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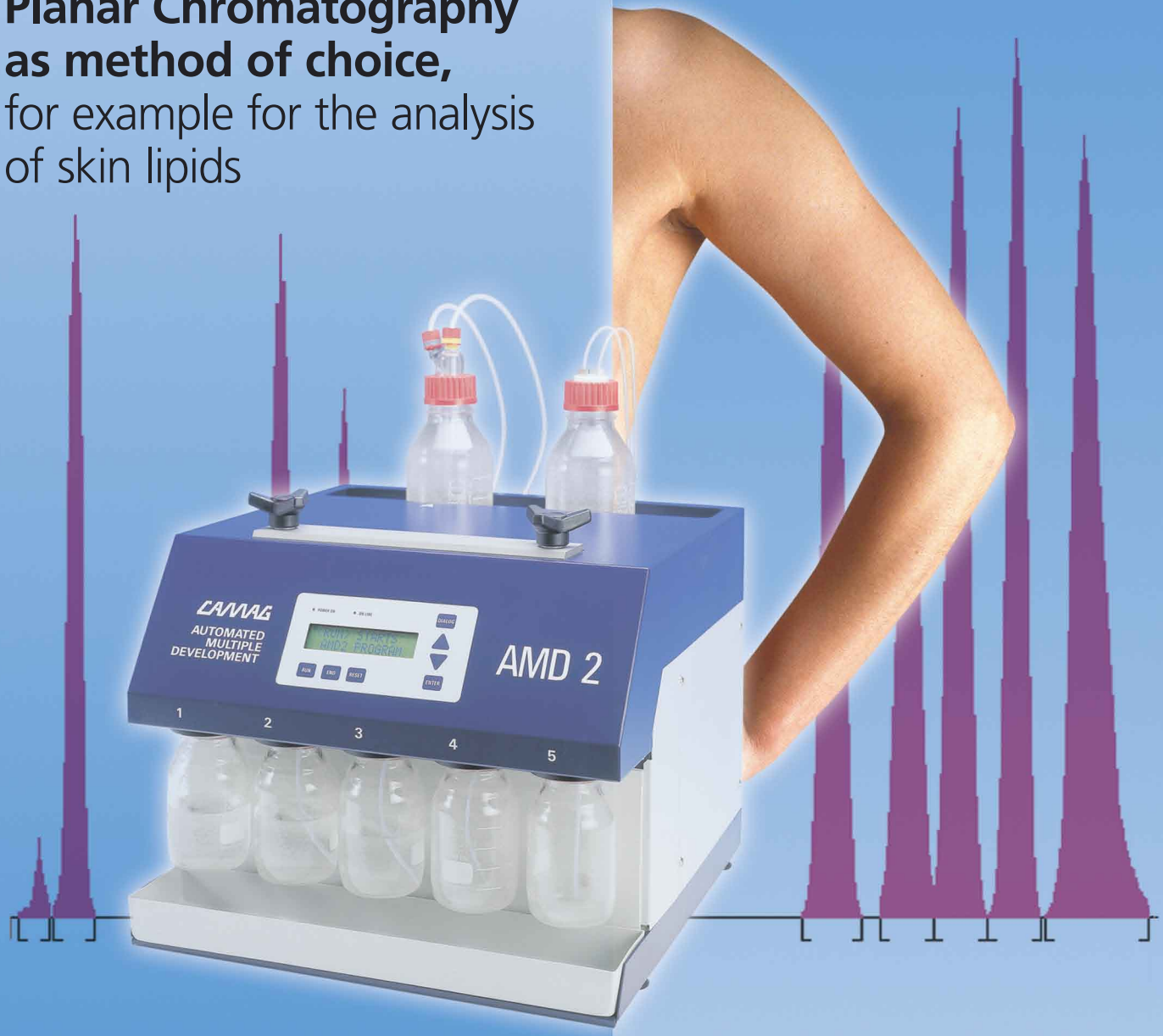
PLANAR CHROMATOGRAPHY

Planar Chromatography as method of choice, for example for the analysis of skin lipids



Important Date:

International Symposium for High Performance Thin-Layer Chromatography, Lyon, October 15–18, 2003

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Improved analysis of skin lipids by AMD

▲ *Prof. Neubert, H. Farwanah, Dr. Raith (left to right)*

▲ *Dr. Zellmer*

The research group of Prof. Neubert works on the development of colloidal drug dosage forms, penetration and permeation of drugs after topical application as well as on quantitation and characterization of active substances and excipients in complex biological matrices. For almost 10 years the group is involved with characterization of natural products such as skin lipids.

Introduction

For better understanding of skin disorders such as psoriasis, atopic dermatitis, ichthyosis, or xerosis, it is important to know the composition and the influence of skin lipids in relation to the degree of the disorders. For this purpose lipids are extracted in vivo from a defined skin zone and analyzed with respect to the composition of lipids, especially ceramides, fatty acids and cholesterol. Already six years ago in CBS 77 we have reported about an interesting paper on this topic by F. Bonte, P. Pinguet, J.M. Chevalier and A. Meybeck. The new method by H. Farwanah, R. Neubert, S. Zellmer and K. Raith* presented here has advantages over the former. Not only can the most important lipids of the stratum corneum be separated and quantified but also the seven known ceramide classes in the same chromatographic run.

Unlike in RP-HPLC which generates a not so comfortable elution characteristic according to chain length, separation of ceramide classes according to number and position of hydroxy groups is possible. Planar chromatography offers clear benefits for this special field of analysis in contrast to HPLC. It is more robust with respect to matrix, faster (time of chromatography is 10 min per sample), uses less solvent (8 mL per sample) and it is more convenient during detection than other techniques. As method of choice it is successfully used for diagnostic purposes as well as for high-throughput analyses.

Sample preparation

12 cm^2 skin area at the inner forearm is extracted with n-hexane – ethanol (2:1) for 5 min, the extract is evaporated at 50° C, the residue is dried under a stream of argon and is dissolved in 500 µL chloroform –methanol (1:1).

Chromatogram layer

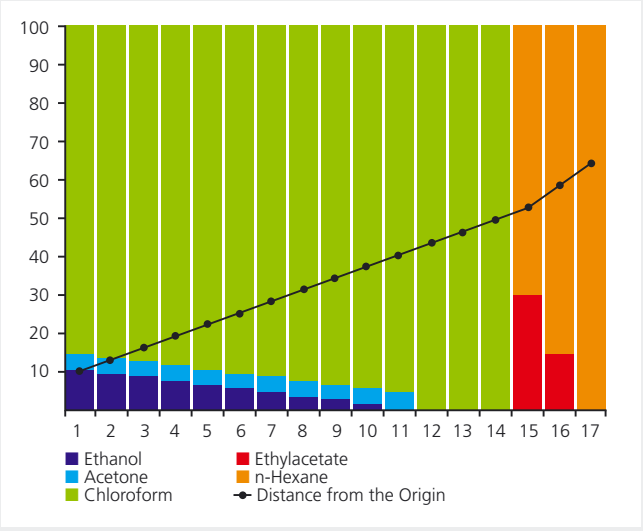
HPTLC plates silica gel 60 F_{254} (Merck), 20 x 10 cm, prewashed

Sample application

With Automatic TLC Sampler 4, 15 tracks as 8 mm bands, application volume 4 and 6 µL, track distance 12 mm, distance from lower edge 8 mm, distance from the side 10 mm

Chromatography

In AMD 2 system using a 17-step gradient on basis of chloroform (see fig. 3), consumption of solvents 120 mL (8 mL per sample), drying step of 90 s and conditioning of the layer with 4 M acetic acid between the single chromatographic runs, developing distance 65 mm, developing time 150 min (10 min per sample).



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▲ *AMD 2 gradient: 11 steps with chloroform – ethanol – acetone and then 3 isocratic steps with chloroform for separation of cholesterol, cholesterol sulfate and the various ceramide classes; for separation of cholesterol, fatty acids, triacylglycerol, cholesteryl esters, and squalene 2 steps with n-hexane – ethyl acetate followed by an isocratic step with n-hexane.*

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CAMAG AMD System
(Automated Multiple Development)

AMD is used when the desired resolution is unattainable over the available separation distance by one step isocratic development. This is often the case for complex samples with high or differing matrix content, mixtures of components with a wide polarity range, or for multi-component mixtures.

For separation of samples with components covering a wide polarity range, a universal gradient reaching from high elution strength to very low elution strength is employed.

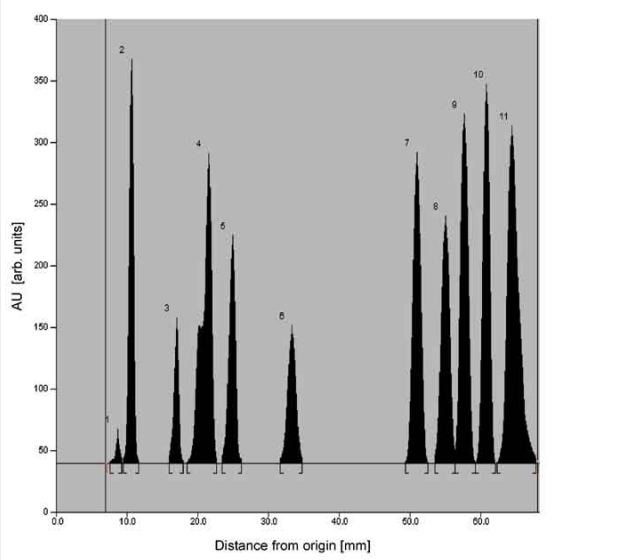
The lipids separated in this application are developed with a gradient based on chloroform. The gradient is shallower compared to that previously decribed by F. Bonte, P. Pinguet, J.M. Chevalier and A. Meybeck concerning the same subject and enables the separation of the ceramide classes according to number and position of hydroxy groups. The first 14 steps with chloroform (from 85% to 100%) are necessary for the separation of cholesterol, cholesterol sulfate and the various ceramide classes. Whereas the last three steps with n-hexane (from 70% to 100%) are employed for separation of cholesterol, fatty acids, triacylglycerol, cholesteryl esters, and squalene.

Postchromatographic derivatization

Dipping in copper sulfate reagent (aqueous solution of 10% copper sulfate, 8% phosphoric acid and 5% methanol) using the Chromatogram Immersion Device, followed by heating for 20 min at 150° C.

Densitometric evaluation

With TLC Scanner 3 and winCATS software, absorption measurement at 546 nm, evaluation of peak area



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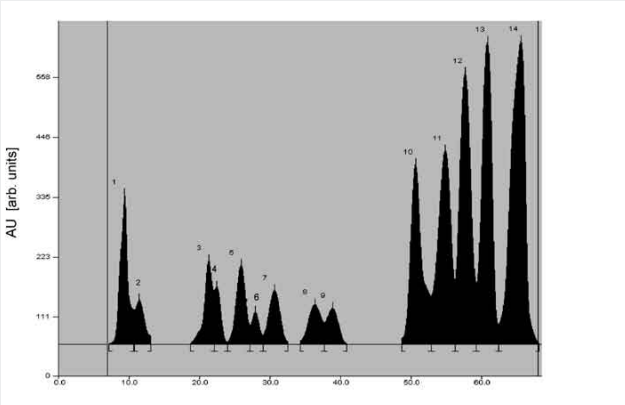
▲ *Densitogram of a lipid standard: 1 = start peak, 2 = cholesterol-3-sulfate, 3 = first peak of synthetic ceramide AP, 4 = double peak of ceramide AS and the second peak of ceramide AP, 5 = ceramide NP, 6 = ceramide NS, 7 = cholesterol, 8 = palmitic acid, 9 = triolein, 10 = cholesteryl oleate, 11 = squalene*



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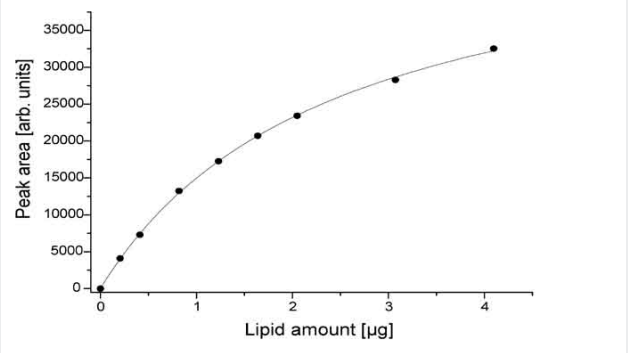
▲ *Densitogram of in-vivo extracted stratum corneum lipids: 1 = start peak, 2 = cholesterol-3-sulfate, 3 = ceramide AH, 4 = ceramide AP, 5 = ceramide AS, 6 = ceramide EOH, 7 = ceramide NP, 8 = ceramide NS, 9 = ceramide EOS, 10 = cholesterol, 11 = free fatty acids, 12 = triacylglycerol, 13 = cholesteryl esters, 14 = squalene*

Results and discussion

The dermatological important skin lipid classes, i.e. ceramides, fatty acids and cholesterol, are well separated. The variation coefficients of the lipids were between 0.2 and 10%. The study reveals that the lipid class composition shows remarkable interindividual variations, whereas the ceramide composition is relatively stable. The relatively soft in-vivo extraction conditions may cause the slightly different ceramide profile as compared to some literature data.



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▲ *Calibration curve (Michaelis-Menten) of ceramide NP, coefficient of correlation 0.9995*

Lipids	µg/cm²	% of recovered ceramides
Ceramide EOS	1.9 ± 0.7	15.7 ± 2.3
Ceramide NS	1.7 ± 0.4	14.1 ± 2.3
Ceramide NP	1.5 ± 0.4	12.1 ± 1.6
Ceramide EOH	1.0 ± 0.3	8.1 ± 0.7
Ceramide AS	2.7 ± 0.7	22.3 ± 1.5
Ceramide AP	0.9 ± 0.4	7.9 ± 3.2
Ceramide AH	2.4 ± 0.6	19.8 ± 1.7

▲ *Profile of the extracted ceramides of six different persons*

A copy of the original publication in J. Chromatogr. B, 1 (2002) 443-450 is available from CAMAG or the authors (raith@pharmazie.uni-halle.de) on request.

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Selective determination of taurine and L-lysine hydrochloride in energy drinks and multi-vitamin syrup

Introduction

Prof. Indrayanto, T.K. Sia, and Y. I. Wibowo are routinely analyzing vitamins in their laboratory by means of an HPLC-UV/-DAD method. However, the HPLC method cannot be used for assay determination of taurine and lysine without elaborate derivatization. For determination of lysine the official method in the British, Indonesian and United States Pharmacopoeia is potentiometric titration. For the determination of taurine no official method exists. Although there are publications about GC and HPLC methods for the assay of taurine in biological fluids, no method is available for simultaneous determination of taurine and lysine, which are both present in multi-vitamin syrup. Analysis of the amino acids by means of an amino acid analyzer is too expensive and time-consuming.

That is why the authors established this simple, rapid and cost efficient planar chromatographic method. Once again the flexibility of planar chromatography is demonstrated. The sample is directly applied onto the layer. Later on, by post chromatographic derivatization with ninhydrin, taurine and lysine are selectively detected in the presence of all other constituents including vitamins.

Chromatogram layer

TLC Aluminium sheets silica gel (Merck), 20 × 10 cm

Sample application

Spotwise with Nanomat, 18 tracks, application volume 2 µL (sample), distance from the lower edge 10 mm, distance from the side 15 mm, track distance 10 mm

Chromatography

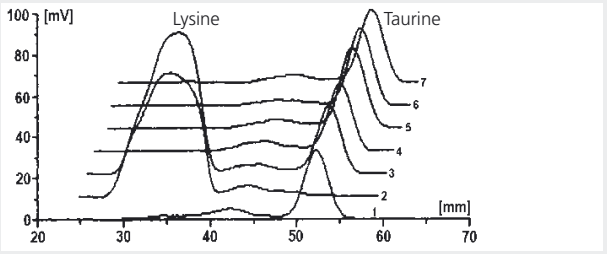
In Twin Trough Chamber with n-butanol – acetic acid – ethanol – water 4:2:3:3, developing distance 80 mm

Postchromatographic derivatization

Dipping in ninhydrin reagent using the Chromatogram Immersion Device, followed by heating for 3–4 min at 100° C.

Densitometric evaluation

TLC Scanner 3 with CATS software, absorption measurement at 490 nm, linear regression of peak area



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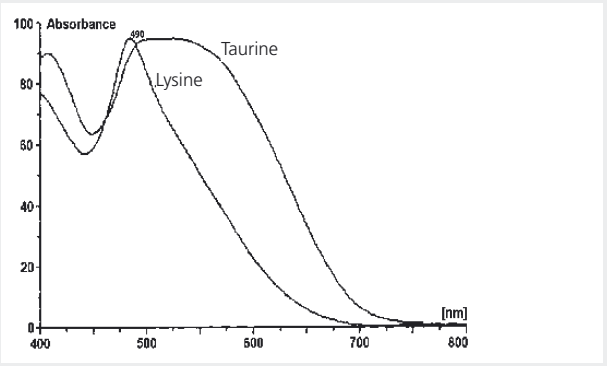
▲ *Densitogram of taurine standard (1), lysine standard (2), multi-vitamin syrup (3) and some energy drinks (4–7)*



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▲ *VIS spectra of taurine and lysine – besides for establishing identity spectra are also used for purity check*

Results and discussion

The complete validation can be reviewed in the original publication. The intermediate precision is better than 1.6%. The average recovery rate at different concentration levels is found to be 99.4 ± 1.7% (n = 13) for taurine and 100 ± 0.9% (n = 5) for lysine.

A copy of the original publication in J. of Planar Chromatogr. 14 (2001) 24–27 is available from CAMAG or the authors (indrayanto@hotmail.com) on request.

* G. Indrayanto, Lab. of Pharm. Biotechnology, Fac. of Pharm., Airlangga University, Jl. Dharmawangsa dalam, Surabaya 60286, and T. K. Sia and Y. I. Wibowo, Research and Development, PT Berofarm Pharmaceutical Comp., Buduran, Sidoarjo 61252, Surabaya, Indonesia

Effective analysis of phospho- and glycolipids in plant lecithins

◀ *K. Schipmann, C. Heift and Dr. R. Lange (from left to right)* 10

C. Heift*, K. Schipmann and R. Lange are working for Degussa Texturant Systems Germany GmbH & Co. KG, Hamburg. As part of the joint project NAPUS 2000 they are involved with clean up and determination of phospho- and glycolipids as well as other minor components of plant lecithins.

The planar chromatographic method presented below was developed by C. Heift, K. Schipmann and R. Lange and is based on HPTLC layers. Compared to classical TLC it allows detection and determination of a larger range of compounds at higher sensitivity. The HPTLC method shows very good separation power and selectivity for all phospholipids. Aside from the actual phospholipids also the lyso compounds can be separated completely in the same run and thus quantitated without difficulties. A further advantage of planar chromatography is its visualization as chromatogram (substances remaining at the start zone are visualized compared to HPLC). The HPTLC method is also suitable for separation and determination of glycolipid classes in plant lecithins. This rapid (time required for separation is 30 to 40 s per sample in contrast to HPLC which needs 20 min per sample), powerful, yet easy procedure is used by the authors as method of choice for all lecithin containing substrates. Up to now seeds, leaves, raw oils and fats, lecithins, cell cultures, aqueous extracts, bread dough as well as margarines have been analyzed.

Scope

Phospho- and glycolipids are essential components of all living tissues. Used commercially they play an important role because of their physiological activity as part of food and cosmetics and their medicinal and biotechnological applications. For the analysis of phospho- and glycolipids ^{31}P-NMR, HPLC and planar chromatography can be used. ^{31}P-NMR is applicable for all phospholipids, but is used predominantly in research because of considerable sophistication. HPLC and planar chromatography (mostly still on classical TLC layers) are used for routine analyses. However, due to limited specificity and separation power of the established TLC methods, so far those have only been applicable to certain phospho- and glycolipids.

Sample preparation

Substrates are homogenized and applied as powder, suspension or liquid on cyano propyl-SPE-columns. Triglycerides are eluted with n-hexane, glycolipids with isopropanol/acetone and phospholipids with methanol, evaporated and taken up in chloroform – methanol 2:1.

Chromatogram layer

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

Sample application

With the Automatic TLC Sampler 4, 20 tracks, band length 7 mm, application volume 1 to 5 µL for phospholipids und 0.2 to 2 µL for glycolipids, distance from lower edge 8 mm, distance from the side 10 mm, track distance 9 mm

Chromatography

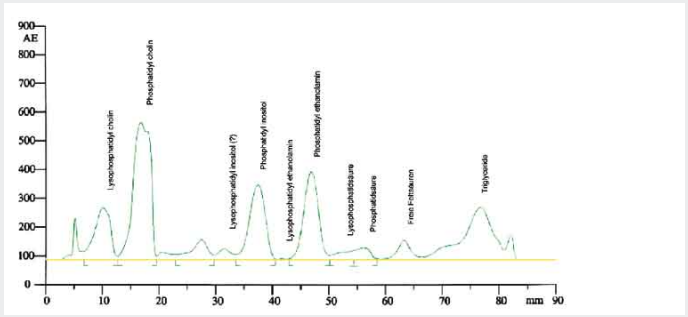
In Horizontal Developing Chamber 20 x 10 cm (tank configuration) with 4 mL chloroform – methanol – acetone – water 18:15:3:1, developing time 15 min for phospholipid separation and with 4 mL acetone – chloroform – water 30:15:2, developing time 10 min for die glycolipid separation, developing distance 70 mm each

Postchromatographic derivatization

Dipping in molybdatophosphoric acid reagent (5% in ethanol) using the Chromatogram Immersion Device, followed by heating at 120° C for 15 min. Phospholipids and glycolipids appear as dark green zone on a light blue background.

Densitometry

TLC Scanner 3 with winCATS software, absorption measurement at 720 nm, evaluation of peak area with linear regression



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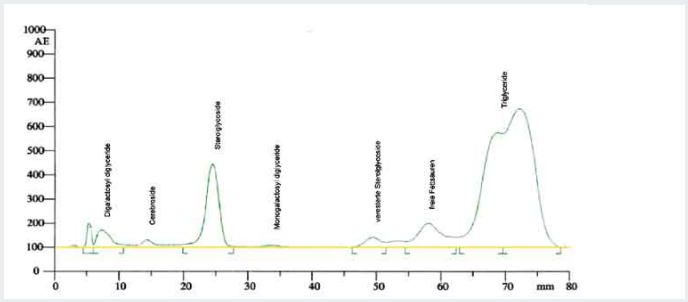
▲ *Densitogram of phospholipids from rape seed**: lyso-phosphatidylcholine 0.2 µg, phosphatidylcholine 2.3 µg, phosphatidylinositol 1.5 µg, phosphatidylethanolamine 1.2 µg, phosphatidic acid 0.3 µg*



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▲ *Densitogram of glycolipids from rape seed**: digalactosyldiglycerid 0.3 µg, cerebroside 0.1 µg, sterylglycoside 0.4 µg, monodigalactosyldiglyceride < 0.1 µg, esterified sterylglycosides 0.1 µg*

Results

This planar chromatographic method adds and especially increases the application spectrum of the established methods (HPLC, ^{31}P-NMR and TLC) for routine analyses of these compounds. Recovery rates for phospholipids are between 96 and 102% and for glycolipid classes between 98 and 103%. Calibration functions of the single phospholipids are linear in the range of 0.09–4.4 µg and of the single glycolipids classes in the range of 0.1–2.0 µg. Coefficient of correlation is found to be between 0.997 and 0.999.

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Chromatogram Immersion Device

All authors of this CBS issue preferred the Chromatogram Immersion Device if postchromatographic derivatization was required (see also page 4 and 5). Compared to spraying, reagent transfer by dipping offers numerous advantages:

- Standardization of the reagent transfer (selectable constant speed of immersion and selectable immersion time)
- Homogeneous reagent transfer all over the layer and thus better reproducibility
- Lower concentration of the active ingredient in the reagent (due to the more efficient liquid transfer of immersion)
- Health risk regarding air contamination

Automatization of the immersion avoids solvent front like tide marks that would disturb densitometric evaluation.

Acknowledgement: Work as part of the joined project Napus 2000 were funded by BMBF.

* Degussa Texturant Systems Germany GmbH & Co KG, Ausschläger Elbdeich 62, D-20539 Hamburg, Germany, contact person: claudia.heift@degussa.com

** Especially in seed analysis this planar chromatographic method is preferred to HPLC because planar chromatography can cope with less amount of sample (analysis of a half grain of seed has to be managed).

CAMAG Distributor Network Worldwide

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In Switzerland we sell our products to customers direct from our headquarters in Muttenz. In Germany and in the United States we sell through our daughter companies in Berlin and in Wilmington, North Carolina. In the newly founded company representing us in France we hold a share minority. In over 70 countries we are represented by carefully selected distributors, most on an exclusive basis.

Every exclusive CAMAG distributor is required to have at least one CAMAG trained product specialist and delegate him or her regularly – usually about every 18 months – to in-house training courses in Muttenz. Alternatively we also offer training courses overseas, e.g. in Far East, which are held by specialists from Switzerland. Task of the CAMAG specialist is to advise customers competently in the selection and application of their CAMAG products and to train them with their operation.

In smaller markets, where the specialist of our distributor cannot maintain full competence, we delegate a specialist from Switzerland for commissioning new systems. In an analogous way we train the service engineers of our distributors and keep them current by training courses in Muttenz.

For customers regulated by GMP/GLP, we offer system qualifications according to IQ/OQ (installation/operation qualification) as a service. In the mean time several of the CAMAG specialists of our distributors have obtained their personal certification to implement IQ/OQ of our systems.

We see ourselves as flexible, customer oriented and scientifically credible organization that in its 45 years of history has earned recognition as a competent partner in all areas of planar chromatography.

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.

Chromatogram development – part 2:

effects of developing distance and position in the chromatogram on resolution

Unlike in HPLC and GC where the mobile phase flow is a parameter which can be easily maintained at a specified rate, in TLC the velocity of the mobile phase generally cannot be controlled unless forced flow development technique is employed[1]. It is affected by the nature of the stationary phase (porosity, packing, particle size, etc.) as well as by properties of the mobile phase (viscosity, surface tension, vapour pressure of the solvents etc.). Generally it is decreasing during development (Fig. 1). Due to the higher resistance against mobile phase flow of a stationary phase packed densely with fine particles, only short developing distances can be utilized on HPTLC plates.

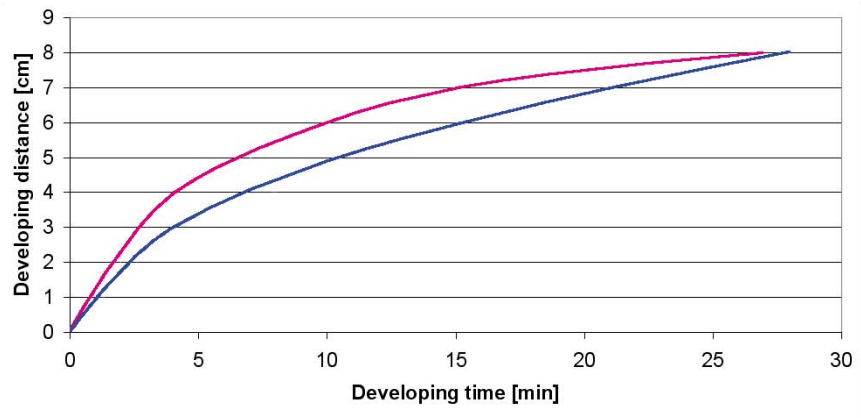


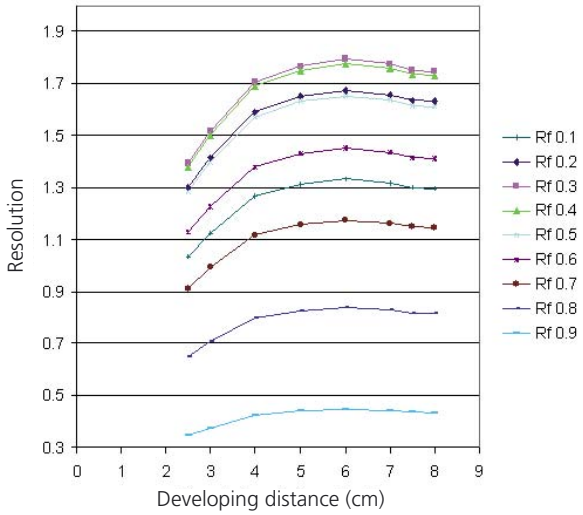
Figure 1 Relationship of developing distance and developing time on HPTLC silica gel 60. Mobile phase: toluene – ethyl acetate 19:1 (red) and ethyl acetate – methanol – water – formic acid 50:2: 3:6 (blue)

[1] *Forced flow development techniques are not discussed here because chromatographic results are different to those obtained by chromatogram development techniques due to absence of the gas phase.*

Keeping all other parameters constant, resolution R_s of two compounds is as much dependent on their relative position in the chromatogram (R_F) as on the migration distance of the front (developing distance).

Figure 2 shows plots of the resolution between two components in an HPTLC system with assumed selectivity of $\alpha = 1.5$ as a function of the developing distance. Resolution was calculated using the equation according to Snyder:

$$R_s = 1/4\,(\alpha - 1)\,(R_F \cdot N)^{1/2} \cdot (1 - R_F)$$



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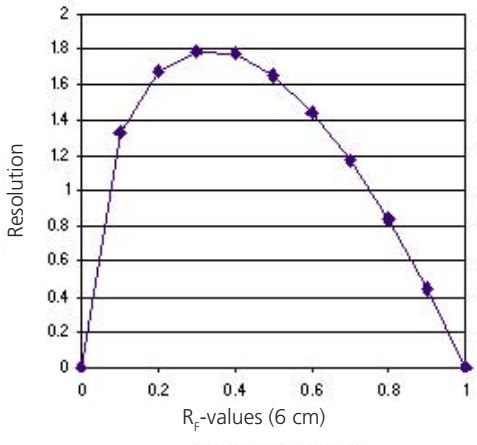

b

Figure 2 Influence of the developing distance and R_F-values (a) and the R_F-values in detail at 6 cm developing distance (b) on the resolution $R_s = 1/4\,(\alpha - 1)\,(R_F \cdot N)^{1/2} \cdot (1 - R_p)$, selectivity α is 1.5, plate number N is taken from[2]

On HPTLC plates the best resolution is obtained at a maximum of 6 cm developing distance (see figure 2a). For most mobile phases on silica gel the development requires 7–20 min. Within a given chromatogram resolution is best in the R_F-range of 0.3–0.4 (see figure 2b). Therefore, the solvent strength of the mobile phase should be adjusted so that a critical substance pair is positioned in this range.

[2] *Poole CF, Poole SK: Chromatography today. Amsterdam: Elsevier Science, 1991, p. 666*

These theoretical predictions can easily be proven experimentally. In Figure 3 the separation of chamomile oil on HPTLC silica gel plates is presented. Based on the substance pair in the R_F range 0.4–0.5 in the chromatograms of Figure 3a, resolution appears to increase as the developing distance is extended. However, if the chromatograms are put on the same scale (Fig. 3b), it is seen that the relative position of the two components does not change. Resolution still goes through a maximum for 6 cm developing distance. Generally, the R_F-values decrease with extended developing distances. This effect can be explained with the increased loading of the plate with volatile components of the developing solvent. The visual impression can be supported by comparison of the analog curves on the same scale (Fig. 3c).

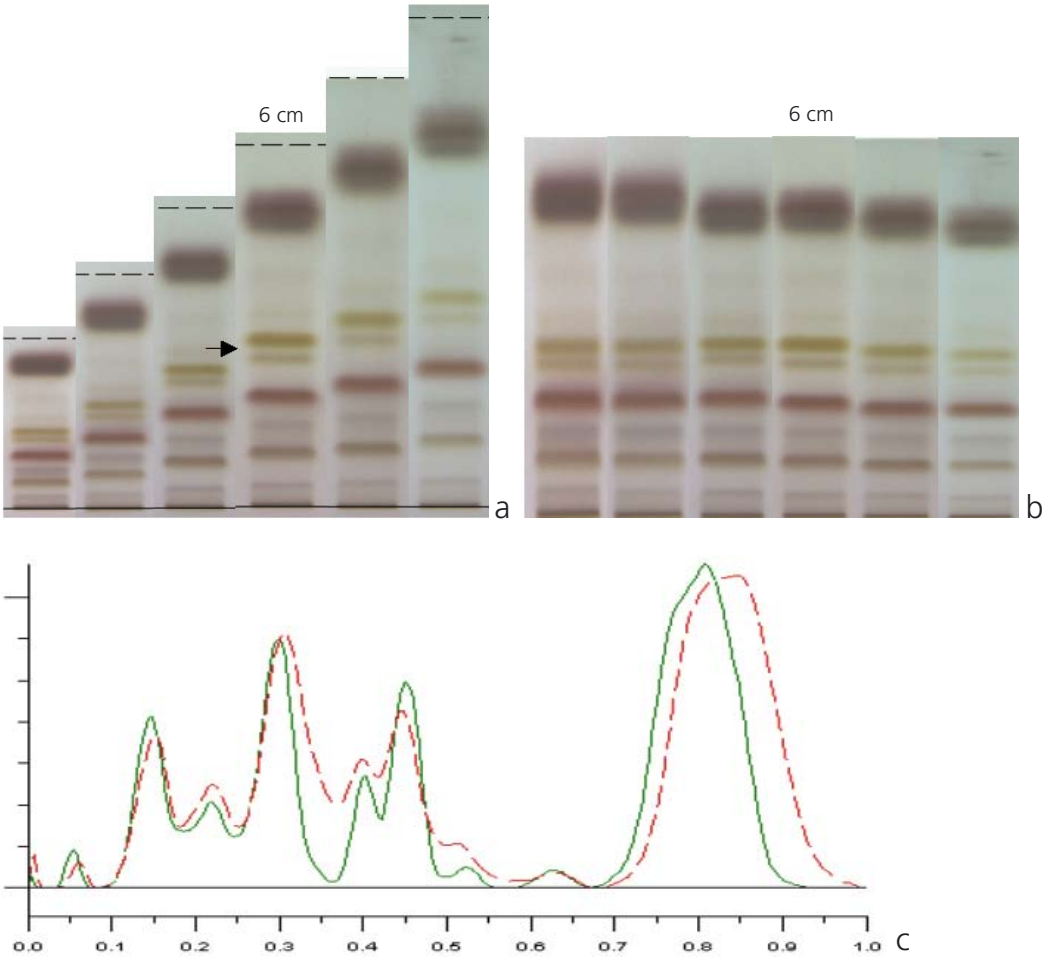

Figure 3 Separation of chamomile oil on HPTLC silica gel 60 plates. Mobile phase: toluene – ethyl acetate 19:1, derivatization by dipping in 10% sulfuric acid in methanol

a) Increasing developing distance (3–8 cm)

b) Chromatograms from a) scaled to the height of track 4 (developing distance of 6 cm)

c) Analog curves of chromatograms over 4 cm (red) and 6 cm (green) developing distance

Practical hints:

It is recommended to generally develop HPTLC plates in a saturated chamber for best reproducibility of the result.

It is advisable to not exceed a developing distance of 6 cm. The components of complex mixtures should be spread as evenly as possible over the entire separation distance. For best separation the most critical substance pair should be maintained at R_F 0.3 for best separation.

The CAMAG Twin Trough Chamber for 10×10, 20×10 and 20×20 cm plates.

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What´s new with the Club de Chromatographie sur Couche Mince (CCCM)?

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◀ *Meeting of CCCM at Lyon in November 2002, in the picture: Prof. Jacques Pothier, Pierre Bernard-Savary, Prof. Friedrich Geiss, Erwin Malzacher (from left to right)*

Four years ago we reported in CBS 83 about the inauguration of the Club of Planar Chromatography in France. It was founded in September 1998 at Pommiers-la-Placette by initiative of Monsieur Pierre Bernard-Savary*. The goal of the club is to raise the level of planar chromatography through exchange of experience among the members. Since then seminars were held twice a year. The club is well-received by the members. Members have presented papers on their fields of research and opinions were exchanged in the discussions. All club members welcome and enjoy this opportunity. The large number of papers expresses the appreciation of planar chromatography. By now they feel strong and confident about using TLC and insist in overcoming missing knowledge among their colleagues. Wherever planar chromatography proves to be the most suitable technique it is used as their method of choice. In November 2002 the now 8th meeting was organized. The following pages refer to papers given at the last two meetings.

The club has about 90 members (70% industry, 20% university and administration, 10% students). The membership fee per year is EUR 150,– (industry), 75,– (university and administration) and 35,– (students) respectively.

Contact: Club de CCM, Mr. P. Bernard-Savary, l´Ancienne Eglise, F-38340 Pommiers-La-Placette, France, pbernardsavary@club-internet.fr or www.clubdeccm.com

* P. Bernard-Savary was the CAMAG product specialist of our former distributor Merck-France. In the mean time Bernard-Savary has started the company Chromacim (means "Peak of Chromatography"), which very successfully represents CAMAG in France.

Finally also an international symposium!

In the tradition of the International Symposia on Instrumental Planar Chromatography held until 1997 in Interlaken, and with the support of the Club CCM Mr. Bernard-Savary is organizing the International Symposium in Lyon, 15–18 October 2003. This is a sign of active club life! The symposium was already announced in CBS 89 (last yellow page). Participants and speakers from all nations are welcome. Lets get excited about new planar chromatographic ideas and elegant solutions for problems, see www.HPTLC.com.

Rapid analysis of indole alkaloids in tissue cultures

Presentation at the 7. meeting of the Club de Chromatographie sur Couche Mince in Tours in June 2002

▲ *Prof. Joel Crèche, Dr. Françoise Andreu, Dr. Martine Courtois (from left to right)*

For more than twenty years Prof. Crèche* and his research team are working on the utilization of plant cell biotechnology in pharmaceutical research. In particular they investigate the biosynthetic pathway of indole alkaloids in cell suspensions of Madagascar Periwinkle.

As method of choice the group prefers planar chromatography, because of its rapidness and efficiency. 50 samples can easily be quantified in 2 hours. The planar chromatographic separation requires 1.7 s per sample whereas an HPLC run lasts about 20 min. Chromatography requires only 0.15 mL solvent per sample. The detection is rather elegant and once again demonstrates the flexibility of planar chromatography. In contrast to other methods ajmalicine can be detected without derivatization and at lower detection limits. Compared to other planar chromatographic procedures based on derivatization with Dragendorff-reagent this newly developed method is more sensitive by a factor of 250, compared to HPLC-UV or PDA detection by a factor of 8 to 10.

Scope

Madagascar Periwinkle (*Catharanthus roseus* [L.] G. Don or *Vinca rosea*) is a tropical plant that represents the most important source of vinca-alkaloids, a class of powerful agents against cancer and especially leukemia. However, the anti-tumor compounds (e.g. vinblastine and vincristine) are produced only in very low amounts. One ton of dried leaves hardly yields 5 mg of vincristine. Alternatively to the poor natural production approaches utilizing biotechnological processes (cultures of periwinkle cells in bioreactors) have been envisioned. Studying the conditions to optimize the biosynthesis requires large series of extracts with very low amounts of analyte to be investigated.

In the following the determination of ajmalicine and serpentine as markers for biosynthesis from cell cultures is described.

Sample preparation

Freeze-dried cells were powdered in a mortar. 25 mg were extracted by sonication with 1 mL methanol for one hour. After centrifugation for 5 min 0.5 mL of the supernatant were transferred into a vial.

Chromatogram layer

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

Sample application

Bandwise with Automatic TLC Sampler, 52 tracks (26 on each side), band length 3 mm, application volume 1 µL, distance from the lower edge 8 mm, distance from the side 10 mm, track distance 7 mm

Chromatography

In Horizontal Developing Chamber 20 × 10 cm from both opposing sides with 4 mL ethyl acetate – diethylamine 9:1 each, developing distance 25 mm, developing time 1.5 min

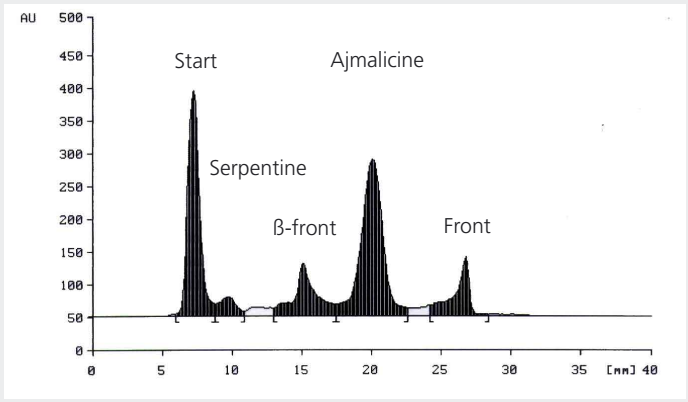
Generation and enhancement of fluorescence

Serpentine shows native fluorescence. Ajmalicine fluoresces (presumably due to photo oxidation) after 4 min of radiation with short-wave UV 254 nm (CAMAG Reprostar or UV-lamp**). This simple intermediate step during which fluorescence is induced by radiation simultaneously to 52 samples on the plate is very time saving.

Note: In case the fluorescence near the detection limit is not sensitive enough (particularly for ajmalicine), the plate can be dipped for 15 s in paraffin oil – n-hexane 1:3 (Chromatogram-Immersion Device). This rapid step enhances fluorescence by a factor 3 thus avoiding the need for repetition of chromatography.

Densitometric evaluation

TLC-Scanner 3 with CATS Software, fluorescence measurement at 254 nm or 365 nm (in the range of the detection limit 254 nm is preferred due to the lower background-fluorescence), linear calibration of peak area and height, detection limit of ajmalicine 2 ng and serpentine 0.5 ng.



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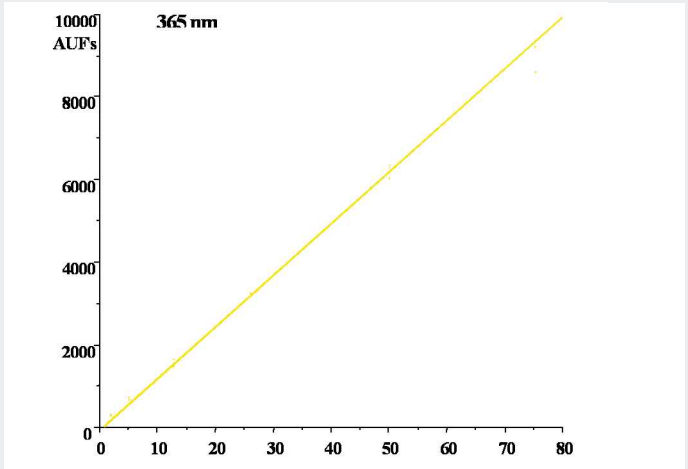
▲ *Densitogram of a cell sample containing ajmalicine and serpentine*



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▲ *Linear calibration of ajmalicine, coefficient of correlation 0.999*

A copy of the presentation is available from the author on request (creche@univ-tours.fr).

* Prof. Joel Crèche, Plant Molecular Biology and Biochemistry Department, EA 2106, Plant Biocompounds and Biotechnology, Faculty of Pharmacy, University of Tours, 31 avenue Monge, F-37200 Tours, France

**Crèche at al. used UV Crosslinker (Amersham Life Science)

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Horizontal Developing Chamber

For the analysis of indole alkaloids chromatography in the Horizontal Developing Chamber (HDC) is very time saving. It allows development of a plate from both opposite sides toward the middle, thus doubling the number of samples per plate compared to conventional developing techniques. In this example 52 samples are simultaneously developed in 1.5 min!

Only 4 mL solvent per side are required for chromatography. Thus, compared to other chamber types the HDC shows less consumption of solvents.

The high grade of flexibility is demonstrated by its easy handling either for conditioning in the tank configuration or for chromatography in the sandwich configuration. The HDC is unsurpassed in flexibility, reproducibility of the results as well as from an economic point of view (see also page 6).

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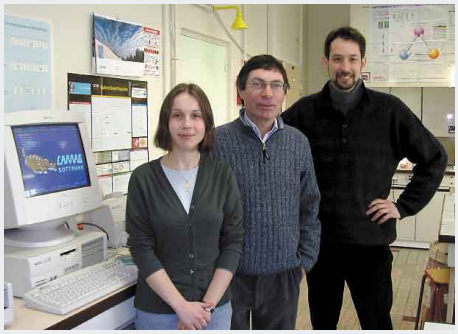
▲ *Madagascar Periwinkle*

Monitoring of proinsecticides (oxazolines) in biological samples

Presentation at the 7. meeting of the Club de Chromatographie sur Couche Mince in Tours in June 2002



▲ *Dr. M. Beaufour, Prof. J.-C. Cherton and A. Carlin-Sinclair (from left to right)*

Planar-Chromatography is unsurpassed with respect to rapidness and particularly well suited as direct analytical method. A special sample clean up is not required, i.e. after dilution samples are directly applied onto the plate. Cross contamination with matrix is avoided because the plates are only used once. A comparison to ^{19}F NMR shows that both procedures lead to the same qualitative results concerning the activation or inhibition of the potential proinsecticides. Both procedures can be used for cross validation with respect to the fluorinated oxazoline Ia.**

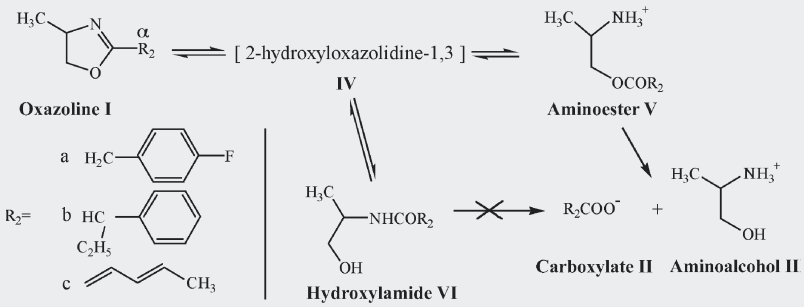
A copy of the original publication in J. Chromatogr. B, 761 (2001) 35–45 is available from CAMAG or the authors (beaufour@chimie.uvsq.fr) on request.

* Dr. Martine Beaufour, Laboratoire Sircob, Bat. Lavoisier, 45 avenue des Etats-Unis, F-78035 Versailles Cedex, France

** Regarding chromatographic separation as well as method development

Scope

M. Beaufour*, J. C. Cherton and A. Carlin-Sinclair are conducting research on proinsecticides for 15 years. The aim of this study is to develop proactive compounds like oxazolines and N-acylaziridines, which are masking active principles with insecticidal activity. Particularly, oxazolines (I) are susceptible to undergo an unmasking which liberates as active principles a carboxylate (II) and an -aminoalcohol (III). The experimental setup assesses whether metabolism of the starting compound occurs when the oxazoline is present in the biological medium of insects. For monitoring HPLC and NMR as direct analytical methods are used. However, in case of non-fluorinated oxazolines the following planar-chromatographic method is preferred.



▲ *Scheme of the hydrolytic and metabolic paths of oxazolines*

Sample preparation

Mesenteron and fat-body of locust are thawed, ground and centrifuged (260 mg fat-body are diluted with 400 µL phosphate buffer before grinding). 5% acetonitrile is added to obtain a final oxazoline concentration between 5×10^{-3} M and 5×10^{-4} M. After different incubation times the samples are diluted 1:4 with a solution of acetonitrile – phosphate buffer (0.1 M, pH 7.4) 5:95, so that the absolute amount of oxazoline on the plate lies in the range of 0.13–1.25 µg.

Chromatogram layer

TLC Aluminium sheets RP-18 (Merck), 20 × 20 cm (cut into 10 × 10 cm pieces)

Sample application

Bandwise with Automatic TLC Sampler, 7 tracks, band length 6 mm, application volume 5 µL, distance from the lower edge 10 mm, distance from the side 20 mm, track distance 10 mm

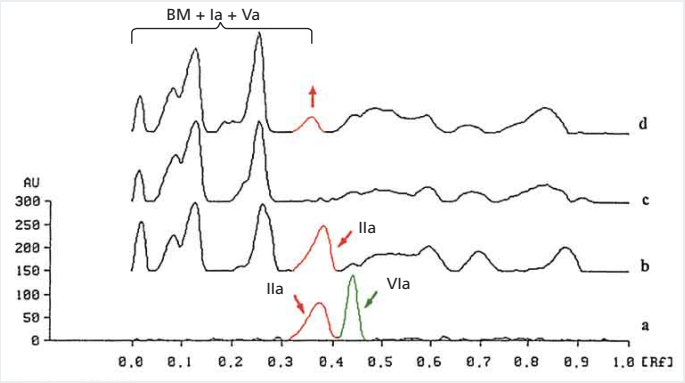
Chromatography

In the Twin Trough Chamber, developing time 30 min (ca. 5 min per sample), monitoring of the metabolism of

- Oxazoline Ia with reversed phase chromatography: first focusing with methanol up to 10 mm, then chromatography with water – acetonitrile 1:1, developing distance 55 mm
- Oxazoline Ia with ion pair chromatography: before application the plate is dipped in 2 mM cetyltrimethylammonium bromide (CTMA Br), then chromatography with phosphate buffer – acetonitrile 1:1, developing distance 60 mm
- Oxazoline Ib and Ic: chromatography with water – acetonitrile – dioxane 4:3:3, developing distance 65 mm

Densitometric evaluation

TLC-Scanner 3 with CATS software, absorption measurement of oxazoline Ia, Ib and related compounds at 200 nm and of oxazoline Ic and related compounds at 262 nm.



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▲ *Monitoring of oxazoline Ia metabolism in locust mesenteron with ion pair chromatography: a = standard IIa and VIa, b = standard IIa in mesenteron, c = biological blank (BM), d = after 3 hours incubation of oxazoline Ia in the biological medium*

UV spectra of carboxylate IIa for identification ▶ *(a = standard, b = sample)*

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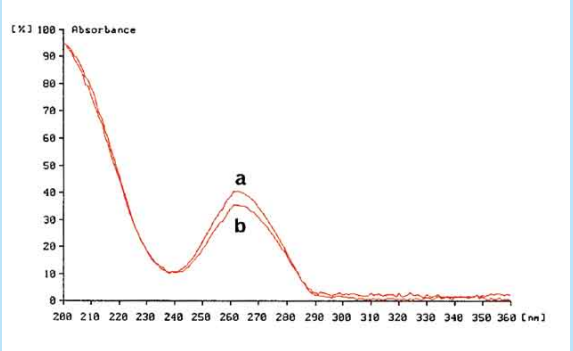
CAMAG Automatic TLC Sampler 4 (ATS 4)

For monitoring of pro-insecticides M. Beaufour et al. are still employing the previous model, the Automatic TLC Sampler 3. In the mean time the instrument was redesigned and optimized. The Automatic TLC Sampler 4 (ATS 4) applies substances under a cover to protect the object from environmental factors. The ATS 4 is controlled by winCATS and easy to operate. It features a self-adjusting object support to accommodate objects of various thicknesses. The spray nozzle does no longer require adjustments. Aside of the standard rack also a special rack holding 96-well-plates can be installed. Automatic sample application increases precision and robustness during routine analysis in a GLP/GMP environment.



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Determination of antibiotics in waste water after biological treatment

Presentation at the 8. meeting of the Club de Chromatographie sur Couche Mince in Lyon in November 2002

25 ▲ *P. Charbinat, L. Vicard, F. Dubost (from left to right)*

Louise Vicard of Aventis Pharma at Neuville sur Saône is in charge of the Site Production Support Lab. Quality and processes improvement, production workshop support, in-process controls, EH&S (environment, hygiene and safety) monitoring on water, air and atmosphere are the lab's objectives.

Scope

Following the authorization of production of a new antibiotic, the authorities ask for the follow-up on this product in the effluents discharged after biological treatment. Controls are to be carried out over one week of discharge once per quarter. The required quantities are of the order of ppm.

For waste water analysis Louise Vicard and her team chose planar chromatography because it is faster than HPLC (time for chromatography is 1 min per sample) and more robust against matrix. Furthermore, sample preparation can be skipped and the waste water directly applied onto the plate. Cross contamination with matrix is avoided because the plate is used only once.

* Louise Vicard, Aventis Pharma S.A, LABS, 31–33 Quai Armand Barbès, F-69583 Neuville-sur-Saône (louise.vicard@aventis.com)

Chromatogram layer

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

Sample application

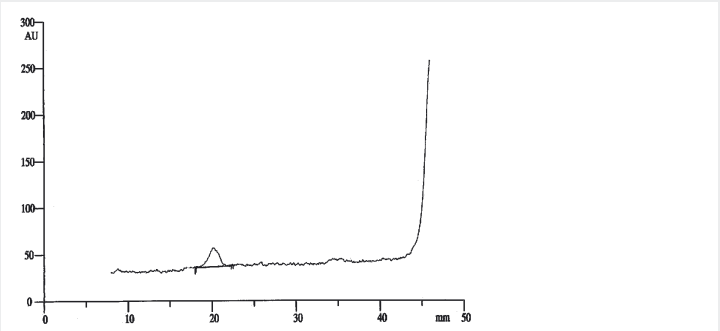
Bandwise with Automatic TLC Sampler, 18 tracks, band length 5 mm, application volume for screening 1.25 and 2.5 µL (standard solution of 0.04 g/L in chloroform) and 20 µL (sample), distance from lower edge 10 mm, distance from the side 15 mm, track distance 10 mm

Chromatography

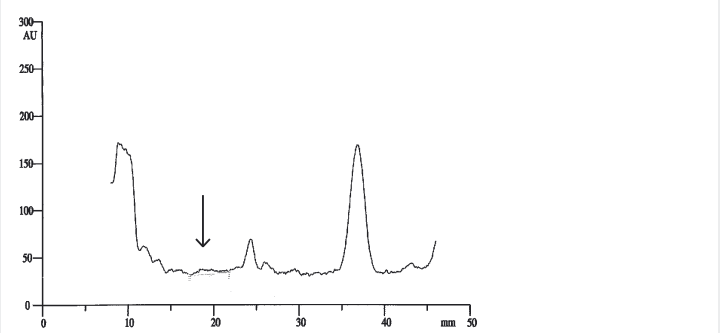
In Automatic Developing Chamber (ADC), 20 x 10 cm, with dichloromethane – methanol – ammonia 90:10:1, developing time 20 min, developing distance 50 mm

Densitometric evaluation

TLC Scanner 3 with CATS software, absorption measurement at UV 270 nm. Michaelis Menten calibration of peak area for determination of detection limit found to be 0.01 µg (1 ppm) and of limit of quantitation found to be 0.02 µg (2 ppm).

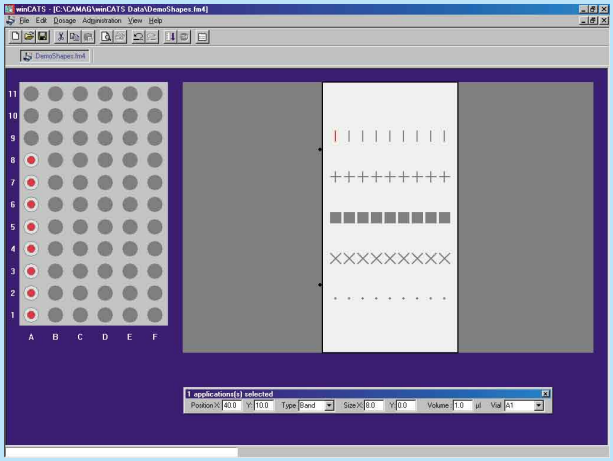


26 ▲ *Standard of 0.05 µg antibiotic (5 ppm)*



27 ▲ *Waste water sample with negative result (detection of the antibiotic below 1 ppm)*

CAMAG Automatic TLC Sampler 4 now available with "FreeMode" option



▲ *FreeMode program window showing a selection of possible application patterns*

The CAMAG Automatic TLC Sampler 4 (ATS4) offers fully automatic sample application for qualitative, quantitative and preparative planar chromatography. It is extremely well suited for screening and mass analysis. The standard program features sample application as spots by contact transfer or as bands using the spray-on technique. Larger volumes can be applied as rectangles. Choices of application patterns will meet the usual planar chromatographic needs.

The option "FreeMode" enables utilization of the Automatic TLC Sampler 4 also for various applications outside of planar chromatography. For application of solution onto any given position of a planar medium FreeMode offers full functionality and free choice of design.

This flexibility of design is used by manufacturers of test kits for application of antigen/antibody solutions onto nitrocellulose membranes. They apply for instance

- Crosses with colored dots in between (as aide for cutting sheets into individual test kits) as well as
- Several long lines side by side (groups consisting of 3 to 5 lines).

Also users of planar chromatography will have several advantages with the FreeMode-option:

- Bands can be applied overlapping. This is advantageous if the migration distance of standard and samples are different due to matrix effects. Overlapping application of sample and standard allows proper identification of zones in the resulting chromatogram.
- In combinatorial chemistry or high throughput screening one library can be applied onto 3 plates 20 x 10 cm respectively 6 plates 10 x 10 cm by a single winCATS data file. Optionally samples can be taken from micro titer plates. For example from a 96-well plate 32 samples each can be applied onto 3 plates (16 samples on opposite sides of each plate).
- On all sides of a plate application for two-dimensional chromatography can be performed. During method development robustness of the procedure can be checked with a two-dimensional chromatography. In this case the plate is developed with the same developing solvent in the second direction after purposefully exposing the plate following the first run. If all standards remain on the diagonal, that means, if they have migrated the same distance regardless of the investigated influence factor, this factor does not affect robustness. For 2-D residue analysis according to Ph. Eur. four samples are applied in the corners of a plate and chromatographed from both opposing sides with two different developing solvents.

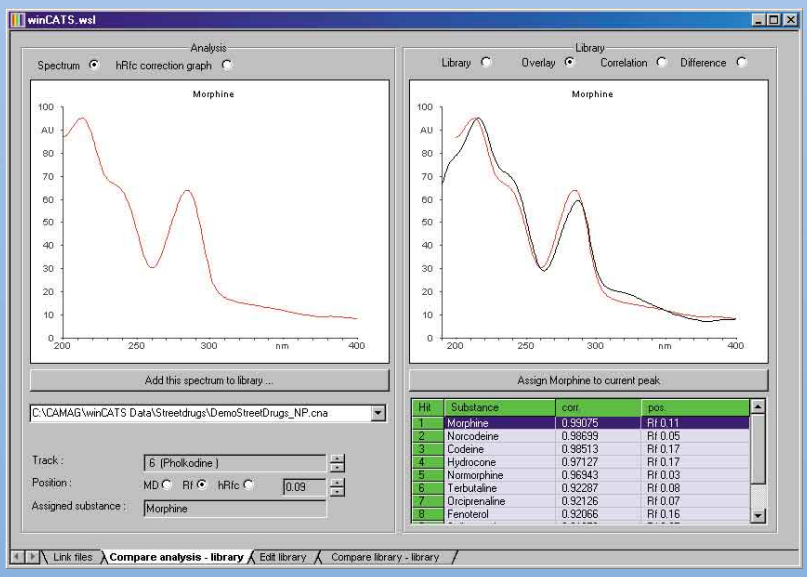
Naturally the programming effort in FreeMode is a bit larger than in the standard program, however, once completed a method can be used and varied as often as desired. Handling through winCATS is comfortable. The application is GLP/GMP compliant.

The special brochure ATS 4 is available at: sales@camag.com

New in winCATS

Planar Chromatography Manager

Spectrum Library



Program window with sample spectrum (left) ▶ and comparison to the highlighted library hit (right)

The spectrum library is now also available in winCATS.

All proven functions of the previous spectrum library have been implemented, including

- Comparison of spectra of unknown substances to that of a library file
- Comparison of spectra from two libraries
- Generation of a hit list with the best matching spectra. As usual the search criteria position (R_F, hR_{Fc}, MD) and correlation can be chosen. Now in addition also chromatographic data (stationary phase, mobile phase, mobile phase type, i.e. alkaline, neutral, acidic) can be included into the search.
- Graphic display of correlation, overlay or difference of both spectra
- Indivial arrangement of the report, e.g. showing the hit list, spectra overlay etc.

What´s new?

- The capacity of the spectrum library is now unlimited regarding number of spectra.
- Search criteria have been extended for chromatographic data (as mentioned before).
- During the assignment of fractions to substances (spot check) the library can be employed for either assignment of unknown zones or confirmation of automatic assignment based on migration distance.
- A subset of spectra can be selected from the comprehensive spectrum library. For this purpose the theme (e.g. cosmetics, food, environmental), stationary phase, mobile phase, mobile phase type can be chosen as search criteria. Thus, it is not necessary to establish different library files e.g. referring to the chromatogram layer used.

Library files already recorded and saved by CATS Spectrum library for MS-Windows (.scl) can be imported and converted into the new format (.wsl). Moreover, former CATS library files (.dsl) can be converted into the new format by CAMAG.

A collection of spectra of 750 basic, amphoteric and quaternary drugs (street drugs) is available from CAMAG. It can be used in toxicological and forensic analysis for screening.

Request the actual winCATS demo CD at: sales@camag.com
It contains examples as well as spectra data.