CBS 118 • MARCH 2017

# CBS 118

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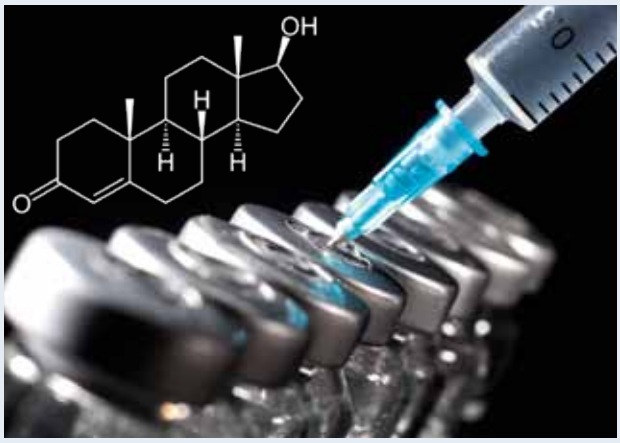
MARCH 2017

# Providing insight into the versatility of HPTLC

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# Detection of steroids and selective androgen receptor modulators

*Dr. Matthias Grill (Lipomed AG), Dr. Melanie Broszat (CAMAG)*

Lipomed AG, a mid-sized company located in Arlesheim, Switzerland, produces reference substances and pharmaceuticals for customers worldwide. The focus of Dr. Matthias Grill is the synthesis and isolation of forensic reference substances. In collaboration with CAMAG a new screening method for steroids and selective androgen receptor modulators (SARMs) in bodybuilding supplements has been developed.

## Introduction

The isolation of endogenous sex hormones was achieved in the 30ies and later, beginning in the 50ies, Djerassi *et al.* were able to synthesize the first orally available progestogen. The search for exclusively anabolic molecules has been a great challenge for many researchers; however, today we know that the anabolic activity of a sex hormone cannot be separated from its androgenic or estrogenic activity. Many of the former patented molecules have disappeared and now doctors mostly use the natural anabolic-androgenic steroid testosterone. The situation is different for the growing black market supply of performance enhancing supplements. Anabolic-androgenic substances were widely used in competitive sports and for body enhancement (muscle building) already in the 50ies. Today in addition to steroids mostly SARMs are developed and marketed as dietary supplements. Most of these new SARM molecules derive from the antiandrogen bicalutamide. To date their long term effects have not yet been thoroughly tested on humans. Adding more concern is the fact that with the increasing demand for new miracle drugs, many new but unapproved substances from the pharmaceutical companies' almost forgotten databases appear in the online market.

**HPTLC is a rapid and simple screening method for steroids and SARMs in bodybuilding supplements. Without tedious sample preparation the substances are directly separated, detected with excellent sensitivity and quantified with the TLC Scanner. The specific derivatization allows for an additional evaluation with the TLC Visualizer. UV spectra and mass spectra help with the identification.**

## Standard solutions

Individual standards in dichloromethane (0.1, 0.01 and 0.001 mg/mL)

## Sample preparation

The active substances methanedienone, ibutamoren and ostarine were extracted with dichloromethane from commercially available dietary supplements (capsules and tablets).

## Chromatogram layer

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

## Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volumes 2.0–10.0 µL

## Chromatography

In the Automatic Developing Chamber (ADC 2) with chamber saturation (with filter paper) for 20 min and after conditioning at 33 % relative humidity for 10 min using a saturated solution of magnesium chloride, development with *n*-heptane – ethyl acetate 1:1 (steroids) and dichloromethane – methanol 9:1 (SARMs) to the migration distance of 70 mm (from lower plate edge), drying for 5 min

## Postchromatographic derivatization

The plate was immersed with the Chromatogram Immersion Device into (1) toluene sulfonic acid reagent (10 % in ethanol; immersion speed 3 cm/s, immersion time 1 s) and heated at 150 °C for 3 min using the TLC Plate Heater or (2) Seebach reagent (5 g phosphomolybdic acid, 2 g cerium sulfate and 12 mL sulfuric acid are made up to a volume of 200 mL with water; immersion speed 3 cm/s, immersion time 1 s) and heated at 110 °C for 5 min using the TLC Plate Heater.

*Note: Both reagents may be sprayed with the CAMAG Derivatizer (3 mL each; (1) blue nozzle, spraying level 3; (2) red nozzle, spraying level 2)*

## Documentation

With the TLC Visualizer under UV 254 nm, UV 366 nm before and after derivatization, and under white light

## Densitometry

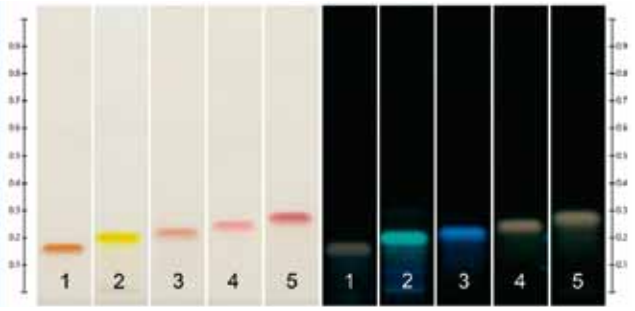
With TLC Scanner 4 and *visionCATS*, spectra recording from 190 to 450 nm, multi-wavelength scan at the respective absorption maxima

## Mass spectrometry

The target zones were eluted with the TLC-MS Interface 2 (oval elution head 4 x 2 mm) at a flow rate of 0.5 mL/min with methanol (with 0.1 % formic acid) into a single quadrupole mass spectrometer (ACQUITY QDa, Waters, USA) and detected in positive ionization mode.

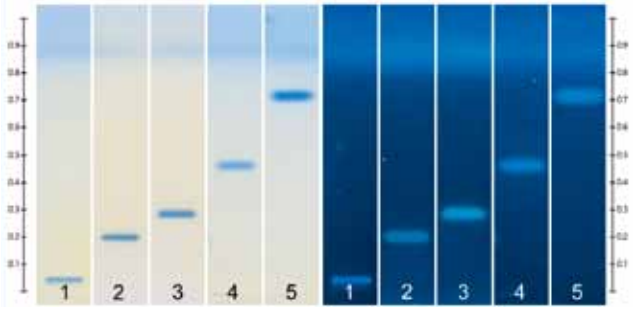
## Results and discussion

With the method presented here bodybuilding supplements can be analyzed for steroids and SARMs using two different mobile phases. Derivatization of the steroids with toluene sulfonic acid reagent provides further information for the identification based on the color differences of the zones. With Seebach reagent the steroids appear as blue zones under white light and UV 366 nm.




1

*HPTLC chromatograms after derivatization with toluene sulfonic acid reagent under white light (left) and UV 366 nm (right); track 1: boldenone, track 2: trenbolone, track 3: nandrolone, track 4: testosterone, track 5: clostebole*




2

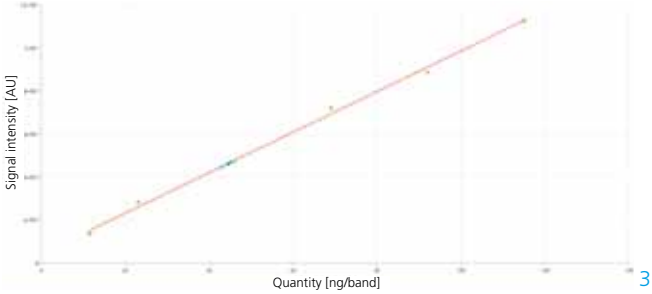
*HPTLC chromatograms after derivatization with Seebach reagent under white light (left) and UV 366 nm (right); track 1: stanozolole, track 2: methanedienone, track 3: methyltestosterone, track 4: drostanolone, track 5: clostebole cypionate*

For the quantitative evaluation of UV active substances, the absorption measurement is performed with the TLC Scanner before derivatization. With a multi-wavelength scan all substances can be quantified in the ng/band range. For the steroid testosterone for example the detection limit is 8 ng/band (absorption measurement at 250 nm).

| Steroid | $hR_F$ | UV 254 nm | UV 366 nm | Seebach reagent | | Toluene sulfonic acid reagent | | Absorption max. (nm) | *m/z* $[M+Na]^+$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | UV 366 | White light | UV 366 | White light | | |
| Stanozolole | 5 | – | – | +* | +* | + | – | – | 329 |
| Boldenone | 16 | + | – | + | + | +* (brown) | +* (orange-brown) | 251 | 309 |
| Methanedienone | 20 | + | – | + | + | +* | +* | 251 | 323 |
| Trenbolone | 21 | + | + | + | + | +* (green) | +* (yellow) | 352 | 293 |
| Nandrolone | 22 | + | – | + | + | +* (blue) | +* (brown) | 248 | 297 |
| Chlordehydro-methyltestosterone | 23 | + | – | + | + | +* | + | 253 | 357 |
| Oxandrolone | 23 | – | – | +* | +* | + | – | – | 329 |
| Testosterone | 24 | + | – | + | + | +* (brown) | +* (pink) | 250 | 311 |
| Clostebole | 27 | + | – | + | + | +* (green-brown) | +* (red) | 264 | 345 |
| Methyltestosterone | 29 | + | – | + | + | +* (blue) | + | 250 | 325 |
| Oxymetholone | 37 | + | – | +* | – | + | – | 295 | 333** |
| Oxystanolone | 39 | – | – | +* | – | + | – | 300 | 319** |
| Trenbolone acetate | 42 | + | + | +* | +* | + | + | 351 | 335 |
| Mestanolone | 44 | – | – | + | + | +* | + | – | 327 |
| Drostanolone | 50 | – | – | + | + | +* | + | – | 327 |
| Methasterone | 50 | – | – | + | + | +* | + | – | 341 |
| Clostebole cypionate | 74 | + | – | + | + | +* | + | 264 | 469 |
| SARMs (no reaction with Seebach and Toluene sulfonic acid reagent) | | | | | | | | | |
| Ibutamoren | 43 | +* | – | | | | | 201 | 551 |
| LGD-2226 | 61 | +* | +* | | | | | 248 | 415 |
| Ostarine | 72 | +* | – | | | | | 268 | 412 |
| LGD-4033 (II) | 78 | +* | + | | | | | 299 | 361 |
| LGD-4033 (I) | 80 | +* | + | | | | | 299 | 361 |

**Recommended detection for visual inspection; **$[M+H]^+$*

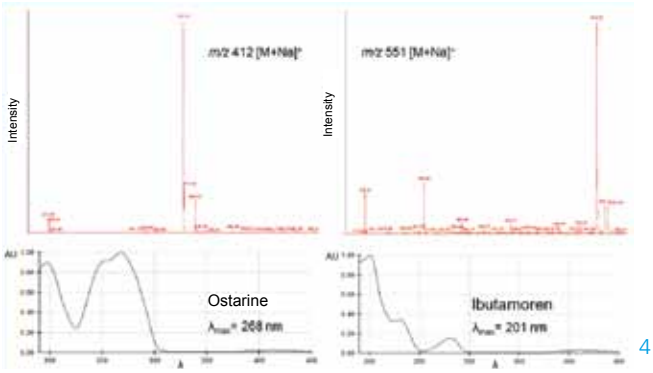
One of the products (tablets) from the black market contained methanedienone as main active ingredient. The content was determined using a calibration curve. The internal standard methyltestosterone was used to correct for variations in the extraction yield.




3

*Calibration of methanedienone (standards: green, samples: blue), absorption measurement at 251 nm*

Other black market products contained ibutamoren or ostarine as main active ingredients. The positive identification was based on UV and mass spectra.




4

*UV and mass spectra of ostarine (left) and ibutamoren (right)*

This rapid and simple method was developed for use in routine analysis. It can be used as an efficient analytical screening method for performance enhancing substances.

[1] CAMAG Application Note A-105.1: Detection of steroids and SARMs, www.camag.com

Further information is available on request from the authors.

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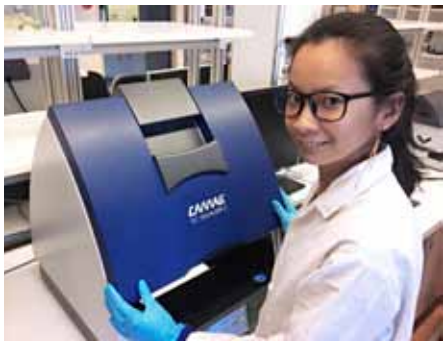
# Quality control of cosmetic products by HPTLC



*Dr. Tiên Do, CAMAG*

CAMAG has started a collaborative project with DSM (Switzerland), Waters (USA), Sederma (France), and Extrasynthese (France) on the analysis of cosmetic products by HPTLC. Two different analytical tasks are described here, detection of UV filter substances with different lipophilicity and identification of plant extracts used as ingredients in cosmeceuticals.

## Introduction

Cosmetics represent a huge market which is in constant evolution, always looking for innovation. Synthetic or natural, hydrophobic or hydrophilic actives are commonly incorporated into cosmetic matrices to create and/or improve products with properties desirable for the consumer. Cosmetic products are often complex mixtures, difficult to analyze because of possible interferences by their different components. Therefore, the incorporation of active ingredients into the various formulations requires the development of suitable analytic methods for quality control. In the past years regulations (*e. g.* EC 1223/2009 in Europe) have continuously tightened and portend to become even more restrictive [1]. The safety of a cosmetic product is obviously based on the safety of its ingredients in the finished product, and also on their safety at different stages of the manufacturing process. Hence toxicity testing has been focused on ingredients, particularly on those that are intended to react with their biological matrices [2]. The first example (A, [3]) is a general method for detection and identification of UV filter substances in sun cream by HPTLC and HPTLC-MS.

Example B describes a method for the detection of the major phenolic markers specific to Edelweiss species (*Leontopodium spp.*) in order to qualify different sources and grades of raw materials as well as glycerol-based cosmetic ingredients [4]. Since the Alpine Edelweiss is protected, the cosmetic industry uses cultivated plant material of which mainly antioxidant compounds are extracted.

**For cosmetics both methods are fast and easy for characterization of UV filters and plant material, even those from different origins and qualities.**

## Chromatogram layer

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

## Sample preparation

(A): 100 mg sun cream were mixed with 5 mL of THF. The mixture was homogenized for 30 s by vortexing and extracted in an ultrasonic bath for 10 min at room temperature. 2 mL water and 3 mL methanol were added and the mixture vortexed again for 30 s. After centrifugation for 10 min at 25 °C the supernatant was collected for application.

(B): 500 mg powdered Edelweiss sample (or 125 mg dry extract) was suspended in 5 mL methanol, sonicated for 10 min, centrifuged for 5 min and the supernatant applied. Glycerol sample (1 g) was extracted with 4 mL water, stirred vigorously and centrifuged for 15 min. The supernatant was loaded on an Oasis HLB SPE cartridge (Waters; conditioned with 5 mL ethanol and equilibrated with 5 mL water), washed with 10 mL water and eluted with 4 mL ethanol. This extract was filled up to 5 mL with ethanol and used for application.

## Standards

(A) Octocrylene, avobenzone, octisalate, and ensulizole in tetrahydrofuran (2 mg/mL)

(B) Methanolic solutions of chlorogenic acid, apigenin, luteolin, luteolin-4-O-glucoside, and luteoline-7-O-glucoside are prepared at 1 mg/mL in methanol. Leontopodic acids A and B, cynarine, and 3,5-dicaffeoylquinic acid (each 0.75 mg/mL)

## Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2 µL

## Chromatography

In the ADC 2 with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min (using a saturated solution of magnesium chloride), migration distance 70 mm (from the lower edge), drying for 5 min

(A) Development with heptane – ethyl acetate 8:2, then second development without saturation and conditioning with isopropanol to a migration distance of 28 mm

(B) Development with butyl acetate – formic acid – water 28:10:0.3

*Note: During method development a better separation of Leontopodic acids A and B was obtained when a small amount of water was added as modifier. Larger amounts are not miscible with butyl acetate.*

## Postchromatographic derivatization

(B) The plate was heated at 100 °C for 3 min and while still hot immersed into Natural Products reagent (1.0 g of 2-aminoethyl diphenylborinate dissolved in 200 mL ethyl acetate; (immersion speed 3 cm/s, immersion time 0 s).

## Documentation

With TLC Visualizer (A) under UV 254 nm and (B) under UV 366 nm after derivatization

## Densitometry

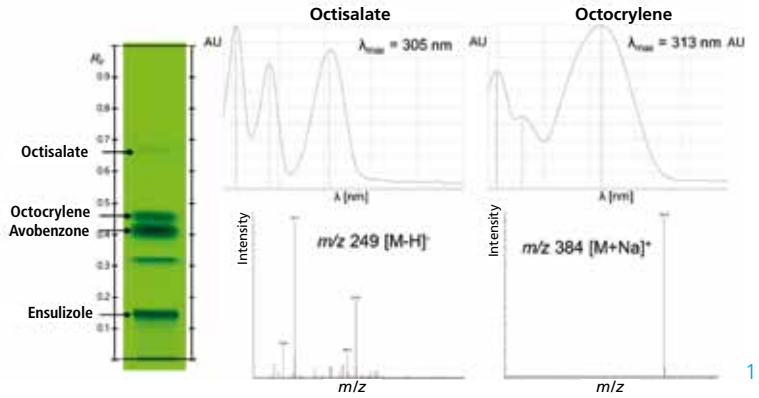
TLC Scanner 4 with *visionCATS*, absorption measurement at 254 nm, slit dimension 5.00 × 0.30 mm, scanning speed 20 mm/s, spectra recording from 190 to 450 nm

## Mass spectrometry

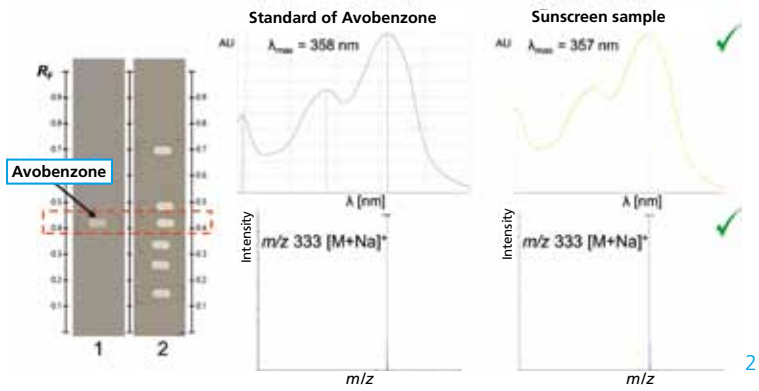
Elution of zones with TLC-MS Interface 2 (oval elution head) at a flow rate of 0.5 mL/min acetonitrile – water 95:5 (with 0.1% formic acid) into a single quadrupole mass spectrometer (ACQUITY QDa, Waters, USA) and detected in the positive and negative ionization mode. Data processing and evaluation of mass spectra with Empower (Waters).

## Results and discussion

(A) A sunscreen sample as well as the standards were analyzed and identified by MS.

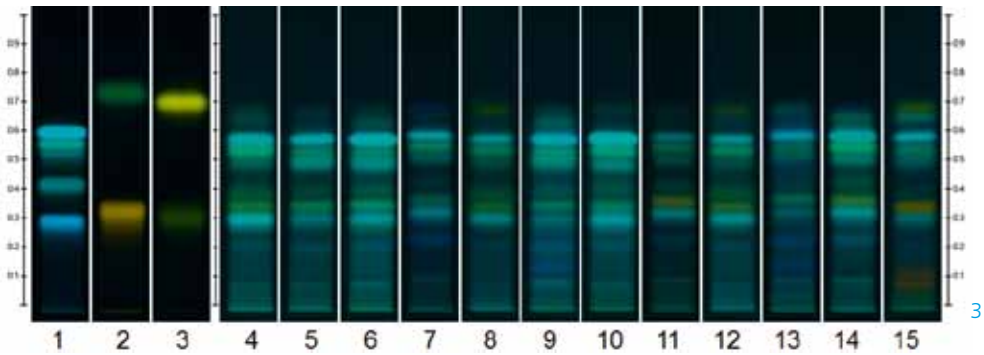



*HPTLC chromatogram under UV 254 nm; UV spectra (190–450 nm) and mass spectra (m/z 50–500) of the standards*




*HPTLC chromatograms under white light, UV spectra (190–450 nm) and mass spectra (m/z 50–500) of avobenzone (track 1) and target zone (track 2)*

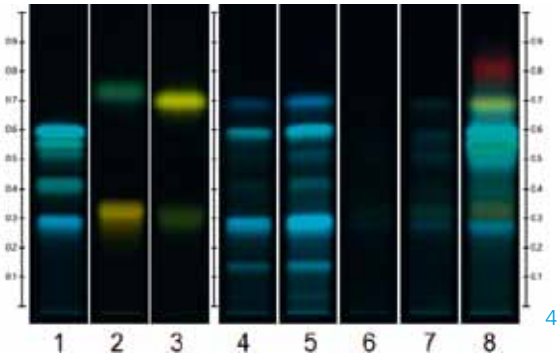
(B) A set of reference substances and powdered samples of Edelweiss were analyzed in order to check the specificity of these markers for the different Edelweiss species.




3

*HPTLC chromatograms under UV 366 nm after derivatization. Track 1: chlorogenic acid, cynarine, leontopodic acid B, leontopodic acid A, 3,5-dicaffeoylquinic acid, track 2: luteolin-7-O-glucoside, apigenin, track 3: luteoline-4-O-glucoside, luteolin (each with increasing $R_F$), tracks 4–15: powdered samples of different Edelweiss species*

Two authentic glycerol based samples (Majestem® Sederma and another manufacturer) and a powdered dry extract were analyzed. The profile of the purchased glycerol sample (tracks 6–7) has shown a very low content of Edelweiss extract (less intense zones).




4

*HPTLC chromatograms under UV 366 nm after derivatization. Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-dicaffeoylquinic acid, track 2: luteolin-7-O-glucoside, apigenin, track 3: luteoline-4-O-glucoside, luteolin (each with increasing $R_F$), tracks 4–5: glycerol sample (Majestem®, 2 and 5 µL), tracks 6–7: purchased glycerol sample (2 and 5 µL), track 8: powdered dry extract (Extrasynthese)*

Both methods proved suitable for analysis of the ingredients of interest. The recommended mobile phase for UV filter substances ensures a sufficient separation of the active substances from other ingredients. Method B demonstrates an excellent resolution of complex cosmeceutical plant extracts with simple sample preparation.

[1] Council Regulation No. 358/2014, Official Journal of the European Union, 2014.

[2] W. Schwack and C. Stiefel, CBS 111 (2013) 7 and 9

[3] CAMAG Application Note A-103.1: Detection of UV filters in cosmetic products (sunscreen) by HPTLC and confirmation by HPTLC-MS

[4] CAMAG Application Note A-104.1: HPTLC fingerprint of Edelweiss plants and extracts used as ingredients in cosmeceuticals

Further information is available on request from the author.

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# Knowledge Transfer Worldwide

Planar Chromatography is a versatile analytical method with many advantages and is appreciated by analysts worldwide. To benefit from optimum and reproducible results, it is not only important to use instruments such as those from CAMAG, but essential to have "knowledge transfer" and expert training.

In 2016 CAMAG offered a multitude of HPTLC courses and workshops in Europe, Asia, USA, Latin America and Africa. In Germany alone more than 1500 participants have been trained in-house in 170 companies since the year 2000. The feedback has always been very positive.

There was a broad range of seminars held in various countries together with the CAMAG distributors and partners, *e. g.* Merck and Waters. In addition we were invited to speak, demonstrate instrumentation, hold workshops at several congresses and at universities.

In 2017 we will continue – CAMAG offers several English courses in Switzerland: ***HPTLC Today, Analysis of Botanicals, Method Development and Validation***; and the HPTLC Course of the Swiss Chemical Society in German. Worldwide many additional courses will be held by our distributors. For further information on our trainings visit **http://www.camag.com/courses**.

The HPTLC symposium 2017 (July 4–8, 2017) in Berlin, Germany, offers to analysts worldwide the best platform for HPTLC training for all of 2017. On the 4$^{th}$ of July three workshops will be held: ***HPTLC-MS for the Chemists and Beyond, Lipid Identification and Quantitative Analysis; and Botanical Analysis in Compliance with USP <203> and Ph.Eur. 2.8.25***.

We are looking forward to meeting you and everyone who is interested in HPTLC in Berlin. Register at **www.hptlc.com**.

# CBS 118

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MARCH 2017


**Remarks about abstracts newly added to the CCBS database with this CBS issue**

The CCBS collection contains now 11'533 abstracts of which 93 have been added with this issue (CBS 118). Trending as with the previous issue, among TLC/HPTLC publications cited are reviews from a variety of fields, whereby microchip liquid chromatography and capillary electrophoresis are noteworthy. This makes the point that usage of TLC/HPTLC is extensive and widely accepted. A recent review by Rabel and Sherma highlighting advances of two-dimensional separations (J. Liq. Chromatogr. Relat. Technol. 39, 627–639, 2016) deserves special interest.

Over the years, reading and abstracting many TLC/HPTLC papers for the CBS let it appear that the art of writing scientific papers took a backseat. The paper flood and pipeline production were often given priority, causing the neglect of vital checks such as: Is the substantial progress evident for the reader? Is the outcome clearly addressed and discussed in the context of the present literature? Are the results concise and clearly presented in figures and tables? Is the significance of digits respected? If no filling word can be skipped anymore, the message gets clear. In any case, at HPTLC 2017, there will be a key lecture by Verpoorte "How to write a world-class scientific paper in HPTLC". For those who cannot attend the symposium, the content will be made accessible at www.hptlc.com.

Dear friends

If analysts in the choice of methods follow the mainstream, *i. e.* do not include HPTLC in their considerations, they may be missing powerful sources of innovation. In this CBS issue focus is laid on applications from differing fields, all taking advantage of the unique feature of planar chromatography that all fractions are stored on the plate and thus are readily accessible for sequential detection steps (image acquisition and scanning densitometry before and/or after postchromatographic derivatization).

One application concerns the analysis of steroids in forensic reference substances, *e. g.* to identify unauthorized substances in black market stimulants. Another focuses on ingredients of cosmetics, and a further application concerns the analysis of anthroposophic and homoeopathic drugs. Finally quantification of nicotin in E-cigarettes is addressed in the fourth method reported.

There is a unique opportunity to discuss your challenges and their possible solution by planar chromatography at the HPTLC 2017 Symposium in Berlin, 4–8 July. Hope to see you there!

Kind regards


Gertrud Morlock
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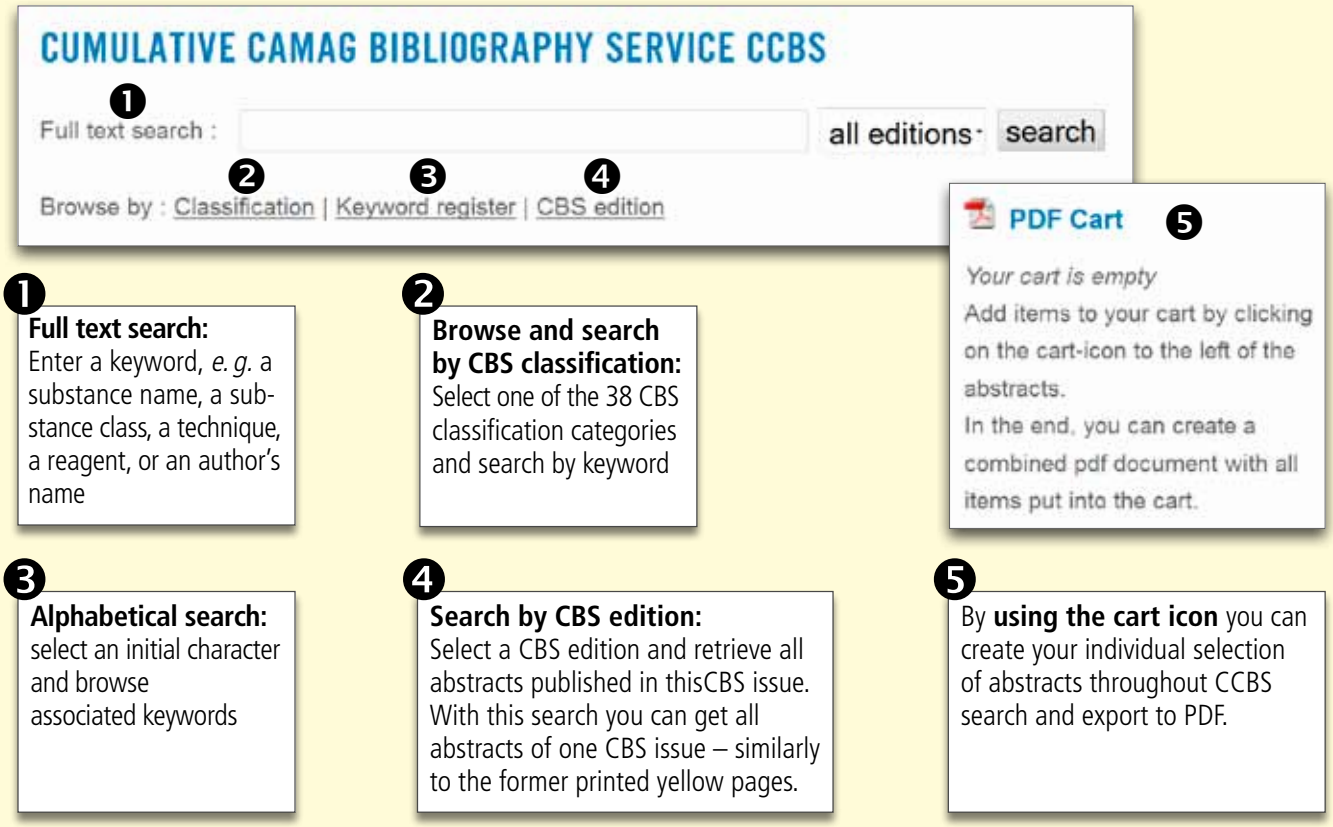
# THE CBS CLASSIFICATION SYSTEM

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

# Cumulative CAMAG Bibliography Service (CCBS) Online Search




With the Cumulative CAMAG Bibliography Service (CCBS) Online Search, you can directly search for information within the CAMAG website. The CCBS covers more than 11'000 abstracts of TLC/HPTLC publications between 1982 and today. Providing reports was established by Dr. Dieter Jänchen in 1965 and the workflow and form evolved over the years. Manually written and transferred into the printed CBS form, it was set-up as a database in 1997. With CBS 93 in 2004, the electronic database for download was introduced and with the CBS 113 in 2014, the online search.

The database covers most relevant scientific journals in the field of Planar Chromatography including also various non-English publications in German, French, Spanish, Portuguese and Chinese. The CCBS features additional practical information for the analyst in the lab, for example details on the mobile phase or the detection. With CCBS the analyst is able to find relevant TLC/HPTLC publications which might be helpful for solving a particular analytical question.

Visit **www.camag.com/ccbs** and choose one of the following search options: full text search or search by CBS classification system or by alphabetical register or by CBS edition. For classical full text search, just enter a keyword in the search box, *e. g.*, a substance name, a substance class, an analytical technique, a reagent, or an author's name, and find all related publications throughout the CCBS.

Alternatively, you can choose to browse by one of the 38 CBS classification categories and search by keyword. The alphabetical search allows selecting an initial character and browsing associated keywords. When browsing by CBS edition, you can retrieve all abstracts published in the corresponding CBS issue, formerly printed as yellow pages.

To create your individual selection of TLC/HPTLC abstracts add your preferred publications to the PDF cart and download these selected articles in one single PDF file.

# Reminder
# International Symposium for High-Performance Thin-Layer Chromatography
# Berlin, 4–8 July 2017

The Scientific Committee cordially invites you to attend HPTLC 2017 in Berlin. It is not too late to participate as a poster presenter at the symposium. A number of world renowned experts in HPTLC have already agreed to participate and an amazing number of young researchers will present their latest work in HPTLC. A program outline is available at www.hptlc.com and a detailed final program will be available end of March 2017.

If you are interested in what HPTLC could do for your laboratory, or simply want to update your understanding of methods employing this technique, then HPTLC 2017 is the venue for you. The symposium combines the attributes of showcasing the most up-to-date research methods with the latest proven applications; presents the advances in instrumentation, automation and data analysis; offers advanced training in cutting edge techniques in a series of targeted short courses with laboratory instruction by experts; all combined with a social program to promote networking and contact with peer mentors. Scientists of all ability levels are welcome and will find something of interest that they can immediately use when they return to their laboratories.

The scientific program will be exciting and diverse. We look forward to seeing you in Berlin and learning from your latest experiences with HPTLC and your ideas for the advancement of HPTLC.

## Deadlines

Poster abstract submission: 31 May 2017
Final registration: 31 May 2017

## Free social events

5 July Welcome cocktail

6 July Symposium dinner

8 July Visiting the Reichstag, Berlin sightseeing tour or boat tour on the Spree

## Location

Maritim proArte Hotel Berlin, Friedrichstrasse 151, 10117 Berlin

## Fees

Including lunches, coffee breaks, welcome cocktail, symposium dinner and social events

- Industrial 700 €
- Academic 500 €
- Students 300 €

## More information

info@hptlc.com, www.hptlc.com

# Comparison of conventional TLC and HPTLC for identity testing of herbal medicinal extracts

*From left: Dr. Margit Müller, Jennifer Macho, apl. Prof. Dr. Dietmar Kammerer*

WALA Heilmittel GmbH has been manufacturing anthroposophical and homoeopathic medicines based on medicinal plants as well as on substances of animal and mineral origin since 1935. The Analytical Development/Research Department is responsible for the development of qualitative and quantitative analytical methods and the establishment of the quality requirements for about 900 different medicinal products, their raw materials, and active ingredients. TLC is the most frequently used method for qualitative analysis of herbal components. In phytochemical analysis it is particularly advantageous, as heat sensitive plant components are not affected and analytes devoid of chromophores can be analyzed after postchromatographic derivatization. TLC has been widely used for identity testing of herbal active substances, although increasingly, it is HPTLC that is generally accepted in pharmacopoeias.

## Introduction

**In research laboratories, HPTLC is preferred in lieu of TLC [1] due to its improved efficiency, reproducibility and automation of relevant steps in the procedure. Furthermore, it saves time and is economical in solvent consumption. The new European Pharmacopoeia (Ph. Eur. 9.0) HPTLC chapter 2.8.25 to become valid in 2017, will include herbal drug-specific monographs such as *birch leaves* (01/2017:1174) illustrating the increasing relevance of HPTLC on a pharmacopoeia level. The method transfer from TLC to HPTLC is at the same time connected with the contemporary conversion from manual sample application and derivatization to automated operations.**

For pharmaceutical manufacturers like WALA Heilmittel GmbH, method changes require extensive consideration of not only regulatory issues, *e. g.* cGMP, but also marketing authorization and financial aspects. Therefore, these changes can only be implemented after thorough investigations. The Analytical Development/Research Department is currently evaluating selected medicinal products and their active ingredients whether a change from TLC to HPTLC is possible with a universal adaptation utilizing HPTLC plates with fluorescence indicator, same mobile phase composition, migration distances (MD) and application volumes. The overall aim is to change the method for identity testing from TLC to HPTLC without affecting the specified zones of the chromatographic fingerprint.

## Chromatogram layer

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

## Standard solutions

Methanolic solutions of quinine hydrochloride, hyperoside, caffeic acid and rutin (each 1 mg/mL), fructose and caffeic acid (each 2 mg/mL) and noscapine hydrochloride (4 mg/mL)

## Sample preparation

Four products were selected: (1) An aqueous-ethanolic extract of kidney vetch was treated by liquid-liquid extraction with ethyl acetate, evaporated to dryness and dissolved in 70% ethanol. (2) Suppositories containing caraway extract were melted, mixed with water and degreased by alternation of centrifugation and cooling. Subsequently, the sample was extracted with ethyl acetate, evaporated to dryness and dissolved in methanol. Both aqueously fermented root extracts of (3) barberry and (4) Solomon's seal were directly applied.

## Sample application

TLC: manual application with band length, distance from the left and lower edge of the plate 15 mm each, track distance 25 mm, application volumes for reference solutions 10 µL and for sample extracts (1) 15 µL, (2) 50 µL, (3) 20 µL and (4) 30 µL

HPTLC: bandwise with Automatic TLC Sampler (ATS 4), band length 8 mm, distance from lower edge 10 mm, distance from left edge 16 mm, track distance 11 mm, application volumes 20 % of those of TLC

## Chromatography

TLC: in twin trough chamber 20 x 20 cm after saturation for 30 min (1) with chloroform – methanol – water 70:30:5 up to 165 mm after 3-h preconditioning at a relative humidity of 75 % (saturated sodium chloride solution); (2) with ethyl acetate – anhydrous formic acid – water 84:8:8 up to 115 mm; (3) with ethyl acetate – anhydrous formic acid – water 80:10:10 up to 95 mm and (4) with chloroform – methanol – water 50:42:8 up to 165 mm

HPTLC: in twin trough chamber 10 x 10 cm after saturation for 10 min with the respective solvent system up to 70 mm (from lower plate edge)

## Postchromatographic derivatization

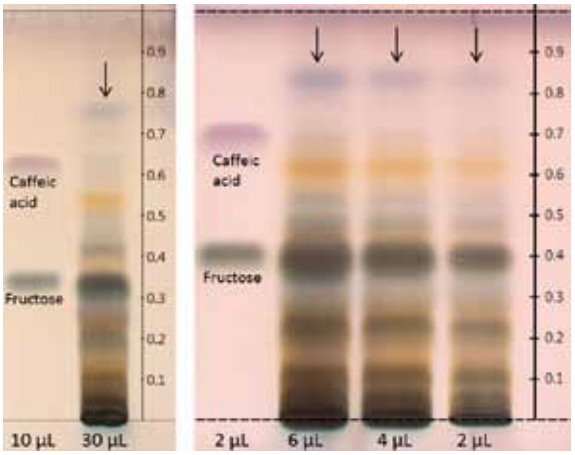
Plates were sprayed manually with (1) a solution of 20 % antimony(III) chloride in chloroform and heated at 105 °C for 30 min; (2) with a 1 % methanolic solution of diphenylboric acid aminoethyl ester (natural product reagent), followed by a 5 % methanolic polyethylene glycol (macrogol) 400 solution and detection at UV 366 nm after 30 min; (3) with a bismuthate reagent (mixture of 0.85 g alkaline bismuth nitrate, 40 mL water, 10 mL acetic acid (99 %), and 20 mL potassium iodide solution (400 g/L), glacial acetic acid and water, 1:2:10); (4) with the Derivatizer and 4 mL of a 1:1 mixture of 5 % sulphuric acid in ethanol and 2 % vanillin solution in ethanol (yellow nozzle, spray level 3) and heated for 15 min at 105 °C.

## Documentation

With TLC Visualizer and *visionCATS*

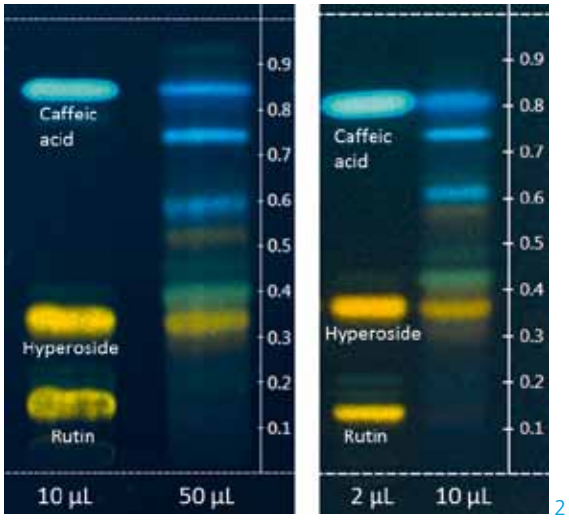
## Results and discussion

Some effects related to the change from TLC to HPTLC were observed. Referring to Solomon's seal extract, the reduced application volumes (20 % of those of TLC) led to similarly blurred zones on HPTLC plates, but the overloading was necessary for the detection of the specific weak blue zone at $hR_F$ 85. Developing times were substantially decreased from 70 min TLC to 20 min HPTLC. Whereas these were roughly the same for suppository and barberry extracts. Only a moderate shift in $hR_F$ values was observed, most pronounced for the Solomon's seal extract.



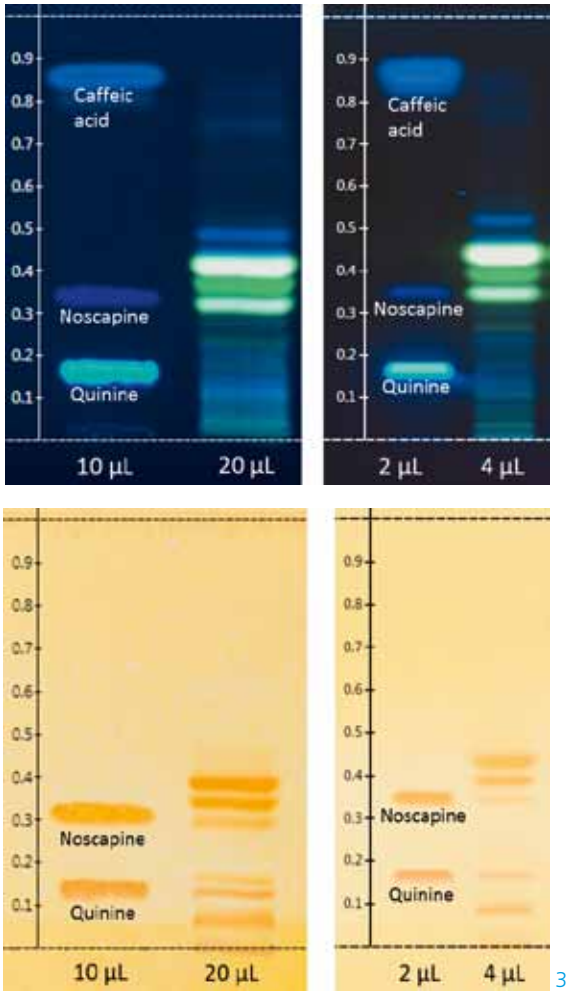

1

*TLC (left) versus HPTLC (right) chromatograms of Solomon's seal extract under white light after derivatization with sulphuric acid vanillin reagent (normalized on MD)*



2

*TLC (left) versus HPTLC (right) chromatograms of suppository extracts under UV 366 nm after derivatization with natural product reagent and polyethylene glycol (normalized on MD)*



3

*TLC (left) versus HPTLC (right) chromatograms of barberry extract under UV 366 nm, and after derivatization with bismuthate reagent under white light (normalized on MD)*


4

## CAMAG Derivatizer

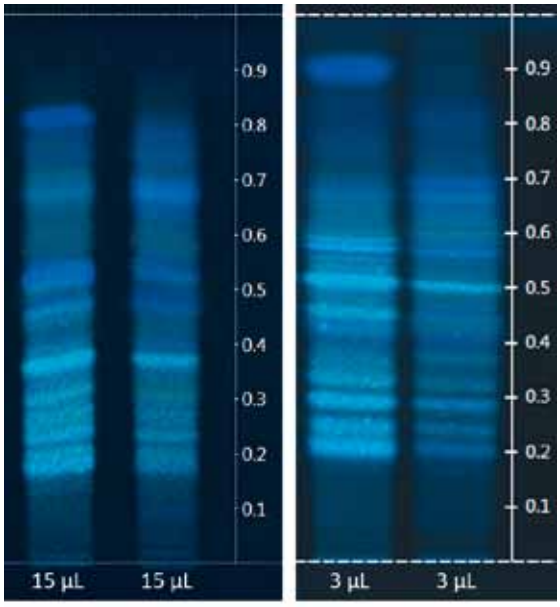
The Derivatizer is used for automated reagent transfer in the derivatization of thin-layer chromatograms. Thanks to its unique "micro droplet" spraying technology the Derivatizer ensures homogeneity and reproducibility in applying derivatization reagents, using the most common reagents.

To meet the diverging physicochemical properties of different reagents, *e. g.* acidity, viscosity, four different color-coded spray nozzles are employed with six spraying modes available to the user.

In addition to the significantly increased homogeneous reagent distribution, the Derivatizer offers other advantages compared to manual spraying:

- Environmentally friendly safe handling through a closed system
- Intuitive handling and easy cleaning
- Low reagent consumption through efficient operation (4 mL for 20 x 20 cm and 2 mL for 20 x 10 cm plates), which is particularly beneficial when expensive reagents are used
- Reproducible and user-independent results

The chromatograms of kidney vetch extract demonstrated the higher separation power of HPTLC, as more zones were separated ($hR_F$ 20–70). Also separation times were different (TLC 60 min *versus* HPTLC 15 min).



5

*TLC (left) versus HPTLC (right) chromatograms of kidney vetch extract under UV 366 nm after derivatization with antimony(III)-chloride reagent (normalized on MD)*

In summary, the change from TLC to HPTLC by a universal adaptation appears promising. Zones were sharper in HPTLC chromatograms compared to TLC. Specified zones are evaluated in relation to a reference. Hence, a moderate shift of $hR_F$ values is no limitation for the method transfer. As demonstrated by the kidney vetch extract, adaptations may be necessary in individual cases. Impact on specified zones as well as changes in composition of the mobile phase should be minor to avoid validations for batches of different harvest years. Such regulatory and expensive efforts are compensated by the advantages of HPTLC. The increasing relevance of HPTLC in pharmacopoeiae encourages the Analytical Development/Research Department to proceed with the method transfer from TLC to HPTLC. Other requirements, such as a system-specific suitability test or intensity markers, must be addressed as well.

*Note: According to the general chapter 2.8.25 the standard HPTLC plate format is 20 x 10 cm. For the sample application, the distance from left edge should be 20 mm and from the lower edge 8 mm. In a non-humidity controlled lab the HPTLC plate is conditioned at 33 % relative humidity until an equilibrium is reached. Time for chamber saturation is 20 min. CAMAG recommends the development in the Automatic Developing Chamber (ADC 2).*

[1] Morlock, G. E., Schwack, W., J. Chromatogr. A 1217 (2010) 6600–6609

Further information is available on request from the authors.

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# HPTLC for the quantitation of nicotine in liquids for electronic cigarettes

*From left: Florian Têtard, Stéphanie Lo, Romain Haize, Jean-Marie Jacquelin*

Headquartered in Angerville, south of Paris, Fabster develops and manufactures flavors and dyes. Flavors are used in a wide range of products, especially in foodstuffs. Fabster has specialized in vanilla extracts and employs HPTLC for quality assurance [1]. Liquids for electronic cigarettes (e-liquids) contain in addition to nicotine various flavors. Nicotine levels are also analyzed by HPTLC. Fabster offers more than 200 e-liquids with different nicotine levels from 3 to 16 mg/mL. Approximately 30 e-liquid samples are analyzed per week.

## Introduction

E-liquids are a mixture of propylene glycol (< 70%), glycerol (30 to 80%), flavors and nicotine. As reported in CBS 116 (pp. 13–15), the nicotine level must not exceed 20 mg/mL to be in compliance with the European regulations. The new contribution focuses on the current need for a sensitivity level, which is lower than the one addressed in the first article.

A low nicotine level (3 mg/mL) must be reliably determined in substance mixtures under real conditions in routine production, whereas the transfer from the laboratory to the industrial approach is the major challenge. The new contribution proves the suitability of the method for routine control.

**Since many samples can be analyzed in parallel without the need of time-consuming sample preparation, quantification of nicotine by HPTLC is highly efficient. Under the following conditions nicotine is baseline-separated from all other substances.**

## Chromatogram layer

HPTLC plates Nano-ADAMANT $UV_{254}$ (Macherey-Nagel), 10 x 10 cm

## Standard solution

4 g nicotine was dissolved in 50 g propylene glycol and 46 g glycerol, followed by dilution of an aliquot with ethanol (0.1 mg/mL).

## Sample preparation

Samples (0.62 to 3.30 g) were weighed in depending on the expected nicotine level of 0.9 mg/mL and diluted with ethanol ad 100 mL.

## Sample application

Bandwise with Linomat 5, band length 4 mm, distance from the side 15 mm, distance from lower edge 8 mm, application volume 1–12 µL for the standard and 2.5 µL for sample solutions (applied in duplicate)

## Chromatography

In the Automatic Development Chamber (ADC 2) with chamber saturation (with filter paper) and after conditioning for 15 min at 33% relative humidity using a saturated solution of magnesium chloride, development with dichloromethane – methanol – ammonia (28%) 89.6:10:0.4 to the migration distance of 70 mm (from the lower edge), drying for 5 min.

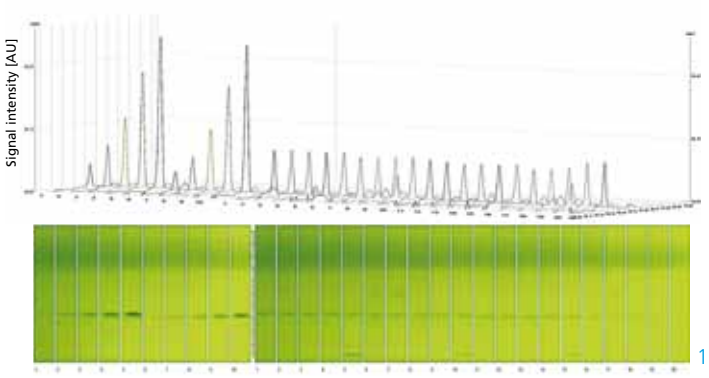
## Densitometry

TLC Scanner 3 and *visionCATS*, absorption measurement at 260 nm, slit dimension 4.00 x 0.30 mm, measurement speed 5 mm/s, data resolution 50 µm/step

## Results and discussion

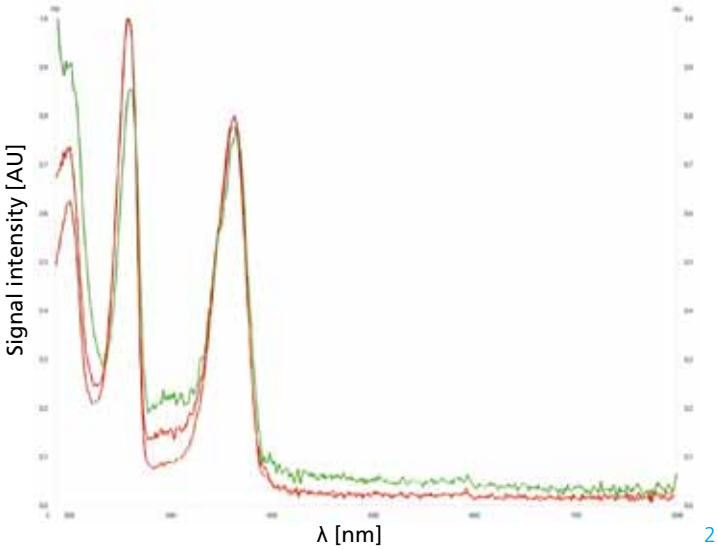
The developed method allows the industrial quality control of the nicotine levels in various e-liquids. The results of the analysis of nicotine in the e-liquid samples prepared at the laboratory scale of around 100 g are fully in accordance with the expected values (bias ≤ 3% with regard to samples containing 3 to 16 mg/mL), showing good reproducibility. Some measurements made on e-liquid samples produced on a 30-kg scale showed higher nicotine bias (≥ 1 mg/mL) and outlined the importance of in-process quality control. The higher bias for industrially produced samples was caused by the inhomogeneity of the mixture prepared. Briefly, the flavor concentrate was first solubilized in propylene glycol, nicotine pre-diluted in propylene glycol was added and glycerol was poured into this mixture. The mixture was stirred until it appeared to be homogeneous. However the viscosity difference of propylene glycol and glycerol at temperatures < 15 °C led to a heterogenic distribution of nicotine in the liquid and thus a higher bias for some samples. The flavor compounds in e-liquids did not contribute to the bias, as the absorption measurement showed much higher $hR_F$ values of the following compounds, if compared to nicotine.

| Substance | $hR_F$ value | max. content [mg/mL] |
|---|---|---|
| **Nicotine** | **38** | **3–16** |
| Ethyl maltol | 53 | 20 |
| Vanillin | 58 | 5 |
| Ethyl vanillin | 65 | 5 |
| Acetyl pyrazine | 69 | 0.2 |
| Acetyl thiazole | 62 | 0.2 |
| Piperonal | 74 | 0.5 |
| Anethol | 77 | 0.2 |
| Methyl nicotinate | 65 | 5 |
| Methyl cyclopentenolone | 58 | 5 |
| Furaneol | 55 | 0.5 |



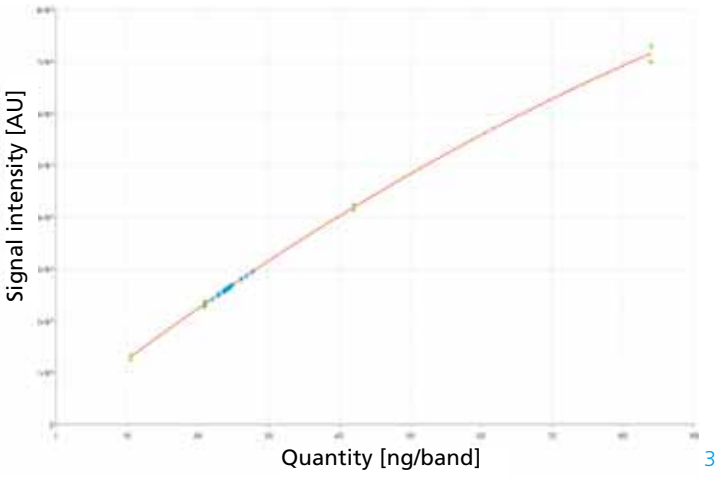

1

*30 densitograms of standards and e-liquid samples scanned at 260 nm and the respective chromatograms under UV 254 nm (left: standards, right: samples, tracks were applied by data-pair technique and rearranged for visualization with the Comparison Viewer in visionCATS)*




2

*Overlay of the UV spectra of standards (red) and sample (green)*




3

*Calibration curve of standards (green) and samples (blue)*

The inhomogeneity can be prevented or at least significantly reduced through process optimization (*e. g.* stirring time adaptation). The developed method is suitable to control the efficiency of homogenization in the e-liquid production process. The quantification of nicotine in e-liquids by HPTLC has proven as fairly uncomplicated and fast. In routine, more than 100 samples per week can be unproblematically analyzed.

[1] J.-M. Jacquelin, Dosage des composes d'intérêt dans des extraits de vanille, Vortrag, Club de Chromatographie sur Couche Mince, Bolbec, France, 09.06.2016, clubdeccm.com

Further information is available on request from the authors.

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4

**CAMAG Automatic Developing Chamber ADC 2**

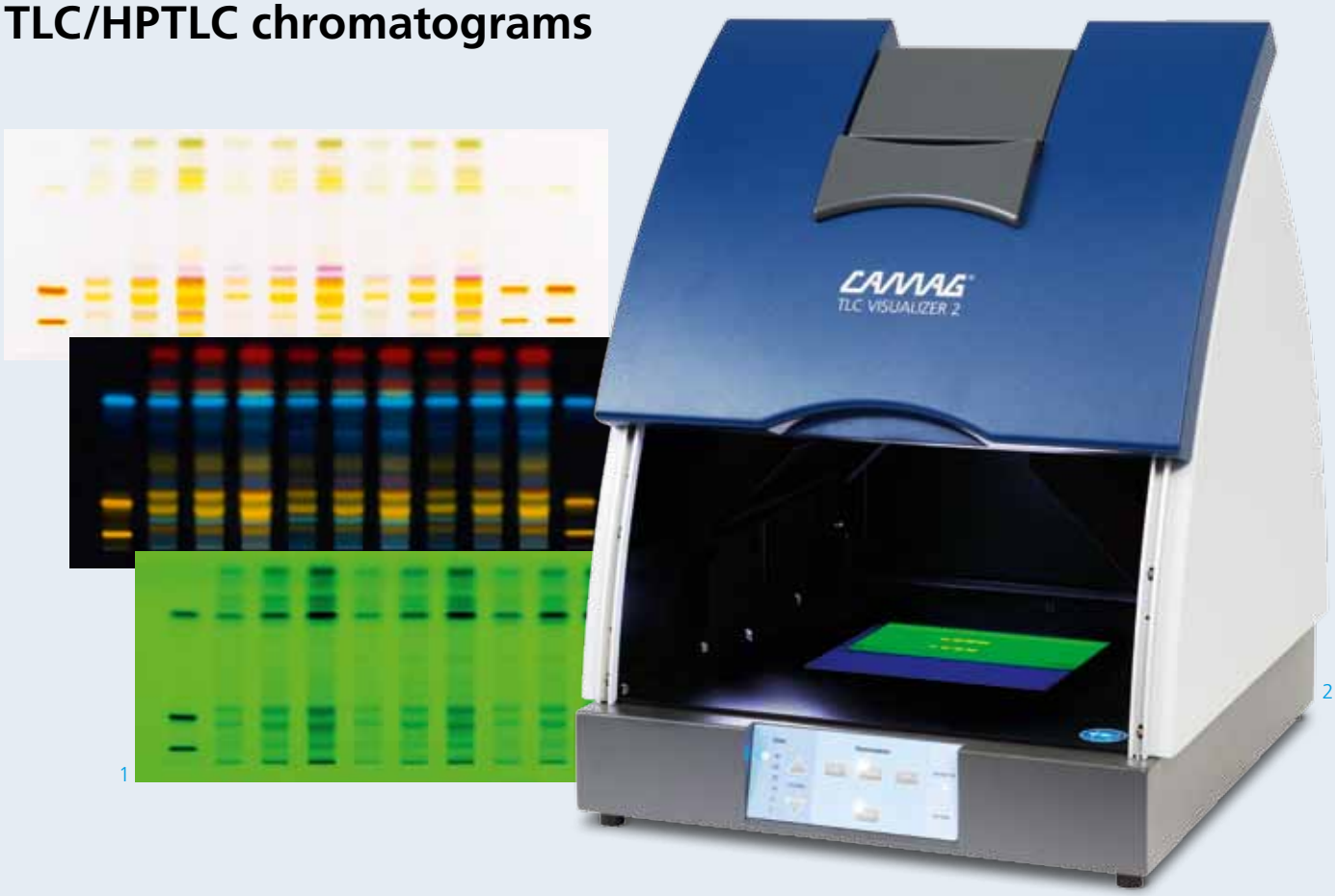
The Automatic Developing Chamber offers convenience, safety and reproducibility for the isocratic development of HPTLC plates preferably 20 x 10 cm, with limitations also 10 x 10 cm.

- Due to the chamber geometry and homogeneity of the gasphase, identical developing distances from plate to plate are secured and thus reproducible results. Chromatography occurs in a closed system and is therefore independent of environmental conditions.
- The actual developing chamber is identical with a regular CAMAG Twin Trough Chamber, so that analytical procedures can be readily transferred in both directions.
- Preconditioning of the layer, the chamber saturation as well as final drying is fully automatic with pre-set parameters.
- The user is freed from all process monitoring responsibilities, a CCD sensor surveys the solvent migration distance.
- The option "Humidity Control" offers investigation of the influence of relative humidity during method development.

Further information on **www.camag.com/adc2**

# CAMAG TLC Visualizer 2

## Professional imaging and documentation system for TLC/HPTLC chromatograms




1 2

The TLC Visualizer 2 is equipped with more technical innovations, offering better image quality than its predecessor:

- Digital CCD camera with a maximum resolution of 82 µm on the plate
- USB 3.0 for easy PC connection and rapid data transfer
- Simple operation under *visionCATS* (from version 2.3 on)

Further information:
**www.camag.com/tlcvisualizer2**

With this high-end imaging system, reproducible images of superior quality are obtained under different illumination modes – white light, UV 366 nm, and UV 254 nm. The software-controlled operation offers:

- Image enhancement tools – Spot Amplification, Clean Plate Correction and Exposure Normalization
- Evaluation possibilities – Semi-quantitative evaluation of images and "Comparison Viewer" of tracks from different plates and/or different illumination modes

The TLC Visualizer 2 meets all requirements to be utilized in a cGMP/cGLP environment.

For *visionCATS* 2.3 the Service Pack 1 has been available since January 2017 on CAMAG's website.

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