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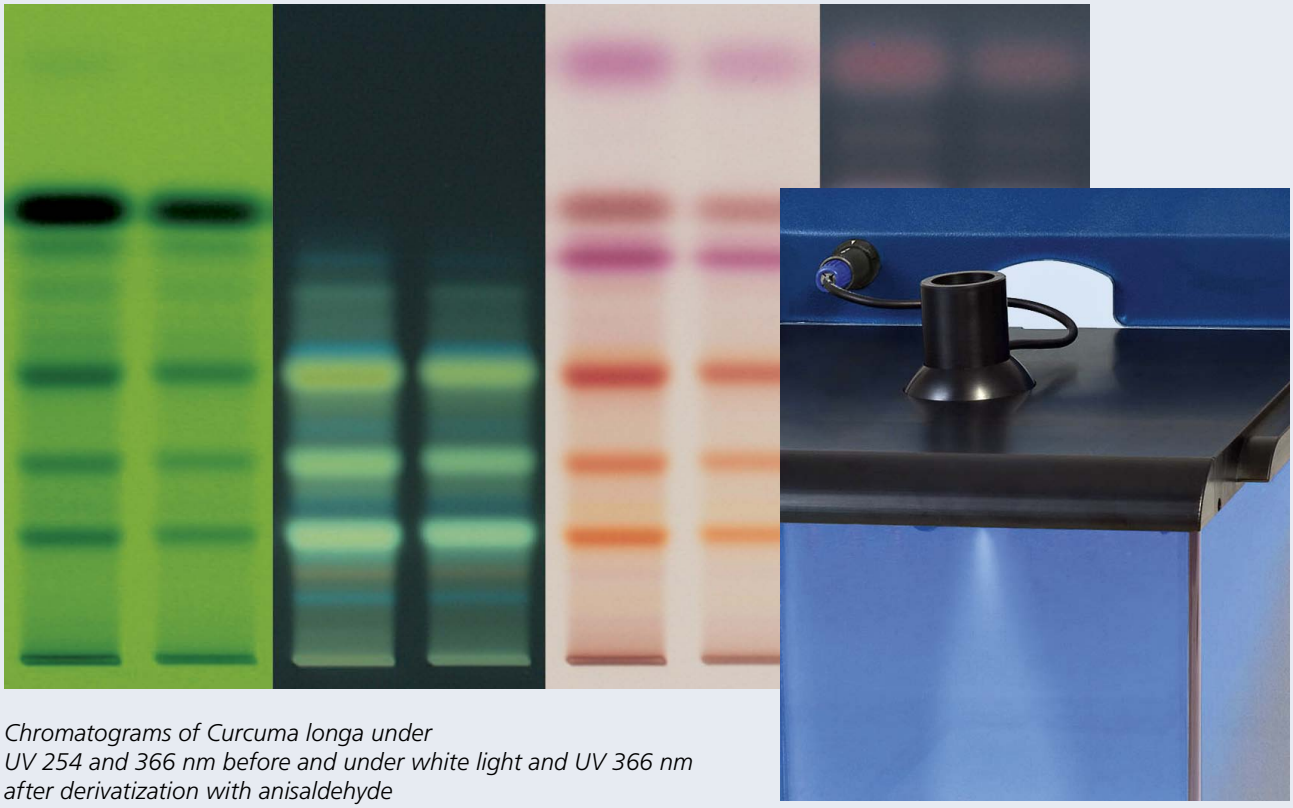
SEPTEMBER 2016



Chromatograms of Curcuma longa under UV 254 and 366 nm before and under white light and UV 366 nm after derivatization with anisaldehyde

Uncomplicated postchromatographic derivatization, an option unique to Planar Chromatography

CAMAG®

WORLD LEADER IN PLANAR CHROMATOGRAPHY

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No. 117, September 2016
CAMAG Bibliography Service
Planar Chromatography
Edited by Gertrud Morlock
cbs@camag.com
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IN THIS ISSUE

Procedures, applications

Products featured in this issue

Column: Know CAMAG

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Printed on FSC paper

CAMAG Derivatizer – New spraying device for the automated derivatization of TLC plates

1 *CAMAG Derivatizer for 20 x 20 cm plates*

With the **Derivatizer** CAMAG presents an automated spraying device which sets a new standard of reproducibility in the reagent transfer onto TLC plates by employing a unique "micro droplet" spraying technology (patent pending). The CAMAG Derivatizer ensures homogeneous and reproducible application of all common reagents. To meet the diverging physicochemical properties of the different reagents, *e.g.* acidity or viscosity, four different color-coded spray nozzles are employed, and the user can select from six spraying modes.

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

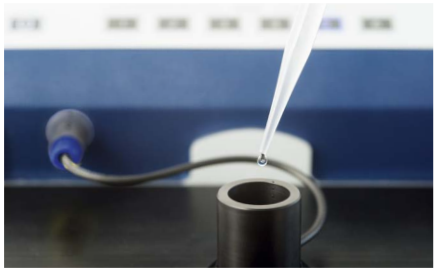
- Environmentally friendly and safe handling through a closed system
- Intuitive handling and easy cleaning
- Low reagent consumption through efficient operation (4 mL for 20 x 20 cm plates and 2 mL for 20 x 10 cm plates)
- Reproducible and user-independent results

How it works

- The transparent hood lifts automatically after pushing a button. The plate tray is removed, the plate arranged and the tray reinserted.

2

- By lowering the hood it completely seals to prevent aerosols/spraying reagent from leaking to the outside.
- The reagent is filled into the appropriate nozzle.



3

- The spraying program is selected and the spraying process started. The nozzle generates an extremely fine reagent mist, which evenly distributes in the chamber and gradually condenses on the TLC plate.

4

- Residues remaining in the gas phase are automatically aspirated and collected in the wash bottle.
- The spraying process is completed. At the push of a button the hood lifts and the TLC plate can be removed.

Derivatization means another step in the process, consequently causing an increase in the variance. If an experienced technician manually sprays, the relative standard deviation of the measured values significantly rises up to 12.0%. In contrast with the CAMAG Derivatizer, the standard deviation slightly increases between 2.5 and 4.5%. Moreover, these results are user-independent and reproducible!

The following most common reagents have been tested and validated by the CAMAG laboratory for use with the CAMAG Derivatizer:

- Sulfuric acid reagent (10% in methanol)
- Anisaldehyde reagent
- Natural product reagent
- Polyethylene glycol solution
- Iodine solution (0.5% in ethanol)
- Dragendorff reagent
- Fast blue salt B reagent
- Ehrlich's reagent
- Phosphomolybdic acid reagent
- Ninhydrin reagent
- Copper (II) sulfate reagent
- Aniline-diphenylamine-phosphoric acid reagent
- Vanillin reagent
- Potassium hydroxide solution (5% in methanol)
- Aqueous solutions (enzymatic solutions, *etc.*)

The CAMAG Derivatizer will be available in October 2016 for two different formats (20 x 20 and 20 x 10 cm). Visit **www.camag.com/derivatizer** for more information.

Quality control of pigment formulations

In cooperation with the company Siegwerk one of the leading international manufacturers of printing inks for packaging and other kinds of printing material, this method was developed at the Chair of Food Science, Justus Liebig University Giessen [1].

Introduction

Printing ink formulations are complex mixtures, which consist of pigments and/or colorants, solvents, resins, and additives such as UV absorbers or plasticizers. Chromatographic methods have not been considered for substances of low solubility. However, HPTLC seems to be a promising analytical tool, due to the single use of the plate. Pigment components of poor solubility stay at the starting zone without disturbing the separated zones. Also sample preparation can be kept minimal because of the one-time use of the plate.

Sample preparation

Depending on the respective pigment formulation 5, 10 or 30 mg of each sample were dissolved in 1.0 mL tetrahydrofuran, methanol, dimethylformamide or a mixture of these solvents [1], sonicated (15 min), and centrifuged (5 min, 10000 g).

Sample application and layer

Bandwise with Automatic TLC Sampler (ATS 4) HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm, band length 6 mm, track distance 9.5 mm, distance from lower edge 9 mm, application distance from left edge 14 mm, application volume 1–10 µL.

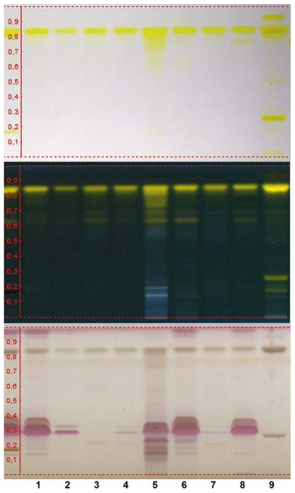
Chromatography

In the AMD 2 system using a 9-step gradient with ethyl acetate, methanol, water, and toluene with alkaline conditioning (1 N aqueous ammonia solution), AMD time 80 min, migration distance 56 mm.

Derivatization and documentation

The plate was immersed into a 10% aqueous sulfuric acid solution using the Chromatogram Immersion Device (immersion time 0 s, immersion speed 3 cm/s) and heated at 110 °C for 5 min using the TLC Plate Heater, documentation with the TLC Visualizer under UV 254 nm, 366 nm, and white light.

Note: This derivatization is now also possible with the Derivatizer.



AMD 2 chromatograms of 9 different batches of pigment yellow 12 under white light, UV 366 nm, and white light after derivatization; tracks 1 and 2 same supplier; tracks 3–9 different suppliers

Results and discussion

The developed generic method allowed the industrial quality control of pigment formulations (p. 16). There were not only differences between pigment formulations of different manufacturers but also between different batches of the same supplier. A total of 124 samples (18 different pigment formulations and up to 20 batches of the same pigment formulation) were investigated. It was possible to compare 18 pigment samples against a benchmark during one run. The analysis time for one sample was less than 5 min and the solvent consumption was below 10 mL. Due to the use of multi-detection, differences between pigment batches were detectable. Post-chromatographic derivatization with sulfuric acid reagent gave further information and showed particular differences regarding the binder and coating materials in the samples.

[1] C. Stiefel, S. Dietzel, M. Endress, G. Morlock, J. Chromatogr. A 1462 (2016) 134–145

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Dextrin profiles of starch digested with different amylases

Grégory Baeyens

As a key player in the global yeast production and experts in fermentation processes, Lesaffre designs, manufactures and markets innovative solutions for baking, food taste, health care and biotechnology.

The enzymology group in R&D focuses on enzymes used in baking. Such enzymes can be used to optimize the quality of bread and baking products with regard to volume, texture, shelf-life, color and appearance of crumb and crust. One of the main missions is to identify and characterize different enzymes for designing a bread improver with a balanced combination of baking ingredients that will result in better baking products.

Introduction

Amylases are starch degrading enzymes particularly used for baking. Within the last two years, several new amylases have been commercialized. Despite our deep knowledge of enzyme characterization, we face difficulties in differentiating them by traditional in vitro enzymatic assays (measurement of products formed over time with selected substrates, pH and temperature conditions). Indeed, the activity values of these new amylases were close to each other.

The idea was to assay from the starch substrate the various amylase-produced dextrins ranging from glucose to maltoheptose. The dextrin profile supported understanding of enzyme specificity and effects during baking. HPTLC was a valuable tool to follow several products in parallel. In this specific case, HPTLC did not show any matrix effects and was five times faster compared to the HPLC run of 35 min per sample.

Chromatogram layer

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

Standard solution

The methanolic stock solution mixture (1 mg/mL each) of glucose (DP1), maltose (DP2), maltotriose (DP3), maltotetrose (DP4), maltopentose (DP5), maltohexose (DP6) and maltoheptose (DP7) was diluted 1:20 with methanol.

Sample preparation

The aqueous enzyme solution (1%) was stirred for 20 min, starch solution (4%) was added 1:1 and incubated at 25 °C for 40 min. Heating at 100 °C for 5 min stopped the reaction; if necessary, dilution in methanol.

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), band length 6.0 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volumes between 0.3 and 4.0 µL

Chromatography

In the Automatic Development Chamber (ADC 2) with chamber saturation (with filter paper) and after conditioning for 10 min at 47 % relative humidity using a saturated solution of potassium thiocyanate, development with acetonitrile – acetone – water 3:3:2 up to a migration distance of 60 mm (from the lower edge), drying for 5 min

Postchromatographic derivatization

The plate was immersed into aniline-diphenylamine-phosphoric acid reagent (2 g diphenylamine and 2 mL aniline in 80 mL methanol, 10 mL phosphoric acid, 85%, ad 100 mL methanol) using the Chromatogram Immersion Device (immersion time 1 s and immersion speed 5 cm/s) and heated at 120 °C for 5 min using the TLC Plate Heater

Documentation

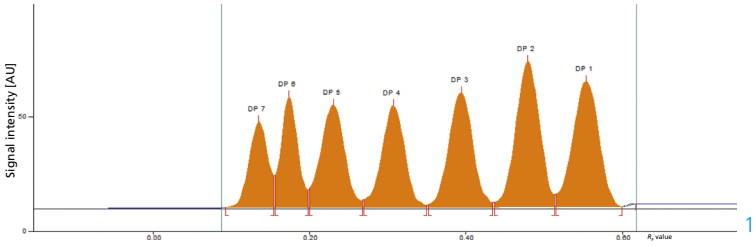
With TLC Visualizer under white light illumination (transmission)

Densitometry

TLC Scanner 4 and winCATS, absorption measurement at 500 nm, slit dimension 4.00 x 0.30 mm, scanning speed 20 mm/s, evaluation via peak area

Results and discussion

The good resolution in the densitogram of the standard mixture DP1 to DP7 allowed a quantification of the formed dextrin products (DPs). The evaluation was done in the polynomial working range (40.0–210.0 ng/band).



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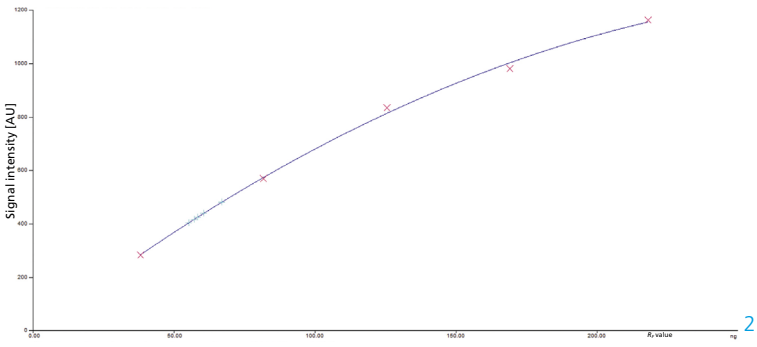
Densitogram of the standard mixture DP1-DP7

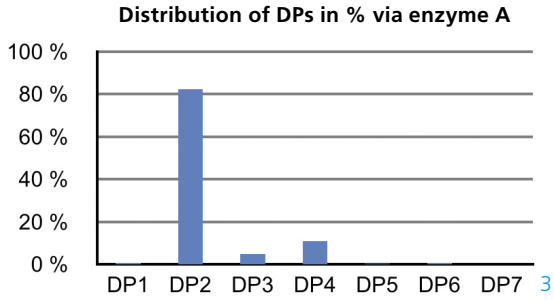


2

Calibration curve of DP2 as example

After the enzymatic reaction, the product profiles of four different enzyme broths were analyzed. The concentration and percentage of each DP within the product profiles were calculated.

Interestingly, very different DP patterns were observed for the enzymes. For example, with enzyme A mainly DP2 was formed, whereas DP4 was the most abundant product for enzyme B. With enzyme C the major product obtained was DP1. Enzyme D was the best enzyme regarding the distribution among the various DPs with regard to DP1, DP2, DP4 and DP6.



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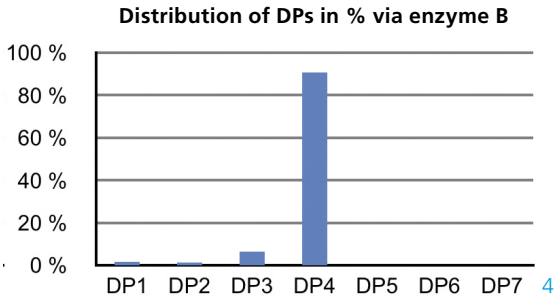

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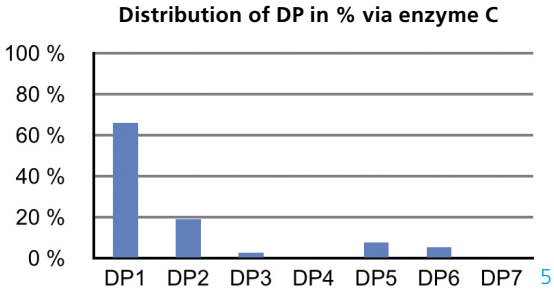

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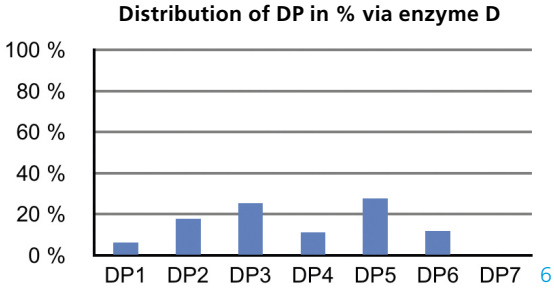

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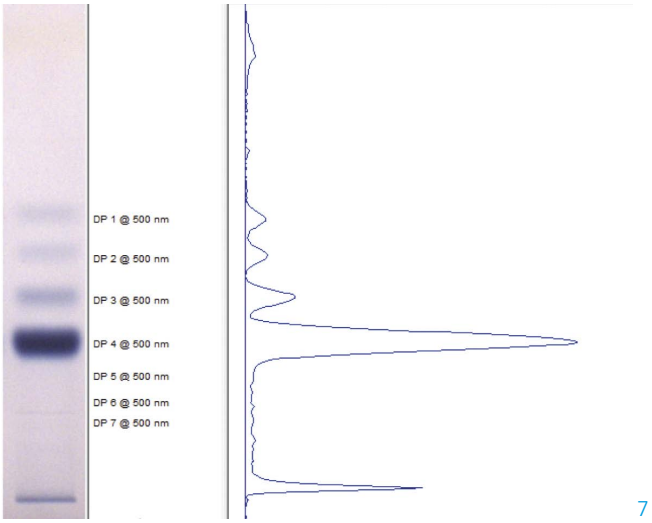

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Chromatogram and densitogram at UV 500 nm of a starch sample digested with enzyme B

A fast and robust method for the quantification of DPs was developed. The method was well suited for the characterization of different enzymes by their formed product profiles. By comparing patterns, enzymes can be differentiated and selected due to their properties. A better understanding of enzyme substrate/product specificity was reached.

Further information is available on request from the authors.

Contacts: Grégory Baeyens and Marie-Pierre Lejeune, Lesaffre International, 147 rue Gabriel Péri, 59700 Marcq-en-Baroeul, France, bsg@lesaffre.fr or mpl@lesaffre.fr

8

New: CAMAG TLC Visualizer 2

The professional documentation and evaluation system for Planar Chromatography

The TLC 2 Visualizer is equipped with technical innovations and offers even 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
- Simple operation under *visionCATS* (from version 2.3)

The TLC Visualizer 2 is further 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. The software controlled operation offers a broad 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 »Image Comparison«.

The CAMAG TLC Visualizer 2 will be available from October 2016.

Order processing at CAMAG

Our highest priority is the satisfaction of our customers. This constitutes one of the central pillars of CAMAG's longstanding business success.

As a globally exporting company we often face changing conditions and increased demands. This requires a high level of experience, expertise, flexibility and organizational competence, which particularly applies to our coworkers in sales administration and logistics, whom we would like to introduce.

From left:
Josef Krummenacher,
Carmen Ruf,
Daniel Bütikofer,
Mario Aren,
sitting in front:
Tobias Hohler

Sales administration

Carmen Ruf has been working at CAMAG since 2000. She is responsible for the smooth running of order processing.

Tobias Hohler started in 2010 and mainly handles quotations. He also supports his colleagues in administrative matters.

Logistics

Josef Krummenacher started 31 years ago and is responsible for the Logistics division. Through his extensive experience, he always finds practical solutions to safely deliver our equipment to the most remote places on earth. **Daniel Bütikofer** started at CAMAG in 2003. Together with **Mario Aren**, who has been part of the CAMAG staff since 2001, Daniel Bütikofer is responsible for storing the wide variety of incoming goods and directing them to the Production Department. The difficult task of preparing shipments to customers worldwide is their daily business, which includes time tested, precision packing to ensure safe delivery.

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

130 abstracts have been added to the CCBS collection by this issue. They include reviews on the analysis of inks in forensic applications and food dyes as well as on multivariate analysis of TLC/HPTLC data. The latter review accounts for an increasing number of papers and thus abstracts that deal with videodensitometry, image evaluation and chemovariate analysis of TLC/HPTLC data. In other reviews on biogenic amines in foodstuffs and on aflatoxin M1 and its fate during processing of milk and dairy products, TLC/HPTLC is one preferred method. A good source of information is also the biennial review on planar chromatography papers, which was recently published for the last two years.

According to IUPAC, the term chromatography stands for "a physical method of separation in which the components to be separated are distributed between two phases". Hence, we can only write TLC or HPTLC (the last C stands for chromatography) in a title or paper if a development step took place. If the samples were just applied and detected (often an effect-directed detection was used on the spot, *e. g.* using DPPH radical reagent or a bioassay), it may be called effect-directed detection or analysis, but not TLC/HPTLC, and in case of bioassays, not bioautography. Please be aware using the correct term, as this is the basis of our scientific communication.

Dear friends

It is a great pleasure to present in this issue a new concept of reagent transfer for postchromatographic derivatization of planar chromatograms. The user can monitor the formation of the extremely fine reagent aerosol and its precipitation onto the chromatogram plate. Consumption of reagent solution is extremely low and the whole process happens in a closed system, both contributing to environmental protection.

In the focus of this CBS issue are applications in the field of food quality control, food safety and authentication of supplements. The quality control of amylases used in baking industry, analysis of certain fish species with regard to their wax ester content, and analysis of rye flour as to traces of ergot alkaloids are such subjects. A generic AMD 2 method is reported for quality control of pigment formulations used in printing inks for food packages. A screening method for St. John's Wort products regarding adulteration is presented as well.

Please observe the announcement of the next International Symposium for HPTLC, held in Berlin 5th–8th July, 2017. Starting from 1980 in Bad Dürkheim, the International Symposia on Planar Chromatography count 23 events, taking place on average every second year. Details for the next one are on the last yellow page of this CBS issue. You are cordially invited to contribute with a poster or lecture by submitting an abstract at www.hptlc.com. Be sure, lively discussions in the HPTLC community will inspire your research.

Kind regards

G. Morlock

Gertrud Morlock
cbs@camag.com

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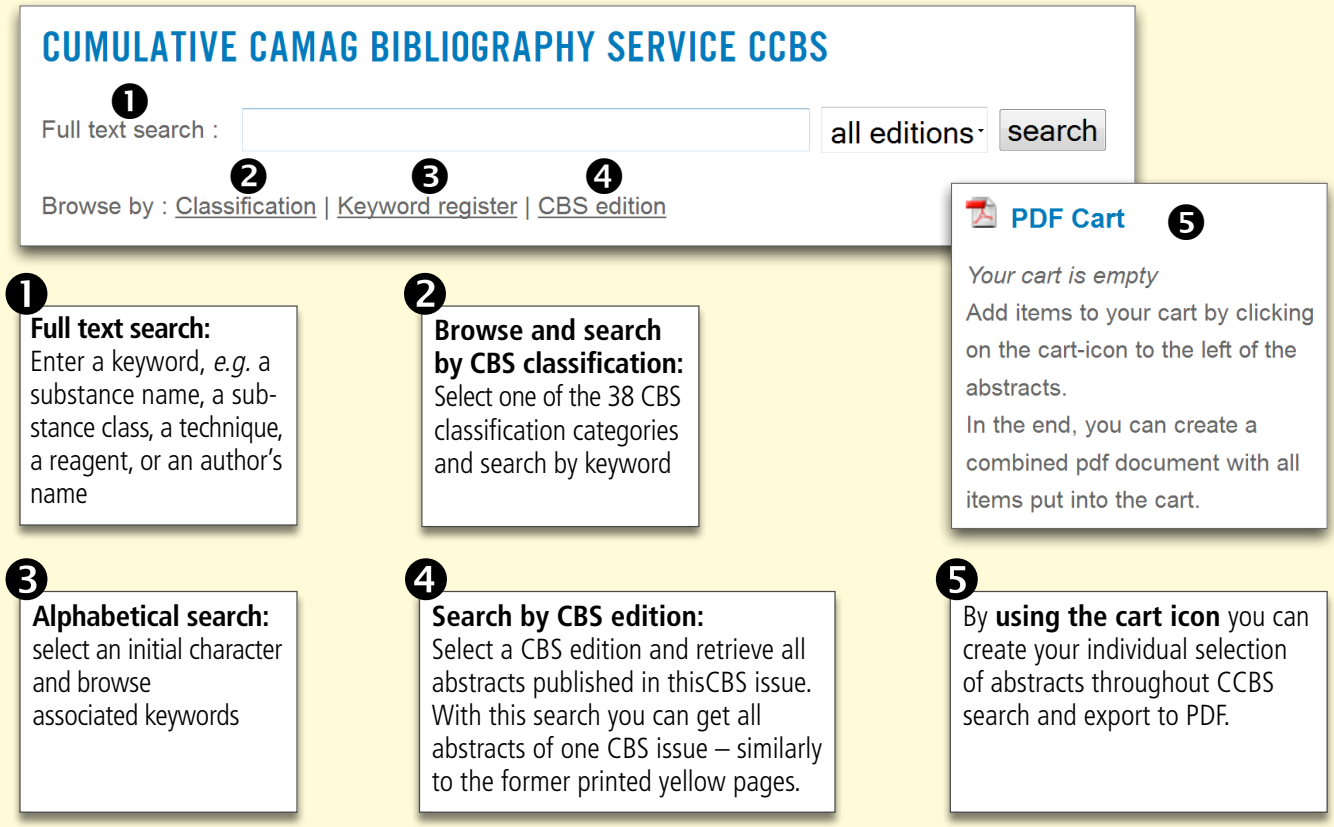
THE CBS CLASSIFICATION SYSTEM

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**

Cumulative CAMAG Bibliography Service (CCBS) Online Search



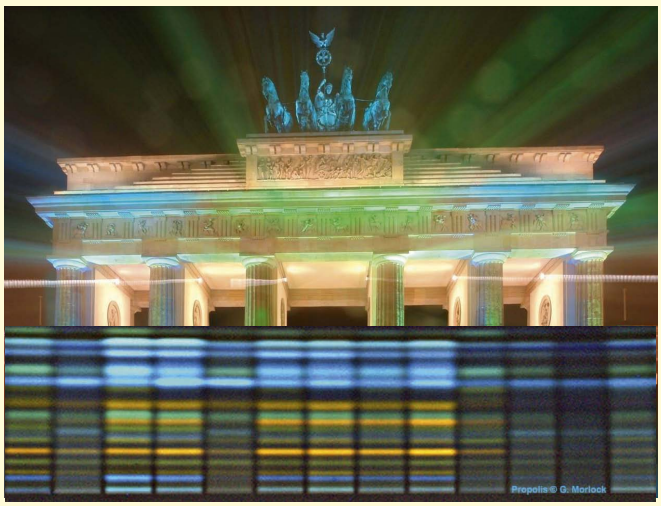
With the Cumulative CAMAG Bibliography Service (CCBS) Online Search, you can directly search for information within the CAMAG website. The CCBS covers more than 11'000 abstracts of TLC/HPTLC publications between 1982 and today. Providing reports was established by Dr. Dieter Jänchen in 1965 and the workflow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



International Symposium for High Performance Thin-Layer Chromatography Berlin, 5–8 July 2017

Call for abstracts

The Scientific Committee of the HPTLC 2017 welcomes you to the beautiful city of Berlin. It is not too late to participate as a speaker or poster presenter at the symposium (deadline for abstract submission March 1st, 2017). 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. The scientific program will be diverse with 11 sessions, 4 tutorials, a panel discussion, young scientist and poster awards as well as an active social program. We look forward to seeing you in Berlin and learning from your latest experiences with HPTLC and your ideas for the advancement of HPTLC. Do check out our website for the latest information on the scientific program and to obtain a discount for early bird registration until March 31st.

Deadlines

Abstract submission (oral and poster): March 1st 2017
Final registration: May 31th 2017

Confirmed invited speakers

Prof. Dr. Rob Verpoorte, The Netherlands: How to write a world-class scientific paper in HPTLC?

Dr. Heinrich Luftmann, Germany: How to assign the unknown compounds via HPTLC–MS?

Prof. Dr. Gertrud Morlock, Germany and Prof. Dr. Colin Poole, USA: Reduced sample preparation without pitfalls in HPTLC?

Prof. Dr. Snezana Agatonovic-Kustrin, Australia: Evaluation of polyphenolic fingerprints and antioxidant profiles of Victorian marine algae with HPTLC and multivariate analysis

Dr. Wanchai De-Eknamkul, Thailand: HPTLC analysis of estrogenic-like constituents in *Pueraria mirifica* root

Prof. Dr. Matthew Lindford, USA: Microfabricated TLC plates based on carbon templates

Dr. Agnes Moricz, Hungary: Layer chromatography hyphenations assisted screening, characterization and isolation of bioactive plant components

Prof. Dr. Susan Olesik, USA: Latest developments of electrospun layers

Prof. Dr. Jentaie Shiea, Taiwan: Interfacing flame-atmospheric pressure chemical ionization mass spectrometry with HPTLC for the characterization of mixed lipids

Prof. Dr. Wolfgang Schwack, Germany: Determination of total glucosinolates in *Brassica* crops

Prof. Dr. Colin Poole, USA: What every chromatographer should know about solvents

Adulteration of St. John's Wort Products

Débora A. Frommenwiler, CAMAG

Introduction

Hypericum perforatum L., known as St. John's Wort, is the principal active ingredient of Herbal Medicinal Products commonly used for treating depression. In 2012 products made with powdered St. John's Wort plant material and/or extracts were in the top ten biggest selling botanical dietary supplement (DS) in the USA's mass market channels. Due to their popularity there is potential for the profit motive being the driving force in the adulteration of St. John's Wort products.

Any analytical method used for quality control under cGMP should not only be specific enough to confidently pass or fail samples during identification, but also be able to identify presence of a different species or any other adulterants. Below an easy HPTLC method to detect such adulteration is presented [1].

Sample preparation

St. John's Wort extract (0.5 g) or powdered St. John's Wort drug (1.0 g) were suspended in 10 mL methanol and sonicated for 10 min at 60 °C. The suspensions were centrifuged for 5 min and the supernatants used for analysis.

Standard solutions

Stock solutions were prepared individually in methanol at a concentration of 0.5 mg/mL.

Chromatography layer

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

(B) HPTLC plates silica gel 60 RP-18 W (Merck), 20 × 10 cm

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8.0 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volume 2.0 µL

Chromatography

In the Automatic Developing Chamber (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, development with ethyl acetate – dichloromethane – formic acid – acetic acid – water 100:25:10:10:11 (A) and methanol – 5% aqueous sodium sulfate 3:4 (B) to the migration distance of 70 mm (from the lower edge), drying for 5 min

Postchromatographic derivatization

The plate (A) was heated at 100 °C for 3 min and while still hot immersed into Natural Products reagent (NP, 1.0 g of 2-aminoethyl diphenylborinate dissolved in 200 mL ethyl acetate), dried and immersed into polyethylene glycol reagent (PEG, 10 g of polyethylene glycol 400 dissolved in 200 mL dichloromethane); for both, immersion speed 3 cm/s, immersion time 0 s

Documentation

With the TLC Visualizer under white light illumination and at UV 366 nm prior to and after derivatization

Densitometry

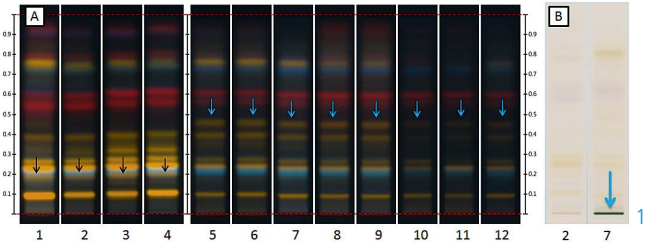
TLC Scanner 4 and visionCATS, absorption measurement at 433 nm (Tartrazine), 524 nm (Amaranth), 480 nm (Sunset yellow) and 632 nm (Brilliant blue), spectra recording between 400 and 800 nm

Mass spectrometry

Elution of zones with TLC-MS Interface 2 (oval elution head, 4.0 x 2.0 mm) at a flow rate of 0.5 mL/min with methanol (with 0.1% ammonium hydroxide) into an ESI-MS (ACQUITY QDa, Waters, USA) and detected in negative ionization mode.

Results and discussion

During a routine investigation of St. John's Wort ingredients and products, the HPTLC Association method for identification of St. John's Wort was used [2]. Some HPTLC fingerprints featured an additional yellow fluorescent zone with variable intensity between the R_F 0.4 and 0.5 at UV 366 nm after derivatization. This zone was absent in the reference samples of extracts and raw materials. At the same time a yellow fluorescent zone, which was present in the reference materials, was absent in those samples. Furthermore the overall intensity of several zones in the test samples was lower than that of corresponding zones of materials with confirmed identity. A closer inspection of the chromatogram under white light illumination prior to derivatization revealed a bluish zone at the application position in the test samples but not in the reference materials.



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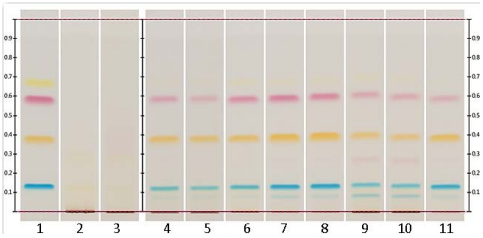
St. John's Wort chromatograms at UV 366 nm after derivatization with NP/PEG reagent (A) and under white light illumination prior to derivatization (B); tracks 1 and 2 dry extracts, tracks 3 and 4 herb reference materials, tracks 5–12 extract with an additional yellow fluorescent zone of variable intensity (blue arrow)

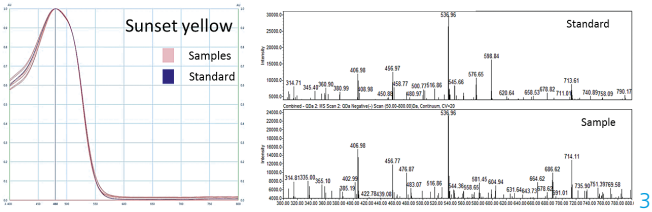
This blue zone suggested the presence of an adulterant, possibly a dye in the samples in question. The hypothesis was tested by employing a method for the analysis of water-soluble food colorants [3] using a wettable reverse phase (RP-18 W). Investigated under white light illumination, the extracts showed zones corresponding in position and color to those of Tartrazine, Amaranth, Sunset yellow and Brilliant blue, in contrast to reference materials.



2

St. John's Wort chromatograms under white light illumination; track 1: Tartrazine, Amaranth, Sunset yellow, Brilliant blue (with decreasing R_F values), tracks 2 and 3: extracts, tracks 4–11: adulterated extracts

With the aim of confirming the identity of the dyes detected in some St. John's Wort samples, UV and MS spectra of the separated zones were recorded and compared to those obtained from standards with matching R_F values. Good correlation was observed. Quantitative evaluation of the amount of individual dyes and their ratio in various samples suggested the presence of a fixed dye mixture.



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Left: comparison of the UV spectra of Sunset yellow with the corresponding zone in an adulterated sample; right: mass spectra of amaranth and the corresponding zone in an adulterated sample (m/z 537 [M-3Na+2H]⁻)

The current HPTLC Association method is suitable for identifying St. John's Wort ingredients and products. Other types of St. John's Wort that show differences in the flavonoid fingerprint can be discriminated. In this study 8 out of 37 samples of St. John's Wort were found adulterated with dyes and contained material with a different fingerprint.

Further information is available from the author.

[1] D. Frommenwiler *et al.*, J. AOAC 99 (2016) 1204–1212

[2] HPTLC identification method for St. John's wort herb (*Hypericum perforatum*), HPTLC Association, www.hptlc-association.org

[3] M. Werther, CBS 88 (2002) 7

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Quantification of wax ester content in escolar

Dr. Andreas Miller

The Bavarian Health and Food Safety Authority (LGL) analyses and assesses food samples in the framework of the official food control. The HPTLC method presented here has been developed in collaboration between Dr. Andreas Miller, LGL, and Prof. Dr. Gertrud Morlock of the Justus Liebig University Giessen.

1

Left: filets of escolar ("Buttermakrele"; Lepidocybium flavobrunneum); right: gunnel ("Butterfisch", Pholidae)

Introduction

The fish species escolar (*Lepidocybium flavobrunneum*) is offered to the consumer as filets for roasting/barbecue as well as ready-to-eat smoked fish filets. Due to the high fish oil content (ca. 18–21 %) escolar exhibits a very juicy and delicious flesh. However, this fish oil consists of indigestive wax esters (> 90 %) which can lead to acute gastro-intestinal symptoms when products of this fish species are consumed.

The wax esters are carboxylic esters consisting of long-chain fatty acids esterified to fatty alcohols. Gastro-intestinal symptoms include cramps and oily diarrhea from the undigested wax esters. Individuals differ in their sensitivity to the consumption of escolar [1]. According to European legislation, food business operators have to inform the consumer about the risk of developing gastro-intestinal symptoms when offering escolar or other fish species belonging to the family *Gempylidae* [2]. Nevertheless, escolar is frequently offered without this mandated information or it is put on the market under a wrong trade name. Especially in sushi restaurants, escolar is offered not under its correct German trade name "Buttermakrele" but as "Butterfisch". However, "Butterfisch" is the German trade name for *Peprilus spp., Poronotus spp.* and *Psenopsis spp.* These fish species do not contain indigestible wax esters and therefore do not require a warning.

A cost-efficient and reliable HPTLC method with uncomplicated sample preparation has been developed for the rapid control of this fish species by determination of the indigestible wax esters [3]. The fish sample is simply homogenized and extracted. Quantification is performed after selective derivatization with the Rhodamine B reagent and subsequent fluorescence measurement.

Sample preparation

Homogenized fish (0.5 g) was mixed with 3 mL *n*-hexane for 2 min using a vortex. After centrifugation the supernatant was collected in a 10 mL volume flask. This extraction process was performed three times and the volume flask containing the three extracts was filled-up with *n*-hexane.

Standard solution

Stearyl stearate and oleyl oleate (100 mg each) were dissolved together in *n*-hexane and filled up ad 10 mL. This stock solution was diluted 1:10 with *n*-hexane (1 µg/µL).

Layer

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

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), band length 6.0 mm, distance from the lower edge 8.0 mm, distance from the left side 13.0 mm, application volume 2.0–15.0 µL for standard solution and 2.0 µL for extracts

Chromatography

In the twin-trough chamber with *n*-hexane – toluene 7:3 after 10 min pre-saturation (using wetted filter paper), migration distance 60 mm (ca. 13 min).

Postchromatographic derivatization

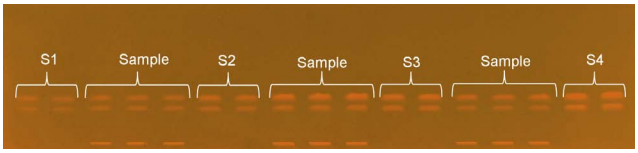
Dipping in aqueous Rhodamine B reagent (0.025 %) using the Chromatogram Immersion Device; detection can be improved by using the 5-fold more sensitive Rhodamine 6G reagent [4].

Documentation and densitometry

With TLC Visualizer under UV 366 nm, densitometry using TLC Scanner 4 and winCATS, fluorescence detection at 366/>400 nm (Hg lamp)

Results and discussion

In preliminary tests gas chromatography (GC) had been used to analyze sample extracts. GC separated the class of wax esters into several individual peaks. However, signal intensity in the flame ionization detector (FID) was influenced by the structure of the individual wax ester: oleyl oleate exhibited ca. 1.8-fold larger peak area compared to stearyl stearate. Therefore, quantification of the wax ester content by GC-FID would only be possible, if individual wax ester peaks in the sample are identified and each wax ester is quantified individually. Using the described HPTLC method oleyl oleate (hR_F 30) and stearyl stearate (hR_F 40) exhibited nearly identical signal intensities. Slightly different chemical structures and chain lengths have little effect on the derivatization with Rhodamine B reagent and consequently on the signal intensities [4]. Therefore, quantification of the wax ester content is possible although analytical standards may not be available for each individual ester. The content of wax esters can be quantified via stearyl stearate or oleyl oleate. After derivatization with Rhodamine B reagent wax esters exhibit an orange fluorescence at UV 366 nm. The mean recovery rate (104 % ± 3 %) was determined by analyzing salmon samples spiked to contain 20% wax ester (3-fold analysis on one day and repetition on another day).



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HPTLC chromatogram under UV 366 nm of wax esters in salmon samples spiked to contain 20 % wax esters (n = 3) and standard solutions (S1–S4) of oleyl oleate (hR_F 30) and stearyl stearate (hR_F 40) after derivatization with Rhodamine B reagent

The three-fold repeated analysis of an escolar sample revealed wax ester contents of 18–22 %. This was in accordance to literature [1]. The repeatability of each analysis was below 5 % and the mean laboratory precision was 3 %. In the framework of official food control this HPTLC method enables the economic analysis of routine samples due to its high sample throughput (parallel analysis of up to 20 samples). This rapid HPTLC method may help to identify the cause of gastro-intestinal symptoms occurring after fish consumption.

Thanks to Dr. Iris Kraemer and Dr. Ingrid Huber (identification of fish species) as well as to Dr. Ulrich Schwank (GC).

[1] The EFSA Journal 92 (2004) 1

[2] Verordnung (EG) Nr. 853/2004

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Further information is available on request from the authors.

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Rapid screening for ergot alkaloids in rye flour by planar solid phase extraction (pSPE)

Dr. Claudia Oellig

In the course of her doctorial thesis at the Institute of Food Chemistry, University of Hohenheim, Stuttgart in the research group of Professor Schwack, Ms Claudia Oellig developed a method for the clean-up of pesticides residues in fruits and vegetables. The method was based on the use of planar solid phase extraction (pSPE). The concept has also been found to be applicable to other areas and a promising and sustainable approach for performing rapid screenings.

Introduction

Ergots (*Secale cornutum*) are the permanent form of the ergot fungus *Claviceps purpurea*. The parasitic fungus grows mainly on cereals, particularly on rye. *Secale cornutum* are responsible for toxicological effects caused by ergot alkaloids commonly produced by *Claviceps purpurea*. About 50 ergot alkaloids from *Secale cornutum* are known; the total alkaloid content varies considerably between 0.01% and 0.5%, depending on the origin. Despite the known toxicity and the infestation of rye grain and rye flours with *Secale cornutum*, there are no maximum limits established for ergot alkaloids in grain and grain-based food [1]. Nevertheless, a maximum level for the total ergot alkaloid content of relevant food categories will be considered soon by the European Union. In this respect the selective and sensitive detection of the alkaloids as the total is a meaningful new approach. Therefore, planar solid phase extraction (pSPE) [2–4] was used in this article for a rapid ergot alkaloid screening, based on HPTLC.

The new ergot alkaloid screening was successfully developed and evaluated for the quantification of the total ergot alkaloid content in rye flour. After extraction with acetonitrile and ammonium acetate buffer as well as liquid-liquid partition in toluene, determination was performed by chromatographic concentration of alkaloids in a single target zone. For detection, the native fluorescence at UV 254/>400 nm offers selective determination and high sensitivity. HPTLC–MS of the target zone allows both a rapid identification of ergot alkaloids present in a sample and the determination of their quantitative ratios [5].

Note: During the whole analysis samples and standards need to be protected against light, because ergot alkaloids are strongly prone to photooxidation.

Sample preparation

Rye flour and finely milled whole rye (6 g) were extracted with acetonitrile (10 mL) and 0.5 M ammonium acetate buffer pH 6.5 (20 mL) on a horizontal shaker for 30 min before being centrifuged. 1 mL of the extract was shaken with 1 mL of toluene, and afterwards 0.3 g sodium chloride was added for phase separation [5].

Standards

For quantitation, an ergocristine standard solution in methanol (0.2 ng/µL) was used. Spiked samples for recovery experiments were prepared by adding finely milled *Secale cornutum* (<0.5 mm) of known alkaloid composition to the rye samples, corresponding to 0.50, 0.75, and 1.50 mg alkaloids/kg rye.

Chromatogram layer

HPTLC silica gel 60 NH_2 plates (Merck), 20 × 10 cm, prewashed with methanol and dried at room temperature inside a fume-hood for 30 min

Sample application

22 rectangular applications (6.0 × 3.0 mm) of gallic acid* (0.05% in methanol, 10 µL) with Automatic TLC Sampler 4 (ATS 4), track distance 8.5 mm, distance from the left side 10.0 mm, distance from

the left side 10.0 mm, distance from lower edge 10.0 mm, drying in the ATS 4 for 10 min, and secondly application volume 20.0 µL for samples and 2.0–35.0 µL for the ergocristine standard.

**Note: Gallic acid is used as antioxidant. After the application of gallic acid, the plate was left inside the ATS 4 (equipped with a light protecting cover) for 10 min to evaporate residual solvent. Afterwards the samples and the standard were applied with the ATS 4.*

Planar solid phase extraction (pSPE)

In the Automatic Developing Chamber (ADC 2) with 10 mL methanol, migration distance 50 mm (migration time 8 min), drying time 3 min. The plate was pre-conditioned for 15 min and the plate activity was equilibrated for 5 min ($MgCl_2$, 33% relative humidity).

Fluorescence enhancement

Dipping the plate in *n*-hexane – paraffin 2:1 with the Chromatogram Immersion Device, immersion speed 3.0 cm/s, immersion time 3.0 s

Documentation

With TLC Visualizer under UV 254 nm and UV 366 nm

Densitometry

With TLC-Scanner 4 in the fluorescence mode at UV 254*/>400 nm (mercury lamp), scanning speed 20 mm/s, slit dimension 4.00 x 0.45 mm. Quantification was performed using peak areas.

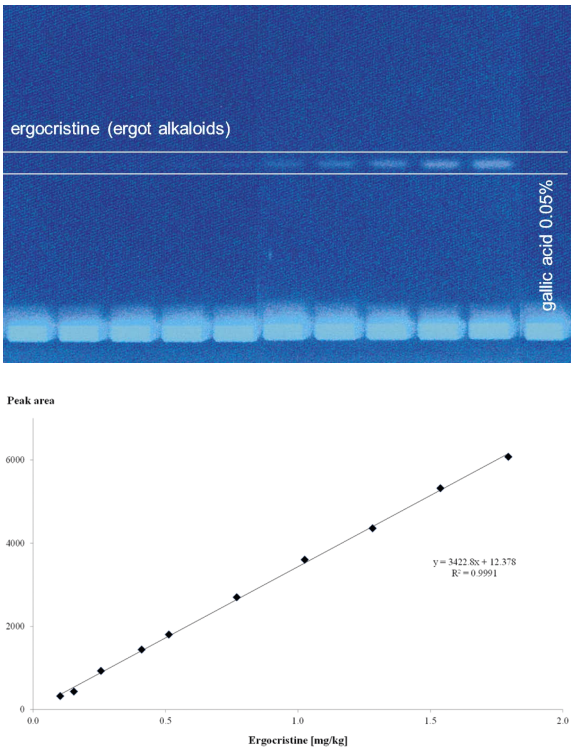
**Note: For the highest fluorescence intensity the optimal excitation wavelength needs to be selected, for this application 254 nm.*

HPTLC–MS

Elution of the target analyte zone with the TLC–MS Interface (with circular elution head) for 60 s with methanol – 0.1% formic 90:10 at a flow rate of 200 µL/min, coupled to a quadrupole mass spectrometer. Measurement was performed in the ESI^+-mode and full-scan data were recorded.

Results and discussion

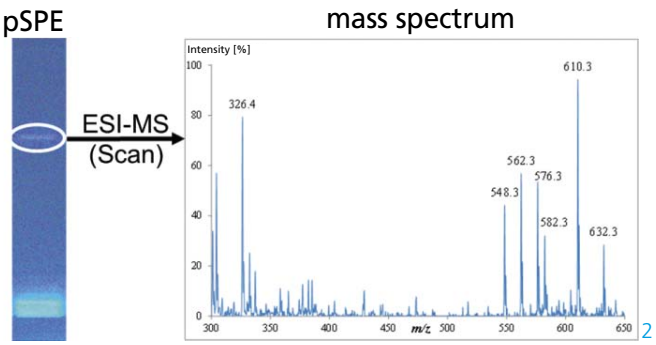
According to the pSPE concept, the determination of ergot alkaloids was performed as the sum of all alkaloids. After a single development with methanol on amino modified silica gel plates, all alkaloids were collected in a target zone. For quantification, the native fluorescence was used at 254/>400 nm after post-chromatographic enhancement. Limits of detection and quantitation (LOD and LOQ) were determined according to DIN 32645 calibration method to be 70.0 and 240.0 µg/kg rye, respectively, expressed as ergocristine.



pSPE-FLD screening of ergocristine under UV 254 nm after pSPE on amino modified silica gel (top), corresponding calibration graph after fluorescence scan at UV 254/>400 nm, 0.4–7.2 ng/zone, displayed as 0.1–1.8 mg/kg rye (bottom).

Method performance of the pSPE-FLD screening was successfully proven by *Secale cornutum* spiked rye flours (containing alkaloids at 0.50, 0.75 und 1.50 mg/kg). Recoveries for ergot alkaloids were determined close to 100% with precisions below 4% (*%RSD*, $n = 5$).

HPTLC–MS additionally enabled an easy characterization of *Secale cornutum* contaminations. The received mass spectrum offered a rapid identification of the ergot alkaloids present in a sample. The relative alkaloid composition was depicted by the quantitative ratio of the mass signals. Thereby a single mass spectrum (fingerprint) allowed the fast and simple characterization of *Secale cornutum* samples.



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Identification of ergot alkaloids in Secale cornutum by HPTLC-MS: pSPE-FLD screening under UV 254 nm (left), and corresponding mass spectrum (right), extracted of the total ion chronogram (ESI$^+$-MS) of the target zone (ergometrine ([M+H]$^+$ 326.4), ergosine ([M+H]$^+$ 548.3), ergocornine ([M+H]$^+$ 562.3), ergocryptine ([M+H]$^+$ 576.3), ergotamine ([M+H]$^+$ 582.3), ergocristine ([M+H]$^+$ 610.3, [M+Na]$^+$ 632.3), ~2.5 mg/kg alkaloids in rye).

The pSPE-FLD screening was found to be an efficient and reliable method for the determination of the total ergot alkaloids in rye, omitting time consuming HPLC analyses of individual alkaloids and calculating the sum. LOD und LOQ were well below the currently applied quality criterion limit for alkaloids in rye of 1 mg/kg. Therefore, the screening was a suitable approach to monitor the exposure to ergot alkaloids. An optional HPTLC–MS measurement enabled the rapid identification of the ergot alkaloids present in a sample by a single mass spectrum, when used as a fingerprint, offering an easy differentiation of *Secale cornutum* of different origins.

[1] Einzelfall-Bewertung von Ergotalkaloid-Gehalten in Roggenmehl und Roggenbroten, Stellungnahme des BfR vom 7. November 2012

[2] Oellig, C., Schwack, W., J. Chromatogr. A 1218 (2011) 6540–6547

[3] Oellig, C., Schwack, W., J. Chromatogr. A 1260 (2012) 42–53

[4] Oellig, C., Schwack, W., J. Chromatogr. A 1351 (2014) 1–11

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3

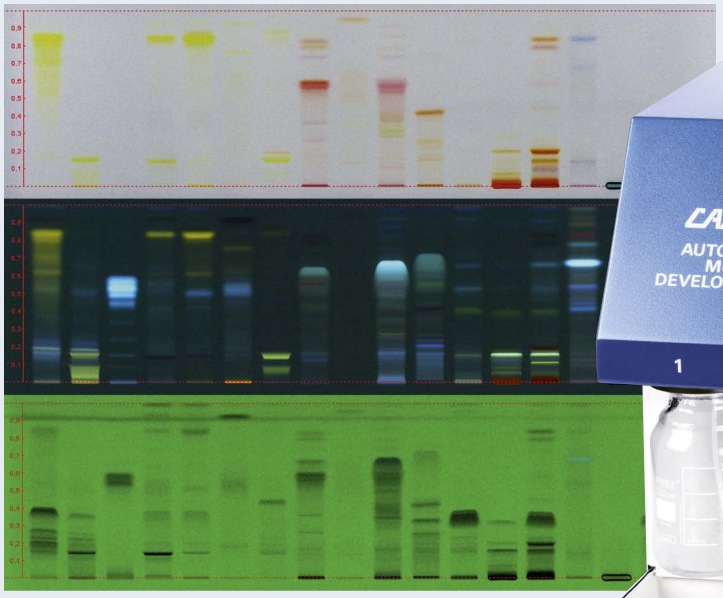
CAMAG TLC Plate Heater

The opportunity to derivatize substances directly on the TLC plate is a major benefit of planar chromatography. Thus, separation zones can be made detectable or the selectivity and/or sensitivity can be increased. Transfer of the required reagent onto the plate can be accomplished by immersion of the plate with the **Chromatogram Immersion Device** or by using a suitable spraying device. To achieve reproducible results by spraying, CAMAG offers the new automated **Derivatizer**, which ensures extremely homogeneous reagent distribution on the TLC plate. (p. 2–3).

Most derivatization reagents require controlled, uniform heating of the plate after the reagent has been applied. For an even reaction of the separated substances over the entire plate, CAMAG offers the **TLC Plate Heater**, which permits visual monitoring of the reaction to determine optimum heating duration and temperature. The temperature is selectable between 25 and 200 °C. The CERAN® heating surface of the device is resistant to all common reagents and can easily be cleaned. The surface of the TLC Plate Heater has a grid for correct plate positioning.

CAMAG AMD 2 System

Automated Multiple Development with AMD 2 – now controlled by *visionCATS*



1

2

AMD 2 chromatogram of 18 organic pigments under white light, UV 366 nm and UV 254 nm; reprinted from [1], Copyright 2016 Elsevier; AMD 2-System
(see application example on p. 4 in this CBS issue)

The separation of complex samples is a challenging task for every chromatographic system, particularly when they span a wide polarity range. The CAMAG AMD procedure offers an excellent solution as it allows stepwise gradient elution over increasing separation distances. As a result acids, bases, neutral, hydrophilic and lipophilic substances can be separated in a single AMD run. This makes AMD suitable for a variety of fields of applications. The method is frequently used in lipid analysis and in routine analysis of drinking water. Pigment formulations with a complex composition, resins as well as additives of mineral oil products are other typical applications of AMD analysis.

The principle

- Multiple development in the same direction with increasing separation distances
- Each successive run uses a solvent of lower elution strength than the previous
- Between runs the layer is dried under vacuum

The result

- Extremely narrow bands due to gradient elution with simultaneous focusing effect
- Enhanced separation capacity with base line separation of up to 40 components within a separation distance of 80 mm

Further informationen: **www.camag.com/amd2**

[1] C. Stiefel, S. Dietzel, M. Endress, G. Morlock, J. Chromatogr. A 1462 (2016) 134–145

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