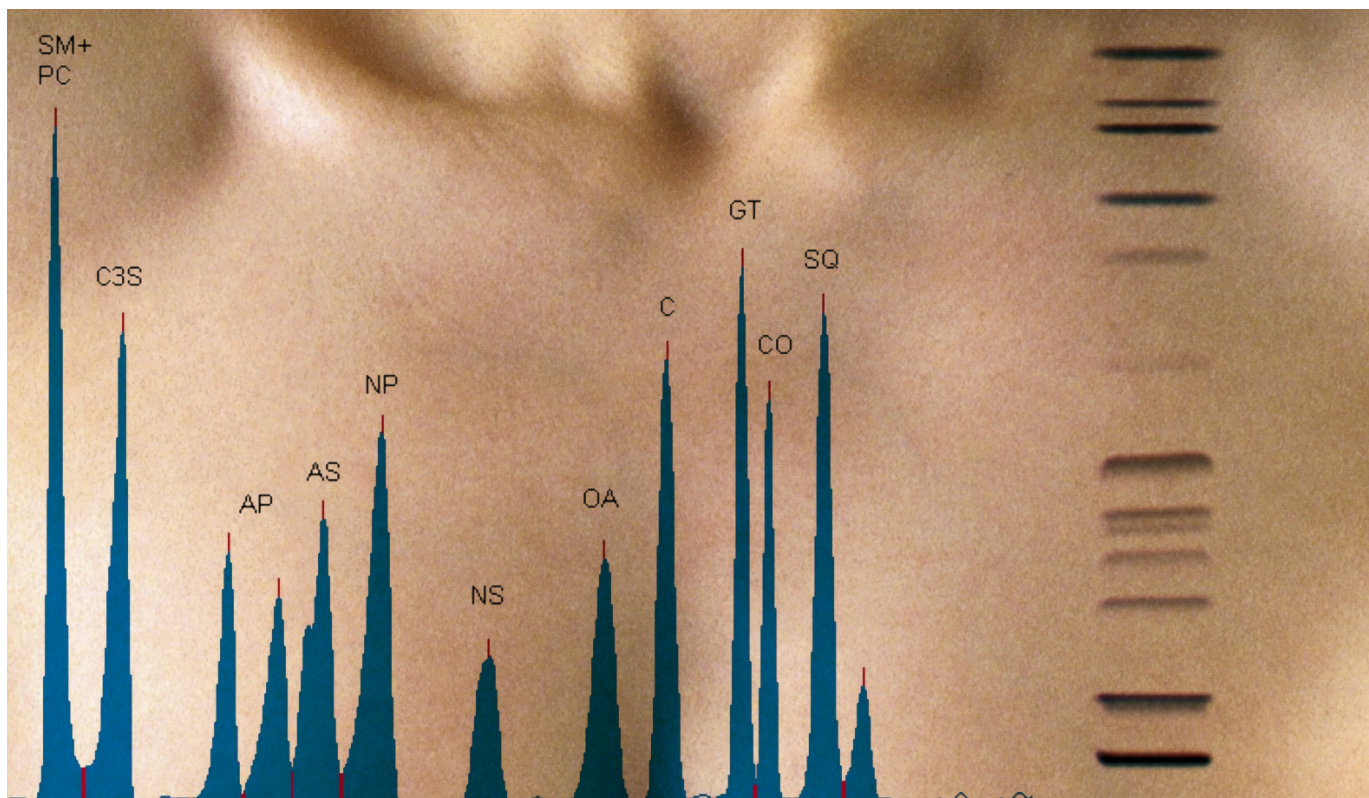


CBS

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AMD chromatogram of Stratum corneum lipids

The versatility of HPTLC – From Bio Analysis to the Detection of Pollutants in Water

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Planar Chromatography in Practice

TLC/HPTLC-ELSD-MS coupling



Mr. François Bretin and Dr. Francis Maquin (right)

The Lead Generation to Candidate Realization (LGCR) platform identifies small molecule leads and progresses them up to registration. LGCR Analytical Sciences (AnSci) is a new department in Sanofi-Aventis R&D, which is dedicated to support Business Divisions and Therapeutics Units. For LGCR-AnSci, Dr. Maquin* and F. Bretin in the research center in Vitry-sur-Seine use various analytical techniques (further locations are in Strasbourg, Chilly-Mazarin, Toulouse and Montpellier).

Introduction

MS coupled to chromatography supports the chemist's decision for compound identification or follow-up in a synthesis mixture. Therefore, HPLC/UPLC-MS is mainly used, but TLC/HPTLC is also widely applied by the chemists as a rapid and reliable method to follow reaction processes.

This is especially true for cases, when compounds remain on the column, due to high polarity or weak solubility, or when analyte detection is poor (no chromophore). In such cases, the HPTLC/MS platform with the new TLC-MS Interface is ideal for structural analysis. The TLC-MS Interface was complemented by a MS software controlled valve, adding some automation to the process, and by an Evaporative Light Scattering Detector (ELSD). The latter enables the analyst to distinguish between compounds that were not eluted from the plate from those that were not sufficiently ionized for MS detection.

Sample preparation

The samples were taken directly from the reactor and diluted with an appropriate solvent, usually an organic solvent of medium polarity.

Standard preparation

Educts or known intermediate products were dissolved and diluted with an appropriate solvent.

Chromatogram layers

TLC and HPTLC plates silica gel 60 F₂₅₄ 10 x 20 and 10 x 10 cm, respectively. If required, the plate size was reduced with the Smartcut.

Sample application

Manually with disposable micropipettes of 5 to 20 μL volumes.

Note: The Nanomat 4 is recommended to ensure precise positioning without damage of the layer. Especially for polar extracts, small volumes should be applied (<2 μL to obtain sharp start zones).

Chromatography

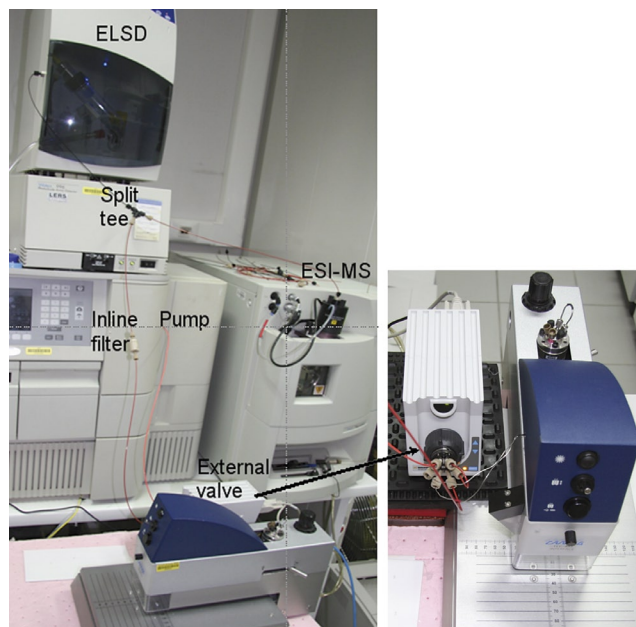
In a twin-through chamber, e.g., with mixtures of methanol and dichloromethane/ethyl acetate, or ethyl acetate and heptane/cyclohexane; the ratios depend on the compound mixtures.

Derivatization

Compounds with neither UV/Vis-activity nor native fluorescence can be derivatized by non-destructive derivatization reagents, e.g., the primuline or berberine reagents for lipophilic compounds, and directly eluted with the TLC-MS interface into the MS. However, for destructive derivatizations, e.g., based on strong acidic carbonization reactions, on both plate sides, the outer track was cut, derivatized, and the respective bands were marked by extrapolation.

Recording of MS spectra

The flow rate of the eluent (methanol – water 95:5, 0.4 mL/min) was split by a tee, and 0.2 mL/min were pumped to the MS (Micromass ZQ, Waters) and 0.2 mL/min to the ELSD (Sedere Sedex 85 LT). The TLC-MS Interface was equipped with either a round or oval elution head and connected via an external automated valve (MXP7900-000, Rheodyne) to the pump (Alliance 2695, Waters) and ESI-MS. In the transfer tube to the detectors, an inline filter was integrated (frit porosity 0.2 μm , A 356/504, Upchurch IDEX). The recording of mass spectra was performed in the positive/negative electrospray mode; for evaluation the MS software (Mass Lynx V4.0/Open Lynx, Waters) was used.

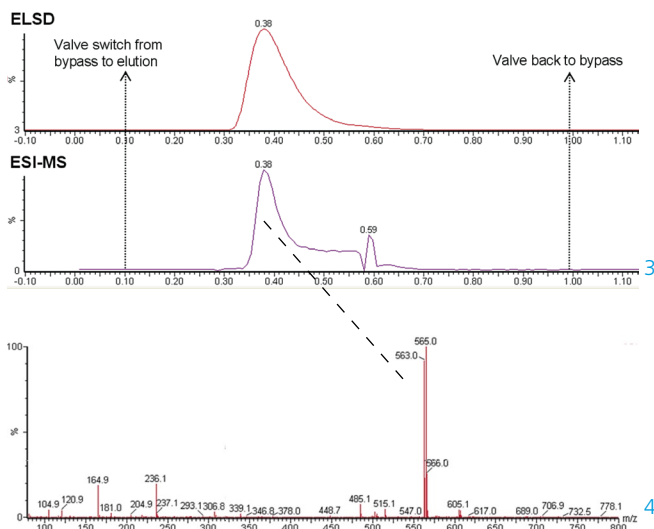


TLC/HPTLC-ELSD-MS system configuration

Results and discussion

In our experiments, a lot of organic structures were not detected by DAD, so the capability of detection of the TLC/HPTLC-DAD-MS configuration was improved by substituting the DAD with the ELSD. As universal detector, the ELSD confirms more reliably the elution of compounds that are not UV/Vis-active.

Once the elution head was lowered onto the zone of interest and an ID number was entered in the software, valve switching was effected remotely, followed by an automatic acquisition of both the MS and ELSD signal. This minor automation resulted in the zones being eluted into the detector, and then the eluent being subsequently switched back to bypass (waste). The following protocol made cross contamination, which of course is dependent on the substance structure and zone concentration, less likely. For the given connecting tube length and internal diameter, the external valve was set 10 s in bypass, then 1.5 min in elution mode and finally 15 s in bypass. For a high throughput, the elution time was set below one minute.



Elution profiles obtained by ELSD and ESI-MS (top) and mass spectrum of the eluted zone (bottom)

An intensive use of the TLC/HPTLC-ELSD-MS instrumentation requires frit cleaning twice a week. The requirement of a cleaning cycle is indicated by an increased pump pressure likely from clogging of the inline filter frit (> 10 MPa).

To conclude, our experience underlines the interest in HPTLC/TLC-MS in an advanced analytical environment. The automatization, reliability of the data, and speed were the convincing arguments, which fully comply with our routine analytical needs.

Further information is available on request from the authors.

Contacts

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- Mr. Pierre Bernard-Savary, Chromacim SAS, Pommiers la Placette, France
- Mr. Henri Gangloff, Sedere SA, Alfortville, France
- Mrs. Véronique de Nailly, BCP Instruments, Irigny, France



TLC Scanner 4

Classical densitometry uses monochromatic light in the form of a slit of selectable length and width to scan the tracks of a chromatogram, measuring the diffusely reflected light. The TLC Scanner 4 – the successor of TLC Scanner 3 – is the most advanced workstation for densitometric evaluation of Thin-Layer Chromatograms currently available.

Analysts can now benefit from a wider spectral range for measurements from 190 to 900 nm. The optimized positioning stage and its open access allow for robust use.

For the screening of water samples in this application, densitometric determination is performed by the multi-wavelength scan covering the wavelength range between 190 and 300 nm. In combination with AMD the whole polarity range of UV-active compounds is detected. This procedure is proven to be helpful in routine search for potential water contaminants.

Determination of the glycoalkaloids α -solanine and α -chaconine in potatoes at different steps of potato processing



6

Dr. Jens Mäder, Prof. Dr. Lothar W. Kroh

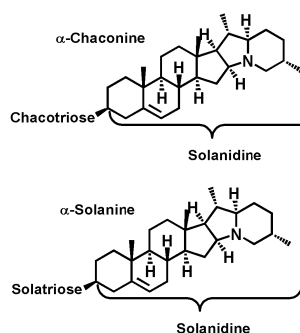


7

Prof. Dr. Kroh*, TU Berlin, and his group, concentrate on carbohydrate chemistry and food analysis, especially in Maillard reaction chemistry, caramelization and melanoidin formation. A further research field of both authors is the characterization of bioactive compounds of processed and unprocessed food. Dr. Mäder is head of product development at Milchwerke Mittelbe, Stendal. This study [1] was performed by Dr. Mäder, Ms Hanschen and Ms Zietz (both not pictured) during their graduation.

Introduction

The potato as a plant of the *Solanaceae* family contains bioactive steroidal alkaloids, which are deterrent to herbivores and pathogens and reported to be membranotoxic, embryotoxic and teratogenic for mammals. They influence the flavor of fresh and processed potatoes from a bitter taste toward a burning sensation at higher concentrations [2]. At the moment no limit values are fixed in Europe, and potatoes, as the most important food source of glycoalkaloids, are regarded as safe for humans if they do not contain more than 200 mg total alkaloids per kg of fresh weight. Both main alkaloids α -solanine and α -chaconine exist at high levels in the tuber peel in a ratio of 1:2 to 1:7.



Chemical structure of the potato alkaloids α -solanine and α -chaconine

The potato is one of the world's most important food crops. Quality-management systems demand food safety and traceability as they often follow the *from farm to fork policy* of regulatory authorities. Therefore, easy to use, inexpensive, and reliable analytical methods are needed to evaluate the content of toxicologically relevant compounds like glycoalkaloids after harvest and during potato storage and processing.

HPTLC is especially suited for effective and rapid determination of alkaloids [4] because up to 16 samples can be simultaneously separated without any matrix influence on the quality of the results. Consequently, all processing stages and by-products from one batch can be monitored and traced simultaneously within one analysis on one plate.

Sample preparation

Samples were collected at different stages of the production chain in a commercial potato flake process. After fourfold extraction of the lyophilized, ground potato powder with methanol - acetic acid 99:5, the extracts were concentrated and directly applied.

Standard solutions

5 mg each of α -solanine and α -chaconine were dissolved in 25 mL methanol - acetic acid 99:1, mixed 1:1 and diluted 1:20.

Chromatogram layer

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

Sample application

Bandwise with Automatic TLC Sampler 4, band length 5 mm, track distance 9.2 mm, distance from the lower edge 8 mm, application speed 70 nL/s, application volume 2–22 µL

Chromatography

In the Horizontal Developing Chamber after 15 min chamber saturation with dichloromethane – methanol – ammonia (2.5 %) 70:30:4.4, migration distance of 75 mm from the lower edge of plate

Post-chromatographic derivatization

With the Chromatogram Immersion Device III the plate was dipped after 25 min drying time at 90 °C into the Carr-Price reagent (70 g SbCl₃ is dissolved in 250 mL of a mixture of acetic acid – dichloromethane 1:3), followed by heating on the TLC Plate Heater at 110 °C for 3 min.

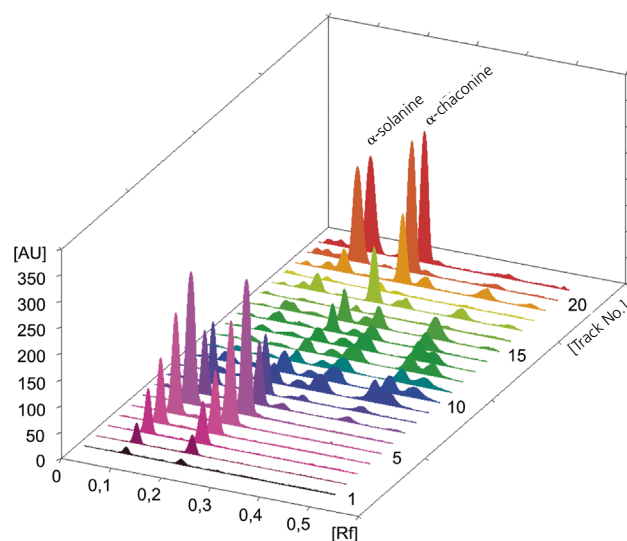
Densitometry

Absorption measurement at 560 nm with TLC Scanner 3 and winCATS software within 15 min after derivatization, before the glycoalkaloid zones change the color from red to violet.

Results and discussion

The densitogram clearly shows the good separation of α -solanine and α -chaconine in the different sample extracts. The extremely sensitive and selective coloring of both glycoalkaloids by dipping into the Carr-Price reagent allowed a limit of detection and quantification of 5–15 (depending on the matrix) and 30 ng/band, respectively, with a recovery rate between 94 and 105 %. Linear calibration was very satisfactory in the range of 30 to 700 ng/band ($r = 0.9998$, $sdv = 2.5\%$), polynomial even up to 1500 ng/band ($r = 0.9999$, $sdv = 1.5\%$).

In conclusion, this method ideally complies with the analytical demands mentioned in the introduction.



Densitogram of standard substances (track 1–6) and sample extracts of different steps of potato processing (track 7–21)

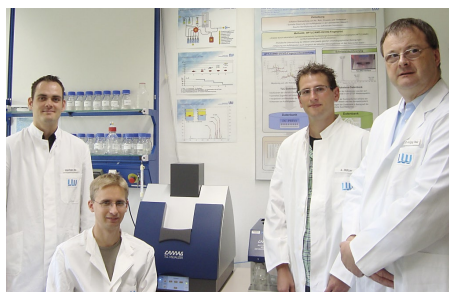
Further information is available from the author on request.

*Prof. Dr. Lothar W. Kroh, Institut für Lebensmitteltechnologie und Lebensmittelchemie, TU Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, lothar.kroh@tu-berlin.de

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- [1] J. Mäder *et al.* J Planar Chromatogr 22 (2009) 43.
- [2] S.L. Sinden, K.L. Deahl and B.B. Aulenbach, J Food Sci 41 (1976) 520.
- [3] A.J.A. Essers *et al.* Environ Toxicol Phar 5 (1998) 155.
- [4] J. Mäder, H. Rawel and L.W. Kroh, J Agric Food Chem 57 (2009) 6292.

1H-Benzotriazole and tolyltriazole in the aquatic environment



9
Dr. Wolfram Seitz, Stefan C. Weiss, Alexander Müller and Dr. Wolfgang Schulz (from left to right)



10
Dr. Walter H. Weber

Lead by Dr. Weber* the laboratory for operation control and research of Zweckverband Landeswasserversorgung (LW) in Langenau, Germany, is active in the analysis of drinking water as well as in the monitoring of ground and waste water from the water protection area Donauried-Hürbe and of surface water from the surrounding area. HPTLC, preferably with automated multiple development (AMD), and online hyphenation of HPTLC-MS are part of the methods applied in routine analysis [1–3].

Introduction

Within the scope of a monitoring program of raw water resources used by LW, comprehensive chemical, physico-chemical and microbiological analyses are performed regularly. In addition non-target screening analyses for the detection of not yet considered contaminants or unknown substances were performed.

In one of the HPTLC/AMD screening tests of extracts from secondary effluents and of surface and ground water samples the resulting chromatograms showed a zone which could not be identified as any of the substances known from target analysis at LW.

Sample preparation

Analyte enrichment from a 100 mL water sample was done by solid phase extraction (SPE) using 0.2 g of Iso-lute ENV+ sorbent at pH 3 or 7. The SPE sorbent was conditioned consecutively with 6 mL each of *n*-hexane, acetone, methanol and water (pH = 3 or 7). After drying 6 mL of methanol were used for elution and after eva-

poration to dryness the residue was taken up in 200 μ L of methanol.

Layer

HPTLC plates Lichrospher F₂₅₄, 20 x 10 cm (Merck) were pre-cleaned with 2-propanol and dried for 30 min at 120 °C on the TLC Plate Heater.

Sample application

With Automatic TLC Sampler (ATS4) as area of 6 x 3 mm

Chromatography

In the AMD 2 system with a 25-step gradient starting in isocratic mode with 5 steps acetonitrile – dichloromethane 1:1 for focusing, followed by 15 steps from acetonitrile – dichloromethane 1:1 to dichloromethane and then in 5 steps from dichloromethane – *n*-hexane 4:1 to *n*-hexane. The final migration distance was 80 mm.

Densitometric evaluation

Multi-wavelength scan at 190, 200, 220, 240, 260, 280 and 300 nm with the TLC Scanner and winCATS software

Documentation

Under UV 254 nm with the TLC Visualizer

Results and discussion

During routine screening of extracts from secondary effluents as well as of surface and ground water samples a high peak was observed in the chromatograms. To further investigate this peak the unknown zone was eluted from the HPTLC plate by means of the TLC-MS Interface and analyzed by Nano LC followed by QTOF-MS (ESI⁺). The analysis revealed that the zone consisted of two substances, peak A at RT = 3.2 min and peak B at RT = 3.4 min.

continuation on page 9

Know CAMAG

One of CAMAG's goals is to provide worldwide in a variety of venues, a continuous flow of information on the state of up to date knowledge of contemporary planar chromatography (see editorial CBS 104). Through one-day workshops at universities, technical training schools, official governmental institutes, and at private companies through in-house training of laboratory personnel, this goal is a reality.

In Germany, CAMAG Berlin has held such seminars for over ten years, now extended into Austria and Benelux. The USA has a similar program and India has an even more aggressive program. In all other countries these are organized by CAMAG Switzerland. If you are interested, contact info@camag.com or telephone +41 61 4673434.

About one such seminars, recently held by CAMAG Berlin, Professor Manfred Gey of the University of Applied Sciences Zittau/Görlitz reports:



Prof. Gey, Ms Werther, Ms Dr. Gey, Dr. Zieloff (from left)



Practical session

1. The beginning: How we got started!

Lectures and practical sessions on analytical chemistry for our students concentrated on Separation Sciences, i.e. instrumental bio-analysis, protein and carbohydrate analysis, hyphenating techniques, etc. Meanwhile, contemporary TLC/HPTLC has enhanced the scope of our "HPLC oriented" laboratories considerably.

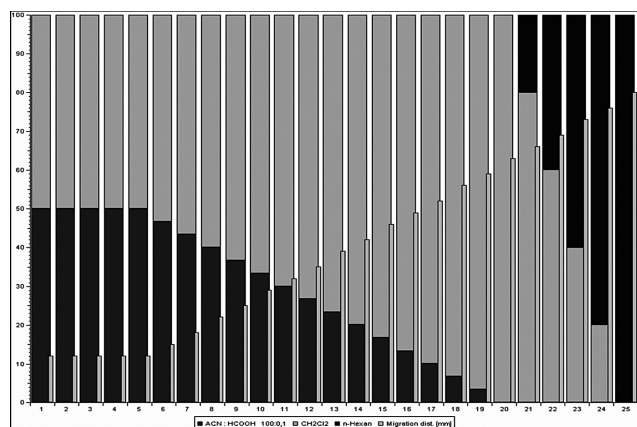
A related institute offered us their CAMAG equipment, which fortunately for us had been mistakenly considered to be non-functional. CAMAG provided generous and efficient support. First they checked the TLC Scanner free of charge, and found it fully functional. Then they instructed our personnel in the operation of the equipment and the use of the software. For this help we are thankful to Dr. Natsias, Ms Werther, Dr. Zieloff and Mr. Nachtwey, all from CAMAG Berlin.

2. Workshop on contemporary TLC/HPTLC 17./18. May 2010 in Zittau

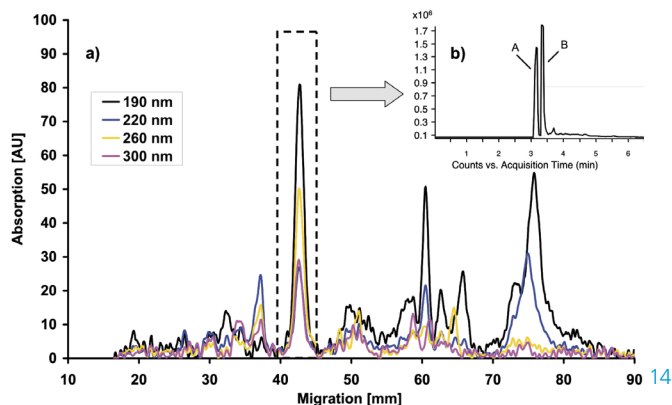
Very soon our cooperation with CAMAG led to the plan to organize a workshop on TLC/HPTLC for students and co-workers from our Faculty for Mathematics & Natural Sciences. There were 30 participants, considered a very good turnout, since all regular lectures and practical sessions ran on in parallel. Dr. Zieloff and Ms Werther demonstrated their outstanding knowledge in their lectures and experimental sessions. The participants were given the opportunity to optimize separations themselves. All results were critically evaluated and discussed. Meanwhile the workshop has now been incorporated in our teaching program as a fixed element.

All in all, the event was considered a great success.

Once again a big thank you to CAMAG!



25-step AMD 2 gradient for the screening of water samples

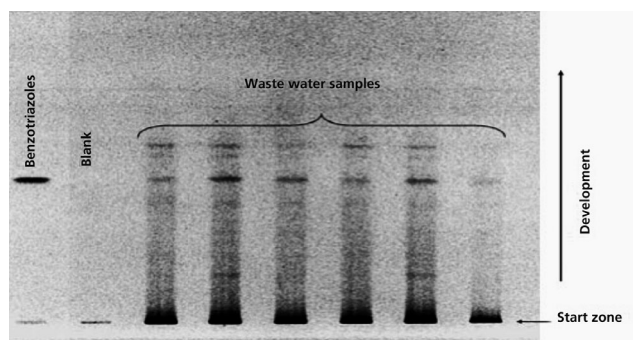


HPTLC/AMD multi-wavelength scan of an extract from secondary effluents (a), Nano LC/QTOF-MS chromatogram of the extracted zone, peak A: 1H-benzotriazole; peak B: tolyl-triazole (b)

For the identification of the two unknown analytes a substance library (approx. 300 entries) created at the laboratory for operation control and research of the LW was used, which features molecular formula and exact mass of potential environmental contaminants described in literature. The unknown analytes were identified as 1H-benzotriazole and a mixture of 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole.

Benzotriazoles are ubiquitous in the aquatic environment because they are used in a broad range of applications: in coatings and paints, as anticorrosive in copper and copper alloys, in coolants and lubricants for engines, as silver protection in detergents, as antifreeze and as aircraft de-icing fluid.

In 14 % of ground water samples ($n = 74$) 1H-benzotriazole was detected at a maximum of 173 ng/L and in 18 % of samples tolyl-triazoles were detected at a maximum of 75 ng/L. Concentrations in the



HPTLC/AMD chromatogram of SPE extracts from various secondary effluents and of the standard mixture 1H-benzotriazole/tolyl-triazole

river Danube and in some of its feeding rivers in the Ulm area were between 100 and 500 ng/L. In secondary effluents, the main source of benzotriazoles in the environment, concentrations of 1H-benzotriazole and tolyl-triazoles can even exceed 10 µg/L. Comparison of water sampled from wastewater treatment plants, surface water and ground water showed that the ratio of the investigated substances shifted towards 1H-benzotriazole.

Further information is available from the authors on request.

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

- [1] W.H. Weber *et al.* Vom Wasser 105 (1) (2007) 7
- [2] W.H. Weber *et al.* Vom Wasser 107 (4) (2009) 16
- [3] A. Müller *et al.* Rapid Commun Mass Spectrom 24 (2010) 659

Optimization of an AMD 2 method for determination of *stratum corneum* lipids



Prof. Dr. Ingo Schellenberg and Dr. Kathrin Kabrodt

The workgroup of Prof. Schellenberg at the Institute of Bioanalytical Sciences (IBAS) is engaged in the isolation and production of plant extracts, which are used in foodstuffs, in nutraceuticals, as well as in cosmetic and pharmaceutical products. Further core competencies are the encapsulation of bioactive ingredients, lipids and flavors, flavors also investigated as to their importance for sensory quality. Parts of these investigations were done by Ms Julia Lüttich in the course of her Bachelor thesis.

Introduction

Lipids are substances that are soluble in organic solvents due to their non polar properties. In *stratum corneum*, which represents the outer layer of skin, lipids play a key role in maintaining the barrier function of the skin, i.e. protection. Predominantly lipids of *stratum corneum* are ceramides, fatty acids and cholesterol. Ceramides consist of long-chain hydroxylated amine base called sphingoid, which are amide-linked to fatty acids. By various combinations of fatty acids and base types there are different ceramide groups. In human *stratum corneum* 9 ceramide groups have been identified up to now.

An optimized AMD2 separation of various lipid standards that are found in native *stratum corneum* is presented below. The AMD system was already successfully used for these analyses (see CBS 77, 90 and 93). However, the de-

scribed methods were rather time and solvent consuming.

Standard solutions

Standard substances	Abbreviation	Supplier	Sample amount (mg)	Concentration of standard solution (mg/mL)
Ceramide NS	NS	Sederma*	14,0	0,35
Ceramide NP	NP	Evonik*	4,8	0,12
Ceramide AS	AS	Sigma-Aldrich	4,8	0,12
Ceramide AP	AP	Evonik*	14,0	0,35
Cholesteryl-3-sulfate	C3S	Sigma-Aldrich	8,0	0,20
Cholesterol	C	Sigma-Aldrich	5,6	0,14
Cholesteryloleate	CO	Sigma-Aldrich	4,0	0,10
Glyceryl trioleate	GT	Sigma-Aldrich	6,0	0,15
Phosphatidylcholine	PC	Sigma-Aldrich	4,0	0,10
Oleic acid	OA	Sigma-Aldrich	3,6	0,09
Squalene	S	Sigma-Aldrich	8,0	0,20
Sphingomyeline	SM	Sigma-Aldrich	5,6	0,14

*Thanks to Sederma and Evonik for free supply of standards.

Each standard substance was weighted into a 2 mL volumetric flask each and filled up with methanol – chloroform 1:1 (stock solution). 500 µL of each stock solution were diluted to 10 mL with the same solvent (standard solution). 500 µL of each stock solution were put into a 10 mL volumetric flask and filled up with the same solvent (standard solution mix).

Chromatogram layer

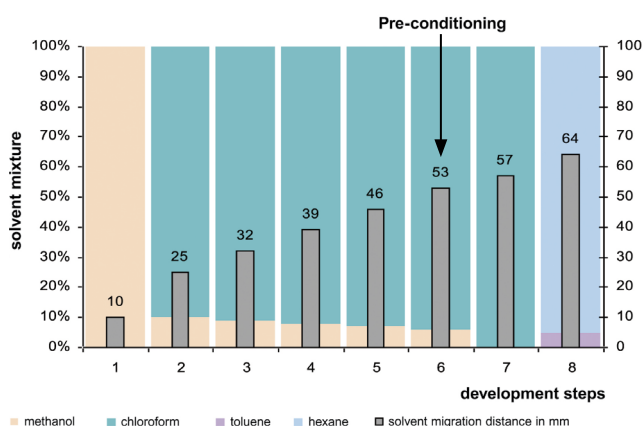
HPTLC plates 20 x 10 cm, silica gel 60 F₂₅₄, 0.1 mm for AMD (Merck); pre-washed twice with chloroform – methanol 2:1 (v/v) and dried at 120 °C in a drying oven for 30 min; storage in an exsiccator

Sample application

Bandwise with Automatic TLC Sampler 4, 13 tracks, band length 8 mm, distance from the side 14 mm, distance from lower edge 8 mm, track distance 13.6 mm (automatically calculated), application volume 5 µL

Chromatography

8-step gradient in AMD 2 system, pre-conditioning before step 6 with 4 M acetic acid, drying time 2 min, maximum migration distance 64 mm, duration 1.5 h, solvent consumption 60 mL



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Post-chromatographic derivatization

With the Chromatogram Immersion Device III the plate was immersed in copper(II)sulfate reagent (aqueous solution of 10% copper(II)sulfate and 8% orthophosphoric acid), then dried and heated at 170 °C on the TLC Plate Heater for 8 min.

Densitometry

Absorption measurement at 600 nm by TLC Scanner 3 with winCATS software

Documentation

TLC Visualizer under white light (reflection mode)

Results and discussion

The first step of the 8-step gradient (100 % methanol) was used to elute all polar substances and to focus them to a sharp line. Steps two to six separated cholesterol-3-sulfate and the different ceramides. Step seven (100 % chloroform) was used to separate cholesteryl oleate from glyceryl trioleate. In the last step with *n*-hexane – toluene 19:1, squalene was separated from cholesteryl oleate. To focus oleic acid, a pre-conditioning of the HPTLC plate with 4 molar acetic acid was necessary before step six was started.

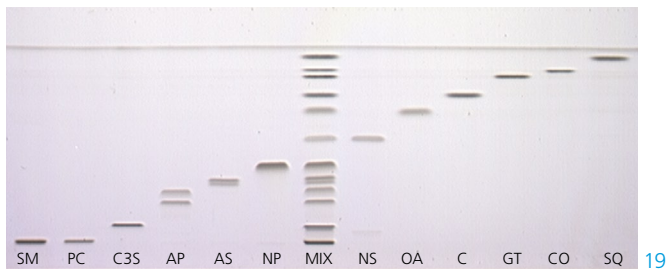


CAMAG AMD 2 System (Automated Multiple Development)

AMD is used when the desired resolution is unattainable over the available separation distance by one step isocratic development. The combination of multiple and gradient development, and the individual pH adjustment of the layer (here just before step 6) leads to a focusing effect of the zones. Peak sharpness and resolution are improved.

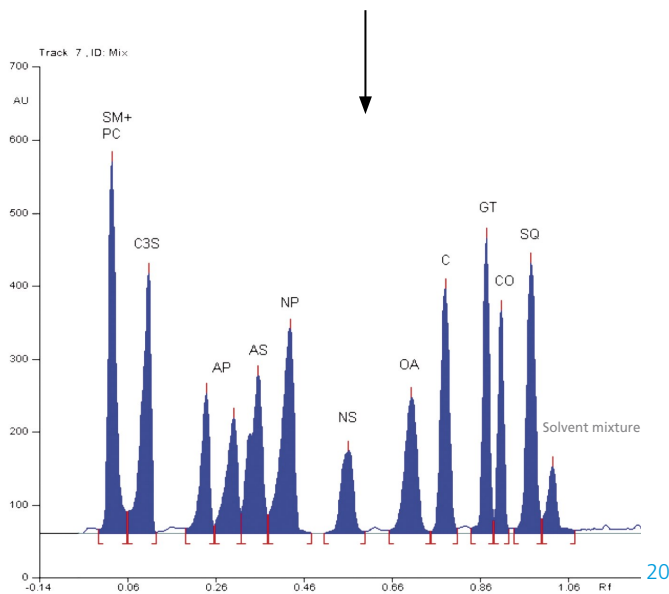
In general, AMD is used for mixtures of components with a wide polarity range. But for this AMD application in the field of lipid analysis, especially the unpolar region of the gradient is of interest. From step to step, the polarity change of the solvent (only 2 %) is minor. This way, also compounds of slight polarity differences are separated. The migration distance increments of mostly 7 mm are high compared to 2 to 4 mm increments usually. For a baseline separation using 4 mm increments, a reduced substance amount per zone (e.g. by a factor of 4) would be necessary. Additionally, a final migration distance of 40 mm would significantly reduce the gradient time further on.

Phosphatidylcholine and sphingomyeline (at the start) could not be separated with this gradient. However, if one wants to separate both lipids, it is possible to supplement some polar steps at the beginning of the gradient.



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AMD2 chromatogram using the 8-step gradient

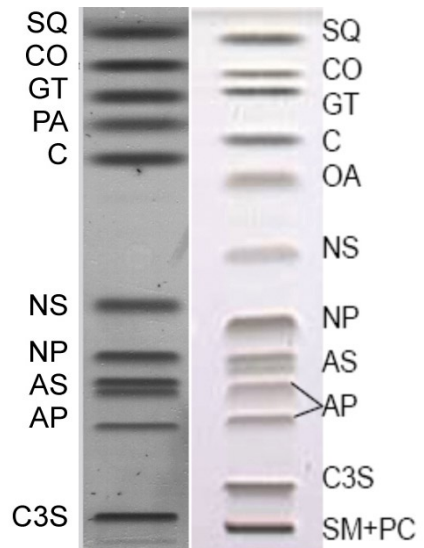


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Densitogram of the standard mixture

Just phosphatidylcholine and sphingomyeline (at the start position) were not separated by the new gradient. This was also not achieved by Bonté *et al.* [1] with their 26-step gradient with a duration of 6.8 h. Zellmer *et al.* [2] and Farwanah *et al.* [3] did not consider these substances in their methods.

A chromatogram comparison of the 17-step gradient by Farwanah *et al.* [3] and the optimized 8-step gradient shows a comparable resolution despite the reduced number of steps. The gradient time was reduced by one hour (from 2.5 h to 1.5 h) and the derivatization time was reduced from 20 min (at 150 °C) to 8 min (at 170 °C).



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Chromatogram comparison of the 17-step gradient by Farwanah *et al.* ([3], left) and the optimized 8-step gradient (right)

At present this method is in the validation process and *in vivo*-extraction of defined skin zones is optimized to separate and quantify the most important skin lipids.

Further information is available from the authors on request.

[1] F. Bonté *et al.* J Chromatogr B 664 (1995) 311

[2] S. Zellmer *et al.* J Chromatogr B 691 (1997) 321

[3] H. Farwanah *et al.* J Chromatography B 780 (2002) 443

Contact: Dr. Kathrin Kabrodt, Prof. Dr. Ingo Schellenberg, Anhalt University of Applied Sciences, Center of Life Sciences, Institute of Bioanalytical Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany, k.kabrodt@loel.hs-anhalt.de

Determination of additives in plastic foils



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Elisabeth Dytkiewitz, Prof. Dr. Wolfgang Schwack

At the Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany, efficient analytical work is conceptualized and then developed and implemented. In doing so, planar chromatography is often used due to its pragmatism in solving analytical tasks. The following example concerning the analysis of food packaging materials is part of the doctoral thesis of Elisabeth Dytkiewitz.

Introduction

Plastic foils of polyvinyl chloride (PVC) used for packaging meat, cheese, fresh vegetables, etc., contain plasticizers and other additives in high percentages. Most of them have in common that they are not chemically bonded to the polymer, making them potentially free for migration into the packaged food. According to the European legislation, migrating constituents must not endanger human health [1]. Therefore migrating studies with food simulating solvents must be performed to check that the composition is compliant. An effect directed analysis (EDA) brings the bioactivity of substances into focus. Using EDA for the analysis of migrating additives was the aim of this study.

After separation by HPTLC, additives present in migrates are solvent-free accessible, making detection with bioluminescent *Vibrio fischeri*

bacteria practicable. Therefore many samples can be run in parallel, revealing not only the presence of toxicologically relevant compounds but also information as to their strength of effect. In contrast to common mixed mode cuvette tests, HPTLC-EDA enables the detection of both inhibition and enhancement of luminescence. Detected additives can be identified from the same plate by coupling of HPTLC with mass spectrometry via the TLC-MS Interface. MassWorks software delivers exact masses and molecular formulae even from a mass spectrometer of low resolution. Hence, HPTLC-bioactivity-MS enables rapid analysis of plastic migrate components based on their toxicological effects.

Concerning rapid screenings with focus on major components, the TLC-MS Interface is also suitable for the direct extraction of additives from the packaging films followed by online mass spectrometric analysis.

Sample preparation

Foil samples (0.8 g) were extracted by 150 mL ethanol (95 % vol) in a screw capped bottle for 4 h at 60 °C. After rotary evaporation of the solvent, the residues were taken up in 2 mL toluene. The solutions were directly used for HPTLC after membrane filtration.

Layer

HPTLC plates silica gel 60 F₂₅₄, Merck, 20 × 10 cm, prewashed by development with methanol, dried for 15 min in a drying oven at 100 °C.

Sample application

Bandwise with ATS4, band length 6 mm, track distance 18 mm, distance from lower plate edge 8 mm, distance from the edges min. 20 mm, application volumes of sample solutions 5–10 µL.

Chromatography

In the ADC2 with isooctane – toluene – diethyl ether – ethyl acetate 8:7:4:1 after chamber saturation for 10 min up to a migration distance of 65 mm. Plate activity was controlled by saturated magnesium chloride solution for 3 min (42 % relative humidity). Plate drying was automatically performed for 30 min.

Detection with biological activity

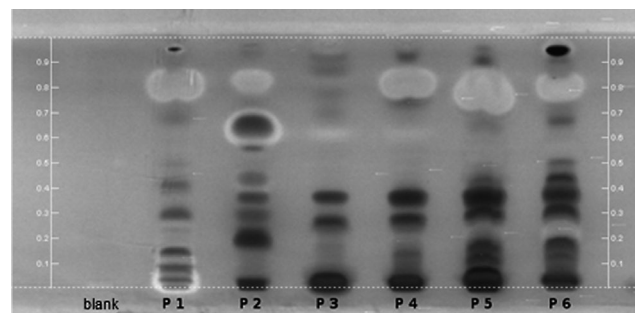
Using the TLC Chromatogram Immersion Device (vertical speed 3 cm/s, immersion time setting 0 s), the plate was dipped in a suspension of *Vibrio fischeri* bacteria (BioLuminex kit, ChromaDex, Boulder, USA) and documented with the Bioluminizer (exposure time 50 s) directly after dipping and after 5 and 10 min.

Mass spectrometry

TLC-MS Interface (oval elution head 4 × 2 mm) coupled to an Agilent 1100 LC/MSD system, operating in positive ESI mode, zone elution with ethanol (95 %vol) at a flow rate of 0.2 mL/min during 10 s. Exact masses were calculated with MassWorks software (Cerno Bioscience, Danbury, CT, USA).

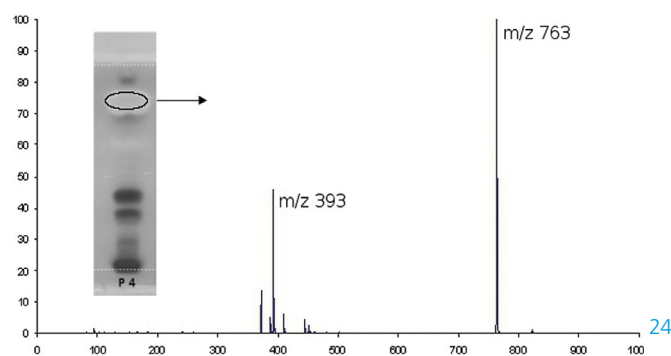
Results and discussion

Ethanol (95 %vol) as a fatty food simulating solvent was used for extraction of additives from plastic foils [2]. These migrates were directly used for HPTLC, which was performed by automated standardized development in the ADC 2 to ensure repeatable results. After some modification of the mobile phase [3] a good separation was obtained. Detection with bioluminescent bacteria showed numerous substances with inhibiting effects in the samples. Even in a plastic wrap of polyethylene (PE), luminescence inhibiting substances were detected. Remarkably, some of the zones caused an activating effect on the bacteria's luminescence, a notable advantage given by the previous chromatography. A cuvette test of the whole migrate would not reveal this property.



Separation and detection of 6 packaging foils; inhibition of bioluminescence displayed by dark zones, enhancement of bioluminescence displayed by light zones. Track assignment: blank: ethanol, P1 and P2: commercial PVC cling film, P3: commercial PE cling film, P4: foil of a mushrooms' packaging, P5: packaging foil for meat, and P6: packaging foil for cheese.

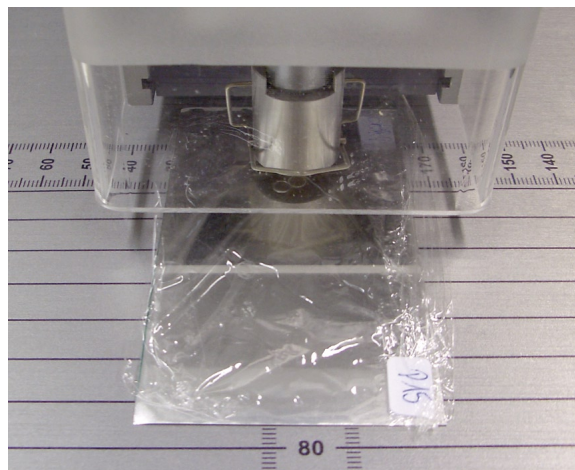
For the identification of EDA detected zones, mass spectra were recorded using the TLC-MS Interface. Ethanol was used to elute the compounds into the ion source. With an elution time of just 10 s, the mass spectrum of the sample zone was achieved immediately. Sodium adducts are caused by the saline bacteria suspension remaining at the HPTLC plate surface. After calculation by MassWorks software, the signals from a low resolution mass spectrometer were improved. Thus, exact masses and molecular formula were obtained enabling the identification of additives.



HPTLC-ESI/MS spectra of the light zone at hR_f 84 of a mushroom packaging foil (P4).

For a rapid screening of additives extractable from packaging foils, the TLC-MS Interface was directly applied for the transfer of migrates from the foil to the MS, i.e. without chromatography. To accomplish this, the sample foil was placed on the back of a TLC aluminum foil. After tightening the

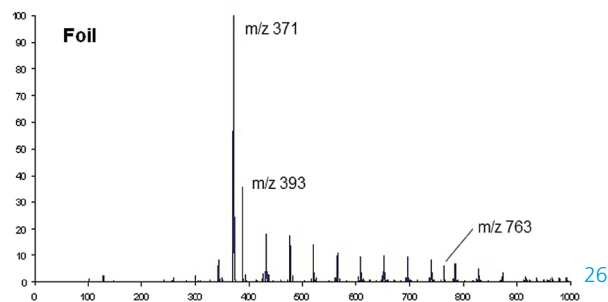
elution head, the mass spectrum was recorded within 10 s.



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Direct extraction of a PVC plastic wrap by the TLC-MS Interface. The film was placed planar on the back of a TLC aluminum foil.

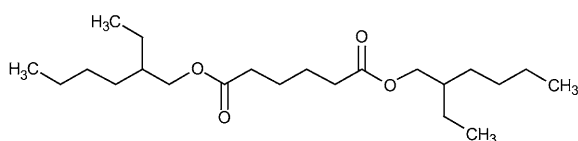
Using this short elution time, the highly soluble and more concentrated additives could easily be identified by mass spectrometry, protonated molecules being generally detected.



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ESI-MS spectrum of compounds directly extractable from a foil by the TLC-MS Interface (P4)

Applying mass spectrometry, bis(2-ethylhexyl) adipate could exemplarily be identified with marginal deviations from the theoretical masses.



Bis(2-ethylhexyl) adipate

MS-Signals of	Mass determined	Theoretical mass	Δ (ppm)	Molecular formula	Assigned to
Plastic foil	371,3174	371,3161	-3,4071	$C_{22}H_{43}O_4$	$[M+H]^+$
HPTLC plate	393,2985	393,2981	-1,0691	$C_{22}H_{42}O_4Na$	$[M+Na]^+$
	763,6077	763,6064	-1,7164	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$

Other substances showing inhibiting effects by *Vibrio fischeri* are not present in this spectrum. The low concentration of some substances and the short time of just 10 s for direct foil extraction are the reasons for the reduced elution. Of course, substances eluted into the food simulating solvent could be measured more intensely after a longer contact time simulated by an intermediate flow stop; however, this would also reduce sample throughput.

Direct foil extraction is useful for rapid screening, but the ultimate procedure which produces additional information is chromatography of migrates, obtained after prolonged heat treatment (4 h at 60 °C) and concentration (by a factor of 75). Thus, information is more comprehensive. *Vibrio fischeri* is ideal for detecting other components with toxicological potential, not just major components. It is a specific benefit that from the wealth of these migrating compounds just bio-actives can be detected and identified.

[1] Regulation (EC) No. 1935/2004 as amended on 18.06.2009

[2] Attachment 10 for §11 BedarfsgegenständeVO as amended on 23.09.2009

[3] H. Chen, Y. Wang, R. Zhu, Chinese J Chromatogr A 24 (2006) 69

Further information is available from the author on request.

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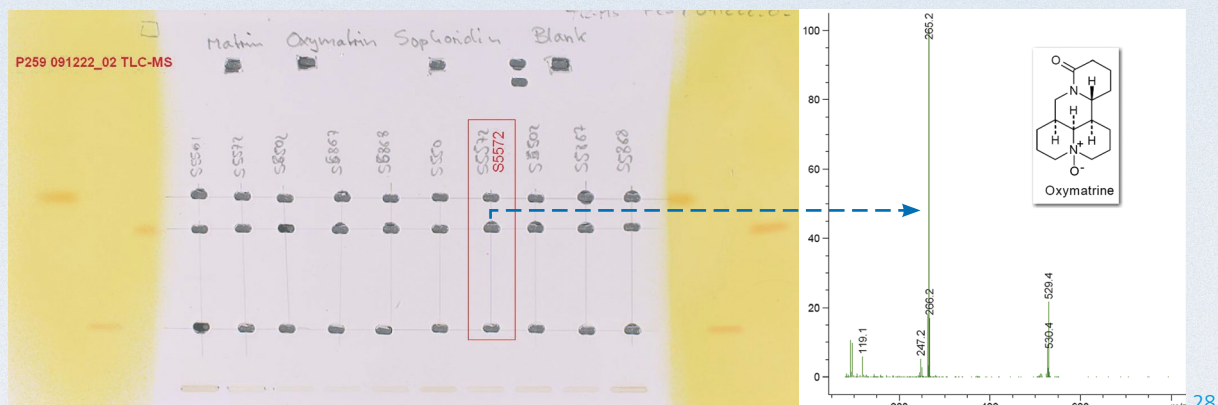
TLC-MS Interface

World wide used
for identification and elucidation
of unknown substances
in research, forensic and
environmental fields

Identification of alkaloids
oxymatrine, sophoridine and
matrine in *Sophora flavescens*
extract*



Cut and milled *Sophora flavescens* root parts



HPTLC plate after extraction of zones with the TLC-MS Interface

Confirmation of oxymatrine, m/z 265 $[M+H]^+$

Further information at: www.camag.com/tlc-ms

*Diploma thesis R. Vizzini

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