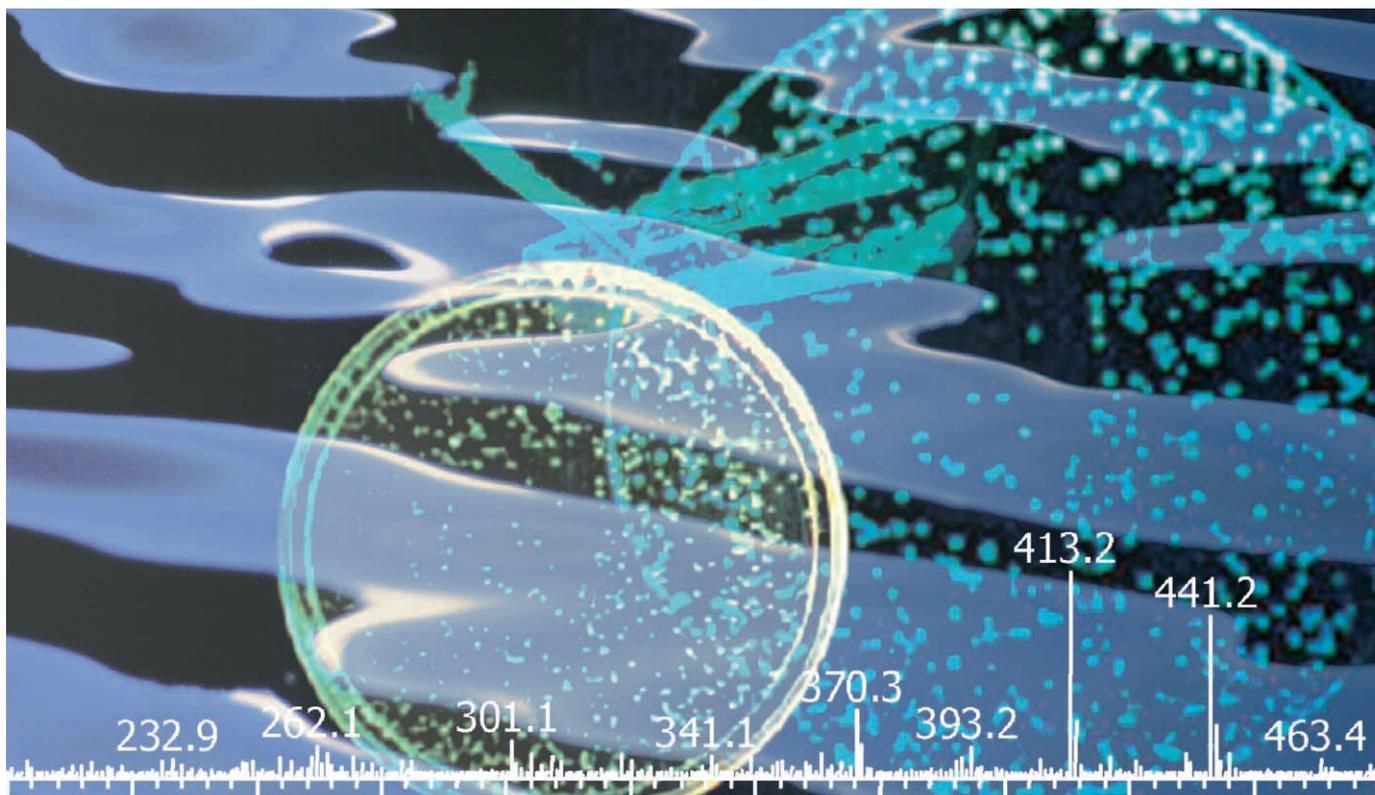


# CBS

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**Specific modes of detection –  
merits of planar chromatography**

**CAMAG**

**94**

No. 94, March 2005

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Planar Chromatography  
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# Planar Chromatography in Practice

## Luminographic detection of toxicity with *Vibrio fischeri* (luminescent bacteria)



▲ Dr. Walter Weber\*



▲ from left to right: Wolfram Seitz, Anna Aichinger, Roger Albert

In addition to chemical, physico-chemical and microbiological monitoring of drinking water production and distribution to more than 3 million customers, the operations and research laboratory of the Landeswasserversorgung, a long-distance water supplier located in Langenau (Germany), headed by Dr. Weber, is conducting extensive analytical and water-chemical research projects.

Automated multiple development TLC (AMD) is used besides GC/MS and HPLC/MS procedures predominantly for determination of some pesticides like glyphosate, AMPA or amitrole, which show only limited detectability by HPLC and GC. The introduction of toxicity tests on developed HPTLC plates has opened possibilities for using further effective methods for evaluation of water quality with respect to biologically active substances. The following article describes the use of luminographic detection of toxicity with luminescent bacteria (*Vibrio fischeri*) in TLC.

### Introduction

For the Landeswasserversorgung, one of the oldest long-distance water suppliers in Germany (founded in 1912), it is very important to know about the presence of environmental toxins and other pollutants (in the following called harmful substances) in raw waters and to exclude them from drinking water. For the detection of harmful substances, besides chemical, physico-chemical, and microbiological tests, recently techniques were introduced, which use activity related detection systems ("bio test systems") such as luminographic detection of toxicity with luminescent bacteria or the determination of acetylcholine esterase inhibition.

Unlike in common analyses, where single substances are measured or sum parameters are detected, biological tests can determine toxic effects. Activity parameters include acute toxicity (e. g. death, luminescence inhibition), chronic toxicity (e. g. growth inhibition), and genetic toxicity (e. g. mutations). Luminographic detection of toxicity determines acute effects.

Bio test systems offer the advantage of detecting also substances unknown to the analyst. With a range of more than 30.000 relevant chemicals and degradation products single substance analysis with physico-chemical methods is clearly overcharged, because only those substances will be found, which the analyst is searching for and for which reference substances are available. Bio test systems within their limits of detection can, to a certain extent, close the gap, which always remains open during risk assessment of samples with complex composition, even with extensive utilization of chemical analysis. Luminographic detection of toxicity with *Vibrio fischeri* is commonly used as test in a cuvette in waste water analysis. The measured activity is the integral of all effects of the sample components, including synergistic and antagonistic effects.

**Performing luminographic detection of toxicity on HPTLC plates following chromatography with AMD combines the advantages of physico-chemical analysis with the registration of toxic effects. Contrary to classic toxicity tests the separation of substance mixtures allows the evaluation of the individual components. In addition some basic statements can be made about the chemical structure of the compound based on their migration behavior.**

### Sample preparation

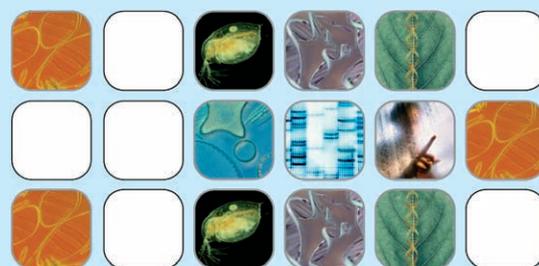
1 L water sample is percolated through the solid phase extraction material Isolute ENV+. The adsorbed substances are then eluted with methanol. The concentrated extract is dissolved in 200 µL methanol.



**Bioluminex™** is a new assay for biological activity and toxicity screening of complex mixtures such as waste water, food & beverage samples, and natural product extracts. This unique and effective technology was invented by Bayer to combine Thin Layer Chromatography (TLC) and the natural bioluminescent properties of *Vibrio fischeri*, a marine bacterium.

### What is the Bioluminex™?

TLC is used to initially separate multi-component mixtures into discrete substance zones. The TLC plate is then coated with bioluminescent *Vibrio fischeri*, which respond within seconds. Zones of toxic compounds are then easily identified as dark spots on a luminescent background, the regions where the toxins or other negatively acting agents reduce the metabolic activity of the bacteria. Rapid response time avoids the inevitable deterioration from diffusion caused by the prolonged incubation periods of other TLC-bioassay systems



**ChromaDex™**

sales@chromadex.com

www.chromadex.com

## Layer

HPTLC plates silica gel 60 F<sub>254</sub> (Merck), 20×10 cm, layer thickness 0,1 mm, pre-washed with 2-propanol (immersion for 24 h) and dried at 100 °C for 30 min on the TLC plate heater under a stream of nitrogen

## Sample application

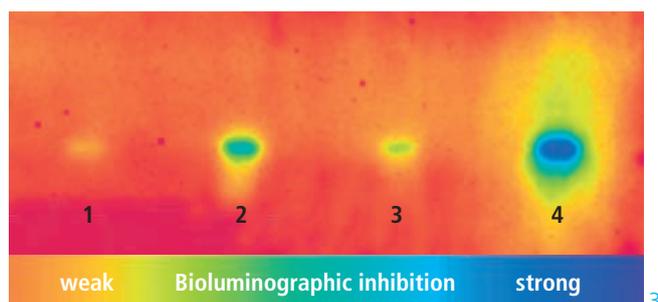
As bands with Automatic TLC Sampler 4, 12 tracks, application volume 80 µL, band length 6 mm, distance from lower edge of plate 8 mm, distance from the sides at least 20 mm, track distance 12 mm

## Chromatography

Chromatography with AMD2 seems promising using a 25 step gradient based on acetonitrile – formic acid – dichloromethane, max. developing distance 80 mm. Up to now humic acids in the matrix were problematic. These compounds can also inhibit luminescence and show tailing, which is not observed during the usual UV/VIS detection. It is attempted to solve this problem with an optimized gradient or improved sample preparation.

## Luminographic detection

Immersion of the developed HPTLC plates with the CAMAG Immersion Device III for 1 s into suspension of luminescent bacteria (*Vibrio fischeri*)



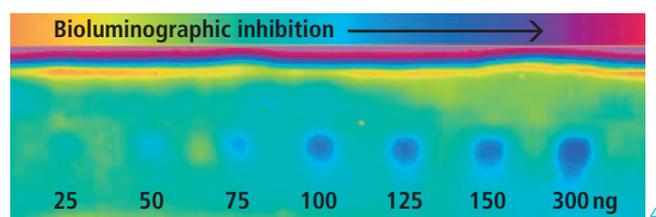
▲ Luminographic detection of four different active pharmaceuticals on an HPTLC plate silica gel 60 F<sub>254</sub>, tracks 1 – 4 show the different inhibition of bioluminescence of the four compounds (600 ng each per zone, without chromatography)

## Evaluation

With CCD-camera, exposure time 40 s, integrated intensity over 2×2 pixel (pixel binning), inversion and scaling of exposure in pseudocolors

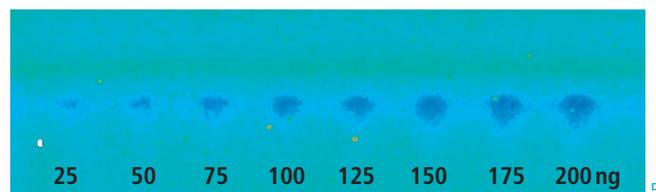
## Results and discussion

Selected pharmaceuticals, which have been detected at ng levels in surface waters, show different inhibition of bioluminescence. In addition to pharmaceuticals, a number of chemically diverse compounds, for examples pesticides, is detected by the described test system.



▲ Luminographic detection of a substance active against luminescent bacteria at 25 to 300 ng per zone on an HPTLC plate silica gel 60 F<sub>254</sub> (without chromatography)

First investigations of extracts from surface water clearly show inhibition of bioluminescence. For optimization of the chromatographic separation of water extracts into individual substance zones by AMD, suitable sample preparation techniques must be developed to remove the matrix. This could possibly be achieved by removal of humic acids by size exclusion chromatography.



▲ Luminographic detection of a substance active against luminescent bacteria on a chromatographed HPTLC plate silica gel 60 F<sub>254</sub>, track loading: 25 to 200 ng substance per zone

The results after HPTLC separation of mixtures show that bio based analysis with luminescence bacteria is applicable for harmful substances in concentrations in the low ng range.

Further information is available on request from the authors.

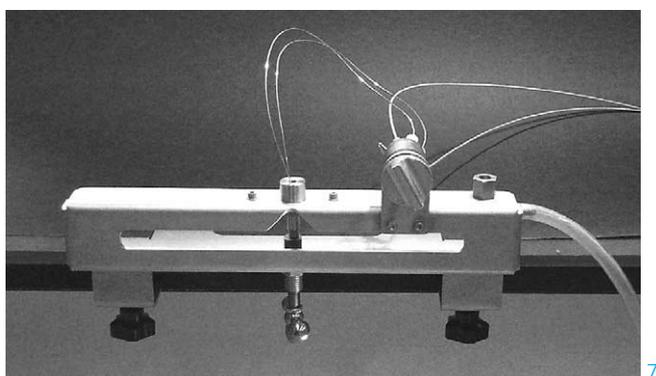
\*Dr. Walter Weber, Zweckverband Landeswasserversorgung, Betriebs- und Forschungslaboratorium, Am Spitzigen Berg 1, D-89129 Langenau, Germany, weber.w@lw-online.de

## Hyphenated HPTLC-MS as rapid method for elucidation of synthesis mixtures



▲ Prof. Dr. Dieter Hoppe, Jochen Becker, Dr. Heinrich Luftmann

The group of Prof. Hoppe, Institute of Organic Chemistry of the Westphalian Wilhelm's-University Muenster, has been working on development of strategies for enantio-selective syntheses since many years. The utilization of metal-complexing ligands for control of base induced hydroxyalkylation is realized for example in the Hoppe-homo-aldol-reaction<sup>1</sup>. Dr. Luftmann\*, head of the department of mass spectrometry, developed a device for rapid and contamination free extraction of substance zones from the layer<sup>2,3</sup>. From the device, called ChromeX-trakt<sup>4</sup>, the eluate of a chromatogram zone can be taken on-line into the electrospray mass spectrometer (ESI-MS). In CBS 93 we already reported about this on-line hyphenation, used for determination of heterocyclic aromatic amines by the group of Prof. Schwack, University of Hohenheim.



▲ ChromeXtrakt for the elution of HPTLC zones

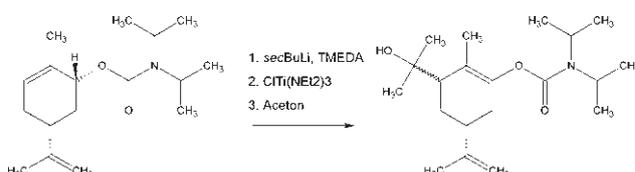
\*Dr. Heinrich Luftmann, Organisch-Chemisches Institut, Westfälische Wilhelms-Universität, Corrensstr. 40, D-48149 Münster, Germany, luftman@uni-muenster.de

### Introduction

In a university synthesis laboratory planar chromatography is readily employed, because here the advantages of the technique can be fully utilized:

- TLC is a method on site - it can be performed in parallel to other laboratory work without problems.
- TLC is rapid – a typical separation does not take more than 10 min and sample preparation is not necessary.
- TLC saves solvents – a separation chamber only requires about 5 mL solvent.
- TLC is inexpensive – costs for a TLC aluminum foil which can be used for several separations is about 0.6 €.
- TLC works in parallel – due to limited requirements concerning instruments several separations can be performed in parallel.

In the early stage of a synthesis development predominantly qualitative analysis is in demand. This includes elucidation of structures of individual components and their chromatographic properties in order to evaluate the success of a reaction or to select the conditions of subsequent preparative separation. The substance amounts separated on the layer are usually sufficient for most MS measurements. Online extraction is demonstrated below for a synthesis approach.



(-)-Carveyl-N,N-diisopropylcarbamate is deprotonated with sec-butyllithium on the  $\alpha$ -carbon atom. The lithium cation is bound on the upper side of the molecule as contact ion pair. Through lithium-titanium exchange an inversion of the steric center is achieved. The titanium is now located on the lower side and guides new substituents to attack from the lower side (Zimmerman-Traxler transition state<sup>5</sup>).

**To optimize such reactions often new reagents and conditions have to be investigated. Monitoring is done by TLC. For identification online hyphenation with mass spectrometry is employed. Time input of the mass spectrometric detector is minor because only MS spectra of zones of interest are recorded.**

### Sample preparation

50  $\mu$ L of the reaction solution are taken out and diluted with diethyl ether to 1 mL. The clear supernatant is used for chromatography (ca. 3  $\mu$ g product mixture per 1  $\mu$ L).

### Layer

TLC aluminum foil silica gel 60 F<sub>254</sub> (Merck), 5×7,5 cm

### Sample application

A 45 mm band is sprayed on, distance from lower edge 10 mm, distance from the side 2.5 mm, application volume 20  $\mu$ L

### Chromatography

Twin trough chamber (10×10 cm) with pentane – tert. butylmethyl ether 3:1, with chamber saturation

### Derivatization

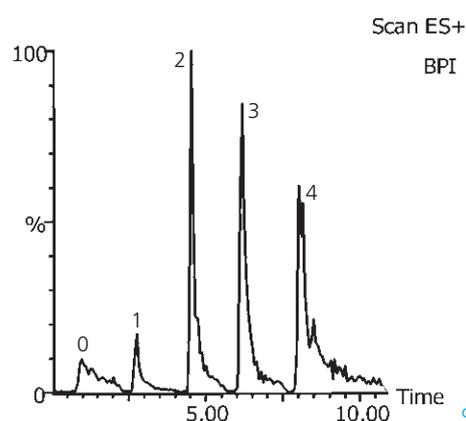
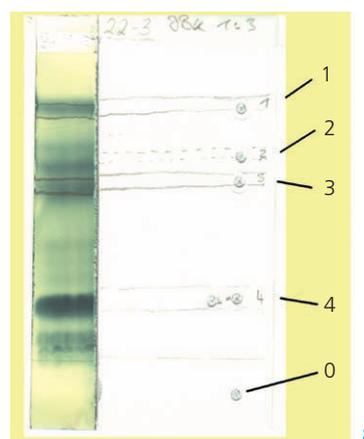
On the left side of the chromatograms a 1 cm strip is cut off, sprayed with phosphomolybdic acid reagent (0.4 g in 100 mL ethanol), and dried in hot air. The position of the detected zone is marked on the underivatized part of the chromatogram with a soft pencil.

### Online-extraction and recording of mass spectra

The extraction head with a diameter of 2 mm is positioned and fixed on the zone of interest. In-situ extraction is done with methanol – chloroform 1:1 at a flow rate of 0.1 mL/min. The outlet capillary of the extractor is connected directly to the ESI-MS1. The mass range  $m/z$  100–600 is measured in 2 s cycles.

### Results and discussion

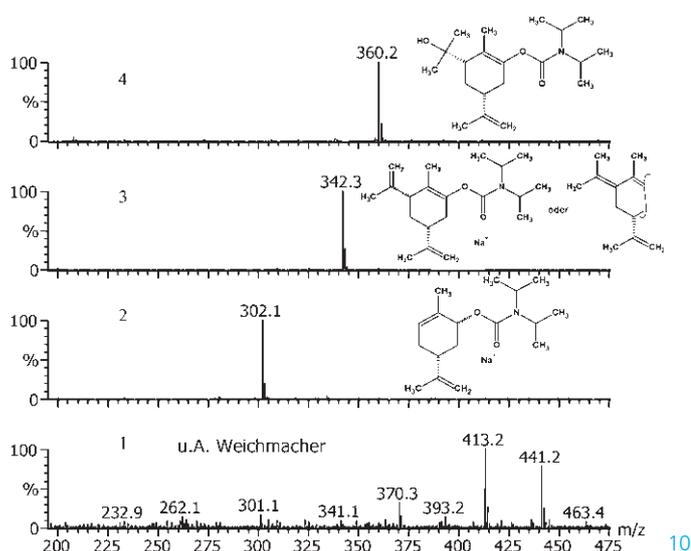
The four extraction positions are visible on the marked zones on the right. The numbers correspond to those in the basepeak chromatogram (BPI).



▲ Chromatogram on derivatized strip, cut off on the left, for marking the zone position (left) and BPI-signal of the four extracted zones (right)

The ESI mass spectra shown are taken from the range of the peak maxima of the BPI signal. They allow the following interpretation:

- Zone 1: among others softeners from the solvents employed MW 390 (Di-iso-octylphthalate) seen in the ESI spectrum as  $[M+Na]^+$  signal at  $m/z$  413.2
- Zone 2: adduct MW 279;  $[M+Na]^+$  signal at  $m/z$  302.1
- Zone 3: homoaldol product, but after elimination of water MW 319;  $[M+Na]^+$  signal at  $m/z$  342.3
- Zone 4: desired product of synthesis MW 337;  $[M+Na]^+$ -signal at  $m/z$  360.2



▲ ESI mass spectra of the the four eluted zones

The four ESI mass spectra of the separated compounds were measured within 10 min. Identity of the spots was deduced from the masses of the  $[M+Na]^+$  ions. The online hyphenation of planar chromatography with mass spectrometry using this extractor has proven efficient for many synthesis applications.



### CAMAG Linomat 5

The CAMAG Linomat 5 has been designed for the application of samples in the form of narrow bands. Particularly for the analysis of synthesis mixtures, it is advantageous to start with compact, narrow sample application zones as they guarantee optimum resolution for a given planar chromatographic system.

The CAMAG Linomat 5 uses the spray-on technique for applying samples onto the chromatogram layer as narrow bands. This permits the application of larger sample volumes than is possible with contact sample transfer, as the solvent almost completely evaporates during the process. Even when strongly polar solvents are used, e.g. methanolic or aqueous solutions, the application zones remain compact and narrow. When larger volumes are required especially in preparative applications, a 500  $\mu$ L syringe can be used instead of the standard 100  $\mu$ L dosage syringe.

A further advantage of the Linomat 5 is its self-adjusting plate support. It allows the use of layers differing in thickness without re-adjusting the spray nozzle. This feature makes the Linomat 5 particularly attractive for preparative applications, when several operators employing different layers may want to use the same sample application device.

- <sup>1</sup>M. Özlügedik, J. Kristensen, J. Reuber, R. Fröhlich und D. Hoppe, *Synthesis* 14 (2004) 2303-2316.
- <sup>2</sup>H. Luftmann, *Anal. Bioanal. Chem.* 378 (2004) 964-968.
- <sup>3</sup>Deutsches Patent 100 36 293. 1.52
- <sup>4</sup>Beziehbar bei ChromAn GmbH, 04288 Holzhausen, chroman@chroman.de
- <sup>5</sup>H. E. Zimmerman, M. D. Traxler, *J. Am. Chem. Soc.* 79 (1957) 1920-1923.
- <sup>6</sup>Quattro LCZ, Waters-Micromass, Manchester, UK

## Customer support world-wide lectures – seminars – service

In 2004 not only our Laboratory Manager, Dr. Eike Reich, often supported by Ms. Anne Schibli-Blatter, was on lecture and seminar tour in the US and Asia, but also other members of the CAMAG team have highly endorsed customers abroad. Here is a summary of their activities outside of Europe.

**André Gschwind, our Chief Service Engineer**, visited Dakar, Senegal in spring and Chile in September. The latter journey was requested by a customer in Concepcion, who is performing with enthusiasm and success HPTLC analyses of antibiotics in fish – we reported about the method in CBS 75 (1995). At that occasion Mr. Gschwind held a 4-day service training for specialists of our partner organizations in Argentine, Brazil, Chile and Peru. The training at the University of Concepcion was under the auspices of our good friend Professor Mario Vega. Visits to customers supporting our distributor Merck-Chile made the assignment complete.



▲ Snap shot of the service training at the University of Concepcion, Chile

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The record in traveling abroad was again set by **Senior Product Manager Jan Masthoff**, our »flying Dutchman«, who in 2004 completed 6 missions: February Bangkok, March Peru and Brazil, April Malaysia, June Taiwan, July China, December China and Korea.

Especially mentioned should be a seminar on HPTLC-Analysis of TCM (Traditional Chinese Medicine) at the Urumuqi Drug Control Institute. 56 interested analysts participated. Urumuqi is located 4 hours by plane west of Beijing. Jan Masthoff had the impression to be the first West-European coming to this place. It is remarkable that CAMAG instruments have made it into this remote corner of the world: an accomplishment of our great partners in China.

On the way back Jan Masthoff conducted seminars at the Korean Department of Agriculture and at the Korean Customs, who is using a CAMAG system for the investigation of confiscated plant and drug samples.



▲ Snap shot of the seminar in Urumuqi, China

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**CAMAG LITERATURDIENST  
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PLANAR CHROMATOGRAPHY**

# CBS



Liebe Freunde

Den CBS gibt es nunmehr seit 40 Jahren! Ich nehme das zum Anlass, mich noch einmal zurück zu melden. Im Mai 1965 erschien die erste Ausgabe. Sie hatte 4 Seiten und referierte 48 Publikationen über Dünnschicht-Chromatographie. Inzwischen haben sich sowohl Umfang als auch Erscheinungsbild des CBS gewandelt. Mit der Institution dieser CAMAG Hauszeitschrift hatten wir uns zum Ziel gesetzt, die Kenntnis über Technik und Anwendung der Dünnschicht-Chromatographie zu verbreiten. Ich hoffe, dass Sie diesen Versuch als gelungen ansehen.

Bis einschließlich CBS 84 (März 2000) zeichnete ich als verantwortlicher Herausgeber. Ich habe aber auch danach nicht aufgehört, die Entstehung jeder CBS-Ausgabe aktiv zu begleiten. Diese Nummer wird allerdings die letzte sein, bei der ich auf diese Weise mitgewirkt habe. Ich bin sehr froh, in Frau Dr. Gerda Morlock eine kompetente und hochmotivierte Nachfolgerin gefunden zu haben. Ihr gebührt höchste Anerkennung, dass sie diese Aufgabe neben ihren anderen Tätigkeiten souverän löst. Ich wünsche mir – und damit vielleicht auch Ihnen – dass sie noch lange Jahre die Herausgabe des CBS übernimmt.

Die aktuelle CBS-Ausgabe präsentiert Ihnen vorteilhafte Detektionen als Besonderheit der Planar-Chromatographie. Im Feld der analytischen Verfahren verkörpert die Planar-Chromatographie in idealer Weise das zukunftsweisende Struktur-Wirkungs-Denken, ein Hauptansatz der Green Chemistry. Die Seiten 2–4 demonstrieren dies am Beispiel der Toxizitätsermittlung durch *Vibrio fischeri*, die Seiten 11–13 durch Immunoreaktion mit spezifischen Antikörpern.

Herzlichst Ihr

Dieter Jänchen  
Dieter.Jaenchen@camag.com

Dear friends

CBS has been in existence now for 40 years! I would like to take this opportunity to report back to you one more time. The first issue appeared in May 1965; it had 4 pages and referenced 48 publications about thin-layer chromatography. Volume and appearance have changed quite a bit over the years. It had been my objective to establish a forum for spreading knowledge about TLC and its application. I hope you appreciate my intention and consider it a success.

I was the official editor through CBS 84 (March 2000), but in subsequent issues, I have assisted with advice and various contributions. This issue, however, will be the last one in which I am actively involved. I am very happy that we have found Dr. Gerda Morlock as a competent and highly motivated successor for the position of CBS editor. She deserves great appreciation for mastering this task, while handling her many other obligations. I hope – and maybe you do as well – that she will continue as editor of CBS for many years!

This particular issue highlights advantageous detection methods as an inherent asset of TLC. In the field of analytical procedures planar chromatography ideally represents prospective structure-reaction-possibilities, an important approach in green chemistry. Pages 2–4 feature detection of toxicity with *Vibrio fischeri*, pages 10–12 immunoassay detection with specific antibodies.

Sincerely,

Dieter Jänchen  
Dieter.Jaenchen@camag.com

# CAMAG

**MÄRZ  
MARCH  
2005** **94**

# THE CBS CLASSIFICATION SYSTEM

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

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

## 1. Reviews and books

94 009 V. G. BEREZKIN et al., see section 3d

94 064 S. GOCAN et al., see section 32e

94 046 J. SHERMA, see section 29

## 2. Fundamentals, theory and general

94 001 E. FORGÁCS\*, T. CSERHÁTI, O. FARKAS, A. ECKHARDT, I. MIKSIK, Z. DEYL (\*Research Laboratory of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P. O. Box 17, 1525 Budapest, Hungary): Interaction between cholesterol and non-ionic surfactants studied by thin-layer chromatography. *J. Liq. Chrom. Rel. Technol.* 27, 1981-1992 (2004). Study of the interaction between cholesterol and non-ionic surfactants by reversed-phase TLC using cholesterol-impregnated TLC plates and methanol - water mixtures. TLC on aluminum oxide impregnated by overnight pre-development in a solution of cholesterol dissolved in chloroform - acetone 1:1. 20 surfactants were tested (Arcopal, Sapogenat, Tween, Genapol, Myrj, and Brij). The intercept obtained from linear regression anal. (RM0), being characteristic for the strength of interaction, and the slope (b), being related to the surface area of surfactants in contact with cholesterol, were detected. Stepwise regression anal. (SRA) was performed to find relation between the structural parameters of surfactants and strength of interaction. Stacking interaction exists between cholesterol and the aromatic ring of the surfactants. The number of ethylene oxide units and length of the carbon chain in the surfactant molecules have significant effect on the strength of the interaction between the compounds studied.

Food analysis, cosmetics, qualitative identification

2c

94 045 S. W. HUSAIN et al., see section 28a

94 024 K. KACZMARSKI et al., see section 11a

94 014 H. MAJSTOROVIC et al., see section 7

94 015 Alina PYKA et al., see section 7

94 004 M. SAJEWICZ, A. PIENIAK, R. PIETKA, K. KACZMARSKI, T. KOWALSKA\* (\*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): Densitometric comparison of the performance of Stahl-type and sandwich-type planar chromatographic chambers. *J. Liq. Chrom. Rel. Technol.* 27, 2019-2030 (2004). Comparison of the N-type and S-type chambers in terms of mobile phase flow rate, the R<sub>f</sub> values obtained, and the densitometrically established concentration profiles of the test analytes. Selected were several phenyl-substituted alcohols and acids, low activity adsorbents (e. g. cellulose powder), and low-polarity solvents (n-octane and decalin) as the components of the chromatographic systems investigated. The overall conclusion from the results obtained is that the physico-chemical performance of the S-chamber is comparable with that of the N-chamber, and that the only indisputable superiority of the S-chamber is the more economical use of the mobile phase.

Densitometry

2d, 2a

94 005 C. SULLIVAN, J. SHERMA\* (\*Department of Chemistry, Lafayette College, Easton, PA 18042, USA): Comparative evaluation of TLC and HPTLC plates containing standard and enhanced UV indicators for efficiency, resolution, detection, and densitometric quantification using fluorescence quenching. *J. Liq. Chrom. Rel. Technol.* 27, 1993-2002 (2004). Commercially pre-coated TLC and HPTLC silica gel plates, with enhanced brightness UV indicator were compared with comparable plates formulated with standard indicators. Results showed that the increased UV indicator plates had visually brighter backgrounds, however, the limits of detection did not improve. HPTLC and TLC on silica gel of caffeine and acetaminophen with ethyl acetate - glacial acetic acid 19:1, and salicylamide with methylene chloride - acetone 4:1 in a twin-trough chamber containing a saturation pad. Quantitative determination at 254 nm.

2d

94 002 Kathrin KOLL\*, E. REICH, A. BLATTER, M. VEIT (\* Forschungsvereinigung der Arzneimittel-Hersteller e. V. (FAH), Kranzweiherweg 10, 53489 Sinzig, Germany): Validation of standardized high-performance thin-layer chromatographic methods for quality control and stability testing of herbals. *J. Assoc. Off. Anal. Chem.* 86, 909-915 (2003). In quality control and stability testing of herbal medicinal products, fingerprint chromatograms are used as powerful tools to evaluate and compare the composition of compounds in such products. To fulfill the ICH- and GMP-based regulatory requirements in pharmaceutical QC, chromatographic fingerprint analysis needs to be validated. By considering the stationary phase, sample application, developing solvents, chromatogram development, plate labeling, derivatization, documentation, and chromatographic equipment the paper provides a comprehensive concept for evaluating validation parameters for planar chromatographic fingerprinting based on a standardized methodology. Validation parameters addressed include stability of the analyte, selectivity, robustness testing, and method reproducibility.

Quality control, herbal, HPTLC, densitometry, quantitative analysis, fundamental considerations

2f, 32e

94 003 M. PROSEK\*, A. GOLC-WONDRA, T. MAVER, M. FIR (\* National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia): Analytical uncertainty in modern quantitative TLC. *J. Planar Chromatogr.* 17, 102-108 (2004). For sample preparation a methanolic salicylic acid solution was sprayed onto a 40x40 cm glass plate. Samples from different positions of the test plate were extracted with methanol and analyzed by HPTLC and HPLC. HPTLC of salicylic acid on silica gel with cyclohexane - isopropanol - chloroform - acetic acid 12:1:1:2. Quantitation by densitometry. Measurement uncertainty in HPTLC and HPLC is relatively small and has no effect on recovery. To come close to the true value the analytical procedure must be managed by well-considered selection of number and positions of sampling locations. TLC is an excellent analytical technique which gives reliable results.

HPTLC

2f

### 3. General techniques

94 008 V. G. BEREZKIN\*, A. O. BALUSHKIN, B. V. TYAGLOV, J. K. ROJILO, I. MALINOWSKA (\*Univ. of Oil Synthesis, Moscow, Russia): (Electro-osmotic thin layer chromatography with covered layer.) (Russian). *Proc. Ros. Acad. Sci.* 399 (3), 362-365 (2004). Determination of colorants (rhodamine B, brilliant green and rhodamine G) on silica gel (sorbphil, Sorbopolymer, Russia) with ethanol (amperage 220  $\mu$ A), ethanol and 0,01 % LiCl (475  $\mu$ A), acetonitril - water 9:1 (430  $\mu$ A), acetonitril - water 9:1 and 0,01 % LiCl (830  $\mu$ A) or silica gel (Merck) with ethanol

(amperage 240  $\mu$ A), ethanol and 0,01 % LiCl (650  $\mu$ A), acetonitril - water 9:1 (360  $\mu$ A), acetonitril - water 9:1 and 0,01 % LiCl (950  $\mu$ A). Frequently used voltage is 1 kV. All the plates are covered with polyethylene film to prevent evaporation of mobile phase.

Pharmaceutical research, qualitative identification, electro-osmotic-flow, colorants, veterinary medication e.g. tilan 3d, 30a

- 94 009 V. G. BEREZKIN\*, E. F. LITVIN, A. O. BALUSHKIN, J. K. ROJILLO, I. MALINOWSKA (\*Univ. of Oil Synthesis, Moscow, Russia): (Electro-osmotic thin layer chromatography (overview). (Russian). Firm laboratory. Evaluation of materials 12 (70), 3-13 (2004). The state of the art and the historical development of EO-TLC is described. Determination of polysaccharides, steroids, colorants, pirimicarb and its analogs (with additional methyl, methoxy and carbamate groups) on silica gel with ethanol or RP18 with water - ethanol 1:4, water- acetonitril 11:9. Frequently used voltages are in the range of 1 - 0,1 kV/cm. Detection of polysaccharides with potassium-iodine reagent. Monitoring of electro-osmotic flow by fluorescence detection (addition of pyrene into mobile phase) or in the vis range (addition of  $\beta$ -carotene into mobile phase).

Review, electro-osmotic-flow, polysaccharides, steroids, colorants, pirimicarb and his analogs 3d, 1

- 94 006 K. BURGER (Bayerwerk, D-41538 Dormagen, Germany): Thin Layer Chromatography with Automated Multiple Development (AMD-TLC). Chemistry of Plant Protection 12 , 181-195 (1995). AMD as a TLC technique, which combines the wide range of polarity gradient elution on silica with a separation power similar to HPLC. Application to parallel analysis of 12 samples for some dozens of pesticides. Limit of quantification is 0.05  $\mu$ g active ingredient per liter. First step allows screening for active ingredients, second step confirms positive results by gradient separation and comparison of UV spectra of sample and standard. Offline-coupling with MS. Standardization as DIN method 38407-part 11 for determination of pesticides in ground and drinking water.

Environmental, AMD, quantitative analysis, densitometry, HPTLC, pesticides 3d

- 94 010 S. GOCAN (Department of Analytical Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania): Two-dimensional thin-layer chromatography. J. Liq. Chrom. Rel. Technol. 27 , 1105-1113 (2004). Two-dimensional (2D) TLC has been mostly used for the separation of a large number of compounds that cannot be separated in a single dimension TLC experiment. In 2D TLC, separation is on one surface spread along the entire area of the plate. The resolving power of the 2D TLC has great application, especially in the areas of biochemistry, biology, natural products, pharmaceuticals, and environmental analysis. Detailed description of two-dimensional development, two-dimensional development on bilayers, two-dimensional separation by TLC/ electrophoresis, and chromatogram evaluation.

Clinical chemistry research, reprint from the Encyclopedia of Chromatography (Marcel Dekker Inc., 2003) 3d

- 94 007 C. E. PALAMAREV, M. I. KANDINSKA, M. D. PALAMAREVA\* (\*Department of Chemistry, University of Sofia, 1, James Bouchier Avenue, Sofia 1164, Bulgaria): Automatic selection of mobile phases. IV. Thin-layer chromatography on silica of simple and complex organic compounds. J. Liq. Chrom. Rel. Technol. 27 , 2003-2018 (2004). The LSChrom software was applied to organic compounds with simple and complex structure being cis or trans olefinic derivatives and substituted tetrahydroisoquinolines, respectively. Lists of recommended mobile phase were obtained, avoiding any preliminary trial and error experiments. The procedure is based on the structure of any compound, literature data for adsorption properties of the relevant

groups available in the structure, and numerous calculations. The experiments with 12 arbitrarily selected eluents showed, for about 150 measurements, the proper retention of any compound and good separation of the cis-trans isomers.

3d

#### 4. Special techniques

- 94 011 Gerda MORLOCK\*, S. HÄBERLE, U. JAUTZ, W. SCHWACK (\* University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, 70599 Stuttgart, Germany, gmorlock@uni-hohenheim.de): New HPTLC-MS method for determination of heterocyclic aromatic amines. CBS 93 , 14-15 (2004). HPTLC of heterocyclic aromatic amines on silica gel WRF (prewashed with methanol by chromatography followed by drying at 120 °C for 30 min) with a 6 step gradient with diethyl ether, methanol and chloroform, with alkaline conditioning via gas phase over max. 60 mm. Quantitative determination by multi-wavelength scan in absorbance mode at 252, 262, 316 nm and fluorescence measurement at 366/>400 nm. Polynomial evaluation via peak area and height. Limit of quantitation is between 1-45 ng absolute on the plate. Online coupling with MS by use of a new extractor developed by Luftmann.

Food analysis, AMD, HPTLC, densitometry, quantitative analysis, heterocyclic aromatic amines  
4e, 23e

#### 7. Phenols

- 94 012 J. FLIEGER\*, H. SZUMILO, M. TATARCZAK, D. MATOSIUK (\*Department of Inorganic and Analytical Chemistry, Medical Academy of Lublin, Staszica 6, 20-081 Lublin, Poland): Effect of impregnation of silica gel with different zinc salts on the TLC behavior of aromatic hydrocarbons with polar groups. J. Planar Chromatogr. 17 , 65-71 (2004). TLC of 13 phenols, 15 quinolines, and 9 anilines on silica gel impregnated with zinc nitrate, bromide, chloride, acetate, and sulfate. Binary non-aqueous mixtures of 2-propanol or ethyl acetate with heptane were used. Development after pre-saturation in horizontal DS chambers. Detection under UV light at 254 nm. The impregnation with zinc acetate leads to improvement of spot shape and separation selectivity, especially for quinoline derivatives.

Qualitative identification

7, 23, 17

- 94 013 Zsuzsa KIRÁLY-VÉGHÉLY\*, G. KÁTAY, E. TYIHÁK, J.-M. MERILLON (\*Research Institute for Viticulture and Enology of the Agricultural Ministry, Experimental Wine Cellar, Maláta u. 4, Budapest, H-1105 Hungary): Separation of stilbene isomers from red wine by overpressured-layer chromatography. J. Planar Chromatogr. 17 , 4-8 (2004). OPLC and TLC of the isomers of trans-resveratrol and related compounds (e. g. piceatannol, cis-resveratrol, cis- and trans-piceid) on RP-18 using dilute acetic acid (pH 2.5) - acetonitrile 19:6. Densitometry at 310 nm.

Food analysis, quantitative analysis, qualitative identification, densitometry

7

- 94 014 H. MAJSTOROVIC, D. RATKOV-ZEBELJAN, Z. Lj. TESIC, Dusanka M. MILOJKOVIC-OPSENICA\* (\*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): Interpretation of the mechanisms of chromatographic separation on CN-silica. Part II. TLC of some phenols. J. Planar Chromatogr. 17 , 9-13 (2004). TLC separation of some phenolic compounds (phloroglucinol, 2-aminophenol, 4-hydroxybenzaldehyde, 4-methoxyphenol, salicylic acid, phenol, 4-tert-butylphenol, 2,4-dichlorophenol, 2,6-dimethylphenol)

on CN-phases, silica gel and polyacrylonitrile with 14 different mobile phases at ambient temperature in a HPTLC horizontal developing chamber. Detection by spraying with 0.05 % fluorescein solution in ethanol and subsequent exposure to UV light at 366 nm.

Qualitative identification 7, 2c

94 029 Neli-Kinga OLAH et al., see section 11a

94 015 Alina PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4, Jagiellonska Street, 41-200 Sosnowiec, Poland): New method of calculation of the partition coefficients of selected alkoxyphenols investigated by RPTLC. *J. Planar Chromatogr.* 17, 58-60 (2004). TLC of 14 meta- and para-alkoxyphenols (methoxy-, ethoxy-, propoxy-, butoxy-, pentoxy-, hexoxy-, and heptoxyphenol) on cellulose impregnated with a 0.75 % solution of ethyl oleate in diethyl ether with ethanol - water 2:3 in a twin-trough chamber with saturation for 30 min. Detection by spraying with aqueous alkaline permanganate solution.

Qualitative identification 7, 2c

94 016 Vesna RASTIJA\*, A. MORNAR, I. JASPRICA, G. SRECNIK, M. MEDIC-SARIC (\*Faculty of Agriculture, University J. J. Strossmayer, Trg Sv. Trojstva 3, 31 000 Osijek, Croatia): Analysis of phenolic components in Croatian red wine by thin-layer chromatography. *J. Planar Chromatogr.* 17, 26-31 (2004). TLC of caffeic acid, gallic acid, p-coumaric acid, kaempferol, apigenin, and naringenin on silica gel with eleven mobile phases; the best mobile phase for separation of phenolic compounds was benzene - ethyl acetate - formic acid 6:3:1. Detection after treatment with ammonia vapor under visible light or by fluorescence in UV light at 254 and 366 nm before and after spraying with 1 % ethanolic aluminum chloride solution.

Qualitative identification 7

94 017 E. TYIHÁK\*, P. OTT, Á. M. MÓRICZ, G. KÁTAY, Z. KIRÁLY-VÉGHÉLY (\*Plant Protection Institute, Hungarian Academy of Sciences, Budapest, POB 102, H-1525 Hungary): Antibiosis, antibiotics, and the formaldehyde cycle: The unique importance of planar chromatographic techniques to progress in these fields. *J. Planar Chromatogr.* 17, 84-88 (2004). OPLC of trans-resveratrol on silica gel with chloroform - methanol 10:1 after preconditioning of the plates for 3 h at 120 °C. Detection by immersion of the dried plates in a bacterial suspension (*Pseudomonas savastanoi* pv. *phaseolicola* race 6) for 20 s and MTT staining (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; 80 mg MTT and 100 mg Triton X-100 in 100 mL water) either after a short draining period or after overnight incubation; after staining the time for evaluation varied (e. g. from 1 h to 6 - 9 days or more). First results of the investigation of a complex bioautographic system with special emphasis on the direct effect of the antibiotic trans-resveratrol (fitoalexin) and the function of formaldehyde in relation to antibiotics.

Pharmaceutical research, qualitative identification, biochemistry 7, 28a

## 8. Substances containing heterocyclic oxygen

94 018 S. GAFNER\*, C. BERGERON, L. L. BATCHA, C. K. ANGERHOFER, S. SUDBERG, É. M. SUDBERG, H. GUINAUDEAU, R. GAUTHIER (\*Tom's of Maine, PO Box 710, Kennebunk, ME 04043, USA): Analysis of *Scutellaria lateriflora* and its adulterants *Teucrium canadense* and *Teucrium chamaedrys* by LC-UV/MS, TLC, and digital photomicroscopy. *J. Assoc. Off. Anal. Chem.* 86, 453-460 (2003). HPTLC of baicalein, baicalin, ikonnokoside I, verbascoside, and

teucroside on pre-washed silica gel with ethyl acetate - formic acid - acetic acid - water 100:11:11:25 in a twin trough chamber. Evaluation under UV light at 254 and 365 nm. Detection by spraying with NP (Natural Products) reagent (0.5 g diphenylboric acid aminoethylester dissolved in 50 mL methanol) and evaluation under UV 365 nm, followed by spraying with PEG (2.5 mL PEG dissolved in 50 mL ethanol), drying for 5 min, and evaluation under UV 365 nm.

Herbal, traditional medicine, HPTLC, qualitative identification

8a

- 94 019 Z. JANECZKO\*, J. KRZEK, E. PISULEWSKA, D. SOBOLEWSKA, M. DABROWSKA-TYLKA, U. HUBICKA, I. PODOLAK (\*Department of Pharmacognosy, Collegium Medicum, Jagiellonian University, Medyczna 9, 30-688 Cracow, Poland): Densitometric determination of genistin and daidzin in different cultivars of soy (*Glycine max*). *J. Planar Chromatogr.* 17, 32-35 (2004). HPTLC of e. g. genistin and daidzin on silica gel with chloroform - methanol - water 23:8:1. Quantitative determination at 260 nm. Detection limits were 25 ng; calculated mean percentage recovery was 99.4 %; standard deviation was 1.55; relative standard deviation was 1.56 %.
- Quantitative analysis, densitometry

8a

- 94 020 I. JASPRICA, A. SMOLCIC-BUBALO, A. MORNAR, Marica MEDIC-SARIC\* (\*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kvoacica 1, 10 000 Zagreb, Croatia): Investigation of the flavonoids in Croatian propolis by thin-layer chromatography. *J. Planar Chromatogr.* 17, 95-101 (2004). TLC of 15 flavonoids (flavanone, naringenin, flavone, 3-, 6-, 6'-, 7-hydroxyflavone, 3,6-, 3,7-dihydroxyflavone, morin, chrysin, quercetin, galangin, apigenin, kaempferol) and 4 phenolic acids (o-, p-coumaric acid, caffeic acid, ferulic acid) on silica gel after prewashing with methanol with 11 mobile phases. Chloroform - methanol - 98-100 % formic acid 441:30:235 and n-hexane - ethyl acetate - glacial acetic acid 31:14:5 were the most appropriate eluents. Detection under UV light at 254 and 366 nm or after spraying with 1 % methanolic diphenylboryloxyethylamine and 5 % ethanolic poly(ethylene glycol) 4000, and under UV light at 366 nm after spraying with 1 % ethanolic aluminum chloride solution.

Traditional medicine, qualitative identification

8a

- 94 021 M. NIKOLOVA\*, S. BERKOV, S. IVANCHEVA (\*Dep. Appl. Botany, Inst. Botany, Bulgarian Acad. Sci., Acad. G. Bonchev Str. 23, 1113 Sofia, Bulgaria): A rapid TLC method for analysis of external flavonoid aglycones in plant exudates. *Acta Chromatographica* 14, 110-114 (2004). TLC of external flavonoid aglycones on silica gel with toluene - dioxane - acetic acid 95:25:4 for *Veronica* exudates, and toluene - acetic acid 2:1 for *Artemisia* exudates. Detection by spraying with "Naturstoffreagenz A" reagent. Documentation of apigenin and quercetin under UV 366 nm by digital camera. Quantitative determination by comparison with the peak areas of the standards. Evaluation of suitability of the method for routine assays with large number of samples.
- Quantitative analysis, densitometry, external flavonoid aglycones

8a

## 11. Organic acids and lipids

- 94 022 C. CRUZ-HERNANDEZ, ZEYAN DENG, JIANQIANG ZHOU, A. R. HILL, M. P. YURAWECZ, P. DELMONTE, M. M. MOSSOBA, M. E. R. DUGAN, J. K. G. KRAMER\* (\* Agriculture and Agri-Food Canada, Food Research Program, 93 Stone Rd West, Guelph, Ontario, Canada): Methods for the analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas

chromatography, and silver-ion liquid chromatography. *J. Assoc. Off. Anal. Chem.* 87, 545-562 (2004). TLC of CLA (conjugated linoleic acids; octadienoic acids 18:2 with a conjugated double-bond system) on silica gel with hexane - diethyl ether - acetic acid 85:15:1. Visualization after spraying with methanol-sulfuric acid 2:1.

Qualitative identification

11a

- 94 023 Urszula HACHULA\*, S. ANIKIEL, M. POLOWNIAK (\*Institute of Chemistry, Department of Analytical Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): Determination of gallic acid after thin-layer chromatographic and paper chromatographic separation. *J. Planar Chromatogr.* 17, 51-53 (2004). TLC of gallic acid, salicylic acid and tannic acid on silica gel with chloroform - ethyl acetate - formic acid 5:4:1. Detection with the iron(III) - 2,2'-dipyridyl system (iron ammonium sulfate and sulfuric acid in water and 2,2'-dipyridyl in hydrochloric acid). Quantitative determination at 280 nm. Limit of detection 0.1 µg per spot.

Quality control, quantitative analysis, densitometry, qualitative identification

11a

- 94 024 K. KACZMARSKI, M. SAJEWICZ, A. PIENIAK, R. PIETKA, Teresa KOWALSKA\* (\*Institute of Chemistry, Silesian University, 9 Szkolna Street, Katowice, Poland): Comparison of lateral interactions with monocarboxylic and  $\alpha, \omega$ -dicarboxylic acids. *J. Liq. Chrom. Rel. Technol.* 27, 1967-1980 (2004). Part of a study originally devoted to investigating lateral interactions of monocarboxylic and  $\alpha, \omega$ -dicarboxylic acids analyzed on a low-activity stationary phase (cellulose powder) with low-polarity monocomponent mobile phases (decalin for monocarboxylic acids and 1,4-dioxan for  $\alpha, \omega$ -dicarboxylic acids). TLC of lauric, myristic, palmitic, 3-phenylpropionic, 2-phenylbutyric, and 4-phenylbutyric acid with decalin and of succinic, adipic, and suberic acid with 1,4-dioxan on cellulose. Detection with an ethanolic solution of bromocresol green. Quantitative determination at 625 nm.

Quantitative analysis, densitometry

11a, 2c

- 94 027 A. NIESTROJ, Alina PYKA\*, J. SLIWIOK (\*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska Street, 41-200 Sosnowiec, Poland): RP TLC evaluation of log P for higher fatty acids, hydroxy acids and their esters. *J. Planar Chromatogr.* 17, 143-146 (2004). TLC of oleic, elaidic, palmitic, stearic, ricinoleic acid, methyl ricinoleate, alpha-hydroxypalmitic acid, methyl alpha-hydroxypalmitate, 12-hydroxystearic acid, methyl 12-hydroxystearate, 9,10-dihydroxystearic acid, methyl 9,10-dihydroxystearate on RP-18 with methanol - water 19:1 and 9:1. Detection with iodine vapor.

Qualitative identification

11a

- 94 026 W. M. NIMAL RATNAYAKE (Health Canada, Health Products and Food Branch, Food Directorate, Nutrition Research Division, PL 2203C Banting Bldg, Ottawa, Ontario, Canada K1A 0L2): Overview of methods for the determination of trans fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. *J. Assoc. Off. Anal. Chem.* 87, 523-539 (2004). TLC of e. g. trans- and cis-18:1 isomers of fatty acids on silica gel impregnated with silver nitrate (10 % solution of silver nitrate in acetonitrile) with toluene, benzene or mixtures of toluene - hexane 1:1, hexane - diethyl ether 9:1, and hexane - chloroform. The plates are normally developed at room temperature in a dark place. The resolution of some positional 18:1 and 18:2 isomers may be improved by development at about -20° or -25 °C. Detection by spraying with 0.05 % rhodamine B in ethanol. GC after extraction.

Quantitative analysis, qualitative identification

11a

- 94 028 Joanna NOWAKOWSKA (Medical University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, Al. Gen. J. Hallera 107, 80-416 Gdansk, Poland): Normal and reversed-phase TLC determination of rosmarinic and valerenic acids. *J. Planar Chromatogr.* 17 , 149-153 (2004). TLC on silica gel and RP-18 after pre-saturation with methanol - water, ethanol - water, propanol - water, acetonitrile - water, acetonitrile - methanol, THF - water, acetonitrile - buffer, and methanol - buffer in which the concentration of organic modifier was varied from 0 to 100 %. Detection by spraying with conc. sulfuric acid - methanol 1:4 followed by heating for 10 min at 120 °C and illumination with UV light.
- Herbal, qualitative identification 11a
- 94 029 Neli-Kinga OLAH\*, D. HANGANU, R. OPREAN, C. MOGOSAN, N. DUBEI, S. GOCAN (\*SC Plantextract SRL, 407059-Radaia, Cluj, Romania): Selective extraction of caffeic acid derivatives from *Orthosiphon stamineus* Benth. (Laminaceae) leaves. *J. Planar Chromatogr.* 17 , 18-21 (2004). TLC of polyphenols and rosmarinic acid on silica gel with 1) toluene - methyl acetate - formic acid 5:4:1; 2) ethyl acetate - methanol - water 77:13:10; 3) ethyl acetate - diethyl ether 8:2. Detection a) at 254 nm, b) in fluorescence after spraying with Neu-PEG reagent, and c) in visible light after spraying with 10 % iron(III) chloride solution. Quantitative determination by densitometry at 254 nm and spectra recording from 200 to 500 nm for identification.
- Pharmaceutical research, densitometry, quantitative analysis 11a, 7
- 94 025 J. L. SCHNECK, S. R. BANDSTRA, B. FRIED\*, J. SHERMA (\*Department of Biology, Lafayette College, Easton, PA 18042, USA): Thin layer chromatographic analysis of neutral lipids in snail conditioned water and feces of *Biomphalaria glabrata* infected with *Echinostoma caproni*. *J. Liq. Chrom. Rel. Technol.* 27 , 2039-2045 (2004). HPTLC of cholesteryl oleate, methyl oleate, triolein, oleic acid, and cholesterol on pre-washed silica gel with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 in a twin-trough chamber with saturation. Detection with phosphomolybdic acid reagent. Quantitative determination at 610 nm.
- HPTLC, densitometry, quantitative analysis, qualitative identification, biological material 11c

### 13. Steroids

- 94 030 A. JAMSHIDI (Department of Novel Drug Delivery Systems, Iran Polymer and Petrochemical Institute, P.O. Box 14965/115, Tehran, Iran, a.jamshidi@ippi.ac.ir): Determination of progesterone in drug release media. *CBS* 93 , 10-12 (2004). HPTLC of progesterone on silica gel pre-washed by development in AMD2 first with chloroform - methanol 1:1 and then with the mobile phase, followed by drying at 80 °C for 15 min. Development in AMD2 with toluene - 2-propanol 10:1 without preconditioning over 60 mm. Quantitative determination by absorbance measurement at 252 nm followed by spectra recording from 200 to 360 nm. The linear working range is 25.7-154.5 ng/zone. Repeatability (standard deviation calculated from the amounts of seven simulated progesterone samples determined on the same plate at three concentration levels in the lower, middle and upper range) is 0.26-1.29 %. Recovery is 99.88-100.97 %. Reproducibility was performed with recycled HPTLC plates.
- Pharmaceutical research, quality control, AMD, HPTLC, densitometry, quantitative analysis, progesterone 13a, 32a

- 94 031 Alina PYKA\*, M. DOLOWY (\*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska St., Pl. -41-200 Sosnowiec, Poland): Separation of selected bile acids by TLC. II. One-dimensional and two-dimensional TLC. *J. Liq. Chrom. Rel. Technol.* 27 , 2031-2038 (2004). TLC of 7 bile acids (chenodeoxycholic, deoxycholic, cholic, glycocholic, lithocholic, glycodeoxycholic, and glycolithocholic acid) on silica gel with n-hexane - ethyl acetate - acetic acid 5:4:1 in the first dimension and chloroform - n-butanol - acetic acid - water 1:16:1:1 in the second dimension. Detection by spraying with 10 % sulfuric acid followed by heating at 120 °C for 20 min. The 2D technique allowed the complete separation of all the bile acids studied.
- Qualitative identification, biological material 13c

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

- 94 032 Izabela FECKA\*, A. KOWALCZYK, W. CISOWSKI (\*Department of Pharmacognosy, Wrocław Medical University, pl. Nankiera 1, 50-140 Wrocław, Poland): Optimization of the separation of flavonoid glycosides and rosmarinic acid from *Mentha piperita* on HPTLC plates. *J. Planar Chromatogr.* 17 , 22-25 (2004). HPTLC and TLC of caffeic acid, rosmarinic acid, caffeetannins and flavonoids on silica gel, amino-, cyano-, and RP-18-phases in a horizontal DS-chamber with a variety of mobile phases at room temperature. Acetone - acetic acid 17:3 was best mobile phase on the aminopropyl layer; water - methanol 3:2 on RP 18. Detection of the colored compounds under UV light at 365 nm before and after spraying with 2 % methanolic aluminum chloride solution, or in visible light after treatment with bis-diazotized sulfanilamide.
- Pharmaceutical research, herbal, qualitative identification 14
- 94 033 S. L. VISHWAKARMA, M. S. BAGUL, M. RAJANI\*, R. K. GOYAL (\*Pharmacognosy and Phytochemistry Department, B. V. Patel Pharmaceutical Education and Research Development Center, Thaltej, Ahmedabad-380054, India): A sensitive HPTLC method for estimation of swertiamarin in *Enicostemma littorale* Blume, *Swertia chirata* (Wall) Clarke, and in formulations containing *E. littorale*. *J. Planar Chromatogr.* 17 , 128-131 (2004). HPTLC of the secoiridoid glucoside swertiamarin on silica gel with ethyl acetate - methanol - water 77:15:5 after pre-saturation for 30 min. Quantitative determination at 250 nm. The method was validated for precision, repeatability, and accuracy and found to be precise.
- Quality control, herbal, quantitative analysis, densitometry, HPTLC 14

#### 17. Amines, amides and related nitrogen compounds

- 94 012 J. FLIEGER et al., see section 7
- 94 034 H. HOFMAN (Stada R&D GmbH, Analytical Development, Sladastrasse 2, D-61118 Bad Vilbel, Germany, herbert.hofmann@stada.de): From visual evaluation according to the pharmacopoeia to exact analytical results. *CBS* 93 , 5-7 (2004). HPTLC of N,N-diethylethane-1,2-diamine in metoclopramide finished products on silica gel with 32 % ammonia - methanol - dichloromethane 3:15:80 over 40 mm with chamber saturation. Detection by dipping in 0.2 % ethanolic ninhydrin solution for 1 s, followed by drying at 120 °C for 5 min. Quantitative determination by absorbance measurement at 480 nm and evaluation of peak area with polynomial regression. The correlation coefficient of the calibration curve is 0.9995, the residual standard deviation 2.19 %.

Intermediate precision is 1.65 %. Recovery for 0.2-1.0 % impurity is 100.5 %. Limit of quantitation is 0.05 % impurity.

Pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis, metoclopramide, N,N-diethylethane-1,2-diamine 17a, 32a

- 94 035 A. TIMMER\*, Daniela BRAMMERTZ, Susanne GRETHER-BECK (\*Environmental Health Research Institute of Heinrich-Heine-Universität Düsseldorf gGmbH, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany, Grether-Beck@uni-duesseldorf.de, Andreas.Timmer@ish.de): Quantification of signaling ceramides in primary keratinocytes. CBS 93, 2-4 (2004). HPTLC of signaling ceramides on silica gel with a 7 step gradient from methanol over dichloromethane to n-hexane over 42 min. Detection by dipping in manganese chloride reagent for 1 s, followed by drying at 120 °C for 20 min. Quantitative determination by absorbance measurement at 550 nm and Michaelis-Menten regression 2 via peak area. Signaling ceramides are separated from other lipids (shingomyelin, phosphatidylcholine, cholesterol) contained in cellular lipid extracts. Comparison with determination of ceramide formation via isotope labeled standards and conventional TLC method.

Environmental, pharmaceutical research, clinical chemistry research, HPTLC, AMD, densitometry, comparison of methods, quantitative analysis, ceramides, keratinocytes, 17c, 32f

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

- 94 036 V. D. KRASIKOV\*, I. I. MALAKHOVA, E. V. DEGTEREV, B. V. TYAGLOV (\*The Scientific and Production Center Lenchrom, V. O. Bolshoy pr. 31, 199004 St Petersburg, Russia): Planar chromatography of free industrial amino acids. J. Planar Chromatogr. 17, 113-122 (2004). HPTLC of serine, threonine, phenylalanine, tryptophan, lysine, ornithine, arginine, valine, and leucine on silica gel with A) 2-propanol - ethyl acetate - 25 % ammonia - water 40:40:3:50; B) 2-propanol - acetone - water - 25 % ammonia 25:25:7:6; C) 2-propanol - 25 % ammonia 7:3; D) chloroform - 96 % ethanol - acetic acid - water (52.5-54.0):(26.0-27.5):(8.9-9.6):(3.5-4.2); E) 1-propanol - 25 % ammonia 11:9; F) 1-propanol - 25 % ammonia - water (3.3-4.1):(0.9-1.5):(7.5-9.0); G) t-amyl alcohol - 2-butanone - water 22:6:5, upper layer, in a presaturated twin-trough chamber. Detection by dipping into a ninhydrin solution. Quantitative determination by reflectance measurement at 500 nm.

Quantitative analysis, densitometry, pharmaceutical production

18a

## 22. Alkaloids

- 94 037 M. GLENSK\*, B. ZBIKOWSKA, W. CISOWSKI (\*Department of Pharmacognosy, University of Medicine, pl. Nankiera1, 50-140 Wroclaw, Poland): TLC separation of *Unicaria tomentosa* alkaloids on chemically modified stationary phases. J. Planar Chromatogr. 17, 14-17 (2004). HPTLC of oxindole alkaloids on silica gel, Diol-, CN-, NH 2-, and RP-18-phases. Normal-phase TLC with 1) ethyl acetate - methanol - water 200:27:20; 2) ethyl acetate - methanol - water - acetic acid 100:2.7:5:3; 3) ethyl acetate - methanol - water - formic acid 100:2.7:5:3; 4) ethyl acetate - isopropanol - ammonia 100:2:1. RP TLC with water - methanol 7:3 containing 0.25 % ammonia; development in horizontal chamber (sandwich configuration) at room temperature. Two-dimensional TLC with mobile phase 2 in the first direction and, after drying, with mobile phase 3 in the second direction. Evaluation under UV 254 nm and after derivatization with iodine

reagent or with Dragendorff reagent. TLC separations were documented by scanning. Silica and cyanopropyl layers with acidic aqueous mobile phases gave the best separation.

Pharmaceutical research, traditional medicine, herbal, qualitative identification 22

- 94 038 J. K. LALLA\*, P. D. HAMRAPURKAR, S. A. MUKHERJEE (\*Sanskriti Bldg. No. 20, Flat No. 701/702, Thakur Complex, Kandivli (E), Mumbai 400 101, India): Estimation of the saussurine content of root powder and a tablet formulation prepared from *Saussurea lappa*. *J. Planar Chromatogr.* 17, 36-39 (2004). TLC of saussurine on RP-18 after preconditioning for 15 min with e. g. methanol - water 2:1, tetrahydrofuran - chloroform 4:1, and methanol - water - glacial acetic acid 80:4:1; the last was found to enable the optimum separation. Detection by treatment with Dragendorff's reagent. Quantitative determination at 540 nm. Limit of detection 3 µg.

Quality control, traditional medicine, densitometry, quantitative analysis, qualitative identification 22

- 94 039 Maria LUCZKIEWICZ\*, P. MIGAS, A. KOKOTKIEWICZ, M. WALIJEWSKA, W. CISOWSKI (\*Department of Pharmacognosy, Medical University of Gdansk, al. Gen. J. Hallera 107, 80-416 Gdansk, Poland): Two-dimensional TLC with adsorbent gradient for separation of quinolizidine alkaloids in the herb and in-vitro cultures of several *Genista* species. *J. Planar Chromatogr.* 17, 65-71 (2004). TLC of sparteine, alpha-isosparteine, retamine, hydroxylupanine, cytisine, methylcytisine, sophocarpine and lupanine on silica gel or diol phases with 1) chloroform - methanol - 25 % ammonia 85:15:1 in the first direction and, after drying, with 2) acetonitrile - water - 36 % hydrochloric acid 30:100:7 in the second direction. In adsorbent-gradient TLC diol plates were used in the first direction with mobile phase 1. After the first development the alkaloids were transferred to the second RP-18 plate with mobile phase 2. This mobile phase was then used for the second, perpendicular development. The connection of the stationary phases (2 mm overlap) was carried out by means of a specially designed device. Detection by spraying with Dragendorff reagent.

Pharmaceutical research, HPTLC

22

### 23. Other substances containing heterocyclic nitrogen

- 94 012 J. FLIEGER et al., see section 7

- 94 041 M. STEFANIAK, P. KUS, J. SLIWIOK\* (\*Department of Chemistry, Silesian University, 9, Szkolna Street, 40-006 Katowice, Poland): TLC and RPTLC separation of porphine, selected porphyrins, and their metalloderivatives. *J. Planar Chromatogr.* 17, 72-74 (2004). TLC of porphine, meso-mono-tert-butylporphyrin, meso-tetra-tert-butylporphyrin and their respective Zn(II), Ni(II), and Cu(II) derivatives on silica gel with n-hexane - chloroform 7:3, and on RP-18 with methanol or methanol - water 9:1 after presaturation of the plates for 30 min. The porphyrins were detected visually.

Qualitative identification, comparison of methods

23a

- 94 011 Gerda MORLOCK et al., see section 4e

- 94 040 T. SLAWIK\*, B. PAW (\*Department of Medicinal Chemistry, Pharmaceutical Faculty, Medical University of Lublin, Chodzki 6, Lublin 20-093, Poland): Lipophilicity of some N- and O-sub-

stituted alkanolic acids of 1,2-benzisothiazol-3(2H)-one determined by reversed-phase thin layer chromatography. *J. Liq. Chrom. Rel. Technol.* 27 , 1043-1055 (2004). TLC of 11 N- and O-substituted alkanolic acids of 1,2-benzisothiazol-3(2H)-one on RP-18 in horizontal chambers using methanol - water, methanol - glycine buffer pH 1.95, methanol - glycine buffer pH 10.0 mixtures of varying composition. Evaluation under UV light at 254 nm.

Qualitative identification 23e

- 94 042 S. ZAREBA\*, T. WAWRZYNOWICZ (\*Department of Food and Nutrition, Medical University, Faculty of Pharmacy, Lublin, Poland): Thin-layer reversed-phase chromatography of some heteroazophenols in ion-association systems. *J. Liq. Chrom. Rel. Technol.* 27 , 425-435 (2004). TLC of 9 heterocyclic azophenols (e. g. azo derivatives of 2-amino-1,3,4-oxadiazole and 1,2,4-triazole) on RP-2 in flat sandwich chambers with a mixture of phosphate buffer solution + water (or aqueous solution of ion-pairing reagent dimethylaurylbenzylammonium bromide, Sterinol) + methanol + acetonitrile, e. g. phosphate buffer - water - methanol - acetonitrile 4:4:12:5. Dimethylaurylbenzylammonium bromide may be used as a counterion for chromatography of ionizable, but coloured, substances.

Qualitative identification 23e

## 27. Vitamins and various growth regulators

- 94 043 F. WATANABE\*, T. MICHIHATA, S. TAKENAKA, H. KITAKA-KATSURA, T. ENOMOTO, E. MIYAMOTO, S. ADACHI (\*Department of Health Science, Kochi Women's University, Kochi 780-8515 Japan): Purification and characterization of corrinoid compounds from a Japanese fish sauce. *J. Liq. Chrom. Rel. Technol.* 27 , 2113-2119 (2004). TLC of corrinoid compounds, authentic B12 and cyanocobamides (benzimidazolyl-, 5-hydroxy-benzimidazolyl- and 7-adenylcyanocobamides) on silica gel with 2-propanol - 28 % ammonia - water 7:1:2 in the dark at 25 °C. Detection under daylight as pink-colored spots.

Food analysis, quality control, qualitative identification 27

## 28. Antibiotics, Mycotoxins

- 94 045 S. W. HUSAIN\*, V. GHOULIPOUR, H. SEPAHRIAN (\*Dep. Appl. Chem., Fac. Chem., Univ. Tarbiat Moallem, 49 Mofatteh Avenue, Tehran-15614, Iran): Chromatographic behavior of antibiotics on thin layers of an inorganic ion-exchanger. *Acta Chromatographica* 14 , 102-109 (2004). Investigation of the chromatographic behavior of amoxicillin, ampicillin, cephalixin, cloxacillin, doxycycline, tetracycline, erythromycin, gentamycin, streptomycin, and co-trimoxazole on titanite silicate inorganic ion-exchanger with organic, aqueous, and mixed aqueous organic mobile phases. Achievement of rapid separation of one antibiotic from numerous other antibiotics, as have many binary and ternary separations. Demonstration of the dependence of R<sub>f</sub> values on the salt concentration of the mobile phase, and the existence of a linear relationship between FM and molarity of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> for some antibiotics in salting-out TLC using aqueous ammonium sulfate solution. Study of the effect of varying volume ratios of the binary mobile phase methanol - 0.1 M formic acid on the R<sub>f</sub> values.

Pharmaceutical research, antibiotics 28a, 2c

94 017 E. TYIHÁK et al., see section 7

94 044 J. STROKA\*, L. DASKO, B. SPANGENBERG, E. ANKLAM (\*European Commission, Directorate General, Joint Research Centre, Institute for Reference Materials and Measurement, Food Safety and Quality Unit, Retieseweg, B-2440 Geel, Belgium): Determination of the mycotoxin, sterigmatocystin, by thin-layer chromatography and reagent-free derivatization. *J. Liq. Chrom. Rel. Technol.* 27 , 2101-2111 (2004). HPTLC of sterigmatocystin on amino phases with toluene - acetone 20:1 followed by drying and heating at 150 °C for 15 min. Quantitative determination at 366 nm (yellowish fluorescent spots).

HPTLC, densitometry

28b

## 29. Pesticides and other agrochemicals

94 046 J. SHERMA (Lafayette College, Department of Chemistry, Easton, PA 18042-1782, USA): Recent advances in the thin-layer chromatography of pesticides: A review. *J. Assoc. Off. Anal. Chem.* 86 , 602-611 (2003). Review of the advances in the applications of TLC and HPTLC for the separation, detection, identification, and determination of pesticides, other agrochemicals, and related compounds for the period 2000-2002. Analyses are described for a variety of samples, such as food, biological, and environmental samples, and for residues of pesticides of various types, including insecticides, herbicides, and fungicides, belonging to different chemical classes. 88 references are included for residue analysis, hydrophobicity studies, and the use of TLC and thin-layer radio-chromatography for studies of pesticide metabolism, degradation, uptake, and related topics.

Agricultural, environmental, food analysis, review

29, 1

94 047 G. ZHONG (Zhong Guohua)\*, M. HU (Hu Meiyang), X. LIU (Liu Xiaojin), C. PENG (Peng Cuyan) (\*Dep. Insect Toxicology, Southern China Univ. Agr., Guangzhou 510642, China): (Quantification of rhodojaponin III in pesticides rooted in some plants by thin-layer chromatography) (Chinese). *Chinese J. Chromatogr. (Sepu)* 22 (3), 296-297 (2004). TLC of rhodojaponin III on silica gel with chloroform - methanol 9:1. Detection by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heating. Identification by comparison with the standard. Quantification by densitometry at 550 nm. Validation of the method by investigating its linearity range (0.1-4.0 µg/spot, r=0.997), precision (RSD=1.30-3.34 %, n=8), and recovery (95.4-101.5 %, RSD=1.97 %, n=5).

Pharmaceutical research, agricultural, traditional medicine, quantitative analysis, densitometry, qualitative identification, rhodojaponin III

29f

## 30. Synthetic and natural dyes

94 048 Irena BARANOWSKA\*, M. ZYDRON, K. SZCZEPANIK (\*Department of Analytical and General Chemistry, Silesian Technical University, 7 M. Strzody Street, 44-100 Gliwice, Poland): TLC in the analysis of food additives. *J. Planar Chromatogr.* 17 , 54-57 (2004). TLC of food pigments (patent blue V, quinoline yellow, brilliant blue FCF, tartrazine, azorubine, ponceau 4 R, curcumine, indigo carmine, cochineal, methyl violet, mixed carotenes, plain caramel, erythrosine B, orange yellow S) and artificial sweeteners (aspartame, acesulfame, sodium cyclamine) and benzoic acid on silica gel. Pigments were developed with isopropanol - 12.5 % ammonia 5:1; sweeteners and benzoic acid were developed with ethanol - isopropanol - 12.5 % ammonia 10:

40:1. Derivatization with a solution of 0.04 g bromocresol green in 100 mL ethanol containing 0.1 M sodium hydroxide.

Qualitative identification

30, 35c

94 008 V. G. BEREZKIN et al., see section 3d

### 32. Pharmaceutical and biomedical applications

94 051 Himani AGARWAL, Neeraj KAUL, A. R. PARADKAR, K. R. MAHADIK (Department of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune 411038, India): HPTLC method for guggulsterone. Quantitative determination of E- and Z-guggulsterone in herbal extract and pharmaceutical dosage form. *J. Pharm. Biomed. Anal.* 36 , 33-41 (2004). A sensitive selective, precise and robust HPTLC method for the analysis of E and Z stereoisomers of guggulsterone (the hypolipidemic agent in the gum-resin exudates of *Commiphora mukul*) both as a bulk drug and in formulations was developed and validated. Separation on silica gel with toluene - acetone 9:1. Quantitative determination by absorbance measurement at 250 nm. This system was found to give compact spots for E- and Z-guggulsterone ( $R_f$  value of  $0.38 \pm 0.02$  and  $0.46 \pm 0.02$  respectively) following double development with the same mobile phase. Both E- and Z-guggulsterone showed good linearity in the concentration range of 100-6000 ng/spot. The method was validated for precision, robustness, recovery, and specificity. The proposed HPTLC method is suitable for the identification and quantitation of these isomers in herbal extracts and pharmaceutical dosage forms.

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC 32a

94 055 J. BOULIGAND\*, A. PACI, L. MERCIERA, G. VASSALB, P. BOURGETA (\*Dept. of Clinical Pharmacy, Institut Gustave Roussy, 39 Rue Camille Desmoulins, 94800 Villejuif, France): High performance thin layer chromatography with a derivatization procedure, a suitable method for the identification and the quantitation of busulfan in various pharmaceutical products. *J. Pharm. Biomed. Anal.* 34 , 525-530 (2004). HPTLC of busulfan, an alkylating agent in pharmaceutical preparations, on Lichrospher silica gel with ethyl acetate - chloroform - methanol 13:4:3 in horizontal sandwich chamber. After development the plate was soaked in 2 % ethanolic solution of 4-nitro benzyl pyridine, followed by heating at 193 °C for 10 min. Quantitative determination by absorbance measurement at 600 nm. The method was validated for accuracy, precision, linearity (100-500 µg/mL), and specificity. The method was suitable for stability studies of busulfan in pharmaceutical preparations.

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization 32a

94 056 E. BRZEZINSKA\*, G. KOSKA (\*Department of Analytical Chemistry, Medical University of Lodz, Muszynskiego 1, 90-151 Lodz, Poland): TLC data in QSAR assay of thiazole and benzothiazole derivatives with H1-antihistamine activity. Part 2. *J. Planar Chromatogr.* 17 , 40-45 (2004). Investigation of the quantitative structure-activity relationships (QSAR) between H1-histaminergic activity and chromatographic data for derivatives of 2-[2-(phenylamino)thiazol-4-yl]ethanamine, 2-(2-benzyl-4-thiazolyl)ethanamine, 2-(2-benzhydrylthiazol-4-yl)ethanamine, 2-(1-piperazinyl)benzothiazole, and 2-(hexahydro-1H-1,4-diazepin-1-yl)benzothiazole. TLC on silica gel, impregnated with solutions of amino acid analogs (propionic acid, propionamide, and n-amylamine) and their mixtures, with acetonitrile - methanol - buffer 2:2:1 and acetonitrile -

methanol -dichloromethane - buffer 6:1:1:2. Detection under UV light at 254 nm.

Pharmaceutical research, qualitative identification 32a

- 94 077 I. M. CHOMA\*, A. CHOMA, I. KOMANIECKA, K. PILORZ, K. STASZCZUK (\*Department of Chemical Physics and Physicochemical Separation Methods, M. Curie-Skłodowska University, M. Skłodowska Sq. 3, 20-031 Lublin, Poland): Semiquantitative estimation of enrofloxacin and ciprofloxacin by thin-layer chromatography - direct bioautography. *J. Liq. Chrom. Rel. Technol.* 27, 2071-2085 (2004). HPTLC of enrofloxacin and ciprofloxacin on silica gel using dichloromethane - methanol - 2-propanol - 25 % ammonia 3:3:5:2. Bioautography with Chrom Biodip® Antibiotics Test Kit. The developed and dried plate was immersed briefly in the microorganism solution and incubated overnight at 28 °C. After incubation the plate was sprayed with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-solution and incubated for about 30 min. The dried plates were scanned for documentation. The areas of the inhibition zones were then measured with a planimeter.

HPTLC, semiquantitative analysis 32a

- 94 059 B. ERDÉLYI\*, A. SZABÓ, L. BIRINCSIK, G. SERES, J. SALÁT, J. IVANICS, A. KÓNYA (\*IVAX Drug Research Institute Ltd., P. O. Box 82, Budapest, Hungary): TLC/HPTLC and HPLC methods for monitoring microbial transformation processes. *J. Planar Chromatogr.* 17, 132-136 (2004). TLC and HPTLC of pravastatin, compactin and by-products (e. g. epi-pravastatin sodium salt and pravastatin-lactone) on silica gel in a twin-trough chamber with toluene - dichloromethane - tetrahydrofuran 23:1:1 for samples from ketone reduction and toluene - acetone - glacial acetic acid 20:30:4 for samples from compactin hydroxylation. Quantitative determination by reflectance measurement at 275 and 238 nm respectively.

HPTLC, quantitative analysis, densitometry, biochemical production 32a

- 94 100 S. Y. GANDHE, A. D. SATHAYE, S. V. PIMPLE, M. A. JOSHI (Emcure R & D Centre, T-184 MIDC Bhosari, Pune - 411026, India): Simultaneous estimation of ginkgo biloba and ginseng from pharmaceutical solid dosage form by HPTLC. *Indian Drugs* 41 (6), 362-365 (2004). HPTLC on silica gel with n-butane - n-propanol - glacial acetic acid - water - chloroform 4:1:1:1:2 with addition of formic acid in proportion of 1:0.001. Densitometric evaluation of ginkgo biloba extract by absorbance measurement at 254 nm. The same plate was used for the quantitative determination of ginseng at 520 nm after spraying with ninhydrin reagent. A simple, fast and precise HPTLC method has been developed for the simultaneous determination of ginkgo biloba and ginseng in solid dosage form.

Pharmaceutical research, quality control, qualitative identification, densitometry, postchromatographic derivatization, comparison of methods, HPTLC 32a

- 94 065 Tat'ána GONDOVÁ\*, M. VINCOVÁ, K. FLÓRIÁN (\*P. J. Safárik University, Faculty of Sciences, Department Physical and Analytical Chemistry, Moyzesova 11, 040 01 Kosice, Slovakia): Determination of the lipophilicity of xanthenes by reversed-phase liquid chromatography. *J. Planar Chromatogr.* 17, 156-158 (2004). HPTLC of eight 8-alkyltheophyllines (theophylline, methyl-, propyl-, butyl-, heptyl-, tridecyl-, benzyl-, phenylethyltheophylline) on RP18 with mixtures of methanol and phosphate buffer pH 7.5 (containing between 50 and 90 % methanol). Detection under UV at 280 nm.

Pharmaceutical research, HPTLC, qualitative identification 32a

- 94 066 Anna GUMIENICZEK\*, H. HOPKALA, A. BERECKA (\*Department of Medicinal Chemistry, Medical University of Lublin, Chodzki 6, 20-093 Lublin, Poland): Reversed-phase thin-layer

chromatography of three new oral antidiabetics and densitometric determination of pioglitazone. *J. Liq. Chrom. Rel. Technol.* 27 , 2057-2070 (2004). HPTLC of pioglitazone hydrochloride ((±)-5-[p-[2-(ethyl-2-pyridinyl)ethoxy]-benzyl]-2,4-thiazolidinedione hydrochloride), rosiglitazone maleate ((±)-5-[4-[2-[N-methyl-N-(2-pyridinyl)amino]-ethoxy]benzyl]-2,4-thiazolidinedione maleate), and repaglinide S(+)-2-ethoxy-4-[N-[1-(2-piperidinophenyl)-3-methyl-1-butyl]-amino carbonylmethyl]benzoic acid on cyano-phase with 1,4-dioxane - buffer pH 2.8/4.4/6.4/7.9 1:4; 2:3; 1:1; 3:2; 2:1 in horizontal chambers without saturation. Quantitative determination of pioglitazone by reflectance/transmittance measurement at 266 nm.

Quantitative analysis, densitometry, HPTLC

32a

- 94 067 K.R. GUPTA\*, S.G. WADODKAR (\*Department of Pharmaceutical Sciences, Nagpur University, Nagpur, India): HPTLC estimation of ofloxacin & tinidazole from pharmaceutical dosage form. *Indian Drugs* 41 (3), 160-164 (2004). Combinations of ofloxacin and tinidazole (1:3) are available as tablet dosage forms. Stability indicating HPTLC of ofloxacin and tinidazole on silica gel with dioxan - ethyl acetate - toluene - acetic acid - water 5.5:5:3.2:2:2 . Quantification by absorbance measurement at 307 nm. The R<sub>f</sub> value of ofloxacin and tinidazole was found to be 0.12 and 0.85 respectively. Recovery was 99.85 % and 99.45 % for ofloxacin and tinidazole respectively. The proposed method was quantitatively evaluated in terms of calibration of concentration range, stability, and accuracy. It can be adopted for routine analysis of the formulation.

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC

32a

- 94 034 H. HOFMAN et al., see section 17a

- 94 089 O. S. IDOWU\*, O. A. ADEGOKE, A. A. OLANYI (\*University of Ibadan, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Orita UI, Ibadan-Oyo Rd, Nigeria): Colorimetric assay of propranolol tablets by derivatization: Novel application of diazotized 4-amino-3,5-dinitrobenzoic acid (ADBA). *J. Assoc. Off. Anal. Chem.* 87 , 573- 578 (2004). TLC of propranolol/reagent azo adduct (with diazotized 4-amino-3,5-dinitrobenzoic acid) on silica gel with ethyl acetate - methanol 9:1, chloroform - methanol 4:1, methanol - ammonia 200:3 (plate was impregnated with 0.1 M methanolic KOH before spotting). Evaluation under UV light at 254 nm.

Quality control, qualitative identification

32a

- 94 030 A. JAMSHIDI et al., see section 13a

- 94 070 J. KRZEK\*, M. STAREK (\*Jagiellonian University, Collegium Medicum, Department of Inorganic and Analytical Chemistry, 9 Medyczna Str., 30-688 Kraków, Poland): Densitometric determination of naproxen, and of naproxen methyl ester, its impurity, in pharmaceutical preparations. *J. Planar Chromatogr.* 17 , 137-142 (2004). TLC of naproxen in pharmaceutical preparations (Naprosyn, Apranax, Aleve, Naproxen) on silica gel with cyclohexane - chloroform - methanol 12:6:1. Quantitative determination at 223 nm. Limit of detection for naproxen is 30 ng. Recovery and repeatability were satisfactory.

Quality control, quantitative analysis, densitometry

32a

- 94 079 K.R. MAHADIK, A. R. PARADKAR, H. AGARWAL, N. KAUL (Department of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune 41038, India): Validated HPTLC content uniformity test for the determination of tizanidine hydrochloride in tablet dosage form. *Indian Drugs* 41 (8), 482-487 (2004). HPTLC on silica gel with toluene - acetone - ammonia 50:50:1. Quantitative determination by absorbance

measurement at 315 nm. The method was validated in terms of linearity (300-1000 ng/spot), precision, accuracy, and specificity. For the content uniformity test the tizanidine hydrochloride content of 10 individual tablet units of two market formulations was determined after extracting with methanol. Both formulations complied with the USP specification. The proposed content uniformity allows the parallel analysis of ten tablets on a single plate and provides a faster and cost-effective quality control tool for routine analysis. A simple, sensitive HPTLC content uniformity test was developed and validated for the analysis of tizanidine hydrochloride in its commercial single component tablet formulations (2mg/tablet).

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC 32a

94 080 K. MANOJ, P. MUTHUSAMY, S. ANBAZHAGAN (Department of Pharmaceutical Analysis, C. L. Baid Mehta College of Pharmacy, Old Mahabalipuram Road, Jyothi nagar, Thorapakkam, Chennai 600096, India): HPTLC method for estimation of pioglitazone hydrochloride from tablet formulation. *Indian Drugs* 41 (6), 354-357 (2004). HPTLC on silica gel with toluene - methanol - acetone - 10 % ammonia 80:40:20:1. Quantitative determination by absorbance measurement at 254 nm. The R<sub>f</sub> value of pioglitazone hydrochloride was found to lie between 0.49-0.55. The linear dynamic response was found to be 2.0-4.0 µg/spot for pioglitazone hydrochloride. The results of the analysis have been validated statistically and by recovery studies. A simple, fast, specific and precise HPTLC method has been developed for the estimation of pioglitazone hydrochloride in its tablet dosage form.

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC 32a

94 082 Genowefa MISZTAL\*, L. KOMSTA, D. CICHECKA (\* Department of Medicinal Chemistry, Medical University, 6 Chodzki, 20-093 Lublin, Poland): Reversed-phase chromatographic retention behavior of some fibrate-type antihyperlipidemic drugs. *J. Planar Chromatogr.* 17, 123-127 (2004). TLC of 5 fibrate-type drugs (bezafibrate, ciprofibrate, clofibrate, clofibric acid, fenofibrate, and gemfibrozil) on RP 18-, RP 8- and cyano-phase in horizontal chambers with chamber saturation with a phosphate buffer and different amounts of six modifiers, i.e. acetone, acetonitrile, dioxane, isopropanol, methanol, and tetrahydrofuran. The best separation of all drugs investigated was obtained with dioxane - pH 7.60 phosphate buffer 7:3. Visualization under UV light at 254 nm. Quantitative determination at 227 nm.

Quality control, quantitative analysis, densitometry 32a

94 078 K. M. PATIL, A. K. AGGARWAL, S. L. BODHANKAR (Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune 411038, India): Validated HPTLC method for estimation of lamotrigine in tablets. *Indian J. Pharm. Sci.* 66 (3), 283-286 (2004). TLC of lamotrigine in its tablet dosage form on silica gel with acetone - toluene - ammonia 14:6:1. Quantitative determination by absorbance measurement at 312 nm. The method was quantitatively evaluated in terms of linearity, accuracy, precision, repeatability, and specificity to prove its utility in the analysis of tablet dosage form.

Pharmaceutical research, quality control, densitometry, qualitative identification, comparison of methods, postchromatographic derivatization 32a

94 085 Gordana POPOVIC\*, M. CAKAR, K. VUCICEVIC, S. VLADIMIROV, D. AGBABA (\*Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P. O. Box 146, 11000 Belgrade, Serbia and Montenegro): Comparison of HPTLC and HPLC for determination of econazole nitrate in topical dosage forms. *J. Planar Chromatogr.* 17, 109-112 (2004). HPTLC of econazole nitrate (1-[2-[4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate) on

- silica gel in a twin-trough chamber with n-butyl acetate - carbon tetrachloride - methanol - diethylamine 6:12:5:1. Quantitative determination by reflectance/absorbance measurement at 230 nm.  
Quantitative analysis, densitometry 32a
- 94 086 V. PURATCHIMANI, S. JHA (Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835215, India): HPTLC standardization of *Gymnema sylvestre* R. Br. using gymnestrogenin as reference. *Indian J. Pharm. Sci.* 66 (2), 242-244 (2004). HPTLC of gymnestrogenin in *Gymnema sylvestre* on silica gel with chloroform - methanol 9:1. Quantitative determination by absorbance measurement at 293 nm. Linearity was in the range of 4-10 µg. A gymnestrogenin content of 1.11 % was found in the test sample. Average percentage recovery was 99.1 ± 0.27. The proposed method is precise and sensitive and can be used for detection, monitoring, and quantification of gymnestrogenin in *Gymnema sylvestre*.  
Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC 32a
- 94 093 R. T. SANE, A. MENEZES\*, M. MOTE, A. MOGHE, G. GUNDI (\*TDM laboratories, Plot no. 194, Scheme no. 6, Road no. 15, Sion (E), Koliwada, Mumbai-22, India): HPTLC determination of montelukast sodium in bulk drug and in pharmaceutical preparations. *J. Planar Chromatogr.* 17, 75-78 (2004). HPTLC of montelukast sodium (1-[[[(1R)-1-[3-[(1E)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]-propyl]thio]methyl] cyclopropane acetic acid monosodium salt) and nimesulide (N-(4-nitro-2-phenoxyphenyl)methane sulfonamide or 4-nitro-2-phoxymethanesulfonamide) as internal standard on pre-washed silica gel with toluene - ethyl acetate - glacial acetic acid 60:34:1 in a twin-trough chamber. Quantitative determination at 344 nm.  
Quality control, quantitative analysis, densitometry 32a
- 94 094 R. T. SANE, S. PANDIT, S. KHEDKAR\* (\*S. P. Mandali's Ramnarian Ruia College, Matunga, Mumbai 400 019, India): High-performance chromatographic determination of celecoxib in its dosage form. *J. Planar Chromatogr.* 17, 61-64 (2004). HPTLC of celecoxib and loratadine (as internal standard) on silica gel after prewashing and bandwise application with n-hexane - ethyl acetate 3:2 in a twin-trough chamber and equilibration for 10 min. Quantitative determination at 262 nm. Mean recovery 100.03 %.  
Pharmaceutical research, quality control, densitometry, quantitative analysis, HPTLC 32a
- 94 092 R. T. SANE MENON, S. INAMDAR\*, M. MOTE, A. MENEZES (\*TDM laboratories, Plot No. 194, Scheme No. 6, Road No. 15, Sion (E), Koliwada, Mumbai-22, India): Simultaneous determination of pioglitazone and glimepiride by high-performance thin-layer chromatography. *J. Planar Chromatogr.* 17, 154-156 (2004). HPTLC of pioglitazone hydrochloride ((±)-5-{p-[2-(5-ethyl-2-pyridyl)-ethoxy]benzyl}-2,4-thiazolidinedione hydrochloride) and glimepiride (trans-3-ethyl-2,5-dihydroxy-4-methyl-N-[2-[4-[[[(4-methylcyclohexyl)amino]-carbonyl]amino]sulfonyl]phenyl]ethyl]-2-oxo-1H-pyrrole-1-carboxamide) and atorvastatin as internal standard on silica gel after pre-washing in a twin-trough chamber after pre-saturation with toluene - methanol - ethyl acetate - formic acid 70:20:15:0.1. Evaluation by densitometry at 235 nm.  
Quality control, quantitative analysis, densitometry 32a
- 94 050 S. A. SHAH, I. S. RATHOD, B. N. SUHAGIA, Meeta BALDANIYA (Department of Quality Assurance, L. M. College of Pharmacy, Navarangpura, Ahmedabad 380009, India): A simple and sensitive HPTLC method for estimation of gatifloxacin in tablet dosage forms. *Indian J. Pharm. Sci.* 66 (3), 306-308 (2004). HPTLC on silica gel with n-butanol - methanol - 6 M ammonia 5:

1:2. Gatifloxacin showed Rf values of  $0.47 \pm 0.03$ . Quantitative determination by absorbance measurement at 292 nm. The method was validated in terms of linearity (400-1200 ng/spot), precision (intra-day variation 1.3 to 3.2 %, inter-day variation 3.9 to 5.0 %), accuracy (93.3 to 99.4 %), and specificity. The limit of detection and limit of quantification for gatifloxacin were found to be 10 ng/spot and 50 ng/spot respectively. The method is simple, sensitive, precise, and can be used for the routine quality control testing of marketed tablet formulations (400 mg).

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC 32a

- 94 088 K. R. SIREESHA, Deepali V. MHASKE, S. S. KADAM, S. R. DHANESHWAR (Department of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune 411038, India): Development & validation of a HPTLC method for the simultaneous estimation of cefuroxime axetil and probenecid. *Indian J. Pharm. Sci.* 66 (3), 278-282 (2004). HPTLC on silica gel with chloroform - acetonitrile - toluene - acetate buffer (pH 6.0) 50:40:10:3. Quantitative determination by absorbance measurement at 266 nm. The method was validated in terms of linearity, accuracy, precision, and specificity. The limit of detection and the limit of quantification were found to be 50 ng/spot and 100 ng/spot respectively. A simple, precise, accurate and rapid HPTLC method has been developed and validated for the simultaneous determination of cefuroxime axetil and probenecid in combined dosage form.

Pharmaceutical research, quality control, densitometry, qualitative identification, comparison of methods, postchromatographic derivatization, HPTLC 32a

- 94 091 E. SUMARLIK, G. INDRAYANTO\* (\*Assessment Service Unit, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia): TLC densitometric determination of bromhexine hydrochloride in pharmaceuticals, and its validation. *J. Liq. Chrom. Rel. Technol.* 27, 2047-2056 (2004). TLC of bromhexin on silica gel with n-butanol - glacial acetic acid - water 260:77:75 in a twin-trough chamber after at least 3 h of saturation. Quantitative determination at 325 nm by absorbance measurement. The method is selective, precise, and accurate and can be used for routine analysis of pharmaceutical preparations in pharmaceutical industry quality control laboratories.

Quantitative analysis, densitometry 32a

- 94 061 P. G. SUNITHA, V. VAIDHYALINGAM, A. SHANTA, R. P. EZHIL MUTHU (Department of Pharmaceutical Chemistry, Madras Medical College, Chennai 600003, India): Development and validation of HPLC and HPTLC methods for quantification of ebastine in tablets. *Indian Drugs* 41 (3), 149-152 (2004). HPTLC on silica gel with toluene - ethyl acetate 1:24. Quantitative determination by absorbance measurement at 260 nm. The linearity range is 100-500  $\mu\text{g}/\mu\text{L}$  and recovery is 100.7 %. The method was validated and compared with HPLC. The results were comparable. Advantages of HPTLC in terms of handling many samples. Thus, a simple, fast and precise HPTLC method was developed for quantification of ebastine in tablets.

Pharmaceutical research, quality control, qualitative identification, densitometry, comparison of methods, postchromatographic derivatization, HPTLC 32a

- 94 095 T. TUZIMSKI (Department of Inorganic and Analytical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Two-dimensional thin-layer chromatography of eight cephalosporins on silica gel layers. *J. Planar Chromatogr.* 17, 46-50 (2004). Two-dimensional TLC and HPTLC of cephalosporins (cefaclor, cefaperazone sodium, cefazolin sodium, cefotaxime sodium, cefoxitin sodium, cefuroxime sodium, cephalothin sodium, p-chlorophenacyl cephalotin ester) on silica gel in a horizontal chamber with methanol - 98 % formic acid - ethyl acetate 29:1:70 in the first dimension followed by methanol - toluene - ethyl acetate - 98 % formic acid 1:4:13:2 in the se-

cond dimension after drying for 1 h . Detection under UV light at 254 and 366 nm. Quantitative determination at 240 nm.

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

- 94 096 S. G. WADODKAR, S. G. WANKHEDE, K. R. GUPTA\* (\*Department of Pharmaceutical Sciences, Nagpur University, Nagpur, India): A validated HPTLC determination of stavudine in capsules. *Indian Drugs* 41 (5), 300-302 (2004). Validated HPTLC method for the estimation of stavudine in capsules on silica gel with toluene - methanol 3:1. Quantitative determination by absorbance measurement at 270 nm. The linearity range of stavudine was 1.4 µg to 3.7 µg. The amount of stavudine estimated was 40.08 mg and 40.01 mg (calculated by peak height and peak area respectively) in the average weight capsule. Recovery was 99.67 % and 99.26 % calculated by peak height and peak area respectively. The proposed method is accurate, precise, selective, and reproducible and can be used for the routine analysis of stavudine.

Pharmaceutical research, quality control, qualitative identification, densitometry, postchromatographic derivatization, comparison of methods, HPTLC 32a

- 94 060 Nadia F. YOUSSEF (National Organization for Drug Control and Research, 6 Abo Hazem St. Pyramids Ave, PO Box 29, Cairo, Egypt): Spectrophotometric, spectrofluorimetric, and densitometric methods for the determination of indapamide. *J. Assoc. Off. Anal. Chem.* 86 , 935-940 (2003). TLC of indapamide (4-chloro-N-(2-methylindolin-1-yl)-3-sulfamoylbenzamide), its degradation product, and related substance 2-methylnitrosoindoline on silica gel using toluene - ethyl acetate - glacial acetic acid 69:30:1 after pre-saturation for 1 h. Detection under UV light at 254 nm. Quantitative determination at 424 nm. Limit of detection 0.11 µg/spot. The method was validated according to the guidelines of the USP.

Quality control, quantitative analysis, densitometry 32a

- 94 053 X. BIAN (Bian Xiaolan)\*, Q. ZHAI (Zhai Qing), L. Zheng (Zheng Lan) (\*Ruijin Hosp. Affil. to Shanghai No.2 Univ. Med., Shanghai 200025, China): (Manufacture technology and clinical application of Naoxinkang pills.) (Chinese). *Chinese J. Trad. Patent Med. (Zhongchengyao)* 26 (8), Appendix 22-24 (2004). Study of the quality standard of Naoxinkang pills by TLC in order to optimize the manufacture technology and clinical application. TLC of Naoxinkang pill extracts on silica gel with 1) the lower phase of chloroform - methanol - water 13:7:2; 2) chloroform - methanol - water 28:8:1; 3) cyclohexane - ethyl acetate 9:1. Detection 1) by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol followed by heating at 105 °C; 2) under UV 365 nm. Identification by fingerprint technique. Discussion of employing the procedures in the optimization of the manufacture technology and its clinical application for the medicine.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, ferulic acid 32c

- 94 057 X. CHEN (Chen Xiaolin) (Xiamen TCM Co., Ltd., Xiamen, Fujian 361009, China): (Study of the quality control of Xinyihao capsules.) (Chinese). *Chinese J. Trad. Patent Med. (Zhongchengyao)* 26 (6), Appendix 7-8 (2004). TLC of Xinyihao capsule extracts on silica gel with 1) chloroform - methanol - water 13:7:2; cyclohexane - acetone - chloroform 5:3:3. Detection 1) by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> solution in ethanol and heating at 110 °C ; 2) under UV 365 nm. Identification by fingerprint techniques. Quantification of borneol by GC with method validation.

Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, borneol 32c

- 94 058 X. DING (Ding Xiaoyan), S. YANG (Yang Songqing), W. Jiang (Jiang Weike), T. ZHOU (Zhou Tao) (Guiyang Dechangxiang Pharm. Co. Ltd., Guiyang, Guizhou 550001, China): (Study of the quality standard for Xiaozhi tablets.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (5), 371-373 (2004). TLC of Xiaozhi tablet extracts on silica gel with 1) ethyl acetate - formic acid - water 5:2:1; 2) benzene - acetone - ethyl acetate - ammonia 4:6:8:1; 3) chloroform - methanol - ammonia 40:10:1. Detection 1) under UV light; 2) by spraying with 5 % solution of potassium iodobismuthate. Identification by fingerprint techniques. Quantification of matrine by HPLC with method validation.  
Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, matrine 32c
- 94 062 G. GAO (Gan Guoping)\*, ZH. WANG (Wang Zhonghong), J. ZAN (Zan Junfeng) (\*Hubei Coll. TCM, Wuhan, Hubei 430061, China): (Study of the quality standard for Qirong medicinal wine.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (2), 113-115 (2004). TLC of Qirong medicinal wine on silica gel with 1) n-hexane - ethyl acetate 4:1; 2) petroleum ether (30-60 °C) - ethyl acetate - formic acid 15:5:1; 3) chloroform - methanol 9:1; 4) ethyl acetate - acetone - formic acid - water 5:5:1:1. Detection 1) under UV 365 nm, and by spraying with 5 % KOH in methanol and under UV 365 nm. Identification by fingerprint techniques. Quantification of psoralen and isopsoralen by HPLC.  
Pharmaceutical research, quality control, qualitative identification, psoralen, isopsoralen 32c
- 94 063 L. GAO (Gao Li)\*, Y. ZHANG (Zhang Yongping), L. ZHOU (Zhou Lan) (\*Zunyi Wancai Pharm. Co., Ltd., Zunyi, Guizhou 563000, China): (Study of the quality standard for Jixiangcao buccal tablets.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (2), 110-113 (2004). TLC of Jixiangcao buccal tablet extracts on silica gel with 1) petroleum ether (30-60 °C) - ethyl acetate 8:1 and 2) toluene - acetone - ethanol - ammonia 20:20:3:1. Detection 1) under UV 365 nm; 2) by spraying with 5 % potassium iodobismuthate solution and sodium nitrite solution. Identification by fingerprint technique. Quantification of ephedrine hydrochloride by HPLC.  
Pharmaceutical research, quality control, qualitative identification, ephedrine hydrochloride 32c
- 93 068 G. HAN (Han Guiru) (Hebei Provin. Inst. Drug Cont. Shijiazhuang, Hebei 050011, China): (Determination of berberine in Dabaidu capsules by thin-layer chromatography.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (4), Appendix 5-6 (2004). TLC of Dabaidu capsules on silica gel with benzene - ethyl acetate - methanol - isopropanol - ammonia 12:6:3:3:1. Detection under UV light. Identification by comparison with the standard. Quantification by fluorescence measurement at 366 nm. Validation of the method by investigation of precision (RSD<2 %), linearity range, repeatability, reproducibility, recovery, and limit of detection (1.2 ng).  
Pharmaceutical research, traditional medicine, quality control, densitometry, quantitative analysis, qualitative identification, berberine 32c
- 94 069 N. HUANG (Huang Nojia) (Shantou Municip. Inst. Drug Cont., Shantou, Guangdong 515041, China): (Studies of the quality standard for Qufeng medicinal wine.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (3), 192-195 (2004). TLC of Qufeng medicinal wine on silica gel with 1) n-hexane - ethyl acetate 9:1; 2) ethyl acetate - methanol - water 100:17:13; 3) toluene - ethyl acetate - formic acid - water 20:10:1:1; 4) chloroform - methanol - water 40:10:1; 5) n-hexane - ethyl acetate - chloroform - glacial acetic acid 50:15:5:1. Detection 1) under UV 365 nm; 2) by spraying with 1 % AlCl<sub>3</sub> in methanol and under UV 365 nm; 3) by spraying with 5 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heating at 105 °C; 4) by exposing to ammonia vapor. Identification by fingerprint technique. Quantification of emodin by densitometry at 460 nm. Validation of the me-

thod by investigation of its linearity range (0.133-1.330 µg/spot,  $r=0.9999$ ), precision (RSD=1.44 %,  $n=6$ ), recovery (99.24 %, RSD=0.74 %,  $n=12$ ).

Pharmaceutical research, herbal, traditional medicine, qualitative identification, emodin  
32c

- 94 071 B. LI (Li Bin), ZH. LI (Li Zhengxiang), R. REN (Ren Rong), X. WANG (Wang Xin) (Pharm. Dep., General Hosp., Tianjin Univ., Med., Tianjin 300052, China): (Study of the quality standard for Bunao pills.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (5) 364-367, (2004). TLC of Bunao pill extracts on silica gel with 1) dichloromethane - methanol 10:1; 2) acetone - benzene - hydrochloric acid (3 mol/L) 80:30:1. Detection 1) by spraying with 5 % phosphomolybdic acid in ethanol and heating at 120 °C ; 2) by spraying with hypo acetamide solution and heating at 80-100 °C. Identification by fingerprint techniques. Quantification of tanshinone II by HPLC with method validation.

Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, tanshinonell  
32c

- 94 072 F. LI (Li Fengqin) (Puyang Inst. Drug Cont., Puyang, Henan 457000, China): (Identification of Tongluo Xiaoshuan capsules by thin-layer chromatography.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (7), Appendix 12-14 (2004). TLC of Tongluo Xiaoshuan capsule extracts on silica gel with 1) chloroform - methanol - water 60:72:1; 2) n-hexane - ethyl acetate 3:1. Detection 1) by spraying with phosphomolybdic acid solution in ethanol and heating at 105 °C for several min; 2) under UV 365 nm. Identification by fingerprint technique.

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

- 94 073 P. LI (Li Ping)\*, B. JIA (Jia Baoquan), Y. LI (Li Yumin), J. ZHANG (Zhang Jianlin) (\*Affiliated Hosp., Lanzhou Med. Coll., Lanzhou, Gansu 730000, China): (Study of the quality standard for Lidan capsules.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (4), Appendix 2-5 (2004). TLC of Lidan capsule extracts on silica gel with 1) ethyl acetate - butanone - formic acid - water 5:3:1:1; 2) chloroform - methanol - water 30:10:1; 3) petroleum ether (60-90 °C) - ethyl acetate - benzene 14:3:3; 4) n-hexane - chloroform - methanol 15:8:2. Detection 1) by spraying with 1 % FeCl<sub>3</sub> in ethanol; 2) by spraying with a solution of P-dimethylaminobenzaldehyde - ethanol - H<sub>2</sub>SO<sub>4</sub> 41:100:10; 3) by spraying with 5 % vanillin - H<sub>2</sub>SO<sub>4</sub> solution followed by heating; 4) by exposing to iodine vapor. Identification by fingerprint technique. Quantification of emodin and chrysophanol by HPLC.

Pharmaceutical research, quality control, traditional medicine, quantitative analysis, qualitative identification, emodin, chrysophanol  
32c

- 94 074 Y. LIANG (Liang Yi), X. CHEN (Chen Xiang), W. LI (Li Wnashou), B. LI (Li Bin) (Sichuan Provin. Inst. TCM, Chengdu, Sichuan 610041, China): (Quantitative determination of astragaloside in Huanwu capsules.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (5), 430-432 (2004). TLC of Huanwu capsules on silica gel with chloroform - methanol - water 13:6:2 after being placed over night at a temperature belowe 10 °C. Detection by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heating at 105 °C for 5 min. Identification by comparison with the standard. Quantification by densitometry at 530 nm. Validation of the method by investigation of the linear range of the calibration curve (0.98-6.86 µg/spot,  $R=0.998$ ), precision (RSD=1.0 %,  $n=6$ ), reproducibility (RSD=0.8 %,  $n=5$ ), and standard addition recovery (98.9 %,  $n=6$ ). Application of the method on real-life samples.

Pharmaceutical research, traditional medicine, herbal, densitometry, quantitative analysis, astragaloside  
32c

- 94 075 Y. LIU (Liu Yuanrui), X. SUN (Sun Xiaoyan), X. GUANG (Guang Xinlan) (Beijing Constabulary Inst. Drug Cont., Beijing 102613, China) : (Quantitative determination of ephedrine chloride in Tongxuan Lifei pills by thin-layer chromatography.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (4), 343-345 (2004). TLC of ephedrine chloride on silica gel with chloroform - methanol - ammonia 200:35:6. Detection by spraying with 0.5 % ninhydrin in ethanol followed by heating at 105 °C for a few minutes. Quantitative determination at 510 nm. Validation of the method by investigation of linear range of the calibration curve (0.42 - 2.10 µg/spot, R = 0.999), precision (RSD = 2.1 %, n=6 within plate and 2.7 %, n = 5 plate-to-plate), reproducibility (RSD = 2.3 %, n = 5), and recovery (98.1 %, n = 6). The results obtained by using the method are given towards some real-life samples.

Pharmaceutical research, traditional medicine, herbal, densitometry, quantitative analysis, ephedrine chloride 32c

- 94 081 J. MENG (Meng Jun)\*, Y. GONG (Gong Yun), Y. DING (Ding Ye) (\*Beijing Jiaotong Univ., Beijing 100009, China): (Study of the quality standard for Fuketiaoqing tablets.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (7), 541-544 (2004). TLC of Fuketiaoqing tablet extracts on silica gel with 1) benzene - ethyl acetate - glacial acetic acid 2:1:1; 2) benzene - ethyl acetate - glacial acetic acid 92:5:5; 3) cyclo hexane - ethyl acetate 7:3; 4) chloroform - methanol - water 30:10:1. Detection 1) under UV 254 nm; 2) by spraying with p-dimethylaminobenzaldehyde-H<sub>2</sub>SO<sub>4</sub> solution and heating at 130 °C and under UV 365 nm; 3) by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> solution in ethanol and heating at 105 °C; 4) by spraying with 5 % vanillin-H<sub>2</sub>SO<sub>4</sub> solution and heating at 105 °C. Identification by fingerprint technique. Quantification of ferulic acid by HPLC.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, ferulic acid 32c

- 94 084 R. PENG (Peng Rongzhen), Y. WU (Wu Yi) (Med. Coll., No.6 Hosp. Affil. Jinan Univ., Jianmen, Guangdong 529000, China): (Study of the quality Standard for Quzhi capsules.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (7), 544-547 (2004). TLC of Quzhi capsule extracts on silica gel with 1) toluene - chloroform - acetone 8:15:5; 2) petroleum ether (60-90 °C) - ethyl acetate 20:1. Detection 1) by exposing to ammonia vapors and under UV 365 nm; 2) by spraying with p-dimethylaminobenzaldehyde in 10 % H<sub>2</sub>SO<sub>4</sub> - ethanol solution followed by heating. Identification by fingerprint technique. Quantification of chrysophanol by HPLC. Discussion of applying the procedures for the quality control of the medicine.

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

- 94 087 H. QIN (Qin Huazhen)\*, J. DENG (Deng Jiagang), Y. CHEN (Chen Yong), ZH XIE (Xie Zhen), Y. LI (Li Yansheng) (\* Pharm. Coll., Guangxi Univ. TCM, Nanning, Guangxi 530001, China): (Study of the quality standard for Huanglong Ganzhixiao granules.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (7), 535 - 538 (2004). TLC of Huanglong Ganzhixiao granule extracts on silica gel with 1) chloroform - butanone - methanol - formic acid - water 22:20:11:2:2; 2) chloroform - methanol - water 13:7:2; 3) chloroform - methanol 99:1; 4) petroleum ether (60 - 90 °C) - ethyl acetate 5:1. Detection 1) by spraying with 10 % AlCl<sub>3</sub> in ethanol and under UV 365 nm; 2) by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol followed by heating at 105 °C, and under UV 365 nm; 3) by spraying with 10 % phosphomolybdic acid in ethanol followed by heating at 105 °C. Identification by fingerprint technique. Quantification of loureirin B by HPLC. Discussion of procedure optimization for individual component drug and of using the procedures in the quality control of the medicine.

Traditional medicine, quality control, pharmaceutical research, herbal, quantitative analysis, qualitative identification, loureirin B 32c

- H. SONG (Song Hongxiang)\*, M. WANG (WANG MEI), W HOU (HOU WENFENG) (\*Henan Coll. TCM, Zhengzhou, Henan 450004, China): (Determination of peimine in Fukang Xiaozhong pills by thin-layer chromatography.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (2), 164-165 (2004). TLC of Fukang Xiaozhong pills on silica gel with the lower phase of chloroform - ethyl acetate - methanol - water 6:8:3:2. Detection by spraying with 5 % potassium iodobismuthate solution. Identification by comparison with the standard. Quantification by densitometry at 502 nm. Validation of the method by investigation of its linearity range (0.41-2.05 µg/spot,  $r = 0.9999$ ); precision (RSD=1.66 %  $n=5$  within plate and 1.88 %  $n=5$  plate-to-plate); reproducibility and standard addition recovery (98.9 % RSD=1.9 %  $n=5$ ).
- Pharmaceutical research, quantitative analysis, densitometry, peimine 32c
- 94 097 L. WANG (Wang Ling)\*, SH. JI (Ji Shen), Y. CHEN (Chen Yihong) (\*Shanghai Inst. Of Drug Cont., Shanghai 200233, China): (Study of the quality standard for Qingdai powder.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (5), 374-376 (2004). TLC of Qingdai powder extract on silica gel with 1) benzene - chloroform - acetone 5:4:1; 2) petroleum ether (60-90 °C) - benzene - ethyl acetate 9:2:1; 3) benzene - ethyl acetate - isopropanol - methanol - water 60:30:10:50:15:3; 4) petroleum ether (30-60 °C) - benzene - ethyl acetate - glacial acetic acid 20:40:14:1. Detection 1) under day light; 2) by spraying with 5 % vanillin-H 2SO 4 solution and heating at 105 °C ; 3) under UV 365 nm; 4) under UV 254 nm. Identification by fingerprint techniques. Quantification of indigo by HPLC with method validation.
- Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, indigo 32c
- 94 098 P. WANG (Wang Yiping), H. FENG (Feng Haixia) (Chongqing Wanzhou Inst. Drog Cont., Chongqing 404000, China): (Identification of Buzhongyiqi pills by using an improved thin-layer chromatographic procedure.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (4), Appendix 8-9 (2004). TLC of Buzhongyiqi pill extracts on silica gel with ethyl acetate - formic acid - glacial acetic acid 15:1:1. Detection by spraying with 10 % H 2SO 4 in ethanol, followed by heating at 105 °C. Evaluation under UV 365 nm. Identification by fingerprint techniques.
- Pharmaceutical research, quality control, traditional medicine, qualitative identification 32c
- 94 099 T. WU (Wu Tao), Y. ZHANG (Zhang Yun), J. XING (Xing Jianguo), A. A. HAJI (Haji Aikerbair. Aisa), L. LIAO (Liao Lixin) (Xinjiang Inst. Pharm., Urumqi, Xinjiang 830011, China): (Study of the quality standard for Xiatare Baixuan tablets.) (Chinese.) Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (6), 463-465 (2004). TLC of Xiatare Baixuan tablet extracts on silica gel with 1) toluene - chloroform - acetone - formic acid 8:5:7:1; chloroform - methanol - formic acid 38:2:1. Detection 1) by spraying with 1 % AlCl 3 in ethanol and under UV 365 nm; 2) under UV light. Identification by fingerprint techniques. Quantification of aloin by HPLC with method validation.
- Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, aloin 32c
- 94 101 P. YU (Yu Pengzhen)\*, H. ZHANG (Zhang Hong), X. NIU (Niu Xizhen), Z. XU (Xu Zuhong), ZH. LI (Li Zhien) (\*Inst. Oceanol. Chinese Acad. Sci., Qingdao Shandong 266071, China): (Optimum extraction of polysaccharide from Ulva pertusa established by orthogonal test.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (1), 16-19 (2004). TLC of neutral sugars, rhamnose, xylose, and glucose, extracted from Ulva pertusa alga, on silica gel with n-butanol - ethyl acetate - isopropanol - acetic acid - water - pyridine 7:20:12:7:6:6. Detection by spraying with a solution of aniline hydrogen phthalate (0.93 g) - phthalic acid (1.66 g) - water saturated n-butanol (100 mL), followed by heating at 110 °C for 10 min. Identification and quantification by

comparison with the standards. Application of an orthogonal test to investigate the effects of four factors (volume of water, temperature, extraction time, and pH) on the yield of the compounds, and determination of the optimum extraction conditions.

Pharmaceutical research, densitometry, quantitative analysis 32c

- 94 102 W. ZENG (Zeng Weijie), N. CAI (Cai Naman), Q. WU (Wu Qunsong), J. ZHU (Zhu Jishi) (Guangdong Chaozhou Inst. Drug Cont., Chaozhou, Guangdong 521011, China): (Study of the quality Standard for Shangbiao oil.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (6), 452-455 (2004). TLC of Shangbiao oil extracts on silica gel with 1) n-heptane-ethyl acetate 17:3; 2) n-heptane - ethyl acetate 9:1; 3). Detection 1) by spraying with 2,4-dinitro-phenylhydrazine solution in ethanol; 2) by spraying with 5 % vanillin-H<sub>2</sub>SO<sub>4</sub> solution and heating at 110 °C for 5 min. Identification by fingerprint techniques. Quantification of menthol and camphor by GC.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, cinnamyl aldehyde, eugenol, camphor, menthol 32c

- 94 103 H. ZHANG (Zhang Huizhong)\*, M. PAN (Pan Mingyun), J. ZHANG (Zhang Jianli), SH. WANG (Wang Shushi), CH. LI (Li Chengshen) (\* Liaoning Prov. Inst. TCM, Shenyang, Liaoning 110034, China): (Study of the quality standard for Qieban Zhiyang ointment.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (7), 597-600 (2004). TLC of Qieban Zhiyang ointment extracts on silica gel with 1) benzene - ethyl acetate 10:1; 2) petroleum ether (60-90 °C) - ethyl formate - formic acid 15:5:1; 3) chloroform - methanol - ammonia 40:3:1; 4) benzene - chloroform - acetone 5:4:1; 5) chloroform - methanol 19:1. Detection 1) under UV 365 nm; 2) by exposing to ammonia vapor; 3) by spraying with potassium iodobismuthate solution; 4) under daylight. Identification by fingerprint technique. Quantification of icariine by HPLC.

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

- 94 104 SH. ZHANG (Zhang Shudan), Q. XU (Xu Qiang) (Zhejiang Provin. Inst. TCM, Hanzhou, Zhejiang 210023, China) : (Determination of stachydrine in Xuanmuyi capsules by thin-layer chromatography.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (4), Appendix 7-8 (2004). TLC of Xuanmuyi capsule extracts on silica gel with n-butanol - hydrochloric acid - water 8:2:10:1. Detection by spraying with 5 % potassium iodobismuthate. Identification by fingerprint technique. Quantification by densitometry at 510 nm. Validation of the method by investigation of its linearity range (10-50 µg/spot, r=0.9999), precision (RSD=2.1 %, n=6), standard addition recovery (100.7 %, RSD=3.8 %, n= 5).

Pharmaceutical research, traditional medicine, quality control, quantitative analysis, densitometry, stachydrine 32c

- 94 105 Y. ZHANG (Zhang Yuejun)\*, Y. TAN (Tan Yongxia), Q. LI (Li Qilan), ZH. GAO (Gao Zhongrong) (\*Hubei Provin.TCM Hosp., Wuhan, Hubei 430061, China): (Determination of matrine in mildew-inhibiting detergents by thin-layer chromatography.) (Chinese). Chinese J. Hosp. Pharm. (Zhongguo Yiyuan Yaoxue Zazhi) 24 (5), 315-316 (2004). TLC of matrine on silica gel with benzene - acetone - ethyl acetate - ammonia 10:15:20:1. Detection by spraying with 5 % potassium iodobismuthate and 0.05 mol/L sodium nitrite in ethanol. Identification by comparison with the standard. Quantification by densitometry at 500 nm. Validation of the method by investigation of the linearity range of the calibration curve (2.14-10.70 µg/spot, r=0.999), precision (RSD=1.71 %, n=5), reproducibility (RSD=2.22 %, n=5), recovery (99.58 %, RSD 1.01 %, n=5), and interferences.

Environmental, quantitative analysis, densitometry, matrine, mildew-inhibiting detergents 32c

- 94 106 CH. ZHAO (Zhao Chunxiang)\*, L. SHA (Sha Lutan), Y. ZHANG (Zhangyanling), ZH. JIANG (Jiang Zhuosong) (\*Jilin Inst. Drug Cont., Cahngchun, Jilin 130062, China): (Study of the methods for the quality control of Changchun Hongyao capsules.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (4), 341-343 (2004). TLC of Changchun Hongyao capsule extracts on silica gel with n-hexane - ethyl acetate 1:1. Detection by spraying with 5 % solution of potassium iodobismuthate. Identification by fingerprint techniques. Quantification of aconitine by HPLC.  
Pharmaceutical research, quality control, traditional medicine, quantitative analysis, qualitative identification, aconitine 32c
- 94 107 J. ZHOU (Zhou Junhui), X. LIU (Liu Xiaomin), W. SUN (Sun Wenji) (Coll. Life Sci., North West China Univ., Xian, Shanxi 710069, China): (Determination of astragaloside in compound Xiaqing injections by thin-layer chromatography.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (3), 248-249 (2004). TLC of astragaloside on silica gel with the lower phase of chloroform - methanol - water 13:6:2. Detection by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heating at 105 °C. Identification by comparison with the standard. Quantification by densitometry at 530 nm. Validation of the method by investigation of precision (RSD=2.2 % n=5 within plate and 3.0 % n=5 plate-to-plate), linearity range (0.45-2.28 µg/spot r=0.998), repeatability, reproducibility, and recovery (98.1 %, n=5, RSD=3.4 %).  
Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, densitometry, astragaloside 32c
- 94 108 T. ZHU (Zhu Tianming), Q. CHEN (Chen Qijin) (Yangzhou Baoying Inst. Drug Cont., Baoying, Jiansu 225800, China): (Identification of Shenbao capsules.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (8), Appendix 16-18 (2004). Study of the identification of Shenbao capsules by TLC, in addition to microscopy and chemical methods. TLC of Shenbao capsule extracts on silica gel with 1) the lower phase of chloroform - ethyl acetate - methanol - water 15:40:22:10; 2) toluene - methanol 4:1. Detection 1) by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol followed by heating at 105 °C; 2) under UV light. Identification by fingerprint technique. Combination of the procedure with microscopy and chemical methods allows identification of all drug ingredients.  
Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC 32c
- 94 109 X. ZHU (Zhu Xuan), H. TANG (Tnag Haiying), M. CHEN (Chen Man) (Jiangsu Xiansheng Pharm. Co., Ltd., Nanjing, Jiangsu 210042, China): (Study of the quality standard for sugar-free Qixuekang oral liquid.) (Chinese). Chinese J. Trad. Patent Med. (Zhongchengyao) 26 (6), 449-452 (2004). TLC of Qixuekang oral liquid extract on silica gel with 1) chloroform - ethyl acetate - methanol - water 15:40:22:10; 2) chloroform - methanol - water 28:10:1. Detection 1) by spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heating; 2) under UV 365 nm. Identification by fingerprint techniques. Quantification of notoginsenoside R1 by HPLC with method validation.  
Pharmaceutical research, traditional medicine, quality control, quantitative analysis, qualitative identification, notoginsenoside R1, puerain, ginsenoside Rg1 32c
- 94 054 A. BLATTER, E. REICH\* (\*CAMAG Laboratory, Sonnenmattstr. 11, CH-4132 Muttenz, Switzerland): Qualitative and quantitative HPTLC methods for quality control of *Stephania tetrandra*. J. Liq. Chrom. Rel. Technol. 27 , 2087-2100 (2004). HPTLC of tetrandrine on silica gel with toluene - ethyl acetate - methanol - 28 % ammonia 100:100:50:3. Detection under UV light at 366 nm, and under white light after reaction with iodine (yellowish zones). If the plate is subsequently derivatized with anisaldehyde solution, the alkaloids show a strong blue fluorescence under UV light at 366 nm. Quantitative determination at 210 nm. The calibration curve is linear for 50 - 112.5 ng tetrandrine per zone. Also HPTLC of aristolochic acids (AAs) on silica gel with

the upper phase of toluene - ethyl acetate - water - formic acid 20:10:1:1 and derivatization with tin(II) chloride (to be prepared freshly: dissolve 1 g tin(II) chloride\*2H<sub>2</sub>O in 1.5 mL 36 % hydrochloric acid diluted with 8 mL water), followed by heating at 100 °C for 1 min and evaluation under UV light at 366 nm. The method allows detection of 1 ppm of AA.

Herbal, traditional medicine, HPTLC, densitometry, quantitative analysis, qualitative identification 32e

- 94 064 S. GOCAN\*, G. CIMPAN (\*Department of Analytical Chemistry, "Babes-Bolyai" University Cluj-Napoca, Romania): Review of the analysis of medicinal plants by TLC: Modern approaches. *J. Liq. Chrom. Rel. Technol.* 27 , 1377-1411 (2004). The review includes 117 recent references regarding modern and efficient TLC methods like: gradient elution (GE) TLC, automated multiple development (AMD), and over-pressured layer chromatography (OPLC). Detailed discussions are included about the mobile phase optimization by the "Prisma" method and other mathematical models. The analysis and quantification by photodensitometry of flavonoids, coumarins, saponins, alkaloids, and other classes of compounds include detailed experimental conditions, stationary and mobile phase, development methods and quantitative determination by densitometric procedures.

Herbal, review 32e, 1

- 94 002 Kathrin KOLL et al., see section 2f

- 94 076 J. LUO (Luo Jie) (Guangzhou Baiyunshan TCM Factory, Guangzhou 510510, China): (Separation of *Aristolochia fangchi* Y. C. Wuex L. D. Chou et S. M. Hwang and *Rubia cordifolia* L by thin-layer chromatography.) (Chinese). *Chinese J. Trad. Patent Med. (Zhongchengyao)* 26 (3), 238-239 (2004). TLC of the extracts of *Aristolochia fangchi* and *Rubia cordifolia* on silica gel with 1) toluene - ethyl acetate - methanol - formic acid 20:10:1:1; 2) benzene - acetone - formic acid 32:8:1; 3) ethyl acetate - methanol - water - ammonia 30:5:1:2. Detection 1) under UV 254 nm and 365 nm; 2) by spraying with 5 % AlCl<sub>3</sub> in ethanol. Identification by fingerprint technique.

Pharmaceutical research, herbal, traditional medicine, quality control, qualitative identification, medicinal herbs 32e

- 94 083 Maria-Ioana MOISE\*. C. MARUTOIU, D. N. BADEA, C.-A. GAVRILA, C. PATROESCU (\*Lucian Blaga University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, 7 - 9 Dr Ioan Ratiu St, 2400 Sibiu, Romania): Application of TLC and GC-MS to the detection of capsaicin from hot peppers (*Capsicum annuum*). *J. Planar Chromatogr.* 17 , 147-148 (2004). TLC of capsaicin on silica gel with concentration zone with toluene - acetone - chloroform 8:7:5 and 9:6:5. Detection by spraying with a 0.25 % ethanolic solution of 2,6-dichloroquinone-4-chlorimide or by exposure to iodine vapor.

Food analysis, quality control, qualitative identification 32e

- 94 049 S. A. NABI\*, E. LAIQ, A. ISLAM (\*Anal. Res. Lab., Dep. Chem., Aligarh Muslim Univ., Aligarh-202002, India): Selective separation and determination of cephalosporins by TLC on stannic oxide layers. *Acta Chromatographica* 14 , 92-101 (2004). Study of the chromatographic behavior of some cephalosporins on synthetic inorganic ion-exchange (stannic oxide) layers with citrate and borate buffers as mobile phases. Separation of cephalosporins in synthetic mixtures and quantification by spectrophotometry. Quantitative determination of cephalosporins in blood serum samples from patients. Statistical discussion of recovery, accuracy, and reproducibility of the method.

Clinical chemistry research, quantitative analysis, cephalosporins 32f

93 035 A. TIMMER et al., see section 17c

### **35. Other technical products and complex mixtures**

94 048 Irena BARANOWSKA et al., see section 30

94 110 A. MATUSZEWSKA\*, A. JOHN (\*Dep. Geochem., Mineralogy, and Petrography, Fac. Earth Sci., Silesian Univ., 60 Bedzinska Str., 41-200 Sosnowiec, Poland): Some possibility of Thin-layer chromatographic analysis of Baltic amber and other natural resins. *Acta Chromatographica* 14, 82-91 (2004). Comparative investigation of the group composition of ethanol extracts obtained from natural resins of different origin and geological age by TLC on silica gel with dichloromethane - methanol 10:1 for carboxylic acids and  $\alpha$ -hydroxy and  $\alpha$ -keto acids, and with hexane - benzene - methanol 2:6:1 for other compounds. Detection with phosphoric and sulfuric acid and under UV 254 nm for additional identification of unsaturated compounds. Discussion of the potential of using the characteristics of the chromatograms as an auxiliary tool for classification of natural resins and, especially important, fossil resins.

Baltic amber, natural resins

35d



# INTERNATIONAL SYMPOSIUM ON PLANAR CHROMATOGRAPHY – INSTRUMENTAL THIN-LAYER CHROMATOGRAPHY

Berlin, 9–11 October 2006

## First Circular and Call for Papers

Planar-Chromatographie bzw. Instrumentelle Dünnschicht-Chromatographie ist nach wie vor eine unverzichtbare Methode der Routine-Analytik. Dieses Symposium soll deutlich machen, auf welchen Gebieten heute die Schwerpunkte ihrer Anwendung liegen, welche Entwicklungen die Methode in den letzten Jahren genommen hat, und worin ihre wichtigsten Vorteile liegen – nämlich Flexibilität, Schnelligkeit und Kosteneffizienz.

Das Symposium soll ein Forum sein zum wissenschaftlichen Erfahrungsaustausch und zur Vorstellung neuer, innovativer Wege. Auch Anwender anderer chromatographischer Methoden haben Gelegenheit, sich über den Stand der Technik der modernen DC/HPTLC zu informieren.

**Das wissenschaftliche Programm** wird aus Vortrags-, Diskussions- und Posterbeiträgen bestehen. Als Symposiumssprachen sind Englisch (bevorzugt) und Deutsch zugelassen.

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Als Themenbereiche werden angeboten:

### Pharma

- Qualitätskontrolle
- Content Uniformity Test (CUT)
- Identitäts- und Reinheitsprüfungen
- Stabilitäts- und Haltbarkeitsprüfungen
- Pflanzeninhaltsstoffe / Phytopharmaka

### Lebensmittel/Futtermittel/Kosmetika/Tenside

- Qualitätskontrolle
- Haltbarkeitsprüfungen
- Zusatzstoffe
- Pestizide

### Umwelt

- Rückstandsanalytik
- Metabolismusstudien

### Klinische/forensische Anwendungen

- Metabolismusstudien
- Verträglichkeitsstudien (z. B. Hautlipide)
- Drogenscreening
- Nachweis von Dokumentenfälschungen (z. B. Farbstoffanalysen)

### Industrielle Anwendungen

- Prozessüberwachung und Optimierung
- Reinigungsvalidierung
- Kombinatorische Chemie
- Additive in Mineralölen und Schmierstoffen

### Kombinierte Techniken

- Kopplung HPTLC-MS
- Wirkungsbezogene Analytik (z. B. Biolumineszenz)

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### Call for Papers

Interessierte Wissenschaftler sind eingeladen, Beiträge aus ihren Anwendungsgebieten in Form von Vorträgen und/oder Posterpräsentationen anzumelden:

Titel, Autorennamen (Postadresse mit Telefon und e-mail), Kurzfassung (englisch und/oder deutsch, maximal 250 Wörter/2 Abbildungen, in MS-Word Format) bis 15. Oktober 2005 an [committee@hptlc.com](mailto:committee@hptlc.com) mit Angabe, ob Vortrag oder Poster gewünscht wird.

Alle Anmeldungen werden vom wissenschaftlichen Komitee unter der Leitung von Prof. Dr. Lothar W. Kroh, Institut für Lebensmittelchemie, TU Berlin geprüft und die Entscheidung bis 15. November 2005 dem Autor mitgeteilt.

### Tagungsort

Best Western Hotel – Steglitz International  
Albrechtstrasse 2, D-12165 Berlin

Zentrale Lage, mit öffentlichen Verkehrsmitteln gut erreichbar, problemlose Parkmöglichkeiten

### Korrespondenz an:

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# INTERNATIONAL SYMPOSIUM ON PLANAR CHROMATOGRAPHY – INSTRUMENTAL THIN-LAYER CHROMATOGRAPHY

Berlin, 9–11 October 2006

## First Circular and Call for Papers

Planar Chromatography or Instrumental Thin-Layer Chromatography has its rightful place in contemporary analytical laboratories. The objective of this symposium is to provide a forum for an open discussion as to which fields of application are most common these days, what recent developments the technique has undergone, and what advantages it has to offer, i.e. unsurpassed flexibility, speed and cost-effectiveness.

This symposium is an opportunity to exchange knowledge, experience, and new ideas related to this powerful tool. It is likewise an opportunity for adepts of other chromatographic methods to acquaint themselves with the state-of-the-art of modern TLC/HPTLC.

**The Scientific Program** will consist of oral and poster presentations and discussions.

Symposium languages are English (preferred) and German.

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Suggested subjects are:

### Pharmaceutical applications

- Quality assurance
- Content uniformity tests
- Identity and purity control
- Stability tests
- Plant ingredients/herbals

### Food, animal feed, cosmetics, tensides

- Quality control
- Stability tests
- Additives
- Pesticides

### Clinical/forensic applications

- Metabolism studies
- Lipids/body tissue
- Drug screening
- Document falsifications (e.g. ink analysis)

### Environmental

- Residue analysis
- Metabolism studies

### Industrial applications

- Process control
- Cleaning validation
- Combinatorial chemistry
- Additives in mineral oils and lubricants

### Hyphenated techniques

- HPTLC/MS coupling
- Activity related detection (e.g. bioluminescence)

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### Call for Papers

Scientists are invited to contribute a paper from their field of application in the form of oral and/or poster presentation. Authors are asked to submit title, author(s) (with postal and e-mail address, telephone), abstract (maximum 250 words/2 illustrations – MS-Word format in English or German) by 15. October 2005 to [committee@hptlc.com](mailto:committee@hptlc.com). Authors may indicate their preference for an oral or poster presentation.

The final decision on acceptance and form of presentation will be made by the scientific committee chaired by Prof. Dr. Lothar Kroh, Institute for Food Chemistry at the Technical University Berlin. Authors will be informed by 15. November 2005.

### Symposium Location

Best Western Hotel – Steglitz International  
Albrechtstrasse 2, D-12165 Berlin

The hotel is centrally located with parking facilities and is readily accessible by public transport.

Please direct all correspondence and/or inquiries to the Chairman of the Scientific Committee:

Professor Dr. Lothar W. Kroh  
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Institute for Food Chemistry  
Gustav-Meyer-Allee 25, D-13355 Berlin

Telephone: +49 30/314-725 84

E-mail: [lothar.kroh@tu-berlin.de](mailto:lothar.kroh@tu-berlin.de)

# DigiStore 2, a new Documentation System with high resolution 12bit CCD Camera and winCATS

The new CCD camera combines the spatial resolution of about  $0.1 \text{ mm}^2$  with exceptional color fidelity, a linear 12bit digitalization (4095 levels per color channel) and a low noise electronics circuitry. The high dynamic range available with this camera makes it possible to detect e.g. impurities at ppm range – an important feature for TLC documentation since it makes an important difference if digitalization allows linear steps of 0.025 % (this camera) or non-linear steps of approx. 0.1 % resolution only (8bit consumer camera). Another important feature is the exceptional reproducibility of the image, which can only be achieved with a highly linear CCD permitting extremely precise control of exposure times.

The following important features also need mentioning:

- The highly linear 12bit CCD camera is connected via a FireWire (IEEE1394a) cable to the PC and achieves image transfer rates of less than a second.
- Automatic optimization of exposure time in steps of  $230 \mu\text{s}$  up to 20 s
- The camera is controlled by winCATS.
- Even the basic version of winCATS supports useful routines for documentation of thin-layer chromatograms, e.g. functions for optimization of exposure time and correction of illumination.
- To ensure the desired high reproducibility of images, the system operates with fixed capture parameters such as focal length, focus, and aperture. As a result, each image is sharp and highly reproducible from plate to plate.



### Automatic optimization of exposure time

The highly linear response of this CCD camera and the excellent reproducibility of the acquired images, combined with fast data transfer via FireWire, made it possible to implement software routines for automatic image optimization. This means for the operator that just by pressing the START button, images can be acquired with WinCATS, regardless of the type of light illumination. The capturing process begins and the image is optimized to best utilize the high (12bit) dynamic range of the camera. Exposure time, lens setting, electronic amplification, etc. need not to be manually optimized by trial-and-error.

The sequence of the process:

Step 1: winCATS acquires several images in order to establish the best exposure time.

Step 2: An optimized image is captured and displayed.

Step 3: Only if necessary: The operator can now define the region of interest (ROI) to use for a second optimization process. This can be very useful, especially with fluorescence images, where some very bright zones may be of less interest and thus are allowed to go "off scale".

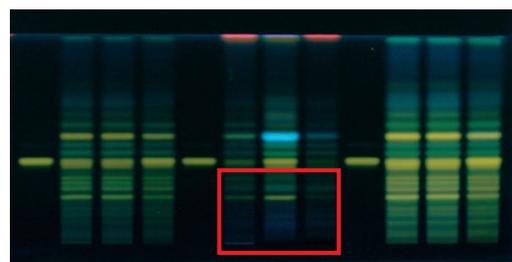
### Flatfield correction

With this documentation system you have the option to make image corrections made necessary by variations in light arriving at the CCD surface. We refer to this as flatfield correction and for each light type, winCATS supports 3 modes: No Correction – Standard Correction – Individual Correction (Option: 027:6372).

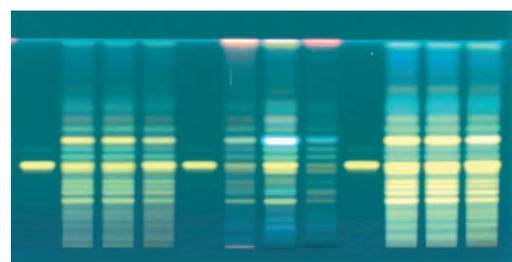
Non-homogeneity of illumination at the edges of a typical image amounts to 15–20 % (the darkest "white" range of the image has about 85 % of the brightest "white" range, see left chromatogram and curve above). Such unavoidable non-homogeneities are caused by the illumination unit, the camera lens, and the CCD detector. With the help of the flatfield correction, which is a winCATS standard feature, this non-homogeneity can be reduced to about 5 % (middle chromatogram). For further correction (right chromatogram) an individual measurement of the given documentation system, including lens setting and light type, is necessary. For this the winCATS Option (027.6372) and a regular qualification on site are required.



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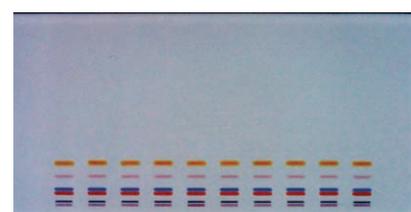
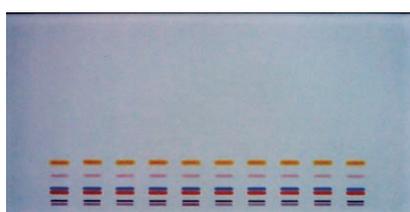
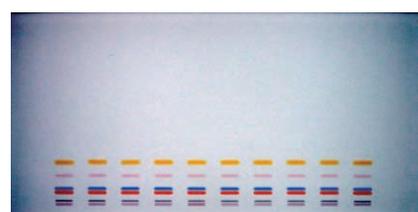


16



17

▲ Usage of ROI: Chromatogram at UV 366 nm with marker showing zone of highest emission of light – optimized image with region of interest (ROI) set – image re-optimized based on ROI.



18

▲ Flatfield correction for white light: No Correction – Standard Correction – Individual Correction (Option: 027.6372)

## Immunoassay detection of gangliosides by specific antibodies



▲ HPTLC team (from left to right): Dr. Iuliana Popa, Dr. Jacques Portoukalian, Ms. Marie-Christine Pernin, Dr. Marie-Jeanne David

The collaboration between the teams of Dr. Iuliana Popa\* and Dr. Marie-Jeanne David\*\* of the Edouard Herriot Hospital in Lyon – the second largest French hospital by the number of beds for patients – led to the set up of the detection of specific anti-ganglioside antibodies in patients sera with neurological diseases for routine clinical applications. This methodology is particularly useful for detection of specific antibodies to

- GM1, GT1b and GQ1b in patients sera with Miller-Fisher syndrome,
- GM1, GD1a, GD1b, GT1b, GD3, GD2, SPG and SGPG in patients sera with peripheral neuropathy and
- GD3 in patients sera with melanoma tumors and retinoblastoma (1).

These antigens are present in the total ganglioside fraction extracted from human sciatic nerves and spinal cord, chromatographed on HPTLC plates and detected by immunostaining.

**Although most hospital laboratories carry out immunoassays on ELISA microtiter plates, Dr. Popa and her team decided to use HPTLC. The major advantage over ELISA is the visualization of single immunostained spots; this allows the detection of multiple antigens in sera of patients suffering from peripheral neuropathy. As further advantage of immuno detection sample preparation is reduced compared to classical detection. Compounds present in minute amounts can be**

**detected whenever a specific antibody is available. With a high affinity antibody, the detection limit is in the nanogram range. Likewise, proteins with high affinity to lipids, such as the cholera toxin, can react with an amount as low as 0.1 ng of GM1 ganglioside (2).**

### Introduction

Gangliosides are a class of glycosphingolipids which have important roles in cell-cell recognition, cell adhesion, modulation of cell proliferation and differentiation and they are expressed at high levels on the surface of human cells, mostly in nerve tissues. Immunoassay detection of gangliosides on HPTLC plates was performed in the laboratory of the Edouard Herriot Hospital by using monoclonal antibodies (Mab) generated after immunization of mice with purified gangliosides from human melanoma and other cancer cells. In this study 3D2, 2C6 and 4F6 have been used as specific antibodies which recognize GD3 ganglioside.

### Sample preparation

Gangliosides were purified by extraction from tissues (melanoma tumors, sciatic nerves and brain) with chloroform – methanol 1:1, liquid liquid extraction, recovery on copolymer columns (3) and fractionation by preparative HPLC (4) and finally taken up in chloroform – methanol 1:1. SGPG (sulphoglucuronyl paragloboside) and SPG (sialyl paragloboside) were purified respectively from sciatic nerves and red cells.

Human sera containing antibodies were obtained after centrifugation (at 2000 rpm for 20 min) of blood samples from patients with peripheral neuropathy or Miller-Fisher syndrome and were stored at  $-80^{\circ}\text{C}$ .

## Standard solution

Gangliosides are dissolved in chloroform – methanol 1:1 (0.1 mg/mL).

## Chromatogram layer

HPTLC plates silica gel 60 (Merck), 10×10 cm

## Sample application

With Linomat, varying number of tracks, application volume 100  $\mu$ L, band length 5 mm, distance from lower edge 10 mm, distance from the side 20 mm, track distance 8 and 10 mm, respectively

## Chromatography

In a flat bottom chamber with chloroform – methanol – 0.2 % aqueous  $\text{CaCl}_2$  11:9:2, developing distance 60 mm from lower edge of the plate

## Immunoassay detection

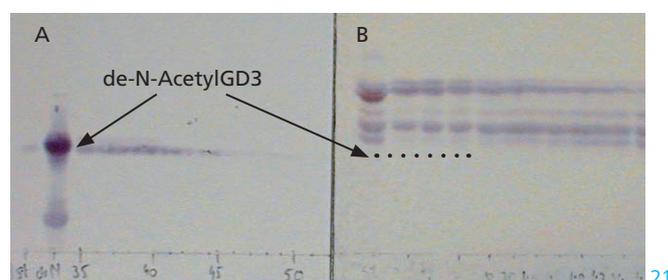
The zones were fixed by dipping the plate in a polyisobutylmethacrylate solution. For endcapping of the active silica gel surface the plate is immersed in a BSA (bovine serum albumine) solution at room temperature for 1 h. After washing with PBS (phosphate-buffered saline), the plates were immersed in the antibody-containing supernatant or in patient's sera containing antibodies at 4 °C overnight. After three washings with PBS, Mab binding was detected by stepwise incubation with biotinylated chain-specific anti-mouse immunoglobulin, followed by streptavidin-horseradish peroxidase complex. After extensive washings with PBS, the bound peroxidase was visualized with chloro-4-naphthol reagent.

## Comparison to chemical derivatization

For chemical detection a second plate (chromatographed in the same way) was sprayed with resorcinol-HCl reagent and heated at 100 °C for 5 min.

## Results and discussion

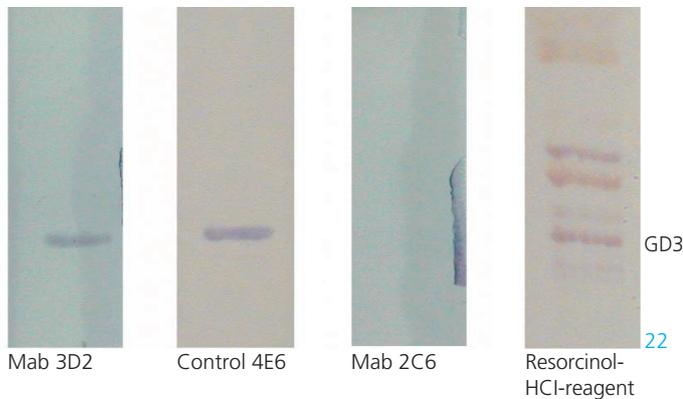
By comparison of the chemically derivatized HPTLC plate of gangliosides purified from melanoma tumors with a second HPTLC plate stained with the antibody SGR37 specific for de-N-acetylGD3, we were able to detect a compound reacting with the antibody in a pool of melanoma gangliosides, although it was not detectable with the resorcinol-HCl reagent.



▲ A: Immunostaining with the specific antibody SGR37 to de-N-AcetylGD3; B: Visualization of the same fractions with resorcinol-HCl reagent. The dotted line shows the migration of de-N-acetylGD3 not visualized with the chemical reagent (too low amount).

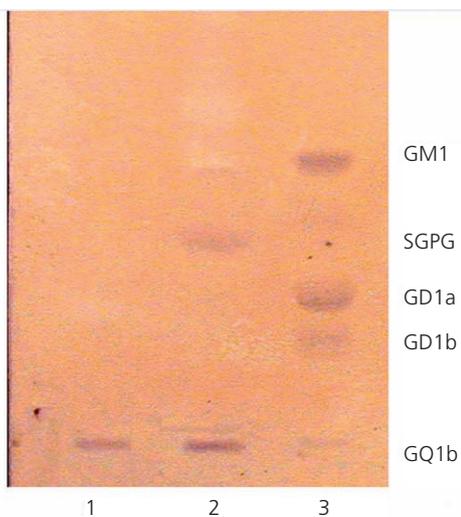
As further advantage of immuno detection sample preparation is reduced. For chemical detection disialogangliosides have to be separated by preparative HPLC from a pool of melanoma gangliosides whereas this step is not necessary for immuno-staining.

The binding of GD3-specific antibodies was dependent on the concentration of the supernatants; the binding activity of the antibody 3D2 to GD3 was found as twice of the binding activity of 2C6. Binding of the Mabs was assessed by immuno-staining on thin-layer plates using a ganglioside fraction purified from melanoma tumors. Mabs 3D2 and 4E6 bound specifically to GD3 and not to any other tested ganglioside, whereas no ganglioside was recognized by Mab 2C6.



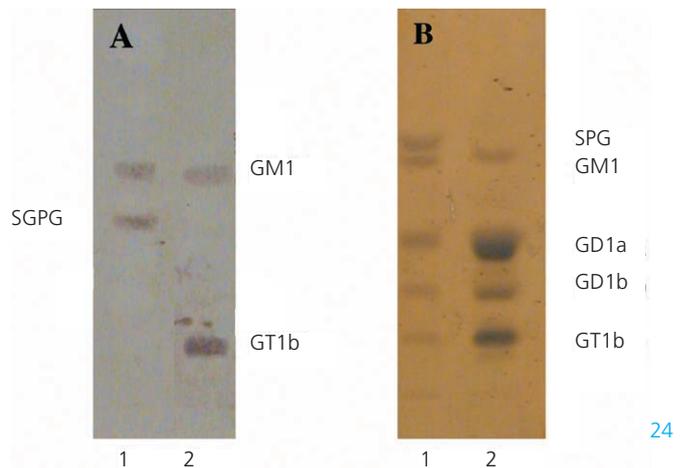
▲ HPTLC image of melanoma gangliosides with 3D2 and 2C6 antiGD3 Mabs compared with the positive control 4E6 Mab and resorcinol-HCl derivatization of total melanoma gangliosides

The advantage of immuno detection on HPTLC plates over ELISA microtiter plates is illustrated in the following figures which clearly show multiple spots stained by antibodies from single patients. With the binding of the patients serum antibodies to GQ1b ganglioside, a diagnosis of Miller-Fisher syndrome can be confirmed, but the simultaneous binding to other gangliosides gives additional information of high interest which would have been overlooked using ELISA assay with GQ1b as antigen.



▲ Immunostaining of sera antibodies of patients with Miller-Fisher syndrome binding to GM1 and GQ1b; track 1: GQ1b ganglioside standard, track 2: human sciatic nerve gangliosides, track 3: bovine brain gangliosides

The same advantages apply to the binding of nerve gangliosides by antibodies from the sera of patients suffering of peripheral neuropathy.



▲ Identification of specific antibodies in sera of patients with atypical neuropathies to gangliosides GM1, GT1b and SGPG; A: Visualization of ganglioside-bound antibodies by using an antiserum to total human immunoglobulins. B: Visualization by resorcinol-HCl reagent; track 1: human nerve gangliosides, track 2: bovine brain gangliosides

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## Amino phases for derivatization of sucralose in milk-based confection



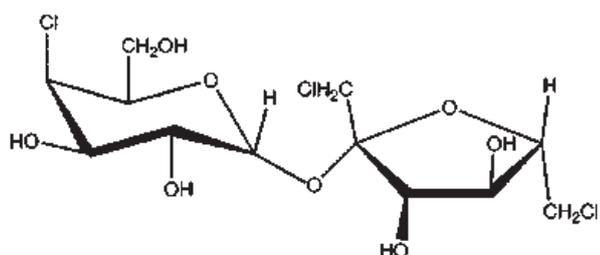
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▲ HPTLC team: Shashi Prabha, Dr. Gerda Morlock

In the last issue of CBS we outlined the working group of Prof. Schwack at University of Hohenheim, Stuttgart. In this study planar chromatography was preferred for analysis of sucralose due to the advantages offered by the derivatization step.

### Introduction

Sucralose is a relatively new sweetener with a sweetening intensity of 600–650 as compared to sucrose, having no calories and a taste profile very similar to sugar without any aftertaste. Because of its exceptional heat stability, excellent solubility characteristics, and high compatibility with commonly used food ingredients, it is employed especially in USA for many low-calorie products. Since 1998 it has been approved by more than 40 countries and it is approved as an additive (E955) for use in European Countries since 2005.



▲ Structure of sucralose (4,1',6'-trichlorogalactosucrose): 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-(2-1)-4-chloro-4-deoxy-α-D-galactopyranoside

Regarding minor UV absorbance (< 200 nm) detection of sucralose is performed by derivatization, refractive index, pulsed amperometric detection or mass selective detector. There is evidence that planar chromatography is advantageous in this case. The amino phase itself reacts as derivatization so that no separate transfer step is needed. By just heating the plate after chromatography sucralose reacts with the amino groups of the layer to fluorescent zones (1). The derivatization step to fluorescent zones can rapidly and simultaneously be performed for all substance zones.

Sucralose was determined in Burfi, a popular Indian ethnic milk delicacy, which is produced in all kinds of modes with various food ingredients. Low-calorie burfi is produced by heat desiccation of milk accompanied by continuous stirring and addition of sucralose, maltodextrin and sorbitol instead of sucrose. Stability has been established for various storage conditions in milk-based confection.



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▲ Burfi containing pistachios, coco pulp or butter

### Sample preparation

5 g Burfi were suspended in water by shaking; after precipitation of proteins and extraction in a ultrasonic bath, the extract was centrifuged and filtered.

### Standard solution

Sucralose dissolved in methanol (0,15 mg/100 mL)

### Layer

HPTLC plates NH<sub>2</sub> F<sub>254</sub> (Merck), 20×10 cm

### Sample application

Bandwise with Automatic TLC Sampler 4, 22 tracks, application volume 4 μL of sample and 2–10 μL of standard solution, band length 5 mm, track distance 7.8 mm, distance from lower edge 8 mm, distance from the side 15 mm

## Chromatography

In horizontal developing chamber with acetonitrile – water 4:1, migration distance 70 mm from lower edge, migration time about 15 min, after chromatography the plate must be dried for 5 min in a stream of warm air

## Derivatization

Heating of the plate for 20 min at 190 °C with TLC plate heater III

## Densitometry

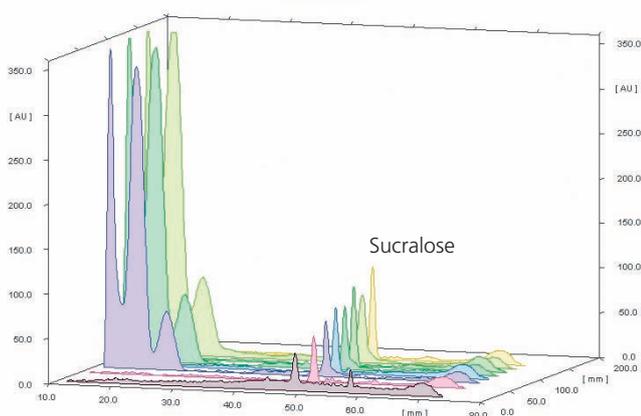
TLC Scanner 3 with winCATS software, fluorescence measurement at UV 366/>400 nm, linear calibration via peak height

## Documentation

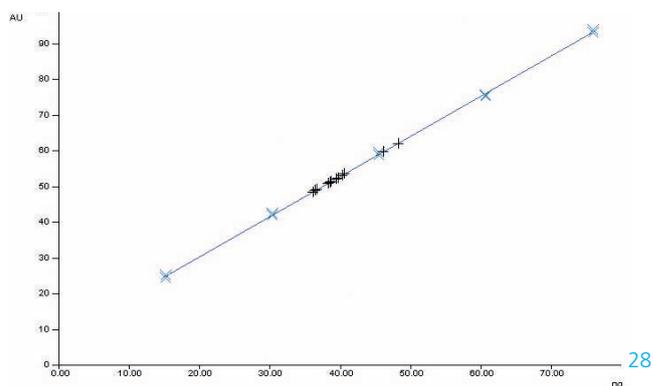
With DigiStore documentation system under UV 366/>400 nm

## Results and discussion

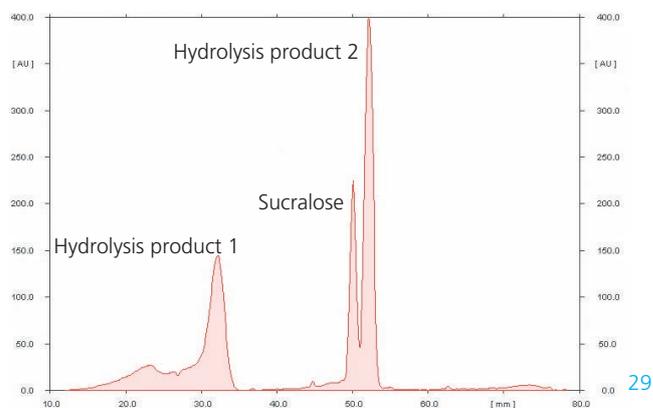
The limit of quantitation of sucralose is found to be in the lower ng range. Calibration is linear (sdv = 0.97 %,  $r = 0.99977$ ) with results falling well within the acceptable working range. Two hydrolysis products are well separated from sucralose, thereby efficiently monitoring the degradation of sucralose in burfi samples stored under different conditions.



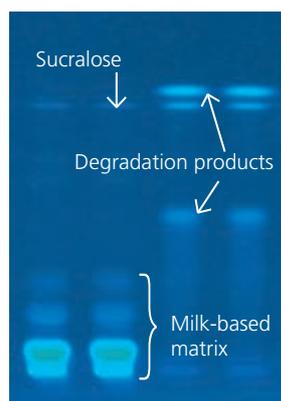
▲ 3D-graphic of standard and sample tracks



▲ Linear calibration of sucralose ( $y = 1,124x + 7,817$ ,  $sdv = 0.97\%$ ,  $r = 0.99977$ )



▲ Track display of a hydrolyzed solution of sucralose showing sucralose and degradation products



▲ Documentation under UV 366/>400 nm; track 1 and 2: sample, track 3 and 4: hydrolyzed solution of sucralose

Further information is available from the authors on request.

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(1) B. Spangenberg, J. Stroka, I. Arranz, E. Anklam, J. Liquid. Chrom. 2003, 26 (16) 2729-2739.

# Automatic Developing Chamber ADC 2

The Automatic Developing Chamber offers convenience, safety and reproducibility for isocratic developments of TLC/HPTLC plates and foils with formats 20×10 und 10×10 cm.

*In this CBS the ADC 2 could be employed for determination of sucralose (p. 14–15) and for separation of gangliosides (p. 11–13). Especially for chromatography of gangliosides a mobile phase system is used containing chloroform besides others. For such solvents reproducible chamber saturation is crucial. The automated and reproducible performance of the separation would be advantageous in this case.*

Chromatogram development is the most critical step of Thin-Layer Chromatography. In the Automatic Developing Chamber ADC 2 this step is fully automatic and reproducible, independent of environmental effects. The activity and pre-conditioning of the layer, chamber saturation, developing distance and final drying can be pre-set and automatically monitored by the ADC 2. Two modes of operation are possible: stand-alone with input of parameters via keypad, or remote operation from winCATS with process monitoring, documentation of operating parameters and reporting.

The ADC 2 with winCATS is compliant with the requirements of GMP/GLP and can be IQ/OQ qualified. If you want to use the instrument in a 21 CFR Part 11 environment, the option 21 CFR Part 11 “compliance ready” is required for each winCATS workstation.



## Key features of the Automatic Developing Chamber ADC 2 at a glance:

- Fully automatic development of 20×10 cm and 10×10 cm TLC/HPTLC plates
- Operation in stand-alone mode or under winCATS
- The user is freed of all process monitoring responsibilities, operation is fully traceable
- The option “Humidity Control” allows reproducible chromatography at defined activity of the layer.

**Further information** can be found in the special brochure “Automatic Developing Chamber ADC 2” or on our website [www.camag.com](http://www.camag.com)