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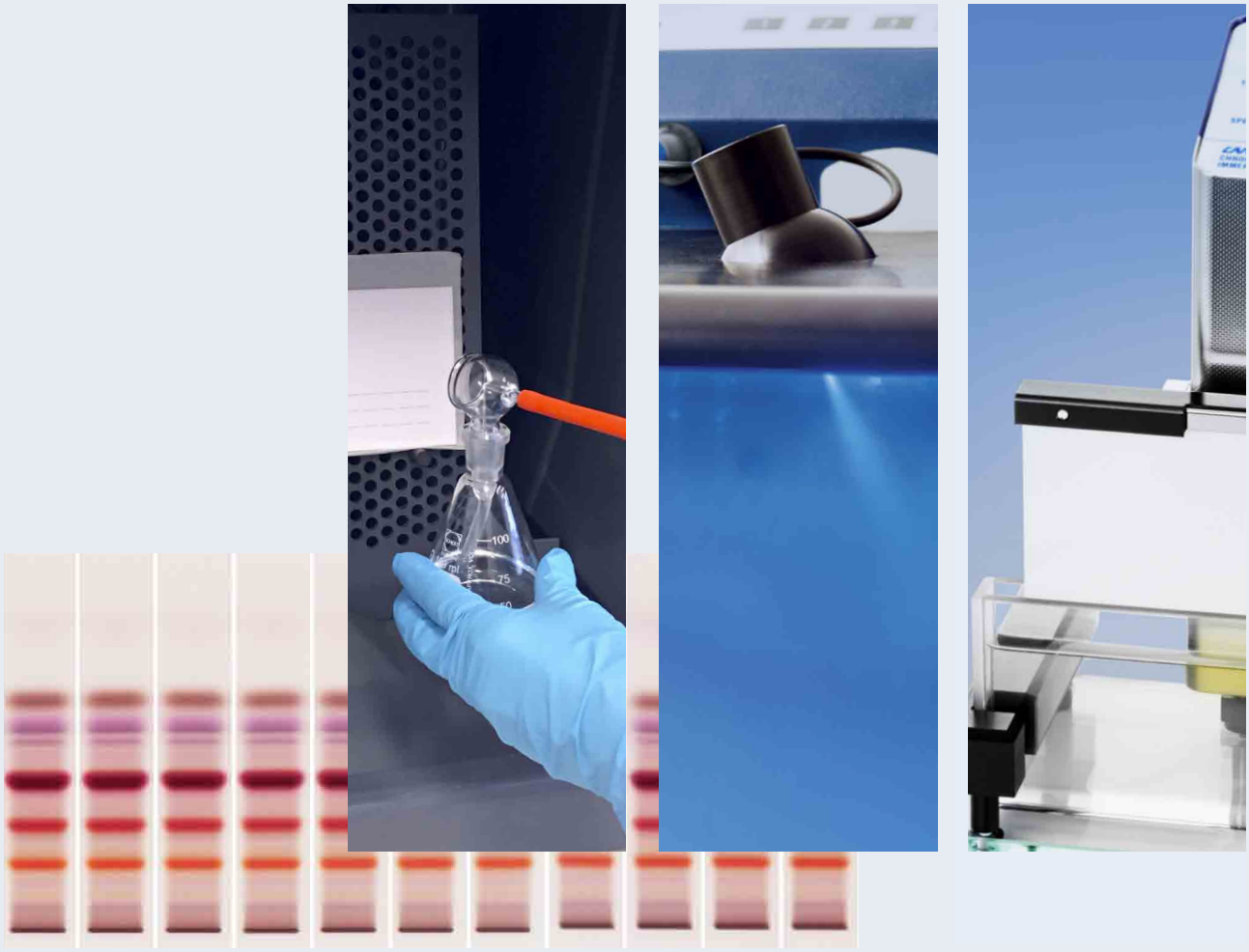

Comparison of different derivatization techniques

Other topics of this issue:
Ricinoleic acid as marker for *Secale cornutum* impurities
Quantification of steviol glycosides
Bitter acids in hops
Tetrahydrocannabinol in Cannabis

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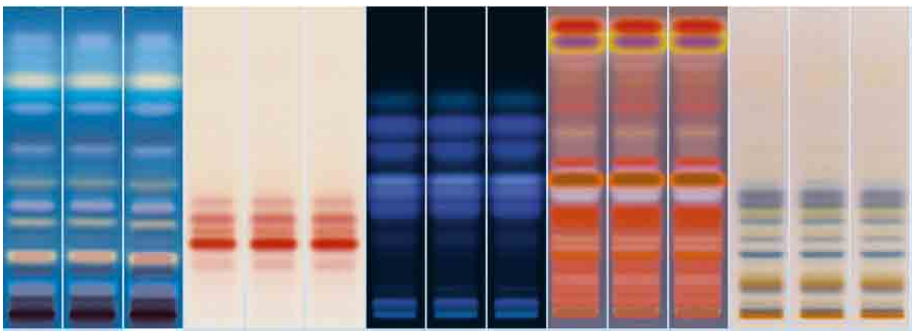
Comparison of different derivatization techniques

Introduction

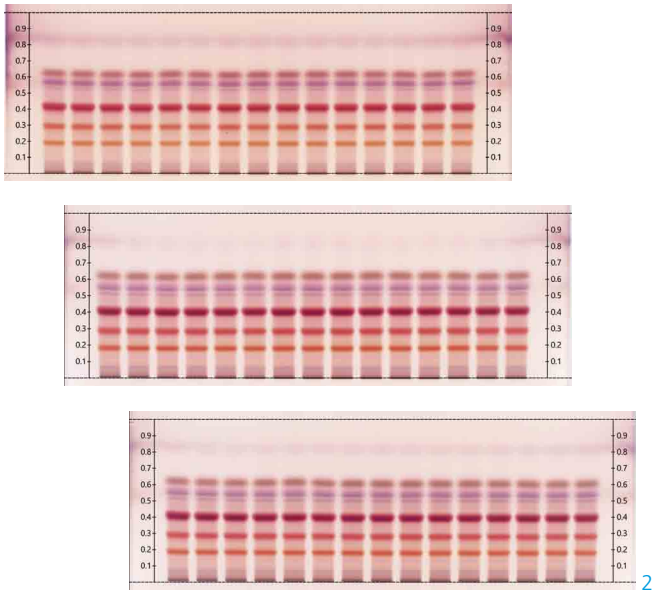
For the postchromatographic derivatization in TLC/HPTLC there are different techniques to transfer liquid reagents onto the plate, *e. g.* manual spraying, automated spraying and immersion (dipping). Immersion has been considered as the technique leading to the most homogeneous reagent distribution; however it has the disadvantage of relatively high reagent consumption. With the design of the Derivatizer, CAMAG has set new standards in terms of homogeneity of reagent distribution and ease of use.

The CAMAG Derivatizer is based on a new and unique micro-droplet spraying technology* for reagent transfer onto TLC/HPTLC plates. All common reagents can be homogeneously and reproducibly sprayed with the Derivatizer. The closed system reduces the contamination of the working area, which has been a problem with manual spraying. In our study we evaluated the three techniques – manual spraying, automated spraying and immersion.



Reagent transfer of different reagents with the Derivatizer: (A) Rhodiola sample derivatized with sulfuric acid reagent (UV 366 nm); (B) Fenugreek sample derivatized with ninhydrin reagent (under white light); (C) Angelica sample derivatized with KOH reagent (under UV 366 nm); (D) olive leaf sample derivatized with anisaldehyde reagent (under UV 366 nm); (E) Rhodiola sample derivatized with aniline-diphenylamine-phosphoric acid reagent (under white light); information on further tested reagents at www.camag.com/derivatizer

*Patent pending

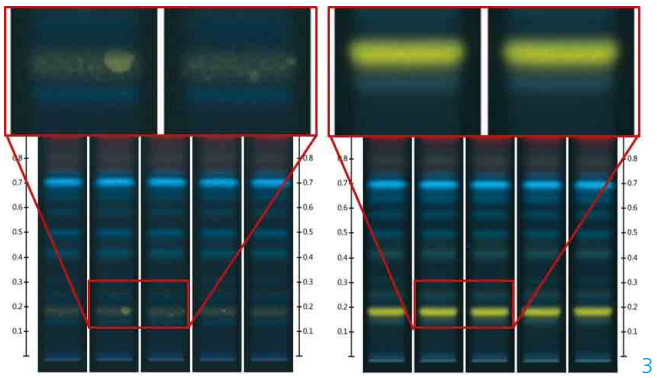


2

Curcuma sample applied multiple times on three HPTLC plates and derivatized after chromatography with anisaldehyde reagent using the Derivatizer (under white light); the results were reproducible from plate to plate and independent of the user

Manual spraying *versus* automated spraying

An experienced user might achieve comparable results by manual and automated spraying with the Derivatizer, which is shown by no significant differences in the color of the zones and the background. In any case, with the Derivatizer the reagent is distributed more homogeneously on the plate because the small size of the micro-droplets leads to a uniform wetting of the plate. This example illustrates that by spraying with the Derivatizer, all parts of the plate were homogeneously derivatized.



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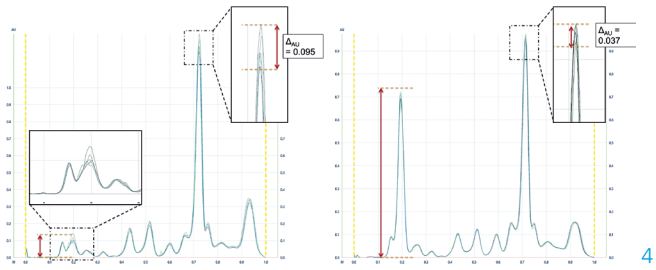
Visual comparison of reagent transfer: Chromatograms under UV 366 nm of a Basil sample after derivatization with Natural products reagent by manual spraying (left) and automated spraying (right)



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Respective densitometric comparison: the yellow fraction (hR_F 18) was only partially converted by manual spraying; the blue fraction (hR_F 70) did not show visual differences. On the other hand, the densitometric values showed an obviously larger variance with manual spraying.

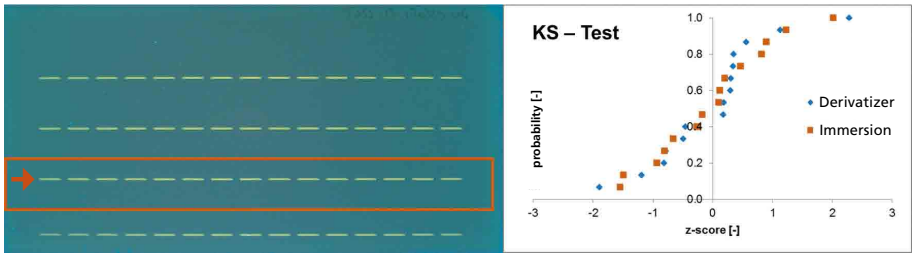
Immersion *versus* automated spraying

Hitherto, reliable quantification after derivatization was only thought possible if the immersion technique was used to transfer the reagent onto the plate. For this comparison, the plate was dipped with the Chromatogram Immersion Device into 200 mL of the reagent solution. With the Derivatizer only 3 mL of the same reagent solution were needed. To compare the data sets of both techniques quantitatively and without influence by other process steps (application and development), we performed the following measurements:

- UV-active substances were applied on four different application positions (y-position) on 15 tracks (rutin as example).
- Each test was repeated in triplicate by three different participants.
- All tests were conducted under standard conditions (temperatures between 20 and 25°C at relative humidity of 35–50%).
- The plates were measured before and after derivatization with Natural products reagent in absorbance mode with the TLC Scanner 4 and the results were calculated via normalized peak heights.

Visual comparison of the results obtained by immersion or automated spraying showed no difference in the color of zones and the plate background. For the statistical analysis the normalized peak heights of both techniques were compared in the Kolmogorov-Smirnov test (KS test). The test compares the match of the distributions of two data sets. Within the 95% confidence interval no difference was observed between the two distributions, so the homogeneity

of the derivatization over the plate was comparable for both techniques. The precisions (measured on three plates) were always below 5% for both automated spraying and immersion. For manual spraying the precision was as high as 12% per plate.



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Homogeneity of the derivatization: Image under UV 366 nm of rutin start positions on 15 tracks applied at 4 different y-positions, derivatized with Natural products reagent (left; reagent transfer via Derivatizer); statistical distribution (KS test) of peak heights of the derivatization techniques immersion vs. automated spraying with the Derivatizer (right)

Results and discussion

With the CAMAG Derivatizer all common derivatization reagents can be sprayed homogeneously, reproducibly and flexibly. In terms of safety and reproducibility of results, spraying with the Derivatizer is clearly superior to manual spraying. The results by automated spraying and immersion are comparable, however immersion requires larger reagent volumes. In numerous other tests we compared the limits of detection for different substances and reagents. Similarly the results by automated spraying and immersion were comparable. In addition, the Derivatizer is advantageous if there is the risk of partially eluting the chromatogram zones during the immersion step.

A practical example for the application of the Derivatizer is shown on pages 14–15 of this CBS.

Additional information is available at www.camag.com/derivatizer and on request from CAMAG.

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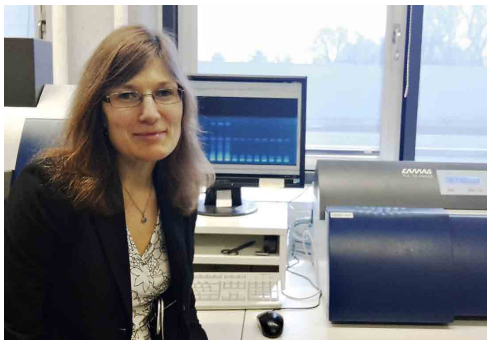
Screening for ricinoleic acid as marker for *Secale cornutum* impurities in rye



Dr. Claudia Oellig

The development of fast screening methods for the determination of *Secale cornutum* impurities in rye and rye products with HPTLC is one of the research topics of Dr. Claudia Oellig at the Institute of Food Chemistry, University of Hohenheim, Stuttgart. In this study a method for the selective determination of ricinoleic acid as a marker for *Secale cornutum* impurities in rye is shown.

Introduction

Secale cornutum (ergot), the overwintering body of the parasitic fungus *Claviceps purpurea* mainly grows on rye, but also on other cereals. The presence of the permanent form is responsible for different toxic effects in mammals, caused by ergot alkaloids. Regardless of the infestation of rye grain with ergot, there are no maximum limits established for ergot alkaloids in grain-based food [1], only for *Secale cornutum*. For food, a maximum limit of 0.05% *Secale cornutum* is laid down in regulation 2015/1940/EU. Due to the significant variability of the total ergot alkaloid content in *Secale cornutum* (0.01–0.5%, which is origin dependent), this quantity is not suitable for the determination of the *Secale cornutum* content. Apart from the ergot alkaloids, *Secale cornutum* is also comprised of characteristic ergot lipids (30%) with 30% of ricinoleic acid. This fatty acid is a useful chemical marker for *Secale cornutum* impurities in cereal [2]. For the screening method presented here for ricinoleic acid, cost-effective HPTLC was used, which guarantees rapid and reliable results for *Secale cornutum* impurities in many samples in parallel.

The ricinoleic acid screening for the determination of the *Secale cornutum* content was successfully developed and evaluated for several rye flours. After lipid extraction with toluene and hydrochloric acid, transesterification with 1% sulfuric acid in methanol and clean-up on Ag-ion cartridges, the ricinoleic acid is selectively derivatized with 2-naphthoyl chloride and analyzed by HPTLC. Sensitive fluorescence detection is performed at UV 280/>340 nm. With limit of detection and quantitation of 0.0001 und 0.0004 % *Secale cornutum* in rye flour, monitoring of the maximum admitted level of 0.05 % is guaranteed [3].

Sample preparation

Rye flour and finely milled whole rye (2 g) were extracted with 4 M hydrochloric acid (14 mL) and toluene (5 mL) for 2 h at 120 °C. 250 µL of the toluene phase (lipids) was evaporated and transesterified with 1 mL of 1% sulfuric acid in methanol for 3 h at 80 °C in a thermomixer and after addition of 1 mL of water and 1 mL of saturated sodium chloride solution liquid-liquid partition was performed with 2 mL of *n*-hexane, followed by SPE clean-up with a 500-µL aliquot of the *n*-hexane phase on preconditioned Ag-ion cartridges. After rinsing with 18 mL of *n*-hexane – acetone 7:3, the ricinoleic acid methyl ester was eluted with 7.5 mL of *n*-hexane – acetone 13:7 [3].

Derivatization

Derivatizing reagent solution (200 µL; 40 mg 4-dimethylaminopyridine and 7.5 mg 2-naphthoyl chloride/mL methylene chloride, anhydrous) was added to the evaporated SPE extract and acylation was done for 30 min at ambient temperature. After addition of 800 µL of methylene chloride, dispersive SPE was performed by vigorously shaking for 1 min with 120 mg of primary secondary amine (PSA) and 90 mg of SiliaBond carboxylic Acid (SiCAA). The clear methylene chloride solution was used for HPTLC [3].

Standard

For quantitation, a ricinoleic acid methyl ester standard in acetonitrile (45 ng/µL) was used. A 25-µL aliquot was evaporated and derivatized according to the outlined procedure (1.125 ng/µL).

Chromatogram layer

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

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 22 tracks, band length 6 mm, track distance 8.7 mm, distance from the left side 8 mm, distance from the lower edge 8.0 mm, drying in a fume-hood for 5 min, application volume 20.0 µL for samples and 0.5–30.0 µL for the ricinoleic acid methyl ester standard

Chromatography

In the Automatic Developing Chamber (ADC 2), conditioning the plate at 33% relative humidity for 5 min using a saturated solution of magnesium chloride, development with 10 mL cyclohexane – diisopropyl ether – formic acid 86:14:1 to the migration distance of 70 mm from the lower edge and drying for 5 min

Fluorescence enhancement

Dipping the plate in *n*-hexane – paraffin 2:1 with the Chromatogram Immersion Device, immersion speed 2.0 cm/s, immersion time 3 s

Documentation

With TLC Visualizer under UV 254 nm and UV 366 nm

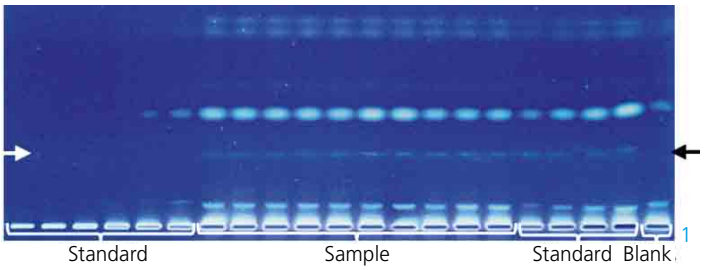
Densitometry

With TLC-Scanner 4 in the fluorescence mode at UV 280/>340 nm (mercury lamp), scanning speed 20 mm/s, data resolution 100 µm/step, slit dimension 4.00 x 0.45 mm. The acquisition was performed with the following manual detector settings: distance from the lower edge for zero adjustment 22.0 mm (track of the lowest standard), quick scan range 25–35 mm (track of the highest standard). Quantitation was performed via peak areas.

Note: The manual detector settings are mandatory to gain highest sensitivity.

Results and discussion

The determination of *Secale cornutum* impurities in rye was performed through the analysis of ricinoleic acid in form of the ricinoleic acid methyl ester that was selectively fluorescent-labeled. Chromatographic separation on silica gel with cyclohexane – diisopropyl ether – formic acid 86:14:1 resulted in a sharp target zone at hR_F 33 without interferences from the rye matrix and fluorescent labeling reagent. For quantitation of ricinoleic acid, the enhanced fluorescence was scanned at UV 280/>340 nm. The relatively stable ricinoleic acid content in *Secale cornutum* of 10% [2] was used for the determination of the *Secale cornutum* amount in the sample. According to the DIN calibration method, limits of detection and quantitation (LOD and LOQ) were calculated to be 0.0001 and 0.0004% *Secale cornutum* for rye cereals.



HPTLC Chromatogram under UV 254 nm. Samples: spiked rye flour (0.02 and 0.05% Secale cornutum), standard: ricinoleic acid methyl ester (marked by arrow) with 0.6 – 33.8 ng/zone, blank: blank rye flour. Reprinted with permission from [3].

Method performance of the HPTLC screening was assessed by *Secale cornutum* spiked rye flours (0.02 and 0.05% *Secale cornutum*). Recoveries for ricinoleic acid was close to 100% and relative standard deviations were below 5% (n = 5).

Finally, the method was applied to several rye flours from local supermarkets to get an overview of the current contamination of rye cereals with *Secale cornutum* in Germany. In all samples low quantities of ricinoleic acid were detected. The maximum *Secale cornutum* quantity, found for a rye flour of the German type 1150 with 0.025%, was clearly below the currently set maximum limit of 0.05%.

Ricinoleic acid and Secale cornutum contents in eight samples. Reprinted with permission from [3].

Rye flour	No.	Ricinoleic acid [mg/kg]	*Secale cornutum* [%]	*%RSD* (*n* = 3)
German type 997	1	7.1	0.0068	*1.6*
German type 1150	2	6.4	0.0062	*2.4*
	3	2.7	0.0026	*1.5*
	4	25.9	0.0250	*1.7*
Whole rye flour, finely milled	5	2.3	0.0022	*3.4*
	6	3.2	0.0030	*4.1*
Whole rye, finely milled	7	3.9	0.0038	*3.6*
	8	3.5	0.0033	*3.6*

HPTLC proved to be an efficient and reliable screening tool for the determination of *Secale cornutum* contaminations in rye through the analysis of the ricinoleic acid, making time-consuming GC–FID analyses unnessary. LOD and LOQ were far below the approved maximum limit of 0.05 % *Secale cornutum* for food, demonstrating the suitablilty and reliability for its broad usage for instance for quality control or routine monitoring in food and feed mills.

[1] www.bfr.bund.de/cm/343/einzelfallbewertung-von-ergot-alkaloid-gehalten-in-roggenmehl-und-roggenbroten.pdf.

[2] Franzmann, C. *et al.* J. Agric. Food Chem. 58 (2010) 4223–4229

[3] Oellig, C., J. Agric. Food Chem. 64 (2016) 8246–8253

Further information is available on request from the author.

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2

CAMAG TLC Visualizer 2

The professional documentation and evaluation system for Planar Chromatography

The TLC Visualizer 2 is optimized for the documentation of TLC and HPTLC plates. Through the highest possible homogeneity under all illumination modes – UV 254 nm, UV 366 nm, and white light – ideal and reproducible images are obtained. Due to the new digital CCD camera with a maximum resolution of 82 µm on the plate, the TLC Visualizer offers even better image quality than its predecessor. The integrated USB 3.0 port ensures easy PC connection and fast data transfer. The easy and intuitive operation with *visionCATS* HPTLC software (from version 2.3 on) offers a wide range of image processing tools and evaluation possibilities, such as a semi-quantitative evaluation of image profiles and a comparison of tracks from different plates with the »Comparison Viewer«.

Further information:
www.camag.com/tlcvisualizer2

To the 90th birthday of Dr. Dieter Jänchen

Dr. Dieter Jänchen, the founder of CAMAG and still active senior boss celebrated his 90th birthday on 6 June 2017. Dr. Jänchen looks back to an impressive life's work. He founded the company CAMAG end of 1958 and soon entered the field of thin-layer chromatography. With his commitment and determination he helped the company gain international acceptance. Today the employees in the CAMAG headquarters in Muttenz and the daughter companies in Germany and the USA do excellent work. In more than 70 countries CAMAG is represented through selected companies.

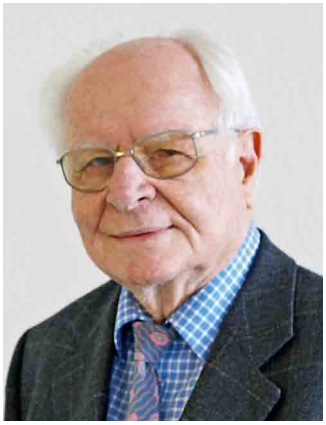
It all started with the production of aluminum oxide, though soon "the boss" decided to enter the instrument business. It is Dr. Jänchen's merit, that we CAMAG employees are proudly part of a company which has established instrumental thin-layer chromatography worldwide as a modern and powerful technique.

It was a long journey, full of courage, diligence, enthusiasm and unshakeable optimism.

Today "Dr. Jänchen's" CAMAG is a worldwide reputable developer, manufacturer and distributor of instrument systems for this method.

With day one he trusted in the creativity and inventiveness of his employees in research and development and actively assisted them with his own ideas. Still hardware and software development take place exclusively within the company. Gradually instruments of highest quality and precision have been developed, first using simple modules, then using microprocessors and the corresponding software.

In time he recognized the necessity to accompany the distributed instrument systems with application methods. In the start-up phase of the company Dr. Jänchen was not only the architect and designer of his company, but also his own sales manager, who as a chemist quickly identified the needs of his customers on-site and was able to consult. He also saw the need to build an application laboratory,



Dr. Dieter Jänchen, Founder of CAMAG, "Father" of the CBS

which has been lending valuable support to this day. Over the years a great collection of reproducible methods, several of them validated, has added up, which is a valuable source of information for our customers.

He created the half-yearly published CAMAG Bibliography Service (CBS) which is much more than the usual company magazine. The collection of extensive publication abstracts (CCBS) has developed into a database of international rank. Still today Dr. Jänchen consults during the elaboration of every new CBS issue.

Dr. Jänchen's hobby was glider and motor flying, which he practiced on a high level. In 26 years as a dedicated glider instructor he passed on his experiences as a successful pilot to his student pilots. His most spectacular experience was a transatlantic ferry flight with a small single-engine aircraft. Dr. Jänchen's friends and employees keep the flights with his private aircraft in good memory.

We have come to know Dr. Jänchen as a humorous, caring and if necessary resolute/authorative "boss". We are happy to have him as a mentor and advisor on our side. To his 90th birthday we wish him good health, vitality and long-lasting creativity!

Dr. Konstantinos Natsias
Chairman of the Board

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

With this CBS issue 110 abstracts on TLC/HPTLC literature have been added to the CCBS database, among these an increasing number related to coupling with mass spectrometry. From the perspective of fundamentals, different techniques have been investigated to improve the outcome of sample analyses by TLC/HPTLC-MS. For example, laser desorption-ion mobility spectrometry sampled the compounds on the TLC chromatogram within 80 s without the need of any further preparation. The TLC plate was moved by the motorized micro-positioning stage towards the fixed laser (J. Chromatogr. A 1459, 145–151, 2016). Another effective technique was the combining of TLC with glow discharge-matrix assisted infrared desorption ionization (J. Chromatogr. A 1460, 181–189, 2016). It is remarkable that automation of HPTLC-MS is more and more an integrative part and major feature of these diverse newer approaches.

On the white CBS pages, selected instrumental applications of HPTLC are presented that attract interest due to noteworthy benefits through the use of HPTLC in a special analytical chemistry field. The impact of HPTLC is outlined along with the resulting outcome by solving a particular analytical task by HPTLC. If you are interested in contributing, feel free to contact cbs@camag.com.

Dear friends

Planar chromatographic techniques have successfully supported chemists for almost eight decades now. After each decade half of the earlier achievements and knowledge is forgotten, or at least, that's the way it feels. For elder scientists it might be something to chuckle about when the younger generation discovers the immense potential and benefits of HPTLC or laugh out loud, when they discover that the performance of HPTLC is equal to or even outperforms HPLC and UHPLC. How often experienced scientists like Dr. Jänchen might have smiled?

There are ample, successful, practical applications for HPTLC which cannot be beaten by other techniques in such an effective and streamlined way. With this evolutionary history it is an everlasting re-discovery and sometimes re-invention, though in a differently fashioned mode. Diverse applications in this CBS issue outline this potential. An even broader field of applications was evident at the recent international symposium on HPTLC in Berlin. Some highlights are presented on the last yellow page.

Kind regards

Gertrud Morlock
cbs@camag.com

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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**

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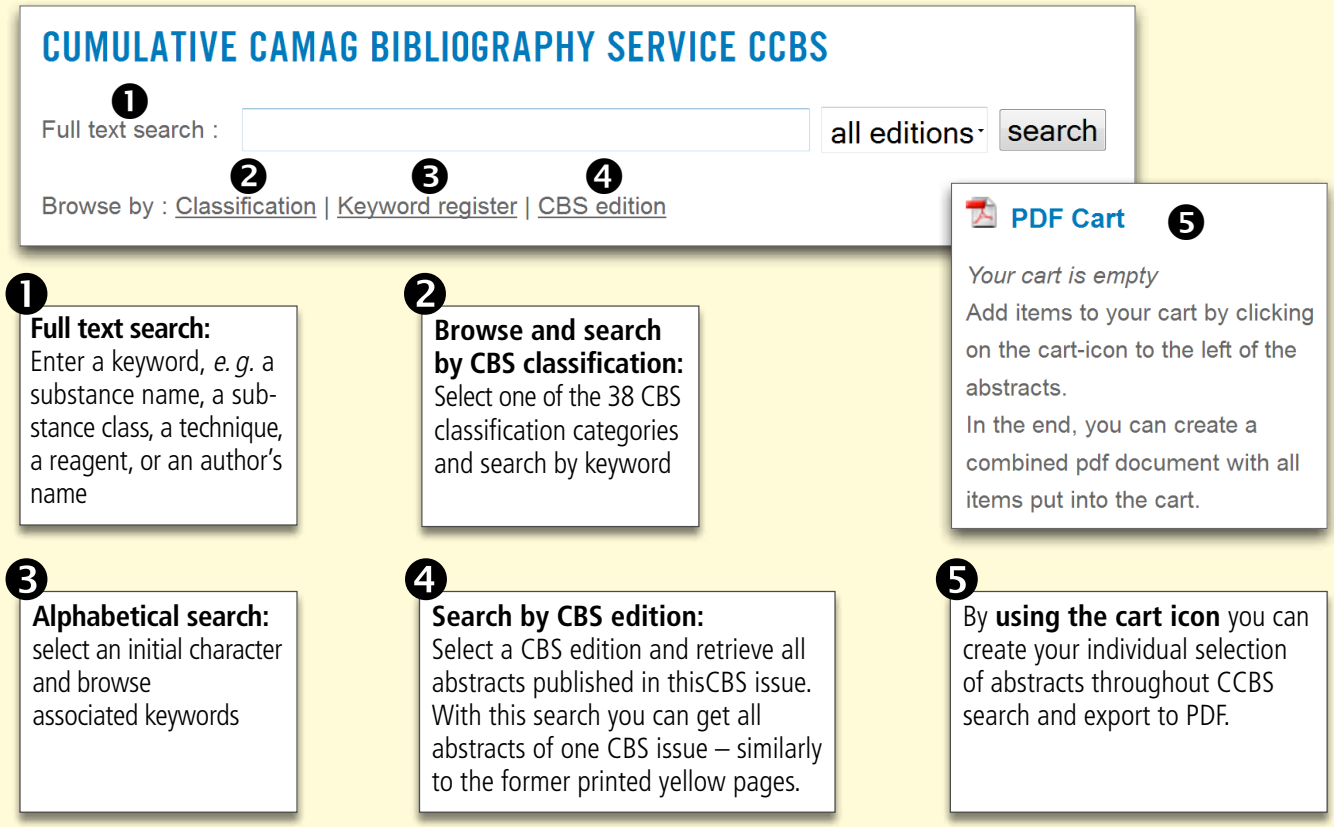

With the Cumulative CAMAG Bibliography Service (CCBS) Online Search, you can directly search for information within the CAMAG website. The CCBS covers more than 11'000 abstracts of TLC/HPTLC publications between 1982 and today. Providing reports was established by Dr. Dieter Jänchen in 1965 and the workflow 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.

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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.

HPTLC at its best!

International Symposium for High-Performance Thin-Layer Chromatography 2017 in Berlin

The recent International Symposium for HPTLC in Berlin, 2–4 July 2017, was a great success. Berlin was chosen because of its central location in the heart of Europe. Thank is owed to the sponsors and to all presenters who made HPTLC at its best! The scientific program attracted all together over 210 scientists from more than 30 nations from around the world.

The program with almost 60 oral presentations and 111 poster presentations triggered many questions and fruitful discussions. It built a striking platform for learning and sharing knowledge, experiences and the latest research in HPTLC. The panel discussion of manufacturers resulted in an exchange of ideas on needs, very helpful for progress in HPTLC which is strongly supported by them. Three short courses offered as a pre-program, were well accepted by the attendees.

Among the audience of 55% academia, 25% industry and 20% students, 22 of the young researchers (aged < 40 years) competed with their oral presentations for the three Young Researcher Awards. Dr. Yisheng Chen from the Jiangnan University, Wuxi, China, won the *Dieter Jänchen Award for the Young Researcher* for his talk on "HPTLC + SERS > HPLC + MS." Dr. Vandana Gawande from Sinhgad Institute of Pharmacy, Pune, India, was the recipient of the *Young Researcher Award dedicated to Hellmut Jork*, for her presentation on "Preparative isolation and characterization of degradation products of cefixime and azithromycin." Dimitri Fichou from JLU Giessen, Germany, won the *Young Researcher Award dedicated to Friedrich Geiss* for his lecture on "Office Chromatography."

From left: Dr. Yisheng Chen, Pierre Bernard-Savary, Louise Bernard-Savary, Dimitri Fichou*, Prof. Dr. Colin Poole, Dr. Vandana Gawande*, Dr. Konstantinos Natsias, Prof. Dr. Rudolf Kaiser (*Winner Young Researcher Awards)*

The six poster prizes were awarded to the presenting author, representative for all authors of these outstanding posters:

Bronze

Prof. Dr. Teresa Kowalska *et al.* for "Application of TLC to ecotoxicological study with the *Steatoda grossa* spider web model" (P–93)

Prof. Dr. Sigrid Mennickent *et al.* for "Quantitative determination of topiramate in human breast milk by HPTLC" (P–103)

Dr. Tim Häbe *et al.* for "Direct bioautography with subsequent DART-MS" (P–18)

Silver

Vesna Glavnik *et al.* for "Comparison of HPTLC-MS methods on silica gel and diol plates for determination of proanthocyanidins in Japanese knotweed" (P–92)

Dimitri Fichou *et al.* for "Open-source developments for Office Chromatography" (P–3)

Gold

Urska Jug *et al.* for "Optimization of HPTLC and HPTLC-MS methods for analysis of flavonoids and phenolic acids" (P–35)

It was not only this award ceremony, the social dinner was highly appreciated. Also, new contacts were made, knowledge exchanged and the next meetings eagerly planned to be: 2018 in Bangkok, Thailand, 2019 in USA, and 2020 in Ljubljana, Slovenia. More information will soon be available at www.hptlc.com.

Quantification of steviol glycosides and steviol/isosteviol

Julian Wald

The analysis of steviol glycosides in food and *Stevia* products presented in CBS 109 has been expanded not only to carbohydrate-rich food, but also to the breakdown products steviol/isosteviol, confirmation by HPTLC-MS and proof of product falsification [1]. This research was performed by Julian Wald and Prof. Dr. Gertrud Morlock at the Justus Liebig University Giessen, Germany.

Introduction

In the EU, the sweetener steviol glycosides (E 960) isolated from the plant *Stevia rebaudiana* has been permitted for use as food additive since December 2011, and food products containing E 960 are increasingly launched on the European market. The broad variety of matrices requires a streamlined, but robust method to screen marketed samples that may be adulterated with cheaper synthetic sweeteners. These guidelines were paramount in developing the method presented here.

This HPTLC method proved to be robust with regard to varying sample matrices and resolution between steviol glycosides was improved. Up to 23 different samples can be separated on one plate, which took 1 h (2.6 min/sample) with solvent consumption of only 0.4 mL/sample. Through derivatization with the 2-naphthol reagent, the detection was selective and inexpensive. Additionally, steviol and isosteviol were detected on the same plate using the primuline reagent. The latter reagent was superior for sugar-containing samples, though detectability was not as good for steviol glycosides. HPTLC-ESI-MS spectra were only recorded from the zones of interest, not from matrix or background, which had prolonged the runtime between MS cleaning cycles. Among the samples analyzed a falsified marketed sample was discovered, qualifying this method for food control.

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 x 10 cm, if required, prewashed with methanol and dried (100 °C, 30 min)

Standard solution

Steviol glycosides (33 ng/µL) and steviol/isosteviol (333 ng/µL) dissolved as methanolic mixture; further solutions in [1]

Sample preparation

Table-top sweetener powders (25–50 mg) and tablets (75 mg) were dissolved in methanol (5 mL) via 5-min ultrasonication, whereas tinctures and liquids were diluted with water (0.5 mL/10 mL). Tea formulations (0.3–3.0 g) and dried/pulverized *Stevia* leaves were boiled for 10 min (0.5 g/30 mL). These suspensions were filtered into a volumetric flask to be filled up to the 50-mL mark with water and diluted 1:10 with methanol. Chocolates were dissolved with ethanol set at 50 °C for 15 min, treated with 0.5 mL Carrez 1/2, filled up to be 4 g/25 mL, centrifuged (3 min, 3000 x g), defatted by freezing (–18 °C, 5 h) and filtered; further preparations in [1].

Sample application

Bandwise with Automatic TLC Sampler 4, 23 tracks, band length 6 mm, track distance 7 mm, distance from lower edge 8 mm and side edge 24 mm, application volumes 1–20 µL for standards and samples

Chromatography

In Automatic Developing Chamber (ADC 2) with 10 mL ethyl acetate – methanol – formic acid 93:40:1 after adjustment of the plate activity for 5 min with lithium chloride (830 g/L) or magnesium chloride (540 g/L), migration distance 60 mm (15 min), drying times 0.5 min before and 3.0 min after development

Baseline separation of steviol and isosteviol on the cut upper plate part at 52 mm in the Twin-Trough Chamber with 7 mL *n*-hexane – acetic acid 19:1 up to 45 mm (10 min)

Mass spectrometry

Underivatized zones were marked and directly eluted with the oval elution head (4 mm × 2 mm) of the TLC-MS Interface using methanol (0.2 mL/min) into the electrospray ionization mass spectrometer (ESI-MS, Agilent Technologies, Waldbronn, Germany).

Postchromatographic derivatization

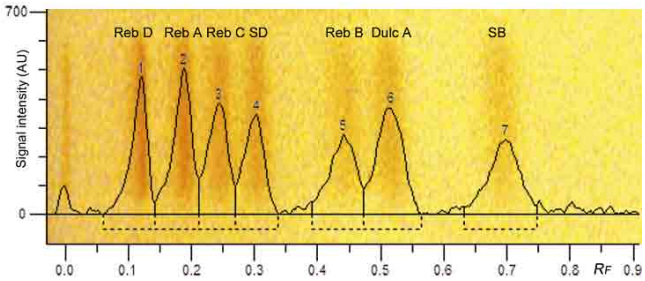
With Chromatogram Immersion Device (immersion speed 3 cm/s, immersion time 0 s), the HPTLC plate was first half-immersed in 135 mL primuline reagent (100 mg primuline in 200 mL acetone – water, 4:1; for isosteviol/steviol detection) from the opposite plate side and dried. Second, the plate was half-immersed in the direction of development in 135 mL 2-naphthol reagent (2 g 2-naphthol in 180 mL ethanol and 12 mL 50% sulfuric acid) and heated on the TLC Plate Heater (120 °C, 5 min). Alternatively, both reagents were used on separate plate halves after plate cut and baseline separation of steviol and isosteviol. Stored in the refrigerator, both reagents were stable for months.

Documentation

Chromatograms were documented at UV 366 nm (primuline reagent) and under white light illumination in transmission/reflection mode (2-naphthol reagent) using the TLC Visualizer.

Densitometry

TLC Scanner 3 with winCATS software, absorption measurement at 500 nm, slit dimension 4.0 mm × 0.3 mm, scanning speed 20 mm/s; VideoScan software was used as alternative.

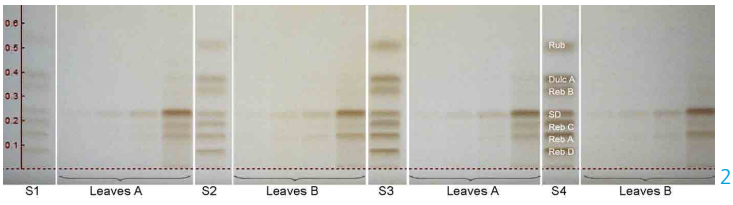


1

Videodensitogram of the underlaid steviol glycoside separation after derivatization with 2-naphthol reagent (60 ng/band each, Rebaudioside: Reb; Stevioside: SD; Dulcoside: Dulc; Steviolbioside: SB); reprinted with permission from [1]

Results and discussion

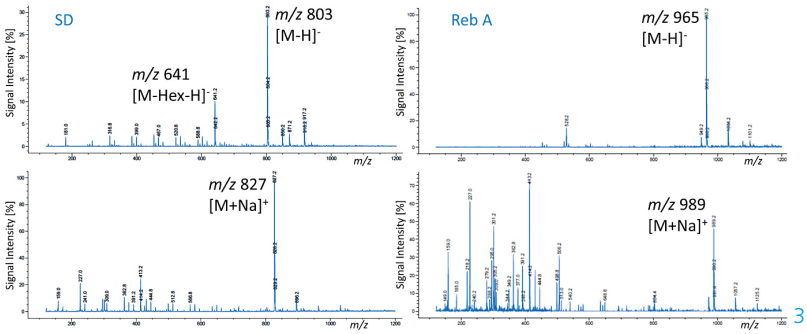
First, the resolution of the separation of the steviol glycosides was improved to separate seven on the same track. Up to 23 samples were separated in parallel within 15 min. The steviol glycosides were detectable down to 2–5 ng/band by absorbance measurement at 500 nm after derivatization with the 2-naphthol reagent. Calibration curves showed correlation coefficients between 0.9983 and 0.9995 and relative standard deviations between 2.7% and 4.8%. Sample results were confirmed by HPTLC-ESI-MS. Also various products were analyzed, resulting in the discovery of a product falsification, containing no steviol glycosides, but instead containing the inexpensive synthetic sweeteners sodium cyclamate and saccharine [1].



2

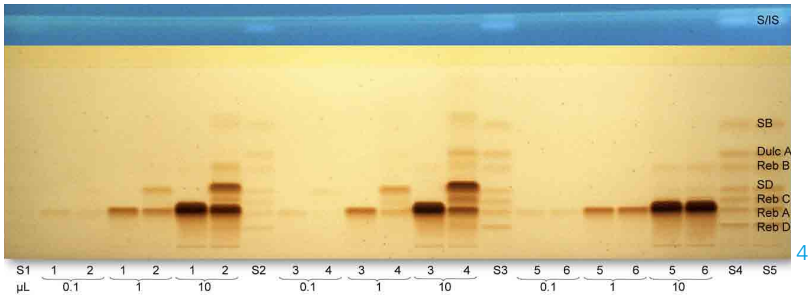
Chromatogram of Stevia rebaudiana leave extracts A and B (0.5, 1, 2 and 10 µL/band) besides standard solution (S1–S4; 33–198 ng/band; rubusoside: Rub) derivatized with the 2-naphthol reagent (white light illumination in reflectance mode); reprinted with permission from [1]

Although the use of steviol glycosides is strictly regulated for products, consumers often use E 960 in an unregulated fashion as evident on the internet. Isosteviol was described to be physiologically active [2] and may be formed in an acidic food matrix at elevated temperatures via acid hydrolysis of E 960. Thus secondly, the detection of the breakdown products steviol/isosteviol was integrated in this method. After two derivatizations from opposing sides, all steviol glycosides were detected between hR_F 11 and 56 and steviol/isosteviol at hR_F >95 as sum parameter.



3

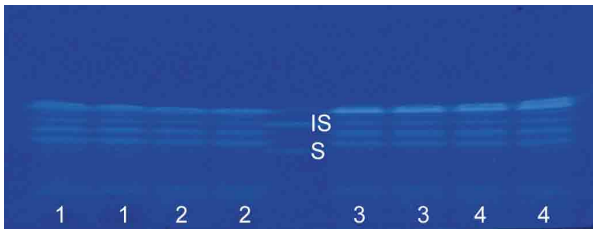
HPTLC-ESI-MS spectra for stevioside (SD, left) and rebausioside A (Reb A, right); reprinted with permission from [1]



4

Chromatogram of Stevia formulations 1-6 besides standard solution (S1–S5; 12–120 ng/band) after derivatization with 2-naphthol reagent (white light illumination in transmission mode) and primuline reagent for detection of steviol/isosteviol (S/IS, UV 366 nm); relevant parts of both images depicted together; reprinted with permission from [1]

As breakdown products are formed only under certain conditions with regard to matrix and food processing, the sum parameter should be a sufficient tool. However, for a baseline resolution of steviol and isosteviol, the following workflow is recommended: after chromatography and half-immersion (50 mm) into the 2-naphthol reagent (automated dipping/fixation of the whole plate was easier), the plate was cut at 52 mm. The upper non-derivatized plate part was further developed with *n*-hexane – acetic acid 19:1 up to 45 mm taking 10 min, followed by derivatization with the primuline reagent.



5

Baseline separation of steviol (S) and isosteviol (IS) of chocolates 1–4 after plate cut, chromatography (10 min) and derivatization with primuline reagent (UV 366 nm); reprinted with permission from [1]

To conclude, the performance data proved the quantitative HPTLC method to be highly suited for food control, along with its capacity for high sample throughput, robustness regarding varying matrices, cost-efficiency and fast analyses.

[1] J. P. Wald, G. Morlock, J. Chromatogr. A 1506 (2017) 109–119

[2] K. L. Wong *et al.* Planta Med. 70 (2004) 108–112

Thanks to PhytoLab, Vestenbergsgreuth, Germany, for standard compounds.

Further information is available from the authors on request.

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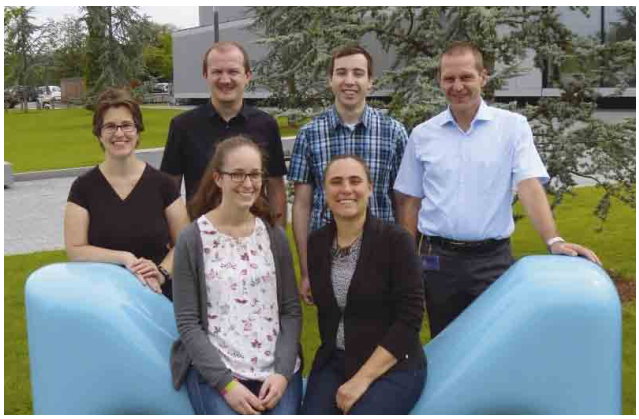
Quantification of Bitter Acids in Hops



From left: Vanessa Pilakowski, Hans Griesinger, Janina Engemann, Markus Burholt, Michaela Oberle and Michael Schulz

TLC has a wide range of qualitative and quantitative application fields. The Instrumental Analytics group of Michael Schulz at Merck in Darmstadt, Germany, develops new chromatographic TLC and HPTLC layers. In this context the group works on new application fields for the use of these techniques.

Introduction

The amount of bitter acids in hops is a very important parameter for beer production. Different types of hops contain different quantities of α- and ß-acids responsible for the bitterness of the beer. In general hops are divided into aromatic hops (<10% α-acids) and bitter hops (>10% α-acids).

In this study, a total of 12 samples were analyzed, *i.e.* four aromatic hops, four bitter hops and four hops of the same variety, but from different regions. The sample extracts were applied on HPTLC plates silica gel 60 F_{254} MS-grade, developed and separated with a gradient using the Automated Multiple Development (AMD 2) System and their fluorescence was measured at 360/>400 nm.

Sample preparation

Crumbled hop pellets (5 g) suspended in 10 mL methanol, 50 mL diethyl ether and 20 mL 0.1 M hydrochloric acid solution were stirred for 40 min. 2 mL of the upper ether phase were transferred to a 20-mL volumetric flask and filled up to the mark with methanol (10 mg/mL). An aliquot of this extract was filtered via a 0.45-µm membrane filter into a sampler vial.

Standard solution

The International Calibration Extract (ICE) 3 contained 44.6% α-acids, 24.3% ß-acids and 31.1% other ingredients dissolved in methanol (1 mg/mL).

Chromatogram layer

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

Note: HPTLC plates for mass spectrometry are characterized by a higher purity of the layer and a thinner layer thickness (100 µm). This allows a more sensitive measurement compared to the standard plate. The suitability for MS is also shown in the certificates of analysis.

Sample application

With Automatic TLC Sampler (ATS 4) as 6 mm bands, application volumes were 0.5–1.6 µL/band for standard and 0.3–1.0 µL/band for sample solutions.

Chromatography

Automated multiple development using a 9-step AMD 2 gradient based on ethyl acetate – methanol – *n*-heptane followed by drying for 2 min after each step

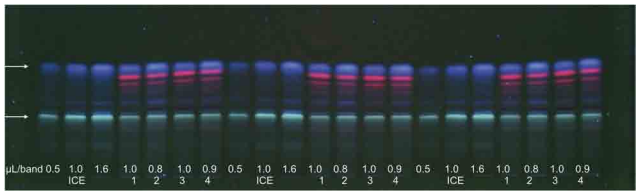
Step	Ethyl acetate (Vol%)	Methanol (Vol%)	*n*-Heptane (Vol%)	Migration distance (mm)
1	50	50	0	12
2	40	40	20	18
3	35	35	30	24
4	30	30	40	30
5	25	25	50	36
6	20	20	60	42
7	15	15	70	48
8	10	10	80	54
9	5	5	90	60

Densitometry and documentation

With TLC Scanner 3 and winCATS, fluorescence measurement at 360/>400 nm (deuterium lamp) and evaluation via polynomial regression; documentation at UV 366 nm via TLC Visualizer

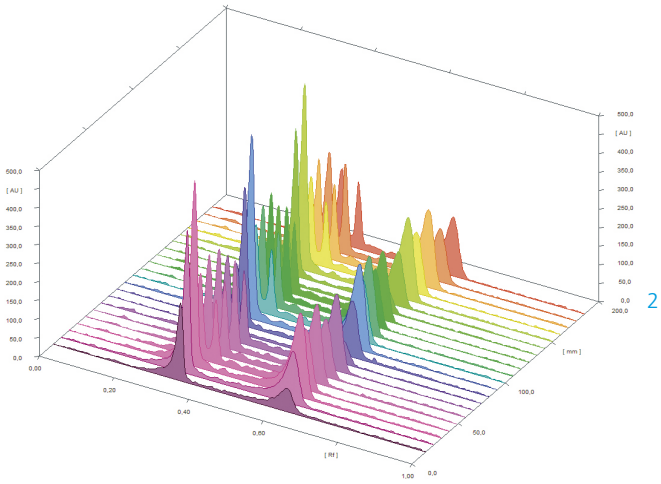
Results and discussion

Using an AMD 2 gradient separation on HPTLC plates MS-grade, it was possible to separate the α-acids (hR_F 36) and ß-acids (hR_F 65) from the hop matrix, showing the difference in the bitter acid content in the various hops. Although hops contain a high amount of matrix, both types of bitter acids were well separated and quantified by fluorescence measurement at 360/>400 nm.



1

AMD 2 chromatogram at UV 366 nm of ICE 3 (three different volumes) and the 4 aromatic hops (no. 1–4, threefold applied n = 3)



2

Fluorescence measurement at 360/>400 nm

With this study the differences in the bitter acid content of both regional and varietal hops was determined. In most cases, the bitter hops contained considerably more bitter acids than aromatic hops. The content of bitter acids differed for different regional origins of the same varietal hop.

Determined content of bitter acids in 12 hops

No.	Hops	Content [%] of α-acids	Content [%] of ß-acids
	Aromatic hops		
1	Mittelfrüh Hallertau	2.7	3.0
2	Spalt Spalter	3.8	5.4
3	Saazer	3.2	2.8
4	Tettnanger	3.1	3.6
	Bitter hops		
5	Apollo	13.5	5.8
6	Green Bullet	9.1	6.0
7	Hallertau Herkules	4.0	3.3
8	Topaz	16.2	7.2
	Regional Hops		
9	Cascade NZ	4.0	3.9
10	Cascade USA	8.1	8.0
11	Cascade Hallertau D	6.5	6.1
12	Cascade Lemondrop USA	4.1	4.2

[1] Adrian Forster *et al.* (2012). Hopfen. Vom Anbau bis zum Bier, Carl, Nürnberg

[2] B. Engelhard, A. Lutz, E. Seigner (2011). Hopfen für alle Biere der Welt, www.lfl.bayern.de

Further information is available on request from the authors.

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Quantification of tetrahydrocannabinol in *Cannabis sativa*

Dr. Melanie Broszat and Eliezer Ceniviva, CAMAG

Introduction

Cannabis sativa, the hemp plant, is one of the oldest crops of mankind and an important resource for the production of textile fibers, food products and medical drugs. Cannabinoids are the substances of medical interest in Cannabis, such as the analgesic cannabidiol (CBD). The cannabinoid tetrahydrocannabinol (THC), however, has an intoxicating effect, for which Cannabis is used as a narcotic drug. In the USA and Switzerland, industrial hemp may not contain more than 1% of THC. In Europe the limit is set to 0.2%. Based on the different intended usage, numerous genetic strains of *Cannabis sativa* have been developed, in which the content of cannabinoids varies greatly. This leads to different analytical tasks [1,2]. CBD, THC and cannabinol (CBN) are the three best studied cannabinoids and have been chosen for the System Suitability Test (SST). CBD and THC contents are used for classification in three main types: THC-rich (type 1), THC content similar to CBD (type 2), CBD-rich (type 3) [2]. CBN is produced during aging and is therefore regarded as a quality feature.

HPTLC is a fast and simple solution for the analysis of Cannabis, especially in regard to different analytical goals. In a limit test numerous samples can be assessed in parallel as to their classification as narcotic drug. Detection and a precise assay of individual cannabinoids is also possible.

Standard solutions

Standards individually in methanol (10 and 100 ng/µL), for the SST a mixture of CBD, THC, and CBN in methanol (each 100 ng/µL).

Sample preparation

500 mg of dried, powdered *Cannabis sativa* were mixed with 5 mL of methanol – *n*-hexane 9:1 and sonicated for 15 min. The mixture was centrifuged for 5 min and the supernatant was used for analysis. For the quantitative assay the extracts were diluted 1:10 with methanol – *n*-hexane.

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 conditioning of the plate at 33% relative humidity for 10 min (using a saturated solution of magnesium chloride), development with *n*-heptane – diethyl ether – formic acid 75:25:0.3, migration distance 70 mm from lower plate edge, drying for 5 min

Densitometry

TLC Scanner 4 with *visionCATS*, absorption measurement at 210 nm prior to derivatization (for cannabinoid acids 285 nm [2])

Postchromatographic derivatization

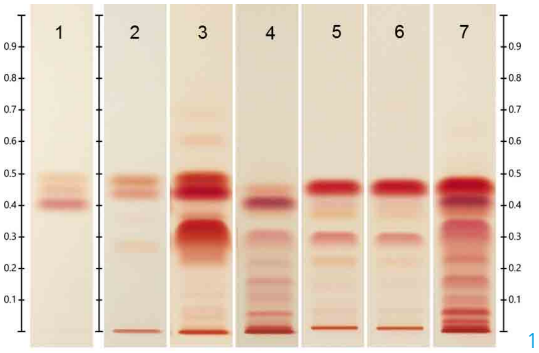
Spraying with Derivatizer (green nozzle, level 3) with Fast Blue salt B reagent (250 mg of *o*-dianisidine bis(diazotized) zinc double salt dissolved in 10 mL of water, 25 mL of methanol and 15 mL of dichloromethane added)

Documentation

With TLC Visualizer under white light after derivatization

Results and discussion

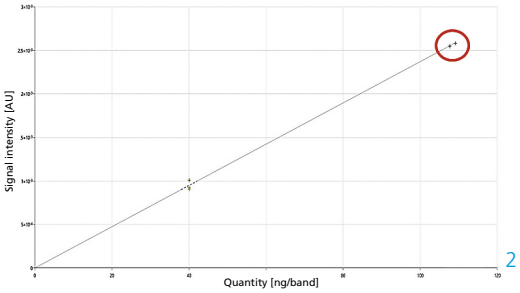
HPTLC is the method of choice for the prompt analysis of numerous Cannabis samples. The SST and 2 µL of each undiluted sample extract are applied. The identification is based on the HPTLC chromatogram. After derivatization with Fast Blue salt B reagent the cannabinoids are detected as colored zones.



1

HPTLC-Chromatogramm nach Derivatisierung mit Echtblausalz HPTLC chromatogram after derivatization with Fast Blue salt B reagent under white light, track 1: SST (CBN, THC and CBD, with increasing hR_F value), tracks 2–7: different Cannabis samples

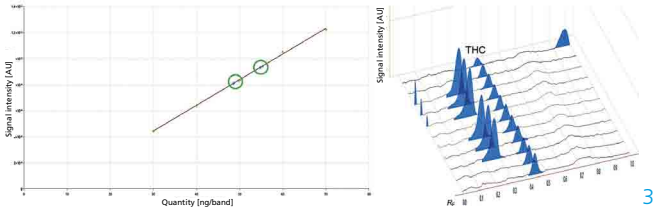
For the screening of THC-free samples the limit test can be used. The sample extracts (diluted 1:10) and as standard solution, the limit amount of THC is applied at least in duplicate. The limit of 0.2% required by the EU is easily detected with or without derivatization. The standard deviation of the assay prior to derivatization is in this example 1.5% and after derivatization with the Derivatizer only 2.1%.



2

Limit test of a sample (duplicate, red circle) which exceeds the EU limit (≥ 0.2% THC), absorption measurement at 210 nm, evaluation via peak area with linear regression (linear-1); single level calibration

For a highly precise assay we recommend multilevel calibration. Also in this case good quantitative results prior to and after derivatization with the Derivatizer can be achieved. Prior to derivatization the standard deviation was 1.1% and after derivatization 2.8%. As the limit of detection was 10 ng/zone for both modes, the derivatization step is only advantageous for image evaluation. The following example shows the quantification of highly potent THC-containing samples with no or hardly any CBD. The extracts were applied 1:10 diluted to evaluate the samples in the linear working range.



3

Quantification of THC in 2 Cannabis samples (triplicates, green circles) by a 5-level calibration (left), evaluation via peak area with linear regression (linear-2); densitograms (right) after absorption measurement at 210 nm

The described method is suitable for the qualitative and quantitative determination of cannabinoids in *Cannabis sativa*. Additionally, the easy, reproducible and cost efficient analysis of intermediate and finished products in the food and drug industry is possible [2]. Depending on the analytical goal, an optimization of the mobile phase or separation on RP-18 phase might be necessary for non baseline separated cannabinoids [2]. For the unequivocal detection of cannabinoid zones mass spectrometry can be used [1,2].

[1] CAMAG Application Note A-98.1: Confirming the presence of cannabinoids in *Cannabis sativa* by HPTLC-MS, www.camag.com

[2] CAMAG Application Note A-108.1: Identification and quantification of different cannabinoids in *Cannabis sativa,* www.camag.com

Further information is available on request from the authors.

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CAMAG
Automatic TLC Sampler (ATS 4)

Fully automatic sample application for all kinds of modern Thin-Layer Chromatography

- **Quantitative analysis**
- **Qualitative analysis, screening, high throughput analysis**
- **Preparative separations**

The cover

is designed to protect the object from environmental influences during sample application.

Spray nozzle

The ATS 4 can be equipped with a normal nozzle or a heated spray nozzle. Heating assists the application of larger sample volumes, particularly with solvents of low volatility.

Exchangeable sample rack

The standard rack has 66 positions for standard 2 mL vials (12 × 32 mm) sealed with normal rubber septa.

The self-adjusting object support

enables sample application onto objects of various thickness (up to 4 mm) without any adjustment to the spray nozzle.

1

Automatic sample application is a key factor for high precision and productivity in routine analysis. With the ATS 4 the samples are either applied as spots through contact transfer (0.1–5 µL) or as bands using the spray-on technique (between 0.5 and > 50 µL). Starting zones in the form of narrow bands offer the best resolution attainable with a given chromatographic system.

Large sample volumes can be sprayed-on in the form of rectangles which are focused to narrow bands prior to chromatography.

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