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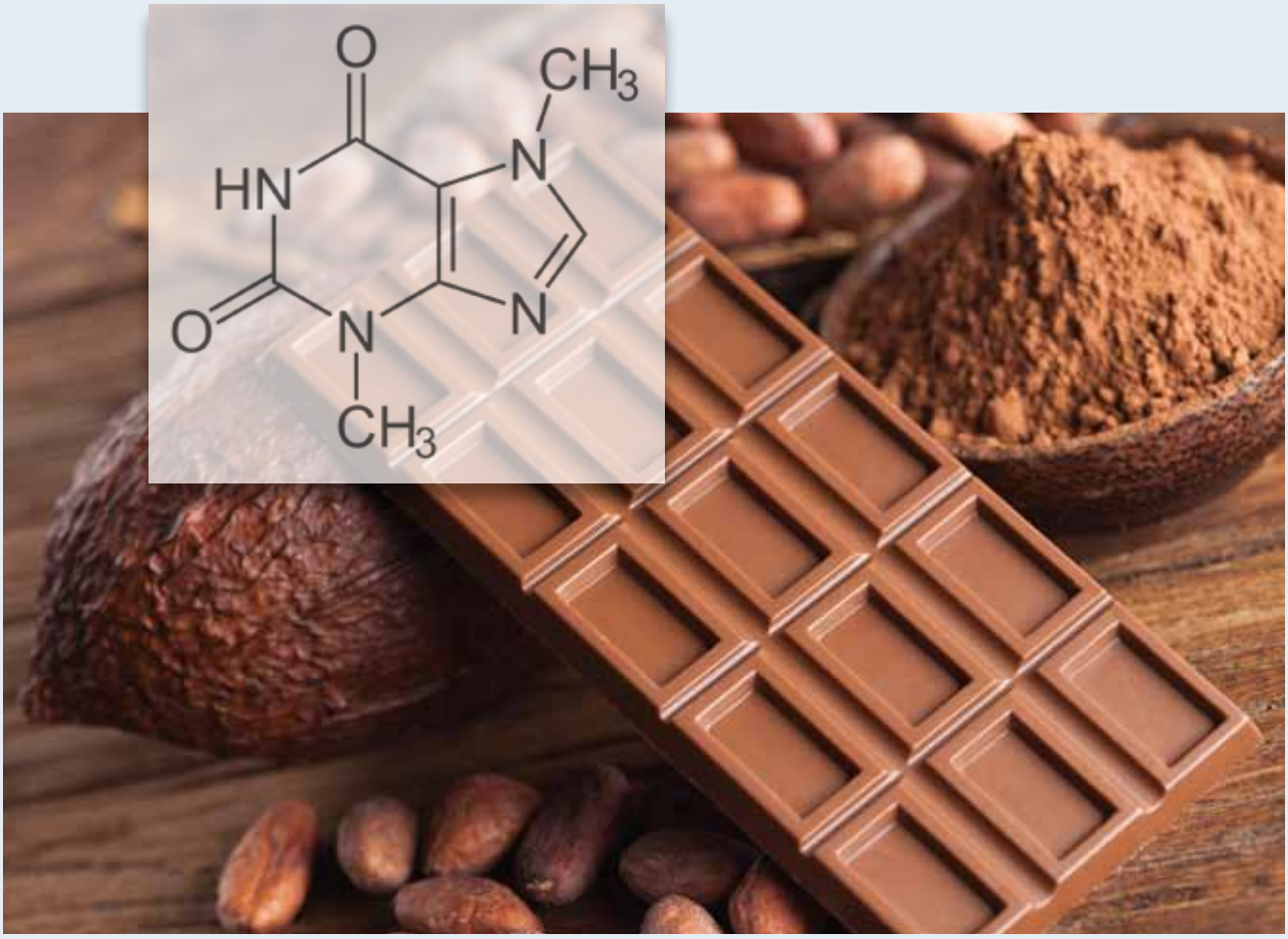

HPTLC quantification of cocoa ingredients and their changes during different chocolate manufacturing steps

Other topics of this issue:

- **AMD separation of biocides**
- **Identification of constituents in broad-leaved dock**
- **Quantitation of 18β-glycyrrhizic acid in licorice root**
- **Degradation profiling of cefixime and azithromycin**

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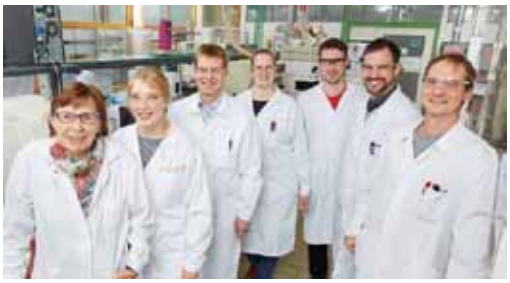
AMD analysis and determination of biocides in lens cleaning fluid



From the left: Karin Czaja, Franziska Budweg, Dr. Hans-Christoph Weiss, Svenja Mülfarth, Sebastian Brinkmann, Christian Zöll and Andreas Kinast

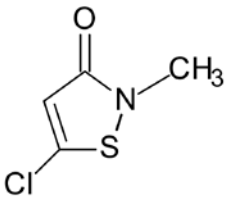
The AMD Laboratory of Currenta has been working on High-Performance Thin-Layer Chromatography since the mid-70s. At that time, belonging to BAYER AG and headed by Dr. Klaus Burger, the AMD technique was developed and has been employed in daily routine since. Up to six systems are working simultaneously to serve many diverse tasks. The analytics of crop protection products, essentially the broad application of biocides has been one of the main tasks of the AMD lab, due to its good reproducibility under standardized conditions. An in-house cumulative chromatographic database with information of spectroscopic and chemical-chromatographic properties of nearly 2000 different active components and metabolites additionally contributed to this longevity. Biocides, even though appearing in various product categories, can be analyzed reliably.

Introduction

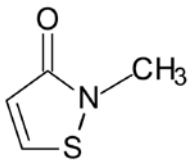
A crucial benefit the AMD technique provides is that for complex samples, no extensive sample preparation for identification and determination of components is needed. The vast majority of these components is suitable for water-free normal-phase chromatography via AMD technique.

These can be algaecides in paints, fungicides in seed stainings or microbicides as preservatives in cosmetics. To the last mentioned belongs the group of isothiazolones like the 5-chloro-2-methyl-isothiazolin-3-one (CMIT) and 2-methyl-isothiazolin-3-one (MIT). They protect aqueous products from microbial degradation. Since early 2017 these substances have been banned from cosmetic leave-on products or are severely restricted with mandatory labeling due to their strong allergenic effect.

The following describes a fast and cost efficient HPTLC analysis of biocide components in lens cleaning fluid in one run. Up to 14 samples can be handled with a consumption of only 73 mL of organic solvents in a total development time of less than one hour, which means 5 min and 5 mL solvent per sample.



CMIT



MIT

Sample preparation

The aqueous lens cleaning fluid can be applied directly.

Standards

The standard can be a 1.5% aqueous mixture of MIT and CMIT which can be obtained from diverse suppliers under different trade names. In this aqueous formulation, the suppliers added 2–3% $MgNO_3$, resulting in an additional peak in the AMD chromatogram. For the analysis, a dilution in purified water was prepared, *e.g.*, 1:1000 (15 mg/L).

Chromatogram layer

HPTLC plates LiChrospher silica gel 60 F_{254} (Merck), 20 x 10 cm, prewashed with methanol – formic acid 99:1

Sample application

Bandwise with Linomat 5 or Automatic TLC Sampler (ATS 4), 18 bands, band length 7 mm, track distance 10 mm, distance from lower edge 8 mm and side edge 12 mm, application speed 100 nL/s, rectangle application (7 x 4 mm) with a speed of 250 nL/s, application volumes 5–10 µL of standard and 10 µL of sample solutions

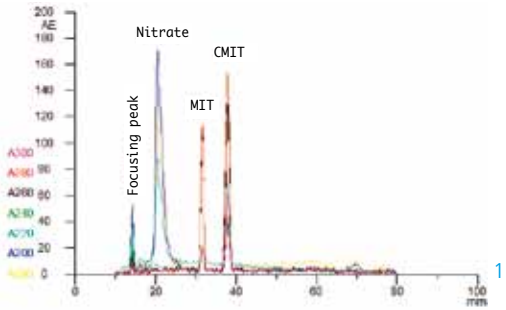
Chromatography

Short 7-step AMD gradient over a total migration distance of 70 mm (duration 1 h)

Step	Methanol (saturated with NH_3) [Vol-%]	Dichloro-methane [Vol-%]	*n*-Hexane [Vol-%]	Migration distance [mm]	Drying time [min]
1	100	0	0	15	3
2	30	70	0	20	3
3	20	80	0	30	2
4	10	90	0	40	2
5	0	100	0	50	1
6	0	100	0	60	1
7	0	33	67	70	5

Densitometry

TLC Scanner 3 with winCATS, multi-wavelength scan or absorption measurement at 280 nm, measurement slit dimension 4.0 x 0.3 mm, scanning speed 20 mm/s, linear regression, evaluation via peak height, optional spectra recording from 190 to 300 nm



Densitogram (multi-wavelength-scan) of calibration standard (150 ng/band, fits threshold)

Biological detection

Bioluminescence detection with *Aliivibrio fischeri* bioassay [1]

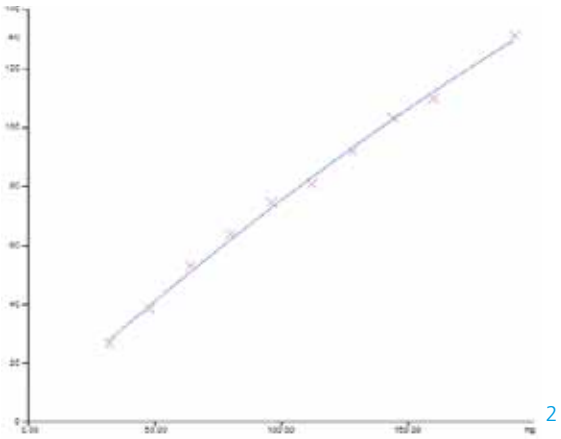
Mass spectrometry

HPTLC-ESI$^+$-MS with TLC-MS Interface (oval elution head 4 x 2 mm), zones were eluted into the MS at a flow rate of 0.2 mL/min with methanol containing 0.1% formic acid

Results and discussion

Both active compounds (MIT and CMIT) were well separated in this optimized gradient. There was no interference with the sample matrix. The threshold value is 15 mg/kg for rinse-off products. It lied within the polynomial regression (r = 0.9989, standard deviation 2.3%) for a 10-µL sample volume applied.

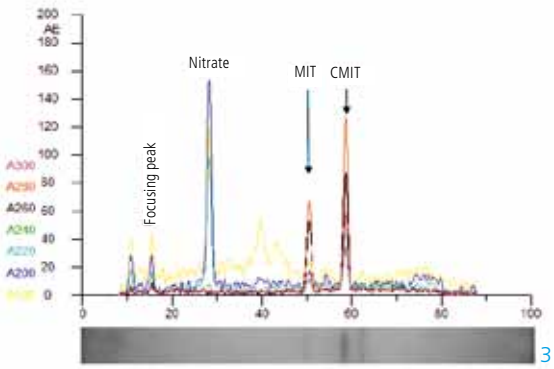
The repeatability of the determination was < 5.0 %, which made the method suitable for trace analysis. If the limit of detection is lower than 1 mg/kg, a higher volume of sample application can be used. *Vice versa* a reduced volume is used if the amount of analytes is higher than 20 mg/kg.



2

Polynomial regression of MIT / CMIT

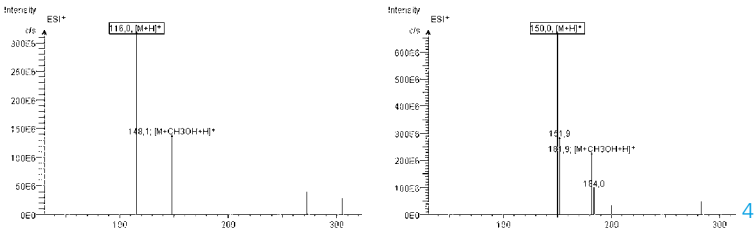
The initially used alkaline universal gradient was optimized with regard to less solvent consumption and shorter development times (1 h in total) as described above. The identification of the analytes was obtained by comparing the migration distance and the UV-absorption with the standard, sufficient for routine analysis. For further confirmation, ESI-MS, chemical derivatization and biochemical staining can be used.



3

Densitogram (multi-wavelength scan) of a sample with 15 mg/L MIT/CMIT, bioautogram after bioluminescence detection with Aliivibrio fischeri [1] in initially used alkaline universal gradient

The mass spectrum (HPTLC-ESI$^+$-MS) confirms CMIT and MIT in the lens cleaning fluid via the protonated molecules $[M+H]^+$ of CMIT (*m/z* 150) and MIT (*m/z* 116) as well as their corresponding methanol adducts $[CMIT+CH_3OH+H]^+$ (*m/z* 182) and $[MIT+CH_3OH+H]^+$ (*m/z* 148).



4

HPTLC-ESI$^+$-MS (zoomed) of the sample zone, confirming MIT (left) and CMIT (right)

With this example cost efficiency and speed of this method are demonstrated. The applicability or selectivity for other matrices has to be verified by spiking with different volumes of the standard solution.

[1] A. Dorn, Bachelor Thesis, Hochschule Niederrhein, Krefeld, 2015

Further information is available on request from the authors.

Contact: Dr. Hans-Christoph Weiss, Franziska Budweg, Christian Zöll and Andreas Kinast, Currenta GmbH & Co. OHG, 41538 Dormagen Chempark, Germany, andreas.kinast@currenta.de

Optimized HPTLC-MS method for identification of constituents in broad-leaved dock

From left: Dr. Kathrin Kabrodt, M.Sc. Silvia Ballert and Prof. Dr. Ingo Schellenberg

The research group *Institute of Bioanalytical Sciences* (IBAS) of the Anhalt University of Applied Sciences in Bernburg, under the direction of Prof. Ingo Schellenberg, is engaged in the production of plant extracts with a defined activity spectrum. The focus is on their utilization in cosmetic and pharmaceutical products as well as in plant protection.

Introduction

The subterranean parts of various plants of the knotweed family (Polygonaceae), for instance rhubarb and dock (*Rheum/Rumex* species), contain a large number of polyphenolic compounds such as flavan-3-ols, stilbenes, naphthyl glycosides and anthraquinones as well as their derivatives [1]. The objective of this work was to analyze active substances of broad-leaved dock (*Rumex obtusifolius* L.). Due to the complexity of its root extracts, preparative reversed-phase HPLC was first performed to obtain fractions with a defined polyphenol pattern. Subsequently, the normal-phase HPTLC with the variety of mobile phases that can be used offers the optimal procedure for separating the fractions into pure substances per zone.

In addition to optimizing the HPTLC procedure, focus was laid on the localization of target zones in preparation for MS detection. Previous methods for determining the position of substances without a chromophore or fluorophore were to apply the samples several times on different plate halves and to spray or immerse one half manually after the development. This usually allowed only one derivatization per plate. Using the Derivatizer, individual parts of the plate can now be sprayed with different derivatization reagents.

Sample preparation

A method patented by IBAS was used to extract the roots. After drying the extracts, their preparative fractionation was carried out by RP-HPLC. 32 fractions, and for comparison, the whole extract was freeze-dried and dissolved in methanol (1 mg/mL).

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm, prewashed by developing with isopropanol followed by drying for 30 min at 120 °C in the drying oven

Sample application

Automatic TLC Sampler (ATS 4), bandwise application, 21 tracks, band length 6.0 mm, track distance 7.5 mm, distance from left edge 22.0 mm, distance from lower edge 6.0 mm, application volume 4.0–10.0 µL for sample solutions

Chromatography

In the Automatic Development Chamber (ADC) with preconditioning for 20 min, development with toluene – ethyl acetate – formic acid 1:8:1, migration distance 75 mm from lower plate edge, drying for 10 min with cold air, followed by drying for 2 min at 50 °C (in the ADC)

Postchromatographic derivatization

Three-step derivatization using the Derivatizer, step 1: natural product reagent (NPR), step 2: in combination with polyethylene glycol solution (PEG), step 3: vanillin-sulfuric acid reagent (VSR) followed by heating on the TLC Plate Heater (100 °C, 3 min)

To derivatize areas of the same plate with different reagents, the areas, which were to be evaluated underivatized by TLC-MS, were covered with a suitable segment of a used HPTLC plate, the layer turned upwards. The turned up (dried) silica gel prevented the creeping of spray reagent under the cover plate.

Documentation

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

Densitometry

TLC Scanner 3 and winCATS, absorption measurement at 280 nm, slit dimension 6.00 × 0.40 mm, scanning speed 20 mm/s, spectra recording from 200 to 700 nm

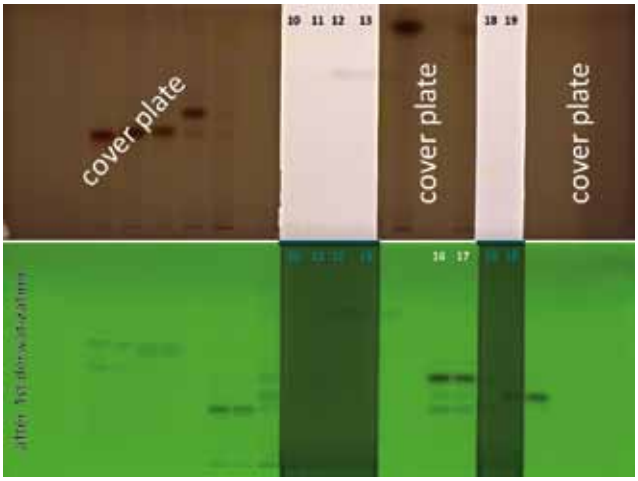
Mass spectrometry

Elution of zones with TLC-MS Interface 2 (oval elution head) at a flow rate of 0.1 mL/min with methanol into a triple quadrupole mass spectrometer with electrospray ionization (4000QTrap, Sciex) and recording in the negative ionization mode.

Results and discussion

Based on the method established at IBAS [1], the mobile phase was optimized for *Rumex obtusifolius* and its fractions, and the development was automated. After the confirmation of a comparable derivatization by both immersion and automatic spraying [2] in preliminary experiments, the partial derivatization of the plate was effected with the Derivatizer. An example of partial covering of the chromatogram plate and its effectiveness can be seen in the following chromatogram.

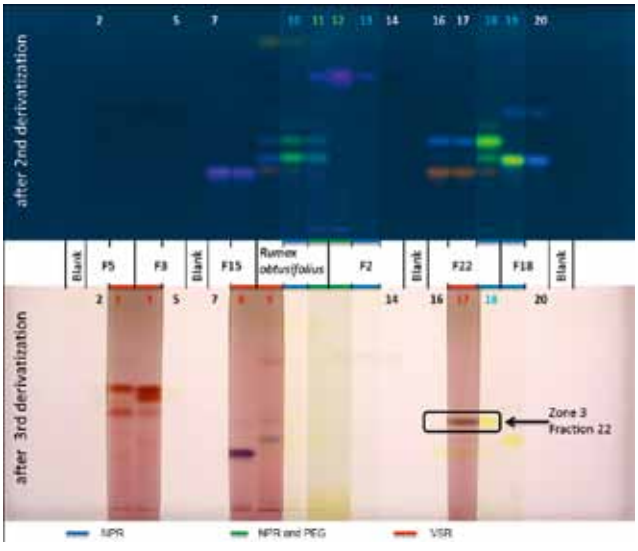
The chromatogram under UV 254 nm after first derivatization shows clearly defined regions having been derivatized without affecting adjacent tracks.



1

Chromatograms of broad-leaved dock extracts before derivatization (under white light) with covered areas and after derivatization of tracks 10 to 13 as well as 18 and 19 with NPR under UV 254 nm (Usually, plates derivatized with NPR are detected under UV 366 nm. In this case, under UV 254 nm it can be clearly seen which parts have been derivatized or not.)

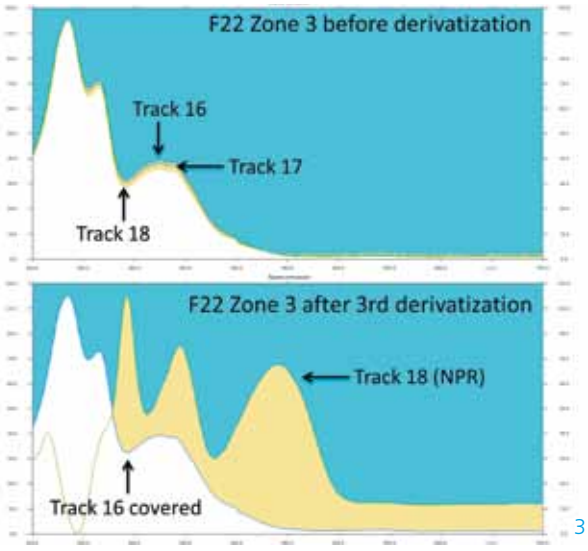
Since both the total extract of *Rumex obtusifolius* as well as some of the fractions contain various substance classes specifically stained by different derivatization reagents, further derivatization was carried out with step 2 (NPR + PEG) and then with step 3 (VSR) on the same plate.



2

Chromatograms of Rumex extracts after the first (NPR, tracks 10–13, 18–19), second (PEG, tracks 11–12) and third derivatization (VSR, tracks 3–4, 8–9 and 17) under UV 366 nm and white light (methanol as blank)

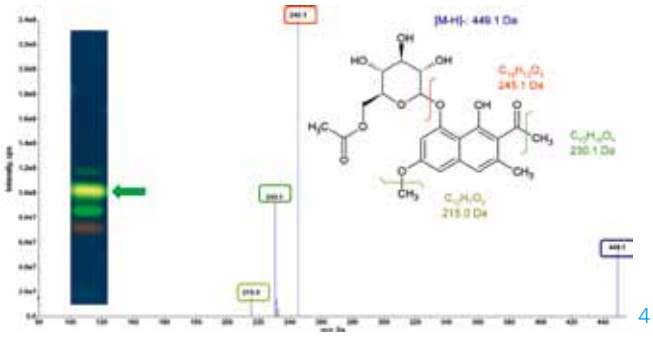
Areas for the MS examination (sample tracks 2, 5, 7, 14, 16 and 20 as well as the blanks) were permanently covered and thus remained unchanged. This is also confirmed by the spectra recorded before and after derivatization. Before derivatization, the spectra of the third zone of fraction 22 are congruent on all three tracks (16, 17 and 18). After derivatization, it is clear to see that the spectrum of the covered track 16 is unchanged. This zone can thus be examined by MS.



3

UV/Vis spectra of zone 3 of fraction 22 prior to (above) and after derivatization (below), track 16 was covered and thus not derivatized, track 18 (NPR), track 17 (VSR) not shown

The mass spectrum of the substance that accounts for the above mentioned zone 3 (main substance in fraction 22 on track 16) is shown below. It was identified as torachrysone acetylglucoside [3], which belongs to the group of naphthyl glycosides.


4

Mass spectrum of zone 3 (track 16, fraction 22) showing torachrysone acetylglucoside

The TLC-MS Interface allowed a quick and easy screening of the constituents of the *Rumex* fractions. Overall, many flavan-3-ols, procyanidins, anthraquinones and naphthyl glycosides could be detected in *Rumex obtusifolius*. The stilbenes frequently found in other Polygonaceae (*Rheum* spec.) were present only in small quantities. Previously, derivatization methods required a large distance between the tracks to be derivatized and those for the MS investigation to eliminate diffusion effects. With the Derivatizer, it was possible to compare samples derivatized and non-derivatized directly next to each other on the same plate, thereby facilitating an easier localization of the target zones.

[1] Kabrodt, K., Schellenberg I., CBS 109 (2012) 5-7

[2] Do, T., CBS 119 (2017) 1-3

[3] Ye, M. *et al.*, J Am Soc Mass Spectrom 18 (2007) 82-91

Further information is available on request from the authors.

Contact: S. Ballert, K. Kabrodt, I. Schellenberg, Hochschule Anhalt, Institute of Bioanalytical Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany, silvia.ballert@hs-anhalt.de

Planar Chromatography in the pharmacopoeias A personal reflection and look into the future

Dr. Eike Reich,
Head of CAMAG-Laboratory
and member of
the management

About fifty years ago, and as one of the first chromatographic methods, Thin-Layer Chromatography (TLC) found entry into the monographs of various pharmacopoeias. Identification of active pharmaceutical ingredients and herbal drugs, and semi-quantitative determination of impurities were the primary applications. For many years prior to the turn of the century, plates for High-Performance Thin-Layer Chromatography (HPTLC) have not yet been mentioned. Also all progress made in the development of Planar Chromatography, particularly with respect to quantitative evaluations, was almost completely ignored. Not even the most powerful devices for Instrumental TLC were able to change this situation. Despite the fact that horizontal development and densitometric evaluation were described in the general chapter of the European (PhEur) and the United States Pharmacopoeia (USP), there were, and still are, hardly any monographs with quantitative planar-chromatographic tests. One exception is the Chinese Pharmacopoeia (ChP), which at that time included about twenty quantitative methods for determination of markers in plant drugs. International harmonization between PhEur, USP, and the Japanese Pharmacopoeia (JP) led to the, fortunately only temporary, decision to completely replace thin-layer chromatographic identifications by IR spectroscopy (for identity of chemical substances) or by HPLC (for impurities, assays, identity of plant drugs).

In 2003, an article published in Pharmeuropa (the public discussion forum of the PhEur) introduced HPTLC for the first time as a separate concept beyond the use of special plates and compared it to classical TLC. That article was also the basis of a draft proposal, published in the same issue of Pharmeuropa, for a complete revision of the Pharmacopoeia's general chapter 2.2.27 "Thin-Layer Chromatography". The revised chapter became official in 2005. This is how rethinking began and opinions about the usefulness of Planar Chromatography changed. All of the leading pharmacopoeias now accepted HPTLC plates and adapted parameters as possible alternatives to classic TLC plates. Parallel to this development, the elaboration of current Good Manufacturing Practices (cGMP) for botanical dietary supplements had begun in the United States. For identification of botanical raw materials and ingredients, the cGMPs mentioned explicitly the use of HPTLC as it had been defined in a scientific paper published in the Journal of AOAC, and included in the monographs of the American Herbal Pharmacopoeia. Regulators and industry realized the necessity for implementation of standardized methods. In 2012, the International Association for the Advancement of HPTLC (HPTLC Association) published a definition of HPTLC and a standard operating procedure (SOP) for identification of herbal drugs by HPTLC. This was adopted into the then newly developed general HPTLC chapters of the USP (chapter <203> High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin) and the PhEur (chapter 2.8.25 High-Performance Thin-Layer Chromatography of Herbal Drugs and Herbal Drug Preparations, which after much discussion among experts of many countries, became official in 2015 and 2017 respectively. From that time on, Planar Chromatography of the 21st century had a name: "HPTLC". It also got an "official look" because Pharmeuropa as well as the EDQM Knowledge Database since then provide electronic images of HPTLC chromatograms for all new monographs. For this spring, USP plans the publication of a new edition of its Dietary Supplement Compendium (DSC) including standardized HPTLC data. However, that is only the beginning. Soon we will find new quantitative applications of HPTLC in Pharmacopoeia monographs on herbal drugs. In addition, there are international efforts going on to create an electronic atlas of HPTC fingerprints of herbal drugs. Such atlas could become a fundament for identification of plant materials along global supply chains. We look forward to seeing that the ChP restores its previous role as a champion with respect to planar chromatographic assays and that the Indian Pharmacopoeia embraces HPTLC.

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

With this issue another 102 abstracts on TLC/HPTLC literature have been added to the CCBS database. Among the added abstracts, a chemometric approach proved that the RP-8 phase can also behave as normal-phase adsorbent depending on the chromatographic system chosen (J. Planar Chromatogr. 30, 401–404, 2017). Remember this paper in case you face an unexpected elution order of compounds via a reversed phase separation. Due to the open system and several factors of influence on the solvent system during development, a combination of separation principles can become effective, though in most cases one is dominant. The only sure aspect of the solvent system is its potential for change. This is essential to understand.

More and more scientists recognize the ease of performing lipid separations by HPTLC. It is not only more simple in the workflow, but may also offer orthogonal separation results. Several papers were reported, in which the results of the HPTLC separation of lipids were proven to be comparable to those of the HPLC method (*e. g.*, J. Chromatogr. A 1515, 232–244, 2017). As another example of a method comparison, a degradation study of a pharmaceutical formulation showed that there was no difference even between a TLC and an ultra-performance liquid chromatography method regarding both, accuracy and precision (J. Chromatogr. Sci. 55, 961–968, 2017).

Dear friends

Planar chromatographic techniques are now successfully used all over the world. The next international HPTLC symposium, hosted in Bangkok, will sustain this flow of knowledge leading to even greater acceptance of HPTLC. Take advantage of this unique opportunity to discuss your challenges and their possible solutions with planar chromatography, 28th–30th November 2018. Options for discussions either at this symposium or further courses are summarized on the last yellow page.

In this CBS issue, several outstanding applications are presented. The analysis of biocides in lens cleaning fluid was performed using the automated multiple development (AMD) technique in combination with mass spectrometry. HPTLC-MS was also used for identification of constituents in broad-leaved dock. The quantification of cocoa ingredients and their changes during different steps of chocolate manufacturing shows that HPTLC is a powerful method to control the processing of food. Other contributions feature the successful use of HPTLC for the degradation study on cefixime and azithromycin as well as the profiling of licorice root species and quantification of 18ß-glycyrrhizic acid.

More applications in Bangkok!

Kind regards

Gertrud Morlock
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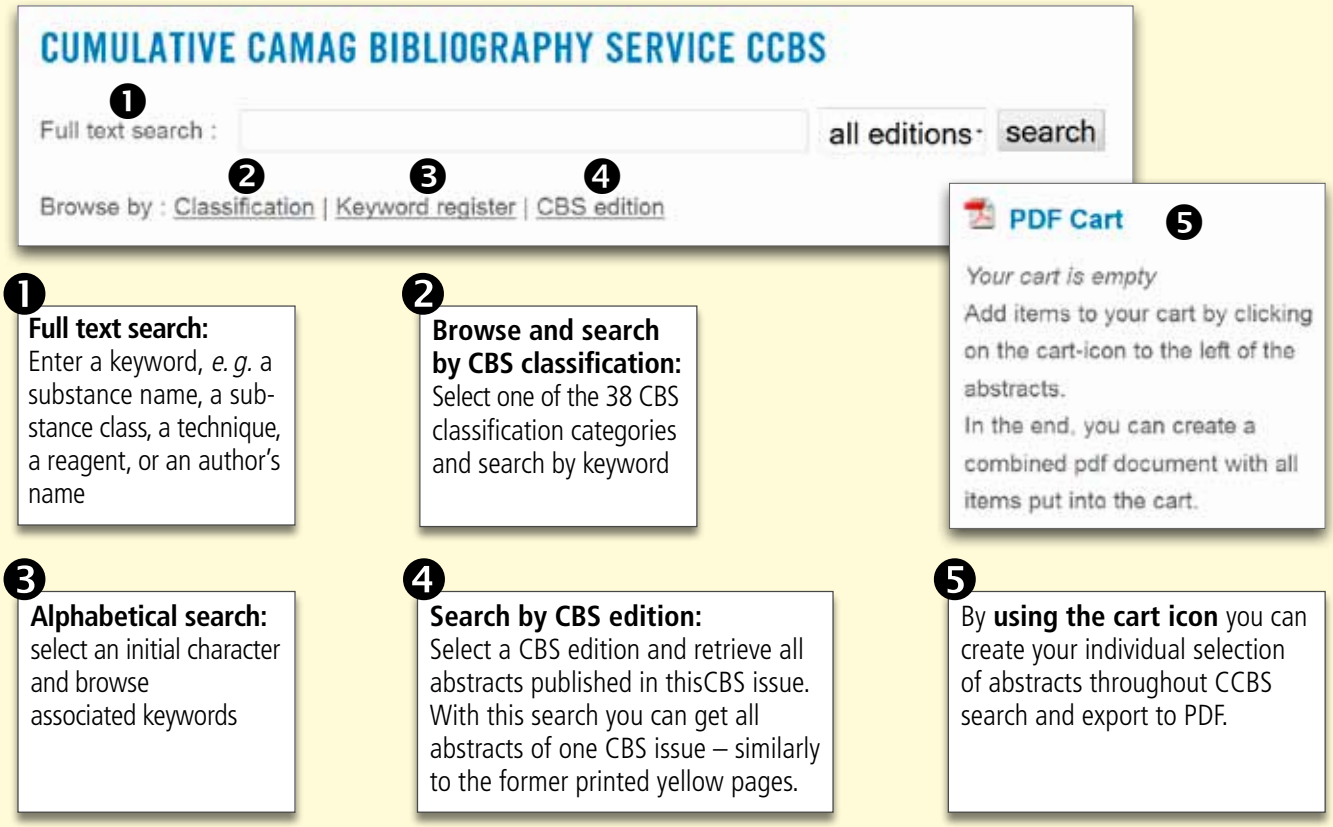
THE CBS CLASSIFICATION SYSTEM

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

Cumulative CAMAG Bibliography Service (CCBS) Online Search



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

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

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

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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.

Training offered worldwide

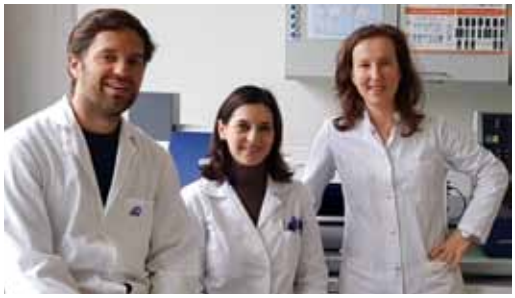
Analysts worldwide appreciate the versatility of Planar Chromatography. Their demand for advanced training opportunities is steadily increasing. The exchange of ideas with experts and the sharing of knowledge are very important for users to achieve optimal results and to advance HPTLC developments.

CAMAG and CAMAG-associated courses on HPTLC will be offered in The Netherlands, France, the United States, Thailand and Switzerland. Courses are held by various organisations and companies like the Swiss Chemical Society (SCG), the centre de compétences en Chimie et Toxicologie Analytiques (ccCTA) and the American Herbal Product Association (AHPA). Further information on these courses is available at www.camag.com/courses.

As a perfect International platform following HPTLC Berlin 2017, we would like to announce HPTLC Bangkok 2018. This 24th international symposium on HPTLC is taking place in Thailand on November 28–30. For latest information on the symposium and the four workshops see www.hptlc.com.

HPTLC combined with effect-directed assays and HRMS is used to point to bioactive compounds in complex samples. The whole workflow will be shown in a 5-day modular course at the Justus Liebig University Giessen in Germany on 28 February – 3 March, 2019. Single days that focus on special assays can be booked individually, see flyer at www.uni-giessen.de/food.

HPTLC quantification of cocoa ingredients and their changes during different chocolate manufacturing steps



Carlo Weber, Katrin Jedrys and Dr. Vasilisa Pedan

The Institute of Food and Beverage Innovation of the Zurich University of Applied Sciences in Wädenswil deals with delicious, healthy, safe and sustainable food. Besides the education of food technology engineers, practical work with the food industry is fundamental to its strength in innovation. The research group, Food Chemistry analyzes food ingredients using different analytical techniques like HPLC-MS, FT-IR and HPTLC-MS. One of the research objectives is the determination of secondary plant metabolites like alkaloids and polyphenols in food. Monitoring the changes through the entire value chain is of primary importance. In this study, determination of secondary metabolites was performed on fresh cocoa beans and diverse intermediate products of the chocolate production process.

Introduction

Polyphenols are not only known for their astringency but also for their great antioxidant potential. They are found in large amounts in cocoa and its derived product chocolate. The characterization and determination of high molecular oligomeric proanthocyanidins (PA) are of great interest because of their higher antioxidant activity compared to low molecular monomeric flavan-3-ols [1]. The changes of the PA profile are also essential for the organoleptic evaluation, which shows that monomeric PAs are more responsible for the bitter taste in contrast to oligomeric PAs, which are responsible for the astringency. Also of great interest are the two alkaloids caffeine and theobromine, not only because of their bitter taste but also for their stimulating and "prime pumping" effect. Anthocyanins can be used as general indicator for determining the degree of cocoa fermentation.

In the following application, HPTLC was used to determine oligomeric PAs, alkaloids and anthocyanins through the entire value chain – from fresh cocoa beans, through roasted cocoa, cocoa mass and up to molded chocolate bars. The established method has been proven as a suitable tool for comprehensive compound analysis in laboratories with high sample throughput. Besides the acquisition of the characteristic HPTLC fingerprint for the specific manufacturing step, the visual information can be used to build up an imaging database.

Chromatogram layer

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

Samples

One single batch of 10 kg fresh cocoa beans was studied across different processing steps [2]. Changes in the PA profile were recorded on a lab-scale model and may not be fully in line with real-life crop or industrial scale processing. Nevertheless, one batch was processed and aliquots of about 50 g were sampled from each processing step. Samples were collected from (1) raw fresh cocoa beans, (2) fermented, dried cocoa beans, (3) roasted cocoa mass, (4) 1 h conched cocoa mass, and (5) molded chocolate bars.

Sample preparation

1 g of the fine grinded and defatted cocoa powder was extracted three times with 3 mL acetone – water 1:1. The combined supernatant was diluted 1:10 with acetone – water 1:1.

Standard solutions

Anthocyanin standard solution (0.01 mg/mL in methanol) with cyanidin-3-*O*-arabinoside (cn-ara) und cyanidin-3-*O*-glucoside (cn-glc); alkaloid standard solution (0.2 mg/mL in acetone – water 1:1) with

caffeine and theobromine; PA standard solution (0.1 mg/mL in methanol) with (-)-epicatechin (EC), proanthocyanidins B2 (PA B2) and C1 (PA C1) as well as cinnamtannin A2 (Cinn A2)

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, distance from left edge 20 mm, distance from the lower edge 8 mm, application volume between 5 and 10 µL for standard solutions and 2 and 10 µL for the sample solutions

Chromatography

In the Automatic Developing Chamber (ADC 2) with chamber saturation (with filter paper) for 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride, development with ethyl formate – formic acid – water – toluene 30:4:3:1.5 to the migration distance of 70 mm (from the lower edge), drying for 5 min

Postchromatographic derivatization

The plate was heated at 100 °C and immersed with Chromatogram Immersion Device (immersion speed 5 cm/s, immersion time 0 s) in Fast Blue Salt B reagent (140 mg Fast Blue Salt B in 140 mL methanol, 10 mL water, and 50 mL dichloromethane), followed by 30 s drying in a cold air stream. Alternatively, the Derivatizer can be used.

Documentation

TLC Visualizer under UV 254 nm and white light, also after derivatization

Densitometry

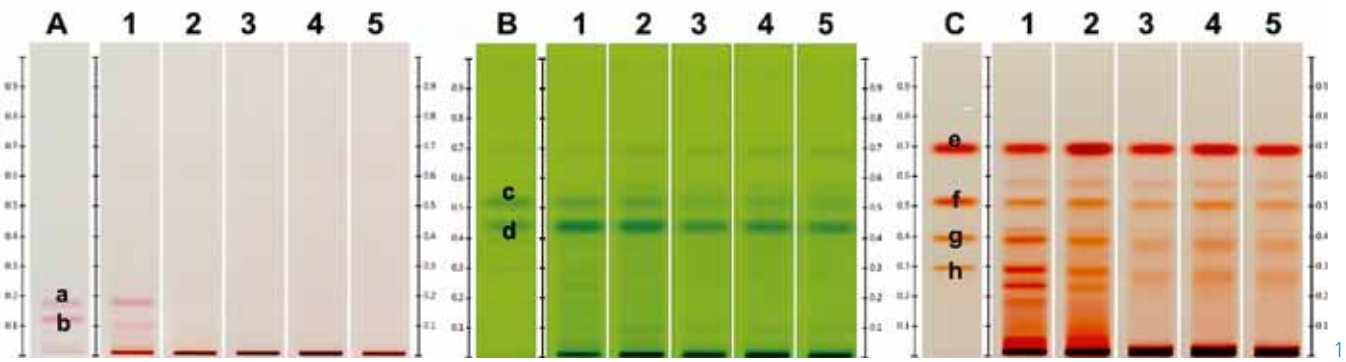
TLC Scanner 4 and *visionCATS*, absorption measurement at 280 nm for alkaloids and 510 nm for anthocyanins and derivatized PA, slit dimension 5.00 × 0.20 mm, scanning speed 50 mm/s, evaluation via peak area, polynomial regression, spectrum recording from 190 to 600 nm

Mass spectrometry

Elution of target zones was done after derivatization with Fast Blue Salt B with the oval elution head (4 × 2 mm) of the TLC-MS Interface. Hereby, acetone – water 1:1 was used as elution solvent at a flow rate of 0.1 mL/min using a HPLC pump. The recording of mass spectra was performed in the positive ionization mode.

Results and discussion

The developed method showed a good separation and a quick quantification of the four oligomeric PAs, the two alkaloids and the two anthocyanins. The present study showed diminishing levels of the alkaloids and especially oligomeric PAs, especially during the fermentation and roasting process. Higher oligomeric PAs are presumed to decrease faster than monomeric PAs. In addition, the alkaloid content decreased throughout the manufacturing, whereas during fermentation, alkaloids may diffuse through the porous surface of the testa cotyledon [3]. During the roasting process, alkaloid content decreased due to diffusion into fat. Anthocyanins are responsible for the reddish coloring of the cotyledon. In general, the content of anthocyanins decreased constantly during all stages of fermentation and drops further during drying. Here, the initial



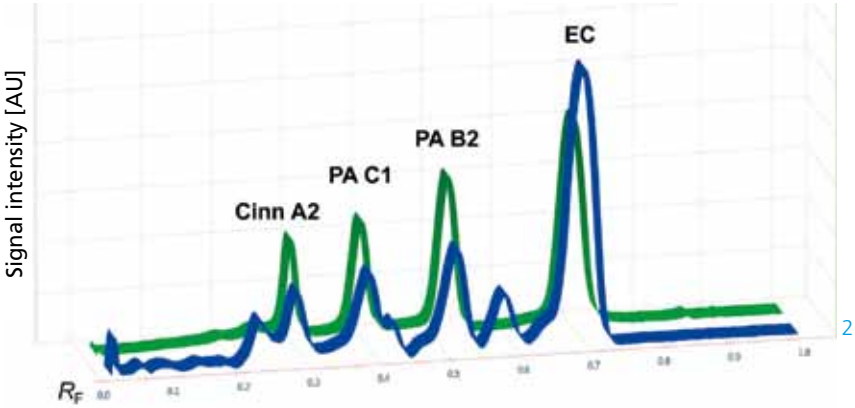
1

HPTLC chromatogram of different standard mixtures for (A) anthocyanins (a: cn-ara, b: cn-glc) illuminated under white light; (B) alkaloids (c: caffeine and d: theobromine) under UV 254 nm and (C) PAs (e: EC, f: PA B2, g: PA C1 and h: Cinn A2) under white light after derivatization as well as products from different steps of the chocolate manufacturing with 1: raw fresh cocoa beans, 2: fermented, dried cocoa beans, 3: roasted cocoa mass, 4: 1 h conched cocoa mass and 5: molded chocolate bars

content in fresh cocoa beans for cn-ara with 0.81 ± 0.01 mg/g and 0.40 ± 0.01 mg/g for cn-glc dropped drastically below the detection limit after six days of fermentation.

Polyphenol, alkaloid and anthocyanin mean content (mg/g non-fat dry matter, reproducibility inclusive sample preparation, n = 3) for products of five different chocolate manufacturing steps

Content [mg/g]	Caffeine	Theobromine	EC	PA B2	PA C1	Cinn A2
1	7.79 ± 0.70	16.38 ± 4.20	7.09 ± 1.09	2.98 ± 1.21	4.40 ± 0.08	5.06 ± 0.82
2	4.48 ± 0.64	11.12 ± 0.86	5.63 ± 0.45	2.04 ± 0.22	2.37 ± 0.22	2.01 ± 0.22
3	2.89 ± 0.39	7.42 ± 1.37	4.33 ± 0.36	1.72 ± 0.19	1.66 ± 0.19	1.10 ± 0.13
4	3.36 ± 0.19	9.00 ± 1.02	5.00 ± 0.15	2.23 ± 0.12	2.44 ± 0.26	1.35 ± 0.16
5	2.75 ± 0.17	7.41 ± 0.76	4.37 ± 0.04	1.81 ± 0.03	1.50 ± 0.20	0.92 ± 0.12



2

Densitogram of the raw fresh cocoa beans extract (blue) and PA standard solution (green), absorption measurement at 510 nm after derivatization

The quantification allows the determination of valuable ingredients through each specific processing step and can lead to optimization of the chocolate process.

[1] Pedan, V. *et al.* S. Food Res. Int. 89 (2016) 890–900

[2] Pedan, V. *et al.* Food Chem. 214 (2017) 523–532

[3] Timbe, D. *et al.* Food Sci. 43 (1978) 560–562

Further information is available on request from the authors.

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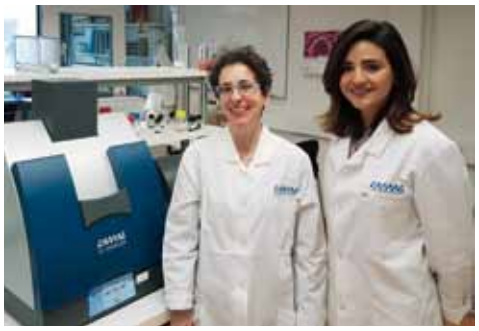
Qualitative and quantitative HPTLC analysis of licorice root



From left: Valeria Maire-Widmer and Débora Arruda Frommenwiler, CAMAG Laboratory

Introduction

Licorice root is one of the top-selling herbal drugs in Europe and the US. Due to its expectorant activity it is used in numerous herbal products against coughs and, because of its sweet taste, is added to many herbal tea mixtures for better flavor.

There are monographs on licorice root in all major pharmacopoeias worldwide. Several describe a quantitative assay of the marker substance 18β-glycyrrhizic acid. According to the pharmacopoeias, licorice root may be derived from at least three species of the *Glycyrrhiza* genus, which are morphologically, chemically, pharmacologically, and toxicologically similar.

To ensure that a herbal ingredient has a specified minimum strength, pharmacopoeias include HPLC assays of 18β-glycyrrhizic acid. The United States Pharmacopoeia (USP) specifies no less than 2.5 % of 18β-glycyrrhizic acid, while other pharmacopoeias accept different contents.

An HPTLC method was developed for the differentiation of licorice roots of the species *Glycyrrhiza glabra* and *Glycyrrhiza uralensis* based on their fingerprints, which is also suitable for the quantitative determination of the minimum content of 18β-glycyrrhizic acid according to USP.

With this method both the assay of the marker substance and the identification of the species is possible on one plate. The quantification of 18β-glycyrrhizic acid is performed by densitometry, whereas identity confirmation is done after derivatization under UV 366 nm and white light [1].

Standard solution

0.125 mg/mL of 18β-glycyrrhizic acid in 70% ethanol

Sample preparation

500 mg of powdered licorice root are mixed with 10 mL 70% ethanol and extracted by sonication for 10 min at room temperature. Following centrifugation for 5 min, 1 mL of the supernatant is diluted with 9 mL methanol.

Chromatogram layer

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

Sample application

Automatic TLC Sampler (ATS 4), bandwise application, 15 tracks, band length 8 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2.0 µL

Chromatography

In the ADC 2 with chamber saturation (with filter paper) for 20 min and after conditioning of the plate at 33% relative humidity for 10 min (with a saturated solution of magnesium chloride), development with dichloromethane – methanol – water – formic acid 120:75:15:1 to the migration distance of 70 mm (from the lower edge), drying for 5 min

Densitometry

TLC Scanner 4 and winCATS, absorption measurement at 254 nm, slit dimension 5.0 mm x 0.2 mm, scanning speed 20 mm/s

Note: The absorption maximum was determined from the UV spectrum on the plate. By coincidence, it matches the intense spectral line of the mercury lamp and thus the optimum excitation wavelength of the fluorescence indicator.

Postchromatographic derivatization

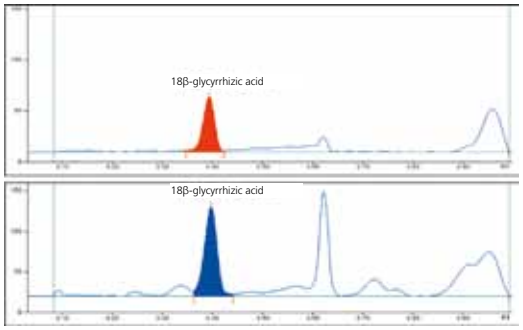
The plate was immersed in 10% sulfuric acid in methanol using the Chromatogram Immersion Device, immersion speed 5 cm/s, immersion time 1 s, and heated at 100 °C for 10 min using the TLC Plate Heater.

Documentation

TLC Visualizer under UV 254 nm (prior to derivatization), UV 366 nm and white light (after derivatization).

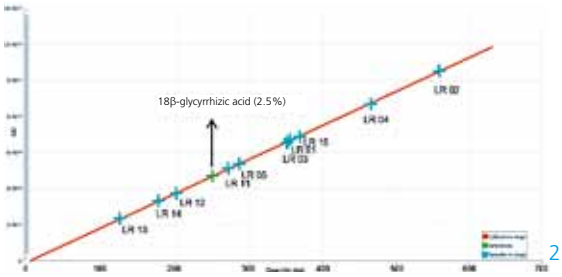
Results and discussion

The current GMP rules require proper identification of each incoming raw material and determination of its strength as proof of quality for an herbal product. The determination of the strength is typically accomplished by assaying the content of one or several selected marker compounds, with the goal to ensure that the herbal ingredient has at least the same potency as specified in the monograph. The current monographs on licorice root in most pharmacopoeias include HPLC assays of 18β-glycyrrhizic acid. In addition to the identification test, another assay has to be performed, which requires more time, material and equipment. Also expertise in sample preparation, product analysis and results interpretation are required. Consequently, the cost of ensuring good quality products increases significantly. To simplify the quality control process, a single HPTLC method for the simultaneous quantification of 18β-glycyrrhizic acid in licorice root and the identification of the species is proposed.



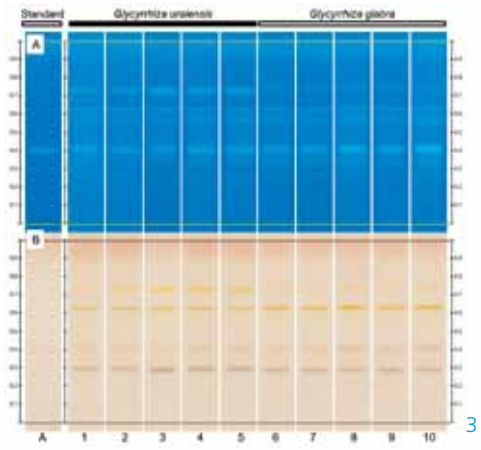
1

Densitograms (absorption measurement at UV 254 nm) of 0.125 mg/mL 18β-glycyrrhizic acid (red) and a root extract of Glycyrrhiza glabra (blue)



2

Single level calibration for the determination of 18β-glycyrrhizic acid (linearity was confirmed by multi level calibration): Licorice root extracts LR13, LR14 and LR12 contain less than 2.5% (green cross) of 18β-glycyrrhizic acid and do not comply with the USP monograph.



3

Identification of species based on comparison of selected fingerprints after derivatization with sulfuric acid under UV 366 nm (A) and white light (B); track A: 18β-glycyrrhizic acid, tracks 1–5: samples of Glycyrrhiza uralensis root, tracks 6–10: samples of Glycyrrhiza glabra roots.

As an alternative to densitometric evaluation with the TLC Scanner, the images under UV 254 nm can be converted into peak profiles [1]. This HPTLC method was used for the analysis of numerous samples of dried licorice root and products thereof. It proved to be reliable and reproducible, and the HPTLC data correlated well with the HPLC data according to the USP monograph. Therefore, the HPTLC method is well suited for the rapid and convenient quantitative and qualitative analysis of licorice root.

[1] Frommenwiler, D. A. *et al.* J. Planar Chromatogr. 30 (2017) 6, 467–473

Further information is available on request from the author.

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Degradation profiling of cefixime and azithromycin (antibiotics)

Dr. Gawande Vandana T.

Dr. Gawande, Assistant Professor in the Quality Assurance Techniques at Sinhgad Institute of Pharmacy, Pune, Maharashtra, India, is involved in the development and validation of chromatographic methods for stability of pharmaceutical drugs, both standalone and fixed dose combinations.

Introduction

Stability is the capacity of a drug product to remain within specification for a given time. It is a prime requirement to ensure its identity, strength, quality and purity. Forced degradation studies are an integral part of drug development programs. For detecting the number and types of degradation products that are formed under various conditions, different chromatographic techniques in conjunction with UV and MS are used.

HPTLC is especially beneficial for stability testing due to its disposable stationary phase, as well as in the case of forced degradation, when appreciable amounts of acidic, alkaline and peroxide reagents are used. Furthermore, HPTLC permits the analysis of a large number of samples in a short time. There are also multiple derivatization and detection options that can help to characterize degradation products. Apart from this, simplicity and low cost of analysis add to the benefits.

Standard solutions

10 mg of cefixime trihydrate (CEFI) or azithromycin dihydrate (AZI) dissolved in 10 mL methanol

Sample preparation

1) Hydrolytic degradation: 1 mL of drug standard solution was mixed with 1 mL water or 0.5 N HCl or 0.5 N NaOH, kept for 1 h at RT and neutralized with acid or base
2) Oxidative degradation: 1 mL of drug standard solution was mixed with 1 mL of H_2O_2 (3 and 30 %) and kept for 1 h at RT
3) Thermal degradation: The drug powder placed in a sealed glass ampoule was heated at 100 °C and 200 °C for 1 h and 2 h in a hot oven
4) Photolytic degradation (according to ICH guidance Q1B, option 2): A thin layer of solid drug powder was exposed with fluorescent cold white light (1.25 million lux h) and UV light (200 Whm^{-2}) in a photo stability chamber

All degradation samples were dissolved (solid samples) or diluted with methanol to a final concentration of 100 µg/mL.

Chromatogram layer

HPTLC plates silica gel 60 F_{254}, aluminium backed (Merck), 20 x 10 cm, prewashed by developing first with methanol and then with the mobile phase followed by drying for 15 min with cold air

Sample application

Bandwise application with Linomat 5 or 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 10.0 µL for sample solutions and 2.0 and 5.0 µL for standard solutions (CEFI resp. AZI); for preparative isolation: band length 180 mm and application volume 200.0 µL

Chromatography

In the Twin Trough Chamber 20 x 10 cm with chamber saturation (with filter paper) for 20 min, development with ethyl acetate – methanol – acetone – toluene – ammonia 2:10:14:1:1 to the migration distance of 80 mm (from the lower edge), drying for 15 min with cold air

Postchromatographic derivatization

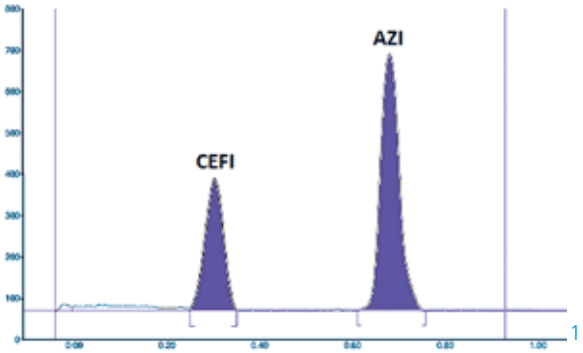
For detection of AZI, the plate was immersed with the Chromatogram Immersion Device into sulfuric acid reagent (1:4 in ethanol; immersion speed 3 cm/s, immersion time 6 s), dried for 30 s with cold air, and heated at 100 °C for 5 min using the TLC Plate Heater.

Documentation

TLC Visualizer under UV 254 nm, UV 366 nm, and white light

Densitometry

TLC Scanner 3 and winCATS, absorption measurement at 235 nm for CEFI and 530 nm for AZI, slit dimension 6.00 × 0.45 mm, scanning speed 20 mm/s, spectra recording from 190 to 550 nm



1 *Densitogram (absorption measurement) at 254 nm of the standard mixture; reprinted from [1]*

Mass spectrometry

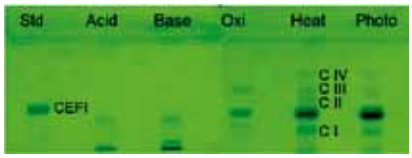
The target zones were marked with a pencil, and plates were cut carefully to separate different bands. Individual bands were cut and sonicated with methanol for extraction of degradation products. Methanol fractions were concentrated and evaporated to obtain solid residues which were analysed by Q-TOF and Ion Trap mass spectrometry in the positive ionization mode.

Results and discussion

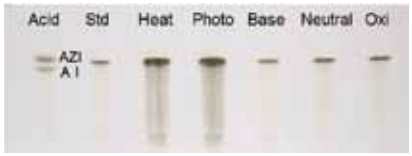
The developed method was validated according to the International Conference on Harmonization guideline Q2 (R1). All parameters were within the acceptance criteria, indicating that the method is suitable for the analysis of AZI and CEFI.

Parameter		Result	
		CEFI	AZI
Linearity	Linear calibration	y = 2.1614x+1002	y = 27.89x-313
	Determination coefficient r^2	0.9924	0.9952
	Range	500–2500 (ng/band)	50–250 (ng/band)
Sensitivity	LOD (ng/band)	58	3
	LOQ (ng/band)	175	10
Trueness and precision	Level spiked: mean amount (ng/band) ± intra-day precision (*%RSD*)	800: 799.3 ± 0.3 1000:1000.6 ± 0.4 1200:1201.4 ± 0.4	100: 99.5 ± 0.3 150:149.7 ± 0.4 200:200.6 ± 0.5
	Level spiked: mean amount (ng/band) ± inter-day precision (*%RSD*)	800: 798.6 ± 0.9 1000: 999.0 ± 0.9 1200:1199.1 ± 0.7	100: 98.9 ± 0.7 150:148.8 ± 0.9 200:199.9 ± 0.8
Accuracy	Level spiked: mean recovery (%) ± precision (*%RSD*)	800: 98.9 ± 1.3 1000: 99.9 ± 0.2 1200:101.1 ± 0.8	100: 99.5 ± 0.3 150:100.7 ± 0.5 200:100.1 ± 0.3
Robustness	Precision (*%RSD*)	< 2	< 2

In the forced degradation studies, CEFI was found to degrade to 4 major products (CI – IV) at different stress conditions. AZI showed only one additional peak (AI) upon acid and neutral hydrolysis. The specificity of the method was verified by comparing the hR_F values and UV spectra of the standard solutions with the degraded samples. Peak purities were investigated by spectrum scans at three positions within each zone and calculating their correlations (peak purities between 0.9993 and 0.9997).



2 *Degradation study of CEFI ; reprinted from [1]*



3 *Degradation study of AZI; reprinted from [1]*

Four degradation products for CEFI and one degradation product for AZI were isolated by preparative TLC and subjected to MS/MS for characterization. The β-lactam ring of CEFI did open under all conditions (chemical structures at [1]). AZI degraded to azithralosamine (loss of cladinose ring).

[1] V. Gawande *et al.* Acta Chromatographica, 2017, DOI: 10.1556/1326.2017.00199

Further information is available on request from the author.

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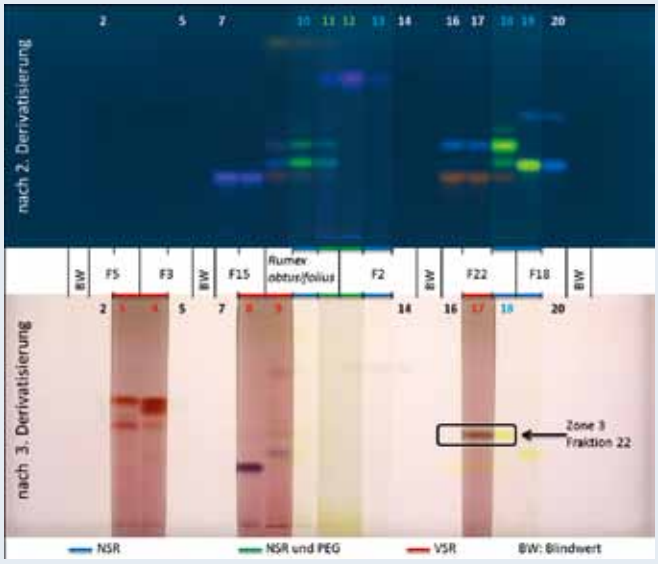
CAMAG Derivatizer

Automated spraying device for reagent transfer in derivatization of TLC/HPTLC chromatograms

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

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



1

A practical example of the versatile use of the Derivatizer is shown on pp. 5–7 in this issue. By covering individual plate areas a partial derivatization with different reagents is possible.

2

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 (2–4 mL) through efficient operation
- Reproducible and user-independent results

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

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