CBS 112 • MARCH 2014

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# 112

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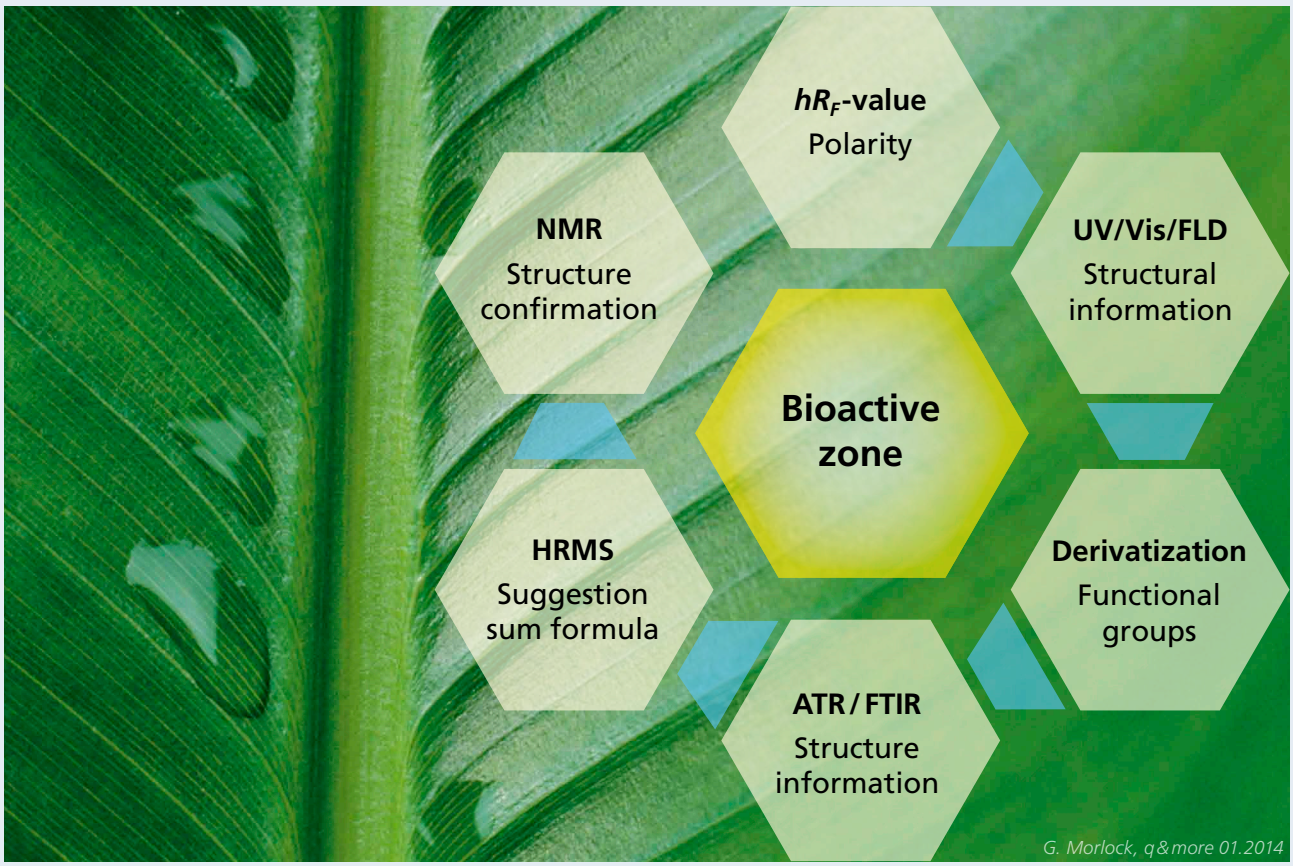


# From the bioactive zone to the structure – hyphenations for effect-directed analysis at an analytical level

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# Bioassay-guided isolation of plant antibiotics

*Dr. Ágnes Móricz, Dr. Péter Ott*

Dr. Ágnes Móricz, analytical biochemist at Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, employs liquid chromatographic techniques especially planar chromatography to separate bioactive natural products. She is working together with her biologist colleague Dr. Péter Ott on coupling planar chromatography with bioassays, focusing on the search and isolation of antibacterial compounds from plant sources.

## Introduction

There is a great demand for new, easy-to-obtain, bioactive agents in the fight against various human, animal and plant diseases. This is especially true for the antimicrobials, since infections are extremely difficult to control, even in the finest hospitals because of the over use of antimicrobials. As a consequence, resistance in pathogens is much more common than ever before. Plants producing an arsenal of secondary metabolites are used for modern drug discovery as an untapped source of bioactive substances. However, the isolation of pure compounds having the desired activity is time-consuming and expensive. Therefore there is a tendency to focus on the isolation and determination of only the active components. For this purpose the effect-directed analyses are essential, as they ensure that only the active fractions are led along the process, discarding the uninteresting ones. Bioassay-guided isolation usually incorporates extraction, fractionation and purification steps and requires continuous bio-monitoring as guidance, for which planar chromatography hyphenated with bioassays is especially suited [1-5]. This study is focused on the usability of TLC/HPTLC-direct bioautography (DB) as guidance in the search for antibacterial plant ingredients.

**TLC/HPTLC-DB is a high-throughput, rapid, relatively simple though reliable method that fulfills the requirements needed for continuous bio-monitoring. It is less time-consuming than the commonly applied agar-diffusion and agar-dilution antimicrobial tests. In contrast to the latter tests as well as to spectrophotometric methods (in liquid phase using vital dyes), the advantage of TLC/HPTLC-DB is the analysis of single components in various matrices, thus eliminating synergistic and antagonistic effects.**

## Chromatogram layer
TLC and HPTLC plates silica gel 60 $F_{254}$ (Merck)

## Standard solutions
Ethanolic solutions of chamomile essential oil (4 mg/mL), herniarin (1 mg/mL), umbelliferone (1 mg/mL), alpha-bisabolol (0.25 mg/mL), and spiroethers obtained by flash chromatography (0.2 mg/mL).

## Sample preparation
1.5 g of chamomile (*Matricaria recutita* L.) flowers were macerated for 48 h with 10 mL of ethanol – water 1:1 or pure ethanol or methanol in 20 mL screw-capped glass bottles. Samples were vortexed for 30 s. The filtered supernatant was directly used for application.

For flash chromatography, methanolic extraction was scaled up to 200 g of chamomile flowers. The concentrated extract was fractionated using preparative silica gel column with chloroform as mobile phase. Percolation was accelerated by nitrogen gas.

## Sample application
With Linomat IV the flash chromatographic fractions were applied spotwise spaced 4 mm, the other extracts and standard solutions were applied as 5-mm bands with a track distance of 7 mm, distance from bottom edge 8 mm. Application volumes ranged from 1 to 10 µL.

## Chromatography
In 20 x 10 cm Twin-Trough Chamber with chloroform – acetone 99:1 (v/v) or dichloromethane up to a migration distance of 90 mm (TLC) and 75 mm (HPTLC); drying time 5 min

## Post-chromatographic (chemical) derivatization
After documentation under UV 254 and 366 nm derivatization was performed by dipping in vanillin reagent (200 mg vanillin and 1 mL sulphuric acid in 50 mL ethanol) or DPPH• reagent (0.02 % 2,2-diphenyl-1-picrylhydrazyl in methanol) for information on radical scavenging activity.

## Biological detection (bioassay)
Immersion of a plate section in a suspension of the soil bacterium *Bacillus subtilis* (Bs), the marine bacterium *Aliivibrio fischeri* (Af), and the plant pathogenic bacteria *Pseudomonas syringae* pv. *maculicola* (Pm) and *Xanthomonas vesicatoria* (Xv).

1

**CAMAG AMD 2 System**
Automated Multiple Development of thin-layer chromatograms

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

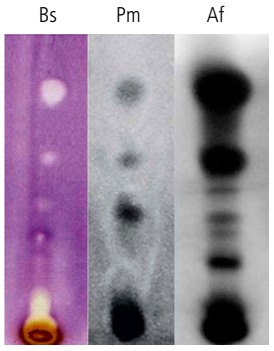
**Result**
- Due to the stepwise elution gradient, combined with the focussing effect of the subsequent runs, extremely narrow bands are formed with typical peak widths of about 1 mm.
- Over the available separation distance of 80 mm more than 40 components can be baseline separated.
- This ensures the highest resolution that can be attained with a planar chromatography system.
- The system meets all the requirements of GLP/GMP and 21 CFR Part 11.

**Applications of the AMD method most often used**
- Environmental protection – Impurities in water, contaminations in soil
- Lipids, phospholipids
- Ingredients of plants and other natural products

*In the next CBS issue an interesting AMD application in water analysis will be presented.*

## Results and discussion

In TLC/HPTLC-DB-directed isolation each step of the process, including extraction, separation, fractionation, and purification, was systematically followed by DB, and the next step was arranged according to its result. The inhibition effect of separated chamomile components against Bs, Pm and Af is shown. The Bs bioautogram was visualized by a yellow tetrazolium salt (vital dye) that is reduced to bluish formazan in the presence of metabolically active bacteria. So the bright spots against the blue background indicate the antibacterial activity. With luminescent bacteria the antibacterial activity could be directly observed as dark zones on Pm and Af bioautograms. Some inhibition zones at the same $hR_F$ were revealed by all three bacteria strains.

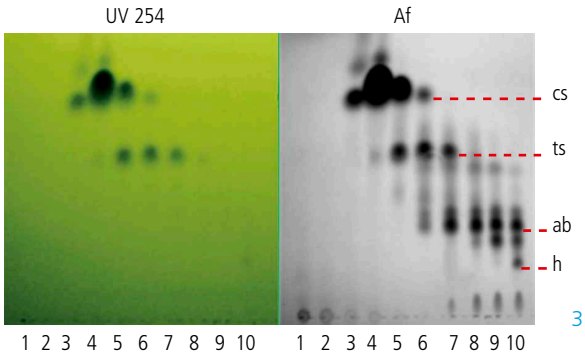


2

*TLC-DB results of chamomile extract separated with chloroform – acetone 99:1*

The chamomile extract was fractionated using flash chromatography and the fractions were analyzed by TLC. The eluted active compounds were identified by GC-MS as cis-spiroether (cs), trans-spiroether (ts), alpha-bisabolol (ab), herniarin (h), bisabolol oxides (bos) and umbelliferone (u).

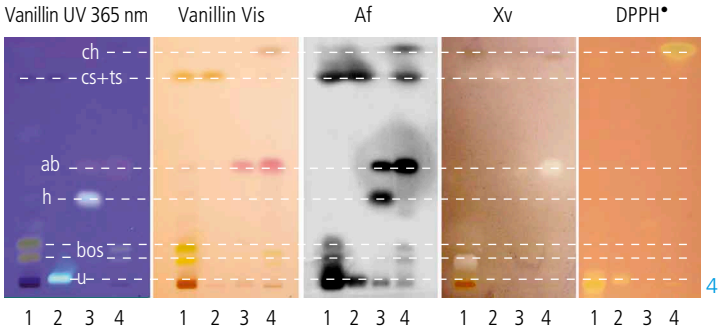
*Editor´s note: Bandwise application would improve resolution between adjacent spots also for TLC.*



3

*TLC-DB of chamomile fractions 1–10 after flash chromatography separated with chloroform*

For verifying the antibacterial activity of the identified active chamomile components, further effect-directed investigations were carried out by HPTLC-DB and HPTLC-DPPH•. The essential oil component chamazulene (ch, below solvent front), artificially produced from matricine during steam distillation, showed the strongest antioxidative capacity. It was revealed as bright zone after derivatization with the DPPH• reagent.



4

*Chromatograms of chamomile extract (1), pure components (2, 3) and essential oil (4) separated with dichloromethane and detected with various reagents and bioassays*

The basic aim of screening plant extracts is finding new effective agents. However, it is unavoidable to discover active components that have already been described. But the possibility of running many samples and standards in parallel ensures a rapid detection of known components simultaneously with sample analysis. To conclude, this fast and reliable HPTLC-DB is considered as effective tool for bio-monitoring of the isolation process of antibacterial plant components.

Further information is available on request from the authors.

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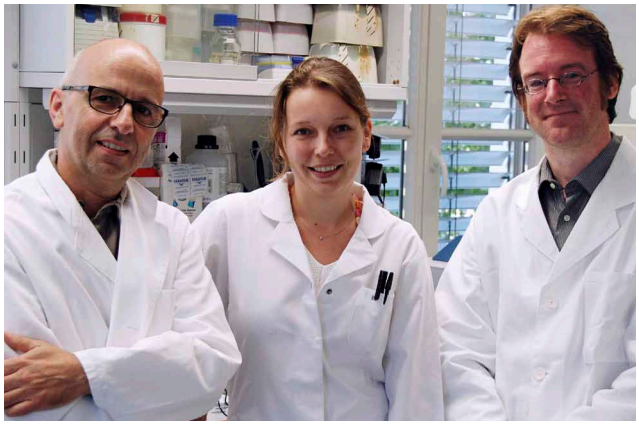
# Effect-directed analysis of environmental samples



*From left: Dr. Georg Reifferscheid, Denise Spira, Dr. Sebastian Buchinger*

The department Biochemistry & Ecotoxicology of the German Federal Institute of Hydrology (BfG) in Koblenz works on the development, validation and standardization of bioassays and bioanalytical instruments as a prerequisite for their implementation in water regulation. A current project contributes to the standardization of methods for the detection of estrogenic effects in water samples. Estrogenic compounds might enter surface waters by emissions of waste water treatment plants or might be caused as well by leaching of agricultural areas. The estrogenic effects can be caused by natural estrogens (for example 17ß-estradiole or its degradation product estrone) but also by xenoestrogens (*e.g.* nonylphenole from washing powder). One of the most common methods for the detection of estrogenic effects is a yeast cell based reporter gene assay (Yeast Estrogen Screen). The work of the department is linked to the ecotoxicological risk assessment of compounds in the water circle with special focus on river waters.

## Introduction

Environmental samples like water and sediments as well as their extracts, eluates or pore waters contain a huge number of compounds. Some of these compounds may cause adverse effects on the environment. Such ecotoxicological effects can affect the whole organism or might cause specific subacute effects such as mutagenicity, immunotoxicity or endocrine disruption (hormone like effects).

One of the major challenges in ecotoxicology is the identification of substances with undesirable effects in complex mixtures. The effect-directed analysis combines chemical separation methods with bioassays. Already well described is the coupling of planar-chromatography with the luminescent bacteria test [1]. In contrast, only some initial results for the coupling of planar-chromatography with the Yeast Estrogen Screen (planar-YES, p-YES) were published until now [2]. This kind of bioassay is currently developed in an expert group* aiming at (inter)national standardization. The present article describes the application of the p-YES bioassay [3] for the analysis of sediment extracts.

**The direct combination of planar-chromatography with specific bioassays offers a number of advantages. Due to the separation it is possible to analyze samples with complex matrices and even samples with acute toxic effects for estrogenic effects which is not possible with the common YES in microtiter plates. The classic test using microtiter plates detects estrogenic effects as a sum parameter which might be influenced by matrix effects. Compared with alternative separation methods such as HPLC it is advantageous that after the chromatographic separation of the sample on the plate the mobile phase evaporates rapidly and completely. A further advantage is the direct accessibility of the analytes for subsequent analysis, *e.g.* by mass spectrometry. The presented method is very robust and detects the estrogenic potential of a sample by means of an activity profile which may *e.g.* allow the evaluation of the elimination performance of a waste water treatment plant [4].**

## Standards

As positive control for estrogenic activity solutions of the standard compounds 17α-ethinylestradiole (EE2), 17ß-estradiole (E2), estrone (E1) and bisphenol A (BPA) in methanol or ethanol were used. (Concentrations range between 20 pg/mL and 200 µg/mL).

## Sample preparation

Sediment samples from a river section were transported at 4 °C, freeze dried after arrival, sieved <2 mm and ground with a ball mill. 10 g of each sample were extracted by fast solvent extraction (ASE method) with *n*-heptane – acetone 1:1. Removal of sulphur was done by tetrabutylammonium sulphite precipitation. The extracts were evaporated and re-dissolved in ethanol (2.5 g sediment equivalents per mL).

## Layer

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

## Sample application

Bandwise with Automatic TLC Sampler (ATS 4), band length 8 mm, distance from lower edge 10 mm, distance from the left and right side 15 mm, track distance minimum 10 mm, application volume 5 µL for of the standard solutions and for sample solutions 3 and 5 µL; depending on the sample volume and sample matrix rectangular application can be performed alternatively

## Chromatography

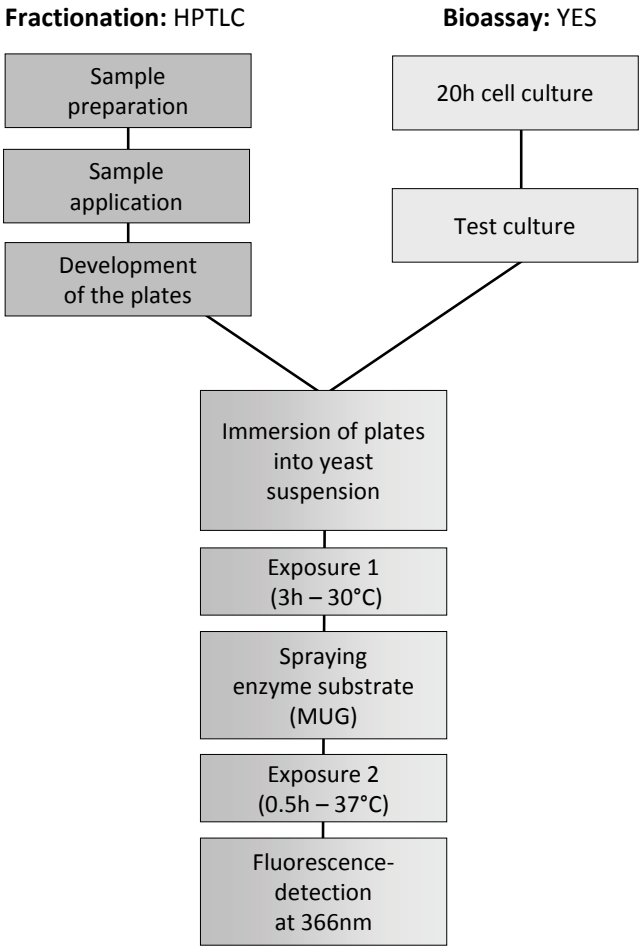
With the Automated Multiple Development AMD 2 System, first step with methanol to 20 mm followed by a second step with ethyl acetate – *n*-hexane 1:1 (v/v) to 80 mm

*Editor´s note: For short and fast focussing of the zones with methanol and the following development a Twin Trough Chamber can be used alternatively.*

## Detection with the p-YES-bioassay

The plate was immersed for 1 s in the prepared yeast cell suspension (vertical speed 5 cm/s) using the Chromatogram Immersion Device. The cell number of the genetically modified yeast strain (*saccharomyces cerevisiae* BJ3505) [5] was determined by photometric absorption measurement and diluted to 150 ± 50 Formazin Attenuation Units (FAU).

Subsequently, the immersed plates were incubated for three hours at 30 °C in a saturated steam atmosphere. For the development of the chromatogram an enzyme substrate solution (0.5 mg/mL 4-methylumbelliferyl-ß-D-galactopyranoside (MUG) solution: 50 mg/mL dissolved in DMSO and diluted 1:100 with LacZ-buffer pH 7) was sprayed homogeneously on the surface of the TLC-plate followed by a second incubation for 30 min at 37 °C. The activation of the reporter gene in the yeast cells, *i.e.* the




1

*Whole process of the p-YES-bioassay*

expression of the reporter enzyme ß-galactosidase is induced by estrogen like compounds on the surface of the plate. The enzyme is detectable by the application of the MUG-solution on the surface of the TLC-plate.

## Documentation

With TLC-Visualizer at 254 nm and 366 nm

## Densitometry

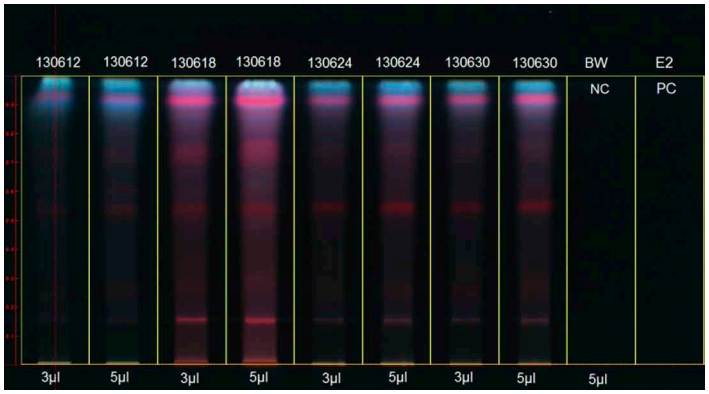
With TLC Scanner 4 under winCATS, fluorescent measurement at 320/> 400 nm with D2-lamp, slit dimension 6 x 0.3 mm, scanning speed 20 mm/s, evaluation by peak area

*Editor´s note: Normally the Hg-lamp is used for fluorescent measurement because of its higher emission intensity, thus alternatively the Hg-lamp at 313 nm can be chosen.*

## Results and discussion

Four different sediment extracts were analyzed for estrogenic effects using the p-YES bioassay. The

enzyme ß-galactosidase cleaves MUG resulting in the generation of a fluorophor. Blue fluorescent spots indicate compounds with estrogenic activity.

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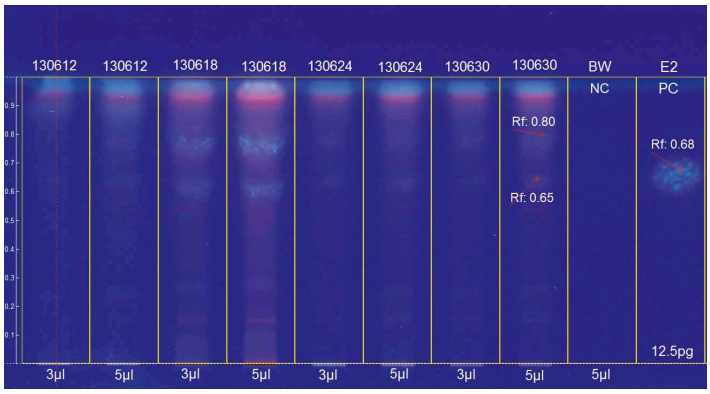


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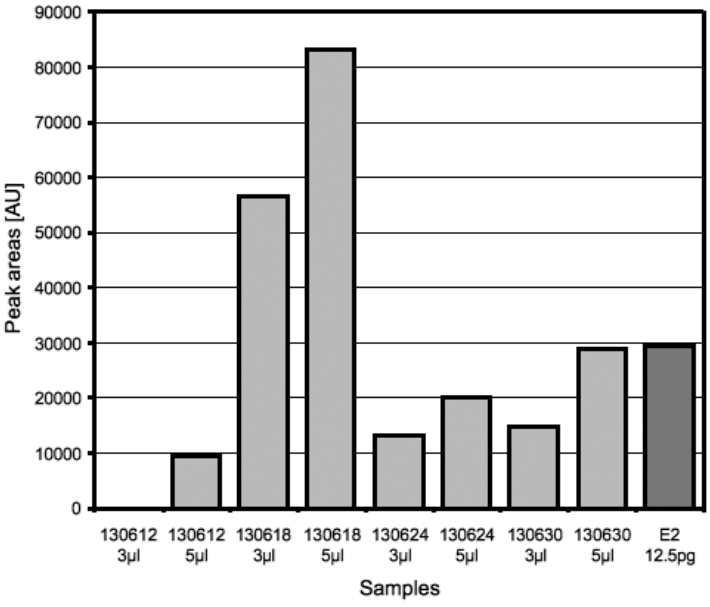
*HPTLC-chromatograms (UV 366 nm) of four ethanolic sediment extracts (track 1–8) plus blank value (track 9) and positive control 17ß-estradiole (track 10) before (top) and after the p-YES-bioassay (bottom)*




4

*Graph of the summed up peak areas for quantitative analysis*

The peak areas of the blue fluorescent spots ($hR_F$ 65 and 80) were summed up and compared to the positive control. The measured peak area is a dimension for the estrogenic activity of the samples. The diagram shows the expected dependency of the detected endocrine effects from the application volume. The estrogenic potential of the samples can be quantified approximately. The extract 130618 shows an elevated estrogenic activity compared to the other samples. However, the similar activity profiles of the four extracts indicate that the same compounds might be causing the detected estrogenic effects. The obtained results suggest that along the investigated river section the endocrine substances are similar in quality but differ in their quantity. The different overall estrogenic potential of the samples was also shown by the classic YES which was performed in parallel.

In sum the presented p-YES bioassay is a fast and robust screening method which can be used in effect-directed analysis of demanding complex mixtures for the detection of estrogenic activity. Major advantages o f the p-YES bioassay are its high tolerance for demanding matrices and the possible assignment of biological effects to individual compounds.

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Further information is available on request from the authors.

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*If interested in active participation in the expert group please contact ch.weins@gmail.com; further working groups involved: Prof. Dr. Schwack, University Hohenheim; Dr. Schönborn, ZHAW Wädenswill; Prof. Dr. Spangenberg, HS Offenburg; Dr. Weins, WBA Saarbrücken; Dr. Ankli, CAMAG; Prof. Dr. Morlock, JLU Gießen.

# Research and Development Department

*Dr. Matthias Loppacher Head of Research and Development*

Unlike with many companies all CAMAG instruments have been developed exclusively at the Swiss headquarters. When it is essential to guarantee that hardware mechanics and electronics are in harmony with instrument controlling software development, there is no substitute for an in-house team, a team of competent and creative members like the ones that compose CAMAG Research and Development.

Before joining CAMAG many of our team members had gained wide experience in research, project management and product management with some acquiring advanced technical and business management training. Also there is experience working in globally active technology groups, medical technology companies, consulting, Swiss hospitals, technical colleges and universities.

As most team members are highly qualified engineers from various fields, interdisciplinary cooperation is a must. Thanks to our good team spirit we have developed high quality instruments for planar chromatography which have been successful in global markets for decades.

Particularly noteworthy are the manifold suggestions by our customers integrated in the development process. This ensures the necessary worldwide acceptance of CAMAG instruments and enables the user to meet the requirements of daily lab work in research and routine analysis.

The greatest reward for our work is the happiness of our customers who remain loyal to CAMAG for years.

*Mechanical Design:*
*Mark Sturgess and Beatrice Käser*

*Software:*
*Nicolas Richerdt, Pierre-Yann Bridé and Dany Damaj*

*Electronics and Firmware:*
*Christoph Fankhauser and Diego Haldemann*

*Testing:*
*Sirin Zur Werra*

*Optics, Image Processing and Instruments:*
*Hansruedi Brugger*

# CBS 112

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MARCH 2014


Liebe Freunde

Wirkungsbezogene Analytik (effect-directed analysis, EDA) ist das Thema dieser CBS-Ausgabe. Der durch HPTLC-EDA mögliche Informationsgewinn umfasst die Identifizierung und Charakterisierung wirksamer Zonen bis hin zur Strukturaufklärung analytischer Mengen, wie auf der Titelseite skizziert. Im Gegensatz zu säulenchromatographischen Trenntechniken kann HPTLC leicht mit EDA kombiniert werden, weil das offene planare System mit unterschiedlichen Detektionsmethoden kompatibel ist. Durch die modulare Instrumentalisierung bleibt es trotz der vielen Kombinationsmöglichkeiten mit spektroskopischen/-metrischen Detektoren ein einfach zu handhabendes System. Auch die Datenmenge ist durch die zielgerichtete Detektion überschaubar. Ein grosser Vorteil ist die eluentenfreie Detektion. Nach der Chromatographie wird das Fliessmittel abgedampft, und das für den jeweiligen Detektor optimale Lösungsmittel kann eingesetzt werden. Dies ist besonders vorteilhaft für die EDA mit Bioassays, wie in zwei aktuellen Beiträgen gezeigt wird.

Das Programm für das *International Symposium for HPTLC* in Lyon vom 2.–4. Juli 2014 wird im April erscheinen (www.hptlc.com). Registrierung sowie Einreichung von Abstracts für *last minute poster* sind noch möglich – Details dazu finden Sie auf der letzten gelben Seite. Wir heissen Sie herzlich in Lyon willkommen! Ergreifen Sie die Gelegenheit, sich mit anerkannten Spezialisten auszutauschen und so Teil der wachsenden HPTLC-Expertengruppe zu werden.

Herzlichst


Gerda Morlock
cbs@camag.com


Dear friends

Focus of this issue is laid on effect-directed analysis (EDA). The information obtained by HPTLC-EDA comprises the identification and characterization of bioactive zones until the elucidation of their structures at an analytical level, which is illustrated on the front page. In contrast to column-derived techniques, EDA can easily be combined with HPTLC: The open planar system is adjustable to different detectabilities. Despite its hyphenation with various spectroscopic/-metric detectors, it remains simple through its modular instrumentation, and its production of low data amounts due to the targeted detection. One major advantage is the eluent-free detection. After chromatography, the eluent is evaporated and an optimal solvent with regard to the detector can be selected. The latter is advantageous for EDA with bioassays, shown in two contributions of this issue.

The program for the *International Symposium for HPTLC* in Lyon, 2–4 July 2014 will be available in April (www.hptlc.com). Registration and abstract submission for *last minute posters* are still open, for details see last yellow page. We would appreciate seeing you there! Take the opportunity to interact with recognized scientists and join the experts.

Sincerely,


Gerda Morlock
cbs@camag.com



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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

112 001 Danica AGBABA, J. SHERMA* (*Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA): Chromatographic methods of analysis: Thin layer chromatography. Encyclopedia of Pharmaceutical Science and Technology, fourth edition. Taylor and Francis: New York (2013) 450-462. This encyclopedia chapter describes the steps and equipment of TLC and HPTLC, such as sample preparation, stationary phases, mobile phases, sample application, chromatogram development, detection (zone visualization), documentation of chromatograms, quantitative analysis, method validation. Examples of applications in pharmaceutical and drug analysis are given. Special techniques are discussed: determination of lipophilicity, preparative layer chromatography, hyphenation with spectrometric methods or bioassays. The chapter features a comprehensive references/bibliography section.

pharmaceutical research, HPTLC, review 1b

112 002 J. SHERMA (Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA): Biennial review of planar chromatography: 2011-2013. Cent. Eur. J. Chem. 12(4), 427-452 (2014). This review summarizes and discussed the most important advances in planar chromatography published between November 1, 2011 and November 1, 2013. Included are an introduction to the current status of the field; student experiments, books, and reviews; theory and fundamental studies; apparatus and techniques for sample preparation, sample application, plate development; detection and identification of separated zones (chemical and biological detection, TLC-MS and TLC coupled with other spectrometric methods); techniques and instruments for quantitative analysis; preparative layer chromatography; thin-layer radiochromatography. The review features numerous applications on various substances and sample matrices and a comprehensive list of 250 references.

review 1

## 2. Fundamentals, theory and general

112 003 V. BEREZKIN*, S. KHREBTOVA (*Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences, 29, Leninsky pr., Moscow, 119991, Russia, berezkin@ips.ac.ru): Thin-layer chromatographic chambers with small and zero gas volume. J. Planar Chromatogr. 26, 386-394 (2013). Review of the role of TLC chambers in planar chromatography, and the merits of S-chambers in terms of reproducibility and higher peak resolution, allowing to implement 30 % faster separation and 20-30 % higher efficiency compared with unsaturated N-chamber.

pharmaceutical research, HPTLC, review 2a

112 004 L. KOMSTA*, R. SKIBINSKI, E. GOWIN, P. MACZKA (*Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland, lukasz.komsta@umlub.pl): Exploring hidden trends in classic and micellar thin-layer chromatographic retention of model compounds by chemometric methods. J. Liq. Chromatogr. Relat. Technol. 36, 2348-2362 (2013). Nonmicellar 2(1) and micellar (2) TLC of 35 model compounds with different functional groups on silica gel, RP-18 and CN phase with modifiers in hexane and water for (1) and on RP-8 with three surfactants, namely cetyltrimetylammoniumbromide, sodium dodecylsulfate, and Triton-X100 dissolved in water above CMC for (2). A principal component analysis (PCA) allowed for a molecular structure-retention relationship study.

pharmaceutical research, HPTLC, qualitative identification 2c

112 005 P. KRIZMAN, K. CERNELIC, A. WONDRA, Z. RODIC*, Ma. PROSEK, Mi. PROSEK (*National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia, zoran.rodic@ki.si): The importance of standardization in quantitative thin-layer chromatography - retrospective and case studies. J. Planar Chromatogr. 26, 299-305 (2013). The review described the main reasons that transformed standard TLC into a reliable and regulatory acceptable technique. Different aspects that allowed the incorporation of quantitative TLC to the QA systems such as automation, standardization of analytical procedures and operational qualification and performance qualification, as well as development of digital technology and new instruments are discussed.

quality control, review, HPTLC 2a

112 006 G. OROS, T. CSERHATI* (*Research Center for Natural Sciences, Hungarian Academy of Sciences, 1025, Pusztaszeri u. 59-67, Budapest, Hungary, szogyim@t-online.hu): Support related differental impact of substituents on performance of (alkoxy-phenyl)benzamides in normal phase TLC. J. Liq. Chromatogr. Relat. Technol. 36, 2363-2377 (2013). The impact of substituent of benzanilide moiety on retention of 14 (alkoxy-phenyl)benzamides was assessed by TLC on silica gel and aluminium oxide with various mixtures of *n*-pentane, benzene and acetone. The TLC method allowed for quantitative structure relationship (QSRR) studies with benzanilide derivatives.

pharmaceutical research, HPTLC, qualitative identification, QSSR 2c

112 007 Z. ROZMER, P. PERJESI* (*Institute of Pharmaceutical Chemistry, University of Pécs, Rókus u. 2, 7624 Pécs, Hungary, pal.perjesi@aok.pte.hu): (E)-2-benzylidenebenzocyclanones: Part X. determination of log P of (E)-3-benzylidene-2,3-dihydro-1-benzopyran-4-ones by RP-TLC. Effect on log P of incorporation of oxygen atom into carbocyclic chalcone analogues. J. Planar Chromatogr. 26, 284-288 (2013). Log P-TLC of (E)-3-benzylidene-2,3-dihydro-1-benzopyran-4-ones on silica gel with methanol - water 3:2. Detection under UV light at 254 nm. The effect of positions (ortho, meta, and para) of the aromatic substituents in the compounds was evaluated.

pharmaceutical research, qualitative identification preparative TLC, structure-lipophilicity relationship 2c

## 3. General techniques

112 008 Aneta GRYSINSKA*, P. PLOCHARZ, R. SZCZAP, T. DZIDO (*Department of Physical Chemistry, Medical University of Lublin, Lublin 20-093, Poland, aneta.halka@umlub.pl): Optimization of some variables of on-line injection in pressurized planar electrochromatography. J. Liq. Chromatogr. Relat. Technol. 36, 2512-2523 (2013). The paper described the influence of several operating variables of online injection into the pressurized planar electrochromatography (PPEC) separation system. Variables that enable narrow and symmetrical peaks such as sample volume, flow velocity, shapes of the grooves and polarization potential were discussed.

review, pressurized planar electrochromatography 3c

112 009 O. KAYNAR*, A. HAYIRLI (*Department of Biochemistry, Faculty of Veterinary Medicine, Atatürk University, Erzurum 25240, Turkey, okaynar@atauni.edu.tr): Evaluation of computational modifications in HPTLC with gel analysis software and flatbed scanner for lipid separation. J. Planar Chromatogr. 26, 202-208 (2013). HPTLC of egg yolk lipid fractions on silica gel with hexane - diethyl ether - formic acid 40:10:1. Detection by spraying with 10 % copper(II)

sulfate in 8 % phosphoric acid. Quantitation by scanning with a flatbed scanner and a gel analysis software. Intermediate/interday/intra-day precision was below 10 % CV ($n$=6).

quality control, food analysis, quantitative analysis, HPTLC 3f, 11c

112 010 I. KOWALSKA, L. CIESLA*, T. ONISZCZUK, M. HAJNOS, W. OLESZEK, A. STOCHMAL (*Department of Inorganic Chemistry, Medical University of Lublin, 20-093 Lublin, Poland, lukecarpenter@poczta.onet.pl): Comparison of two TLC-DPPH-image processing procedures for studying free radical scavenging activity of compounds from selected varieties of *Medicago sativa*. J. Liq. Chromatogr. Relat. Technol. 36, 2387-2394 (2014). The image processing programs ImageJ and Sorbfil TLC Videodensitometer were compared to study direct antioxidant properties of compounds separated from extracts obtained from *Medicago sativa*. Comparable results were obtained based on built-in functions present in both programs for processing TLC videoscans.

quality control, HPTLC, densitometry, quantitative analysis, comparison of methods 3f

112 011 W. LIU (Liu Wei), H. WU (Wu Haijun), X. WANG (Wang Xiupeng), Q. ZHU (Zhu Qing), Y. KANG (Kang Yanguo), A. HE (He Anqi), SH. WENG (Weng Shifu), ZH. YANG (Yang Zhanlan), J. XIA (Xia Jinming), Y. XU (Xu Yizhuang)*, J. WU (Wu Jinguang) (*State Key Lab. of Rare Earth Mat. Chem. & Appl., Coll. of Chem. & Mol. Engineering, Peking Univ., Bejing 100871, China): (Study on the preparation and application of barium fluoride particles used as the stationary phase in TLC-FTIR analysis) (Chinese). Chem. J. of Chinese Univ. 34 (6), 1347-1352 (2013). TLC is widely applied as an analytical technique due to its distinguishing features like its intuitiveness, high performance, inexpensiveness, etc., however, gives insufficient qualitative information of the separated ingredients. On the contrary, FTIR is a strong technique in functional identification and qualitative analysis towards pure compounds rather than mixtures. TLC-FTIR combines the merits of both techniques and realizes in situ detection of the interested analytes. In the search for a stationary phase suitable for TLC-FTIR the processing procedure for barium fluoride particles was improved. The particle size was minimized to enhance separation performance and to attenuate the spectrum baseline elevation caused by the effect of light scattering. Preparation of barium fluoride particles by reaction of 100 mL of a 0.6 mol/L barium chloride solution and 150 mL of a 0.95 mol/L sodium fluoride solution. The resulting sediment was filtered, washed, and burned in a Muffle furnace at 600 °C for 3 hours, which produced particles with a size of approx. 100 nm. The TLC plates were coated by precipitation/evaporation method: 5 g barium fluoride powder and 50 mL distilled water were mixed to a suspension, which was poured into a vessel containing the basal gold-plated glass sheet placed horizontally. After complete evaporation of the liquid a barium fluoride TLC plate was obtained with a smooth, even and mechanically strong surface. Demonstration by TLC of rhodamine B and malachite green on the new barium fluoride plate with methanol - acetone 3:10 with chamber saturation for 30 min. In situ identification of the separated zones by microscopic reflection FTIR. The results indicated that TLC-FTIR based on barium fluoride plates has wide application prospect.

qualitative identification 3b

112 012 A. SINHABABU*, S. BASU (*Natural Product Laboratory, Department of Chemistry, The University of Burdwan, Burdwan - 713 104, West Bengal, India, sinhababu04@yahoo.co.in): Modified ninhydrin reagents to detect colors of amino acid zones on thin-layer chromatographic plates. J. Planar Chromatogr. 26, 517-520 (2013). TLC of amino acids on silica gel with

1-propanol - water 7:3. Detection by spraying with (1) 1 % 2-hydroxybenzenecarbaldehyde solution in propanone; or (2) 1 % 4-bromobenzenecarbaldehyde solution in propanone (2), followed by heating at 110 ºC for 10 min. After cooling, all plates (treated with (1) or (2)) were sprayed with ninhydrin (indane-1,2,3-trione monohydrate) reagent and air dried. Amino acid zones in various shades from yellow to brownish-red were detected (detailed table provided in paper). Plates were heated again at 110°C for 10 min and color changes were recorded. The LOD were determined visually by comparison of standard solutions (1 mg/L) and further dilution until no zone was seen. LOD for (1) and (2) were in the range of 0.1-1.0 µg/zone and 0.01-1.0 µg/zone, respectively.

pharmaceutical research, HPTLC, postchromatographic derivatization, quantitative analysis 3e, 18a

## 4. Special techniques

112 021 Georgiana CRETU *et al.*, see section 8a

112 013 Q. GODAL, E. FERREIRA, J. WOLFENDER* (*University of Geneva, University of Lausanne, School of Pharmaceutical Sciences, EPGL, 30, Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland, Jean-Luc.Wolfender@unige.ch): Latest developments in assessing antifungal activity using TLC-bioautography: a review. J. AOAC Int. 96, 1175-1188 (2013). Review of different methods for antifungal screening. Latest developments in HPTLC, where the correlation of bioactivity and quantification was needed to evaluate the antifungal potential. TLC-bioautography was presented as a simple way to screen antifungal activities and two visualization approaches for this technique were described, as well as recent TLC antifungal bioautography applications.

review, HPTLC, qualitative identification, bioautography 4e

112 048 G. HORVATH *et al.*, see section 15a

112 014 A. SCHOENBORN*, A. GRIMMER (*Zurich University of Applied Sciences, P.O. Box, 8820 Waedenswil, Switzerland, andreas.schoenborn@zhaw.ch): Coupling sample preparation with effect-directed analysis of estrogenic activity - proposal for a new rapid screening concept for water samples. J. Planar Chromatogr. 26, 402-408 (2013). HPTLC of 17alpha-ethinylestradiol (1) and 17beta-estradiol (2) and estrone (3) on silica gel with chloroform - acetone - petroleum 11:4:5. The method was combined with the Yeast Estrogen Screen for the detection of estrogenic activity in aqueous samples. Quantitative determination by absorbance measurement at 366 nm. The $hR_F$ values of compounds (1) to (3) were 60, 50 and 67, respectively. LOD for (2) was 5 pg/zone.

environmental, HPTLC, quantitative analysis 4e, 13b

112 015 D. SPIRA*, G. REIFFERSCHEID, S. BUCHINGER (*Federal Institute of Hydrology, Am Mainzer Tor 1, 56068 Koblenz, Germany, spira@bafg.de): Combination of high-performance thin-layer chromatography with a specific bioassay - a tool for effect-directed analysis. J. Planar Chromatogr. 26, 395-401 (2013). HPTLC of 17alpha-ethinylestradiol (1), 17beta-estradiol (2) and estrone (3) on silica gel with chloroform - acetone - petroleum 11:4:5. The method was combined with the Yeast Estrogen Screen for the detection of estrogenic effects in sediment extracts of a river. Quantitative determination by absorbance measurement at 320 nm. The $hR_F$ values of compounds (1) to (3) were 74, 61 and 84, respectively. LOD and LOQ were calcu-

lated for the estrogenic model compound (1): LOD was 0.46 pg without prior development of the TLC plate and 0.48 pg after development, LOQ was 0.8 pg without development and 1.6 pg after development.

environmental, HPTLC, quantitative analysis, yeast estrogen screen 4e, 13b

## 5. Hydrocarbons and halogen derivatives

112 016 S. MENNICKENT*, R. FIERRO, M. VEGA, M. DE DIEGO, G. GODOY, C. CIFUENTES, A. MIRANDA (*Department of Pharmacy, Faculty of Pharmacy, University of Concepción, Concepción, Chile, smennick@udec.cl): Quantification of sertraline in human serum by high-performance thin-layer chromatography as a tool for pharmacotherapy adherence evaluation. J. Planar Chromatogr. 26, 358-362 (2013). HPTLC of sertraline in human serum on silica gel with toluene - ethyl acetate - methanol - glacial acetic acid 9:3:2:1. Quantitative determination by absorbance measurement at 220 nm. The $hR_F$ of sertraline was 45. Linearity was between 1 and 70 ng/zone. LOD and LOQ were 120 and 250 pg/zone. Recovery (by standard addition) was found to be 94.6-99.0 %. The % RSD values of intra- and inter-assay were between 0.9-2.8 % and 0.8-3.4 %, respectively.

clinical chemistry, research, HPTLC, quantitative analysis 5c

## 6. Alcohols

112 017 R. PANDEY*, S. SHUKLA, S. SARAF, S. SARAF (*Columbia Institute of Pharmacy, Pt. RSU, Raipur, C.G., India, ravindra56@rediffmail.com): Standardization and validated high-performance thin-layer chromatographic fingerprint method for quantitative determination of plumbagin in a traditional Indian formulation. J. Planar Chromatogr. 26, 440-444 (2013). HPTLC of plumbagin in herbal formulation on silica gel with toluene - ethyl acetate - methanol 8:1:1. Quantitative determination by absorbance measurement at 265 nm. The $hR_F$ value of plumbagin was 70. Linearity was between 200 and 600 ng/zone. LOD and LOQ were 3 and 8 ng/zone. Average recovery (by standard addition) was found to be 100.0 %. Intermediate intra- and inter-day precision ($n$=6) was below 0.05 %.

pharmaceutical research, herbal, HPTLC, quantitative analysis 6

112 018 N. RAMADAN*, H. MOHAMED, A. MOSTAFA (*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini, 11562 Cairo, Egypt, analpharm@yahoo.com): Chromatographic methods for the simultaneous determination of metoprolol tartrate and hydrochlorothiazide in the presence of hydrochlorothiazide degradation product. J. Planar Chromatogr. 26, 510-516 (2013). HPTLC of metoprolol tartrate (1) and hydrochlorothiazide (2) and hydrochlorothiazide degradation product (3) on silica gel with acetate - toluene - methanol - ammonium hydroxide 33 % 10:6:3:1. Quantification by absorbance measurement at 275 nm. The $hR_F$ values for compounds (1) to (3) were 8, 20 and 38, respectively. Linearity was in the range of 5-40 µg/zone for (1) and 3-10 µg/zone for (2). LOD and LOQ were 730 and 2180 ng/zone for (1) and 80 and 250 ng/zone for (2). Recovery was in the range of 98.4-101.9 % for both (1) and (2). Intermediate/interday/intra-day precision was below 1 %. The method showed comparable results with a validated HPLC method.

pharmaceutical research, quality control, quantitative analysis, HPTLC 6

## 8. Substances containing hetrocyclic oxygen

112 019 A. AL-TAWEEL, S. ALQASOUMI, P. ALAM, M. ABDEL-KADER* (*Department of Pharmacognosy, College of Pharmacy, Alexandria University, Alexandria 21215, Egypt, mpharm101@

hotmail.com): Densitometric-high-performance thin-layer chromatographic estimation of diosmin, hesperidin, and ascorbic acid in pharmaceutical formulations. J. Planar Chromatogr. 26, 336-342 (2013). HPTLC of diosmin (1), hesperidin (2), and ascorbic acid (3) on silica gel with ethyl acetate - methanol - water 15:3:2 for (1) and (2) and ethyl acetate - methanol - water - acetic acid 15:10:2:1 for (3). Quantification by absorbance measurement at 340 nm for (1), 286 nm for (2) and 265 nm for (3). The $hR_F$ values for (1), (2) and (3) were 34, 40 and 56, respectively. Linearity was in the range of 100-800 ng/zone for (1) and (2) and 50-400 ng/zone for (3). LOD and LOQ were 6 and 17 ng/zone for (1), 4 and 13 ng/zone for (2) and 4 and 13 ng/zone for (3), respectively. Recoveries were in the range of 98.1-99.3 % for (1), 98.4-99.5 % for (2) and 98.0-99.1 % for (3). Intermediate/interday/intra-day precision was below 2 % ($n$=6).

quality control, pharmaceutical research, HPTLC, quantitative analysis 8a, 11a

112 020 R. ARIMBOOR*, K. SALINI, C. ARUMUGHAN (*Agro processing and Natural Products Division, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram, Keralam, Pin 695019, India, ranjith.arbr@gmail.com): HPTLC estimation of oroxylin A in *Oroxylum indicum* herbal formulations. J. Planar Chromatogr. 26, 274-278 (2013). HPTLC of oroxylin A in *Oroxylum indicum* herbal formulations on silica gel with toluene - ethyl acetate - acetic acid 14:5:1. Quantitative determination by absorbance measurement at 275 nm. The $hR_F$ of oroxylin A was 80. Linearity was in the range of 250-1500 ng/zone. LOQ was 120 ng/zone. Recovery was in the range of 94.5-101.2 %. Intermediate/interday/intra-day precision was below 2 % ($n$=5). Comparable results were obatined with a validated HPLC method.

traditional medicine, quality control, quantitative analysis, HPTLC 8a

112 021 Georgiana CRETU, Gertrud MORLOCK* (*Justus Liebig University Giessen, Institute of Nutritional Science, Chair of Food Science, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany, gertrud.morlock@ernaehrung.uni-giessen.de): Analysis of anthocyanins in powdered berry extracts by planar chromatography linked with bioassay and mass spectrometry. Food Chem. 146, 104-112 (2014). HPTLC-Vis-ESI-MS of major anthocyanins in powder berry extracts of bilberry, blueberry, chokeberry, açai berry and cranberry on silica gel with ethyl acetate - 2-butanone - water - formic acid 35:15:4:6 in the first run (for anthocyanins) and ethyl acetate - toluene - formic acid - water 50:15:6:4 in the second run merely for the cut upper plate part (for anthocyanidins). Quantitation by absorbance measurement using a 4-point calibration at 520, 530 and 555 nm (multiwavelength scan). Correlation coefficients were between 0.9988 and 0.9999. Repeatability of sample analysis ($n$=3) was below 3.6 %. For confirmation of the results or characterisation of unknown anthocyane zones, mass spectra were recorded. Chromatography was directly linked to the effect using DPPH$^{\bullet}$ reagent and the luminescent *Aliivibrio fischeri* bioassay. The method demonstrated to be a rapid, visually appealing alternative to known HPLC methods.

food analysis, HPTLC, quantitative analysis, qualitative identification, comparison of methods 8a, 4e

112 022 V. LEBOT*, T. DO, L. LEGENDRE (*CIRAD, UMR AGAP, PO Box 946, Port-Vila, Vanuatu, lebot@vanuatu.com.vu): Detection of flavokavins (A, B, C) in cultivars of kava (*Piper methysticum*) using high performance thin layer chromatography (HPTLC). Food Chem. 151, 554-560 (2014). HPTLC of flavokavins A, B and C in the roots of kava (*Piper methysticum*) on silica gel with hexane - dioxane 4:1. Identification by absorbance measurement at 366 nm. Ratios between flavokavins (FKA, B, C) and kavalactones allowed an unambiguous identification of the

cultivar noble kava and exclusion of two-days kava in exported material.

herbal, HPTLC, qualitative identification 8b

112 023 P. SATI, V. AGNIHOTRI*, A. PANDEY (*Plant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora 263 643, Uttarakhand, India, vasudhaagnihotri@yahoo.com): Optimization of temperature and time length during post chromatographic derivatization of thin-layer chromatographic separation of ginkgolides and bilobalide standards. J. Planar Chromatogr. 26, 452-454 (2013). Optimization of temperature and duration required for detection of terpene trilactones. TLC of ginkgolides A, B and C and bilobalide on silica gel with ethyl acetate - hexane 9:1. Detection by spraying with acetic anhydride followed by heating at 80, 100, 120 and 140 ºC. The formation of alpha, beta-unsaturated gamma-lactone from ginkgolides was detected even at low temperatures for a respective heating time.

pharmaceutical research, HPTLC, qualitative identification 8b

112 024 R. SHARMA, F. AQIL, J. JEYABALAN, R. GUPTA, I. SINGH* (*Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, Punjab 160062, India, ipsingh@niper.ac.in): Quantitative analysis of *Eugenia jambolana* (Willd. ex O.Berg) for its major anthocyanins by densitometry. J. Planar Chromatogr. 26, 363-369 (2013). HPTLC of delphinidin-3,5-diglucoside (1), petunidin-3,5-diglucoside (2), and malvidin-3,5-diglucoside (3) in the fruits of *Eugenia jambolana* on silica gel with ethyl acetate - formic acid - acetic acid - water - methanol 126:19:10:32:12. Quantification by absorbance measurement at 529 nm. The $hR_F$ values for (1), (2) and (3) were 17, 24 and 34, respectively. Linearity was in the range of 400-1000 ng/zone for (1) and (2) and 200-500 ng/zone for (3). LOD and LOQ were 84 and 283 ng/zone for (1), 72 and 242 ng/zone for (2) and 43 and 154 ng/zone for (3), respectively. Average recoveries were 95.4 % for (1), 96.3 % for (2) and 97.3 % for (3). Intermediate/interday/intra-day precision was below 5 % ($n$=3).

food analysis, quality control, quantitative analysis, HPTLC 8a

112 025 M. SINGH, Y. KAMAL, E. TAMBOLI, R. PARVEEN, S. ANSARI, S. AHMAD* (*Bioactive Natural Product Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Hamdard University, Hamdard Nagar, New Delhi 110062, India, sahmad_jh@yahoo.co.in): Glabridin, a stable flavonoid of *Glycyrrhiza glabra*: HPTLC analysis of the traditional formulation. J. Planar Chromatogr. 26, 267-273 (2013). HPTLC of glabridin in a *Glycyrrhiza glabra* formulation on silica gel with toluene - dichloromethane - ethyl acetate 1:1:1. Quantitative determination by absorbance measurement at 287 nm. The $hR_F$ value for glabridin was 57. Linearity was in the range of 25-500 ng/zone. LOD and LOQ were 10 and 25 ng/zone. Recovery was in the range of 97.3-103.2 %. Intermediate/interday/intra-day precision was below 2 % ($n$=6).

traditional medicine, quality control, quantitative analysis, HPTLC 8a

112 026 A. YADAV, M. GUPTA* (*Analytical Chemistry Department, Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, India, guptammg@rediffmail.com): Quantitation of antitubercular compounds in *Oroxylum indicum*, a thai vegetable used in the Indian system of medicine. J. Planar Chromatogr. 26, 306-311 (2013). HPTLC of baicalein (1), hispidulin (2), chrysin (3), and oroxylin A (4) in different plant parts of *Oroxylum indicum* on silica gel with chloroform - methanol 97:3 + 1 drop water + 1 drop formic acid. Quantitation by absorbance

measurement at 270 nm. The $hR_F$ values for compounds (1) to (4) were 22, 2, 36 and 61, respectively. Linearity was in the range of 500-2500 ng/zone for (1) to (4). LOD and LOQ were 77 and 257 ng/zone for (1), 99 and 330 ng/zone for (2), 69 and 229 ng/zone for (3) and 45 and 151 ng/zone for (4), respectively. Average recoveries were 98.3 % for (1), 95.2 % for (2), 100.2 % for (3) and 98.0 % for (4). Intermediate/interday/intra-day precision was below 5 % ($n$=5).

herbal, quality control, quantitative analysis, HPTLC 8a

## 9. Oxo compounds, ethers and epoxides

112 027 N. ABDELWAHAB*, E. ABDELALEEM (*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Bani-Suef University, Alshaheed Shehata Ahmad Hegazy St., 62514, Beni-Suef, Egypt, nadasayed2003@yahoo.com): TLC-densitometric determination of guaifenesin, pseudoephedrine hydrochloride and guaifenesin related substance (guaiacol). J. Planar Chromatogr. 26, 73-77 (2013). TLC of guaifenesin (1), pseudoephedrine hydrochloride (2) and guaiacol (3) on silica gel with hexane - ethyl acetate - acetone - water - triethylamine 30:30:40:3:1. Quantitative determination by absorbance measurement at 208 nm for (1) and (2) and 278 nm for (3). The $hR_F$ values for (1) to (3) were 34, 12 and 83, respectively. Linearity was in the range of 2-12 µg/zone for (1), 14-25 µg/zone for (2) and 0.1-1.1 µg/zone for (3). LOD and LOQ were established for (3) only: LOD was 40 ng/zone and LOQ 100 ng/zone. Recovery (by standard addition) was between 99.9 and 100.0 %. Intermediate/interday/intra-day precision was below 2 % ($n$=3).

pharmaceutical research, quality control, quantitative analysis, HPTLC 9

## 11. Organic acids and lipids

112 019 A. AL-TAWEEL *et al.*, see section 8a

112 028 D. BEIDEMAN, B. FRIED*, J. SHERMA (*Biology Department at Lafayette College, Easton, PA 18042, USA, friedb@lafayette.edu): Effects of *Schistosoma mansoni* infection on the survival, fecundity and triacylglycerol content of *Biomphalaria glabrata* snails. J Vet Sci Med Diagn 2(3), 1-3 (2013). HPTLC of neutral lipids on silica gel (HLF plates with 19 scored channels of 9 mm width) prewashed with dichloromethane - methanol 1:1 and heated for 30 min at 120 °C, with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 with chamber saturation. Detection of dark zones on a yellow background after spraying with 5 % ethanolic phosphomolybdic acid reagent. Quantification of triacylglycerols by absorption measurement at 610 nm with polynomial regression via peak area.

HPTLC, quantitative analysis, qualitative identification, postchromatographic derivatization 11c

112 029 D. BEIDEMAN, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA, 18042, USA, friedb@lafayette.edu): Effects of coexposure with *Echinostoma caproni* and *Schistosoma mansoni miracidia* on neutral and polar lipids of *Biomphalaria glabrata* as determined by high-performance thin-layer chromatography-densitometry and observations on snail survival and fecundity. J. Liq. Chromatogr. Relat. Technol. 36, 2489-2496 (2013). HPTLC of neutral and polar lipids in *Biomphalaria glabrata* snails subjected to either *Echinostoma caproni* and *Schistosoma mansoni miracidia* coexposure, on silica gel with petroleum ether - diethyl eter 4:1 + 1 drop glacial acetic acid for neutral lipids and chloroform - methanol - water 65:25:4 for phospholipids. Detection by spraying with 5 % ethanolic phosphomolybdic acid for neutral lipids and 10 % cupric sulfate in 8% phosphoric acid for polar lipids. Quantitative de-

termination by absorbance measurement at 610 nm and 370 nm for neutral and polar lipids, respectively.

environmental, HPTLC, qualitative identification, quantitative analysis 11c

112 030 Meghan CICCHI, J. BOLSTRIDGE, Nevena POPOVIC, B. FRIED*, J. SHERMA (*Biology Department at Lafayette College, Easton, PA 18042, USA, friedb@lafayette.edu): High-performance thin-layer chromatographic analysis of the neutral lipid content of urine and feces in mice experimentally infected with *Schistosoma mansoni*. Trends in Chromatography 8, 1-6 (2013). HPTLC of neutral lipids and a neutral lipid standard (consisting of 20 % each of cholesterol, oleic acid, triolein, methyl oleate, and cholesteryl oleate) on silica gel (HLF plates with 19 scored channels of 9 mm width) prewashed with dichloromethane - methanol 1:1 and heated for 30 min at 120 °C, with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 with chamber saturation. Detection by spraying with 5 % ethanolic phosphomolybdic acid reagent and heating at 120 °C for 5 min. Neutral lipids appeared as blue zones on a yellow background. The fractions of free sterols, free fatty acids, triacylglycerols, methyl esters, and steryl esters were identified by comparison with the standard mixture. Quantification by absorption measurement at 610 nm via linear calibration (peak area).

pharmaceutical research, HPTLC, quantitative analysis, qualitative identification, postchromatographic derivatization 11c

112 009 O. KAYNAR *et al.*, see section 3f

112 031 S. DE*, P. NARIYA (*Research and Development Center, Valsad, Gujarat, India, subratde@gmail.com): A new, rapid online-HPTLC method for evaluation of DPPH reduction with ascorbic acid. J. Planar Chromatogr. 26, 21-25 (2013). Online HPTLC of 2,2-Diphenyl-1-picrylhydrazyl (DPPH; 1) and ascorbic acid (2) on silica gel with methanol - water 17:3. Quantitative determination by absorbance measurement at 517 nm. The $hR_F$ values for (1) and (2) were 81 and 86, respectively. Linearity was in the range of 2-6 µg/zone for (1) and 1-5 µg/zone for (2). The LOD and LOQ were 3.9 and 13 ng for (1) and 4.5 and 15 ng for (2), respectively. The method allowed for estimation of reduction of DPPH by ascorbic acid. To investigate the applicability of the online reaction, in one set of experiments the reaction of DPPH and ascorbic acid was carried out in test tubes (external reaction), and in another set the reaction was carried out on TLC plates (online reaction) by spiking through application by overspraying of DPPH and ascorbic acid on the same track.

pharmaceutical research, HPTLC, quantitative analysis 11a

112 032 S. DE*, P. NARIYA, N. JIRANKALGIKAR (*RMD Research and Development Center, Waghaldhara, Valsad, India, subratde@gmail.com): Development of a novel high-performance thin-layer chromatographic-densitometric method for the detection of tallow adulteration in cow ghee. J. Planar Chromatogr. 26, 486-490 (2013). HPTLC of tallow in cow ghee on silica gel with *n*-hexane - diethyl ether 2:3 + 1 drop glacial acetic acid for unsaponifiable fraction (1) and *n*-hexane - diethyl ether 13:7 + 1 drop glacial acetic acid for saponifiable fraction (2). Detection by spraying with 10% methanolic sulfuric acid reagent followed by heating at 110 ºC for 5-10 min. Zones at $hR_F$ values of 9 and 20 were identified as tallow concentration increased.

quality control, food analysis, qualitative identification, HPTLC 11c

112 033 D. GANDHI, P. MEHTA* (*Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University, Ahmedabad 382 481, India, drpritimehta@nirmauni.ac.in): Validated high-performance thin-layer chromatographic method for the quantification of betulinic acid from two indian plants of the species *Dillenia* and *Ziziphus*. J. Planar Chromatogr. 26, 331-335 (2013). HPTLC of betulinic acid in the bark and leaves of *Dillenia indica*, *Dillenia pentagyna*, *Ziziphus jujube* and *Ziziphus mauritiana* on silica gel with toluene - chloroform - ethanol 4:4:1. Detection by dipping into anisaldehyde - sulfuric acid reagent, followed by heating at 80 ºC for 15 min. Quantification by absorbance measurement at 525 nm. The $hR_F$ of betulinic acid was 71. Linearity was in the range of 100-1200 ng/zone. LOD and LOQ were 4 and 11 ng/zone, respectively. Recovery was in the range of 99.5-101.7 %. Intermediate/interday/intra-day precision was below 2 %.

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

112 034 Alexandra HUNSBERGER, B. FRIED*, J. SHERMA (*Biology Department at Lafayette College, Easton, PA 18042, USA, friedb@lafayette.edu): Effects of *Echinostoma caproni miracidia* dose on the neutral and polar lipids of *Biomphalaria glabrata* as determined by high-performance thin-layer chromatography. Acta Parasitologica 58(4), 615-618 (2013). HPTLC on silica gel (HLF plates with 19 scored channels of 9 mm width) for 1) neutral lipids with petroleum ether - diethyl ether - glacial acetic acid 80:20:1, detection by spraying with 5 % ethanolic phosphoric acid; and 2) polar lipids with chloroform - methanol - deionized water 65:25:4, detection by spraying with 10 % cupric sulfate in 8 % phosphoric acid. Quantitative determination by absorption measurement at 610 nm for neutral lipids and 370 nm for polar lipids. Polynomial regression via peak area.

HPTLC, quantitative analysis, postchromatographic derivatization 11c

112 035 A. JAMSHIDI*, N. DARVISHI, H. MAHDAVI (*Novel Drug Delivery Systems Department, Iran Polymer and Petrochemical Institute, P.O. Box 14185/458 Tehran, Iran, a.jamshidi@ippi.ac.ir): High-performance thin-layer chromatographic analysis of salicylic acid in release medium during development of an adhesive topical anti-acne patch. J. Planar Chromatogr. 26, 322-324 (2013). HPTLC of salicylic acid in a release medium of an adhesive patch on silica gel with toluene - methanol - glacial acetic acid 74:25:1. Quantification by absorbance measurement at 270 nm. Linearity was in the range of 57-339 ng/zone. LOD and LOQ were 25 and 75 ng/zone. Recovery was in the range of 96.8-103 %. Precision was below 4 % ($n$=6).

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

112 036 G. JANICSAK, E. RADNAI, R. ENGEL, G. BLUNDEN, I. MATHÉ* (*Institute of Pharmacognosy, University of Szeged, 6270 Szeged, Hungary, mathe@pharm.u-szeged.hu): TLC-densitometry of rosmarinic and caffeic acids in the evaluation of *Lamiaceae* species growing in central Europe. J. Planar Chromatogr. 26, 132-136 (2013). TLC of rosmarinic (1) and caffeic acids (2) in extracts of *Salvia species* on silica gel with toluene - ethyl acetate - formic acid 5:4:1. Quantitative determination by absorbance measurement at 325 nm. The $hR_F$ values for (1) and (2) were 40 and 60, respectively.

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

112 037 R. KANNAN, R. ARUMUGAM, T. THANGARADJOU, P. ANANTHARAMAN* (*Research Centre for Plant Growth and Development, School of Life Sciences, Universityof KwaZulu-

Natal Pietermaritzburg, Private Bag X 01, Scottsville 3209, South Africa, paraman_cas@yahoo.co.in): Phytochemical constituents, antioxidant properties and *p*-coumaric acid analysis in some seagrasses. Food Res. Int. 54, 1229-1236 (2013). HPTLC of *p*-coumaric acid in the leaves of *Enhalus acoroides* (L.f.) Royle, *Thalassia hemprichii* (Ehrenberg) Ascherson, *Halodule pinifolia* (Miki) den Hartog, *Syringodium isoetifolium* (Ascherson) Dandy, *Cymodocea serrulata* (R. Brown) Ascherson & Magnus and *Cymodocea rotundata* Ehrenberg & Hemprich ex Ascherson on silica gel with toluene - ethyl acetate - methanol - glacial acetic acid 16:2:1:1. Quantitative determination by absorbance measurement at 254 nm. The $hR_F$ value of *p*-coumaric acid was 42. Linearity was in the range of 100-500 ng/zone. LOD and LOQ were 15 and 45 ng/zone.

traditional medicine, herbal, quantitative analysis, HPTLC 11a

112 038 L. KOMSTA*, M. KOBYLKA (*Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland, lukasz.komsta@umlub.pl): Application of self modelling multivariate curve resolution to thin-layer chromatographic data obtained by densitometric detection. J. Planar Chromatogr. 26, 232-236 (2013). TLC of acetylsalicylic acid and salicylic acid on silica gel with hexane - ethyl acetate 2:1. Detection by scanning at 101 wavelengths, from 200 to 300 nm. Positive matrix factorization used as a curve resolution approach allowed for the evaluation of the inhomogeneity of the application zone. The method was also applied for the system ciprofibrate/clorfibric acid on silica gel with hexane - tetrahydrofurane 8:2 + 1 drop glacial acetic acid.

pharmaceutical research, preparative TLC, qualitative identification 11a

112 039 A. NIRANJAN*, S. VERMA, A. LEHRI, D. AMIA (*Central Instrumentation Facility, Council of Scientific and Industrial Research, National Botanical Research Institute, Lucknow 226001, India, abhishek_niranjan@yahoo.co.in): High-performance thin-layer chromatographic analysis for the simultaneous quantification of four phenolic compounds in green, red, and black fruits of *Trapa natans var. bispinosa* Roxb. (Singhara). J. Planar Chromatogr. 26, 316-321 (2013). HPTLC of gallic acid (1), caffeic acid (2), quercetin (3) and kaempferol (4) in the green, red, and black fruits of *Trapa natans var. bispinosa* Roxb. (Singhara) on silica gel with toluene - ethyl acetate - formic acid 13:11:2. Quantification by absorbance measurement at 282 nm. The $hR_F$ values for compounds (1) to (4) were 23, 34, 38 and 46, respectively. Linearity was in the range of 1000-1500 ng/zone for (1) to (4). LOD and LOQ were 116 and 351 ng/zone for (1), 135 and 409 ng/zone for (29, 92 and 278 ng/zone for (3) and 87 and 263 ng/zone for (4), respectively. Average recoveries were 98.5 % for (1), 98.2 % for (2), 96.4 % for (3) and 97.3 % for (4). Intermediate/interday/intra-day precision was below 3 % ($n$=6).

food analysis, quality control, quantitative analysis, HPTLC 11a

112 040 Alina PYKA*, P. BOCHENSKA (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, Sosnowiec, 41-200, Poland, apyka@sum.edu.pl): Use of TLC for the quantitative determination of acetylsalicylic acid, caffeine, and ethoxybenzamide in combined tablets. J. Liq. Chromatogr. Relat. Technol. 36, 2405-2421 (2013). HPTLC of acetylsalicylic acid (1), caffeine (2), and ethoxybenzamide (3) in combined tablets on silica gel with *n*-hexane - acetone - glacial acetic acid 7:2:1. Quantitative determination by absorbance measurement at 200 nm for (1), 275 nm for (2) and 300 nm for (3). The $hR_F$ values for (1) to (3) were 44, 29 and 59, respectively. Linearity was in the range of 1.7-15.4 µg/zone for (1), 0.6-4.0 µg/zone for (2) and 1.2-8.0 µg/zone for (3). LOD and LOQ were 130 and

390 ng/zone for (1), 25 and 80 ng/zone for (2) and 60 and 180 for (3), respectively. Recoveries (by standard addition) were in the range of 99-100 %. Intermediate intra- and inter-day precision was below 2 % (*n*=3).

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

112 041 D. SHAH*, D. SUTHAR, C. NAGDA, U. CHHALOTIYA, K. BHATT (*Indukaka Ipcowala College of Pharmacy, Beyond GIDC, P.B. No. 53, Vitthal Udyognagar, 388 11, Gujarat, India, dimalgroup@yahoo.com): Development and validation of HPTLC method for estimation of ibuprofen and famotidine in pharmaceutical dosage form. J. Liq. Chromatogr. Relat. Technol. 37, 941-950 (2014). HPTLC of ibuprofen (1) and famotidine (2) on silica gel with methanol - ethyl acetate - hexane - ammonia 4:12:2:1. Quantitative determination by absorbance measurement at 264 nm. The $hR_F$ values for (1) and (2) were 41 and 69, respectively. Linearity was in the range of 320-9600 ng/zone for (1) and 10-300 ng/zone for (2). LOD and LOQ were 104 and 316 µg/mL for (1) and 3 and 10 µg/mL for (2). Recoveries (by standard addition) were in the range of 100-101 % for both (1) and (2). Intermediate intra- and inter-day precision was below 2 % (*n*=3).

pharmaceutical research, HPTLC, quantitative analysis 11a

## 13. Steroids

112 061 H. AHMED *et al.*, see section 28a

112 042 G. RAUT, A. SHIRKHEDKAR, V. UGALE, S. SURANA (*Department of Pharmaceutical Chemistry, R.C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist. Dhule, (MS) 425405, India, atulshirkhedkar@rediffmail.com): Simultaneous determination of prednisolone acetate and moxifloxacin hydrochloride in bulk and in eye drop using RP-HPTLC. J. Liq. Chromatogr. Relat. Technol. 37, 528-537 (2014). HPTLC of prednisolone acetate (1) and moxifloxacin hydrochloride (2) in pharmaceuticals on RP-18 with methanol - water 7:3 + 1 drop triethylamine. Quantitative determination by absorbance measurement at 274 nm. The $hR_F$ values for (1) and (2) were 50 and 72, respectively. Linearity was in the range of 600-3600 ng/zone for (1) and 300-1800 ng/zone for (2). LOD and LOQ were 57 and 173 ng/zone for (1) and 27 and 83 ng/zone for (2). Recoveries (by standard addition) were in the range of 100-102 % for (1) and (2). Intermediate intra- and inter-day precision was below 2 % (*n*=3).

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

112 043 Katarina REIFFOVA*, E. KUPCOVA (*Pavol Jozef Safárik University, Faculty of Natural Sciences, Institute of Chemistry, Department of Analytical Chemistry, Moyzesova 11, 041 54 Kosice, Slovak Republic, katarina.reiffova@upjs.sk): A rapid test for selection of suitable detection reagents for the postchromatographic detection of estrogens. J. Planar Chromatogr. 26, 375-378 (2013). TLC of estrone, estradiol, and estriol on silica gel with chloroform - ethyl acetate - acetone 6:2:1. Detecion by dipping into (1) 10 % solution of phosphomolybdic acid (PMA) in methanol, followed by heating at 100 ºC for 10 min; (2) 0.2 % ceric ammonium sulfate in phosphoric acid, followed by heating at 110 ºC for 10 min; (3) 0.2 g manganese(II) chloride in 30 mL water, 30 mL methanol and 2 mL concentrated sulfuric acid, followed by heating at 100-120 ºC for 10-15 min; and (4) 1 g of vanillin in 25 mL of ethanol, 25 mL of distilled water, and 35 mL of ortho-phosphoric acid (85 %), followed by heating at 120-160 ºC for 5-15 min. The lowest detection limit for estrone (75 ng/zone) was achieved using (1) and (3), where-

as for estradiol and estriol (both 4.7 ng per zone) by using (2).

pharmaceutical research, quality control, HPTLC, quantitative analysis, postchromatographic derivatization 13b

112 014 A. SCHOENBORN *et al.*, see section 4e

112 015 D. SPIRA *et al.*, see section 4e

## 14. Steroid glycosides, saponins and other terpenoid glycosides

112 044 Irma PODOLAK*, U. HUBICKA, B. WITEK, Z. JANECZKO, J. KRZEK (*Department of Pharmacognosy, Pharmaceutical Faculty, Medical College, Jagiellonian University, Medyczna 9, 30-688 Cracow, Poland, mfpodola@cyf-kr.edu.pl: Quantification of saponins in different plant parts of *Lysimachia* L. species by validated HPTLC-densitometric method. J. Planar Chromatogr. 26, 248-253 (2013). HPTLC of ardisicrispin A in the roots, stems, leaves, fruits and flowers of *Lysimachia nemorum* L. (1), *Lysimachia nummularia* L. (2), *Lysimachia vulgaris* L. (3), *Lysimachia punctata* L. (4), *Lysimachia thyrsiflora* L. (5), *Lysimachia ephemerum* L. (6), *Lysimachia ciliata* L. (7), and *Lysimachia clethroides* Duby (8) on silica gel with chloroform - methanol - water 8:7:1 for (1), (3) and (7) and n-butanol - acetic acid - water 6:1:3 for (2), (4), (5), (6) and (8). Detection by spraying with 25 % sulfuric acid in methanol, followed by heating at 105 ºC for 5 min. Quantitative determination by absorbance measurement at 545 nm. The $hR_F$ values for ardisicrispin A in both development systems were 86 and 36, respectively. Linearity was in the range of 2-13 µg/zone. LOD and LOQ were 620 and 950 ng/zone for the chloroform mobile phase and 1890-2880 ng/zone for the *n*-butanol mobile phase. Average recovery was in the range of 94.3-95.1 %. Intermediate/interday/intra-day precision was below 4 % ($n$=3).

herbal, quality control, quantitative analysis, HPTLC 14

112 045 Z. TURKMEN*, S. MERCAN, S. CENGIZ (*Istanbul University, Institute of Forensic Sciences, Forensic Toxicology Laboratory, Cerrahpaa-Istanbul, Turkey, zeytur@gmail.com): An HPTLC method for the determination of oleandrin in *Nerium* plant extracts and its application to forensic toxicology. J. Planar Chromatogr. 26, 279-283 (2013). HPTLC of oleandrin in the stems of *Nerium oleander* on silica gel with *n*-hexane - ethyl acetate 2:3. Quantitative determination by absorbance measurement at 275 nm. The $hR_F$ of oleandrin was 24. Linearity was in the range of 2-75 ng/zone. LOD and LOQ were 2.2 and 6.7 ng/zone, respectively. Relative recoveries in serum and urine were 83 and 89 %, respectively. Intermediate/interday/intra-day precision was below 9 %.

herbal, quality control, toxicology, quantitative analysis, HPTLC 14

## 15. Terpenes and other volatile plant ingedients

112 046 M. ALAJMI, P. ALAM*, F SHAKEEL (*Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia, alamperwez007@gmail.com): Quantification of bioactive marker beta-amyrin by validated high-performance thin-layer chromatographic-densitometric method in different species of *Maytenus* grown in Saudi Arabia. J. Planar Chromatogr. 26, 475-479 (2013). HPTLC of beta-amyrin in the aerial parts of *Maytenus obscura* and *Maytenus parviflora* on silica gel with hexane - ethyl acetate 3:1. Detection by spraying with *p*-anisaldehyde, followed by heating at 105 ºC for 10 min. Quantification by absorbance measurement at 550 nm. The $hR_F$ of beta-amyrin was 38. Linearity was in the

range of 10-100 ng/zone. LOD and LOQ were 9 and 27 ng/zone. Recovery was in the range of 99.3-99.7 %. Intermediate/interday/intra-day precision was below 2 % (*n*=6).

herbal, quality control, HPTLC, quantitative analysis 15a

112 047 S. ALQASOUMI (Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Kingdom of Saudi Arabia, sqasoumi@ksu.edu.sa): Densitometric HPTLC method for qualitative and quantitative analysis of piperitone. J. Planar Chromatogr. 26, 93-95 (2013). TLC of piperitone in the aerial parts of *Cymbopogon proximus* on silica gel with hexane - ethyl acetate 4:1. Quantitative determination by absorbance measurement at 245 nm. The $hR_F$ of piperitone was 40. Linearity was in the range of 250-1000 ng/zone. Recovery (by standard addition) was between 98.8 and 99.1 %. Intermediate/interday/intra-day precision was below 2 % (*n*=3).

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

112 048 G. HORVATH, K. ACS (*University of Pécs, Medical School, Department of Pharmacognosy, Hungary, gyorgyi.horvath@aok.pte.hu): TLC-direct bioautography for determination of antibacterial activity of *Artemisia adamsii* essential oil. J. AOAC Int. 96, 1209-1213 (2013). TLC of thujone (1) and 1,8-cineole (2) in the leaves of *Artemisia adamsii* essential oil on silica gel with toluene - ethyl acetate 93:7. Detection by dipping into vanillin - sulfuric acid reagent, followed by heating at 90 ºC for 3 min. The $hR_F$ values for (1) and (2) were 56 and 45. Bioautography by dipping into S. aureus suspension for 10 s. After drying for 2 min followed by incubation at 37 ºC for 17 h the plates were dipped in an aqueous solution of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide 50 mg/80 mL) for 10 s and incubated at 37 °C for 2 h. Inhibition zones appeared as pale yellowish zones against blue background.

traditional medicine, HPTLC, qualitative identification, bioautography 15a, 4e

112 049 G. NOWAK*, M. URBANSKA, J. NAWROT, M. BERNARD, R. PAC (*Department of Medicinal and Cosmetic Natural Products, Poznan University of Medical Sciences, Mazowiecka Str. 33, 60-623 Poznan, Poland, gnowak@ump.edu.pl): Color and chemical reactions of selected sesquiterpene lactones and ecdysones from *Asteraceae* on TLC plates. J. Planar Chromatogr. 26, 289-293 (2013). TLC of guaianolides and four other types of sesquiterpenoids, namely germacranolides, seco-pseudoguaianolides, and pseudoguaianolides, as well as two phytoecdysteroids in the aerial parts of Centaurea bella, Dugaldia hoopesii, Inula aschersoniana, Serratula wolffii, Stizolophus balsamita and *Zoegea baldschuanica* on silica gel with mixtures of methylene chloride and acetone in ratios 8:1, 7:1, 5:1, 3:1, 2:1 and 1:1 for sesquiterpenes and methylene chloride - acetone 1:2 as well as methylene chloride - methanol 5:1 for the separation of ecdysones. Detection by spraying with anisaldehyde reagent (anisaldehyde 0.5 mL; acetic acid 10 mL; methanol 85 mL; sulfuric acid 4.5 mL), followed by heating at 103 ºC for 3 min. The color evaluation allowed for correlation with the presence of substituents on the sesquiterpene ring with high probability.

pharmaceutical research, herbal, qualitative identification 15a

112 050 D. YADAV, M. GUPTA* (*Analytical Chemistry Department, Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, India, guptammg@rediffmail.com): Isolation and HPTLC analysis of iridoids in *Premna integrifolia*, an important ingredient of Ayurvedic drug dashmool. J. Planar Chromatogr. 26, 260-266 (2013). HPTLC of of iridoids 10-O-trans-*p*-cou-

maroylcatalpol (1), 4-hydroxy-E-globularinin (2) and premnosidic acid (3) in the stem bark of *Premna integrifolia* on silica gel with ethyl acetate - methanol - water - acetic acid 40:6:3:1. Detection by dipping into vanillin - sulphuric acid reagent (1 % vanillin in ethanol - sulfuric acid 19:1), followed by heating at 110 ºC for 3 min. Quantification by absorbance measurement at 510 nm. The $hR_F$ values for (1) to (3) were 52, 41 and 33, respectively. Linearity was in the range of 1-10 µg/zone for (1) to (3). LOD and LOQ were 198 and 663 ng/zone for (1), 312 and 1040 ng/zone for (2) and 200 and 666 ng/zone for (3), respectively. Average recoveries for (1) to (3) were found to be 97.3 %, 98.3 % and 97.6 %, respectively. Intermediate/interday/intraday precision was below 2 % ($n$=9).

herbal, quality control, quantitative analysis, HPTLC 15a

## 17. Amines, amides and related nitrogen compounds

112 051 I. ALI*, A. HUSSAIN, K. SALEEM, H. ABOUL-ENEIN (*Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi 110025, India, drimran_ali@yahoo.com): Separation and identification of antidepressant drugs in human plasma by solid-phase extraction-thin-layer chromatography. J. Planar Chromatogr. 26, 349-353 (2013). HPTLC of fluvoxamine maleate (1), paroxetine (2), and sertraline (3) on silica gel with 2-propanol - dichloromethane 7:3. Quantification by absorbance measurement at 282 nm. The $hR_F$ values for (1), (2) and (3) were 44, 22 and 68, respectively. LOD was 0.2 ng/zone for (1) and (2) and 0.1 ng/zone for (3). Recoveries for (1) to (3) were 41 %, 35 % and 33 %, respectively.

pharmaceutical research, clinical chemistry, research, HPTLC, quantitative analysis 17a

112 052 P. PUSHPALATHA*, R. KUMAR, M. IDRIS, M. ANAND, T. RAO, S. VARMA (*Central Forensic Science Laboratory, Directorate of Forensic Science, Ministry of Home Affairs, Hyderabad, India, pushpas_710@yahoo.co.in): Determination of free duloxetine in human serum by high-performance thin-layer chromatography. J. Planar Chromatogr. 26, 354-357 (2013). HPTLC of free duloxetine in human serum on silica gel with acetone - benzene - triethylamine 10:9:1. Quantitative determination by absorbance measurement at 235 nm. The $hR_F$ of duloxetine was 32. Linearity was between 35 and 140 ng/zone. LOD and LOQ were 10 and 35 ng/zone. Recovery (by standard addition) was found to be 92.9-97.6 %. Intra- and inter-day precision values were below 1.8 % and 5.7 %, respectively.

clinical chemistry, research, HPTLC, quantitative analysis 17a

112 053 Z. TURKMEN*, S. MERCAN, I. BAVUNOGLU, S. CENGIZ (*Istanbul University, Institute of Forensic Sciences, 34303, Cerrahpasa, Istanbul, Turkey, zeytur@gmail.com): Development and validation of a densitometric-high-performance thin-layer chromatographic method for quantitative analysis of amitriptyline in gastric lavage. J. Planar Chromatogr. 26, 496-501 (2013). HPTLC of amitriptyline in gastric lavage on silica gel with methanol - ammonia (25%) 197:3. Quantitative determination by absorbance measurement at 209 nm. The $hR_F$ value of amitriptyline was 49. Linearity was between 10 and 250 ng/zone. LOD and LOQ were 5 and 17 ng/zone. Recovery (by standard addition) was in the range of 83-92 %. Intermediate intra- and inter-day precision was below 2 %.

pharmaceutical research, toxicology, HPTLC, quantitative analysis 17a

## 18. Amino acids and peptides, chemical structure of proteins

112 054 S. MENNICKENT*, C. RIVAS, M. VEGA, M. DE DIEGO (*Department of Pharmacy, Faculty of Pharmacy, University of Concepción, P.O. Box 237, Concepción, Chile, smennick@udec.cl):

A stability-indicating HPTLC method for quantification of enalapril maleate in tablets. J. Chil. Chem. Soc. 58, 1737-1740 (2013). HPTLC of enalapril maleate in tablets on silica gel with 1-buthanol - glacial acetic acid - water 12:3:5. Quantitative determination by absorbance measurement at 207 nm. The $hR_F$ values of enalapril and its degradation products enalapril diketopiperazine and enalaprilat were 62, 82 and 52, respectively. Linearity was in the range of 200-1200 ng/zone for enalapril. LOD and LOQ were 23 and 72 ng/zone. Intermediate intra- and inter-day precision was below 2 % ($n$=6).

pharmaceutical research, quality control, quantitative analysis, HPTLC 18b

112 012 A. SINHABABU *et al.*, see section 3e

## 19. Proteins

112 135 Laurie-Anne BARRET *et al.*, see section 35a

112 055 R. GWARDA*, A. TOMCZYSZYN, A. MISICKA, T. DZIDO (*Department of Physical Chemistry, Chair of Chemistry, Medical University of Lublin, 4a Chodzki Street, 20-093 Lublin, Poland, radoslaw.gwarda@umlub.pl): Staining of some synthetic oligopeptides using ninhydrin solution. J. Planar Chromatogr. 26, 455-456 (2013). HPTLC of oligopeptides on RP-18 (chromatography described in R. Gwarda, A. Tomczyszyn, A. Misicka, T.H. Dzido, Acta Chromatogr., in print, 2015). Detection by spraying or dipping for 2 s with 2 % ninhydrin solution in 1) acetone - glacial acetic acid 25:1 or 2) acetone - methanol - glacial acetic acid 25:25:2, followed by heating at 80 ºC. Results showed that short dipping of the plate in ninhydrin solution resulted in a better detectability of peptides than spraying it with the same solution. Solution 2) with methanol made the ninhydrin reaction faster, and colored all zones in a distinct pink/red color, unfortunately one peptide (no. 1) showed poor color intensity.

pharmaceutical research, HPTLC, qualitative identification 19

## 22. Alkaloids

112 056 Z. JAREMICZ, Maria LUCZKIEWICZ*, KISIEL, R. ZARATE, N. VAZDEKIS, P. MIGAS (*Department of Pharmacognosy, Medical University of Gdansk, al. gen. J. Hallera 107, 80-416 Gdansk, Poland, mlucz@gumed.edu.pl): Multi-development-HPTLC method for quantitation of hyoscyamine, scopolamine and their biosynthetic precursors in selected *Solanaceae* plants grown in natural conditions and as *in vitro* cultures. Phytochem. Anal. 25, 29-35 (2014). HPTLC of hyoscyamine (1), scopolamine (2) and their biosynthetic precursors anisodamine (3), cuscohygrine (4) and litorine (5) in selected *Solanaceae* plants on silica gel with two-stage multi-development using chloroform - methanol - acetone - 15:3:2 + 1 drop ammonia. Quantitative determination by absorbance measurement at 190 nm for (3) and at 520 nm for (1), (2), (4) and (5) after spraying and derivatising with Dragendorff‘s reagent following a Munier and Macheboeuf modification (Baerheim-Svendsen and Verpoorte, 1983). The $hR_F$ values for compounds (1) to (5) were 41, 78, 24, 30 and 49, respectively. Linearity was in the range of 1000-3000 ng/zone for (3) and 500-3000 ng/zone for (1), (2), (4) and (5). LOD and LOQ were 127 and 425 ng/zone for (1), 100 and 334 ng/zone for (2), 11 and 38 ng/zone for (3), 104 and 413 ng/zone for (4), and 111 and 373 ng/zone for (5), respectively. Recoveries were in the range of 88-91 % for all the analytes. Intermediate intra- and inter-day precision was below 3 % ($n$=3).

herbal, quality control, quantitative analysis, HPTLC 22

### 23. Other substances containing heterocyclic nitrogen

112 057 S. DHOLE*, K. PRAMOD, A. NIKHIL (*Department of Pharmaceutical Chemistry, Sharad Pawar College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-441110, M.S., India, seemadhole@gmail.com): Development and validation of HPTLC densitometry method for simultaneous estimation of rosiglitazone and glimepiride in fixed tablet dosage form. J. Chil. Chem. Soc. 58, 1663-1666 (2013). HPTLC of rosiglitazone (1) and glimepiride (2) in tablet dosage form on silica gel with methanol - toluene - ethyl acetate 1:8:1. Quantitative determination by absorbance measurement at 228 nm. The $hR_F$ values for (1) and (2) were 39 and 20, respectively. Linearity was in the range of 100-1500 ng/zone for (1) and 100-1500 ng/zone for (2). LOD and LOQ were 30 and 35 ng/zone for (1) and 85 and 90 ng/zone for (2), respectively. Recovery was 96 % for (1) and (2). Intermediate intra- and inter-day precision was below 2 % ($n$=6).

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

112 058 A. MOHAMED, F. MOHAMED, S. AHMED, Y. MOHAMED* (*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Egypt, gadeedegypt2008@yahoo.com): New and selective HPTLC-densitometric method for determination of pioglitazone hydrochloride. J. Planar Chromatogr. 26, 209-214 (2013). HPTLC of pioglitazone hydrochloride on silica gel with chloroform - methanol 10:1. Detection by spraying with 0.5 % o-phthalaldehyde in ethanol, followed by heating at 70 ºC for 15 min. Quantitative determination by scanning with a flatbed scanner and a gel analysis software. Linearity was in the range of 200-3000 ng/zone. LOD and LOQ were 65 and 197 ng/zone, respectively. Recovery was in the range of 98-102 %. Intermediate/interday/intra-day precision was below 2 %.

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

### 26. Organometallic and related compounds

112 059 R. ZAKRZEWSKI*, Z. REMBISZ, W. CIESIELSKI, G. CELICHOSKI (*Department of Inorganic and Analytical Chemistry, University of Lodz, Lodz, Poland, robzak@chemia.uni.lodz.pl): Quantification of metal dithiocarbamates by thin-layer chromatography. J. Planar Chromatogr. 26, 502-507 (2013). TLC of metal dithiocarbamates: antimonic(III) dipentyldithiocarbamate (1), zinc dipentyldithiocarbamate (2), zinc dibutyldithiocarbamate (3), ferric(III) dipentyldithiocarbamate (4) and lead(II) dipentyldithiocarbamate (5) on RP-18 with 2-propanol - water 10:1. Detection by spraying with sodium azide 2 % - potassium iodide 0.01 M - starch solution 1 % pH 6.0 and exposure to iodine vapour for 15 s. Linearity for compounds (1) to (5) ranged 100-2000, 50-1000, 50-1000, 50-1000 and 100-3000 pmol/zone, respectively. LOD was in the range of 10 pmol/zone for the compounds.

agricultural, quality control, HPTLC, quantitative analysis, qualitative identification 26a

### 27. Vitamins and various growth regulators

112 060 P. TEO (Teo Peishan), D. LIU (Liu Daicheng)* (*Key Laboratory of Animal Resistance, College of Life Science, Shandong Normal University, 88 East Wenhua Road, Jinan 250014, P. R. China, liudch@sdnu.edu.cn): Determination of biotin in Antarctic krill (*Euphausia superba*) by high-performance TLC with different post-chromatographic derivatizations. J. Sep. Sci. 36, 2703-2708 (2013). HPTLC of biotin in *Euphausia superba* on silica gel with dichloromethane - 2-propanol - methanol 3:3:2 + 1 drop glacial acetic acid. Detection (1) by spraying with 0.1-1 % 4-(dimethylamino)cinnamaldehyde and sulfuric acid in ethanol, followed by drying for 5 min and spraying with liquid paraffin - chloroform 1:10. Alternatively, biotin was detected (2) by spraying with 0.05 % potassium permanganate. Quantitative determination by absorbance

measurement at 530 nm for (1) and 400 nm for (2). The $hR_F$ value of biotin was 50. Linearity was in the range of 340-3310 ng/zone for both (1) and (2). LOD and LOQ were 50 and 90 ng/zone for (1) and 60 and 100 ng/zone for (2). Average recoveries were 99.6 % for (1) and 99.7 % for (2). Intermediate intra- and inter-day precision was below 2 % ($n$=3).

food analysis, HPTLC, quantitative analysis, postchromatographic derivatization 27

## 28. Antibiotics, Mycotoxins

112 061 H. AHMED*, B. MOUSSA, R. EL-BAGARY, M. DARWISH (*National Organization for Drug Control and Research (NODCAR), P.O. Box 29, 12553 Cairo, Egypt, hanan_egypt1@yahoo.com): Three validated methods for simultaneous determination of ofloxacin and dexamethasone in binary mixture. J. Planar Chromatogr. 26, 56-61 (2013). TLC of ofloxacin (1) and dexamethasone (2) on silica gel with methanol - 0.01 M phosphate buffer 2:3 and pH adjusted to 5 with orthophosphoric acid. Quantitative determination by absorbance measurement at 300 nm for (1) and 240 nm for (2). The $hR_F$ values for (1) and (2) were 22 and 60, respectively. Linearity was in the range of 1-6 µg/zone for both (1) and (2). The LOD and LOQ were 260 and 870 ng/zone for (1) and 220 and 740 ng/zone for (2), respectively. Average recovery (by standard addition) for (1) and (2) was 99.2 %. Intermediate/interday/intra-day precision was below 2 % ($n$=3). The method showed comparable results with a validated HPLC method.

pharmaceutical research, quality control, quantitative analysis, HPTLC 28a, 13a

112 062 M. AHMED*, Y. SREE, S. FATTAH, N. HASSAN, M. SAAD (*Department of Food Toxicology and Contaminants, Division of Food Industries & Nutrition, National Research Center, 33 El-Tahrir St., Dokki, Cairo, m_bedear76@yahoo.com): Determination of tylosin, spiramycin, and erythromycin residues in Egyptian buffaloes‘ meat by thin-layer chromatography-bioautography. J. Planar Chromatogr. 26, 409-416 (2013). TLC bioautography of tylosin (1), spiramycin (2), and erythromycin (3) residues in the meat of Egyptian buffaloes on silica gel with methanol - ethyl acetate - acetone 5:3:2. The $hR_F$ values of (1) to (3) were 80, 41 and 20, respectively. Linearity was between 15-120 ng/zone for (1), 50-200 ng/zone for (2) and 1-20 ng/zone for (3). LOD for (1) to (3) were 12, 45 and 2 ng/g, respectively. Recovery (by standard addition) was found to be 84.2-92.2 %. Precision as *%RSD* was below 7.6 %. Bioautography was performed after development according to the method by J. Michard et al. (Method protocol for the detection of tylosin, spiramycin and virginiamycin in animal feedingstuffs by thin layer chromatography. SIMBAG-FEED Report 4.6. RIKILT, 2005). TLC plates were dried at 60 °C for 30 min in a petri dish and then covered to a thickness of 1.5 mm with agar medium containing the bacterial suspension. After incubation over night at 35 °C about 5 mL of 2,3,5-triphenyltetrazolium chloride solution (0.1 g in 100 mL water) was poured on the medium and re-incubated for some minutes. The inhibition zones were measured and their $hR_F$ values were compared with those of antibiotic standards. Detection was considered positive if the $hR_F$ value of the sample‘s inhibition zone is ±5 % of the standard.

food analysis, quantitative analysis, TLC-bioautography 28a

112 063 R. EL-BAGARY, N. ABO-TALIB, M. ELDIN* (*National Organization for Drug Control and Research, Cairo, Egypt, drmohamedbadawi@hotmail.com): Different validated methods for determination of cefditoren pivoxil. J. Planar Chromatogr. 26, 43-55 (2013). TLC of cefditoren pivoxil on silica gel with 1-butanol - acetic acid - water 17:2:1. Quantitative determination by absorbance measurement at 295 nm. The $hR_F$ of cefditoren pivoxil was 70. Linearity was in the range of 600-1600 ng/zone. The LOD and LOQ were 40 and 130 ng/zone, respectively. Recovery (by standard addition) was between 97.8 and 101.8 %. Intermediate/interday/intra-day

precision was below 2 % ($n$=3). The method showed comparable results with a validated HPLC method.

pharmaceutical research, quality control, HPTLC, quantitative analysis, comparison of methods 28a

112 064 A. GORRAN, M. FARZANEH*, M. SHIVAZAD, M. REZAEIAN, A. GHASSEMPOUR (*Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C. Evin, Tehran, Iran, m_farzaneh@sbu.ac.ir): Aflatoxin B1-reduction of *Aspergillus flavus* by three medicinal plants (*Lamiaceae*). Food Control. 31, 218-223 (2013). HPTLC of aflatoxin B1 (AFB1) on silica gel with chloroform - acetone 9:1. Quantitative determination by fluorescence measurement at 366 nm. The method allowed for determination of inhibitory effect of essential oils on AFB-1 production by *A. flavus*.

Traditional medicine, toxicology, quantitative analysis, HPTLC 28b

112 065 E. GRZELAK, W. JESIONEK, B. DZIEDZIC, Irena CHOMA* (*University of Maria Curie-Sklodowska, Department of Chromatographic Methods, Lublin, Polandirena, choma@umcs.lublin.pl): Applications of novel direct bioautography tests for analysis of antimicrobials: a review. J. AOAC Int. 96, 1167-1174 (2013). The review reports various applications of bioautography tests for determination of wide spectrum of antimicrobials. Based on two direct tests using *Bacillus subtilis* and *Escherichia coli*, the review discusses different applications for the screening of analytes in various biological samples. For example, the antibacterial activity of essential oils in conifers is estimated on the basis of the measured area of inhibition zones.

pharmaceutical research, traditional medicine, review, HPTLC, bioautography 28, 4e

112 066 B. NYAMWERU, E. KAALE*, V. MANYANGA, M. CHAMBUSO, T. LAYLOFF (*Pharm R&D Laboratory, School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65013, Dar es Salaam, Tanzania, elia.kaale@lycos.com): Development and validation of a thin-layer chromatographic-densitometric method for the analysis of ciprofloxacin hydrochloride tablets. J. Planar Chromatogr. 26, 370-374 (2013). HPTLC of ciprofloxacin in tablets on silica gel with acetone - water - ammonia 30:3:5. Quantification by absorbance measurement at 280 nm. The $hR_F$ of ciprofloxacin was 27. Linearity was in the range of 250-600 ng/zone. Recovery was in the range of 96-101 %. Intermediate/interday/intra-day precision was below 2 %.

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

112 067 D. RAKSHITH, P. SANTOSH, K. TARMAN, D. GURUDATT, S. SATISH* (*Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore - 570 006, Karnataka, India, satish.micro@gmail.com): Dereplication strategy for antimicrobial metabolite using thin-layer chromatography-bioautography and LC-PDA-MS analysis. J. Planar Chromatogr. 26, 470-474 (2013). TLC-bioautography of antimicrobial metabolites in the extracts of endophytic fungus *Xylaria sp.* on silica gel with toluene - diethyl ether - acetic acid 1:1:1. The plates were transferred to sterile Petri dishes and overlaid with Mueller Hinton (for *Escherichia coli* and *Vibrio parahaemolyticus*), Brain Heart Infusion (for *Staphylococcus aureus* and *Listeria monocytogenes*) medium containing 0.65 % agar incorporating 1 mg/mL 2,3,5-triphenyltetrazolium chloride, and Sabouraud Dextrose Agar medium (for *Candida albicans* and *Aspergillus niger*) inoculated with 1 % standardized microbial inocula. For bacteria plates were incubated for

24 h at 37 °C. For fungi, plates were incubated for 48-72 h at 25 °C and the agar surface was sprayed with a 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and inhibition zones were detected as clear spots against a red and purple background.

pharmaceutical research, qualitative identification, TLC-bioautography 28a

112 068 D. ZHANG (Zhang Dou-Sheng), W. LIU (Liu Wen), Y. LI (Li Ya-Ping), C. HU (Hu Chang-Qin)* (*National Institutes for Food and Drug Control, Beijing 100050, China, hucq@nicpbp.org.cn): Establishment and optimization of an HPTLC method for the analysis of gatifloxacin and related substances by design of experiment. J. Planar Chromatogr. 26, 215-225 (2013). HPTLC of gatifloxacin and 8 related substances on silica gel with methanol - 1,2-dichloroethane - ammonia - acetonitrile 28:72:5:5. Quantitative determination by absorbance measurement at 366 nm. The $hR_F$ value for gatifloxacin was 42. Linearity was in the range of 20-1000 ng/zone. LOD and LOQ were 2.6 and 5.6 ng/zone. Intermediate/interday/intra-day precision was below 2 % ($n$=6). The method showed comparable results with a validated RP-HPLC mehod.

pharmaceutical research, quality control, quantitative analysis, HPTLC, comparison of methods 28a

## 29. Pesticides and other agrochemicals

112 069 B. MALI (Regional Forensic Science Laboratory, State of Maharashtra, Cantonment, Aurangabad - 431 002, India, malibdm@yahoo.co.in): Specific spray reagent for the detection and identification of endosulfan by thin-layer chromatography. J. Planar Chromatogr. 26, 508-509 (2013). TLC of endosulfan on silica gel with *n*-hexane - acetone 4:1. Detection by spraying with sodium hydroxide followed by fuchsin (1) or malachite green (2) reagents (50 mg in 100 mL water), followed by exposure to bromine gas for 3-5 min. The $hR_F$ values of endosulfan detected by (1) and (2) were 50 and 80, respectively.

toxicology, HPTLC, qualitative identification, postchromatographic derivatization 29a

## 30. Syntetic and natural dyes

112 070 J. VLAJKOVIC, F. ANDRIC, P. RISTIVOJEVIC, A. RADOICI, Z. TESIC, D. OPSENICA* (*Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158, Belgrade, Serbia, dusankam@chem.bg.ac.rsk): Development and validation of a TLC method for the analysis of synthetic food-stuff dyes. J. Liq. Chromatogr. Relat. Technol. 36, 2476-2488 (2013). HPTLC of synthetic colorants tartrazine (1), quinoline yellow (2), sunset yellow FCF (3), azorubine (4), amaranth (5), ponceau 4R (6), allura Red AC (7), patent blue V (8), indigo carmine (9), and brilliant blue FCF (10) on RP-18 with 0.5 M ammonium sulfate in 30 % of ethanol - water solution. Quantitative determination by absorbance measurement at 450, 500 and 625 nm. The $hR_F$ values were in the range of 17 and 64. Linearity was in the range of 20-180 ng/zone for (1) to (8) and 35-300 ng/zone for (9) and (10). LOD and LOQ were 2 and 3 ng/zone, respectively. Recovery (by standard addition) was in the range of 81-108 %. Intermediate intra- and inter-day precision was below 5 % ($n$=3).

pharmaceutical research, quality control, HPTLC, quantitative analysis 30a

## 32. Pharmaceutical and biomedical applications

112 071 I. ALI*, A.HUSSAIN, K. SALEEM, H. ABOUL (*Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi 110025, India, drimran_ali@yahoo.com): Separation and identification of antidepressant drugs in human plasma by solid-phase extraction-thin-layer

chromatography. J. Planar Chromatogr. 26, 349-353 (2013). TLC of fluvoxamine maleate (1), paroxetine (2) and sertraline in pharmaceuticals on silica gel with 2-propanol - dichloromethane 7:3. Quantitative determination by absorbance measurement at 366 nm. The $hR_F$ of (1), (2), and (3) was 44, 22 and 68, respectively. Linearity was between 200 and 1600 ng/zone for both (1) and (2). Limits of detection and quantitation were 75 and 100 ng/zone for (1), and 40 and 80 ng/zone for (2), respectively. Recovery (by standard addition) was found to be 96 - 107 %.

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

112 072 S. ALQASOUMI, P. ALAM*, M. ANWER, M. KADER (*Department of Pharmacognosy, College of Pharmacy, Salman Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia, prawez_pharma@yahoo.com): Qualitative and quantitative analysis of khellin in *Ammi visnaga* fruits and pharmaceutical preparations using HPTLC and HPLC. J. Liq. Chromatogr. Relat. Technol. 37, 61-72 (2014). HPTLC of khellin in the fruits of *Ammi visnaga* on silica gel with chloroform - acetone 9:1. Quantitative determination by absorbance measurement at 248 nm. The $hR_F$ of khellin was 29. Linearity was between 50 and 300 ng/zone. LOD and LOQ were 10 and 25 ng/mL. Recovery (by standard addition) was in the range of 98-99 %. Intermediate intra- and inter-day precision was below 1.6 % ($n$=6). The results obtained were comparable with HPLC results.

herbal, HPTLC, quantitative analysis 32e

112 073 J. BHARATE, R. VISHWAKARMA*, S. BHARATE, T. THITE, M. KUSHWAHA, A. GUPTA (*Medicinal Chemistry Division, Indian Institute of Integrative Medicine (CSIR), Jammu 180001, India, ram@iiim.ac.in): Quantification and validation of two isomeric anticancer compounds, garcinol and isogarcinol, in ultrasound-assisted extracts of *Garcinia indica* fruits using high-performance thin-layer chromatography. J. Planar Chromatogr. 26, 480-485 (2013). HPTLC of garcinol (1) and isogarcinol (2), two polyisoprenylated benzophenones in the fruits of *Garcinia indica* on silica gel with *n*-pentane - ethyl acetate 7:3 + 1 drop formic acid. Quantification by absorbance measurement at 327 nm. The $hR_F$ values for compounds (1) and (2) were 72 and 62, respectively. Linearity was in the range of 200-1400 ng/zone for (1) and 990-6900 ng/zone for (2). LOD and LOQ were 65 and 200 ng/zone for (1) and 300 and 900 ng/zone for (2). Recovery was in the range of 98.2-101.7 % for both (1) and (2). Intermediate/interday/intra-day precision was below 2 % ($n$=6).

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

112 074 Y. CHEN (Chen Yunzi)*, D. LIU (Liu Dongwen) (*The Preparation Center, Foshan Munic. Hosp. of Trad. Chinese Med., Guangdong, Foshan 528000, China): (Study on the method for the quality control of Sanyu Xugu Tie plaster by thin-layer chromatography) (Chinese). J. of China Pharm. 22 (9), 14-16 (2013). Sanyu Xugu Tie plaster is a herbal TCM preparation for the treatment of traumatic injury, contusion, stasis of blood and innominate toxic swelling. For quality control, TLC on silica gel 1) for *Radix cynanchi paniculati* and the standard 2‘-hydroxy-4‘-methoxyacetopheone, with cyclohexane - ethyl acetate 3:1, detection by spraying with 5 % ferric chloride in ethanol and heating until the zones are visible in daylight; 2) for *Oleum eucalypti* and the standard cineole, with cyclohexane - ethyl acetate 19:1, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C until the zones are visible in daylight; 3) for *Herba sarcandrae* and the standard isofraxidin, with toluene - ethyl acetate - formic acid 9:4:1, detection under UV 366 nm; 4) for Kusnezoff monkshood root and the standard acetylbenzoylaconine, with cyclohexane - ethyl acetate diethylamine 12:8:1, detection by spraying with 5 %

potassium iodobismuthate in water - hydrochloric acid 200:1 and viewing in daylight.

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

112 075 SH. CHENG (Cheng Shouxian) (Manufacturing Lab. of Liling Municip. Trad. Chinese Med. Hosp., Hunan, Liling 412200, China): (Study of the method for identification of *Flos Lonicerae* and *Astragalus mongholicus* in Shaoshang Heji mixture by thin-layer chromatography) (Chinese). Chinese J. Mod. Drug Appl. 6 (11), 124-125 (2012). Shaoshang Heji mixture is a herbal TCM preparation for treating burns with significant curative effects. For quality control, TLC on silica gel 1) for *Flos Lonicerae* and the standard chlorogenic acid, with ethyl acetate - formic acid - water 16:3:6, detection under UV 366 nm; 2) for *Astragalus mongholicus* 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 at 105 ° for 5 min, evaluation in daylight and under UV 366 nm.

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

112 076 A. DAR, N. ARUMUGAM* (*Department of Biotechnology, School of Life Sciences, Pondicherry University, Kalapet, Puducherry 605 014, India, n_arumugam@hotmail.com): Lignans of sesame: Purification methods, biological activities and biosynthesis - a review. Bioorg. Chem. 50, 1-10 (2013). Review of the current status of research on sesame lignans, regarding analytical methods, biological activities and biosynthesis. The papers described the revolutionary change in the type of techniques used and reported some of the TLC and HPTLC methods that allowed for lignan separation and quantification.

traditional medicine, herbal food analysis, review, HPTLC, quantitative analysis, qualitative identification 32e, 1b

112 077 S. DE*, P. NARIYA, N. JIRANKALGIKAR (*RMD Research and Development Center, Waghaldhara, Valsad 396375, India, subratde@gmail.com): A rapid validated high-performance thin-layer chromatographic-densitometric method for the simultaneous estimation of different chemical-nature compounds piperine and gallic acid in pharmaceutical dosage forms. J. Planar Chromatogr. 26, 325-330 (2013). HPTLC of piperine (1) and gallic acid (2) in pharmaceuticals on silica gel with toluene - ethyl acetate 3:7. Quantitative determination by absorbance measurement at 340 nm for (1) and 254 nm for (2). The $hR_F$ values of (1) and (2) were 55 and 19, respectively. Linearity was between 250 and 1250 ng/zone for (1) and 750 and 1750 ng/zone for (2). LOD and LOQ were 10 and 33 ng/zone for (1) and 25 and 83 ng/zone for (2). Recovery (by standard addition) was in the range of 94-103 %. Intermediate intra- and inter-day precision was below 0.7 % ($n$=3).

pharmaceutical research, HPTLC, quantitative analysis 32a

112 078 X. FENG (Feng Xuahua) (Coll. of Pharm., Anhui Xinhua Univ., Anhui, Hefei 230088, China): (Study of the method for the quality control of Shuangxiang Hewei capsules by thin-layer chromatography). J. of Guangzhou Chem. Engin. 41 (9), 146-147 (2013). Shuangxiang Hewei capsules are a herbal TCM preparation and contain Pogostemon cablin (Blanco) Benth. as the key drug, which is used for treatment of anorexia, vomiting, gastric distention, and limb weakness. For quality control through identification of the key drug Pogostemon cablin, TLC of the drug

extracts and the standard patchouli alcohol on silica gel with cyclohexane - acetone 10:3, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C until the zones are visible in daylight.

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

112 079 G. GAO (Gao Gang)*, H. XU (Xu Hua) (*Baotou Municip. Testing Center for Food & Drug, Inner Mongolia, Baotou 014040, China): (Study of the method for the quality control of Keke Diwan drop pills by thin-layer chromatography) (Chinese). Chinese J. of Northern Pharmacy 10 (10), 9-10 (2013). Keke Diwan drop pills are a herbal TCM preparation for the treatment of cough, dyspnea and shortness of breath. For quality control, TLC on silica gel 1) for *Ephedra sinica* Stapf and the standard ephedrine hydrochloride, with chloroform - methanol - ammonia 100:20:1, detection by spraying with 0.3 % ninhydrin in *n*-butanol - glacial acetic acid 19:1 and heating at 105 °C, viewing in daylight; 2) for *Pericarpium papaveris*, with toluene - acetone - ethanol - concentrated ammonia 20:20:3:1, detection under UV 366 nm; 3) for *Glycyrrhiza uralensis* Fisch., with benzene - chloroform - methanol - glacial acetic acid 40:90:15:1 with preconditioning for 10 min, 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

112 080 N. GENG (Geng Naizhi), SH. Zhang (Zhang Shuoxin), X. WANG (Wang Xiaoli), X. DU (Du Xiaowei), D. JIANG (Jiang Deyou), CH. LIU (Liu Chenggang)* (*Heilongjiang Univ. of Chinese Med., Heilongjiang, Harbin 150040, China): (Study of the method for the quality control of Naodesheng Koufuye oral liquid) (Chinese). J. of Inform. on Trad. Chinese Med. 30 (3), 61-63 (2013). Naodesheng Koufuye oral liquid is a herbal TCM preparation for treatment of cerebral arteriosclerosis and ischemic stroke. For quality control, TLC on silica gel 1) for *Flos Carthami*, with cyclohexane - ethyl acetate 11:6, detection by exposure to ammonia vapors and viewing under UV 366 nm; 2) for *Radix Puerariae* and the standard puerarin, with chloroform - ethyl acetate - methanol - water 15:40:22:10, detection under UV 366 nm; 3) for *Rhizoma Ligustici* Chuanxiong and the standard ferulic acid, with benzene - chloroform - methanol 2:2:1, detection under UV 254 nm. Quantification of ginsenoside Rb, ginsenoside Rg and notoginsenoside R by HPLC.

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

112 081 Y. GUO (Guo Yanling)*, SH. WENG (Weng Shuiwang) ( *Fujian Maternal & Child Health Hosp., Fujian, Fuzhou 350001, China; 2 Fujian Inst. for Drug Contr., Fujian, Fuzhou 350001, China): (Study of the method for the quality control of Shenghua Heji compound oral liquid by thin-layer chromatography) (Chinese). J. Strait Pharm. 24 (7), 75-76 (2012). Shenghua Heji compound oral liquid is a herbal TCM preparation for treatment of postpartum blood circulation, promote uterine contraction, removing blood stasis. For quality control, TLC 1) for *Angelica sinensis*, on silica gel with benzene - ethyl acetate - glacial acetic acid 2:1:1, detection under UV 254 nm; 2) for *Rhizoma chuanxiong*, *Semen persicae*, *Glycyrrhizae* and the standard ferulic acid, on silica gel with benzene - glacial acetic acid - methanol 30:2:3, detection under UV 366 nm and after spraying with 1 % ferric chloride - 1 % potassium ferricyanide 1:1 in daylight; 3) for *Rhizoma chuanxiong* and the standard ferulic acid, on neutral alumina layer

with petroleum ether (60-90 °C) - chloroform 1:1, detection by spraying with 5 % potassium iodobismuthate in water - hydrochloric acid 200:1 and viewing in daylight.

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

112 082 G. HAN (Han Guiru)*, W. ZHANG (Zhang Wenchen), L. AN (An Lina), Y. SHEN (Shen Yulong), P. ZHANG (Zhang Peng) (*Hebei Provinc. Inst. for Drug Contr., Hebei, Shijiazhuang 050010, China): (Improvement and perfect the method for the quality control of Jinmaxing Zhikepian tablets) (Chinese). J. of China Pharm. 21 (14), 41-43 (2013). Jinmaxing Zhikepian tablets are a herbal TCM preparation for the treatment of asthma, and acute and chronic bronchitis. For quality control, TLC on silica gel 1) for *Peucedanum praeruptorum* Dunn, *Scutellaria baicalensis* and *Herba Ephedrae*, with chloroform - methanol - ammonia 40:8:1, detection a) under UV 366 nm and 254 nm, b) by spraying with 2 % ninhydrin in ethanol and heating at 100 °C until the zones are visible in daylight; 2) for Lonicera Japonica, with the upper phase of ethyl acetate - formic acid - water 3:1:1, detection by heating at 100 °C for 5 min and viewing under UV 254 nm; 3) *Eriobotrya japonica* Thunb., with cyclohexane - ethyl acetate - formic acid 150:40:3, detection by spraying with 10 % sulfuric acid in ethanol and heating at 100 °C until the zones are visible in daylight; 4) for *Semen Armeniacae Amarum*, with cyclohexane - ethyl acetate - formic acid 150:20:1, detection by spraying with 2 % phosphomolybdic acid in ethanol and heating at 100 °C until the zones are visible in daylight; 5) for *Platycodon grandiflorus*, with chloroform - ethyl acetate - methanol - ammonia 4:1:4:1, detection by spraying with 5 % ninhydrin in sulfuric acid - ethanol 1:6 and heating until the zones are visible in daylight. Quantification of pseudoephedrine hydrochloride, ephedrine hydrochloride and vitamine B17 by HPLC.

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

112 083 Q. HU (Hu Qing)*, J LI (Li Jianhua), Q. SHEN (Shen Qingliang) (*Henan Hosp. of China Armed Police Forces, Henan, Zhengzhou 450052, China): (Study on the method for the quality control of Shengma Gegen granules) (Chinese). J. of Chinese Med. 26 (169), 716-718 (2012). Shengma Gegen granules are a herbal TCM preparation for the treatment of viral hepatitis type C, drug-induced liver diseases, and postherpetic neuralgia. For quality control, TLC on silica gel 1) for *Rhizoma Cimicifugae*, with benzene - chloroform - glacial acetic acid 12:2:1, detection under UV 366 nm; 2) for *Radix Paeoniae Rubra*, with chloroform - ethyl acetate - methanol - formic acid 200:25:50:1, detection by spraying with 2 % vanillin in sulfuric acid - ethanol 1:200 and heating mildly until the zones are visible in daylight. Quantification of puerarin by HPLC.

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

112 084 S. JHA, A. BHAGWAT, N. PANDITA (*Department of Biological Sciences, School of Science, NMIMS, Mumbai-56, India, skoundilya@gmail.com): Method development and validation of GABA using high-performance thin-layer chromatography in brain homogenate. J. Planar Chromatogr. 26, 417-420 (2013). HPTLC of gamma-amino butyric acid (GABA) in mice brain tissue on silica gel with *n*-butanol - glacial acetic acid - water 65:18:28. Detection by dipping in 0.2 % ninhydrin solution, followed by heating at 65 ºC for 3 min. Quantitative determination by absorbance measurement at 480 nm. Linearity was between 100 and 1000 ng/zone. LOD and LOQ were 50 and 151 ng/zone. Recovery (by standard addition) was found to be 65-78.5 %.

Intra- and inter-day precision values were below 7.7 %.

clinical chemistry, research, HPTLC, quantitative analysis 32f

112 085 ZH. JIANG (Jiang Zhenou), M. LAI (Lai Maoxiang)*, J. YANG (Yang Jiangfei) (*Guangxi Acad. of Trad. Chinese Med. Sci., Guangxi, Nanning 530022, China): (Study of the method for the separation of four kinds of Yao traditional medicinal herbs by thin-layer chromatography) (Chinese). Chinese J. of Guide for Trad. Chinese Med. & Pharm. 19 (4), 70-71 (2013). *Pothos chinensis* (Raf.) Merr. and three other medicinal herbs are used in traditional Yao medicine, they have anti venom, anti tumor, and antioxidant effects and reduce blood sugar. For quality control, TLC of 1) *Pothos chinensis* (Raf.) Merr., on polyamide phase with acetone - water 1:2, detection by spraying with 2 % aluminiumchloride in ethanol and heating at 105°C until the zones are visible in daylight, and under UV 366 nm; 2) for *Mappianthus iodoides* Hand.-Mazz., on silica gel with petroleum ether (60-90 °C) - ethyl acetate 4:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones are visible in daylight, and under UV 366 nm; 3) *Zanthoxylum nitidum* (Roxb.) DC., on silica gel with chloroform - methanol - ammonia 150:5:1, detection under UV 366 nm; 4) for *Saussurea woodiana* Hemsl., on silica gel with petroleum ether (60-90°C) - ethyl formate - formic acid 75:25:2, detection under UV 366 nm.

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

112 086 I. KHAN, P. SANGWAN*, A. DAR, R. RAFIQ, M. FARRUKH, J. DHAR, S. TASDUQ, S. KOUL (*Bioorganic Chemistry Division, CSIR - Indian Institute of Integrative Medicine, Jammu, Jammu and Kashmir, India, skoul@iiim.res.in): A validated high-performance thin-layer chromatography method for the identification and simultaneous quantification of six markers from *Platanus orientalis* and their cytotoxic profiles against skin cancer cell lines. J. Sep. Sci. 36, 2602-2610 (2013). HPTLC of betulinic acid (1), betulinic acid-3-acetate (2), 3-acetyl-betulinaldehyde (3), oleanolic acid-3-acetate (4), 3-beta-hydroxy-28,19-beta-olenolide (5), and beta-sitosterol (6) in the bark of *Platanus orientalis* on silica gel with *n*-hexane - toluene - acetone 12:7:2. Detection by dipping into a ceric ammonium sulfate reagent, followed by heating at 105-110 ºC for 5-10 min. Quantitative determination by absorbance measurement at 420 nm for (1), at 550 nm for (2, 3) and at 500 nm for (4-6). The $hR_F$ values for compounds (1) to (6) were 16, 42, 85, 37, 25 and 29, respectively. Linearity was in the range of 100-600 ng/zone for (1) to (6). LOD and LOQ were 60 and 100 ng/zone for (1), 80 and 100 ng/zone for (2), 70 and 90 ng/zone for (3), 40 and 80 ng/zone for (4), 100 and 100 ng/zone for (5) and 60 and 90 ng/zone for (6), respectively. Recoveries were in the range of 95-99 % for all the analytes. Intermediate intra- and inter-day precision was below 2 % ($n$=3).

herbal, quantitative analysis, HPTLC 32e

112 087 J. LI (Li Jing), Y. GAO (Gao Ying), ZH. WANG (Wang Zhenhua), W. LI (Li Weimin), Z. KONG (Kong Zengke), H. ZHOU (Zhou Haiping)* (*Handan Munic. Health Bureau, Hebei, Handan 056000, China): (Study on the method for the quality control of Radix Bupleuri) (Chinese). Chinese J. of Northern Pharmacy 10 (6), 8-10 (2013). Radix Bupleuri is the dried root of *Bupleurum chinense* Dc. and Bupleurum *scorzonerifolium* Willd. (*Umbelliferae*). The crude drug has antipyretic, antiviral, anti-inflammatory and anti-tumor effects, and reduces blood lipid levels. The quality of the drug differs depending on its origin and harvesting time. For quality control, TLC on silica gel 1) with ethyl acetate - ethanol - water 8:2:1; and 2) with the lower

phase of chloroform - ethyl acetate - methanol - water 15:40:22:10 after laying up at 10 °C. Detection by spraying with 2 % p-dimethylaminobenzaldehyhyde in 40 % sulfuric acid and heating at 60 °C until the zones are visible in daylight and under UV 366 nm. Identification by comparison with the standard *Radix Stellaviae*. Quantification of total *Radix Stellaviae* by UV spectrophotometry with *p*-dimethylaminobenzaldehyhyde - phosphoric acid colorization method at 545 nm. Quantification of *Radix Stellaviae* A and D by HPLC.

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

112 088 K. LI (Li Ke)*, D. CHEN (Chen Dan), Y. HU (Hu Yibing), L. SHI (Shi Leizhe), R. LI (Li Ruocun) (*Hunan Provinc. Acad. of Trad. Chinese Med. Hunan, Changsha 420013, China): (Study of the method for the quality control of *Inula nervosa* Wall, a traditional Chinese medicinal herb) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. (20), 30-32 (2012). *Inula nervosa* Wall is a herbal TCM drug with anti rheumatism effects. It is frequently used as key component in herbal TCM preparations for the treatment of rheumatism. For quality control, TLC of the volatile oil (obtained by steam distillation of the crude drug) diluted 1:100 with acetone, and the standards thymol and thymyl isobutyrate, on homemade layers (coated using a slurry of silica gel in 1 % NaOH solution), with cyclohexane - toluene 3:4, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:200 and heating at 105 °C until the zones are visible in daylight.

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

112 089 X. LI (Li Xixiang), X. LIU (Li Xiaoshuan)*, ZH. XIAO (Xiao Zhengguo), J. LI (Li Jiwen), L. WEI (Wei Lingping) (*Gansu Province Hosp. of Trad. Chinese Med., Gansu, Lanzhou 730050, China): (Study of the method for the quality control of Tongqiao Biyan Wan pills) (Chinese). Chinese J. of Inform. on TCM 19 (3), 47-49 (2012). Tongqiao Biyan Wan pills are a herbal TCM preparation for the treatment of rhinitis, sinusitis, headache, and nasal congestion. For quality control TLC on silica gel 1) for *Ligusticum chuanxiong*, with toluene - ethyl acetate 10:1, detection under UV 254 nm; 2) for Cape Jasmine and the standard gardenoside, with chloroform - methanol 3 : 1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing in daylight; 3) for *Ephedra sinica* Stapf, with chloroform - methanol - concentrated ammonia 100:20:1, detection by spraying with 0.1 % ninhydrin in propanone and heating at 105 °C, viewing in daylight. Quantification of gardenoside by HPLC.

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

112 090 L. LIAO (Liao Liying) (Jiujiang Municip. Inst. for Food & Drug Contr., Jiangxi, Jiujiang 332000, China): (Study of the method for the quality control of Shenshi Keli granules by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 11 (1), 470-472 (2013). Shenshi Keli granule is a herbal TCM preparation for the treatment of urinary calculus. For quality control TLC on silica gel 1) for *Dichondra repens* Forst., with chloroform - acetone 19:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C for 5 min and viewing under UV 366 nm; 2) for Chicken gizzard‘s stomach lining, with n-butanol - glacial acetic acid - water 7:1:1, detection by spraying with 0.3 % ninhydrin in *n*-butanol and heating at 105 °C until the zones are visible in daylight.

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

112 091 L. LIU (Liu Longyou), Q. CHEN (Chen Qingyan), Y. TANG (Tang Yingshuang)* (PLA No. 68306 Hosp., Shanxi, Xian 710600, China): (Study on the method for the quality control of Fufang Kuhuang Penwu spray by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 18 (12), 82-84 (2012). Fufang Kuhuang Penwu spray is a herbal TCM preparation for treatment of eczema, skin itch and solar dermatitis. For quality control, TLC on silica gel 1) for borneol, with cyclohexane - ethyl acetate 33:7, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:4, heating at 105 °C until the zones are visible in daylight; 2) for *Cortex Phellodendri Chinensis* and the standard berberine hydrochloride, with benzene - ethyl acetate - methanol - isopropanol - concentrated ammonia 12:6:3:3:1, detection under UV 366 nm; 3) for *Rheum palmatum* L., *Reynoutria japonica* Houtt, and the standard emodin, with petroleum ether (30-60 °C) - ethyl formate - formic acid 15:5:1, detection under UV 366 nm before and after exposure to ammonia vapors.

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

112 092 Y. LIU (Liu Yilan) (Taizhou Municip. Jiangyan Hosp. of Trad. Chinese Med., Jiangsu, Taizhou 225500, China): (Study of the method for the quality control of Zhuangyao Zhitong Heji compound oral liquid) (Chinese). Chinese J. Mod. Drug Appl. 7 (17), 228-229 (2013). Zhuangyao Zhitong Heji compound oral liquid is a herbal TCM preparation to treat deficiency of the kidney and liver, chills and pain of the waist and knee, wind-cold-dampness arthralgia and low back injury. For quality control, TLC on silica gel with *n*-hexane - ethyl acetate 9:1, detection under UV 366 nm. Identification of *Angelica sinensis, Ligusticum chuanxiong* and *Saposhnikovia divaricata* roots by fingerprint comparison with reference drugs.

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

112 093 N. LU (Lu Ning Wei), W. ZHANG (Zhang Wen Xiang), Q. AN (An Qiong), N. LI (Li Ning), Y. DONG (Dong Yu Ming)* (*School of Pharmacy, Lanzhou University, Lanzhou 730000, Gansu, P. R. China, dongym@lzu.edu.cn): Two-dimensional thin-layer chromatographic fingerprint of *Helleborus thibetanus* Franch. using a polyamide plate with nonaqueous and reversed micellar mobile phases. J. Planar Chromatogr. 26, 463-469 (2013). 2D TLC fingerprint of *Helleborus thibetanus* Franch on polyamide with chloroform - ethyl acetate - methanol 15:40:22 in the first dimension and isooctane - *n*-propyl alcohol - water 20:5:1 + 0.28 M sodium dodecyl sulfate in the second dimension, developing distances were 70 mm for both. Detection under UV 366 nm. Nine zones were detected for the identification of *Helleborus thibetanus* Franch.

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

112 094 Y. LU (Lu Yiling) (Hangzhou Municip. Inst. for Drug Contr., Zhejiang, Hangzhou 310017, China): (Evaluation of the uncertainty of thin-layer chromatographic determination of oleanolic acid in Kangbingdu Jiaonang capsules) (Chinese). Chinese J. of Herald of Med. 32 (2), 242-244 (2013). TLC is widely used for the analysis of active components in TCM preparations. The degree of accuracy of the detection results can be confirmed by uncertainty evaluation, by which the critical value of measurement results are judged. The uncertainty assessment for the measurement process and experimental data is part of the information which has to be submitted with drug inspection reports. In this paper the TLC determination of the oleanolic acid content of Kangbingdu Jiaonang capsules is used as an example to study the uncertainty in order to improve the accuracy and reliability of data analysis and the measuring results. TLC

of the extracts and the standard oleanolic acid, on silica gel with chloroform - methanol 20:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 100 °C until the zones are visible in daylight. Quantification by densitometry at 520 nm. In summary the main factors affecting the uncertainty of the results are 1) the data calculated from the calculation curve, the uncertainty due to the least squares fitting curve, the purity of the standard sample used, weighing operation and the glassware taken; 2) the uncertainty of the sample application volume due to the application tools employed; 3) the uncertainty of the sample dilution factor due to the operation proficiency. A method for the calculation of the uncertainty corresponding to above factors in TLC determination of oleanolic acid in Kangbingdu Jiaonang capsules is presented, so that the accuracy and reliability of the results can be evaluated scientifically and an objective basis is provided to improve the executive procedure employed.

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

112 095 Y. LU (Lu Yuan), Y. HUANG (Huang Yong), L. ZHENG (Zheng Lin), Y. LI (Li Yongjun), A. WANG (Wang Aimin), Y. WANG (Wang Yonglin)* (*School of Pharm., Guiyang Med. Coll., Guizhou, Guiyang 550004, China): (Study of the method for the quality control of Fuketiaojing Jiaonang capsules by thin-layer chromatography) (Chinese). J. of China Pharm. 22 (6), 57-58 (2013). Fuketiaojing Jiaonang capsules are a herbal TCM preparation for the treatment of irregular menstruation and menstrual pain. For quality control, TLC on silica gel 1) for *Angelica sinensis* and *Ligusticum chuanxiong*, with cyclohexane - ethyl acetate 9:1, detection under UV 366 nm; 2) for *Cyperus rotundus*, with chloroform - ethyl acetate - methanol 50:10:1, detection by spraying with 5 % *p*-dimethylaminobenzaldehyde in 10 % sulfuric acid and heating until the zones are visible in daylight; 3) for Corydalis yanhusuo W. T. Wang ex Z. Y. Su et C. Y. Wu, with *n*-butanol - glacial acetic acid - water 7:1:2, detection under UV 366 nm.

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

112 096 A. MAURYA, S. SRIVASTAVA* (*Medicinal Chemistry Department, Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow-226015, India, santoshkumar_1955@yahoo.com): A simple and reliable HPTLC method for the determination of four marker components in the quality control of *Alstonia scholaris*. J. Planar Chromatogr. 26, 254-259 (2013). HPTLC of ursolic acid (1), betulinic acid (2), beta-sitosterol (3), and lupeol (4) in the stem bark, root bark and leaves of *Alstonia scholaris* on silica gel with chloroform - methanol 99:1. Detection by dipping into vanillin-sulfuric acid reagent for 2 s, followed by heating at 96 ºC for 8 min. Quantitative determination by absorbance measurement at 680 nm. The $hR_F$ values for (1) to (4) were 18, 27, 57 and 77, respectively. Linearity was in the range of 2-10 µg/zone for (1) and 84) and 4-20 µg/zone for (2) and (3). LOD and LOQ were 330 and 1100 ng/zone for (1), 530 and 1310 ng/zone (2), 290 and 970 ng/zone for (3) and 340 and 1140 ng/zone for (4), respectively. Average recoveries (by standard addition) for (1) to (4) were between 98.9 and 100.5 %. Intermediate/interday/intra-day precision was below 2 % ($n$=9).

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

112 097 B. NYAMWERU, E. KAALE*, V. MUGOYELA, M. CHAMBUSO (*Medicinal Chemistry and Allied Sciences and Pharm R&D Laboratory, School of Pharmacy, Muhimbili University of Health, Tanzania, wariwari@gmail.com): Development and validation of an HPTLC-densitometric method for simultaneous analysis of lamivudine, tenofovir disoproxil fumarate, and

efavirenz (LTE) in tablets. J. Planar Chromatogr. 26, 226-231 (2013). HPTLC of lamivudine (1), tenofovir disoproxil fumarate (2), and efavirenz (3) in tablets on silica gel with toluene - methanol 27:6. Quantitative determination by absorbance measurement at 254 nm. The $hR_F$ values for compounds (1) to (3) were 12, 16 and 53, respectively. Linearity was in the range of 375-900 ng/zone for (1) and (2) and 750-1800 ng/zone for (3). LOD and LOQ for (3) were 1 and 3 ng/zone. Recovery was in the range of 98.3-102.9 % for (1) to (3). Intermediate/interday/intra-day precision was below 2 % ($n$=6).

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

112 098 M. OCH, A. OCH, L. CIESLA, J. KOCKI, Anna KOCKA* (*Medical University of Lublin, Department of Pharmaceutical Botany, Faculty of Pharmacy, Chodzki 1, 20-093 Lublin, Poland, anna.kocka@o2.pl): Screening various *Juniperus* species for the occurrence of umbelliferone by means of bivariate multiple development thin-layer chromatography. J. Planar Chromatogr. 26, 421-426 (2013). HPTLC of umbelliferone in the leaves of eleven *Juniperus* species on silica gel with *n*-hexane - ethyl acetate 7:3 in the first run and dichloromethane - diethyl ether 4:1 in the second run. Quantitative determination by absorbance measurement at 326 nm. The $hR_F$ of umbelliferone was 30. Linearity was between 10 and 80 ng/zone. LOD and LOQ were 7 and 23 ng/zone. Average recovery (by standard addition) was 96.3 %. Intermediate intra- and inter-day precision ($n$ = 6) was below 7 %.

herbal, HPTLC, quantitative analysis 32e

112 099 J. PENG (Peng Juan), H. YAN (Yan Han), N. GUO (Guo Na), Y. NIE (Nie Yinglan), B. FAN (Fan Bin)* (*Med. Experim. Center, China Acad. Trad. Chinese Med. Sciences, Beijing 100700, China): (Study of the method for the quality control of Yangxue Yishen capsules) (Chinese). Chinese J. of Inform. on TCM 20 (7), 52-54 (2013). Yangxue Yishen capsules are a herbal TCM preparation for treating rough skin, blood scale disease, , alopecia areata and neurodermatitis. For quality control, TLC on silica gel 1) for *Gastrodia elata* Blume and the standard gastrodine, with chloroform - ethyl acetate - methanol - formic acid 80:10:30:1, detection by spraying with 10 % phosphomolybdic acid in ethanol and heating at 110 °C until the zones are visible in daylight; 2) for *Epimedium davidii* Franch and the standard icariin, with benzene - methanol - glacial acetic acid 45:8:4, detection by spraying with 10 % aluminium chloride in ethanol and letting stand for 40 min at room temperature, evaluation under UV 366 nm; 3) for *Salvia miltiorrhiza* and the standard protocatechuic aldehyde, with chloroform - acetone - formic acid 25:10:4, detection by exposure to ammonia vapors for 15 min and viewing under UV 254 nm. Quantification of astragaloside A by HPLC-ELSD.

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

112 100 P. PRAJAPATI*, V. VAGHELA (*Institute of Research and Development, Gujarat Forensic Sciences University, Gandhinagar-382007, Gujarat, India, prajeshprajapati@gmail.com): Densitometric measurement for estimation of ciclesonide in bulk and its dosage form (Rotacap) by high-performance thin-layer chromatography. J. Planar Chromatogr. 26, 435-439 (2013). HPTLC of ciclesonide in bulk and dosage form on silica gel with acetonitrile - 1,4-dioxane - n-hexane 1:1:8 +1 drop ethyl acetate. Quantitative determination by absorbance measurement at 242 nm. The $hR_F$ of ciclesonide was 38. Linearity was between 1 and 6 mg/zone. LOD and LOQ were 100 and 303 ng/zone. Recovery (by standard addition) was found to be 98.9-100.3 %.

Intra- and inter-day precision values were below 0.7 and 1.4 %, respectively.

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

112 101 E. PRIYA*, P. SELVAN, P. PRAKASH (*Department of Pharmaceutical Technology, Anna University of Technology, Tiruchirappalli - 620 024, Tamilnadu, India, sanmug77@gmail.com): HPTLC method development and validation for simultaneous analysis of emodin and chrysophanol in *Cassia tora* Linn methanolic extract. J. Liq. Chromatogr. Relat. Technol. 36, 2525-2533 (2013). HPTLC of emodin (1) and chrysophanol (2) in the seeds of *Cassia tora* L. on silica gel with toluene - ethyl acetate 9:1. Quantitative determination by absorbance measurement at 435 nm. The $hR_F$ values for (1) and (2) were 31 and 85, respectively. Linearity was in the range of 10-300 µg/zone for both (1) and (2). LOD and LOQ were 290 and 990 ng/zone for (1) and 240 and 750 ng/zone for (2). Average recoveries (by standard addition) for (1) and (2) were 94 % and 95 %, respectively. Intermediate intra- and inter-day precision was below 0.8 % ($n$=6).

herbal, HPTLC, quantitative analysis 32e

112 102 K. RAVIKANTH*, A. KANAUJIA, D. THAKUR, P. SINGH, B. GAUTAM (*R&D Centre, Ayurvet Ltd., Village Katha, P.O. Baddi-173205, District Solan, Himachal Pradesh, India, krk@ayurvet.in): Validated high-performance thin-layer chromatographic methods for the quantitative estimation of bioactive constituents in Methiorep Premix, a feed additive polyherbal formulation. J. Planar Chromatogr. 26, 312-315 (2013). HPTLC of L-dopa (1) and rutin (2) in a polyherbal formulation on silica gel with butan-1-ol - acetic acid - water 4:1:1 for (1) and ethyl acetate - butan-1-ol - formic acid - water 5:3:1:1 for (2). Quantification by absorbance measurement at 288 nm for (1) and 254 nm for (2). The $hR_F$ values for compounds (1) and (2) were 45 and 50, respectively. Linearity was in the range of 0.4-1.2 µg/zone for (1) and 0.4-0.9 µg/zone for (2). LOD and LOQ were 64 and 159 ng/zone for (1) and 76 and 228 ng/zone for (2), respectively. Average recoveries were 99.6 % for (1) and 100.1 % for (2). Intermediate/interday/intra-day precision was below 5 % ($n$=8).

food analysis, herbal, HPTLC, quantitative analysis 32e

112 103 J. RUAN (Ruan Jianbing)*, W. ZOU (Zou Wenzhe), X. JIN (Jin Xueping) (*Dep. of Envir. & Biochem. Engineering, Wuhan Career Acad. of Software & Engineering, Hubei, Wuhan 430205, China): (Study of the method for the quality control of Leifengshi Jiaonang capsules) (Chinese). J. of Modern Trad. Chinese Med. 15 (4), 320-323 (2013). Leifengshi Jiaonang capsules are a herbal TCM preparation for the treatment of rheumatism, ankylosing spondylitis, and lupus erythematosus. For quality control, TLC on silica gel 1) for *Astragalus membranaceus* (Fisch.) Bunge and the standard astragaloside A, with chloroform - methanol - water 13:7:2, detection by spraying with 10 % sulfuric acid in ethanol, heating at 105 °C and viewing in daylight and under UV 366 nm; 2) for *Angelica sinensis*, with petroleum ether (60-90 °C ) - diethyl ether - acetic acid 20:20:1, detection under UV 366 nm 3) for *Epimedium sagittatum*, with ethyl acetate - formic acid - butanone - water 10:1:1:1, detection under UV 366 nm. Quantification of ferulic acid by HPLC.

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

112 104 CH. SHI (Shi Changsheng), L. XU (Xu Li)* (*Pharm. Dep. of Hebei Armed Police Corps Hosp., Hebei, Shijiazhuang 050081, China): (Determination of indirubin and indigo in Banlian

Chongji electuary by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 10 (17), 98-99 (2012). Banlian Chongji electuary is herbal TCM preparation for the treatment of virus flu and upper respiratory infections. For quality control the indirubin and indigo contents are determined by TLC on silica gel with petroleum ether (60-90 °C) - ethyl acetate - chloroform 1:1:8. Detection in daylight, quantification by densitometry at 290 nm. The linearity was in the range of 0.1-0.5 µg/zone (*n*=5, r=0.9999) both for indirubin and indigo. The precision (*%RSD*, *n*=5) was 1.2 % for indirubin and 2.2 % for indigo. Recovery by standard addition was 95.5 % (*%RSD*=4.5 %, *n*=5) for indirubin, and 96.0 % (*RSD%*=2.9 %, *n*=5) for indigo.

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

112 105 L. SONG (Song Li)*, H. LIU (Liu Haijing), R. QIAO (Qiao Rongxia) (*Shanxi Provin. Inst. for Food & Drug Contr., Shanxi, Xi'an 710061, China): (Study on the method for the analysis of *Polygonum multiflorum* Thunb. in Shanhaidanpian tablets) (Chinese). J. of China Pharm. 26 (9), 997-999 (2012). Shanhaidanpian tablets are a herbal TCM preparation for the treatment of poor blood flow, blood stasis and chest pain syndrome. For quality control, TLC on silica gel with toluene - ethyl acetate - formic acid 20:2:1, detection by exposure to vapors until the zones are visible in daylight. Quantitative determination of 2,3,5,4'-tetrahydroxystilbene-2-O-beta-D-glucoside by HPLC.

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

112 106 X. SU (Su Xing), X. LI (Li Xiangkun), H. TAO (Tao Hongxun)*, J. ZHOU (Zhou Jinge), G. CHOU (Chou Guixin), Z. HENG (Cheng Zhihong) (*Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P. R. China, laura-wu2000@163.com): Simultaneous isolation of seven compounds from *Glehnia littoralis* roots by off-line overpressured layer chromatography guided by a TLC antioxidant autographic assay. J. Sep. Sci. 36, 3644-3650 (2013). TLC bioautography of superoxide scavengers in the roots of *Glehnia littoralis* on silica gel with petroleum ether - ethyl acetate 4:1. Detection by dipping into 300 mU/mL xanthine oxidase in 100 mM phosphate buffered saline pH 7.9, followed by incubation at 37 ºC for 20 min and dipping into a mixture of 10 mM xanthine and 20 mM nitrotetrazolium blue chloride in 100 mM phosphate buffer (pH 7.9), followed by a second incubation for 20 min at 37 ºC. The method allowed target-directed isolation of compounds.

herbal, qualitative identification, bioautography 32e

112 107 SH. SUN (Sun Shouguo)*, SH. WANG (Wang Shouxu), F. JIA (Jia Furong), Y. GAO (Gao Yingnan), X. JING (Jing Xuejie) (*Anhui Huatuo TCM Co. Ltd., Anhui, Haozhou 236803, China): (Study of the method for the quality control of Zhixie Baotong Keli granules by thin-layer chromatography) (Chinese). Chinese J. of Med. Guide 11 (16), 509-510 (2013). Zhixie Baotong Keli granules are a herbal TCM preparation for the treatment of dysentery, abdominal pain, dry mouth, nausea, and vomiting. For quality control, TLC on silica gel 1) for *Tetradium ruticarpum*, with cyclohexane - ethyl acetate - methanol - triethylamine 15:5:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C for 4 min and viewing under UV 366 nm; 2) for Pogostemon cablin, with petroleum ether (60-90 °C) - ethyl acetate 19:2, detection by spraying with 1 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C until the zones are visible in daylight; 3) for *Fructus Amomi* and the standard bornyl acetate, with cyclohexane - ethyl acetate 22:1, detection by spraying with 5 % vanillin in sulfuric acid - etha-

nol 1:4 and heating at 105 °C until the zones are visible in daylight.

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

112 108 R. THANGAVELU*, S. MAKAPOTHULA, N. GOWDA (*Analytical Research Laboratory, Department of Pharmaceutical Analysis, PES College of Pharmacy, Hanumanthanagar, Rajiv Gandhi University of Health Sciences, Bengaluru-560050, Karnataka, India, nraj_msubaroda@yahoo.co.in): Validated specific densitometric method for simultaneous estimation of telmisartan and atorvastatin in presence of degradation products formed under ICH-recommended stress conditions. J. Planar Chromatogr. 26, 445-451 (2013). HPTLC of telmisartan (1) and atorvastatin (2) on silica gel with methanol - chloroform 1:7. Quantitative determination by absorbance measurement at 280 nm. The $hR_F$ values for (1) and (2) were 64 and 27, respectively. Linearity was between 1.2-7.2 µg/zone for (1) and 0.4-2.4 µg/zone for (2). LOD and LOQ were 3 and 8 ng/zone. Recoveries (by standard addition) were 98-101 % for (1) and 99-101 % for (2). Intermediate intra- and inter-day precision was below 2 %. Comparable results were obtained with HPLC and UV methods.

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

112 109 N. VANI, B. MOHAN, G. NAGENDRAPPA* (*Department of Chemistry, University of Mysore, Manasagangothri, Mysore 570 006, India, gnagendrappa@yahoo.co.in): A new high-performance thin-layer chromatographic method for determination of diazepam in spiked blood samples. J. Planar Chromatogr. 26, 343-348 (2013). HPTLC of diazepam in spiked blood samples on silica gel with hexane - ethyl acetate 7:3. Quantification by absorbance measurement at 230 nm. The $hR_F$ of diazepam was 34. Linearity was in the range of 0.5-20 µg/zone. LOD and LOQ were 0.2 and 0.5 µg, respectively. Recovery was in the range of 94-98 %. Intermediate/interday/intra-day precision was below 2 % ($n$=5).

clinical chemistry research, quantitative analysis, HPTLC 32c

112 110 A. VAYKOLE, S. NIRMAL*, R. JADHAV, S. PATTAN (*Department of Pharmacognosy, Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Loni 413736, Maharashtra, India, nirmalsunil@rediffmail.com): Development and validation of HPTLC method to detect curcumin, piperine, and boswellic acid in polyherbal transdermal patch. J. Liq. Chromatogr. Relat. Technol. 37, 367-378 (2014). TLC of curcumin (1), piperine (2), and boswellic acid (3) in polyherbal transdermal patch on silica gel aluminum foils with chlorofom - ethyl acetate - formic acid 75:60:2. Quantitative determination by absorbance measurement at 540 nm. The $hR_F$ values for (1) to (3) were 48, 52 and 61, respectively. The linear calibration range was selected at very high amounts (1-15 µg/zone for (1) to (3)) despite the low LOD and LOQ of 0.06 and 0.2 ng/zone for (1), 0.31 and 0.95 ng/zone for (2) and 14.22 and 43.10 ng/zone for (3). Average recovery (by standard addition) was in the range of 98-99 % for (1) to (3). Intermediate intra- and inter-day precision was below 0.1 % ($n$=6).

pharmaceutical research, quantitative analysis 32a

112 111 L. WANG (Wang Lifen)*, Y. YE (Ye Yuhua), (*Shaoyang Hosp. of Integrated Trad. Chinese & Western Med., Hunan, Shaoyang 422001, China): (Study of the method for the quality control of Zhining Xiji lotion) (Chinese). Chinese J. of Guide for Trad. Chinese Med. & Pharm. 18

(11), 80-82 (2012). Zhining Xiji lotion is a herbal TCM preparation for treating inflammatory external hemorrhoids, incarcerated hemorrhoids, and the postoperative perianal edema. For quality control, TLC on silica gel 1) for *Radix Sophorae Flavescentis*, with toluene - acetone - methanol 16:6:3 after chamber saturation with ammonia for 15 min, detection by spraying with 5 % potassium iodobismuthate in hydrochloric acid - water 0.5:100 and viewing in daylight; 2) for *Cortex Phellodendri Chinensis*, developed with *n*-butanol - glacial acetic acid - water 7:1:2, detection under UV 366 nm. Quantification of berberine hydrochloride by HPLC.

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

112 112 L. WANG (Wang Lijuan) (Jinke Tibetan Med. Co. Ltd., Qinghai, Xining 810003, China): (Study of method for pharmacognosy identification of *Nardostachys chinensis* Batal. and *Pterocephalus hookeri* (Clarke) Hoeck by thin-layer chromatography) (Chinese). Chinese J. of Ethnopharm. 7 (7), 19-21 (2013). The roots of the grasses *Nardostachys chinensis* Batal. and *Pterocephalus hookeri* (Clarke) Hoeck are herbal Tibetan drug for treating epidemic disease, long fever and dysentery. During investigation of the medicinal plants growing in the Qinghai and Tibetan Plateau the following identification method for the two drugs was developed. TLC on silica gel 1) for *Nardostachys chinensis* Batal., a) with cyclohexane - ethyl acetate 5:1 and b) with petroleum ether (60-90 °C) - acetone 4:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones are visible in daylight. With mobile phase a) 3 zones were separated and with b) 6 zones; 2) for *Pterocephalus hookeri* (Clarke) Hoeck, a) with benzene - ethyl acetate 16:7 and b) with petroleum ether (60-90 °C) - acetone 3:2, all detection under UV 366 nm. With both mobile phases three blue fluorescent zones were detected.

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

112 113 L. WANG (Wang Linli)*, D. MENG (Meng Desheng), Y. PENG (Peng Yixin) (*Dep. of Pharm., Daping Hosp. Affil. to Inst. of field surgery, Chinese PLA Third Milit. Med. Univ., Chongqing 400042, China): (Study of the method for the quality control of Tongfenglishi Koufuye oral liquid) (Chinese). J. of China Pharm. 21 (15), 49-50 (2012). Tongfenglishi Koufuye oral liquid is a herbal TCM preparation for the treatment of rheumatic arthralgia, the numbness of limbs, swollen carbuncles and hyperlipidemia. For quality control, TLC on silica gel 1) for *Radix et Rhizoma Rhei*, with petroleum ether (30-60 °C) - ethyl formate - formic acid 15:5:1, detection in daylight; 2) for *Fructus Crataegi*, with cyclohexane - chloroform - ethyl acetate 20:5:8, detection by spraying with 10 % sulfuric acid in ethanol, heating at 110 °C and viewing in daylight; 3) for *Semen Vaccariae*, with chloroform - methanol - water 15:7:2, detection by spraying with 5 % potassium iodobismuthate in HCl - water 1:200 and viewing in daylight. Quantification of emodin by HPLC.

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

112 114 X. WANG (Wang Xianping)*, SH. ZHANG (Zhang Shengbo), X. BI (Bi Xueyan), Q. Q. ZHANG (Zhang Qingbo) (*Heilongjiang Univ. of Trad. Chinese Med., Heilongjiang, Harbin 150040, China): (Study of the method for the quality control of Gongxueting Keli granules) (Chinese). Chinese J. of Heilongjiang Med. 26 (3), 382-385 (2013). Gongxueting Keli granules are a herbal TCM preparation for treating women‘s asthenia of both the spleen and kidney, and blood stasis due to menstruation and metrorrhagia. For quality control, TLC on silica gel 1)

for *Eclipta* and the standard ecliptasaponin A, with dichloromethane - ethyl acetate - methanol - water 30:40:15:3, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones are visible in daylight; 2) for *Leonurus Artemisia* (Laur.) S. Y. Hu F and the standard stachydrine hydrochloride, with acetone - ethanol - hydrochloric acid 10:6:1, detection by heating at 105 °C for 15 min followed by spraying with 10 % sulfuric acid in ethanol and then heating, followed by spraying with 5 % potassium indobismuthate - 1 % ferric chloride in ethanol 10:1, viewing in daylight.

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

112 115 X. WANG (Wang Xiaochuan), ZH. ZHANG (Zhang Zhenhua) (Neijiang Municip. TCM Hosp., Sichuan, Neijiang 641000, China): (Study of the method for the quality control of Fufang Huoxue Tongluojiu liquor by thin-layer chromatography) (Chinese). Chinese J. of Northern Pharmacy 10 (3), 5-6 (2013). Fufang Huoxue Tongluojiu liquor is a herbal TCM preparation for easing traumatic injury, soft tissue injury, arthralgia, and muscle soreness. For quality control, TLC on silica gel 1) for *Caesalpiniaceae*, with chloroform - acetone - formic acid 8:4:1, detection by heating at 105 °C until the zones are visible in daylight; 2) for *Rhizoma Corydalis* and the standard tetrahydropalmatine, with toluene - acetone 9:2, detection by exposure to iodine vapors for 3 min and viewing under UV 366 nm; 3) for *Flos Carthami*, with chloroform - ethyl acetate - glacial acetic acid 5:4:1, detection under UV 254 nm; 4) for semi quantification of aconitine, with chloroform - ethyl acetate 1:1, detection by saturation with ammonia vapors and spraying with 5 % potassium iodobismuthate in hydrochloric acid - water 1:200 and viewing in daylight. Semi quantitative analysis by comparison with aconitine.

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

112 116 Q. WEI (Wei Qing)*, J. WEI (Wei Jinde), H. KANG (Kang Hongying) (*The First coll. of Clin. Med. Sci., Sanxia Univ., Hubei, Yichang 443003, China): (A method for rapid identification of carmine, etythrosine and brilliant crocein illegally added in *Kadsura longipedunculata* Finet et Gagnep. by thin-layer chromatography) (Chinese). J. of Hubei Univ. of Trad. Chinese Med. 15 (5), 38-40 (2013). *Kadsura longipedunculata* Finet et Gagnep. is the dried and ripe fruit of *Schisandra sphenanthera* Rehd. et Wils., a herbal TCM drug commonly used in TCM preparations for the treatment of cough, spermatorrhea, enuresis, diarrhea, night sweating, palpitation and insomnia. False and inferior species often appear on the market, e.g. inferior plant material was sold as high quality product after adding synthetic dyes which are harmful to human health. As this kind of adulteration is not easily identified by eye, a TLC method for rapid identification of three dyes was developed. TLC of the drug extracts and the standards carmine, erythrosine, and brilliant crocein, on silica gel with ethyl acetate - *n*-butanol - ethanol - ammonia - water 1:3:3:1:1, detection in daylight.

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

112 117 Y. WU (Wu Yin), A. JIA (Jia An), H. ZHOU (Zhou Haifeng), W. NI (Ni Weimin)* (*Shanghai Hosp. of Trad. Chinese Med., Shanghai 200040, China): (Study of the method for the quality control of Sanhuang zhiyang chaji liniment by thin-layer chromatography) (Chinese). Chinese J. of Guide for Trad. Chinese Med. & Pharm. 2 (2), 97-99 (2013). Sanhuang zhiyang chaji liniment is a herbal TCM preparation prescribed for treating skin itching, carbuncle furuncle, acute eczema, impetigo and skin infection. For quality control, TLC on silica gel 1) for *Cortex*

*Phellodendri Chinensis* and the standard berberine hydrochloride, with toluene - ethyl acetate - isopropanol - methanol - ammonia 12:6:3:1:1, detection by exposure to ammonia vapors and viewing under UV 366 nm; 2) for *Radix Scutellariae* and the standard baicalin, with ethyl acetate - butanone - methanol - water 5:3:1:1, detection by spaying with 1 % ferric chloride in ethanol and viewing in daylight; 3) for *Radix et Rhizoma Rhei* and the standard emodin, with petroleum ether (60-90 °C) - ethyl formate - formic acid 15:5:1, detection under UV 366 nm; 4) for *Radix Sophorae Flavescentis* and the standard matrine, with benzene - propanol - ethyl acetate - ammonia 10:15:20:1, detection by spaying with 5 % potassium iodobismuthate in hydrochloric acid - water 1:200 and viewing in daylight.

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

112 118 L. XIAO (Xiao Linlin) (Ganzhou Municip. People's Hosp., Jiangxi, Ganzhou 341000, China): (Study of the method for the quality control of Huaxue Jietonggao ointment by thin-layer chromatography) (Chinese). Chinese J, Mod. Drug Appl. 6 (22), 129-130 (2012). Huaxue Jietonggao ointment is herbal TCM preparation for the treatment of sprain, contusion, rheumatism, lumbago and back ache. For quality control, TLC on silica gel 1) for *Capsicum annuum* and the standard capsaicin, with petroleum ether (30-60 °C) - ethanol 9:1, detection under UV 254 nm; 2) for *Zingiber officinale* Rosc., with petroleum ether (60-90 °C) - ethyl acetate 2:1, detection by spraying with 2 % *p*-dimethylaminobenzaldehyde in 40 % sulfuric acid in ethanol and heating at 105 °C until the zones are visible in daylight; 3) for *Heracleum hemsleyanum*, with *n*-hexane - toluene - ethyl acetate 2:1:1, detection under UV 366 nm.

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

112 119 SH. XIE (Xie Shiwei) (Dongguan Municip. Inst. for Drug Contr., Guangdong, Dongguan 523109, China): (Study of the method for the identification of Chinese *Eupatorium* L. root by thin-layer chromatography) (Chinese). J. of Trad. Chinese Med. & Pharm. Consult. 4 (5), 16-17 (2012). The root of Chinese *Eupatorium* L. is a herbal TCM drug used in various preparations for the treatment of diphtheria, sore throat, fever, cough, traumatic swelling pain and snake bites. For quality control and to prevent counterfeiting TLC of herbal extracts and the standard euparin on silica gel with n-hexane - chloroform - methanol 20:10:1, detection by spraying with 1 % aluminiumchloride in ethanol and heating until the zones are visible in daylight, and under UV 366 nm.

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

112 120 Y. XIE (Xie Yutao)*, W. XIANG (Xiang Wei), J. WU (Wu Jinyang), L. LI (Li Lin), Y. ZHANG (Zhang Yan) (*Dep. Pharm., Nanchong Municip. Central Hosp., Sichuan, Nanchong 637000, China): (Study of the method for the quality control of Xian Rong Bushen Jiu liquor by thin-layer chromatography) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. 13, 31-33 (2012). Xian Rong Bushen Jiu liquor is a herbal TCM preparation containing several TCM herbs effective for improving impotence, premature ejaculation, lassitude and hypogonadism, etc. For quality control, TLC on silica gel 1) for *Epimedium brevicornu* Maxim. and the standard icariin, with the lower phase of chloroform - ethyl acetate - methanol - water 15:40:22:10, detection under UV 366 nm before and after spraying with 1 % aluminium chloride in ethanol; 2) for *Panax ginseng* C. A. Mey. and the standards ginsenoside Rb1, ginsenoside Re and ginsenoside

Rg1, with chloroform - ethyl acetate - methanol - water 10:1:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones are visible in daylight.

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

112 121 J. XING (Xing Jun)*, W. DU (Du Weiliang) (*The Hosp. Affiliated to Luzhou Coll. of Med, Sichuan, Luzhou 646000, China): (Study of the method for the quality control of Shengjing Yusi capsules by thin-layer chromatography) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. (23), 51-52 (2012). Shengjing Yusi capsule is a herbal TCM preparation for treating male infertility. For quality control, TLC on silica gel 1) for *Epimedium davidii* Franch, with chloroform - methanol - water 13:7:2, detection under UV 366 nm; 2) for *Fructus Schisandrae Chinensis*, with petroleum ether (30-60°C) - ethyl acetate - formic acid 15:5:1, detection by spraying with 5 % phosphomolybdic acid in ethanol and heating at 105 °C until the zones are visible in daylight.

pharmaceutical research,traditional medicine,quality control, herbal, q ualitative identification 32e

112 122 J. YANG (Yang Jing)*, N. CHEN (Chen Na), ZH. MA (Ma Zhipeng), H. SUN (Sun Hao), X. CAO (Cao Xiaoxia) (*Tianjin Bohai Career Technical Coll., Tianjin 300402, China): (Study of the method for the quality control of Tiaojing Huayu Wan pills) (Chinese). Chinese J. of Inform. on TCM 19 (7), 46-48 (2012). Tiaojing Huayu Wan pills are a herbal TCM preparation for treatment of menstrual disorders, menstrual abdominal pain, amenorrhea caused by vital energy stagnation and blood stasis. For quality control, TLC on silica gel 1) for *Rhizoma Cyperi*, with benzene - ethyl acetate - glacial acetic acid 92:5:5, detection under UV 254 nm; 2) for *Radix Angelicae Sinensis, Rhizoma Ligustici* Chuanxiong, and the standard ferulic acid with benzene - ethyl acetate - formic acid 40:10:1, detection under UV 366 nm. Quantification of paeoniflorin by HPLC.

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

112 123 R. YOUSSEF*, E. KHAMIS, S. YOUNIS, F. EL-YAZBI (*Faculty of Pharmacy, Department of Pharmaceutical Analytical Chemistry, University of Alexandria, El-Messalah, Alexandria 21521, Egypt, rmmy1973@yahoo.com): Validated high-performance thin-layer chromatographic method for the evaluation of oseltamivir pharmaceutical formulations counterfeited with ascorbic acid compared with a colorimetric method. J. Planar Chromatogr. 26, 427-434 (2013). HPTLC of oseltamivir (1) in pharmaceuticals counterfeited with ascorbic acid (2) on silica gel with methanol - water 3:2 +1 drop ammonia. Quantitative determination by absorbance measurement at 254 nm. The $hR_F$ values for (1) and (2) were 70 and 83, respectively. Linearity was between 5 and 14 µg/zone. LOD and LOQ were 2 and 5 µg/zone. Average recovery (by standard addition) was found to be 100.6 %. Intermediate intra- and inter-day precision was below 1.6 %. Comparing with colorimetric method, HPTLC method provided advantages in terms of speed, lower cost, and environmental protection without sacrificing accuracy.

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

112 124 CH. ZHANG (Zhang Chengguang)*, M. TAN (Tan Meiying), A. XU (Xu Aili), S. LI (Li Sumei) (*Guangdong Prov. 2nd Hosp. of Trad. Chinese Med., Guangdong, Guangzhou 510095, China): (Study of the method for the quality control of Zhongfeng Fuyuan Koufuye oral liquid)

(Chinese). Jiangxi J. of Trad. Chinese Med. 44 (367), 58-60 (2013). Zhongfeng Fuyuan Koufuye oral liquid is a herbal TCM preparation for the treatment of post stroke shoulder hand syndrome during stroke recovery and sequela period, and constipation after stroke. For quality control, TLC on silica gel 1) for *Astragalus membranaceus* (Fisch.) Bunge., 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 and viewing in daylight; 2) for *Pericarpium Citri Reticulatae*, with toluene - ethyl acetate 3:2, detection by heating at 105 °C for 3 min and viewing under UV 366 nm; 3) for *Paeonia veitchii*, with toluene - ethyl acetate - isopropanol - methanol - ammonia 20:10:5:5:1, detection by exposure to ammonia vapors for 15 min and spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C, viewing in daylight. Quantification of astragaloside A by HPLC.

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

112 125 M. ZHANG (Zhang Ming), Y. NI (Ni Ying), W. JIN (Jin Weihua), K. FAN (Fan Kaihua)* (* Dep. of Pharm., General Hosp. of Chengdu Milit. Area Command Sichuan, Chengdu 610083, China): (Study of the method for the quality control of Fufang Xiaoji Zhike Milian Gao ointment) (Chinese). Chinese J. of Med. Guide 10 (20), 99-102 (2013). Fufang Xiaoji Zhike Milian Gao ointment is herbal TCM preparation for clearing the body heat caused by inflammation, resolving phlegm and relieving asthma. For quality control, TLC on silica gel 1) for *Pericarpium Citri Reticulatae* and the standard hesperidin, first over 3 cm with ethyl acetate - methanol - water 99:17:13 and thenover 8 cm with toluene - ethyl acetate - formic acid - water 95:45:5:3, detection by spraying with 2 % aluminium chloride in ethanol and viewing under UV 366 nm; 2) for *Schisandra Chinensis* and the standard schizandrin A, with the upper phase of petroleum ether (30-60 °C) - ethyl formate - formic acid 15:5:1, detection under UV 254 nm; 3) for *Crataegus pinnatifida* and the standard ursolic acid, with toluene - ethyl acetate - formic acid - water 39:8:2:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 80 °C, detection under UV 366 nm. Quantification of tenuifolin by HPLC.

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

12 126 SH. ZHANG (Zhang Shuyu) (Pharm. Dep., General Hosp. of Jinan Milit. Area, Shandong, Jinan 250031, China): (Qualitative and quantitative analysis of tanshinone IIA in Fufang Hanfangji Keli granules) (Chinese). Chinese J. of Pharm. Practice 31 (3), 228- 230 (2013). Fufang Hanfangji Keli granule is a herbal TCM preparation for treatment of acute and chronic hepatitis, fatty liver, gallbladder disease, and for reducing high total serum cholesterol and aortic atherosclerotic plaques. For quality control, the key drug of the preparation, *Salvia miltiorrhiza* Bunge, is analysed based on its content of tanshinone IIA. TLC on silica gel with petroleum ether (60-90 °C) - ethyl acetate 4:1. Quantification of tanshinone IIA by HPLC.

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

112 127 SH. ZHANG (Zhang Shuyu)*, Y. YU (Yu Yanli), SH. LIU (Liu Shijun), Y. BI (Bi Yunsheng), CH. NI (Ni Chenming) (*General Hosp. of Jinan Milit. Area, Shandong, Jinan 250031, China): (Qualitative and quantitative determination of the effective components of *Aristolochia fangchi* in Fufang Hafangji Keli granules) (Chinese). J. of China Pharm. 27 (7), 725-728 (2013). Fufang Hafangji Keli granules, with *Aristolochia fangchi* as key component drug, are a her-

bal TCM preparation for improving the liver function, and are used for treatment of acute and chronic hepatitis, cirrhosis, and fatty liver. TLC of the extracts and standards (+)-tetrandrine and fangchinoline on silica gel with cyclohexane - chloroform - acetone - methanol 10:6:1:1, detection by spraying with (bismuth subnitrate - glacial acetic acid - water 1:10:40) - potassium iodide - glacial acetic acid - water 1:1:4:20. Quantification of (+)-tetrandrine and fangchinoline by HPLC.

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

112 128 X. ZHANG (Zhang Xiaohua)*, X. LI (Li Xiujuan), ZH. JIAO (Jiao Zhenghua) (Gansu Provinc. Hosp. of Trad. Chinese Med., Gansu, Lanzhou 730050, China): (Study of the method for the quality control of Huayu Shengji Gao ointment) (Chinese). Chinese J. of Inform. on TCM 19 (6), 55-57 (2012). Huayu Shengji Gao ointment is a herbal TCM preparation for treating pain of surgery wounds, burn ulcer, and fester. For quality control, TLC on silica gel for 1) *Angelica sinensis*, with *n*-butanol - ethyl acetate 9:1, detection under UV 366 nm; 2) for *Lithospermum erythrorhizon*, with cyclohexane - toluene - ethyl acetate - formic acid 50:50:5:1, detection in daylight; 3) for *A. dahurica* (Fisch.) Benth. et Hook, with petroleum ether (30-60 °C) - diethyl ether 3:2, detection under UV 366 nm. Quantification of ferulic acid by HPLC.

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

112 129 Y. ZHANG (Zhang Yun)*, H. HU (Hu Haiting), Q. LI (Li Qin) (*Pharm. Coll., Henan Univ., Henan, Kaifeng, 475004, China): (Exploration on the procedures for the quality control of Shiming Granules by thin-layer chromatography) (Chinese). Chinese J. of Henan Univ. (Med Sci.) 32 (2), 117-121 (2013). Shiming Granule is a herbal TCM preparation for invigorating blood circulation, improving liver and pulmonary functions and the eyesight. For quality control, TLC on silica gel 1) for *Rhizoma et Radix Notopterygii*, with *n*-hexane - ethyl acetate 7:3, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating until the zones are visible in daylight and under UV 366 nm; 2) for *Bupleurum chinense*, with ethyl acetate - methanol - water 8:2:1, detection by spraying with 2 % *p*-dimethylaminobenzaldehyde in 40 % sulfuric acid and heating at 60 °C, viewing under UV 366 nm; 3) for *Rhizoma Atractylodis*, with petroleum ether (60-90 °C) - ethyl acetate 20:1, detection by spraying with 5 % *p*-dimethylaminobenzaldehyde in 10 % sulfuric acid and heating until the zones are visible in daylight; 4) for *Exocarpium Citri Rubrum*, with ethyl acetate - methanol - water 100:17:10, detection by spraying with 3 % aluminiumchloride in ethanol, viewing under UV 366 nm; 5) for *Radix Saposhnikoviae*, with chloroform - methanol 4:1, detection under UV 254 nm; 6) for *Salvia miltiorrhiza*, with benzene - ethyl acetate 19:1, detection under UV 366 nm; 7) for *Radix Paeoniae Rubra*, with chloroform - methanol - formic acid 200:25:50:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating until the zones are visible in daylight; 8) for *Herba Equiseti Hiemalis*, with cyclohexane - ethyl acetate - formic acid 20:10:1, detection by spraying with 5 % aluminium chloride in ethanol and viewing under UV 366 nm; 9) for *Cuscuta chinensis* Lam., with ethyl acetate - butanone - formic acid - water 11:1:1:1, detection by spraying with 5 % aluminium chloride in ethanol and heating at 105 °C until the zones are visible, and viewing under UV 254 nm; 10) for *Rehmannia glutinosa* Libosch, with chloroform - methanol - ammonia 40:10:1, detection by spraying with 5 % vanillin in sulfuric acid - ethanol 1:4 and heating at 105 °C until the zones are visible in daylight; 11) for *Rhizoma Atractylodis Macrocephalae*, with cyclohexane - ethyl acetate 7:3, detection by spraying with 5 % *p*-dimethylaminobenzaldehyde in sulfuric acid - ethanol 1:10 and heating at 105 °C until the zones arevisible in daylight; 12) for *Radix Glycyrrhizae*, with ethyl acetate - formic acid -

water 15:3:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, viewing under UV 366 nm; 13) for *Radix Achyranthis Bidentatae*, with chloroform - methanol 30:1, detection by spraying with 5 % phosphomolybdic acid in *n*-propanol and heating at 110 °C until the zones are visible in daylight.

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

112 130 J. ZHAO (Zhao Jiping)*, A. LIU (Liu Aiping) (*Xiantao Municip. Inst. for Drug & Food Contr., Hubei, Xiantao 433000, China): (Study of the method for quick determination of rhaponiticin in antibacterial Kangjun Xiaoyan Jiaonang capsules by thin-layer chromatography) (Chinese). J. of China Pharm. 26 (6), 620-622 (2012). *Rheum palmatum* L., a herbal TCM drug for clearing away body heat, eliminating toxin and nourishing skin, is commonly used as a component of herbal TCM preparations. However, a homologous herb, *Rumex madaio Makino* (syn. *Rumex daiwoo Makino*) is sometimes mistaken for *Rheum palmatum* in drug production. For quality control of rhaponiticin in antibacterial Kangjun Xiaoyan Jiaonang capsules, TLC of the extracts and the standard rhaponiticin on silica gel with ethyl acetate - acetone - water 10:7:1, detection under UV 366 nm. Quantification of rhaponiticin by HPLC. The method was successfully applied to nine batches of real life samples from five pharmaceutical factories.

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

112 131 L. ZHENG (Zheng Lihong)*, W. LI (Li Weimin), J. HUANG (Huang Jian), Y. LI (Li Yang), ZH. CHEN (Chen Zhifeng), K. MAO (Mao Kechen) (Beijing Chinese Med. Hosp. Affiliated to Capital Med. Univ., Beijing 100010, China): (Study on the method for the quality control of Qieshisan medical powder) (Chinese). Beijing J. of Trad. Chinese Med. 31 (6), 458-460 (2012). Qieshisan medical powder is a herbal TCM preparation for the treatment of eczema and skin exudate erosion. For quality control 1) microscopy of *Scutellaria baicalensis* and *Rheum palmatum* L. (mixing the powder with chloral hydrate on a slide and heating until the slide looks transparent; 2) TLC of natural Indigo and the standards indirubin and indigo, on silica gel with toluene - chloroform - acetone 5:4:1, detection in daylight; 3) Quantification of baicalin by HPLC.

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

112 132 H. ZHOU (Zhou Hongchao)*, Y. SUN (Sun Yanchao) (*Xuchang Municip. Inst. for Drug Control., Henan, Xuchang 461000, China): (Study of the method for the quality control of Yufukang Jiaonang capsules by thin-layer chromatography) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. (3), 41-43 (2012). Yufukang Jiaonang capsules are a herbal TCM preparation for the treatment of numbness of the tongue, language disadvantage, choke and cough when eating, aphasia, etc. For quality control, identification of the main component crude drug, the root of *Salvia miltiorrhiza* and the standard tanshinone llA by TLC on silica gel with toluene - ethyl acetate 19:1, detection in daylight.

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

112 133 H. ZHOU (Zhou Hongmei)*, F. LU (Lu Fuqiang) (* Heilongjiang Tianlong Pharm. Co. Ltd., Heilongjiang, Harbin 150060, China): (Semiquantitative determination of taurine in Fufang Niaoweoan Diyanye eye drops by thin-layer chromatography) (Chinese). Chinese J. of Heilongji-

ang Med. 26 (2), 189-190 (2013). Fufang Niaoweoan Diyanye eye drops, *i.e.* allantoin/vitamin B6/vitamin E/aminoethylsulfonic acid eye drops, are used for treating eye fatigue and dry eye syndrome. For quality control, semiquantitative determination of taurine, the main active ingredient, by TLC on silica gel with *n*-butanol - ethanol - water - glacial acetic acid 3:1:1:1, detection by spraying with ninhydrin - ethanol 1:1000 and viewing in daylight. Semi-quantification by comparison of the area size and color of the target zone with the standard taurine. The LOD was around 100 ng/zone. The influences of temperature, acidic, alkaline and oxidizing conditions were studied.

quality control, clinical routine analysis, qualitative identification 32c

112 134 P. ZHU (Zhu Pei), H. ZHONG (Zhong Haiyan)*, W. HUANG (Huang Weiwen), B. ZHOU (Zhou Bo), J. GONG (Gong Jijun), Q. ZHAO (Zhao Qingjie) (*Coll. of Food Sci. & Eng., Central South Univ. of Forestry Sci. & Technol., Hunan, Changsha 410004, China): (Determination of polypeptide in protolysate of Camellia oleifera cake by thin-layer chromatography) (Chinese). Chinese J. Econ. Forest Res. 31 (2), 142-145 (2013). Compared to other analytical techniques TLC has the advantage of no/minor sample pretreatment, simultaneous separation and determination of numerous and complex samples. Determination of polypeptide in protolysate of *Camellia oleifera* cake by TLC on silica gel with *n*-butanol - ethanol - water 4:1:1, detection by spraying with 0.5 % ninhydrin in propanone and heating at 105 °C for 15 min and viewing in daylight. Quantification of polypeptide by densitometry at 231 nm using glutathione (a tripeptide composed of glutamic acid, cysteine and glycine) as the external calibration standard. The *%RSD* for repeatability was 0.03 % ($n$=5). Recovery by standard addition was 106.9 % (*%RSD*=0.06 %, $n$=5). The LOQ was 2 µg/zone. The results of the TLC method for a batch of real life samples were comparable with the results by RP-HPLC.

quality control, agricultural, qualitative identification, densitometry, comparison of methods 32e

## 35. Other technical products and complex mixtures

112 135 Laurie-Anne BARRET, Ange POLIDORI, Francoise BONNETÉ, P. BERNARD-SAVARY, Colette JUNGAS* (*CEA, IBEB, Lab Bioenerget Cellulaire, Saint-Paul-lez-Durance, 13108, France): A new high-performance thin layer chromatography-based assay of detergents and surfactants commonly used in membrane protein studies. J. of Chromatogr. A 1281, 135-141 (2013). The use of detergents for the extraction, solubilization and purification of membrane proteins (MPs) is necessary due to their hydrophobic nature. Detergent quantification is essential to routine analysis because the concentration of amphiphiles is crucial in the crystallization process. HPTLC of detergents (in small quantities, bound to solubilized MPs) on silica gel with dichloromethane - methanol - acetic acid 80:19:1. The optimum HPTLC conditions were investigated using *n*-dodecyl-beta-D-maltoside (DDM), the most popular detergent for membrane protein crystallization. Quantification by fluorescence measurement at 366 nm using a Hg lamp. The calibration curve was linear in the range of 100-1600 ng of DDM in water and the limit of detection of was 50 ng/zone, which is the best LOD achieved to date for a routine detergent assay (not modified by the addition of NaCl, commonly used in protein buffers). In comparison with other techniques (colorimetry, GC, and FTIR) the HPTLC method has the advantage of no prior sample treatment for concentration or extraction, and no chemical labeling is required. In comparison with TLC, the HPTLC method is 100 times more sensitive. The HPTLC method is suitable for routine analysis, assay results are obtained within 3 hours and only few microliters of sample are needed.

quality control, HPTLC, qualitative identification, quantitative analysis, densitometry, comparison of methods 35a, 19

112 136 I. REZIC (Laboratory of Analytical Chemistry, Department of Applied Chemistry, Faculty of Textile Technology, University of Zagreb, Croatia, iva_rezic@net.hr): Thin-layer chromatographic monitoring of sonolytic degradation of surfactants in wastewaters. J. Planar Chromatogr. 26, 96-101 (2013). HPTLC of cetylpyridinium chloride (1), sodium dodecyl sulfate (2) and Triton X-100 (3) on silica gel with methanol - water 1:1. Quantitative determination by absorbance measurement at 254 nm. The $hR_F$ values for (1) to (3) were 6, 74 and 91, respectively. Linearity was in the range of 170-1740 µg/zone for (1), 250-1980 µg/zone for (2) and 160-1640 µg/zone for (3). LOQ was 75 ng/zone for (1), 90 ng/zone (2) and 65 ng/zone for (3).

environmental, quality control, HPTLC, quantitative analysis 35a

112 137 S. ZHANG (Zhang Suzhen), H. BIAN (Bian Huan), D. WANG (Wang Daoying), F. LIU (Liu Fang), Y. ZHU (Zhu Yongzhi), W. XU (Xu Weimin), M. ZHANG (Zhang Muhan), H. LIU (Liu Hongjin)*, N. JIANG (Jiang Ning) (*Inst. of Agr. Prod. Processing, Jiangsu Acad. of Agr. Sci., Jiangsu, Nanjing 210014, China): (Study of the procedure for the test of colophony residue in the epidermis of meat ducks unhaired with rosin by thin-layer chromatography) (Chinese). Chinese J. of Jiangxi Agr. Sci. 25 (5), 117-119 (2013). Meat ducks used to be unhaired by employing certain safe depilating agents, however, a hot liquid composed of rosin and paraffin has found to be illegally applied by dipping into the hot liquid, so as to glue the liquid rosin closely onto the duck epidermis, and then by peeling the depilating agent after cooling. In this process some rosin components, such as abietic acid, may remain in the duck epidermis and even permeate the duck meat, which may be harmful to humans if daily intake exceeds 1 mg/kg body weight. Description of a procedure for testing colophony residues in the epidermis of meat ducks unhaired with rosin. TLC of the sample extracts (prepared by SPE), the standard abietic acid and depilating agent components (food grade wax and rosin glycerol ester), on silica gel with petroleum ether (60-90 °C) - ethyl acetate - glacial acetic acid 90:10:1, detection by spraying with 5 % sulfuric acid in ethanol and heating at 85 °C until the zones are visible in daylight. The LOD of abietic acid was 0.04 g/L. The method was successfully applied to the analysis of samples obtained from meat ducks unhaired with the depilating agents A) rosin - food grade wax 29:20, and B) food grade rosin glycerol ester - food grade wax 29:20, in the production conditions simulating a livestock and poultry processing enterprise.

quality control, food analysis, qualitative identification 35

## 38. Chiral separation

112 138 R. BHUSHAN*, S. BATRA (*Department of Chemistry, Indian Institute of Technology, Roorkee, India, rbushfcy@iitr.ernet.in): Direct enantiomeric resolution of (±)-bupropion using chiral liquid chromatography. J. Planar Chromatogr. 26, 491-495 (2013). TLC of enantiomers of (±)-bupropion on silica gel, impregnated with L-Glu, with acetonitrile - methanol - dichloromethane - water 28:5:11:5. Detection by exposition to iodine vapor. LOD was 0.2 µg/zone for each enantiomer.

pharmaceutical research, quality control, HPTLC, quantitative analysis 38


# Preliminary Program and Last Minute Posters

The Scientific Committee of the HPTLC 2014 welcomes you to the beautiful city of Lyon with the well-known French spirit of hospitality, good food and friendship. It is not too late to register your interest to participate with a poster at the symposium. A number of world renowned experts in HPTLC have already agreed to participate and a detailed final program will be available end of March. It will cover an exciting and diverse scientific program, panel discussion, workshops, poster presentations and an active social program. We look forward to seeing you in Lyon and learning from your experiences and ideas for the promotion of HPTLC. Do check out our website *www.hptlc.com* for the latest information on the scientific program and workshops and to obtain a discount for early bird registration until 31 March.

**Confirmed invited speakers so far**

- Prof. Dr. Susan Olesik, USA: Electrospun layers for UTLC
- Prof. Dr. Colin Poole, USA: An interphase model to explain retention in TLC
- Prof. Dr. Matthew Linford, USA: Fast, Microfabricated, normal phase TLC plates based on carbon nanotube forest scaffolds
- Pimolpun Kampalanonwat and Prof. Dr. Pitt Supaphol, Thailand: UTLC-MS on electrospun layers
- Dr. Irena Vovk, Slovenia: Analysis of natural products
- Prof. Dr. Abulimiti Yili, China: Analysis of biopolymers
- Prof. Dr. Wolfgang Schwack and Claudia Oellig, Germany: Planar SPE coupled to flow injection TOF-MS for rapid pesticide screening
- Prof. Dr. Alvaro Viljoen, South Africa: HPTLC in the quality control of African Traditional Medicines – examples from the South African flora
- Prof. Dr. Jentaie Shiea, Taiwan: Hyphenations in TLC/HPTLC-MS
- Elizabeth Crawford, Czech Republic/USA: Planar chromatography meets direct ambient mass spectrometry: current trends
- Dr. Agnes Moricz, Hungary: HPTLC-bioassay-MS, a rapid tool to search and analyze bioactive natural products
- Dr. Vicente Cebolla, Spain: Online, hyphenated AMD-FDIC-MS for quantitation of biomarkers of lysosomal storage diseases in human fluids
- Prof. Dr. Tadeusz Dzido, Poland: Orthogonal pressurized planar electrochromatography
- Tim Häbe and Prof. Dr. Gertrud Morlock, Germany: Improvements toward quantitative, automated HPTLC-DART-MS

**Practical Workshops (in parallel)**
WED, 2 July, 9:00-12:00 at University of Lyon

**Tutorials, including panel discussion**
WED, 2 July, 14:00-17:00 at Congress Center

**Sessions: Oral presentations and Poster Sessions**
THU/FRI 3 - 4 July, 9:00-18:00

**Deadlines**
Last minute poster abstracts: 1 May 2014

**Final registration: 30 May 2014**

**Fee**
Scientific program, lunches, coffee breaks, symposium dinner and social events included (reduction of 100 € with ISPS or CCCM membership).

Industrial 600 €

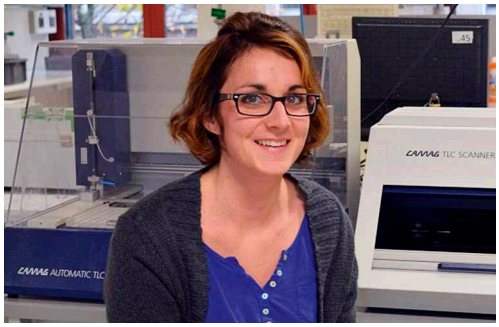
Academic 450 €

Students 200 €

**Extra:** Practical workshop 50 € for symposium registrants, 400 € for non-registrants (Academic/Industrial), 200 € (for Students)

Further announcements and information will be updated at **www.hptlc.com** **info@hptlc.com**

# HPTLC determination of sibutramine in adulterated herbal slimming supplements



*Caroline Mathon*


1

In close collaboration with the CAMAG Laboratory Ms Caroline Mathon developed the described validated and efficient HPTLC method for the rapid quantification of sibutramine in food supplements. This work was part of her PhD thesis, dedicated to the assessment of the composition of food supplements based on botanical ingredients, and performed at the University of Geneva's School of Pharmaceutical Sciences. Ms Mathon worked under the supervision of Dr Philippe Christen (University of Geneva) and Dr Stefan Bieri from the Official Food and Veterinary Control Authority, Geneva.

## Introduction

The market for herbal diet (slimming) food products is vast and accessible for everyone, but it faces quality and safety issues due to possible product adulterations and admixtures of illegal synthetic substances. Herbal slimming food supplements are not regulated by the rigorous drug safety rules therefore illegal admixtures can easily remain undetected and are a potential risk to consumers. Recently the synthetic substance sibutramine was detected in many adulterated slimming products. Until the year 2010 sibutramine was an approved drug used as an appetite suppressant but was withdrawn from European and US markets because of its unacceptable risk/benefit ratio and its potential to cause cardiovascular events or strokes.

**To detect and quantify sibutramine in herbal slimming supplements an HPTLC-UV densitometry method was developed. This method allows for simple, fast and accurate analysis of complex mixtures. For confirmation of positive results by mass spectrometry a TLC-MS interface was used to elute target zones from the HPTLC plate; this was especially useful for samples with low sibutramine concentration.**

With the validated HPTLC-UV method 52 slimming supplements including capsules, tablets, and two instant beverages, all purchased on the internet, were analyzed. 15 samples were quantified using the two orthogonal methods HPLC-UV and HPLC-MS. The statistical comparison of results from all three methods showed no significant differences in the Bland-Altman test.

## Sample preparation

200 mg sample were extracted with 10 mL of methanol for 30 s by vortex mixing followed by extraction in an ultrasonic bath for 10 min at room temperature. After centrifugation at ~3000 g for 10 min at 25°C, the supernatant was collected and directly applied onto HPTLC plates.

## Layer

HPTLC plates silica gel 60 $F_{254}$ 20 × 10 (Merck).

## Sample application

Bandwise with Automatic TLC Sampler (ATS 4), band length 8 mm, distance from lower edge 8 mm, distance from left and right edge 20 mm, track distance 10 mm, application volumes 5 µL for samples and standard solutions.

## Calibration and spiking of samples

Calibration curves were constructed using four concentration levels at 78, 146, 751, and 3023 ng. For quality control spiking with sibutramine was performed with the ATS 4 by overspotting of sample zones on the plate (146, 1170, and 2340 ng on the plate).

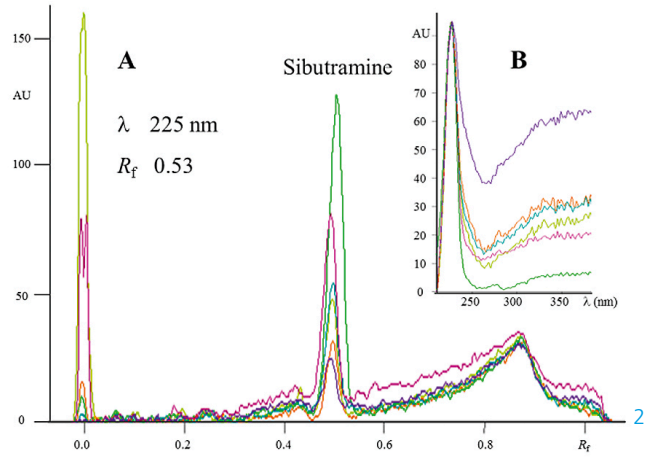
## Chromatography

In the Automatic Developing Chamber (ADC 2) with toluene – methanol 9:1, chamber saturation for 20 min, relative humidity controlled with a saturated solution of $MgCl_2$, migration distance 70 mm, drying 5 min. The average developing time was 15 min.

## Densitometry

Identification and quantification were performed directly after chromatography by using a TLC scanner 3 with winCATS software; measurement in absorption mode at 225 nm, scanning speed 20 mm/s using a slit of 4.0 × 0.3 mm.

For comparison of UV spectra with the sibutramine reference spectrum assigned peaks were scanned between 210 and 700 nm to obtain and compare UV spectra with a sibutramine reference spectrum. Scanning speed 100 nm/s, slit dimensions 4.0 × 0.3 mm.

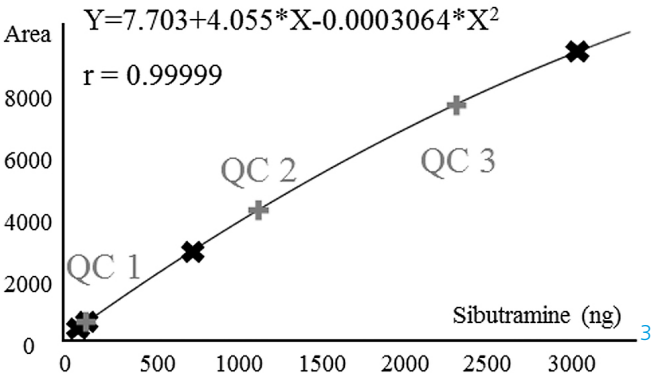



*Peak overlay of individual calibration points and quality control tracks of sibutramine at $R_F$ = 0.53 / 225 nm (A) and corresponding UV spectra (B).*

## Mass spectrometry

Sibutramine zones were eluted from the plate by means of the TLC-MS interface (circular head) with methanol at a flow rate of 0.1 mL/min. To improve the ionization in the ESI-MS the extracts were diluted online with methanol – 5 mM ammonium formate solution 1:1.

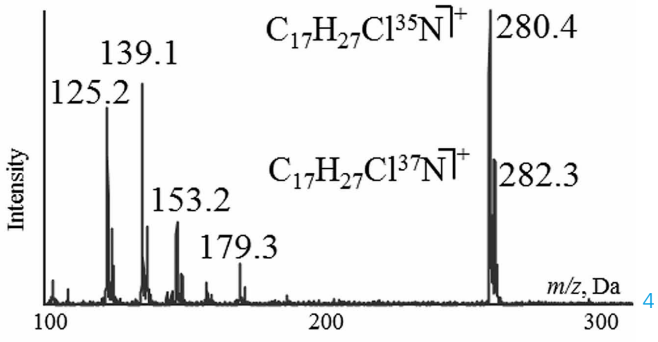
Identification was performed with a 3200™ Q-trap mass spectrometer in $ESI^+$-mode with spectra recording in scan mode or in SRM mode (selected reaction monitoring). For the mass transition 280.2→125.0 thomson (Th) the collision energy was 37 V, for the second mass transition 280.2→139.0 Th it was 21 V. The scan range was between 100 and 1000 Th at 1000 Th/s.




## Results and discussion

Sibutramine was quantified using winCATS software with polynomial regression in the working range from 78 to 3022 ng.

During method validation, intermediate precision was between 2 and 9% and accuracy for the three quality controls (QC) was between 96 and 104%. The lowest QC (146 ng on the plate) is equivalent to a sibutramine amount of 0.3 mg per capsule, which is approx. 30 times less than the former therapeutic dosage of sibutramine (10–15 mg per day). To exclude false positive results the sibutramine zones were eluted from the plate with the TLC-MS interface and analyzed by MS. The pseudo molecular ion $[M+H]^+$ of sibutramine was at 280.4 in the MS scan.



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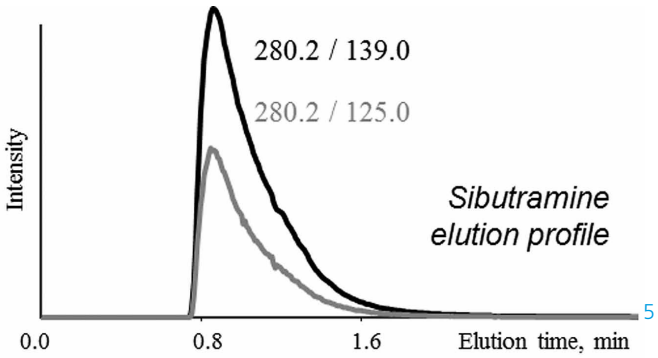
Identification was confirmed by monitoring two specific mass transitions (*i.e.*, 280.2 →139.0 and 280.2 →125.0). None of the samples was false positive.



5

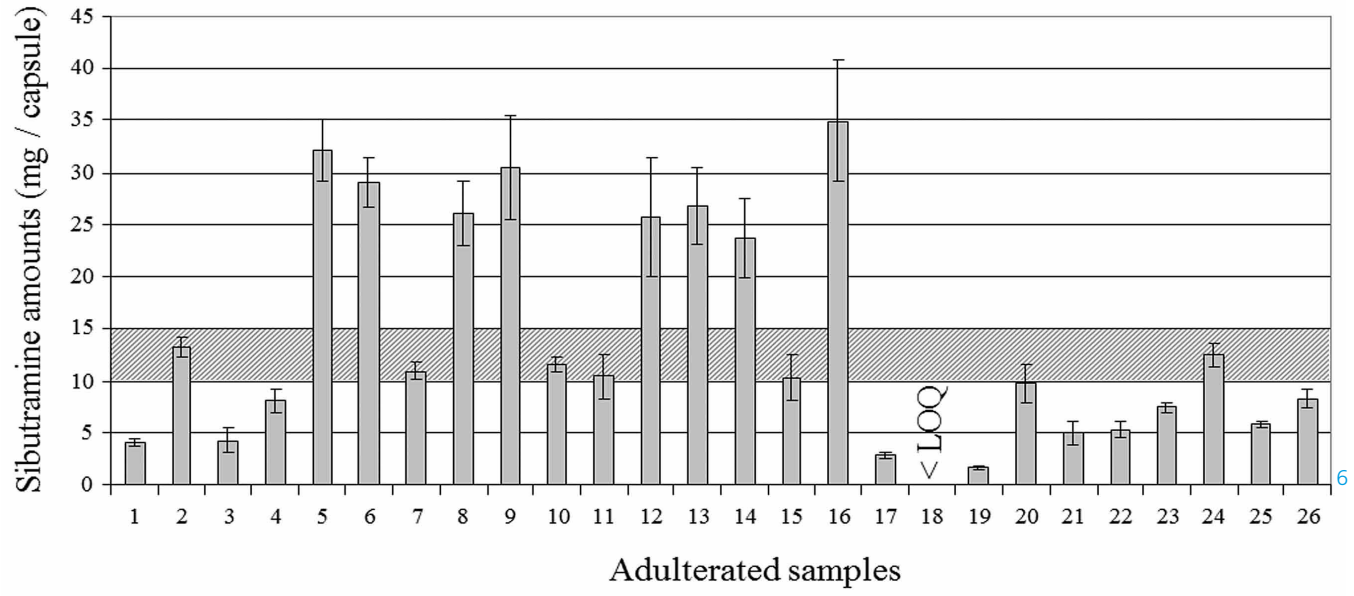
Among the analyzed 52 slimming products 26 were adulterated with sibutramine. Detected amounts ranged from traces to 35 mg per capsule. This corresponds to 3 times the dosage of the formerly approved appetite suppressant drug (10–15 mg/day).

The present study illustrates that the adulteration of herbal food supplements with sibutramine is highly relevant. For detection of adulteration a reliable screening method is required. HPTLC with UV densitometric detection enables easy and rapid quantification of sibutramine which can be identified unequivocally by mass spectrometry using a TLC-MS interface.

A paper describing the results of the work was accepted by the Journal of Food Additives and Contaminants. Details are available on request from the authors.

Contact: Dr Philippe Christen, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland Philippe.Christen@unige.ch

Daniel Handloser, daniel.handloser@camag.com, CAMAG, Sonnenmattstrasse 11, CH 4132, Schweiz



6

# Anthocyanes in food and animal feed by HPTLC-Vis-(EDA-)MS

*Stephanie Krüger*

Ms Stephanie Krüger has developed this rapid HPTLC method to investigate anthocyanes as bioactive compounds in food and feed during her diploma thesis under supervision of Professor Gertrud Morlock.

## Introduction

Anthocyanins are naturally occurring water soluble flavonoid dyes whose colors range from yellow through orange and red to bluish hues. Several health-promoting properties like anti-inflammatory, anti-microbial, antioxidant and cancer preventive activities are attributed to them, as well as a positive influence on stress triggered chronic illnesses like diabetes. [1] As food color E 163, anthocyanin extracts are added to various food stuffs. Also animal feed is supplemented with pomace of anthocyanin rich fruits to enrich the phenolic and therefore antioxidant content.

**Food and animal feed are heavily loaded with matrices. Planar chromatography as a matrix-robust method allows to analyze anthocyanes in such samples without any purification steps. It is an additional asset that several anthocyanes of the same group (*e.g.* aglycones, monoglucosides, diglucosides) can be separated at the same time and, if required, both polar and apolar groups on the same plate. The determination of the 11 individual anthocyanes was performed using three complementary detection methods i.e. direct detection in the visible and UV range, detection of radical scavenging properties by derivatization with DPPH•, and detection of bioactivity after immersion in a suspension of *Aliivibrio fischeri* bacteria. By recording mass spectra after elution with the TLC-MS Interface, it was possible to characterize most of the unknown anthocyanin zones. The method allows a high sample throughput *i.e.* parallel analysis of up to 9 samples in duplicate [2].**

## Chromatogram layer

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

## Standard solutions

Anthocyanes were dissolved in 0.5 % hydrochloric acid in methanol with stock solution concentrations of 1 mg/mL for the 5 anthocyanidins (aglycons) and 0.1 mg/mL for the 6 anthocyanins (glycosides). The stock solutions were pipetted together into one standard mixture in quantities between 6 and 32 ng/µL [2]. All solutions were stored in the dark at –20 °C.

## Sample preparation

The solid samples (grape pomace, animal feed, pomace formulation AntaOx E (used as reference) and grape seed meal) were extracted with acidified methanol and the supernatant centrifuged for 5 min at 1000 g. The belladonna extract was purified using XAD-7 (1 mL equals about 25 g fresh great cherry fruits). Wine and fruit juice samples purchased at local shops in 2012 were centrifuged for 10 min at 10000 g and the supernatant was acidified with 1 % hydrochloric acid. All samples were stored in the dark at –20 °C.

## Sample application

Bandwise (8.0 or 7.5 mm bands) using Automatic TLC Sampler 4 leading to 19 or 22 tracks, respectively, distance from the left side 15 mm, distance from the lower edge 8 mm. For quantitation, standard mix volumes ranged between 2.5 and 12 µL and samples volumes between 0.5 and 15 µL.

## Chromatography

In the Automatic Development Chamber ADC 2 for anthocyanins with ethyl acetate – 2-butanone – formic acid – water 7:3:1.2:0.8. Before development the relative humidity was adjusted to

33 % by a saturated magnesium chloride solution for 2 min. The migration distance was set to 65 mm (from lower plate edge). When anthocyanidins ($hR_F$ > 90) were detected, the lower part of the plate was cut off with the smartCUT Plate Cutter and the upper part was developed with ethyl acetate – toluene – formic acid – water (10:3:1.2:0.8, v/v/v/v). Plates used in the investigation with *A. fischeri* bioassay or DPPH• reagent were dried for 15 min to remove all acidic solvent residues.

## Documentation

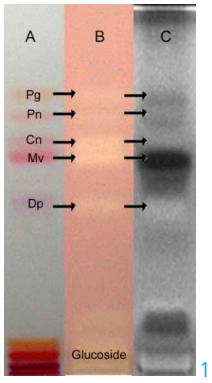
With DigiStore 2 System under UV 254 nm, UV 366 nm and under white light

## Densitometry

With TLC Scanner 3 under winCATS, absorbance measurement using multi-wavelength scan at 505, (510), 520, 530 and 555 nm

## Bioassay detection with *Aliivibrio fischeri*

*A. fischeri* culture medium was prepared according to DIN EN ISO 11348-1, section 5. Immersion with the TLC Chromatogram Immersion Device for 2 s at a speed of 2 cm/s. Immediate documentation with the BioLuminizer (10 images at 3 min time intervals, exposure time 50 s).



1

*Comparison of alternative detection methods: Vis (A), DPPH• reagent (B) and A. fischeri suspension (B), exemplarily shown for the apolar anthocyanidins pelargonidin (pg), peonidin (pn), cyanidin (cn), malvidin (mv) and delphinidin (dp)*

## Postchromatographic derivatization

Plates were dipped into the DPPH• reagent (0.5 mM 2,2-diphenyl-1-picrylhydrazyl radical in methanol) at a speed of 2 cm/s and an immersion time of 5 s using the TLC Chromatogram Immersion Device. Then they were first dried for 90 s in the dark at ambient temperature, followed by heating for 30 s on the TLC Plate Heater at 60 °C. Documentation under white light; the reverse DPPH• chromatogram (bright zones on

2

**CAMAG BioLuminizer**

Hyphenating TLC/HPTLC and bioassay is an excellent tool for identification of single toxic compounds in complex sample matrices.

The BioLuminizer is a compact, user-friendly detection system for capturing bioluminescence images. It shows an exceptional image quality and a high resolution for a short exposure time. The system is comprised of a compartment excluding any extraneous light, climate controlled for extended stability of the plate, and a 16 bit CCD digital camera of high resolution and high quantum efficiency.

In this CBS issue, the BioLuminizer was used to detect the impact of compound zones on the luminescence and bioactivity of selected bacteria. Such a luminescent bioassay detection was used in the contributions on pp. 2–5 and 12–15 for detection of the luminescence inhibition or intensification of *Pseudomonas savastonoi* and *Aliivibrio fischeri* bacteria. As the bioassay indicates single bioactive compound zones, it is an effective strategy for finding bioactive compounds in complex mixtures. It directly links to a bioactive compound.

Further information can be found under **www.camag.com/bioluminizer** or in the special brochure CAMAG BioLuminizer.

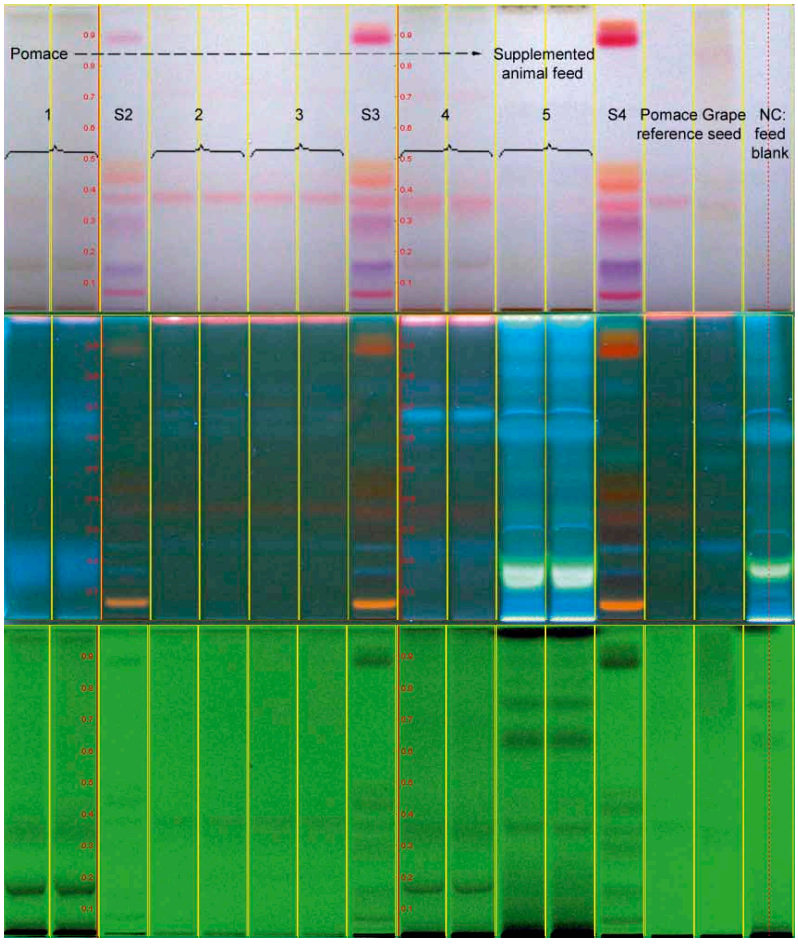
rose-violet background) was scanned at 517 nm by selecting "fluorescence mode" in order to invert the otherwise negative peaks.

## Mass spectrometry

Zones were eluted with the TLC-MS Interface with oval elution head (2 x 4 mm) using methanol at a flow rate of 0.1 mL/min (pump of Agilent HP 1100 Chem-Station) All spectra were recorded with a single-quadrupol MS with electrospray ionization (ESI) in the positive mode (capillary voltage +4 kV, fragmentation voltage 160 eV or 300 eV for anthocyanins and anthocyanidins, respectively). A plate background spectrum recorded aside the analyte zone was subtracted from the analyte spectrum.

## Results and discussion

After minimal sample preparation, the HPTLC separation of the samples took merely 20 min, and in case of presence of anthocyanidins further 13 min. Grape pomace, animal feed, grape juice, wine samples and various other fruit juice samples were analyzed. Anthocyanin patterns corresponded when juices originated from the same fruit.




[3]

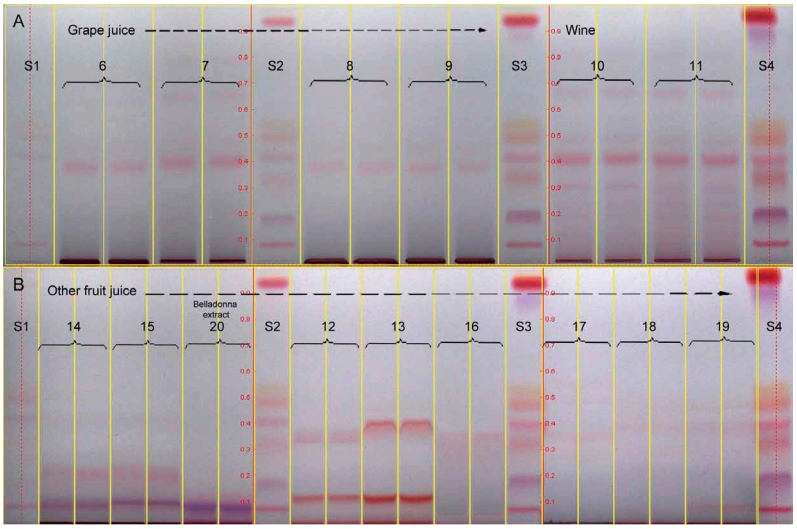
*HPTLC chromatograms of pomace and animal feed samples and standard mixture (S2–S4) under white light, 366 nm and 254 nm (reprinted from [2] with permission)*

The method showed good validation data. The correlations coefficients for all 11 anthocyanes were between 0.9993 and 0.9999. For the analysis of mv-3-glc in grape pomace and animal feed, the mean repeatabilities (*%RDS*, n = 2) were 1.4 %. Intermediate precision (*%RDS*, n = 3) was ≤6.7 % and the method's ruggedness was ≤ 5.5 %. In the visible range LOD and LOQ (signal-to-noise ratio of 3 and 10) were ≤90 ng/zone for all anthocyanes. Interestingly anthocyanins had generally better LODs/LOQs than their aglycones. For example, pn-3-glc and pg-3-glc had LOQ values ≤7 ng/zone, which were better by a factor 3 and 5, respectively, compared to their sugar-free counterparts.

To ascertain anthocyanes' bioactive properties two effect-directed detections were used. For DPPH$^{\bullet}$ the values for LOD and LOQ were slightly increased, but generally still ≤100 ng/zone. The evaluation for *A. fischeri* was done visually, and the LODs for anthocyanidins were in the same range as the other two detection methods. However, the values for anthocyanins were increased up to 20 times (<1550 ng/zone).

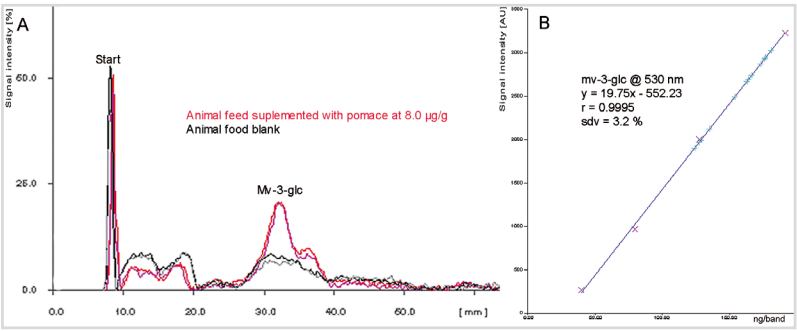
However, it was not always possible to assign sample zones to an anthocyanin of the standard mix. To characterize these zones HPTLC-ESI-MS spectra were recorded. Since little is known about the anthocyanin pattern of the great cherry fruit (belladonna), the identification of the two anthocyanins was of special interest. The mass spectra of the higher, pink colored zone showed an ion at *m/z* 287 corresponding with cyanidin [A$^+$]. With regard to $hR_F$-value one can conclude a multi-glycosylated derivative. The lower, lila colored zone showed two ions, one at *m/z* 317 corresponding with petunidin [A$^+$] and another at *m/z* 657 corresponding with the sodium adduct of the dimer [2A+Na]$^+$. Further sodium adducts

of the monoglycoside $[Hex+Na]^+$ and diglycoside moiety $[Hex_2+Na]^+$ as well as their dimer $[2Hex_2+Na]^+$ also indicated a multiple glycosylated anthocyanin.



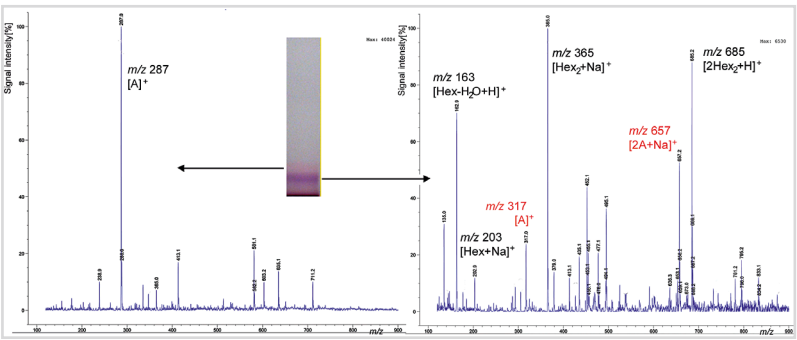

4

*HPTLC Chromatograms of grape juices and wine (A) as well as other fruit juices (B) (reprinted from [2] with permission)*




5

*Overlaid densitograms obtained by absorbance measurement at 530 nm of animal feed supplemented with mv-3-glc pomace at 8.0 µg/g (twofold determination) (A); calibration curve of mv-3-glc (B) (reprinted from [2] with permission)*




6

*HPTLC-ESI+-MS of two unknown anthocyanine zones in a belladonna extract (reprinted from [2] with permission)*


[1] J. Shipp, E.-S. Abdel-Aal, The Open Food Science Journal 4 (2010) 7

[2] S. Krüger, O. Urmann, G.E. Morlock, J. Chromatogr. A 1289 (2013) 105


Further information is available on request from the authors.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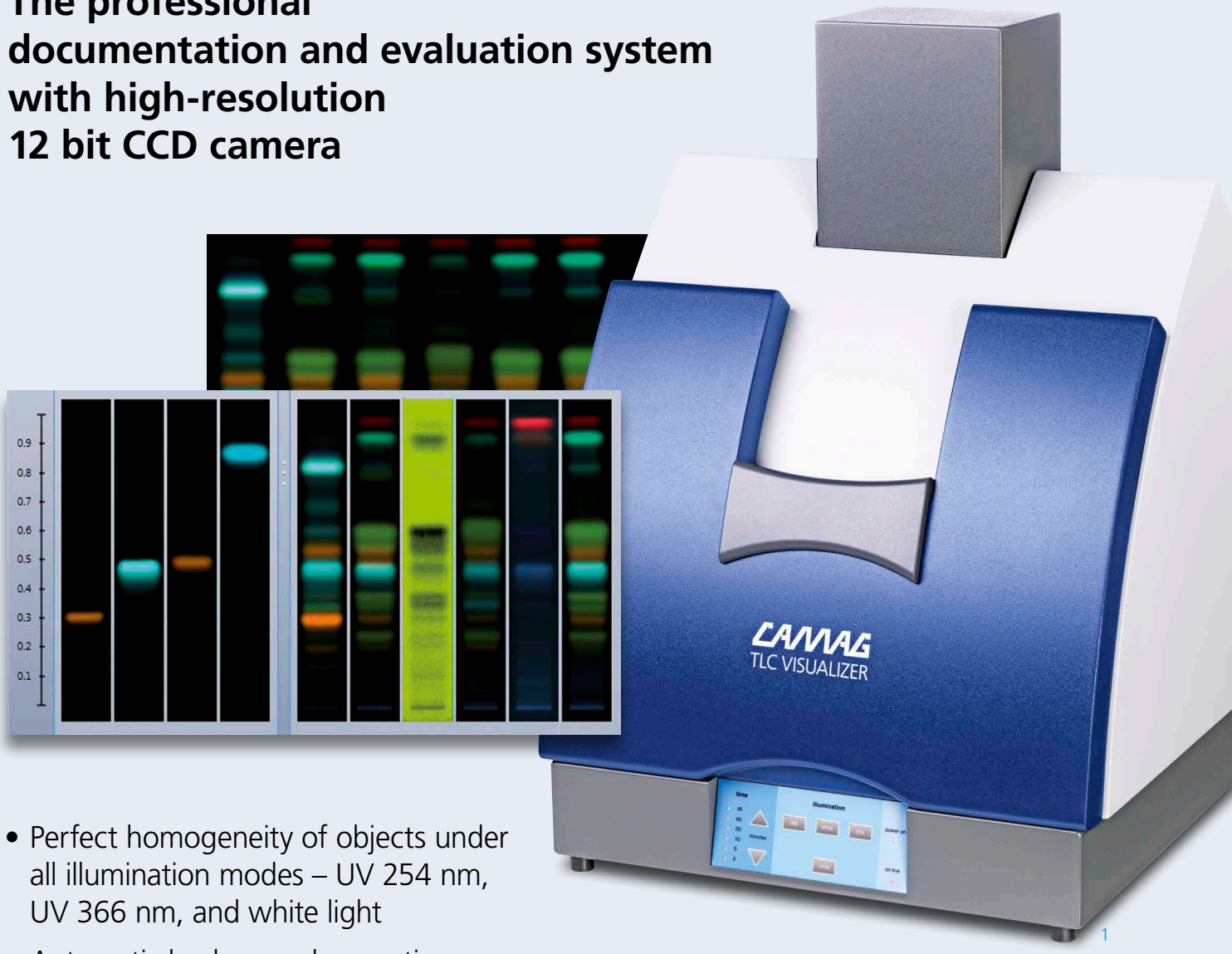


1

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