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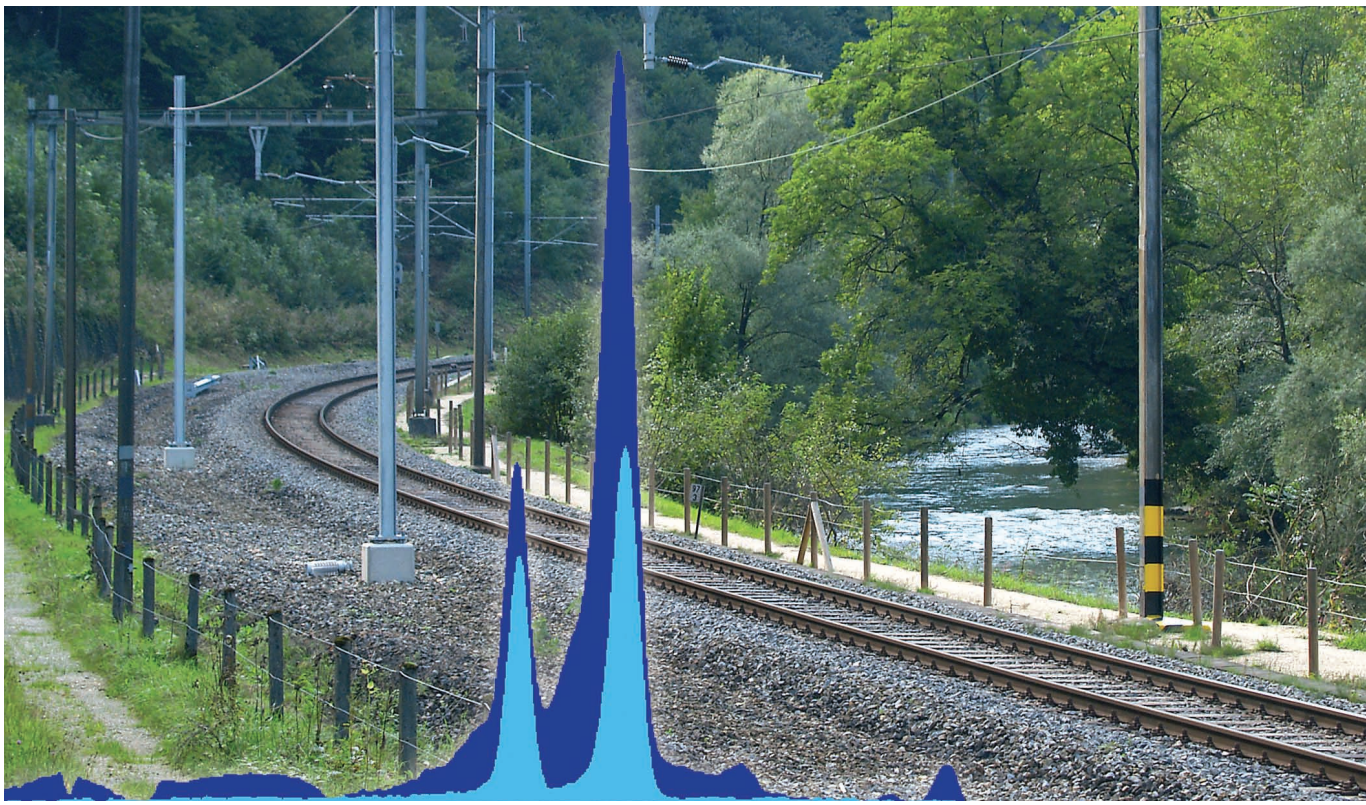
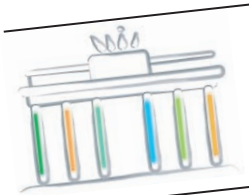


Considering your task – is planar chromatography a better option than HPLC? (see pages 2–7)



INTERNATIONAL SYMPOSIUM ON PLANAR CHROMATOGRAPHY – INSTRUMENTAL THIN-LAYER CHROMATOGRAPHY

Berlin (Germany), 9–11 October 2006

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95

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Determination of histamine and other planar chromatography

1 ▲ *LUA Site Dresden (from left to right): Sven Kretzschmar, Sibylle Neugebauer, Dr. Dieter Hübner*

2 ▲ *Prof. Dr. Karl Speer*

In CBS 83 (September 1999) we reported about the quantitative determination of histamine by planar chromatography at the Institute of Veterinary Pharmacology and Toxicology (IVPT), Bernau. That separation was performed on an RP-18 phase. Pauly's reagent was used for post-chromatographic derivatization.

The following screening method for 7 biogenic amines in fish was developed and compared with other analytical methods at the Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Saxony (LUA), Department of Food Chemistry, Dresden. The pre-chromatographic derivatization with dansyl chloride is based on a publication by the Food Technology and Dairy Science Department in Dokki, Egypt [1]. Mr. Kretzschmar's diploma thesis [2] was supervised by Prof. Speer*) of the Institute of Food Chemistry, Technical University Dresden.

Introduction

During improper storage of fish and fish products increased formation of biogenic amines, particularly histamine, is observed. To protect consumers from poisoning by biogenic amines par. 16 of the fish hygiene act sets limits concerning content (currently only for histamine) for certain kinds of fish.

Because also other biogenic amines are physiologically active, they should be analyzed as well. For this purpose, a screening method was developed which allows handling a large number of samples in a short time. The TLC method presented is well suited for checking the allowed histamine limit. With reduced analytical efforts, the results are comparable to those obtained by employing HPLC, ELISA and fluorimetric methods.

Initially, the samples are extracted with 10% trichloroacetic acid (TCA). The extract is made basic and treated with dansyl chloride. From this mixture the derivatized amines are extracted and separated on a silica gel layer. The evaluation is either performed visually (semi-quantitatively) or densitometrically (quantitatively) at UV 365/>400 nm.

biogenic amines in fish by

Sample preparation

80 mL TCA (10%) are added to 10 g of the homogenized sample. The mixture is homogenized for 90 s at 9000 G. After adjusting to 100 mL with TCA and filtration the filtrate can either be used for derivatization or rather be stored at –20 °C.

Pre-chromatographic derivatization

For pre-chromatographic derivatization 1 mL filtrate is adjusted dropwise with 4N NaOH to pH 8. After adding 1 mL borate-buffer (pH 8.8) and 2 mL dansyl chloride solution (0.5% in acetone) the solution is shaken for 30 s and then incubated for 1 h using a water bath at 40 °C. Then, the final volume is adjusted with water to 10 mL, 5 mL of diethyl ether are added, the mixture is shaken vigorously and then centrifuged. The organic layer is removed and the watery residue then shaken twice more. The combined extracts are reduced to dryness and taken up with 5 mL acetonitrile. 1 mL of each standard solution is derivatized in the same way.

Standard solutions

Hydrochlorides of the biogenic amines putrescine, cadaverine, spermidine, spermine, histamine, tyramine and β-phenylethylamine (0.5 mg/mL in water) remain stable for two weeks in the refrigerator and for at least 6 months in the freezer. 5 mL of each stock solution is diluted with TCA to 100 mL (25 µg/mL). Standard solutions for calibration, which are also derivatized pre-chromatographically, have to be prepared freshly by diluting 200, 400, 600 and 800 µL individually to 1 mL with TCA.

Layer

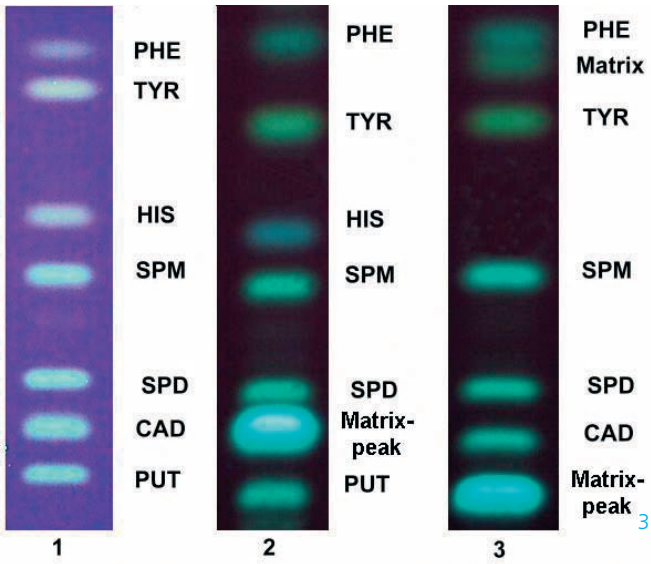
TLC plates silica gel 60 (Merck), 20×10 cm, layer thickness 0.25 mm, pre-washed with developing solvent (by chromatography).

Sample application

Bandwise with Automatic TLC Sampler, 15 tracks, application volume 10 µL, band length 6 mm, distance from lower edge 8 mm, distance from side edge at least 15 mm, track distance 12 mm

Chromatography

In a horizontal developing chamber with benzene – chloroform – triethylamine 10:6:7; the developing distance is 90 mm from the lower edge. For some fish samples cadaverine is obscured by a matrix peak. When changing the mobile phase proportions to 10:6:2, cadaverine can also be determined.



▲ *Selected tracks are detected under UV 365/>400 nm: Track 1 (standard mixture of the 7 biogenic amines β-phenylethylamine, tyramine, histamine, spermine, spermidine, cadaverine and putrescine); Track 2 (not contaminated fish sample spiked with standard mixture); Track 3 (as Track 2, but developed with solvent proportions 10:6:2).*

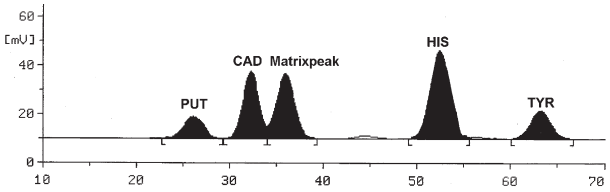
Densitometric evaluation

In the UV-cabinet, applied substance amounts of 10 ng are still visible under UV 365 nm. Quantitative evaluation is performed with a TLC scanner using fluorescence measurement at UV 365/>400 nm. Peak areas are used for calibration.

Results and discussion

With this method, histamine levels between 50–250 mg/kg can be determined. At higher levels, the sample amount can be reduced or the extract can be diluted. Below are the validation data for histamine:

Linearity	50–250 mg/kg
$LOD_{DIN\ 32645}$	17,5 mg/kg
$LOQ_{DIN\ 32645}$	56 mg/kg
Recovery (200 mg/kg)	108 %
Confidence interval	± 9,9 mg



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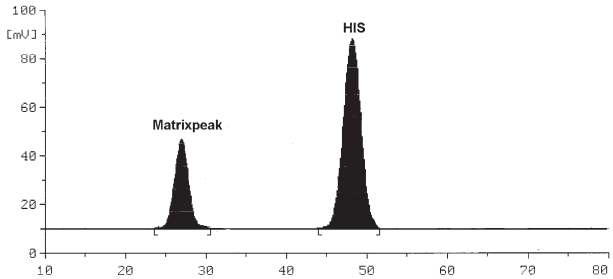
▲ *Densitogram of fish sample "Mackerel smoked" at UV 365/>400 nm*



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▲ *Densitogram of a sample "Tuna in oil" (1:10 diluted) at UV 365/>400 nm*

The fish samples mentioned were investigated with the new TLC screening method as well as with an HPLC method, an ELISA and a fluorimetric method from the LUA Saxony. The results are compared in the table. It becomes clear that the TLC method is suitable for checking the established histamine limits. It yields comparable results with less analytical work.

Levels of biogenic amines in samples of Mackerel and Tuna, obtained with different analytical methods:

Method	Sample	Histamine [mg/kg]	Putrescine [mg/kg]	Cadaverine [mg/kg]	Spermine [mg/kg]	Spermidine [mg/kg]	Tyramine [mg/kg]
TLC	Mack.	180	(20)	60	nd	nd	(30)
	Tuna	2805	nd	nd	nd	nd	nd
HPLC	Mack.	175	10	70	<5	<5	40
	Tuna	2540	25	75	<5	<5	35
ELISA (Histamine)	Mack.	250					
	Tuna	2895					
Fluori-metric	Mack.	170					
	Tuna	3100					

nd = not detectable; levels in parentheses are below the limit of quantification.

Further information is available from the authors on request.

[1] A. R. SHALABY, Food Chemistry 65 (1999) 117-121.

[2] S. Kretzschmar: Entwicklung einer dünnschicht-chromatographischen Screening-Methode für Histamin und weitere biogene Amine in Fisch. Diploma thesis, TU Dresden 2004.

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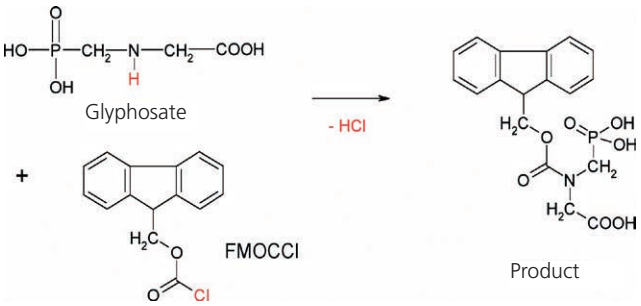
Ultra trace analysis of glyphosate and AMPA in water with HPTLC

▲ *Dr. Walter Weber**

▲ *Wolfram Seitz und Anna Aichinger*

Beside 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 projects.

In addition to GC/MS and HPLC/MS methods modern planar chromatography is used. In the last issue of CBS the luminographic toxicity detection with luminescent bacteria Vibrio fischeri was featured. Analysis of the widely used total herbicide glyphosate and its degradation product AMPA is also performed with HPTLC, because this method has proven to be more robust than an HPLC method and proved well-suited in interlaboratory tests.



▲ *Derivatization of glyphosate with FMOCCl (9-fluorenylmethyl-chloroformiate)*

Introduction

Glyphosate (N-(phosphonomethyl)glycine) is one of the most widely used total herbicides all around the world. For more than 30 years it is used as systemic herbicide against wild herbs and grasses, for example for controlling vegetation on railroad tracks. The principal degradation product of glyphosate is aminomethylphosphonic acid (AMPA). It can also be formed in the degradation of different phosphonic acids.

The German Drinking Water Act sets a limit for plant treatment agents and pesticides of 0.1 µg/L for the individual substance and 0.5 µg/L in total. A reliable determination of glyphosate is therefore indispensable. The investigation of surface waters revealed glyphosate sometimes up to the µg/L level. For the substance AMPA the base contamination of the river Ruhr was found at 0.73 µg/L [1].

Analysis at the ng/L level in water requires a concentration step which is difficult due to the high polarity of the substances. Moreover the absence of analytically important groups like chromo- or fluorophores makes derivatization a necessity.

Up to now analytical methods with GC and HPLC including pre- or post-column derivatization are published. Due to the ionic character of the substances liquid chromatography is given preference. Post column derivatization is more common. Mostly the analytes are extracted from water with a cation exchanger and purified on an anion exchanger. After separation a specific derivatization is performed, which is followed by fluorescence detection.

In the described method for determination of glyphosate and its degradation product AMPA with planar chromatography the analytes are derivatized in water, which leaves them ready for liquid-liquid extraction and subsequent fluorescence detection [2]. By a successful participation in an interlaboratory test conducted by AQS-BW the performance of the method was proven. The herbicide glufosinate can also be determined by this method [3].

Sample preparation

To a 50 mL water sample 8 mL borate buffer (pH = 9) and 50 mL FMOCCl-reagent (16 mg/100 mL acetone) are added. After 30 min reaction time the mixture is extracted three times with 30 mL dichloromethane – 2-propanol (3:1 v/v) each. The organic phase is discarded. Following acidification with sulfuric acid the analytes are extracted two times with 30 mL dichloromethane – 2-propanol (3:1 v/v) each. The extract is first evaporated to dryness using a rotary evaporator, then dissolved in methanol.

Layer

HPTLC plate silica gel 60 F_{254} (Merck) 20 x 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 a TLC plate heater III under a stream of nitrogen.

Sample application

As bands with the Automatic TLC Sampler 4, 18 tracks, application volume 80 µL, band length 5 mm, distance from lower edge of plate 12 mm, distance from the sides at least 15 mm, distance of tracks 10 mm

Chromatography

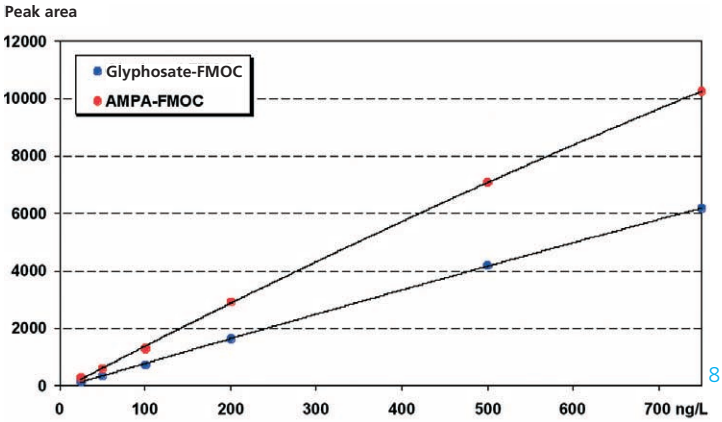
In a twin trough chamber with n-butanol – water – acetic acid 5:4:1. After phase separation the developing solvent is taken from the organic phase. Developing distance 70 mm from lower edge of plate

Densitometric evaluation

TLC Scanner 3 with winCATS software, fluorescence measurement with deuterium lamp at UV 268 nm/secondary filter M 360 nm, linear calibration using peak areas

Results

In the evaluated concentration range the method is very linear ($r^2 > 0.999$). The limit of detection was set to 50 ng/L for the entire procedure including liquid-liquid extraction.



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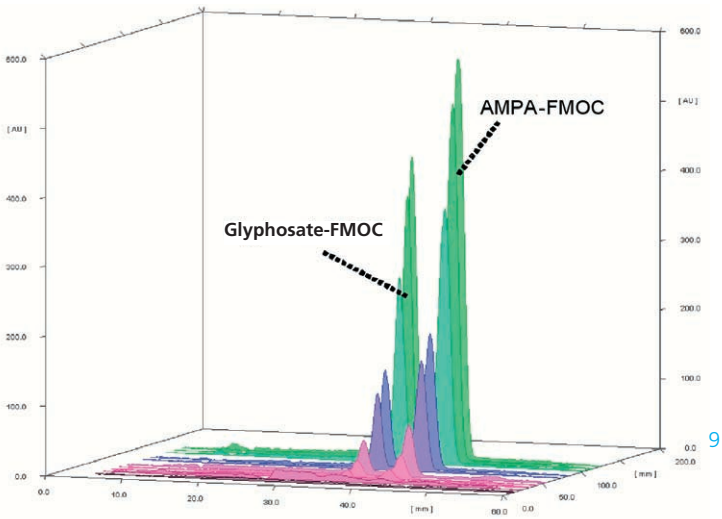
▲ *Linear regression for glyphosate-FMOC and AMPA-FMOC following liquid-liquid extraction from water and HPTLC analysis with fluorescence detection ($r^2 > 0,999$)*



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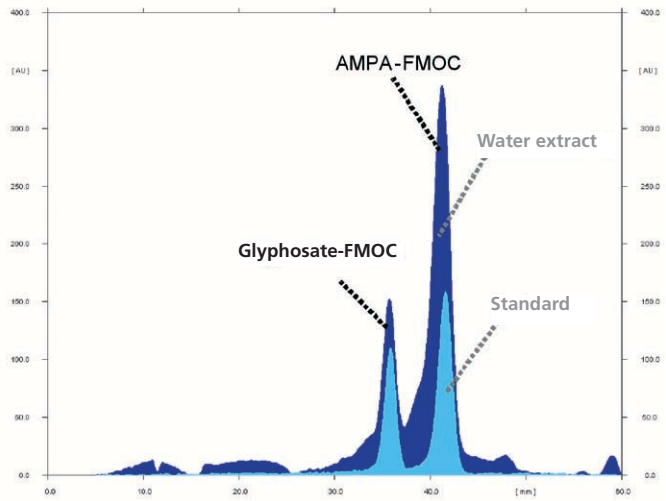
▲ *Fluorescence evaluation at UV 268 nm/M 360 nm of an HPTLC chromatogram of 25 to 750 ng/L glyphosate-FMOC (36 mm) and AMPA-FMOC (42 mm) each, calculated as glyphosate and AMPA*

Investigations of real samples revealed sufficient selectivity for the determination of the analytes. Therefore HPTLC is a good alternative to HPLC for ultra trace analysis of glyphosate and AMPA in water.



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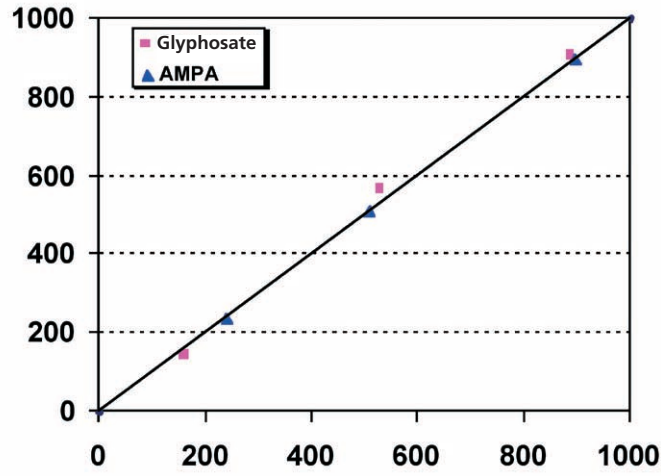
▲ *Comparison of a water extract containing glyphosate-FMOC and AMPA-FMOC with a standard track at 200 ng/L each*

In the interlaboratory trial AQS-BW 2005 the results of glyphosate and AMPA show a quite good correlation (r^2 = 0,986) with the true values. The Z_u score of the three concentration levels is between 0,05 and 0,55 (exclusion value Z_u>2).



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▲ *Correlation of the true value and the results of HPTLC (interlaboratory test AQS-BW 2005)*

Further information is available from the authors on request.

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

[1] R. Reupert, C. Schlett, gwf Wasser Abwasser 138 (1997) 559-563.

[2] R. Gauch, U. Leuenberger, U. Müller, Z. Lebensm. Unters. Forsch. 188 (1989) 36-38.

[3] Unpublished results of Zweckverband Landeswasserversorgung

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CAMAG TLC Scanner 3

Due to modern densitometry planar chromatography can face the method comparison as recently shown for the successful partition of the operations and research laboratory of the Landeswasserversorgung in an interlaboratory test. The CAMAG TLC Scanner 3 is employed for highest accuracy of quantitative evaluation. The use of the optimal measuring wavelength is indispensable when spectral selectivity and sensitivity is essential (see also middle section "Parameters of planar chromatography").

The operations and research laboratory of the Landeswasserversorgung performs the fluorescence measurement in the excitation/emission optimum of the FMOC derivatives using the deuterium lamp at 268 nm combined with a monochromatic filter of 360 nm to cut-off the excitation wavelength. Whereas Dr. Hegewald, general manager of an analytical laboratory in Évora (p. 9), prefers the mercury lamp at UV 265/M 360 nm enabling a more sensitive detection due to the increased radiation intensity of the lamp.

CAMAG
Summer Meeting 2005

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During the week of August 22-28 the General Managers of the top five distribution partners met for their annual conference at the CAMAG headquarters Muttenz. The focus of the meeting was on customer requirements, which we want to transfer into new product ideas and marketing strategies.

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Dilip Charegaonkar of Anchrom, our Indian Partner, was honoured with the freshly invented prize as 'Distributor of the Year' for his successful battle to win a tender concerning 11 complete systems.

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A highlight was the invitation to an open house from Hakama, a leading Swiss specialist for sheet metal products and supplier of housings for CAMAG instruments since 1979. For the first time we could observe how sheet metal is converted into sophisticated products such as the cover of the CAMAG TLC Scanner 3.

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The relaxing part of the summer meeting was spent at La Gruyère, a picturesque and historical village/region, famous for its cheese production. A visit to an Alp-dairy (traditional – certainly not GMP-compliant) and a guided tour of Gruyère castle were among the educational events of this weekend. The entorage went on an extended hike in the area between Les Rosalys, Dent de Lys and Le Moleson producing some sore muscles while increasing the team spirit.

Parameters of Planar Chromatography

The articles in this series are dedicated to the important steps of planar chromatography and their parameters which influence the chromatographic result. Hints for optimization are given to help the reader to use planar chromatography most efficiently.

Collecting these pages is recommended.

Scanning Densitometry – Part 1: scanning modes and multi-wavelength scan

Introduction

The principal benefit of using densitometry is "measuring" and quantifying a thin-layer chromatogram. This was the primary requirement for instrumental HPTLC to become accepted as a reliable quantitative analytical tool, comparable to HPLC and GC. Let us focus on some practical aspects of scanning densitometry.

All these measurements are relative, meaning that the properties of an "unknown" always are compared to those of a "known" compound. With the help of known standards the system can be calibrated on an individual plate with standards and unknowns chromatographed side by side. The reason is that during the measurement the layer acts as the background which the electronic amplification must be adjusted to with respect to light, sensitivity and range before each measurement to give optimum performance.

Scanning densitometry offers the most accurate type of evaluation in HPTLC. It has a great advantage over visual inspection and video densitometry because of its spectral selectivity. Since monochromatic light in the range of 190 to 800 nm can be used and tuned to the absorption/fluorescence excitation maximum of the individual compounds the measurement is very sensitive. Typical detection limits are in the low nanogram range (absorbance) or medium picogram range (fluorescence). Densitometry is usually performed prior to derivatization. Only compounds without chromophoric groups must be chemically altered to render them detectable.

Scanning densitometry can also be used for identification by comparing profiles of the analog curves of individual sample tracks. This includes multi-wavelength scanning, i.e. the sequential scanning of each chromatogram track with up to 31 wavelengths, and the quantitative evaluation of each compound at the optimal wavelength. UV spectra of all relevant compounds can be recorded and used for identification purposes.

Scanning modes

Densitometry can be performed in absorbance or fluorescence mode. During absorbance measurement the high amount of light reflected from the plate (without sample) represents the baseline signal, which is lowered when the light beam hits an absorbing zone of the track. The resulting negative peak is inverted electronically so that absorbance is presented as a positive peak in the densitogram. The fluorescence measurement requires a cut-off filter to efficiently eliminate the reflected UV-light used for excitation. In contrast to the absorbance mode a low light baseline signal is generated and the signal attenuation can be increased.
The fluorescent light emitted at a higher wavelength by the sample passes the filter and reaches the photomultiplier, thus generating a positive peak.

For measurement of UV spectra directly on the plate the light beam is positioned onto the specified zone on the plate and the wavelength is continuously changed to cover the desired spectral range. Usually the UV spectrum is measured at the peak maximum. When additional spectra are taken at the flanks of the peak and correlated with the peak maximum, peak purity can be obtained. This principle is a convenient way of checking a peak for co-eluting compounds. There are two major pieces of information, which can be obtained from the UV spectrum. One is the position of the absorption maximum of the compound in question, the other the general shape of the curve. Both concern identity. By comparing the spectrum of an unknown with those of reference compounds in a library, which was generated under identical conditions, compounds can be identified.

Selection of measurement parameters

It is a good idea to visually inspect the plate prior to scanning for any scratches and chromatographic irregularities. Best results are obtained when the width of the measuring slit is about 60 to 80 % of the length of the zone applied as band or 120 % of the size of the largest spot of the chromatogram (spot application).

In order to optimize available light and resolution of the scan a “Micro” slit of 0.3–0.4 mm height should be selected and scanning speed should be low (e.g. 20 mm/s). Qualitative measurements can use much higher scanning speed. Usually the scan starts before the application zone and ends after the front position of the track. This way the complete information is available for data processing during which the track start and end points can be adjusted to include only the compounds of interest, provided the background permits this.

Example: The 5 sulfonamides sulfamethoxazole, sulfamerazine, sulfamethazine, trimethoprime, dimetridazole can easily be separated on silica gel. When initially scanned at 254 nm the chromatogram in Fig. 1 is obtained. The scanning wavelength of 254 nm is often selected because most substances show at least some absorption and the chromatogram resembles the visual impression of a plate viewed under UV 254 nm (provided it contains fluorescence indicator which is only necessary for the visual impression, not for the scanning).

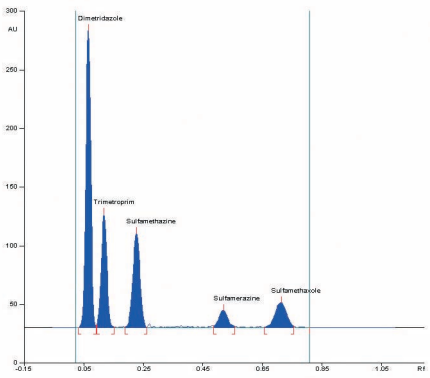

Fig. 1: Separation of five sulfonamides, detection of absorbance at 254 nm

The UV spectra recorded between 200 and 400 nm reveal that each substance has a different absorption maximum although dimetridazole, sulfamethazine and trimethoprime show quite similar UV absorbance (Fig. 2)

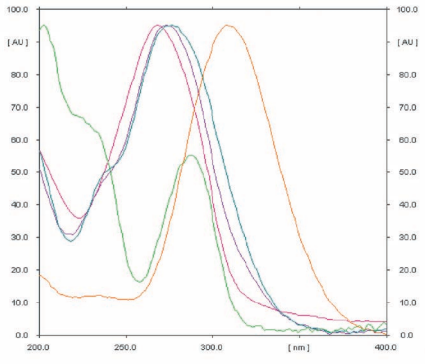

Fig. 2: UV spectra of five sulfonamides: sulfamethoxazole maximum at 308 nm, sulfamerazine maxima at 203 and 288 nm, sulfamethazine maximum at 276 nm, trimethoprime maximum at 273 nm, dimetridazole maximum at 268 nm

For quantitative evaluation the measurement should be performed at the individual absorption maximum of each compound. WinCATS offers the multi-wavelength option for that purpose (Fig. 3).

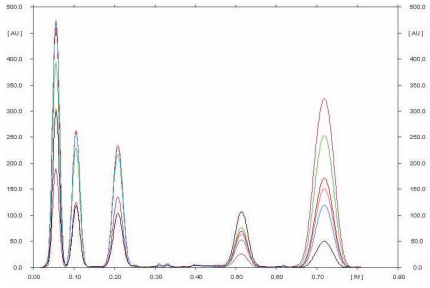

Fig. 3: Multi-wavelength scan for evaluation at the optimum wavelength for each compound: dimetridazole at 268 nm, trimethoprime at 273 nm, sulfamethazine at 276 nm, sulfamerazine at 203 nm and sulfamethoxazole at 308 nm

The chromatogram recorded at 308 nm illustrates the consequence (Fig. 4): the peak for sulfamethoxazole is now much higher relative to those of the other compounds.

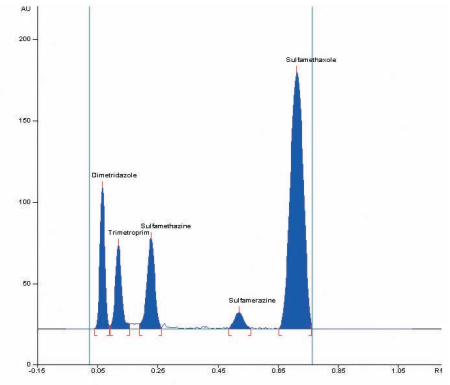

Fig. 4: Same separation of five sulfonamides as in Fig.1, detection of absorbance at 308 nm.

In the case a compound has high absorption over a wide wavelength range or, like sulfamerazine, two maxima, the following aspects should be considered. When evaluating at the first maximum (203 nm) the measurement may not be optimal because the deuterium lamp has low intensity and almost any compound (including traces of the developing solvent) will absorb at this wavelength. When scanned at the second (local) maximum for sulfamerazine (288 nm), the deuterium lamp emits much more light which results in larger peaks, a low signal to noise ratio and thus an excellent fit of the calibration curve (Fig. 5).

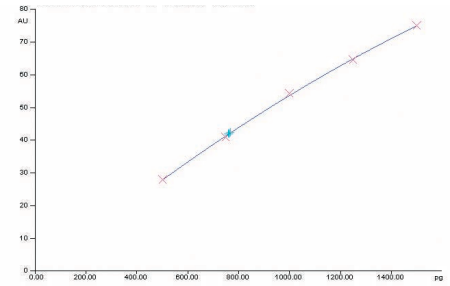

Fig. 5: Calibration curve for sulfamerazine at 288 nm

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PLANAR CHROMATOGRAPHY

CBS

Liebe Freunde

Die Planar-Chromatographie wird weit verbreitet als Screening-Methode eingesetzt. Nicht so oft wird sie in einem Methodenvergleich anderen Verfahren gegenübergestellt. Ein Methodenvergleich ist aufwendig und oft sind in einem Labor nur begrenzt Möglichkeiten für eine alternative Analytik vorhanden.

Aus diesem Grund heben wir Anwendungen in diesem Kontext hervor. Zum Beispiel hat die Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Fachbereich Lebensmittelchemie in Dresden, in Zusammenarbeit mit dem Institut für Lebensmittelchemie der TU Dresden einen Methodenvergleich zur Bestimmung von Histamin in Fisch durchgeführt (S. 2–4).

Die planar-chromatographische Ultraspurenanalytik von Glyphosat und AMPA in Wasser, die im Betriebs- und Forschungslaboratorium des Zweckverbandes Landeswasserversorgung in Langenau durchgeführt wird, konnte ihre Leistungsfähigkeit in einem Ringversuch der AQS-BW unter Beweis stellen (S. 5–7). Interessant hierzu ist auch die Bestimmungsvariante auf Seite 9.

Ein reger Austausch in der Planar-Chromatographie fand auch dieses Jahr statt, sei es auf dem »International Symposium on Planar Chromatography« in Siofok, Ungarn, oder bei den halbjährlichen Treffen des französischen Clubs (vorgestellt im CBS 90). Informieren möchte ich Sie schon heute über das nächste Symposium zur Planar-Chromatographie 2006 in Berlin (siehe letzte gelbe Seite).

Herzlichst Ihre

Gerda Morlock

Gerda Morlock

Dear friends

Planar chromatography is widely used as a screening method, however, not so often confronted with a comparison to other methods. A method comparison is time-consuming and many laboratories have only limited possibilities for alternative confirmation of the results obtained.

For this reason, we lay emphasize on applications in such a context. For example the Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Saxony, Department of Food Chemistry, Dresden, in cooperation with the Institute of Food Chemistry, TU Dresden, conducted a method comparison for the determination of histamine in fish (p. 2-4).

Planar chromatographic ultra-trace analysis of glyphosate and AMPA in water performed in the operations and research laboratory of the Landeswasserversorgung in Langenau assured its competence in an interlaboratory trial of the AQS-BW (p. 5-7). Of interest might also be the alternative derivatization technique on page 9.

Active exchange of ideas in planar chromatographic research was also fruitful this year, either on the International Symposium on Planar Chromatography in Siofok, Hungary, or at the semi-annual meetings of the French Club CCCM (introduced in CBS 90). Thus, I would like to focus your interest on the next International Symposium for Planar Chromatography/ Instrumental HPTLC 2006 in Berlin (see last yellow page).

Sincerely,

Gerda Morlock

Gerda Morlock

CAMAG

SEPTEMBER 2005 95

THE CBS CLASSIFICATION SYSTEM

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

95 001 J. QU (Qu Jianbo)*, H. LOU (Lou Hongxiang), P. FAN (Fan Peihong) (*School Pharm., Shandong Univ., Jinan 250012, China): (Application of TLC-bioautography in drug screening) (Chinese). J. Chinese Trad. and Herb. drugs 36 (1), 132-137 (2005). A review with 22 references on TLC-bioautography, including the screening of natural compounds with antibacterial and/or antifungal activity, cholinesterase inhibitors, free radical eliminators, and antioxidants. Discussion of the advantages of the technique compared to other related techniques.

Traditional medicine, pharmaceutical research, herbal, qualitative identification, autoradiography, review, TLC-bioautography, antibacterial activity, antifungal activity, cholinesterase inhibition, free radical, antioxidation 1, 32

2. Fundamentals, theory and general

95 002 M. FILIP, Virginia COMAN*, R. GRECU, K. Albert, Z. MOLDOVAN (*`Raluca Ripan' Institute for Research in Chemistry, 30 Fantanele Street, P. O. Box 702, RO-400294 Cluj-Napoca, Romania): Characterization of some chemically modified acidic alumina T samples for TLC. J. Planar Chromatogr. 17, 424-430 (2004). Chemically modified acidic alumina T stationary phases have been prepared by organosilylation with the trifunctional organosilicon compounds n-octadecyltrichlorosilane, 3-mercaptopropyltrimethylsilane, and N-(2-aminomethyl)-3-aminopropyltrimethoxysilane. These chemically modified phases were characterized by elemental analysis, measurement of specific surface area, FTIR spectroscopy, 13C CP/MAS NMR spectroscopy, mass spectroscopy, and thermal analysis. The TLC behavior has been tested by separation and identification of some dyes and benzo[a]pyrene derivatives.

Stationary phases 2a

95 003 H. KALÁSZ (Semmelweis University, Department of Pharmacology and Pharmacotherapy, H-1089 Budapest, Nagyvárad tér 4, Hungary): Planar displacement chromatography. J. Planar Chromatogr. 17, 464-467 (2004). Displacement chromatography (DC) has been widely used to separate metabolites with similar chemical characteristics. DC works with highly overloaded sample sizes, which are normal for samples not subjected to clean-up. DC successfully handles samples which contain high concentrations of salts and/or proteins, and results in consecutive steps of displaced compounds rather than Gaussian curves. Planar displacement chromatography (DTLC) is suitable for seeking new metabolites in excreted body fluids. Transfer of the radio labeled methyl group can easily be proven using spacer-displacement planar chromatography. Displacement TLC of L-deprenyl and 14C-L-deprenyl on silica gel with chloroform - triethanolamine 19:1. Detection by X-ray film with an exposure time of 120 h.

Pharmaceutical research, autoradiography 2a, 32a

95 004 E. REICH*, A. SCHIBLI (*CAMAG Laboratory, Sonnenmattstr. 11, CH-4132 Muttenz, Switzerland): A standardized approach to modern high-performance thin-layer chromatography (HPTLC). J. Planar Chromatogr. 17, 438-443 (2004). Proposals for general standardized HPTLC methodology: 1. Plate material (consistent material, prewashing, direction of development, activation of plates, influence of relative humidity). 2. Sample application (precise and accurate volume, solvent, position, spot or bandwise application). 3. Preparation and storage of mobile phases (stability, possible reaction). 4. Development (saturation, use of a twin-trough chamber, influence of the vapor phase, distance, drying). 5. Derivatization (dipping, spraying, heating). 6. Documentation of plates. 7. Labeling (plates, images). 8. Quantitative evaluation 8. Documentation of work.

HPTLC, standardization 2a

95 028 Irena BARANOWSKA et al., see section 17a

95 102 R. M. BAOSIC et al., see section 33a

95 041 Malgorzata JANICKA et al., see section 29d

95 036 M. NATIC et al., see section 24

95 044 Nada U. PERISIC-JANJIC et al., see section 29e

3. General techniques

95 005 V. G. BEREZKIN*, A. O. BALUSHKIN, E. B. NEPOKLONOV, A. V. TOPCHIEV (*Institute of Petrochemical Synthesis, Russian Academy of Sciences, Leninski pr. 29, 119991 Moscow, GSP-1, Russia): Principles of electroosmotic circular thin-layer chromatography. J. Planar Chromatogr. 17, 476-479 (2004). Compared with traditional linear thin-layer chromatography, circular TLC is known to have three advantages: substantially better resolution, lower limits of detection (because of the concentration of the zones), and lower solvent consumption. The results obtained indicate that use of circular electroosmotic TLC (EO-TLC) made the chromatographic process both faster and more efficient. Traditional circular TLC and circular electroosmotic TLC of dyes (rhodamin 6G, brilliant green, sudan III, crystal violet) with DMSO.

Electroosmotic circular thin-layer chromatography 3d

95 006 T. H. DZIDO*, J. MRÓZ, G. W. JÓZWIAK (*Department of Physical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Adaptation of a horizontal DS chamber to planar electrochromatography in a closed system. J. Planar Chromatogr. 17, 404-410 (2004). Highly reproducible retention was achieved when the adsorbent layer of the plate was pre-wetted and equilibrated with the mobile phase after adaptation of a horizontal DS chamber for electrochromatography in a closed (pressurized) system. The disadvantages of the open system, evaporation of the mobile phase from the plate and excessive flow of mobile phase to the surface of the adsorbent layer during development, were eliminated. Separation of a test dye mixture on pre-wetted RP-8 and RP-18 phases with acetonitrile - buffer and application of a potential of 2 kV to create the electric field. Detection by scanning in reflectance mode at 420 or 254 nm.

HPTLC, planar electrochromatography 3d

95 008 E. MINCSOVICS (OPLC-NIT, Andor u. 60, 1119 Budapest, Hungary): Flowing eluent wall processing OPLC: Using segmentation of non-segmented adsorbent layer for single and parallel separations. J. Planar Chromatogr. 17, 411-419 (2004). A new concept, the flowing eluent walls (FEW) process, for segmentation of a non-segmented adsorbent bed, has been used for single- and multi-channel on-line overpressured-layer chromatography, which leads to active and non-active regions on the adsorbent layer during the separation process. Mobile phase only is introduced to the non-active part of the layer whereas mobile phase and sample can be admitted to the active parts. The FEW concept provides the possibility of real multichannel liquid chromatographic separation on a non-segmented layer and column shaped adsorbent bed. Separation of chamomille oil, dye mixtures, ascorbigen standards, and cabbage extracts, were used as examples. The FEW configuration is suitable for rapid isolation of relatively large amounts of a substance.

Flowing eluent walls process, OPLC 3d

95 010 Y. WANG (Yuping Wang), D. WANG* (Dongyuan Wang), J. WANG (Jie Wang), Z. XIONG (Zhili Xiong), H. ZHANG (Hongxia Zhang), G. SHE, (Gaohong She) J. LI (Jian Li), S. XIAO (Shengtao Xiao) (*Department of Analytical Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016 P. R. China): A new instrument for automated multiple development in thin-layer chromatography. J. Planar Chromatogr. 17, 290-296 (2004). Description of a new AMD instrument. Its main advantages are very low cost both of construction and in use. In comparison

with ascending development in conventional instruments, a laboratory-made horizontal sandwich chamber is used for development. With the help of a series of special accessories no obvious mobile phase remains in the distributor after each step thus saving a large amount of solvent. All the components of the instrument are easy to obtain, so the average worker in the laboratory could construct the entire instrument except the control unit. An application of the instrument is described; the results obtained were satisfactory. Compared with the commercial instrument the main differences are 1) a horizontal sandwich chamber with funnel distributor is used as development chamber, 2) the most expensive component, a motor-driven valve, is omitted, 3) a micro air pump (normally used to supply oxygen for goldfish) is used to deliver mobile phase to the chamber. AMD separation of 13 dyes with first acetone - ethyl acetate 1:1 to compress the spots to slim bands, then seven steps with ethyl acetate - chloroform 4:21 to1:9 were completed; then seven steps with chloroform - cyclohexane 17:3 to 67:33. After these fifteen steps of AMD the mixture was separated into eighteen visible spots.

AMD 3d

95 007 S. KHAWAS, D. PANJA, S. LASKAR* (*Natural Products Laboratory, Chemistry Department, University of Burdwan, Burdwan-713104, W. Bengal, India): A new reagent for identification of amino acids on thin-layer chromatography plates. J. Planar Chromatogr. 17, 314-315 (2004). Separation of 22 amino acids on silica gel with n-propanol - water 7:3. Detection by spraying with 1) 5 % 4-hydroxyacetophenone in acetone, followed by drying in air until all solvent had completely evaporated, and heating in an oven at 110 °C for 10 min, and, after cooling, spraying with 2) 0.4 % isatin-5-sulfonic acid (sodium salt) in ethanol - water 4:1, followed by drying in air and heating for 10 min at 110 °C. Detection limits were between 0.1 and 2 µg.

3e, 18

95 009 B. SPANGENBERG*, M. WEYANDT-SPANGENBERG (*University of Applied Sciences Offenburg, Badstrasse 24, 77652 Offenburg, Germany): Fluorescence evaluation using the Kubelka-Munk formula. J. Planar Chromatogr. 17, 164-168 (2004). HPTLC of flupirtine maleate on silica gel with ethyl acetate - methanol - 25 % ammonia 17:2:1 in a saturated developing chamber. Then the developed plate must be dipped for 2 s in 1:3 paraffin - hexane. Dipping increases the fluorescence tenfold and preserves the fluorescence stability for hours. Presentation of a new formula for transforming fluorescence measurements in accordance with Kubelka-Munk theory. The fluorescence signals, the absorption signals, and data from a selected reference are combined in one expression. Only diode-array techniques can measure all the required data simultaneously. The fluorescence calibration curve was linear over the range 300 to 5000 ng per spot.

Quality control, HPTLC, densitometry, quantitative analysis, flupirtine maleate 3f, 32a

95 013 K. L. BUSCH et al., see section 4e

4. Special techniques

95 013 K. L. BUSCH (Wyvern Associates, 4201 Wilson Blvd, 110-440 Arlington, VA 22203, USA): Planar separations and mass spectrometric detection. J. Planar Chromatogr. 17, 398-403 (2004). Review divided into several sections: ‚Summaries' contains a review of some recent research results in TLC-MS and PC-MS (use of a diode IR laser to desorb samples from a thin-layer chromatogram, with ionization of the desorbed gaseous molecules via a corona discharge; description of an interface between TLC and an electrospray ionization (ESI) mass spectrometer; on spot matrix-assisted laser desorption ionization (MALDI) mass spectrometry for TLC-MS); in ‚Assessments and perspectives' new results are detailed, precedent and new instrumental developments are previewed; in the section ‚Interconnections' synergies between mass spectrometry and different approaches to planar separations are explored. Finally, in ‚Forecasts' expectations and future developments, in addition to recent techniques, are described. 11 references.

Review, mass spectrometry, TLC-MS 4e, 3g

95 011 I. HAZAI (IVAX Drug Research Institute, Department for Pharmacokinetics, H-1325 Budapest, P. O. Box 82, Hungary): Use of multiple readings to increase the sensitivity of phosphor image detection in TLC. J. Planar Chromatogr. 17, 449-453 (2004). In thin-layer radiochromatography the high sensitivity of the phosphor imaging analyzer can be further increased by multiple reading of the image plates, because the latent image is not lost quantitatively in the reading process. Summing of the chromatograms obtained in successive readings results in increased signal-to-noise ratio. Thus, by use of this approach either the exposure time can be shortened or higher sensitivity can be achieved. TLC of a 14C-labeled test substance in rat serum on silica gel with chloroform - n-hexane - ethanol - ammonia 75:15:9:1. Detection by radioluminography.

Radioscanning, quantitative analysis 4e

95 012 G. HORVÁTH*, L. G. SZABÓ, É. LEMBERKOVICS, L. BOTZ, B. KOCSIS (*Department of Botany, Faculty of Natural Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary): Characterizaton and TLC-bioautographic detection of essential oils from some Thymus taxa, determination of the activity of the oils and their components against plant pathogenic bacteria. J. Planar Chromatogr. 17, 300-304 (2004). TLC of essential oils and thymol, carvacrol, geraniol as standards and streptomycin and gentamycin as positive controls on silica gel with toluene - ethyl acetate 93:7. Detection with ethanolic vanillin sulfuric acid. Quantitative determination at 500 nm. For bioautography the developed plates were dipped for 10 s in approximately 50 mL culture medium containing the test organism followed by drying for 2 min. After storage of the plates at 26 - 28 °C for 17 h they were dipped for 10 s in an aqueous solution (0.1 g/60 mL) of 3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide (MTT) the layers were incubated at 28 °C for 2 h and then dipped in 70 % ethanol and dried at room temperature.

Herbal, qualitative identification, quantitative analysis, densitometry, Thymus 4e, 32e

95 014 H. LUFTMANN*, D. HOPPE, J. BECKER (*Organisch-Chemisches Institut, Westfälische Wilhelms-Universität, Corrensstr. 40, 48149 Münster, Germany, luftman@uni-muenster.de): Hyphenated HPTLC-MS as rapid method for elucidation of synthesis mixtures. CBS 94, 5-7 (2005). HPTLC of synthesis reaction products on silica gel with pentane - tert. butyl methyl ether 3:1 with chamber saturation. Detection by spraying with phosphomolybdic acid reagent (0.4g in 100 mL ethanol), followed by drying in hot air. Identification by online hyphenation with mass spectrometry. For online extraction with the ChromeXtract device the substance zones are eluted with methanol - chloroform 1:1 (flow rate 0.1mL/min). The outlet capillary is connected directly to the electrospray mass spectrometer (ESI-MS). Measurement in 2 s cycles in a mass range from m/z 100-600.

Qualitative identification, HPTLC, ChromeXtract, TLC-MS online coupling, synthesis 4e

95 015 M. PROSEK*, L. MILIVOJEVIC, M. KRIZMAN, M. FIR (*National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia): On-line TLC-MS. J. Planar Chromatogr. 17, 420-423 (2004). A new on-line TLC-MS interface, with computer-controlled extraction of substances from selected spots on a TLC or HPTLC plate, has been constructed. The controlled collection of the sample and its programmed injection into the mass spectrometer is the advantage of this type of interface. It has been tested and validated with a standard solution of caffeine as test substance. HPTLC of caffeine on silica gel with dichloromethane - methanol 9:1. Quantification with a video-documentation system.

HPTLC, TLC-MS interface 4e

95 016 W. WEBER*, W. SEITZ, Anna AICHINGER, R. ALBERT (*Zweckverband Landeswasserversorgung, Betriebs-und Forschungslaboratorium, Am Spitzigen Berg 1, D-89129 Langenau, Germany, weber.w@lw-online.de): Luminographic detection of toxicity with Vibrio fischeri (luminescent bacteria). CBS 94, 2-4 (2005). HPTLC-AMD of four pharmaceuticals and extracts of surface water on silica gel prewashed with 2-propanol (immersion for 24 h) with a 25-step gradient based on acetonitrile - formic acid - dichloromethane. Luminographic detection at ng-

level by immersion of the developed HPTLC plates into Vibrio fischeri bacteria suspension. Visual evaluation with CCD-camera, exposure time 40 s, inversion and scaling of exposure in pseudocolors. To remove matrix (humic acids) from surface water samples size exclusion chromatography is recommended.

Environmental, toxicology, AMD, HPTLC, qualitative identification, Vibrio fischeri, Bioluminex, luminographic detection, water analysis 4e, 37c

95 104 L. WILLIAMS et al., see section 35b

8. Substances containing heterocyclic oxygen

95 017 T. HOFMANN*, L. ALBERT, T. RÉTFALVI (*University of West Hungary, Institute for Chemistry, Ady Endre u. 5, 9400 Sopron, Hungary): Quantitative TLC analysis of (+)-catechin and (-)-epicatechin from Fagus Sylvatica L. with and without red heartwood. J. Planar Chromatogr. 17, 350-354 (2004). TLC of (+)-catechin and (-)-epicatechin on silica gel with diisopropyl ether - formic acid 9:1. Detection by spraying with vanillin-sulfuric acid reagent and heating at 120 °C for 5 min. Quantitative determination by absorbance measurement at 513 nm.

Quantitative analysis, densitometry, Fagus Sylvatica, catechin, epicatechin 8a

95 018 B. LAPORNIK, Alenka GOLC WONDRA*, M. PROSEK (*National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia) : Comparison of TLC and spectrophotometric methods for evaluation of the antioxidant activity of grape and berry anthocyanins. J. Planar Chromatogr. 17, 207-212 (2004). HPTLC of malvidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, peonidin 3-glucoside, and petunidin 3-glucoside on silica gel with ethyl acetate - formic acid - twice distilled water 17:2:3 in an unsaturated twin trough chamber. After drying detection with methanolic 2,2-diphenyl-1-picrylhydrazyl reagent. Quantitative determination by videodensitometry.

Food analysis, HPTLC, qualitative identification, quantitative analysis, densitometry, anthocyans 8a

95 019 Z. MALES*, M. PLAZIBAT, V. B. VUNDAC, I. ZUNTAR, K. H. PILEPIC (*Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, 10000 Zagreb, Croatia): Thin-layer chromatographic analysis of flavonoids, phenolic acids, and amino acids in some Croatian Hypericum taxa. J. Planar Chromatogr. 17, 280-285 (2004). TLC of flavonoids (quercetin, I3,I8-biapigenin, quercitrin, isoquercitrin, hyperoside, rutin) and phenolic acids (caffeic and chlorogenic acid) on silica gel with ethyl acetate - formic acid - acetic acid - water 100:11:11:26 and ethyl acetate - formic acid - water 8:1:1. Detection by spraying with natural products - polyethylene glycol reagent and observation under UV light at 365 nm. Detection limit for flavonoids was 2.5 µg. Quantitative determination by spectrophotometry, calculated as quercetin. Also TLC of 16 amino acids on cellulose.

Herbal, quantitative analysis, qualitative identification, Hypericum 8a, 11a, 18a, 32e

95 020 Marica MEDIC-SARIC*, I. JASPRICA, A. MORNAR, A. SMOLCIC-BUBALO, P. GOLJA (*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, 10000 Zagreb, Croatia): Quantitative analysis of flavonoids and phenolic acids by two-dimensional thin layer chromatography. J. Planar Chromatogr. 17, 459-463 (2004). TLC of standard solutions of nine flavonoids and six phenolic acids (cinnamic, o-coumaric, m-coumaric, p-coumaric, caffeic, ferulic acid, galangin, quercetin, pinocembrin, naringenin, apigenin, chrysin, kaempferol, morin, acacetin) on silica gel in pre-saturated developing chambers with 1) n-hexane - ethyl acetate - glacial acetic acid 31:14:5, or 2) chloroform - methanol - formic acid 88:7:5. After drying, bands were visualized under short- and long-wavelength UV light. Detection by spraying with 1 % aluminium trichloride solution and evaluation under long-wavelength UV light. Standards were chromatographed again with a propolis extract. First,

plates were developed with mobile phase 1 (or 2), the eluent was evaporated, standard solutions were applied again, and the plate was rotated through 90 ° and chromatographed again with mobile phase 2 (or 1). The presence (or absence) of all standards was determined according to their Rf values and fluorescence colors. Quantitative determination by absorbance measurement at 254 and 366 nm.

Herbal, densitometry, quantitative analysis, qualitative identification, flavonoids, phenolics 8a

95 021 E. SOCZEWINSKI*, M. WOJCIAK-KOSIOR, G. MATYSIK (*Department of Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Analysis of glycosides and aglycones of flavonoid compounds by double-development thin-layer chromatography. J. Planar Chromatogr. 17, 261-263 (2004). HPTLC of aglycones and glycosides (flavone, hesperetin, naringenin, apigenin, kaempferol, quercetin, myricetin, tiliroside, apigenin 7-glucoside, myricitrin, kaempferol 3,7-dirhamnoside, hyperoside, hesperidin, rhoifolin, rutin, naringin) on silica gel by using mixtures of dichloromethane and ethyl acetate for aglycones and mixtures of ethyl acetate and formic acid for glycosides. The plates were conditioned for 15 min and developed face down in a horizontal chamber. To separate mixtures of flavonoids two-step gradient development was used. In the first step (for glycosides) the plates were developed to a distance of 65 mm with ethyl acetate - formic acid - water 170:30:1. In the second step (for aglycones) the plates were developed to a distance of 95 mm with dichloromethane - ethyl acetate - formic acid 170:30:1. Quantitation by densitometry at 254 nm.

Herbal, HPTLC, densitometry, quantitative analysis, glycosides, aglycones 8a

95 022 M. WÓJCIAK-KOSIOR*, G. MATYSIK, A. SKALSKA (*Department of Chemistry, Medical Academy, Staszica 6, 20-081 Lublin, Poland): Densitometric determination of kinetics of hydrolysis of flavonoid glycosides. J. Planar Chromatogr. 17, 286-289 (2004). HPTLC of isoquercitrin, avicularin, rutin, apigenin 7-glucoside, naringin, and hesperidin on silica gel with ethyl acetate - methanol - formic acid 90:10:1. Detection under UV light at 254 nm and by spraying with 1 % methanolic diphenylboric acid beta-ethylamine ester, followed by spraying with 5 % ethanolic polyethylene glycol 4000. Quantitative determination by densitometry at 254 nm. Report of the possibilities and advantages of HPTLC for investigation of hydrolysis.

Herbal, pharmaceutical research, HPTLC, densitometry, quantitative analysis, hydrolysis, flavonoid glycosides 8a

95 032 M. PROSEK et al., see section 20

10. Carbohydrates

95 023 Gerda MORLOCK*, Shashi PRABHA (*University of Hohenheim, Institute of Food Chemistry 170, Garbenstr. 28, 70599 Stuttgart, Germany, gmorlock@uni-hohenheim.de): Amino phases for derivatization of sucralose in milk-based confection. CBS 94, 14-15 (2005). HPTLC of sucralose on amino phases with acetonitrile - water 4:1 over 70 mm. Stability of sucralose in Burfi, a milk-based confection, was determined over a defined time period. The 2 products of hydrolysis have been monitored as well. Detection by heating for 20 min at 190° C. Quantitative determination by fluorescence measurement under UV 366. Limit of quantitation in the lower ng range.

Food analysis, quantitative analysis, HPTLC, densitometry, sucralose 10a

11. Organic acids and lipids

95 019 Z. MALES et al., see section 8a

95 024 J. A. JARUSIEWICZ, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA 18042, USA): High-performance thin-layer chromatographic analysis of neutral li-

pids and phospholipids in the apple snail Pomacea bridgesii. J. Planar Chromatogr. 17, 454-458 (2004). HPTLC of lipids and phospholipids (tricylglycerols, free sterols, free fatty acids, steryl esters, phosphatidylcholine, phosphatidylethanolamine) on silica gel with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 in a presaturated twin-trough chamber. Detection by spraying with 5 % phosphomolybdic acid in ethanol, followed by heating for 10 min at 115 °C. The neutral lipids are visible as blue spots on a yellow background. For polar lipid analysis, plates were developed with chloroform - methanol - water 65:25:4 and sprayed with 10 % cupric sulfate, followed by heating for 10 min at 140 °C, to detect phospholipids as brown spots on a white background. Quantitative determination by densitometric analysis at 610 nm for neutral lipids and at 370 nm for polar lipids.

Agricultural, HPTLC, quantitative analysis, densitometry 11c

95 025 Iuliana POPA*, Marie-Jeanne DAVID** (*EA 37-32, Laboratory of Dermatology, Pav. R, and ** Laboratory of Biochemistry, Edouard Herriot Hospital, 69437 Lyon Cx 03, France, popa@lyon.inserm.fr. Permanent adress of I. Popa: Institute of Macromolecular Chemistry, Aleea Gr. Ghica Voda 41A, Iassy, Romania): Immunoassay detection of gangliosides by specific antibodies. CBS 94, 11-13 (2005). HPTLC of gangliosides extracted from tissues on silica gel with chloroform - methanol - 0.2% aqueous CaCl2 11:9:2 over 60 mm. Immunoassay detection by dipping in polyisobutylmethacrylate solution and bovine serum albumine solution, followed by immersion in anti-body containing supernatant or patient‘s sera at 4° C overnight. After washing with phosphate-buffered saline detection of Mab binding by stepwise incubation with biotinylated chain-specific anti-mouse immunoglobulin, followed by steptavidin-horsradish peroxidase complex. Visualization with chloro-4-naphtol reagent. Immuno detection is better than chemical derivatization with resorcinol-HCl reagent and has advantages over detection on ELISA microtiter plates.

HPTLC, gangliosides, immunoassay 11e

13. Steroids

95 026 Mária BÁTHORI*, A. HUNYADI, G. JANICSÁK, I. MÁTHÉ (*Department of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6, H-6720 Hungary): TLC of ecdysteroids with four mobile phases and three stationary phases. J. Planar Chromatogr. 17, 335-341 (2004). TLC and HPTLC of 29 ecdysteroids (e. g. 20-hydroxyecdysone, polypodine B, 2-deoxyintegristerone, ajugasterone C, isovitexirone, muristerone A, turkesterone, makisterone C, rubrosterone, poststerone, ecdysone, herkesterone) on silica gel, RP-18, and cyano phase with four mobile phases enabling separation of all the ecdysteroids from each other in at least one system. Detection under UV light at 254 nm or by use of vanillin-sulfuric acid spray reagent. After spraying the spots were either observed in daylight or at 366 nm. Quantitative determination by reflectance-absorbance measurement at 254 nm.

Herbal, HPTLC, densitometry, quantitative analysis, qualitative identification, ecdysteroids 13e

14. Steroid glycosides, saponins and other terpenoid glycosides

95 027 R. T. SANE, M. SASIKUMAR, A. Y. DESHPANDE*, A. A. MENEZES, G. GUNDI (*TDM Laboratory, Plot No. 194, Scheme No, 6, Sion East, Mumbai 400022, India): Quantitation of protodioscin in Tribulus terrestris L. fruit powder by reversed-phase high-performance thin-layer chromatography. J. Planar Chromatogr. 17, 379-382 (2004). HPTLC of protodioscin and fruit powder extracts on RP-18 (prewashed with methanol) with 0.1 M potassium dihydrogenphosphate - acetonitrile - methanol - triethylamine 50:40:10:1 in a twin-trough chamber previously saturated with the mobile phase for 10 min. Detection by dipping in 0.1 M sulfuric acid, drying and heating for 10 min at 80 °C. Densitometric evaluation at 366 nm. Detection and quantitation limits were 0.03 µg and 0.05 µg, respectively. Response was linearly dependent on amount of protodioscin in the range 0.05 to 1.00 µg.

Food analysis, HPTLC, densitometry, quantitative analysis, protodioscin, Tribulus terrestris
14

17. Amines, amides and related nitrogen compounds

95 028 Irena BARANOWSKA*, M. ZYDRON (*Department of Analytical and General Chemistry, Silesian University of Technology, 7 M. Strzody Str., 44-100 Gliwice, Poland): Retention-mobile phase relationships for methylxanthines and biogenic amine metabolites in adsorption thin-layer chromatographic systems. J. Planar Chromatogr. 17, 233-237 (2004). TLC of biogenic amines (dopamine, adrenaline, noradrenaline, 3-methoxytyramine, methanephrine, normethanephrine, vanillylmandelic acid, homovanillic acid, 3,4-dihdroxyphenylacetic acid, 3,4-dihydroxyphenylethyleneglycol, 3-methoxy-4-hydroxyphenylethyleneglycol) and methylxanthines (1-methylxanthine, 7-methylxanthine, theophylline, paraxanthine, theobromine, caffeine) on silica gel with mobile phases comprising a non-polar or weakly polar diluent (chloroform, heptane, or hexane) and a polar modifier (tetrahydrofurane, dioxane, acetone, or ethyl acetate). Examination under UV light or detection with a solution of iron(III) chloride (5 g) and iodine (2 g) in 50 mL of 20 % aqueous tartaric acid. Relationship between Rf values and mobile phase composition was investigated.

Clinical chemistry research, qualitative identification, biogenic amines 17a, 23, 2c

95 030 K. LESKÓ, Livia SIMON-SARKADI*, É. STFANOVITS-BÁNYAI, Z. VÉGH, G. GALIBA (*Budapest University for Technology and Economics, Department of Biochemistry and Food Technology, 1111 Budapest, Müegyetem rkp. 3, Hungary): OPLC analysis of polyamines in wheat seedlings under cadmium stress. J. Planar Chromatogr. 17, 435-437 (2004). OPLC of agmatine, spermine, spermidine, putrescine, cadaverine, histamine, and tyramine and homogeneous fresh plant tissue extracts (as dansyl derivatives) on silica gel with 1) hexane - n-butanol - triethylamine 900:100:91, and 2) hexane - n-butanol 4:1 with overrun development and a stepwise gradient. Off-line quantitative evaluation of the dansyl amines by fluorodensitometry at 313/>400 nm.

Agricultural, quantitative analysis, densitometry, OPLC 17a

95 031 Gertrud MORLOCK* (*Institute of Food Chemistry, University of Hohenheim, D-70599 Stuttgart, Germany): New HPTLC method, with systematic mobile-phase optimization, for determination of six apolar heterocyclic aromatic amines. J. Planar Chromatogr. 17, 431-434 (2004). HPTLC of six heterocyclic amines (2-amino-9H-pyrido[2,3-b]indole, 2-amino-3-methyl-9H-pyrido[2,3-b]indole, norharmane, harmane, 2-aminodipyrido[1,2-alpha:3‘,2‘-d]imidazole, 2-amino-6-methyldipyrido[1,2-alpha:3‘,2‘-d]imidazole) on silica gel by multiple development with diethylether. The mobile phase was selected by using a practical and systematic four-level optimization scheme based on the solvent classification system according to Snyder and the PRISMA model of Nyiredy et al. Quantitative determination by fluorescence measurement at 366/>400 nm was performed immediately after development.

Food analysis, HPTLC, quantitative analysis 17a

18. Amino acids and peptides, chemical structure of proteins

95 007 S. KHAWAS et al., see section 3e

95 019 Z. MALES et al., see section 8a

20. Enzymes

95 032 M. PROSEK*, A. SMIDOVNIK, M. FIR, M. STRAZISAR (*National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia): TLC identification and quantification of coenzyme

Q10-beta-cyclodextrin complex. J. Planar Chromatogr. 17, 181-185 (2004). HPTLC of an inclusion complex of coenzyme Q10 with beta-cyclodextrin on silica gel by one-dimensional, two-dimensional, and multi-dimensional separation with 1) dioxane - water 1:1 and 2) chloroform - methanol 11:9. Detection by spraying with 5 % phosphomolybdic acid in ethanol and drying at 110 °C, followed by spraying with 50 % sulfuric acid and heating at 120 °C for 5 min.

Food analysis, HPTLC, quantitative analysis, densitometry, coenzyme Q10 20, 8b

22. Alkaloids

95 033 T. A. LÓPEZ*, M. L. DE LA TORRE, M. S. CID (*Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnologia Agropecuaria (INTA), Animal Toxicology Laboratory, C. C. 276, Balcarce (7620), Buenos Aires, Argentina): An efficient TLC method for the analysis of gamma-coniceine and coniine in Conium maculatum L. foliage. J. Planar Chromatogr. 17, 218-223 (2004). TLC of gamma-coniceine and coniin on silica gel with chloroform - ethanol 13:7. Detection and quantification by spraying with Dragendorff‘s spray reagent and visual comparison of the intensity of the color of the sample spots with that of the spots of the corresponding standards. Detection limits were 1.7 and 0.7 µg per spot for coniine and gamma-coniceine, respectively.

Toxicology, qualitative identification, quantitative analysis, gamma-coniceine, coniin 22

23. Other substances containing heterocyclic nitrogen

95 028 Irena BARANOWSKA et al., see section 17a

95 035 Joanna NOWAKOWSKA (University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, Al. Gen Hallera 107, PL 80-416 Gdansk, Poland): Use of HPTLC with non-aqueous binary mobile phases for determination of selected porphyrins. J. Planar Chromatogr. 17, 388-390 (2004). HPTLC of uroporphyrins I and III, coproporphyrins I and III, and protoporphyrin on silica gel with binary mobile phases prepared by mixing pure esters, ketones, and xylenes with DMSO in proportions from 0 to 100 %. Detection under UV light at 254 nm. The separation of the porphyrins required the presence of DMSO in the mobile phase.

Pharmaceutical research, HPTLC, qualitative identification, porphyrins 23a

95 034 J. D. VELICKOVIC, D. ANDRIC, G. ROGLIC, Z. L. TESIC, Dusanka M. MILOJKOVIC-OPSENICA* (*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): Planar chromatography of some 1-arylpiperazines behaving as dopaminergic ligands. J. Planar Chromatogr. 17, 255-260 (2004). TLC of fourteen 1-arylpiperazine derivatives on silica gel with monocomponent and binary non-polar mobile phases and on RP-18 with binary mobile phases comprising mixtures of methanol, acetone, or dioxane (as organic modifiers) and water in a horizontal chamber after equilibration for 15 min. Detection under UV light at 254 nm.

Clinical chemistry research, qualitative identification 23e, 32a

24. Organic sulfur compounds

95 036 M. NATIC, R. MARKOVIC, K. ANDELKOVIC, D. MILOJKOVIC-OPSENICA, Z. TESIC* (*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): Reversed-phase thin-layer chromatography of stereodefined 2-alkylidene-4-oxothiazolidines and 1,2-dithiols. J. Planar Chromatogr. 17, 323-327 (2004). TLC of three 1,2-dithioles and five 2-alkylidene-4-oxothiazolidines on RP-18 with binary mixtures of methanol and water, tetrahydrofuran and water, and acetone and water in different proportions. Detection with iodine vapor. Good correlation of chromatographically obtained lipophilicity data with calculated log P values.

Clinical chemistry research, qualitative identification 24, 2d

27. Vitamins and various growth regulators

95 037 G. KÁTAY*, Z. NÉMETH, S. SZANI, O. KÖCK, L. ALBERT, E. TYIHÁK (*Plant Protection Institute, Hungarian Academy of Sciences, P. O. Box 102, H-1525 Budapest, Hungary): Overpressured-layer chromatographic determination of ascorbigen (bound vitamin C) in Brassica vegetables. J. Planar Chromatogr. 17, 360-364 (2004). Analytical OPLC of ascorbigen (ASC, 2-C-[(indol-3-yl)methyl]-alpha-l-threo-l-glycero-3-hexulofuranosonic acid lactone) on silica gel by means of two-step development: the first step (n-hexane) served for elimination of the total wetness front, the second (chloroform - methanol 9:1) for the separation. Detection by spraying with 10 mL Procházka's reagent (reaction with formaldehyde), then heated for 5 min at 105 °C. Quantitative determination by densitometry at 460 nm.

Food analysis, agricultural, qualitative identification, quantitative analysis, densitometry, OPLC 27

28. Antibiotics, Mycotoxins

95 039 Joanna NOWAKOWSKA (Medical University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, Al. Gen. J. Hallera 107, PL 80-416, Gdansk, Poland): Analysis of selected macrocyclic antibiotics by HPTLC with non-aqueous binary mobile phases. J. Planar Chromatogr. 17, 200-206 (2004). HPTLC of macrocyclic antibiotics (erythromycin, troleandomycin, tylosin, vancomycin, rifamycin B, and rifampicin) on LiChrospher silica gel. A wide range of mixtures of alcohols and ketones with hexamethyldisiloxane in proportions of 0 to 100 % and with dimethyl sulfoxide in proportions from 0 to 50 % were used as mobile phases. Separations were performed in chromatographic chambers after presaturation with mobile phase vapor. Detection by spraying with a 1:4 mixture of concentrated sulfuric acid and methanol followed by heating for approximately 10 min at 120 °C.

Quality control, qualitative identification, HPTLC, antibiotics 28a

95 038 C. MARUTOIU*, S. PUIU, M. I. MOISE, L. SORAN, O. F. MARUTOIU, L. BOBOS (* „Lucian Blaga" University from Sibiu, Department of Chemistry, 7-9 Ion Ratiu Street, RO-2400 Sibiu, Romania): Optimization of the separation of some aflatoxins by thin-layer chromatography. J. Planar Chromatogr. 17, 372-374 (2004). TLC of aflatoxins B1, B2, G1, and G2 on silica gel with chloroform - acetone 23:2 in unsaturated chambers. Detection under UV light at 366 nm. Selection of the optimum mobile phase composition by software programs.

Food analysis, toxicology, qualitative identification, aflatoxins 28b

29. Pesticides and other agrochemicals

95 043 T. TUZIMSKI* (*Department of Physical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Separation of a mixture of eighteen pesticides by two-dimensional thin-layer chromatography on a cyanopropyl-bonded polar stationary phase. J. Planar Chromatogr. 17, 328-334 (2004). HPTLC of eighteen pesticides (propaquizafob, quizalofop-P, triadimefon, triadimenol, dimethomorf, quinoxyfen, cyromazine, oxyfluorfen, fluoroglycofen, acetochlor, metazachlor, imazapyr, furalaxyl, triclopyr, buprofezin, pyriproxyfen, fenoxycarb, piperonyl butoxide) on cyano phase. The greatest spread of separated compounds was obtained by combining nonaqueous normal-phase mobile phases (tetrahydrofuran or ethyl acetate in n-heptane 1:4 in the first direction and aqueous reversed phases mobile phases (methanol - water 7:3 or acetonitrile - water 1:1) in the second dimension. Detection under UV light at 254 or 366 nm. Videoscanning and densitometry at 254 nm.

Agricultural, densitometry, quantitative analysis, HPTLC, pesticides 29

95 041 Malgorzata JANICKA*, B. OSCIK-MENDYK, B. TARASIUK (*Department of Planar Chromatography, Faculty of Chemistry, M. Curie-Sklodowska University, M. Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland): Planar chromatography in studies of the hydrophobic properties of some new herbicides. J. Planar Chromatogr. 17, 186-191 (2004). TLC of sixteen new herbici-

des (7 2-(chlorophenoxy)acyl derivatives like e.g.methyl 2,4-dichlorophenoxyacetate, methyl 2-(2,4,5-trichlorophenoxy)propionate and 9 N-aryltrichloroacetamides like e.g. N-(4-chlorophen yl)trichloroacetamide, n-trichloroacetanilide, trichoroacetanilide) on RP-18 with aqueous buffer - methanol mixtures in saturated sandwich chambers. A Reprostar 3 video camera and Videostore software were used for visualization and evaluation of chromatograms.

Agricultural, qualitative identification, herbicides 29d, 2d

95 042 Malgorzata JANICKA*, N. U. PERISIC-JANJIC, J. K. RÓZYLO (*Faculty of Chemistry, Department of Planar Chromatography, Maria Curie-Sklodowska University, Maria Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland): Thin-layer and overpressured-layer chromatography for evaluation of the hydrophobicity of s-triazine derivatives. J. Planar Chromatogr. 17, 468-476 (2004). HPTLC of nine s-triazines on RP-18 and cyano phases with aqueous solutions of different organic modifiers (acetone, acetonitrile, tetrahydrofuran, and dioxane). After development the dried plates were examined under UV light at 254 nm. OPLC on RP-8 and RP-18 with water - acetonitrile. The plates were preconditioned in acetonitrile before development.

Qualitative identification, HPTLC 29d

95 044 Nada U. PERISIC-JANJIC*, T. L. DJAKOVICS-SEKULIC, K. POPOV-PERGAL (*Department of Chemistry, Faculty of Science, University of Novi Sad, Trg D, Obradovica 3, 21000 Novi Sad, Serbia and Montenegro): Correlation between the structure of some 2,4-dioxotetrahydro-1,3-thiazoles and TLC retention data. J. Planar Chromatogr. 17, 192-199 (2004). TLC of 3-ethyloxycarbonyl-5-substituted-2,4-dioxotetrahydro-1,3-thiazole derivatives (2‘-fluoro-6‘-chlorobenzylidene, 1‘-naphthylidene, 4‘-methoxybenzylidene, 2‘-oxybenzylidene, 2‘-thienylidene, 4‘-dimethylaminobenzylidene, 1‘-carboethoxy-3‘-indolylidene, 3‘,4‘-dimethoxybenzylidene, 4‘-isopropylbenzylidene, benzylidene) on silica gel with non-polar diluent (hexane) - polar modifier (ethyl acetate, acetone, or dioxane) and on reversed-phase systems of the type rice starch with polar diluent (aqueous ammonia) - polar modifier (methanol, acetone, or dioxane). Examination of the dried plates after development under UV light at 254 nm.

Agricultural, pharmaceutical research, qualitative identification 29e, 2c

95 040 S. GE (Ge Shimei), F. TANG (Tang Feng), Y. YUE* (Yue Yongde), R. HUA (Hua Rimao), R. ZHANG (Zhang Rong) (* International Center for Bamboo and Rattan, 100102 Beijing, China): HPTLC determination of pyrethroid residues in vegetables. J. Planar Chromatogr. 17, 365-368 (2004). HPTLC of deltamethrin, fenpropathrin, bifenthrin on silica gel (prewashed with chloroform - methanol 1:1) with toluene - petroleum ether 8:3 or cyclohexane - chloroform 1:1 in a twin-trough chamber and in a horizontal chamber. Quantitative determination by densitometric scanning at 254 and 366 nm.

Food analysis, HPTLC, densitometry, quantitative analysis, qualitative identification, pyrethroid residues 29f

30. Synthetic and natural dyes

95 045 Temenushka N. KONSTANTINOVA*, A. S. NEICHEVA, A. Y. VENKOVA (*Organic Synthesis Department, University of Chemical Technology and Metallurgy, 8 Ohridsky str., Sofia 1756, Bulgaria): TLC and HPLC studies of new 9-phenylxanthene dyes. J. Planar Chromatogr. 17, 369-371 (2004). TLC of 9-phenylxanthene derivatives (fluorescein, erythrosine, eosin, rhodamine B, and their allyloxy-derivatives) on silica gel with benzene - methanol 5:1, toluene - ethanol 7:1, acetonitrile - water 7:1, toluene - ethyl acetate - methanol 1:5:2. Detection under UV light at 254 nm or with iodine vapor. Quantitation by densitometric scanning.

Food analysis, cosmetics, densitometry, quantitative analysis 30a

95 046 Temenushka N. KONSTANTINOVA*, R. A. LAZAROVA, P. P. MILADINOVA (*Organic Synthesis Department, University of Chemical Technology and Metallurgy, 8 Ohridsky Str., Sofia

1756, Bulgaria): Thin-layer chromatographic study of some dyes and fluorescent brighteners for polymers. J. Planar Chromatogr. 17, 444-448 (2004). TLC of 16 naphthalimide dyes, 17 benzanthrone dyes, 20 triazine dyes, and 17 fluorescent brighteners on (mainly) silica gel with n-heptane - acetone 1:1 and 3:1, chloroform - methanol 1:1 and 2:1, n-heptane - benzene - chloroform 3:2:1, n-heptane - chloroform - acetone 2:2:1, n-butanol - pyridine - 25 % ammonia 1:1:1, chloroform - methanol - 25 % ammonia 11:5:1, n-propanol - 25 % ammonia 1:1 and 2:1, n-butanol - acetic acid - water 4:1:5. Quantitative determination by UV-scanning densitometry.

Environmental, qualitative identification, quantitative analysis, densitometry, dyes 30a

95 047 J. SHERMA*, B. FRIED (*Department of Biology, Lafayette College, Easton, PA 18042, USA): Separation and determination of chloroplast pigments from spinach by thin-layer chromatography: a student laboratory experiment. J. Planar Chromatogr. 17, 309-313 (2004). TLC of extracted pigments from spinach (carotene, chlorophyll a, lutein, chlorophyll b, violaxanthin, neoxanthin) on silica gel with isooctane - acetone - diethyl ether 3:1:1 or on RP-18 with petroleum ether (35 - 60 °C) - acetonitrile - methanol 1:2:2 in a twin- trough chamber covered with aluminum foil, lined with a saturation pad, and equilibrated with the mobile phase for 15 min before insertion of the plate. Quantitative determination by densitometry at 429 nm. A TLC experiment with great value for students.

Food analysis 30b

32. Pharmaceutical and biomedical applications

95 049 S. B. AGARWAL, N. D. GRAMPUROHIT*, A. S. PAREKAR (*C. U. Shah College of Pharmacy, S.N.D.T. Women‘s University, Santacruz (W), Mumbai 400 049, India): Standardization of herbal formulations containing kurchi (Holarrhena antidysenterica). IPC 56th 2004, Abstract No. D-9. HPTLC for the standardization of the alkaloid conessine in several market formulations containing kurchi bark, on silica gel with toluene - methanol - chloroform 1:2:1. Detection by spraying with Dragendorff‘s reagent. Densitometric evaluation at 460 nm. The method was validated for accuracy, precision, linearity range, specificity, LOD, LOQ and found suitable for routine analysis of herbal formulations containing Kurchi as main ingredient.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, conessine 32a

95 050 Danica AGBABA*, D. NOVOVIC, K. KARLJIKOVIC-RAJIC, V. MARINKOVIC (*Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P. O. Box 146, 11000 Serbia and Montenegro): Densitometric determination of omeprazole, pantoprazole, and their impurities in pharmaceuticals. J. Planar Chromatogr. 17, 169-172 (2004). HPTLC of omeprazole and pantoprazole and their impurities omeprazole sulfone and N-methylpantoprazole on silica gel with chloroform - 2-propanol - 25 % ammonia - acetonitrile (108:12:3:40). Detection under UV light at 254 nm. Quantitation of omeprazole and omeprazole sulfone at 300 nm and of pantoprazole and N-methylpantoprazole at 295 nm in reflectance-absorbance mode. Regression coefficients ($r > 0.998$), recovery (90.7 - 120.0 %), and detection limits (0.025 - 0.05 %) were validated and found to be satisfactory.

Quality control, HPTLC, densitometry, quantitative analysis, omeprazole, pantoprazole 32a

95 053 Mugdha BHOSALE*, A. R. PARADKAR**, K. R. MAHADIK**, K.S. JAIN* (*Sinhgad College of Pharmacy, Vadgaon (Bk), Pune 411 041, India) (** Bharati Vidyapeeth Deemed University‘s, Poona College of Pharmacy, Erandwane, Pune 410038, India): Stability indicating HPTLC determination of cefuroxime axetil as bulk drug and in pharmaceutical formulations. 56th IPC 2004, Abstract No. GP-46. Stability indicating HPTLC determination of cefuroxime axetil in bulk drug and in formulations on silica gel with chloroform - methanol 23:2. Quantitative determination by scanning at 278 nm. The sample was subjected to acidic, and alkali hydrolysis, oxidation and photo degradation. The degraded products were well separated. The method

was validated for accuracy, precision, linearity, specificity, ruggedness, and recovery (98–100 %).

Pharmaceutical research, quality control, quantitative analysis, densitometry, postchromatographic derivatization, comparison of methods, HPTLC, cefuroxime axetil 32a

95 055 Mira CAKAR*, G. POPOVIC, S. VLADIMIROV (*Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P. O. Box 146, 11000 Belgrade, Serbia and Montenegro): Simultaneous HPTLC determination of imidazole antimycotics and parabens in creams. J. Planar Chromatogr. 17, 177-180 (2004). HPTLC of bifonazole, econazole nitrate, methyl- and propylparaben on silica gel with ethyl acetate - n-hexane - methanol - ammonia - diethylamine 5:40:8:4:20 in a twin-trough chamber. Quantitation by scanning in reflectance/absorbance mode at 230 nm (econazole nitrate), 250 nm (bifonazole), and 300 nm (parabens).

Quality control, densitometry, HPTLC, quantitative analysis, qualitative identification, antimycotics, parabens 32a

95 083 M. S. CHARDE*, M. J. UMEKAR, S. B. JOSHI, A. V. KASTURE (*Department of Pharmaceutical Sciences, Nagpur University, Nagpur 440033, India): Estimation of ranitidine HCl and domperidone in combined dosage form using HPTLC. IPC 56th 2004, Abstract No. GP-8. Simultaneous HPTLC determination of ranitidine and domperidone on silica gel with methanol - 1, 4-dioxan 2:3. Quantitative determination by densitometric scanning at 282 nm. Rf values were 0.33 for ranitidine and 0.78 for domperidone. Linearity range was 0.5 - 2.5 mg/mL for both of the drugs. The recovery was in the range of 100.25 - 100.78 %. The method is suitable for the analysis of both drugs in combined dosage form.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, ranitidine, domperidone 32a

95 059 Shruti DHURU*, Pratima TATKE, K. K. SINGH (*C.U.Shah College of Pharmacy, S.N.D.T. Women‘s University, Santacruz (West) , Mumbai 400 049, India): Standardization and evaluation of Neem oil in pharmaceutical formulations by HPTLC. IPC 56th 2004, Abstract No. G-28. Neem Oil obtained from the seed kernels of Azardirachla indica (Meliaceae) is a fixed oil known as oil of Margosa. An HPTLC method is reported for the analysis of Neem oil as a bulk drug and formulations containing oil. TLC of neem oil extracted with chloroform, on silica gel with chloroform - n-hexane - methanol 18:2:1. Quantitative determination by scanning at 254 nm. The linearity range was 100 - 500 mg/mL. Formulations were found to contain 0.35 g/g of Neem Oil.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, neem oil 32a

95 051 S. B. GAICA, D. M. OPSENICA, B. A. SOLAJA, Z. L. TESIC, Dusanka M. MILOJKOVIC-OPSENICA* (*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): The effect of the structure of mixed tetraoxanes on their chromatographic behavior on different adsorbents. J. Planar Chromatogr. 17, 342-349 (2004). TLC of 29 1,2,3,4-tetraoxanes on silica gel, cyano phase, and RP-18. The binary mobile phases ethyl acetate - petroleum ether and ethyl acetate - toluene were used under normal-phase conditions, and water - organic modifier (methanol, acetone, dioxane) under reversed-phase conditions. Chromatography was performed using a HPTLC horizontal developing chamber equilibrated for 15 min with the vapor of the mobile phase. Detection by spraying with 50 % sulfuric acid and heating until the spots became visible.

Pharmaceutical research, qualitative identification 32a

95 061 M. GANDHIMATHI, S. C. VIJAY KUMAR*, T. K. RAVI, Shaise JACOB, Lekha MATHEW, S. MALATHI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS,395, Sarojini Naidu Road, Coimbatore 614044, India): Simultaneous estimation of Loratadine and Ambroxol from formulation by HPTLC. IPC 56th 2004, Abstract No. G-20. Simultaneous HPTLC

determination of loratadine and ambroxol in combined dosage form on silica gel with n-hexane - dichloromethane - triethanolamine 11:8:1.The Rf value of loratadine and ambroxal was found to be 0.40 and 0.16 respectively. Quantitative evaluation by scanning at 254 nm. The method was linear in the range of 0.2 -1 mg/spot for loratadine and 1.2 - 6 mg/spot for ambroxol with recovery of 98.2 - 98.5 %. The method was validated for accuracy, precision, linearity, specificity, LOD, and LOQ.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, HPTLC, loratadine, ambroxol 32a

95 082 K. R. GUPTA*, A. N. MALIYE, M. R. TAJNE, S. G. WADODKAR (*Department of Pharmaceutical Sciences, Nagpur 440033, India): Stability indicating HPTLC determination of indapamide in tablets. IPC 56th 2004, Abstract No. GP-11. Stability indicating HPTLC determination of indapamide in tablets on silica gel with toluene - methanol 7:3. Quantitative determination by scanning at 246 nm. Optimization of experimental parameter such as band size, chamber saturation, and slit width. The method was linear in the range of 1.4 - 3.72 g, recovery was 100.01 %. The drug was subjected to stress conditions according to ICH guidelines, degradation products were separated from the pure drug. The method was validated for accuracy, precision, linearity, and specificity.

Pharmaceutical research, quality control, quantitative analysis, densitometry, postchromatographic derivatization, comparison of methods, HPTLC, indapamide 32a

95 066 H. HOPKALA, A. POMYKALSKI* (*Department of Medicinal Chemistry, Faculty of Pharmacy, Prof. Skubiszewski Medical Universit y of Lublin, 6 Chodzki St., 20-093 Lublin, Poland): TLC analysis of non-steroidal anti-inflammatory drugs and videodensitometric determination of fenbufen in tablets. J. Planar Chromatogr. 17, 383-387 (2004). TLC of fenbufen, ibuprofen, ketoprofen, diclofenac sodium, mefenamic acid, and tiaprofenic acid on silica gel by ascending and horizontal techniques, and on RP-18 in horizontal chambers. Good separation was achieved on silica gel by horizontal development with chloroform - methanol - 25 % ammonia 67:25:8; reversed phase chromatography on RP-18 with 0.15 mol/L phosphate buffer, pH 5.73 - 10 % CTMA-Br (N-cetyl-N,N,N-trimethylammonium bromide) in methanol 7:13 enabled better separation of the six drugs. Detection under UV light at 254 nm - for ibuprofen detection was best achieved after normal phase chromatography with 20 % aqueous sodium carbonate solution. A simple videodensitometric procedure was developed and validated. RSD for quantitation of fenbufen was 2.44 - 3.10 %.

Quality control, densitometry, quantitative analysis, non-steroidal anti-inflammatory drugs 32a

95 068 A. JASHIDI (Department of Novel Drug-delivery Systems, Iran Polymer and Petrochemical Institute, P. O. Box 14185/458, Tehran, Iran): A convenient and high throughput HPTLC method for determination of progesterone in release media of silicon-based controlled-release drug-delivery systems. J. Planar Chromatogr. 17, 229-232 (2004). HPTLC of progesterone on silica gel in an automatic multiple development chamber (AMD) with toluene - 2-propanol 9:1 without chamber saturation and with 10 min drying time. Visual inspection under UV light at 254 nm. Quantitative determination in reflectance mode at 252 nm. Limits of quantitation and detection were 25 and 5 ng/zone.

Quality control, AMD, HPTLC, densitometry, quantitative analysis, progesterone 32a

95 069 N.S. JEGANATHAN*, M. RAJ MOHMED, R.MANAVALAN (*Dept.of Pharmacy, Annamalai University, Annamalai Nagar -608002 TN, India): Quantitative determination of piperine in Trikatukuc Curanam by HPTLC. IPC 56th 2004, Abstract No. DP-33. An HPTLC method is reported for the standardization of Trikatukuc Curanam, an Ayurvedic preparation with Piper nigrum and Piper longum, both containing piperine as major alkaloid. HPTLC of piperine on silica gel with toluene - ethyl acetate 7:3. Extraction with methylene chloride, the evaporated residue of

the organic layer was taken in ethyl acetate and subjected to the analysis. The band corresponding to piperine was scanned at 338 nm. Linearity was 8-40 ng with recovery of 99.03 %.

Pharmaceutical research, quality control, postchromatographic derivatization, densitometry comparison of methods, quantitative analysis, piperine 32a

95 003 H. KALÁSZ et al., see section 2a

95 070 N. KAUL, H. AGRAWAL, A. R. PARADKAR, K. R. MAHADIK* (*Department of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune-411038, Maharashtra State, India): Stability-indicating high-performance thin-layer chromatographic determination of zidovudine as the bulk drug and in pharmaceutical dosage forms. J. Planar Chromatogr. 17, 264-274 (2004). HPTLC of zidovudine (3'-azido-3'-deoxythymidine) and degradation products on silica gel with toluene - carbon tetrachloride - methanol - acetone 35:35:20:1. Quantitative determination by absorbance measurement at 270 nm. The method was validated for precision, robustness, and recovery. Limit of detection was 20 ng per spot, limit of quantitation 40 ng.

HPTLC, densitometry, quantitative analysis, zidovudine 32a

95 071 Amandeep KAUR*, Prateek K. JAIN* and R. K. AGRAWAL (*Pharmaceutical Chemistry, Research Laboratory, Department of Pharmaceutical Science, Dr. Hari Singh gour University Sagar m.p. 470003, India): TLC densitometric method for the quantification of conessine in Holarrhena antidysenterica. IPC 56th 2004, Abstract No. G-5. HPTLC of conessine in Holarrhena antidysentrica, an important ayurvedic drug, on silica gel with toluene - ethyl acetate - diethyl amine 13:5:2. Detection by spraying with Dragendorff's reagent. Quantitative determination by densitometric scanning at 520 nm. Different market samples of the drug were found to contain 0.30 - 1.46 % of conessine with recovery of 95.18 - 102.70 %

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, HPTLC, conessine 32a

95 060 M. G. PAI*, Dattesh VEREKAR, K. VENKATESHWAR RAO (*Goa College of Pharmacy, Panaji, Goa, India): Development and validation of a new sensitive method for the simultaneous estimation of amlodipine - atenolol in tablets by HPTLC. IPC 56th 2004, Abstract No. GP-5. Simultaneous HPTLC determination of amlodipine and atenolol on silica gel with ethyl acetate - methanol - ammonia 60:40:3. Quantitative determination by scanning at 254 nm. The method was found linear in the range of 0.5 - 5.0 mg/mL amlodipine and 5.0 mg - 50 mg/mL atenolol. Recovery was 98.11 - 101.5 % for both of the compounds. The method was validated for accuracy, precision, linearity, specificity, LOD, and LOQ.

Pharmaceutical research, quality control, quantitative analysis, postchromatographic derivatization, comparison of methods, densitometry, HPTLC, amlodipine, atenolol 32a

95 078 R. PIETRAS*, H. HOPKALA, D. KOWALCZUK, A. MALYSZA (*Department of Medicinal Chemistry, Medical University, Chodzki 6, 20-093 Lublin, Poland): Normal-phase TLC separation of some antiarrhythmics. Densitometric determination of mexiletine hydrochloride in capsules. J. Planar Chromatogr. 17, 213-217 (2004). TLC of disopyramide, flecainide, mexiletine, tocainide, and verapamil on aluminium oxide and silica gel in horizontal chambers. The best mobile phase for separation on the alumina plates was tetrahydrofuran - hexane - 25 % ammonia 25:24:1 and on silica chloroform - tetrahydrofuran - ethanol - 25 % ammonia 81:19:20:1. Detection under UV light at 210 nm and by use of different reagents. Quantification of mexiletine hydrochloride in capsules was performed densitometrically at 254 nm. Correlation coefficient in the concentration range 20 - 45 µg per band was 0.9974, with RSD of 5.23 %.

Quality control, quantitative analysis, densitometry, antiarrhythmic drugs 32a

95 080 P. N. PRESANNAKUMARAN, Ann Mary ISAAC* (Thejus Tharu. College of Pharmaceutical Sciences, Medical College, Trivandrum, India): Estimation of rabeprazole using HPTLC. 56th IPC 2004, Abstract No. GP-48. HPTLC of rabeprazole sodium in tablet dosage form on silica gel with ethyl acetate - methanol 9:1. Optimization of experimental parameters such as bandwidth, chamber saturation time, solvent front migration, and mobile phase composition. Quantitative determination by scanning at 260 nm. The Rf value was 0.59. The method was linear with a correlation coefficient of 0.99, recovery was 98.81 %.

Pharmaceutical research, quality control, comparison of methods, postchromatographic derivatization, quantitative analysis, densitometry, HPTLC, rabeprazole 32a

95 081 Alina PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4, Jagillonska Street, PL-41200 Sosnowiec, Poland): Study of lipophilicity and application of selected topological indexes in QSAR analysis of nicotinic acid derivatives. Part I. J. Planar Chromatogr. 17, 275-279 (2004). HPTLC of nicotinic acid and selected derivatives (methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, hexyl nicotinate, benzyl nicotinate, nicotinamide, N-methyl nicotinamide) on RP-18 with methanol - water in different volume proportions after chamber saturation for 30 min. Detection under UV light at 254 nm. Investigation of the lipophilicity by TLC and use of the data for quantitative structure-activity relationships.

Pharmaceutical research, HPTLC, qualitative identification, quantitative structure-activity relationships 32a

95 079 T. K. RAVI, Prabhathi KITANIA, M. GANDHIMATHI, P. RAVIMATHI*, Satheesh KUMAR N. (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, 395, Sarojini Naidu Road, Coimbatore 641 044, India): HPTLC method for the estimation of mirtazapine from tablet formulation. IPC 56th 2004, Abstract No. GP-17. HPTLC of mirtazapine in tablet dosage form on silica gel with chloroform - methanol 1:9. The Rf value was 0.50 - 0.52, the linearity range was 0.3 - 1.5 mg/spot. Quantitative determination by scanning at 295 nm. The method was validated for accuracy, precision, linearity, specificity, LOD, and LOQ.

Pharmaceutical research, quality control, comparison of methods, postchromatographic derivatization, quantitative analysis, densitometry, HPTLC, mirtazapine 32a

95 105 M. SAJEWICZ et al., see section 38

95 091 R. T. SANE, S. N. MENON, M. MOTE*, S. INAMDAR, A. MENEZES (*TDM laboratories, Plot No. 194, Scheme No. 15, Road No. 15, Sion (E), Koliwada, Mumbai-22, India): High-performance thin-layer chromatographic determination of aceclofenac in the bulk drug and in pharmaceutical preparations. J. Planar Chromatogr. 17, 238-240 (2004). HPTLC of aceclofenac and mosapride citrate (as internal standard) on silica gel in a twin-trough chamber equilibrated with the mobile phase with toluene - methanol - ethyl acetate - glacial acetic acid 550:250:200:1. Quantitative determination by densitometry at 284 nm.

Quality control, densitometry, HPTLC, quantitative analysis, aceclofenac 32a

95 085 Sapna SHRIKUMAR, A. SAIT, A. JITENDRA*, M. SUKUMAR, T. K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore-614044, India): An HPTLC method for the standardization of Curculigo Orchioides and its formulations for antioxidant activity using gallic acid as standard. IPC 56th 2004, Abstract No. G-18. Curculigo Orchioides (Amary llidaceae) is used in various ayurvedic formulations. The rhizomes contain about 5.78 % total phenolis, gallic acid being the major component of the alcoholic extracts. HPTLC of gallic acid on silica gel with toluene - ethyl acetate - glacial acetic acid 25:15:1. The Rf value of gallic acid was 0.19, the linearity range of 150-750 ng/spot. Rhizomes were found to contain 2.54 % gallic acid, formulations contained 5.13 % gallic acid, recovery was 99.5 %.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, HPTLC, Curculigo Orchioides, gallic acid 32a

95 086 Sapna SHRIKUMAR, A. SAIT, Manju GOPI*, A. SUGANTHI, M. SUKUMAR, T. K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore 641 044, India): HPTLC method for the standardization of Aphanamixis Polystachya for its antioxidant activity using gallic acid as standard. 56th IPC 2004, Abstract No. GP-36. HPTLC for the standardization of gallic acid in alcoholic extracts of Aphanamixis polystachya (Meliceae) on silica gel with toluene - ethyl acetate - formic acid - methanol 15:15:4:1. Rf value of gallic acid was 0.45, linearity was 15 - 75 mg/mL. Formulations were found to contain 9.56 % of gallic acid. Gallic acid is the main phenolic compound and can be used for standardization of the crude drug.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, Aphanamixis polystachya, gallic acid 32a

95 087 Sapna SHRIKUMAR*, S. CICY, M. SUKUMAR, T. K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore 641 044, India): HPTLC method for the estimation and quantification of gallic acid in some ayurvedic formulations of triphala. IPC 56th 2004, Abstract No. GP-26. HPTLC of triphala, an ayurvedic formulation containing about 3.60 % of total phenolics. Separation of alcoholic triphala extracts on silica gel with n-hexane - ethyl acetate 2:1. Rf value of the main spot gallic acid was 0.04 in triphala and its formulation. The method was found to be very specific for gallic acid having a linearity range of 0.2 - 1.6 mg/mL. Several formulations analyzed by HPTLC contained 5.2 - 7.6 % of gallic acid. The reported method is suitable for estimation of gallic acid in raw material and formulations.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, triphala 32a

95 088 R. SKIBINSKI, Genowefa MISZTAL* (*Department of Medicinal Chemistry, Medical University of Lublin): Determination of fluvoxamine and moclobemide in tablets by densitometric and videodensitometric TLC. J. Planar Chromatogr. 17, 224-228 (2004). TLC of fluvoxamine and moclobemide on silica gel in horizontal chambers with benzene - acetone - ethanol - 25 % ammonia 9:7:2:1. Densitometric detection and quantification were performed at 249 nm and 236 nm, respectively. The range of linearity was 1 - 10 µg per spot; the RSD was less than 2.5 % for densitometry and less than 5.1 % for videodensitometry.

Quality control, densitometry, quantitative analysis, fluvoxamine, moclobemide 32a

95 009 B. SPANGENBERG et al., see section 3f

95 089 G. SUBRAMANIAN, CH. SRIDEVI NAIDU, Gautam MISHRA, Varadaraj BHAT, N. UDUPA* (*College of Pharmacy, Manipal, Karnataka, India): Stability indicating HPTLC determination of oxcarbazepine in tablets. 56th IPC 2004, Abstract No. GP-47. Stability indicating HPTLC determination of oxcarbazepine in tablet dosage form on silica gel with toluene - methanol 4:1. The Rf value of oxcarbazepine was 0.17. Quantitative determination by scanning at 255 nm. The compound was subjected to acid and alkali hydrolysis, oxidation, dry heat, and photo degradation. All degraded products were well resolved from the pure drug. The method was validated for accuracy, precision, linearity, robustness, and recovery.

Pharmaceutical research, quality control, postchromatographic derivatization, comparison of methods, quantitative analysis, densitometry, HPTLC, oxcarbazepine 32a

95 090 A. SUGANTHI VIPIN PRAKASH*, Sapna SHRIKUMAR, K. A. Mirkasim, T. K. RAVI (*College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641 044, India): HPTLC method for the simultaneous estimation of valdecoxib and tizanidine hydrochlo-

ride in tablets. IPC 56th 2004, Abstract No. GP-3. Simultaneous HPTLC determination of valdecoxib and tizanidine in tablets on silica gel with n-butyl acetate - formic acid - chloroform 7:3:2. Quantitative determination by densitometric scanning at 283 nm. The Rf values of valdecoxib and tizanidine were 0.78 and 0.39 respectively. Linearity range was 200 - 1000 ng/spot and 60 - 300 ng/spot respectively. Mean recovery for both of the compounds was 99.57 - 101.28 %. The method was validated for accuracy, precision, linearity, LOD, and LOQ.

Pharmaceutical research, quality control, densitometry, quantitative analysis, comparison of methods, postchromatographic derivatization, HPTLC, valdecoxib, tizanidine 32a

95 093 S. TAMBE, S. KALE, P. SHAH, S. CHHAJED* (*M.G.V's Pharmacy College, Panchavati, Nasik, India): HPTLC analysis of beta-carotene in oral solid dosage forms. IPC 56th 2004, Abstract No. CP-32. A stability indicating HPTLC method has been developed for the analysis of solid dosage forms containing beta-carotene. HPTLC of beta-carotene on silica gel with petrol ether (40-60 °C) - methanol - toluene 4:8:1. Rf value of beta-carotene was 0.65-0.70. Quantification by densitometric evaluation at 460 nm. The method was validated for accuracy, precision, linearity, and stability, and can be adopted for routine analysis of beta-carotene in formulations.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, beta-carotene 32a

95 094 S. TAMBE, S. KALE, S. KULKARNI*, S. CHHAJED (*M.G.V's Pharmacy College, Panchavati, Nasik, India): HPTLC analysis of ondansetron in oral solid dosage forms. IPC 56th 2004, Abstract No. GP-15. Stability indicating HPTLC determination of ondansetron in solid oral dosage forms on silica gel with chloroform - methanol 4:1. Quantitative determination by scanning at 310 nm. The Rf value was 0.62 - 0.64, linearity was 40 - 120 ng. The average recovery was 100.01 %. The method was found suitable for routine analysis of formulations containing ondansetron.

Pharmaceutical research, quality control, densitometry, quantitative analysis, comparison of methods, postchromatographic derivatization, HPTLC, ondansetron 32a

95 034 J. D. VELICKOVIC et al., see section 23e

95 056 J. CHEN (Chen Jiatang)*, J. LIU (Liu Junyi), J. SU (Su Juan) (*Nanjing Tongrentang Pharm. Co., Ltd., Nanjing 210012, China): (Study of the quality standard for compound Yiqi granules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 167-169 (2005). TLC on silica gel with 1) ethyl acetate - methyl ethyl ketone - formic acid - water 10:1:1:1; 2) chloroform - methanol 20:1; 3) chloroform - formic acid - water 13:7:2. Detection 1) under UV 254 nm; 2) by spraying with 10 % H2SO4 in ethanol and heating at 105 ºC. Identification by fingerprint technique. Quantification of astragaloside IV by densitometry at 530 nm. Validation of the method by investigation of linearity (1.12 µg - 5.60 µg, r = 0.999); precision (RSD = 1.63 %, n= 5 within plate and RSD = 2.3 % plate to plate); reproducibility of five time assay towards the same sample (RSD = 3.12 %); standard addition recovery (97.69 %, RSD = 2.10 %, n = 5). The results for three real life samples are given.

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

95 057 ZH. CHEN (Chen Zhongyi)*, T. YAO (Yao Tongwei), Y. PENG (Peng Yunzhen), ZH. ZHANG (Zhang Zhijian (*Dep. Pharm. Sci., Zhejiang Univ., Hangzhou, Zhejiang 310006, China): (Assay and related impurity detection for magnesium fructose - diiphosphate) (Chinese). J. Chinese Pharm. Anal. 25 (1), 86-90 (2005). TLC of fructose, fructose-6-phosphate and related impurities on silica gel - carboxy methyl cellulose (CMC) -Na phase with n-butanol - acetone - glacial acetic acid - ammonia - water 35:15:20:3:27. Detection by spraying with 1 % sodium periodate solution followed by spraying with a solution of benzidine - ethanol - acetone - hydrochloric acid

- water 0.8 g:80 mL:30 mL:1.5 mL:70 mL. Identification by fingerprint technique. Quantification by comparison with standards. The detection limits were investigated. In addition, the content of magnesium fructose diphosphate was determined by diphenylamine colorimetric method, and the related impurities are determined with the phosphomolybdic acid colorimetric method.

Pharmaceutical research, herbal, quality control, qualitative identification, quantitative analysis, magnesium fructose, diphosphate, diphenylamine colorimetric method 32c

95 058 J. CUI (Cui Jiucheng)*, X. SONG (Song Xiaomei), Y. CAI (Cai Yan) (*Shanxi Coll. TCM, Xianyang, Shanxi, 712083, China): (Analysis of the processing principle of Fructus Schisandrae Sphenantherae by steaming with wine) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 176-178 (2005). HPTLC on silica gel with 1) cyclohexane; 2) cyclohexane - ethyl acetate 9:1. Detection 1) by spraying with 5 % phosphomolybdic acid in ethanol; 2) by spraying with vanillin - conc. H2SO4 solution. Identification of volatile oil by fingerprint technique. Determination of total lignan content by spectrophotometry. Analysis of the processing principle by comparison of the contents of the volatile oil and lignans in the extracts obtained by using different processing procedures, and discussing of the optimal processing procedures.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, volatile oil 32c

95 062 J. GAO (Gao Jiarong)*, J. ZHANG (Zhang Junru) (*No.1 Affiliated Hosp., Anhui Coll. TCM, Anhui, Hefei 230031, China): (Study of the quality standard for Qieyou Tangjiang extract) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (1), 94-96 (2005). HPTLC on silica gel with 1) n-butanol - glacial acid - water 19:5:5; 2) petroleum ether (30-60 ºC) - formic acetate - formic acid 15:5:1; 3) chloroform - ethyl acetate - methanol - formic acid 200:25:50:1. Detection 1) by spraying with 0.5 % ninhydrin in ethanol; 2) under UV 365 nm; 3) by spraying with vanillin - H2SO4 solution and heating. Identification by fingerprint technique. Quantification of emodin by densitometry at 440 nm. Validation of the method by investigation of linearity (0.1 µg - 0.5 µg, r = 0.998); precision (RSD = 3.8 %, n = 15 within plate and RSD = 3.2 %, n = 5 plate to plate); reproducibility of five time assay towards the same sample (RSD = 4.4 %); standard addition recovery (99.5 %, RSD = 2.2 %, n = 5). The results for some real life samples are given.

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

95 063 CH. GUO (Guo Changqiang)*, J. LIU (Liu Jinxing), M. ZHANG (Zhang Min), Y. LI (Li Yan), CH. ZHOU (Zhou Chuanguo) (*Shandong Acad. TCM, Jinan, Shangdong 250014, China): (Study of the quality standard for Yijing Bushen granules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), 716-719 (2004). TLC on silica gel with 1) ethyl acetate - chloroform - formic acid 3:2:1; 2) chloroform - methanol 7:2; 3) petroleum ether (60-90 ºC) - ethyl acetate 7:3. Detection 1) under UV 365 nm; 2) by spraying with 10 % H2SO4 in ethanol and heating at 105 ºC for 5 min; 3) by spraying with vanillin - H2SO4 solution. Identification by fingerprint technique. Quantification of icarrin by HPLC.

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

95 064 J. GUO (Guo Jingqiang)*, R. NIU (Niu Ruijie), G. HUANG (Huang Guifen) (*Tianjin municip. Inst. Drug Cont., Tianjin 300070, China): (Determination of astragaloside in Zilongjin tablets by thin-layer chromatography) (Chinese). J. Chinese Trad. and Herb. Drugs (Zhongcaoyao), 36 (2), 222-224 (2005). TLC on silica gel with chloroform - methanol - water 70:35:4. Detection by spraying with 10 % H2SO4 in ethanol and heating at 105 °C for 5 min. Identification by fingerprint technique. Quantification by densitometry at 530 nm. Validation of the method by investigation of linearity (0.458 µg - 2.748 µg, r = 0.998); precision (RSD = 2.48 %, n = 5 within plate and RSD = 4.69 % plate to plate); reproducibility of five time assay towards the same sample (RSD = 2.41 %); and standard addition recovery (96.11 %, RSD = 1.91 %, n = 5). The results for

real life samples are given.

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

95 065 W. GUO (Guo Wenping), X. BAI (Bai Xiaoshi)*, L. LI (Li Laixiu) (*Sanmenxia People's Hosp., Sanmenxia, Henan 472000, China): (Preparation of Tianma Toufengling capsules and study of its quality standard) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 246-248 (2005). TLC of extracts prepared by different processing technology on silica gel with 1) petroleum ether (60 - 90 ºC) - chloroform - methanol 10:3:2; 2) chloroform - methanol 5:1; 3) benzene - glacial acetic acid 4:1. Detection 1) under UV 254 nm; 2) by spraying with 1 % vanillin - H2SO4 solution and heating at 105 ºC. Identification by fingerprint technique. Quantification of gastrodine by HPLC. Discussion of using the procedures for monitoring the preparation process and the quality control of the medicine products.

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

95 067 SH. HU (Hu Shuangfeng) (Ningbo Municip. Inst. Drug Cont., Ningbo, Zhejiang 315040, China): (Differentiation and identification of Xanthium sibiricum Patr. seed and the phoney, Xanthium Spinosam L. seed) (Chinese). Chinese J. Hosp. Pharm. (Zhongguo Yiyuan yaoxue Zazhi) 25 (2), 185-187 (2005). HPTLC on silica gel with n-butanol - glacial acetic acid - water 4:1:5. Detection by exposing to ammonia vapors. Identification by fingerprint technique combined with morphological differentiation and UV spectra comparison.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, differentiation and identification 32c

95 072 CH. LI (Li Chuncheng), X. YANG (Yang Xinghao)*, J. CUI (Cui Jinghao, Y. WANG (Wang Yanfei), J. ZHU (Zhu Jia) (*Pharm. R & D Centre, Nanjing Normal Univ., Nanjing 210097, China): (Separation and purification of the active fraction of Sinisan powder with macroporous resins) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (1), 84-87 (2005). TLC screening of Sinisan powder extracts, purified with macroporous resins, on silica gel with 1) n-butanol - ammonia - ethanol 7:3:1; 2) chloroform - methanol 7:1. Detection 1) by spraying with 5 % AlCl3 in ethanol 2) by spraying with 5 % vanillin - H2SO4 solution. Identification by fingerprint technique. Screening of purification conditions by evaluation of the content of the active principle, and yield of the purified products. Type HP20 macroporous resin has been concluded to be the optimum for active fraction of the recipe in purification efficiency.

Herbal, pharmaceutical research, traditional medicine, quality control, qualitative identification, separation and purification 32c

95 073 C. LI (Li Cunman)*, L. LI (Li Lanfang), Q. ZHANG (Zhang Qinzeng) (*Hebei Provin. Acad. TCM, Shijiazhuang, Hebei 050021, China): (Study of the quality standard for complex Xiaojingtong capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (8), 631-634 (2004). TLC on silica gel with 1) n-hexane - ethyl acetate 9:1; 2) chloroform - diethyl ether 1:1; 3) n-butanol - ethyl acetate - water 4:1:5. Detection by 1) exposing to iodine vapors; 2) spraying with 10 % H2SO4 in ethanol and heating at 105 ºC. Identification by fingerprint technique. Quantification of astragaloside by densitometry at 530 nm. Validation of the procedure by investigation of linearity (1.1 - 5.5 µg per spot), precision (RSD = 1.82 %, n = 5 within plate and 1.99 %, n = 5 plate to plate), repeatability by standard addition recovery (100.1 %, RSD = 1.59, n = 6), etc. The results are given for a group of real samples.

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

95 074 F. LI (Li Fengqin) (Puyang Municip. Inst. Drug Cont., Puyang, Henan 457000, China): (Identi-

fication of the main components and the dosage optimization in Shujin Qiefeng capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), app.17-19 (2004). HPTLC on silica gel with 1) toluene - acetone - ethanol - ammonia 20:25:3:2; 2) n-hexane - ethyl acetate - glacial acetic acid 15:5:1; 3) n-hexane - ethyl acetate - ammonia 20:20:1. Detection 1) by spraying with 5 % potassium iodobismuthate solution; 2) by spraying with 1 % potassium permanganate in diluted sulfuric acid followed by heating at 120 ºC, and under UV 360 nm. Identification by fingerprint technique. Determination of the content of aconitine by comparison with standard.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, aconitine 32c

95 076 Q. MENG (Meng Qing)*, H. LIANG (Liang Hanming), G. CHEN (Chen Gengfu), X. GUO (Guo Xiaoling), Y. FENG (Feng Yifan) (*Guangdong Coll. Pharm. Guangzhou 510224, China): (Study of the quality standard for Tongfeng Huadyting tincture) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 158-161 (2005). HPTLC on silica gel with 1) cyclo-hexane - ethyl acetate - methanol 4:5:1; 2) n-hexane - ethyl acetate 3:1; 3) n-hexane - ethyl acetate 9:1; 4) cyclohexane - ethyl acetate - diethylamine 45:20:3. Detection 1) under UV 365 nm; 2) by spraying with 10 % H2SO4 in ethanol and heating; 3) by spraying with diluted potassium iodobismuthate solution followed by spraying with sodium nitrite solution in ethanol. Identification by fingerprint technique. Semi-quantitative determination of aconitine by comparison with the standard. Quantification of strychnine by HPLC. The results for some real life samples are given.

Pharmaceutical research, traditional medicine, quality control, HPTLC, densitometry, quantitative analysis, qualitative identification, aconitine, strychnine 32c

95 077 Q. PENG (Peng Qian)*, H. ZHAO (Zhao Hua), GUO ZHANG (Zhang Guozhu) (*Hanzhong municip. Inst. Drug Cont., Hanzhong, Shanxi 723000, China): (Pharmacognostic identification of Saruma henryi and differentiation of Asarum siebodii) (Chinese). J. Chinese Trad. and Herb. Drugs (Zhongcaoyao), 36 (2), 277-280 (2005). HPTLC on silica gel with toluene - ethyl acetate - water - formic acid 20:10:1:1. Detection under UV 365 nm. Identification of volatile oil by fingerprint technique, combined with microscopy and a chemical method. Quantification of aristolochic acid A by HPLC.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, aristolochic acid A 32c

95 084 L. SHEN (Shen Linni)*, H. YU (Yu Haihong), Y. ZHENG (Zheng Yan) (*Zhejiang Deqing County TCM Hosp., Deqing, Zhejiang 313200, China): (Determination of chlorogenic acid in Liyin tablets by thin-layer chromatography) (Chinese). Chinese J. Hosp. Pharm. (Zhongguo Yiyuan yaoxue Zazhi) 25 (1), 90-92 (2005). TLC on silica gel with chloroform - ethyl acetate - formic acid 2:2:1. Detection under UV light. Identification by fingerprint technique. Quantification by densitometry at 325 nm. Validation of the method by investigation of linearity (0.52 µg - 4.68 µg, r = 0.9992); precision (RSD = 2.20 %, n = 5); reproducibility of five time assay towards the same sample (RSD = 3.10 %); standard addition recovery (99.0 %, RSD = 0.26 %, n = 5). The results for three real life samples are given.

Pharmaceutical research, traditional medicine, quality control, herbal, doping, quantitative analysis, qualitative identification, densitometry, chlorogenic acid 32c

95 095 J. TANG (Tang Jingwen) (Shanghai Shuangji Pharm. Co., Ltd., Shanghai 201319, China): (Study of the quality control for Kangmoling capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 150-154 (2005). TLC on silica gel with 1) chloroform - methanol 15:2; 2) n-hexane - ethyl acetate 9:1; 3) toluene - ethyl acetate - acetone - methanol 50:25:25:3. Detection 1) by spraying with 10 % phosphomolybdic acid and heating; 2) under UV 365 nm; 3) by exposing to acetic anhydride vapors and heating at 140 - 160 ºC and under UV 365 nm. Identification by fingerprint technique. Quantification of flavone glycoside by HPLC. The results for three real life samples are given.

Pharmaceutical research, traditional medicine, quality control, densitometry, quantitative analysis, qualitative identification, flavone glycoside 32c

95 096 X. WANG (Wang Xiaoling) (Luoyang Municip. Inst. Drug Cont., Luoyang, Henan 471003, China): (Identification of the medicinal herb rhubarb and its preparations by thin-layer chromatography) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), app.19-20 (2004). HPTLC on silica gel with petroleum ether (30 - 60 ºC) - formic acetate - formic acid 15:5:1, at 11 ºC and humidity of 40 %. Detection by exposing to ammonia vapors. Identification by fingerprint technique and comparison with the standards.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, emodin 32c

95 097 M. XIN (Xin Meiyu) (Guangdong Wannianqing Pharm. Co., Ltd., Shantou, Guangdong 515031, China): (Study of the quality standard for Buxie Danggui extract) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 225-227 (2004). TLC on silica gel with 1) petroleum ether (30 - 60 ºC) - ethyl acetate 9:1; 2) benzene - glacial acetic acid 4:1. Detection 1) by spraying with 1 % vanillin solution and heating at 105 ºC for 10 min; 2) under UV 365 nm. Identification by fingerprint technique. Quantification of ferulic acid by densitometry at 325 nm. Validation of the method by investigation of linearity (0.16 µg - 1.6 µg, r = 0.9992); precision (RSD = 1.8 %, n= 5 within plate and RSD = 2.3 % plate to plate); reproducibility of five time assay towards the same sample (RSD = 0.5 %); and standard addition recovery (97.17 %, RSD = 0.9 %, n = 5). The results for three real life samples are given.

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

95 098 J. ZHANG (Zhang Junping)*, X. HUANG (Huang Xiaolan), H. LE (Le Haiping) (*Nanchang Municip. Inst. Drug Cont., Nanchang, Jiangxi 330003, China): (Study of the quality control of Kangbingdu oral liquid) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), App.12-14 (2004). TLC on silica gel with 1) n-butanol - glacial - water 19:5:5; 2) chloroform - methanol - glacial acetic acid - 17:2:1; 3) two fold development with benzene - acetone 9:1. Detection 1) by spraying with ninhydrin solution and heating at 105 ºC; 2) by spraying with 5 % vanillin solution and heating; 3) by spraying with a solution of 8 % vanillin in ethanol - H2SO4, and heating at 105 ºC. Identification by fingerprint technique. Quantification of phyllyrin by densitometry at 280 nm. The quantitative procedure is validated by investigating its linearity (1 - 5 µg/spot, r = 0.9998); precision (RSD = 0.36 % n = 5); repeatability (RSD = 2.92 % n = 5) and standard addition recovery (99.6 %, RSD = 2.4 %, n = 5), etc. The determination results are given for a group of real life samples.

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

95 099 Y. ZHANG (Zhang Yujie)*, H. HUANG (Huang Haixin), H. Tian (Tian Hong) (*Nanyang Municip. Inst. Drug Cont., Nanyang, Henan 473061, China): (Study of the quality standard for Pingxiao capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), App.14-16 (2004). TLC on silica gel with 1) toluene - ethyl acetate - formic acid 25:20:4; 2) ethyl acetate - methanol - water 100:17:13. Detection 1) by spraying with diazotized para-nitroaniline solution; 2) by spraying with 5 % AlCl3 in ethanol and under UV 365 nm. Identification by fingerprint technique. Quantification of hesperidin by HPLC.

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

95 100 ZH. ZHAO (Zhao Zhi Qiang) (Shanghai Leiyunshang Pharm. Co., Ltd., Shanghai 201517, China): (Study of the quality standard for Funing granules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), App. 3-5 (2004). TLC on silica gel with 1) n-hexane - ethyl acetate 9:1;

2) chloroform - methanol 5:2; 3) n-hexane - ethyl acetate 3:1. Detection 1) under UV 365 nm; 2) by spraying with 10 % H_2SO_4 in ethanol and heating at 110 ºC; 3) by spraying with 5 % $FeCl_3$ in ethanol. Identification by fingerprint technique. Quantification of icariine by HPLC.

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

95 101 L. ZHOU (Zhou Lingying)*, X. CAO (Cao Xiaolan), X. BAI (Bai Xiaochun) (*Sichuan Enwei Inst. TCM, Chengdu, Sichuan 610041, China): (Study of the quality standard for Jieeryin effervescent tablets) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (1), 34-37 (2005). TLC on silica gel with 1) benzene - acetone - ethyl acetate - ammonia water 10:15:20:1; 2) ethyl acetate - butanone - formic acid - water 5:3:1:1; 3) n-butanol - glacial acetic acid - water 7:1:2. Detection 1) by spraying with potassium iodobismuthate solution; 2) by spraying with 2 % $FeCl_3$ in ethanol; 3) by spraying with vanillin - H_2SO_4 solution and heating; 4) under UV light. Identification by fingerprint technique. Quantification of geniposide by HPLC. The results for ten real life samples are given.

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

95 052 S. BASAR, Angelika KOCH* (*Frohme-Apotheke, Frohmestrasse 14, 22457 Hamburg, Germany): Test of the stability of olibanum resins and extracts. J. Planar Chromatogr. 17, 479-482 (2004). HPTLC of beta-boswellic acid (BA), acetyl-beta-BA, keto-BA, and acetyl-keto-BA and ethanolic extracts of olibanum resin on silica gel with toluene - ethyl acetate - formic acid - heptane 80:20:3:10 in a twin trough chamber without chamber saturation. Quantitative determination by reflectance measurement at 245 and 285 nm. Also two dimensional development with the same mobile phase in the second direction.

Quality control, densitometry, HPTLC, quantitative analysis 32e

95 054 Anne BLATTER*, E. REICH (*CAMAG Laboratory, Sonnenmattstr. 11, CH-4132 Muttenz, Switzerland): High performance thin-layer chromatographic analysis of aristolochic acid in Chinese drugs. J. Planar Chromatogr. 17, 355-359 (2004). HPTLC of aristolochic acid A, B, and C and numerous plant extracts on silica gel in a saturated twin-trough chamber using the upper phase of the mixture toluene - ethyl acetate - water - formic acid 20:10:1:1. Quantitative determination by fluorescence measurement at 366 nm after derivatization with tin(II) chloride reagent. The working range and linearity, LOD and LOQ (based on the calibration plot), and precision (n = 6), were validated with methods described by K. Ferenczi-Fodor et al., J. AOAC Int. 84 (2001) 1265-1276. The stability of the analyte during chromatography was established by two dimensional chromatography. The new method enables visual detection of the acids with certainty at very low levels (400 pg absolute of aristolochic acid A) in plant material and can therefore be used for screening Chinese drugs to ensure their safety on the basis of absence of aristolochic acid.

Quality control, toxicology, HPTLC, quantitative analysis, densitometry, aristolochic acid 32e

95 012 G. HORVÁTH et al., see section 4e

95 075 Q. MA (Ma Quanming)*, SH. LI (Li Shengyou) (*Qinghai Provin. People's Hosp., Xining, Qinghai 810007, China): (Preparation and quality control of Complex Zhike capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (8), Append. 9-11 (2004). TLC on silica gel previously immersed in 0.5 % NaOH solution, developed with 1) ethyl acetate - methanol - water 100:17:13; and 2) with the upper phase of toluene - ethyl acetate - formic acid - water 20:10:1:1. Detection by spraying with $AlCl_3$ solution and under UV 365 nm. Identification by fingerprint technique and comparison with the standard. Quantification of scutellarin by HPLC.

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

95 019 Z. MALES et al., see section 8a

95 092 R. T. SANE*, S. N. MENON, S. SHAILAJAN, K. K. JARIPATKE (*Ramnarain Ruia College, Matunga, Mumbai-19, India): High-performance thin-layer chromatographic analysis of Asteracantha longifolia Nees. for determination of pharmacokinetics. J. Planar Chromatogr. 17, 483-485 (2004). HPTLC of plant and plasma extracts on prewashed silica gel with toluene - ethyl acetate - methanol 30:3:1 in a twin-trough chamber previously equilibrated with the mobile phase. Quantitative determination by fluorescence/reflectance measurement at 366 nm.

Pharmaceutical research, traditional medicine, HPTLC 32e

95 048 E. A. ABOURASHED* (*Department of Pharmacognosy, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh Saudi Arabia 11451): Validation and application of an HPTLC method for the determination of parthenolide in feverfew herbal products. J. Planar Chromatogr. 17, 375-378 (2004). HPTLC of parthenolide and extracts of feverfew capsules on silica gel with ethyl acetate - n-hexane 3:2 in glass chambers presaturated for 30 min. Detection by dipping in p-anisaldehyde reagent and heating at 105 °C for 5 min, followed by immediate densitometric scanning at 543 nm. The method is precise with CV < 5%; calibration recovery of 101.12 +/- 4.11 % and overall accuracy of 101.14 +/- 4.47 %. The levels of parthenolide in the products analyzed ranged from 0.03 to 0.24 %.

Herbal, quality control, traditional medicine, HPTLC, densitometry, quantitative analysis, feverfew, parthenolide 32g

95 001 J. QU et al., see section 1

33. Inorganic substances

95 102 R. M. BAOSIC, D. M. MILOJKOVIC-OPSENICA, Z. L. TESIC* (*Faculty of Chemistry, University of Belgrade, Studentski trg 16, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): The effect of the electronegativity of donor atoms in coordinated beta-diketonato ligands on the chromatographic behavior of metal complexes. J. Planar Chromatogr. 17, 250-254 (2004). TLC of three series of beta - diketonato complexes of the type [M(acac)3-n(phacphac)n], [M(acac)3-n(phacphSac)n], and [M(acac)3-n(phSacphSac)n] (where M represents cobalt(III), chromium(III), or ruthenium(III), acac is the 2,4-pentanedionato ion, phacphac is the 1,3-diphenyl-1,3-propanedionato ion, phacphSac is 3-mercapto-1,3-diphenyl-prop-2-en-1-one, phSacphSac is the 3-mercapto-1,3-diphenyl-prop-2-en-1-thion ion, and n = 0-3) on silica gel with five mono-component (chloroform, toluene, dichloromethane, xylene, tetrahydronaphthalene) and five two-component eluents (n-amyl acetate - carbon tetrachloride 1:1, n-butyl acetate - carbon tetrachloride 2:3, chloroform - carbon tetrachloride 1:1 and 3:7, and dichloromethane - carbon tetrachloride 4:1. Separations were performed in a horizontal chamber after equilibration for 30 min. After development the colored spots were readily visible.

Qualitative identification 33a, 2c

95 103 Iva REZIC*, L. BOKIC, A. J. M. HORVAT (*Laboratory of Analytical Chemistry, Department of Textile Chemistry and Material Testing, Faculty of Textile Technology, University of Zagreb, Pierottieva 6, 10000 Zagreb, Croatia): TLC separation and identification of heavy metals present in cotton material. J. Planar Chromatogr. 17, 305-308 (2004). TLC of manganese(II), chromium(III), nickel(II), cobalt(II), iron(III), and zinc(II) on cellulose with acetonitrile - hydrochloric acid - water 73:15:12 as optimum ternary mobile phase. Detection by spraying with 0.1 g quercetin in 100 mL 2-propanol and 10 g dimethylglyoxime in 100 mL ethanol and exposition to

ammonia vapor. Recording of the colored spots under white light by means of a highly sensitive CCD color video camera.

Environmental, densitometry, qualitative identification, quantitative analysis 33a

35. Other technical products and complex mixtures

95 104 L. WILLIAMS*, R. SJOVIK, M. L. FALCK-PEDERSEN (*SINTEF Applied Chemistry, P. O. Box 124 Blindern, N-0314 Oslo, Norway): ChemScreen: Planar synthesis, separation and screening of antioxidants. J. Planar Chromatogr. 17, 244-249 (2004). TLC of thirty compounds (coumarin derivatives) including by-products on silica gel with ethyl acetate - hexane 2:3 or, for more polar synthesized compounds, with methanol - dichloromethane 7:93 in a saturated chamber. Detection under UV light at 254 nm, and by derivatization if necessary, e. g. by iodine reagent. Quantitative determination by densitometry at 292 - 363 nm. Direct screening of the reaction mixture on the plate for antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test radical.

Environmental, densitometry, quantitative analysis, ChemScreen 35b, 4e

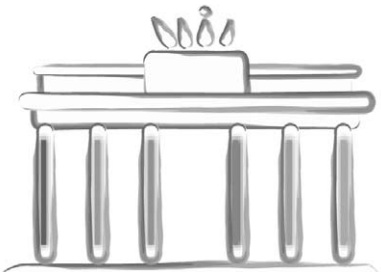
37. Environmental analysis

95 016 W. WEBER et al., see section 4e

38. Chiral separation

95 105 M. SAJEWICZ, R. PIETKA, Teresa KOWALSKA* (*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): Chiral separation of S-(+)- and R-(-)-ibuprofen by thin-layer chromatography. An improved analytical procedure. J. Planar Chromatogr. 17, 173-176 (2004). TLC of R,S-(+-)-ibuprofen and S-(+)-ibuprofen on silica gel prewashed with methanol - water 9:1 and impregnated with L-arginine by conventional dipping for 2 s in a 0.03 mol/L solution of the compound in methanol. One-dimensional development with acetonitrile - methanol - water 5:1:1 plus several drops of acetic acid to adjust the pH to 4.8, and two-dimensional chromatography with the same solvent mixture in two directions. Quantitation by densitometry at 210 nm.

Quality control, densitometry, quantitative analysis, ibuprofen 38, 32a



INTERNATIONAL SYMPOSIUM FOR PLANAR CHROMATOGRAPHY/ INSTRUMENTAL HPTLC

Berlin (Germany), 9–11 October 2006

We are enthusiastic to learn how many analysts are now confronted with situations where Instrumental High-Performance Thin-Layer Chromatography is a suitable solution to their problems and is favored over better known and more widely used analytical methods. At the same time it is rather difficult nowadays to find a place for this technique in the minds of opinion leaders, even if the need exists in analytical laboratories.

This has arisen through inadequate information and training. How to select the method; how to use planar chromatography when it is described as a method of choice; avoidance of usual mistakes; which other samples may be well covered by this technique, etc.

To address these issues an exchange of knowledge is foremost, from which sprang our motivation to hold again an international event with the Interlaken series spirit, last held in 1997.

The first issue, which was held in Lyon (France) in 2003, held its promises with 116 participants from 17 countries, 2 workshops, 15 lectures, 26 posters, and a 4 manufacturers' session. After this success we are happy to welcome you to Berlin from the 9th to the 11th October 2006. Berlin, the capital of Germany, is dynamic, cosmopolitan and creative, allowing for every kind of lifestyle. East meets West in the metropolis at the heart of a changing Europe. Germany's largest city is a city of opportunities just waiting to be seized in all areas, like entertainment, recreation, economy, science and academic life. This appears to us as the best choice for this 2nd issue.

With the firm idea to look straight forward, the program expects to provide exactly what you need. The main symposium, featuring lectures and posters, will provide a real overview of the most recent advances in all the application fields where TLC has a place. Training and the manufacturers' sessions will be held in conjunction with the main symposium but functioning as a separate program. The training program is strongly focused to provide all your needs in a short time. This time a special workshop will be targeted on validation. The best experts will be there to guide you through each step and explain what you need to know, independent of your level of expertise.

Such an event wouldn't be possible without support from the manufacturers. As in all other high technology fields, they are at the forefront of innovation and technical improvement. Their collaboration with world leaders in the field from the early days of TLC has ensured development of Instrumental HPTLC to a modern analytical technique.

We are looking forward to share our knowledge, ideas and enthusiasm!

Call for papers

The scientific program will feature invited keynote speakers, selected submitted lectures and poster presentations. Contributions are invited from all areas of planar chromatography, especially from colleagues working in the pharmaceutical, food, environmental and medical fields.
Papers on theory, method development, validation, instrumental methods, hyphenated techniques, and quantitative applications in all areas of chemistry are most welcome.

Colleagues wishing to participate in the scientific program should submit a brief abstract to
Prof. Dr. Lothar W. Kroh, Institute for Food Chemistry, Technical University Berlin, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany or to
committee@hptlc.com
by 15th June 2006 stating whether they wish to present an oral presentation or a poster.

Abstract should be no more than 250 words and 2 figures/tables in a MS Word file (in Arial, font size 12, justified). The abstract should indicate the title, the author's names (with the presenting author underlined), affiliation (with e-mail address) and a brief description of the work to be presented. All submissions will be reviewed by the scientific organizing committee and authors will be informed of their decision by 31st July 2006.

All accepted contributions will be published in the symposium proceedings. Papers presented at the symposium will be also published after review, in a special issue of an analytical journal. Complete manuscripts must be delivered to the publishers' representative at the time of the symposium. Further details on the format for manuscripts will be obtained from the editorial office of the journal.

Pre-chromatographic in situ derivatization of glyphosate und AMPA

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▲ *Dr. Holger Hegewald*

Dr. Hegewald* is general manager of an analytical laboratory in Évora, Portugal. In research projects with the University of Évora he is analyzing pesticides and plant hormones. In addition he performs analyses of PAH's in water, aflatoxin M1 in milk, as well as anthocyanes and organic acids in wine. For those analyses planar chromatography is used exclusively.

Introduction

In contrast to the previous article, which featured in vitro derivatization, a method including pre-chromatographic in situ derivatization shall be presented here.

This type of derivatization on the application position of the plate (in situ) is only possible in planar chromatography. Advantages are automated, reproducible reaction with minimal reagent consumption and avoidance of chlorinated solvents. This in situ derivatization does not take much longer than normal sample application.

Sample preparation

Is not described in this article. Possible is for example a solid phase extraction using ion exchangers.

Standard solutions

0.5 ng/µL glyphosate and APMA are each dissolved in water – methanol 3:2.

Layer

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

Sample application and derivatization

Sample and standard solutions are applied as rectangles of 7×3 mm. Track distance 10 mm. Then each application position is over-sprayed with 3 µL of 75 mM borate-NaOH buffer pH 10.5 and 3 µL 9-fluorenylmethylchloroformiate (FMOCCl, 2 mg/mL in acetonitrile). Reaction zones are covered with a glass plate and incubated at room temperature for 10 min. Prior to chromatography the zones are dried 5 min with a hairdryer. If necessary, a focussing step with methanol up to the upper end of the starting zone could reduce peak width (not performed here).

Chromatography

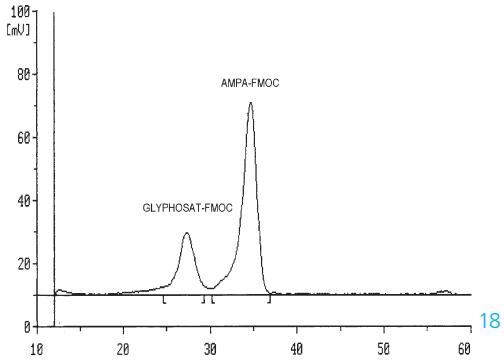
In twin trough chamber without chamber saturation with n-butanol – acetic acid – water 5:1:1, developing distance 70 mm from lower edge of plate. After drying, dipping in or spraying with paraffin – toluene 1:1 enhances fluorescence about 10-fold.

Densitometric evaluation

TLC Scanner with CATS software, fluorescence measurement with Hg lamp (allows more sensitive detection) at UV 265 nm/secondary filter M 360 nm, linear calibration using peak height

Results and discussion

The limits of quantitation are 0.5 ng absolute per substance zone for glyphosate-FMOC and 0.2 ng for AMPA-FMOC. Calibration is linear between 0.25 and 35 ng. This allows quantitation of glyphosate and AMPA with certainty in a water volume of about 15 mL.



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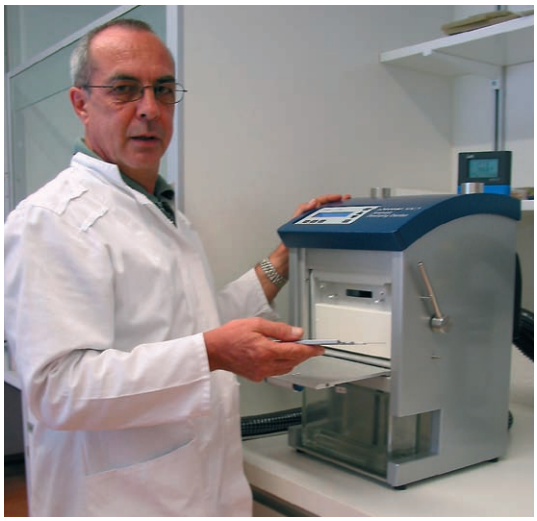
▲ *Densitogram of a standard track 5 ng glyphosate-FMOC and AMPA-FMOC each*

Further information is available from the author on request.

* Dr. Holger Hegewald, Lacrome Lda, Rua César Batista 6 D, P-7000-715 Évora, Portugal, lacrome@clix.pt

Quality and reproducibility of chamber saturation with the new Automatic Developing Chamber ADC 2



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▲ *Daniel Handloser performing the ADC2 experiments in the CAMAG Laboratory*

Based on a separation of sulfonamides and the fingerprint of a rhubarb root extract, the quality and reproducibility of chamber saturation in ADC 2 was compared to the conventional Twin Trough Chamber.

Introduction

The new Automatic Developing Chamber ADC 2 was introduced on the cover page of CBS 94. The ADC 2 allows fully automatic development of TLC/HPTLC plates and thus the most important step of chromatography, development, is executed reproducibly and independently of environmental factors. This is most important, when the developing solvent contains highly volatile components, making the method sensitive to changes in the mobile and the gas phase. A detailed discussion of the processes taking place during chromatogram development including the gas phase can be found in Parameters of Planar Chromatography, Chromatogram development part 1 – chamber type and saturation (CBS 87).

Reproducibility of the chromatographic separation is not only essential for quantification, but also an important element of routine identification of complex fingerprints of plant extracts (botanicals). All necessary parameters can be programmed and automatically monitored with the ADC 2. Thus the unit controls environmental factors, minimizes or eliminates potential manual handling errors, and frees the analyst from monitoring the process. Existing methods can be transferred directly, due to the fact that the ADC2 uses a conventional 20×10 cm Twin Trough Chamber (TTC).

Chromatography of sulfonamides

Reproducibility was investigated with a model system, which is sensitive to changes in the gas phase. The separation of the five sulfonamides with dichloroethane – methanol – 2-propanol – 25% ammonia 25:5:5:1 responds with changes of R_F-values and a wavy solvent front to inhomogeneities and varying saturations of the gas phase. In practice good separation of sulfonamides is achieved with a much simpler developing solvent. 5 plates each were developed under unsaturated and saturated conditions in a conventional Twin Trough Chamber and compared to 5 plates developed in the ADC 2 under similar conditions.

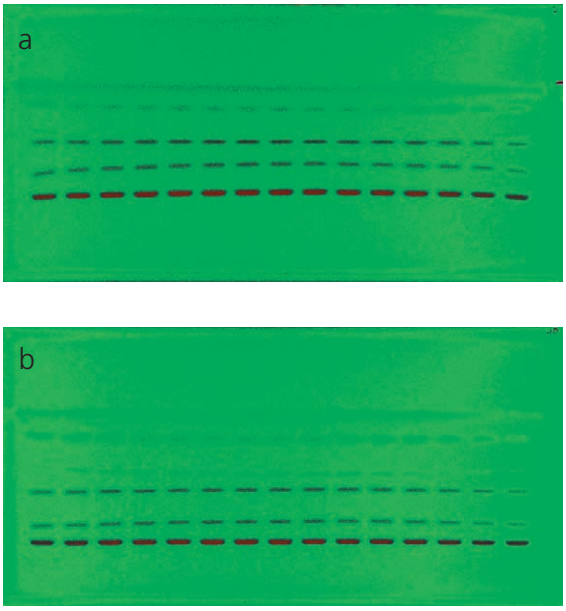
Chromatographic conditions

- Standard solution: sulfamethoxazole (160 ng/µL), sulfamerazine (50 ng/µL), sulfamethazine (50 ng/µL), trimethoprim (32 ng/µL) and dimetridazole (100 ng/µL) in chloroform – methanol 1:1.
- Layer: HPTLC plates silica gel 60 F_{254} (Merck), 20×10 cm
- Sample application: 8 mm bands with Automatic TLC Sampler 4, 15 tracks, application volume 5 µL, distance from lower edge of plate 8 mm, distance from left and right edge 15 mm, distance between tracks 12 mm.
- Chromatography: Developing solvent dichloroethane – methanol – 2-propanol – 25% ammonia 25:5:5:1, development in TTC 20×10 cm and ADC 2 respectively, varied chamber saturation.
- Densitometric evaluation: TLC Scanner 3 with winCATS software, absorption measurement at UV 254 nm.

Results and Discussion

The influence of chamber saturation can be seen in the behavior of the R_F-values and the solvent front. Under saturated conditions, when the layer is loaded with solvent molecules, the R_F-values are lower than those obtained in an unsaturated chamber due to measurement of the virtual front. Comparing identical saturation times the ADC 2 gives lower R_F-values than the TTC, thus the ADC 2 is tighter and guarantees improved saturation conditions.

In the first part of our test a partially saturated system was employed (10 mL developing solvent in one trough only, 10 min waiting time, no filter paper). Although conditions in the TTC and the ADC 2 were comparable, the chromatograms still were different as depicted in the next figure. The lid must be opened in order to introduce the plate into the Twin Trough Chamber. This caused noticeable and non-reproducible disturbance of the saturation condition and R_F-values were higher and wavy at the center. The same experiment in the ADC 2 yielded a higher saturation (lower R_F-values) and a more homogenous chromatogram.



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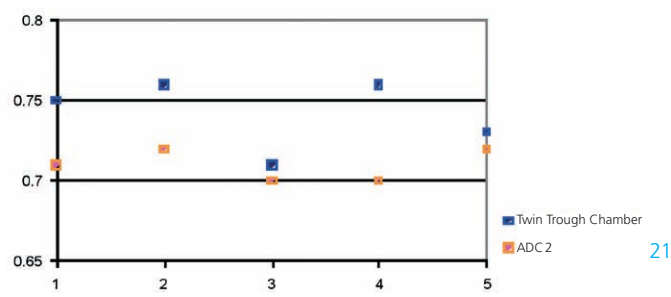
▲ *Separation of a sulfonamide mixture under partially saturated conditions in the Twin Trough Chamber (a) and ADC 2 (b). Images under UV 254 nm.*

In a second experiment the saturation was further increased. Instead of partially saturating the chamber for 10 min prior to development, saturation is now performed over 20 min and 10 mL of developing solvent was charged in both troughs. One trough of the conventional Twin Trough Chamber was equipped with a filter paper wetted with 10 mL of developing solvent and then the chamber was closed. After 20 min the lid was opened and the plate placed in the chamber. This manual operation disturbed the previously established chamber saturation even if it were performed very carefully. A comparison of results from plate to plate revealed that the homogeneity of chromatography across the plate is further increased, but the average R_F-values between plates varied significantly.

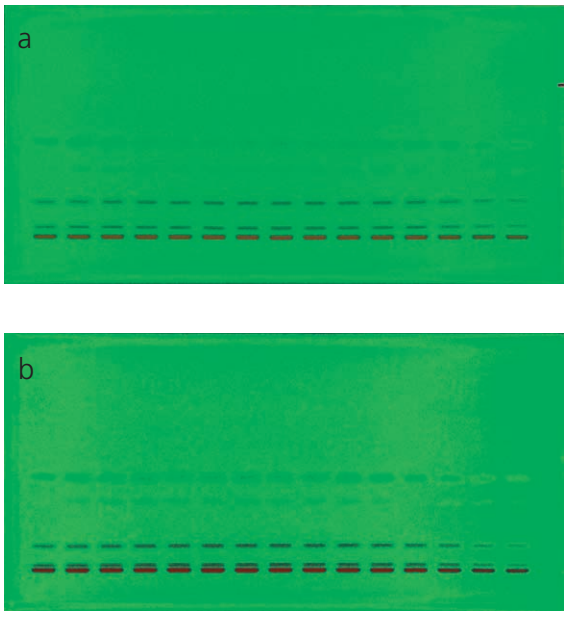
Repeating this experiment in the ADC 2 yielded again more reproducible R_F-values than in the TTC. This can be seen in the next figure for dimetridazole. Because the ADC 2 needs a thicker filter paper, which is wetted with 25 mL instead of 10 mL developing solvent, it can be expected that the saturation will be slightly higher, resulting in somewhat lower R_F-values.



21

▲ *R_F-values (n = 15) of dimetridazole averaged across the plate (values from 5 plates), saturated conditions (20 min, filter paper), no pre-conditioning. Relative standard deviation of the R_F-averages (n = 5) is 2.9 % for the TTC and 1.3 % for the ADC 2.*

An even more stable chromatographic system can be obtained if the plate, prior to development, is pre-conditioned for 10 min in the saturated chamber. R_F-values are further lowered in this mode and at the same time reproducibility of chromatography is improved.

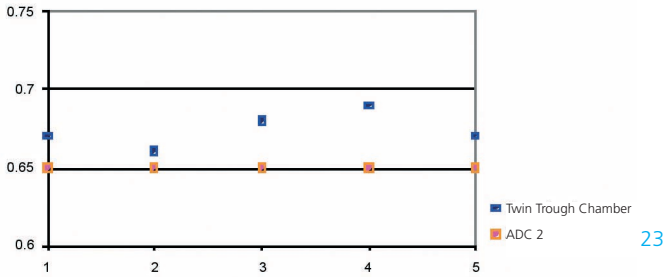


22

▲ *Separation of a sulfonamide mixture in the saturated chamber (20 min, filter paper) with 10 min pre-conditioning of the layer; Twin Trough Chamber (a) and ADC 2 (b). Images under UV 254 nm.*

In the Twin Trough Chamber the filter paper was wetted in one trough with 10 mL developing solvent. The plate was positioned in the empty trough and pre-conditioned during 10 min. Then the chamber must be opened for a moment to charge the empty trough with 10 mL of developing solvent. Because the solvent can only be introduced at one point chromatography does not start simultaneously across the plate. When the lid is open chamber saturation is disturbed seriously.

In the ADC 2 all manual operations are eliminated. Both troughs of the chamber are charged with developing solvent at the same time. For the pre-conditioning the plate is moved into the gas phase of the chamber. After the selected time has elapsed (10 min) it is lowered into the developing solvent. This approach creates a highly reproducible saturated system for each development. The next figure illustrates the improved reproducibility of the R_F-values for dimetridazole.



23

▲ *R_F-values (n = 15) of dimetridazole averaged across the plate (values from 5 plates), saturated 20 min with filter paper plus pre-conditioning 10 min. Relative standard deviation of the R_F-averages (n = 5) now 1.6 % for the TTC and 0 % for the ADC 2.*

Application example: fingerprint of a rhubarb root extract

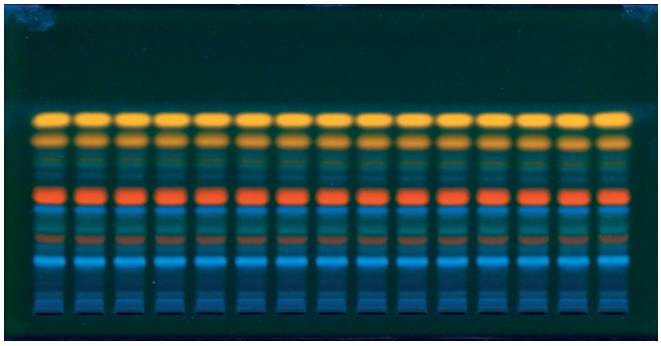
The effects of chamber saturation on stability and reproducibility of the chromatographic system illustrated by the example of a sulfonamide separation are of general practical importance. The chromatogram of a Rhubarb root extract confirms the excellent homogeneity of the R_F-values across the plate. Thanks to the reproducible development in the ADC 2 such fingerprints are not a random result.

Chromatographic conditions

- Sample preparation: 500 mg Rhubarb root was sonicated for 15 min at 60 °C with 5 mL methanol, an then centrifuged.
- Layer: HPTLC silica gel 60 F_{254} (Merck), 20×10 cm
- Sample application: 8 mm bands with Automatic TLC Sampler 4, 15 tracks, application volume 5 µL, distance from lower edge of plate 8 mm, distance

from left and right edge 15 min, distance between tracks 12 mm

- Chromatography: Developing solvent methanol – dichloromethane 1:4, development in ADC 2 with 20 min chamber saturation.



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▲ *Fingerprint of a Rhubarb root extract, image under UV 366 nm.*

Outlook

In addition to improved reproducibility of R_F-values the ADC 2 also controls layer activity and thus ensures reproducible separations, independent of relative humidity of the laboratory environment. Applications of the ADC 2 should be numerous – **we would be excited to learn about your own practical experience with the system.**

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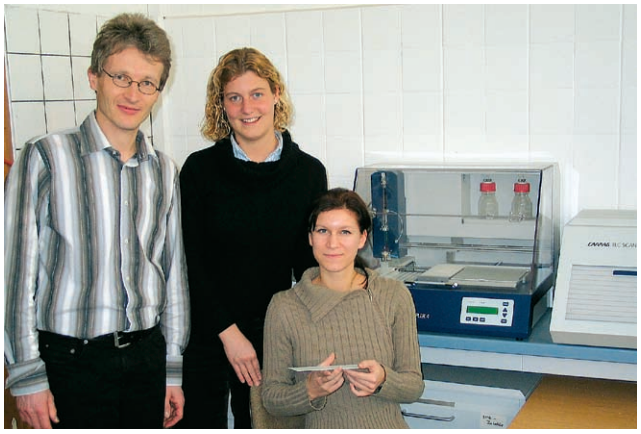
ADC 2 – Reproducibility, Safety and Convenience

The Automatic Developing Chamber ADC 2 is universally applicable and gives results of unsurpassed reproducibility. It is designed to automate all manual operations necessary during chromatogram development. It is one of the strengths of the ADC 2 that it utilizes the CAMAG 20×10 cm Twin Trough Chamber, and thus permits analytical procedures based on such chambers to be employed largely without changes.

The opening of the chamber during manual development as well as all other human and environmental influence factors are controlled or even eliminated in the ADC 2. As soon as the solvent front has reached the pre-defined position (developing distance), the plate is automatically removed from the solvent and efficiently dried under flow-optimized conditions.

Quantitation of in vitro lipolysis products with HPTLC



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▲ *From left to right: Prof. Dr. Karsten Mäder, Andrea Rübe, Sandra Klein*

The group of Professor Mäder*, Institute of Pharmaceutical Technology and Biopharmacy, Martin-Luther-University Halle, is performing research for the production of biodegradable nanocapsules and nanospheres based on lipid carriers. Such formulations can yield higher resorption of less soluble drugs while decreasing the variability. For the mechanism of resorption increase digestion of the drug delivery system plays an important role.

Introduction

For optimization of the drug carrier, test methods are needed which can simulate in vitro the process of degradation of the drug formulation. Artificial digestion with lipases is a common procedure. The drug formulations are incubated with a mixture of pancreatin (as source of lipase and colipase) and bile extract. After completed digestion the individual lipid constituents are extracted, applied onto HPTLC plates, chromatographed and finally quantitatively detected. Planar chromatography is a suitable technique for evaluation of in-vitro assays of digestion processes. In contrast to the commonly used pH-stat method, featuring a titrimetric determination of the produced fatty acids, digestion can be better characterized because the individual lipid classes can be quantified. That way HPTLC contributes to a better understanding of the digestion behavior of colloidal lipid carriers and to the optimization of such drug formulations.

Sample preparation

Samples are first acidified with 1N HCl to assure protonation of dissociated free fatty acids, then homogenized with Ultraturrax. Finally lipids are extracted with chloroform.

Stationary phase

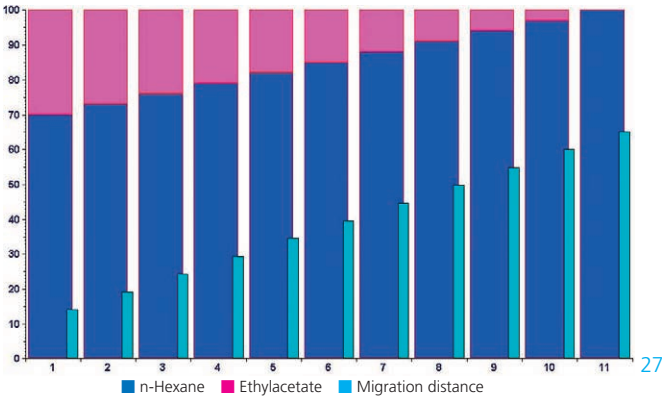
HPTLC plates silica gel 60 F_{254} (Merck) 20×10 cm

Sample application

As bands with Automatic TLC Sampler 4, 18 tracks, application volume variable, band length 8 mm, track distance 10 mm, distance from lower edge of plate 8 mm, distance from the sides at least 15 mm

Chromatography

In AMD2 system using an 11-step gradient based on ethyl acetate. Between the individual steps the plate is dried for 90 s and then conditioned with 4 M acetic acid. The developing distance is max. 65 mm; the time requirement for the gradient is 110 min.



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▲ *AMD2 gradient for separation of lipolysis products*

Post-chromatographic derivatization

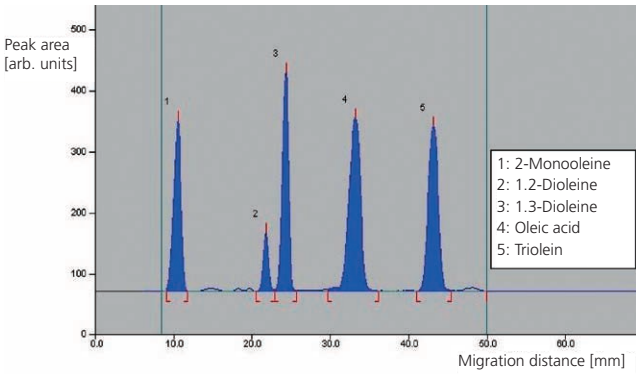
With the Chromatogram Immersion Device the plate is dipped for 20 s into an aqueous copper sulfate solution (10% CuSO4, 8% H3PO4 and 5% methanol) and then heated for 30 min at 150 °C. Long-chain lipids will be colored brown. .

Densitometric evaluation

With TLC Scanner 3 and winCATS software, absorption measurement at 675 nm, evaluation of peak area with calibration according to Hill kinetics

Results and discussion

The following densitogram illustrates the AMD2 separation of a lipid standard mixture.



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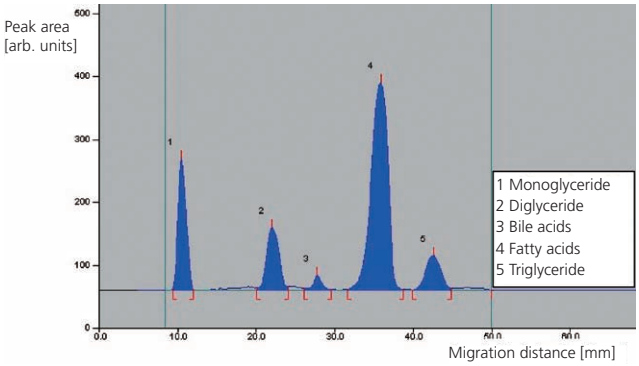
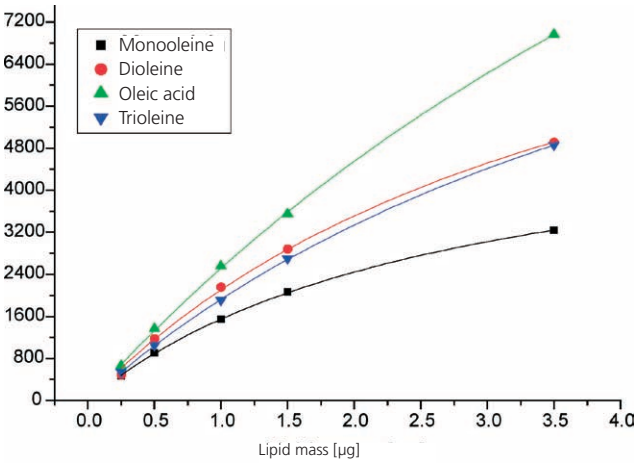
▲ *Densitogram of a lipid standard mixture*

In the densitogram of the extracted lipolysis products 4 species of the standard mixture are found again. Furthermore an additional peak (3), due to bile acids, is seen. The lipid concentrations obtained with the calibration curves describe the digestion behavior of the lipid carrier system.



29

▲ *Densitogram of lipolysis products extracted after artifical digestion*



30

▲ *Calibration curve of the standard mixture (Hill kinetics), correlation coefficients for monooleine 0.996, dioleine 0.996, oleic acid 0.998 and trioleine 0.999*

Further information is available from the authors on request.

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