi, Taiyuan 030001, China): (Study of the method for the quality control of Xiaocaihu tablets by thin-layer chromatography) (Chinese). Chinese J. of Medication & Clinics 12 (11), 1445-1446 (2012). Xiaocaihu tablet is a herbal TCM for treating chills and fever, dry throat, etc. For quality control TLC on silica gel 1) for *Bupleurum chinense*, with ethyl acetate - methanol - water 12:2:1, detection by spraying with 5 % *p*-dimethylaminobenzaldehyde in 10 % sulfuric acid in ethanol and heating mildly, viewing in daylight; 2) for root of *Pilose Asiabell*, with *n*-butanol - glacial acetic acid - ethyl acetate - water 14:2:2:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 100 °C, viewing in daylight.

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

111 139 X. XIN (Xin Xiufang)*, J. ZHENG (Zheng Jianfeng), F. REN (Ren Fengjiao), A. ZHENG (Zheng Ali), L. WU (Wu Lingxia) (*Xifeng Pharm. Co., Ltd., Gansu, Qingyang 745000, China): (Study on the method for the quality control of Yanyan tablets) (Chinese). J. Gansu Coll. of Trad. Chinese Med. 29 (6), 19-22 (2012). Yanyan tablets are a herbal TCM for treating chronic pharyngitis. For quality control, TLC on silica gel 1) for *Radix Stemonae*, with petroleum ether (60-90 °C) - methanol - ethyl acetate - ethylenediamine 20:20:40:1, detection by spraying with 5 % potassium iodobismuthate in HCl - water 1:200 and viewing in daylight; 2) for *Radix Ophiopogonis* and flowers of common coltsfoot, with petroleum ether (60-90 °C) - acetone 6:1, detection under UV 254 nm. Quantification of rutin by HPLC.

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

111 140 J. XU (Xu Jing), W. WANG (Wang Wei), B. SHAO (Shao Bing), Y. LIU (Liu Yan)* (*Inst. of Clinical Pharm. & Drug, The 2nd Afiliated Hosp., Harbin Univ. of Med., Heilongjiang, Harbin 150086, China): (Study of the preparation and the method for the quality control of Ruxian pills) (Chinese). Acta of Trad. Chinese Med. & Pharm. 40 (3), 100-102 (2012). Ruxian pills are a herbal TCM for treatment of abdominal mass accumulation and breast tumors etc. The medicine is prepared by blending the decoction and powders of the component drugs with honey in proper proportion and then processing into pills. For quality control, TLC on silica gel 1) for *Radix Angelicae Sinensis*, with *n*-hexane - ethyl acetate 9:1, detection under UV 366 nm; 2) for *Radix Paeoniae Rubra*, with chloroform - ethyl acetate - methanol - water 200:25:50:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:25 and heating at 105 °C, viewing in daylight; 3) for *Spina gleditsiae*, with toluene - ethyl acetate - formic acid 7:2:1, detection under UV 366 nm. Using this method the optimum extraction conditions for preparing the decoction from the crude drugs as well as the stability during storage of the final preparation were studied.

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

111 141 X. XU (Xu Xinxin), Y. CHENG (Cheng Yi)*, Y. CHEN (Chen Yuchao) (*Guangzhou Univ. of Trad. Chinese Med., Guangdong, Guangzhou 510006, China): (Study of the method for the quality control of Xiaoer Danzhen granules by thin-layer chromatography) (Chinese). Chinese J. of Northern Pharm. 10 (2), 2-3 (2013). Xiaoer Danzhen granules are a herbal TCM for treating children with focal accumulation of heat, aphthae, hot urination, chicken pox, influenza fever, etc. For quality control, TLC on silica gel 1) for *Radix Scutellariae* and the standard astragaloside A, with *n*-butanone - glacial acetic acid - water 7:1:2, detection by spraying with 2 % ferric chloride in 2N HCl - water 1:100, and heating at 105 °C, viewing in daylight; 2) for *Fructus Gardeniae* and the standard gardenoside, with the lower phase of chloroform - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol, and heating at 105 °C, viewing in daylight; 3) for *Radix Angelicae Sinensis*, with *n*-hexane - ethyl acetate - formic acid 45:5:1, detection under UV 366 nm.

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

111 142 Y. XU (Xu Yong)*, D. MAO (Mao Dan), J. LU (Lu Jiwei), Y. CHU (Chu Yanrong), K. WANG (Wang Ke), SH. JI (Ji Shen) (*Shanghai Inst. for Food & Drug Contr., Shanghai 201203, China): (Study of the method for the quality control of Fufang Jugeng Mahuangjian Tangjiang syrup ll) (Chinese). J. of Qilu Med. & Pharm. 31 (4), 574-576 (2012). Fufang Jugeng Mahuangjian Tangjiang syrup ll is a herbal TCM preparation for treating cough caused by asthmatic bronchitis. For quality control and to guarantee the safty and effectiveness of the medicine, TLC on silica gel 1) for ephedrine hydrochloride, with *n*-butanol - formic acid - water 4:1:5, detection by spraying with 0.2 % ninhydrin in acetone and heating at 105 °C, viewing in daylight; 2) for *Platycodon grandiflorus*, with chloroform - diethyl ether 1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight.

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

111 143 M. YANG (Yang Manqin), R. XIE (Xie Ruonan)*, J. MA (Ma Jun), M. XU (Xu Mingwei), D. HU (Hu Die) (*Acupuncture & Moxibustion Hosp. Affiliated to Anhui Coll. of Trad. Chinese Med., Anhui, Hefei 230061, China): (Study on the method for the Quality Control of Shisanwei Hezhong pills) (Chinese). J. of Anhui Coll. of Trad. Chinese Med. 32 (6), 80-82 (2012). Shisanwei Hezhong pills are a herbal TCM formulation for regulating the spleen and stomach and for treating epigastric pain. For quality control, TLC on silica gel 1) for *Rhizoma Corydalis* and the standard tetrahydropalmatine, with *n*-hexane - chloroform - methanol 15:8:2, detection by exposing to iodine vapors and viewing in daylight and under UV 366 nm; 2) for White Paeony Root and the standard paeoniflorin, with chloroform - ethyl acetate - methanol - formic acid 200:25:50:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:10 and heating at 105 °C, viewing in daylight; 3) for *Pericarpium Citri Reticulatae* and the standard hesperidin, with ethyl acetate - methanol - water 100:17:13, detection by spraying with 2 % aluminium chloride and viewing under UV 366 nm. Quantification of naringin by HPLC.

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

111 144 B. YANGZHONG (Tibet Municip. Inst. of Food & Drug inspection, Tibet, Lhasa 850000, China): (Study of the method for the identification of *Radix Angelicae Sinensis* in Shanhu Qishiwan pills by thin-layer chromatography) (Chinese). Chinese J. of Xizang Sci. & Technol.

231, 48-49 (2012). Shanhu Qishiwan pills are a traditional Tibetan herbal medicine used for relieving uneasiness of mind and body, invigoration of blood circulation and relief of pain. For quality control, TLC on silica gel with *n*-hexane - ethyl acetate 4:1, detection under UV 366 nm.

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

111 145 B. Yangzong*, C. Wangmu (*Tibet Municip. Inst. of Food & Drug inspection, Tibet, Lhasa 850000, China): (Study on a complementary method for the quality control of Ershiwuwei Luronghao pills by thin-layer chromatography) (Chinese). Chinese J. Ethnomed. & Ethnopharm. (15), 4-5 (2012). Ershiwuwei Luronghao pills are a traditional Tibetan herbal medicine for treating hepatomegaly, cirrhosis, liver and stomach pain. For quality control, TLC on silica gel 1) for *Meconopsis*, with cyclohexane - ethyl acetate 6:1, detection under UV 366 nm; 2) for *Syzygium aromaticum*, with toluene, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:200 and heating at 105 °C, viewing in daylight; 3) for *Radix Aucklandiae*, with the upper phase of cyclohexane - ethyl formate - formic acid 15:5:1, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:200 and heating at 105 °C, viewing in daylight.

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

111 147 W. YUAN (Yuan Weibin)*, X. HUANG (Huang Xiaobing), J. SHI (Shi Jiaping), X. WU (Wu Xueru) (*Orthopa. & Traumatol. Hosp. Affiliated to Guangzhou Univ. of Trad. Chinese Med., Guangdong, Guangzhou 510240, China): (Study of the method for the quality control of Guanjieyan Babuji preparation by thin-layer chromatography) (Chinese). Chinese J. of Guide for Trad. Chinese Med. & Pharm. 18 (7), 76-78 (2012). Guanjieyan Babuji preparation is a herbal TCM for treating arthritis and joint diseases. For quality control, TLC on silica gel 1) for Amur Corktree Bark and the standard berberine hydrochloride, with toluene - ethyl acetate - methanol - isopropanol - water 60:30:20:15:3, detection by exposing to ammonia vapors and viewing under UV 366 nm; 2) for *Fructus Psoraleae* and the standards psoralen and angelicin, with *n*-hexane - ethyl acetate 4:1, detection by spraying with 10 % KOH in methanol and viewing under UV 366 nm 3) *Radix et Rhizoma Rhei* and the standard emodin, with petroleum ether (30-60 °C) - ethyl formate - formic acid 15:5:1, detection under UV 366 nm; 4) for *Radix Astragali seu Hedysari*, with chloroform - methanol 10:1, detection by exposing to ammonia vapors and viewing under UV 366 nm.

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

111 148 R. ZHANG (Zhang Rui) (Maanshan Municip. Inst. for Food & Drug Contr., Anhui, Maanshan 243000, China): (Determination of leucine in Banlangen granules by thin-layer chromatography) (Chinese). J. Anhui Med. & Pharm. 17 (2), 202-203 (2013). Banlangen granule, processed of *Radix Isatidis*, is a traditional Chinese herbal medicine prescribed clinically to treat sore throat, cheek swelling, acute and chronic tonsillitis and mumps. For quality control a method for the determination of leucine, a main antiviral effective component, is presented. TLC on silica gel with *n*-butanol - glacial acetic acid - water 19:5:5, detection by spraying with 0.1 % ninhydrin in acetone, heating at 105 °C and viewing in daylight. Quantification by densitometry at 509 nm. Validation of the method by investigation of the linearity range (0.10-0.90 µg/zone, $r = 0.998$, n=5), precision (%*RSD* = 2.8 %, n=6), stability (%*RSD* = 0.8 %, n=6, towards single lane within one hour), and reproducibility (%*RSD* = 2.0 %, n=6, within plate). The recovery (by stan-

dard addition) was 98.8 % (%*RSD* = 1.1 %, *n*=6).

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

111 149 W. ZHANG (Zhang Wenwei), L. YAN (Yan Lili), ZH. CHEN (Chen Zhongxin)* (*Heilongjiang Univ. of Trad. Chinese Med., Heilongjiang, Harbin 150040, China): (Study of the method for the quality control of Fangji Fuling Tang decocted extract) (Chinese). J. of Inform. on Trad. Chinese Med. 29 (6), 96-98 (2012). Fangji Fuling Tang decocted extract is a herbal TCM preparation for the treatment of chronic nephritis edema, cardiac edema, etc. For quality control the method of the Chinese Pharmacopeia is improved. TLC on silica gel 1) for *Radix Stephaniae Tetrandrae* and the standards (+)-tetrandrine, hanfangichin B, and fangchinoline, with chloroform - methanol - acetone 6:1:1, detection by spraying with 5 % potassium iodobismuthate in hydrochloric acid - water 1:200 and viewing in daylight; 2) for *Radix Astragali seu Hedysari* and the standard astragaloside A, with the lower phase of chloroform - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol and heating mildly, viewing in daylight; 3) for *Radix Glycyrrhizae*, with the lower phase of ethyl acetate - formic acid - glacial acetic acid - water 15:1:1:1, detection under UV 366 nm. Quantification of (+)-tetrandrine, hanfangichin B, and fangchinoline by HPLC.

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

111 150 Y. ZHANG (Zhang Yuntian)*, Y. GAO (Gao Ying), X. PU (Pu Xianglan), Y. XU (Xu Yiliang) (*Jiangyin Tianjiang Pharm. Co., Ltd., Jiangsu, Wuxi 214434, China): (Study of the method for the quality control of Ganmaoting Peifang granules) (Chinese). J. of China Pharm. 22 (1), 20-22 (2014). Ganmaoting Peifang granule is a herbal TCM for the treatment of cold, influenza, cough, sore throat, etc. For quality control, TLC on silica gel 1) for *Fructus Arctii*, with chloroform - methanol - water 40:8:1, detection by spraying with 10 % sulfuric acid in ethanol and heating mildly, viewing in daylight; 2) for *Radix Isatidis*, with *n*-butanol - glacial acetic acid - water 19:5:5, detection by spraying with 0.1 % ninhydrin in propanone and heating at 105 °C, viewing in daylight. Quantification of arctiin by HPLC.

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

111 151 H. ZHAO (Zhao Haifeng)*, R. ZHAO (Zhao Rongjuan), M. ZHANG (Zhang Meng), J. ZHENG (Zheng Jie), CH. CUI (Cui Chunli) (*Shanxi Coll. of Trad. Chinese Med., Shanxi, Xianyang 712046, China): (Study of the effects of decoction on the medicinal ingredients of *Radix Aconiti Lateralis Preparata* and *Trichosanthes kirilowii* Maxim by thin-layer chromatography) (Chinese). Shanxi J. of Trad. Chinese Med. 33 (12), 1666-1667 (2012). It is highly debatable whether *Radix Aconiti Lateralis Preparata* is compatible with *Trichosanthes kirilowii* Maxim when both needed in processing single formulations. A method is presented to investigate the change of the chemical composition of the extracts obtained by separate and mixed decoction of two kinds of drugs. Preparation of the analytes by decocting, separately, of *Radix Aconiti Lateralis Preparata* (A), *Trichosanthes kirilowii* Maxim (B) and combined decoction of the two (C), then extracting, separately, with petroleum ether (30-60 °C) producing extracts A1, B1, C1 and with *n*-butanol producing extracts A2, B2, C2. TLC on silica gel with the upper phase of *n*-butanol - ethyl acetate - water 4:1:5, detection 1) under UV 366 nm; 2) by spraying with 10 % sulfuric acid in ethanol and viewing under UV 366 nm. By investigation of 5 batches of the drug sample, significant

difference was found between the analytes from single drug decoction and those from mixed drug decoction. The unknown ingredients found in the mixed decoction will be further characterized and analyzed. This study provides an experimental base for exploration of the mechanism of the interaction among the ingredients released from the two drugs.

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

111 152 X. ZHAO (Zhao Xin)*, X. LI (Li Xianyi), P. ZHANG (Zhang Peng) (*Inst. for Drug & Instr. Contr., Joint Logistics Dep., Shenyang Military Region, Chinese PLA, Liaoning, Shenyang 110026, China): (Study of a method for the quality control of Kuanzhong Laokou pills by thin-layer chromatography) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 23 (2), 502-503 (2012). Kuanzhong Laokou pills are a herbal TCM for treating thoracic abdominal bloating and stomach pain. For quality control TLC on silica gel 1) for *Rhizoma atractylodis macrocephalae*, with petroleum ether (60-90 °C) - ethyl acetate 50:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:200 and heating mildly, viewing in daylight; 2) for *Pericarpium citri reticulatae*, with ethyl acetate - butanone - formic acid - water 7:2:1:1, detection by spraying with 1 % ferric chloride in ethanol and viewing under UV 366 nm; 3) for *Radix aucklandiae*, with cyclohexane - ethyl acetate 17:3, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:200 and heating mildly, viewing in daylight; 4) for *Radix et rhizoma rhei*, with petroleum ether (30-60 °C) - ethyl formate - formic acid 15:5:1, detection under UV 366 nm or by exposing to ammonia vapor and viewing in daylight.

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

111 153 G. ZHENG (Zheng Guofeng) (Fuyang Inst. for Food & Drug Contr., Anhui, Fuyang 236015, China): (Study on the method for the quality control of Huoxiang Qushu Soft capsules by thin-layer chromatography) (Chinese). J. Strait Pharm. 24 (9), 53-55 (2012). Huoxiang Qushu Soft capsules are an approved herbal TCM preparation used for promoting perspiration, reducing internal heat, regulating spleen and stomach. For quality control, TLC on silica gel 1) for *Herba Pogostemonis*, with petroleum ether (30-60 ºC) - ethyl acetate - glacial acetic acid 85:15:1, detection by spraying with 5 % ferric chloride in ethanol and heating at 105 °C, viewing in daylight; 2) for *Radix Angelicae Dahuricae*, with petroleum ether (30-60 ºC) - diethyl ether 3:2, detection under UV 366 nm; 3) for *Folium Perillae*, with ethyl acetate - methanol - formic acid - water 18:1:2:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm; 4) for *Rhizoma Atractylodis*, with petroleum ether (30-60 ºC) - acetone 9:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 5) for *Rhizoma Pinelliae Preparatum*, with petroleum ether (30-60 ºC) - ethyl acetate - acetone - formic acid 60:12:10:1, detection under UV 254 nm; 6) for *Poria*, with toluene - ethyl acetate - formic acid 40:10:1, detection by spraying with 2 % vanillin in sulfuric acid - ethanol 1:200 and heating at 105 °C, viewing in daylight; 7) for *Radix Glycyrrhizae*, with ethyl acetate - formic acid - glacial acetic acid - water 15:1:1:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm.

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

111 154 L. ZHENG (Zheng Li), D. CHEN (Chen Dan)*, SH. FAN (Fan Shiming), L. ZENG (Zeng Lingjun), R. REN (Ren Ruiqin), B. WANG (Wang Baorong), SH. CHEN (Chen Shuyun) (*Coll.

of Pharm., Fujian Univ. of Trad. Chinese Med., Fujian, Fuzhou 350122, China): (Study of the method for the identification and quality control of Coix seed from different place of origin) (Chinese). J. of Fujian Univ. of TCM 22 (5), 52-54 (2012). Coix seed is a TCM herb frequently prescribed to treat edema, dermatophytosis, spasm and diarrhea. For quality control of the crude drug available from different places of origin, TLC of the herb extract and standard glycerol trioleate on silica gel with petroleum ether (60-90 °C) - ethyl acetate - glacial acetic acid 25:5:1, detection by spraying with 0.5 % vanillin in sulfuric acid - ethanol 1:200, heating at 105 °C for 5 min and viewing under UV 366 nm. Coix seed from different places of origin contains different amounts of glycerol trioleate (measured by HPLC).

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

111 155 M. ZHU (Zhu Meixiao), Y. CHEN (Chen Yan), J. YI (Yi Jinhai)*, Y. LIU (Liu Yunhua), A. CHEN (Chen Andong), X. LI (Li Xiaoliang) (*Sichuan Provincial Academy of Science for Trad. Chinese Med. & Pharm., Sichuan, Chengdu 610041, China): (Qualitative and quantitative determination of nodakenin in tradtional Chinese medicinal herbal drug *Notopterygium* root) (Chinese). Chinese J. of Lishizhen Trad. Med. & Pharm. 22 (1), 116-117 (2011). *Notopterygium* root is the dried root of *Notopterygium itwisum* Ting ex H. T. Chang and *Notopterygium forbesii* Boiss and is used to relieve rheumatic pains. Development of a method for qualitative and quantitative determination of nodakenin, one of the key active components in the crude drugs for quality control. TLC of the extracts of the drugs on silica gel with chloroform - methanol 4:1, detection at UV 366 nm. Quantification of nodakenin was done by HPLC.

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

111 156 X. ZOU (Zou Xiaohua), CH. ZHAO (Zhao Chao), X. ZHOU (Zhou Xin)* (*The Key Lab. for Inform. System of Mount. Areas & Prot. of Ecol. Environment, Guizhou Normal Univ., Guizhou, Guiyang 550001, China): (Study on the method for the quality control of *Emilia sonchifolia* (Linn.) DC. by thin-layer chromatography) (Chinese). J. of Guizhou Normal Univ. (Natural Sci.) 30 (3), 9-11 (2012). *Emilia sonchifolia* (Linn.) DC. is a TCM herb used in the formulations for treating conjunctivitis, tonsillitis, pneumonia, infectious hepatitis, dysentery, diarrhea, urinary tract infection, etc. For quality control TLC of the herb extracts an the standard emodin on silica gel with chloroform - methanol - formic acid 300:10:1, detection 1) under UV 366 nm; 2) by exposing to ammonia vapors and viewing under UV 366 nm.

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

37. Environmental analysis

111 157 B. MILZ, B. SPANGENBERG* (*University of Offenburg, Institute of Process Engineering, Badstrasse 24, 77652 Offenburg, Germany, spangenberg@hs-offenburg.de): 2D-thin layer chromatography (2D-TLC) flash test of 17alpha-ethinylestradiol and related steroids detected by fluorescence densitometry. J. Liq. Chromatogr. Relat. Technol. 36, 2378-2386 (2013). 2D-HPTLC of testosterone (1), progesterone (2), hydrocortison (3), estriol (4), ethinylestradiol (5), sitosterol (6), estrone (7), prednisolone (8), estradiol (9) and norethindrone (10) in waste water on cyanopropyl silica gel with dichloromethane - methanol - cyclohexane 19:1:12 in the first direction and water - acetonitrile - ethanol - dioxane 8:2:1:1+1 drop ammonia in the second direction. Detection by heating at 110 °C for 1 min followed by dipping into a mixture of sulfuric

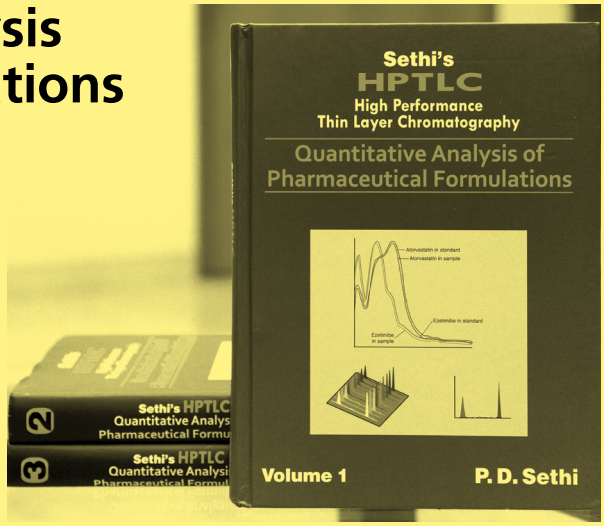
acid 98 % - water 1:49 and heating at 110°C for 10 min. Quantitative determination by absorbance measurement at 366 nm. The hR_F values for the first and second direction were 80 and 5 for (1), 72 and 9 for (2), 32 and 21 for (3), 8 and 31 for (4), 31 and 11 for (5), 80 and 0 for (6), 44 and 12 for (7), 12 and 34 for (8), 71 and 1 for (9) and 62 and 16 for (10). LOD and LOQ for 17alpha-ethinylestradiol were 1 and 2 ng/zone, respectively.

environmental, HPTLC, densitometry, quantitative analysis 37c

111 158 W.H. WEBER*, W. SCHULZ, A. MUELLER, S.C. WEISS (*Zweckverband Landeswasserversorgung, Betriebs- und Forschungslaboratorium, Am Spitzigen Berg 1, 89129 Langenau, Germany, weber.w@lw-online.de): Drinking water treatment - Identification of reaction by-products of 4- and 5-methyl-1H-benzotriazole formed during ozonation. CBS 109, 13-15 (2012). HPTLC-AMD of tolyltriazoles and by-products after ozone treatment of water samples, on LiChrospher silica gel (pre-washed with 2-propanol and dried at 120 °C for 30 min) by automated multiple development (AMD) using a 22-step gradient from methanol - formic acid 200:1 over dichloromethane to *n*-hexane. Detection under UV 254 nm and by spraying with 2,4-dinitrophenylhydrazine reagent, evaluation under white light. Densitometric measurement at 190, 200, 220, 240, 260, 280, and 300 nm before derivatization and at 380, 400, and 420 nm after derivatization. Coupling of HPTLC with HPLC-QTOF/MS using the TLC-MS Interface offline with water - acetonitrile 1:1 with 5 mmol ammonium acetate as extraction solvent and a flow rate of 0.2 mL/min.

environmental, HPTLC, densitometry, quantitative analysis 37c

HPTLC: Quantitative Analysis of Pharmaceutical Formulations



HPTLC:
Quantitative analysis
of pharmaceutical formulations
Volumes: 1 to 3
Pages: 1288
Editor: P. D. Sethi
Published: 2013
Publisher: CBS Publishers & Distributors Pvt ltd,
New Delhi, India

Dr. P. D. Sethi`s latest HPTLC book (vol. 1) titled "HPTLC: Quantitative analysis of pharmaceutical formulations" summarizes the analysis of 120 pharmaceutical formulations, the description of the pharmaceutical ingredients, chromatographic and measurement conditions, as well as spectra and references. This book starts with a general introduction to TLC and HPTLC, summarizing available plates, a description of plate handling as well as a short description of all necessary steps to obtain a good separation. The book also includes sample applications, plate pre-conditioning and development, plate drying, as well as plate detection and visualization. It also provides valuable tips on plate spraying, to stabilize a developed zone or how to select the suitable wavelength for densitometric scanning. Another topic is reversed-phase TLC/HPTLC and how to check sample stability by 2-dimensional chromatography. The reader can also find a chapter on TLC/HPTLC instrumentation and even a glossary of terms used in this field.

Although all work presented is based in CAMAG instrumentation, the reader will be able to get a basic understanding about the potential of HPTLC. The practical tips given at the end of the first chapter are very helpful. The second chapter deals with the very important subject of method validation. The most important parameters on this topic are discussed. For example, using UV-spectra for peak purity checking is presented as well as how to validate accuracy, precision, and reproducibility. Especially, the latter two parameters are often confused with one another. Therefore, it must be positively emphasized that this book clarifies these parameters. Eight pages deal with LOD and LOQ estimations, although these parameters are not so important for a pharmaceutical assay. More relevant are the discussed terms linearity and confidence interval which are clearly presented. The second chapter ends with a valuable practical glossary of terms related to HPTLC/TLC method validations. A strong point of this book is that it not only includes theoretical discussions but also typical examples of TLC and HPTLC method development and validation. Practical examples are given explaining the spirit behind the presented methods.

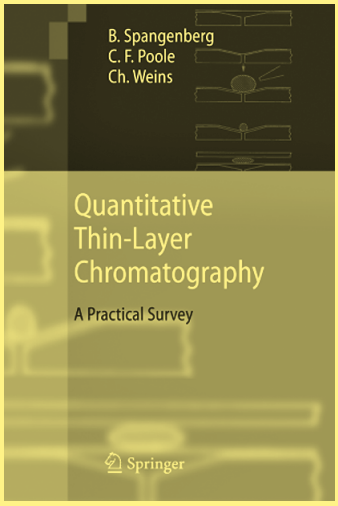
The following chapters describe the planar chromatographic analysis of pharmaceutical mixtures. For each drug or drug combination, the chemical structures of the pharmaceutical active compounds are given, as well as their densitograms and the UV spectra. The assay descriptions always comprise sample pretreatments, chromatographic conditions, densitometric evaluation, references, and comments. Chromatographic conditions stationar and mobile phases are presented, as well as the temperature, relative humidity, saturation conditions, migration distance, and developing time.

The experimental chapters start in chapter 3, including descriptions of analysing beta-blockers, anti-arrhythmic, antianginals, antihypertensive drugs, and diuretics. Chapter 4 in volume 2 presents the analysis of pharmaceutical active compounds in the field of musculoskeletal disorders. The analysis of analgesics, antipyretics, anti-inflammatory drugs and muscle relaxants are also presented here. Chapter 5 includes antibiotics, comprising antitubercular drugs, sulphonamides, penicillin, cephalosporins, anthelmintics, urinary anti-infectives, and antimalarial drugs. Chapter 6 covers expectorants, antitussives, mucolytics, bronchodilators, and antiallergic drugs. Chapter 7 in volume 3 presents antacids, ulcer healing drugs, anti-diarrhea, and digestive enzymes. The topics of chapter 8 are sedatives, tranquilizers, antiemetics, antinauseants, and antimigraine drugs. Chapter 9 presents the analytical methods for antifungals, anti-infectives, keratolytics as well as topical steroidal preparations. You can also find assays on rubefacients and eye, ear, and nasal preparations. Chapter 10 deals with hypolipidemic agents, anti-diabetics, anticoagulants, antithrombotics, antivirals, and oral contraceptives. Thus, all relevant drugs were recognized.

In summary, Dr. Sethi presents 528 planar chromatographic separation methods covering all fields of pharmaceutical preparations, on 1288 pages. This book is to be recommended for all scientists working in this field, because it comprises state-of-the-art planar chromatography in pharmaceutical analysis.

Prof. Dr. Bernd Spangenberg
University of Applied Sciences Offenburg

Quantitative Thin-Layer Chromatography



Quantitative
Thin-Layer Chromatography
A Practical Survey
B. Spangenberg, C.F. Poole, Ch. Weins
Springer-Verlag: Berlin, Heidelberg 2011
ISBN 978-3-642-10727-6
388 pages € 171,19

When in 2001 Szabolcs Nyiredy published his book "Planar Chromatography" subtitled "A retrospective view for the third millennium", he mentioned in the foreword that the time is right for this new book. Since that time, some more books dealing with TLC have been published, but ten years later the time surely was right for a new book entitled "Quantitative Thin-Layer Chromatography". During the last ten years, the instrumental TLC equipment was significantly extended and improved, allowing fully automated and highly reproducible HPTLC in terms of quantitative trace analysis. While the aesthetic plate image based analysis of botanical products is widely used and rather difficult to be such efficiently replaced by HPLC, the present book clearly signals the mostly ignored potential of modern instrumental HPTLC.

The book is organised in 14 chapters, beginning with a brief review of history of planar chromatography followed by the most important theoretical basis of thin layer chromatography, which must be understood by the planar chromatographer to avoid frustrations with TLC. Surveys on TLC layers, the characteristics of the mobile phase, and sample preparation and application techniques are presented in chapter 3, 4 and 5, respectively. More detailed information is given on development techniques (chapter 6) and specific staining reactions, including the preparation of reagents (chapter 7), clearly meeting the subtitle "A Practical Survey"; just post-chromatographic derivatisation makes TLC unrivalled. Chapter 8 adresses the bioeffective-linked analysis that is an upcoming great chance for TLC unlike HPLC. Planar chromatography detectors are surveyed in chapter 9, followed by more theoretical treatment of diffuse reflectance and fluorescence from TLC plates. The final chapters 12, 13 and 14 deal with chemometrics in HPTLC, statistics for quantitative TLC and planning an analysis and validation in TLC, respectively.

The book should clearly have the ability to advise chromatographers of the great potential of quantitative HPTLC and to revise their experience of former education. It is well made and attractively illustrated by a couple of coloured figures. Intensively reading the book is strongly recommended not only to beginners of TLC like students, but also to any chromatographer who is looking for alternatives not only by exchanging the HPLC column.

Gently critical comments apply to the ChromXtractor device mentioned in chapter 9.4, which is not "commercially available" anymore, but a more comfortable TLC-MS interface since 2009. With regard to the book title, quantitative aspects could be more intensively respected in both chapter 8 and 9.4, dealing with hyphenations clearly strengthening TLC.

Prof. Dr. Wolfgang Schwack

Institute of Food Chemistry
University of Hohenheim Stuttgart,
Germany

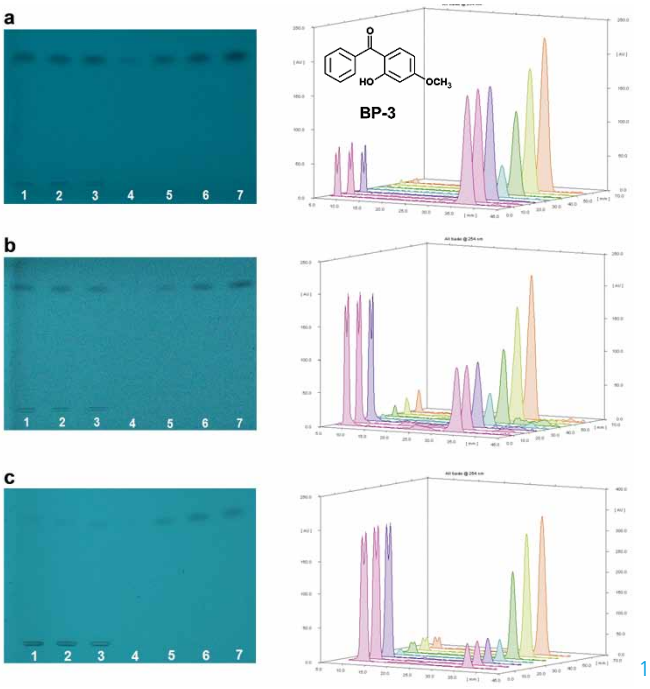
continuation from page 7

Densitometric evaluation

With TLC Scanner 3 at 254 nm, slit 3 x 0.3 mm, scan speed 20 mm/s, evaluation via peak area

Results and discussion

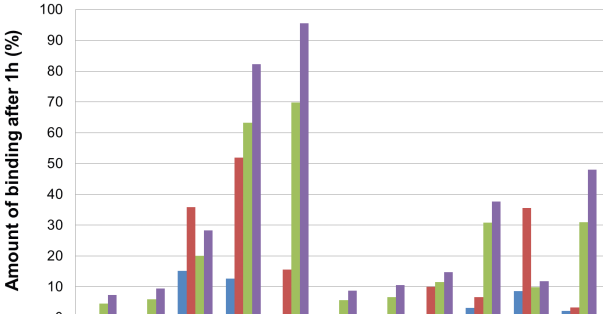
The UV filters that after reaction covalently bonded the amino phase at the start zone, are visible on the plate image, and can be quantified by the amount of the migrated UV filter (difference to the applied quantity).



1

Chromatograms and densitograms of amino plates with BP-3 after storage in the dark at room temperature (a), heating at 33 °C (b), and UV irradiation irradiated at 350 W/m² (c), 1 h each (with permission from [1])

Among the examined UV filters, the group of ketones and diketones showed by far the highest reactivity. As a negative control, silica normal phase and reversed phase plates were applied, when a binding of the UV filters was not observed at all. For all UV filters, there was a linear correlation between the fraction bonded to the amino phase and the reaction time. For BP-3, HMBS and OCR, after only 1 h storage in the dark (at RT), a noticeable amount was covalently bonded to the amino phase. Additional heating at 33 °C increased the adduct formation significantly. This was also observed for BM-DBM. Irradiation under natural sunlight or the sun simulator, initiated a strong reaction with the amino phase in the case of BP-3, HMBS, EHS, OD-PABA and BM-DBM, whereby the latter was nearly completely bonded after 1 h. The important UV filter EHMC was less reactive. However, bonding to the amino phase could be initiated both by heating and irradiation. The camphor derivatives 3-BC and 4-MBC and the triazones DEBT and EHT revealed the least tendency to form adducts.



	3-BC	4-MBC	HMBS	BP-3	BM-DBM	EHT	DEBT	EHMC	EHS	OCR	OD-PABA
Darkness	0,2	0,6	15,1	12,6	1,0	0,0	0,0	0,8	3,2	8,6	2,1
Heating at 33°C	0,7	0,8	35,9	51,9	15,6	0,3	0,4	10,0	6,6	35,6	3,3
Sunlight	4,5	6,0	20,1	63,3	69,8	5,6	6,6	11,5	30,8	9,8	31,0
Suntest CPS+	7,4	9,4	28,3	82,3	95,6	8,8	10,5	14,7	37,6	11,8	48,0

2

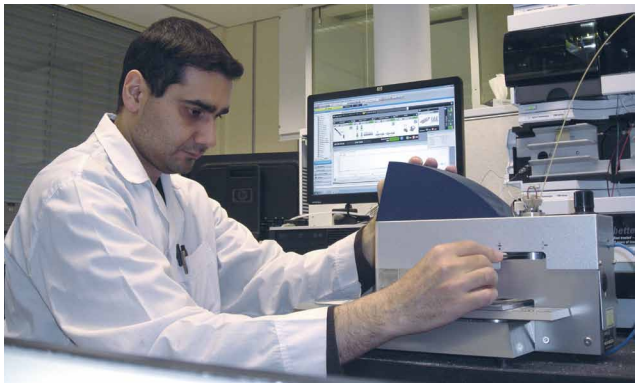
Comparing the results of the HPTLC screening with (photo) patch test data of the dermatological practice, just those UV filters that were known common triggers for allergic and photoallergic reactions, showed the greatest tendency to bond to the amino phase. Thus, the developed screening method is well suited to estimate the potential of different UV filters to form protein adducts and to identify possible skin sensitizers.

[1] C. Stiefel, W. Schwack, Int. J. Cosmet. Sci. (2013), in press

Further information is available on request from the authors.

Contact: Prof. Dr. Wolfgang Schwack, Institute of Food Chemistry, University of Hohenheim, 70599 Stuttgart, Germany, wolfgang.schwack@uni-hohenheim.de

Identification of acetylcholinesterase inhibitors from Galbanum



Hamid-Reza Adhami

As a part of his PhD study in the group of Professor Liselotte Krenn at the Department of Pharmacognosy, University of Vienna (Austria), Hamid-Reza Adhami investigated the combination of bioautography and HPTLC-MS/NMR as a fast method for identification of acetylcholinesterase (AChE) inhibitors from Galbanum [1]. The HPTLC related part of the research was performed at the CAMAG laboratory in Muttenz (Switzerland) while MS/NMR experiments were conducted at the University of Applied Sciences and Arts Northwestern Switzerland in Basel.

Introduction

Acetylcholinesterase inhibitors are used in the treatment of Alzheimer's disease and dementia. In the search for new natural compounds with acetylcholinesterase inhibitory activity Mr. Adhami focused his work on Galbanum, the oleo gum-resin from *Ferula gummosa* Boiss. The plant is native to central Asia and has been used in Iranian traditional medicine.

The isolation of active compounds from plant extracts is often laborious and time-consuming. It is therefore desirable to develop fast and reliable methods for the screening and characterization of bioactive compounds.

In this study the combination of HPTLC bioautography (an analytical technique in which organic compounds are separated by chromatography and identified by studying their effects on microorganisms or enzymes) with HPTLC–MS/NMR was a suitable and rapid approach to identify and characterize promising compounds from Galbanum which showed AChE inhibitory activity in a preliminary screening [2]. For Mr. Adhami's research HPTLC was the method of choice because it provided the necessary flexibility for combining various complementary detection modes.

Sample preparation

30 g of Galbanum were stirred with 200 mL of dichloromethane overnight, then sonicated for 30 min at 40 °C for reducing the extraction time and increasing the extract yield. The extract was concentrated under reduced pressure at 40 °C. Fractionation was performed using vacuum liquid chromatography with silica gel as the stationary phase and gradient elution with hexane and chloroform (20–100%) (preparative column chromatography using vacuum to move mobile phase, enabling high sample loading). Ten fractions were obtained (A1-A10).

Editors remark: 30 g were processed to obtain sufficient material for structure elucidation by NMR.

Standard solutions

For TLC bioautography a methanolic solution of chelidonine (0.35 mg/mL) was used as positive control.

Layer

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

Sample application

Bandwise with ATS4, band length 8 mm, track distance min. 10 mm, distance from lower edge 8 mm, distance from left edge min. 15 mm

Chromatography

In the ADC2 with chamber saturation for 20 min to a migration distance of 70 mm using various mobile phases (see image captions). Separation was affected by the activity of the adsorbent; therefore the plate was conditioned prior to both developments at 33 % relative humidity for 10 min using a saturated solution of magnesium chloride.

Documentation

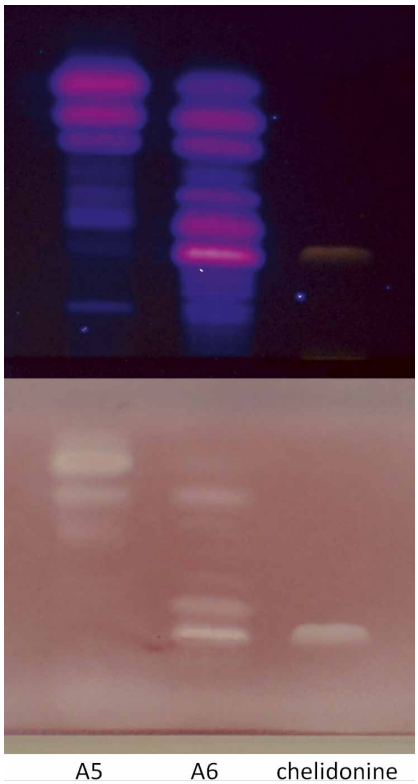
With TLC Visualizer documentation system under UV 366 nm.

Densitometry

TLC Scanner 3 with winCATS software, fluorescence measurement at 366/>400 nm, absorption measurement at 220, 245, 280, 310, and 420 nm (multi-wavelength scan).

HPTLC-MS

With TLC-MS Interface connected to a quaternary pump and an Agilent single quadrupole mass spectrometer with multimode source. Extraction solvent methanol, flow rate 0.4 mL/min for 2 min. Positive APCI mode, drying gas flow 5 L/min, nebulizer gas pressure 20 psi, drying gas temperature 350 °C, vaporizing temperature 250 °C, fragmentor voltage 70 V, capillary 4000 V, corona current 5 µA, charging voltage 2000 V.



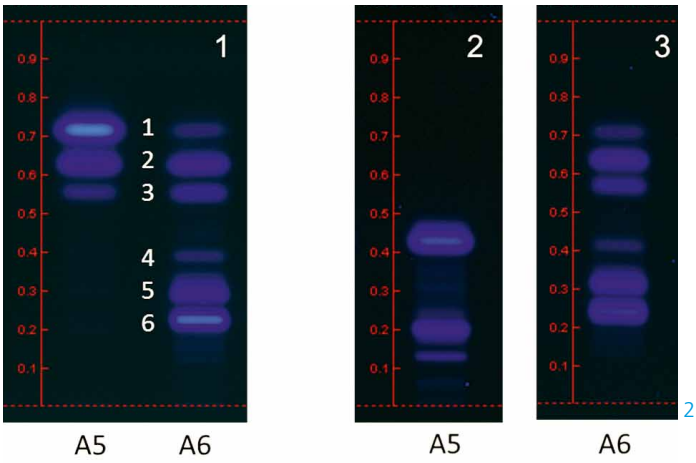
1

TLC separation of the 2 fractions of Galbanum as well as chelidonine standard with chloroform – ethyl acetate – methanol 50:5:1 under UV 366 nm (top) and with AChE inhibition (down)

Results and discussion

Ten fractions from the DCM extract of Galbanum were compared by HPTLC analysis followed by bioautographic detection for acetylcholinesterase inhibitory activity. Fractions A5 and A6 showed the highest activity.

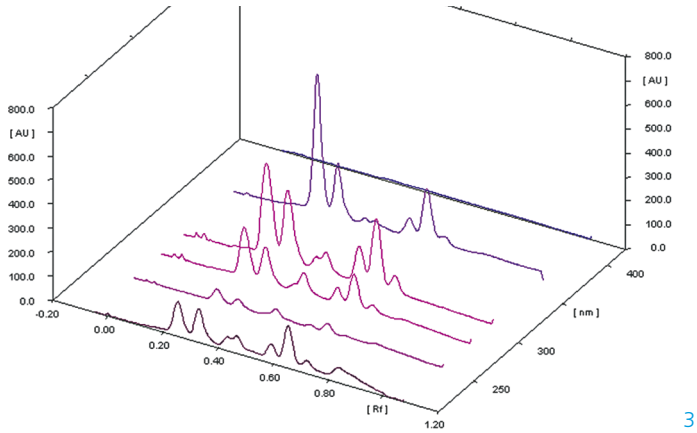
The HPTLC separation of these fractions was optimized according to the published guideline [3].



2

HPTLC separation of fractions and detection under UV 366 nm with
1) chloroform – ethyl acetate – methanol 50:5:1
2) chloroform – methanol 99:1
3) chloroform – ethyl acetate – methanol 95:10:2

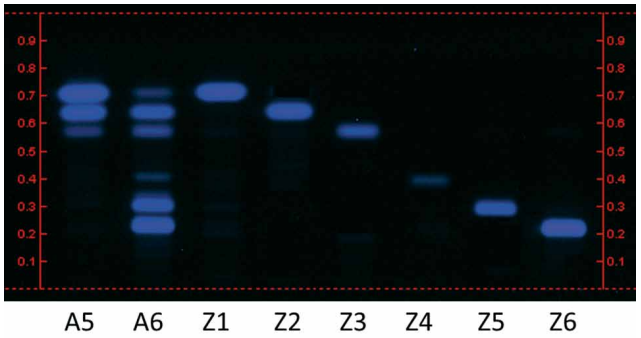
For purity control of the fractions the chromatograms were evaluated densitometrically using multi-wavelength scan.



3

Absorption measurement of A6 at 220, 245, 280, 310 and 420 nm

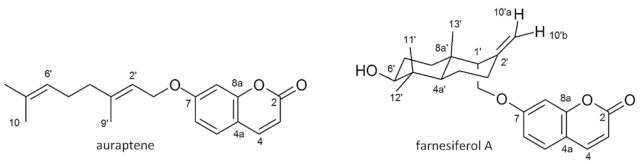
Zones 1 to 6 were extracted using the TLC-MS interface. The eluate of each zone was collected in a vial and concentrated by evaporation of the solvent. The residues were used for optimized HPTLC analysis.



4

HPTLC of fractions A5 and A6 and isolated zones (Z1–Z6) with chloroform – ethyl acetate – methanol 50:5:1, detection under UV 366 nm

Zones 1 and 6 gave the highest yield. For subsequent analysis by NMR spectroscopy and determination of AChE inhibitory effect fractions A5 and A6 were then applied on HPTLC plates with the ATS4 as 16 cm wide bands with concentrations of 5.0 µg/mm. From 20 HPTLC plates 1.58 mg of compound 1 and 1.75 mg of compound 6 were obtained. The molecular structures of these compounds were elucidated by one- and two-dimensional 1H- and 13C-NMR spectroscopy and mass spectrometry using an UHPLC-QTOF MS system. Compound 1 was identified as auraptene and compound 6 as farnesiferol A. The *in vitro* assay of AChE inhibition showed high activity with IC_{50} values of 47.5 µg/mL for auraptene and 17.6 µg/mL for farnesiferol A.



5

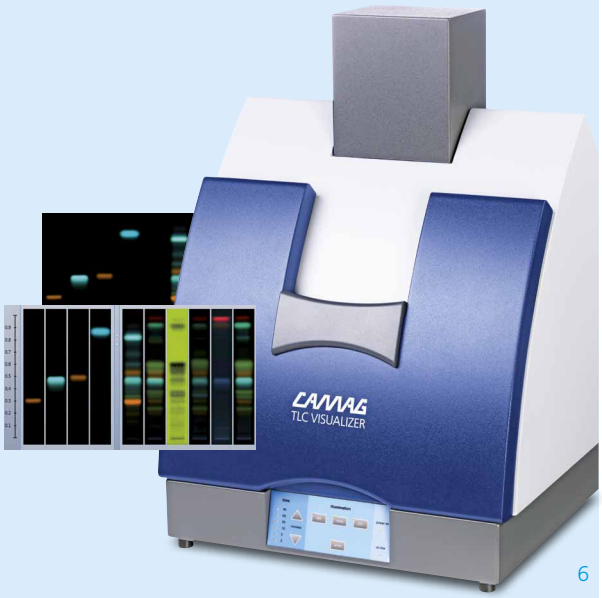
Molecular structures of auraptene and farnesiferol A

Further information is available on request from the author: Dr. H-R. Adhami, Department of Pharmaceutical Science, Tshwane University of Technology, Pretoria, South Africa. Email: AdhamiH@tut.ac.za

[1] H-R. Adhami *et al.*, Phytochem. Anal. (2013) DOI 10.1002/pca.2422.

[2] H-R. Adhami, H. Farsam, L. Krenn, Phytother. Res. 25 (2011) 1148.

[3] E. Reich, A. Schibli, High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Thieme, Stuttgart, 2006, S. 183.



6

CAMAG TLC VISUALIZER

The professional documentation and evaluation system with high-resolution 12 bit CCD camera

- Perfect homogeneity of objects under all illumination modes – UV 254 nm, UV 366 nm, and white light
- Automatic background correction
- Automatic image optimization for quantitative as well as qualitative image evaluation
- Image Comparison Viewer for comparative display of multiple images/plates on the same screen.
- User friendly CAMAG TLC software (winCATS or visionCATS)

Further information: **www.camag.com/tlcvisualizer**

In several of the application examples reported in this CBS the predecessor model CAMAG Digistore was used. The newer Visualizer offers specific advantages:

Compact design with integrated camera ensures improved homogeneity of object illumination.

The caption parameters are optimized and programmed in the IQ/OQ qualification procedure and remain fixed, ensuring high reproducibility of the images from plate to plate.

Viewing window for visual inspection with complete protection of the user from UV irradiation

Planar-chromatographic fingerprint of German propolis

Irina Scholl

As a part of her diploma thesis, Irina Scholl in co-operation with Dr. Annette Schroeder and Nadine Kunz of the Apicultural Institute in Stuttgart and in association with the company, WALA Heilmittel GmbH, has developed this rapid HPTLC method under the supervision of Professor Gertrud Morlock to investigate the profile of phenolic compounds of German propolis.

Introduction

Propolis is a complex product which bees (*Apis mellifera*) collect from resinous buds of different plants and use as a glue in their hive. It is often used in medicinal and cosmetic preparations due to certain distinct properties. Flavonoids and other phenolic compounds are the highly valued components of primary interest in propolis due to their interesting biological activities. The intention was to develop a method for the identification of the plant origin of the propolis samples which would also be suitable for screening the chemical profile of German propolis, which is still unknown.

105 propolis samples from Southern Germany were characterized with regard to their planar-chromatographic fingerprint and compared with 16 foreign propolis samples and 18 plant extracts [1, 2]. The ethanolic extracts of propolis and plant samples were evaporated to dryness, taken up in ethyl acetate and separated with HPTLC. Detection was performed after derivatization with natural product reagent A (Neu`s reagent) and fluorescence enhancement of zones with polyethylene glycol at UV 366 nm. For the high number of samples to be investigated, the HPTLC method proved to be very effective, rapid and cost-saving for control of the product quality.

Chromatogram layer

HPTLC plate silica gel 60 (Merck), 20 × 10 cm

Standard solutions

Methanolic standard mixtures were prepared with final concentrations between 3 and 37 ng/µL depending on substance [2].

Sample preparation

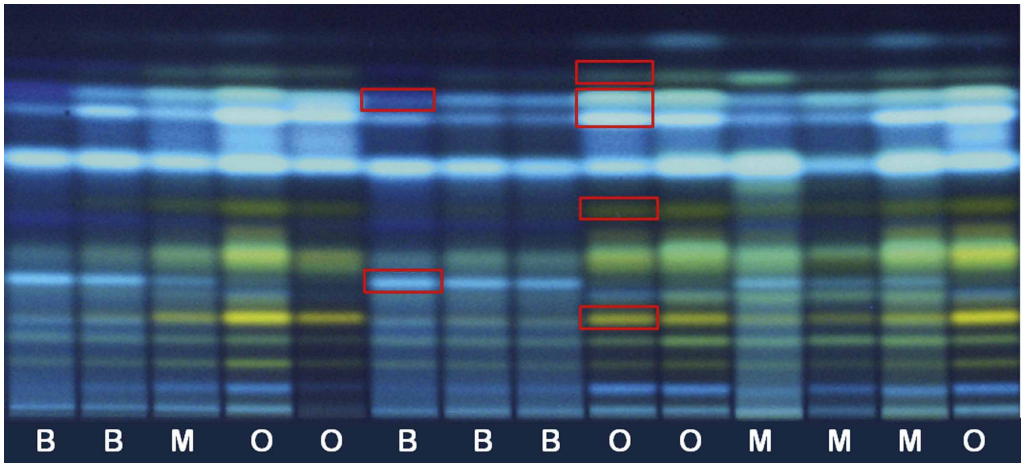
100 mg powdered propolis were heated with 3 mL ethanol for 2 min in a heating block until boiling with two-fold intermediate mixing using the vortex. After addition of 2 mL ethanol, the extract was centrifuged for 5 min at 10 °C and 3.8 g. The ethanol-soluble residue was evaporated to dryness, the residue was taken up in 1 mL and diluted 1:5 (v/v) with ethyl acetate. Samples were stored at 4 °C in the refrigerator.

Sample application

Bandwise with the Automatic TLC Sampler 4, 19 tracks, band length 8 mm, track distance 9.5 mm, distance from lower edge 8 mm, application volumes 0.5–2.5 µL (samples) and 10 µL (standards)

Chromatography

With 8 mL *n*-hexane – ethyl acetate – glacial acetic acid, 5:3:1 (v/v/v) and preconditioning with 5 mL hydrochloric acid (37 %) in the opposite trough of the twin trough chamber (20 × 10 cm). The filter paper was exactly adjusted to the side glass wall to avoid a smiley-effect of the front and wetted with the hydrochloric acid shortly before chromatography. Migration distance was 65 mm from the lower plate edge (migration time 45 min). Drying followed for 3 min.



1

HPTLC chromatogram of German propolis samples of orange (O), blue (B) and mixed (M) types with characteristic markers (marked red)

Postchromatographic derivatization

Immersion into the 0.5 % methanolic 2-aminoethyldiphenylborane reagent using the TLC Immersion Device (vertical speed 3.5 cm/s, immersion time 0 s). After intermediate drying, the plate was dipped into 5 % methanolic polyethylene glycol (PEG) solution for enhancement and stabilization of fluorescent zones. The reagents stored in the refrigerator were stable for at least 2 months.

Documentation

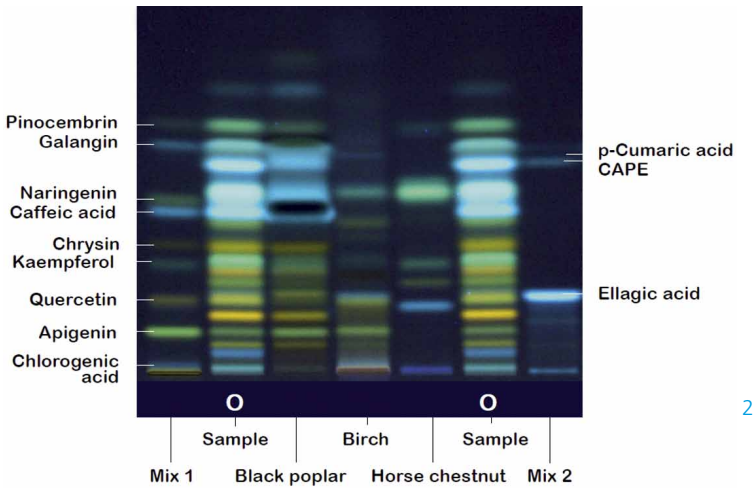
Chromatograms were documented with the DigiStore 2 Documentation System at UV 366 nm.

Results and discussion

Two different types of German propolis were found, *i.e.* an orange (O) and a blue (B) type. Mixed types (M) containing patterns of both O- and B-types were observed as well. Each planar-chromatographic fingerprint type showed a characteristic pattern of markers (marked red).

No correlation was observed between the yellow, red or brown propolis colors and the chromatographic fingerprint types O and B. Propolis extracts of 9 different locations from the years 2008, 2009 and 2010 were compared. 4 locations were dominated by the O type and 2 locations by the B type. At 3 locations just one sample of the B type differed from the normally dominating O type. This implies that the propolis composition of a single location tends to be stable and that *Apis mellifera* uses similar resinous plant origins within the location area.

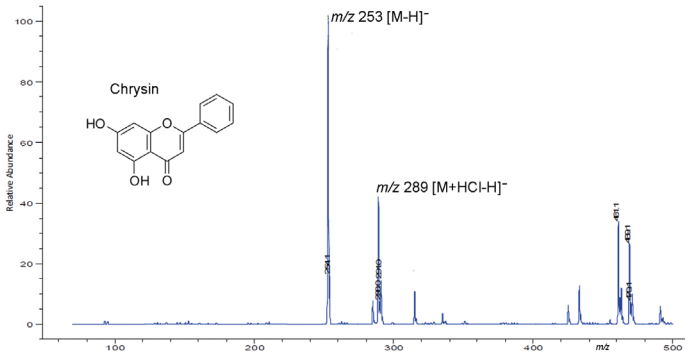
For identification of the plant origins of German propolis, HPTLC separations of plant extracts were performed. Extracts of the black poplar (*Populus nigra* L.) showed a very similar pattern to the O type samples and is most likely to represent the origin of this type. The B type correlated to a certain extent with the aspen (*Populus tremula*) as well as both types with horse chestnut (*Aesculus hippocastanum* L.).



2

HPTLC comparison of O type propolis samples with plant extracts and standards

The following substances were associated with both types by comparisons with mass spectrometric analyses: In the O type, among others, pinocembrin, galangin, CAPE (caffeic acidphenethylester) and chrysin, whereas in the B type *p*-cumaric acid and ellagic were identified. Caffeic acid, quercetin and apigenin appear in both types.

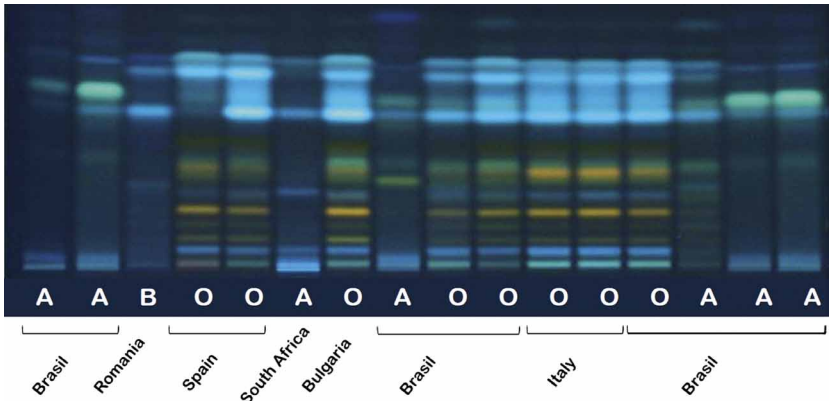


3

HPTLC-MS of a substance zone in an O type propolis sample assigned as chrysin

Propolis samples from other countries predominantly showed the O type and a B type, which slightly differed from the German B type. These samples were also comparable to the German propolis with regard to the sensory profile (color, odor).

Most of the Brasilian propolis samples had a color between green and black and had an unpleasant odor, displaying a different pattern with a bright green zone (assigned as alternative (A) type) that could not be detected in any German or even European propolis samples. The A type was not characteristic for the domestic flora.



4

HPTLC of foreign propolis assigned as orange (O), blue (B) and alternative (A) types

HPTLC was demonstrated to be a highly suited, simple and cost-saving analytical method for characterization and quality control of propolis. Comparisons with plant extracts allowed the identification of the regional or plant origin of propolis. Hyphenated HPTLC-MS supported the identification and assignment of marker compounds.

Further information is available on request from the authors.

[1] N. Kunz, I. Scholl, A. Schroeder, G. Morlock: Planar-chromatographischer Fingerabdruck von deutschem Propolis, Poster auf der AG der Institute für Bienenforschung, 29.–31.03.2011, Berlin

[2] G. Morlock, A. Schroeder, N. Kunz, I. Scholl, submitted

Contact: Prof. Dr. Gertrud Morlock, Justus Liebig University Gießen, Institute of Nutritional Science, IFZ, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, Gertrud.Morlock@ernaehrung.uni-giessen.de

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1

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For all HPTLC methods reported in this CBS the ATS 4 was used for sample application.

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