CBS 122 • MARCH 2019

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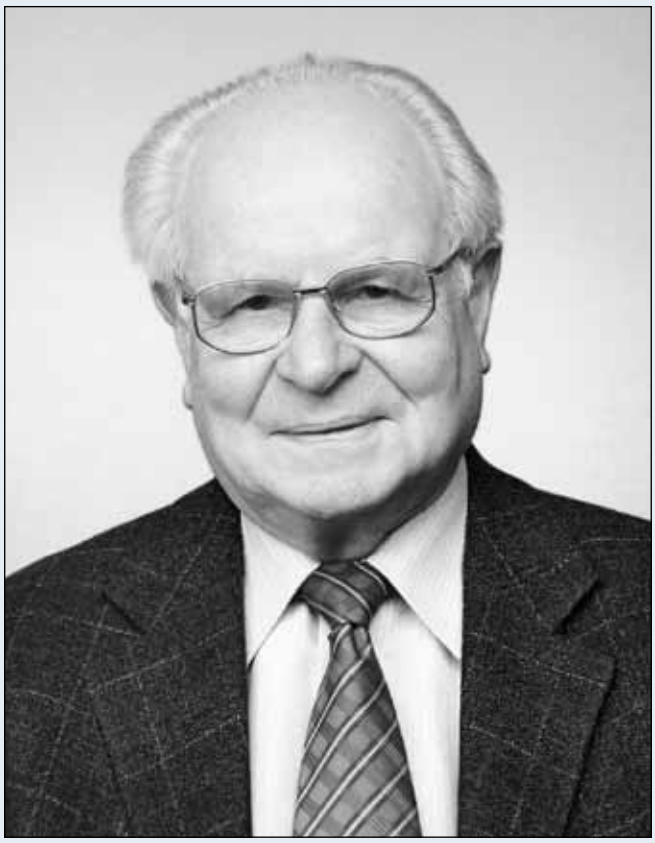
MARCH 2019



1927–2018

In memoriam

This issue is dedicated to Dr. Dieter Jänchen, Founder of CAMAG and pioneer of Instrumental Thin-Layer Chromatography

CAMAG BIBLIOGRAPHY SERVICE PLANAR CHROMATOGRAPHY

CAMAG® WORLD LEADER IN PLANAR CHROMATOGRAPHY

Obituary for Dr. Dieter Jänchen, 1927–2018

CAMAG's founder Dr. Dieter Jänchen died at the age of 91 on December 22, 2018, at the hospital in Liestal, near Muttenz, Switzerland.

We are sad that he is no longer with us. Until the very end, we and the CAMAG employees greatly appreciated his guidance and wisdom. Our comfort is that he did not have to suffer long and that he found his rest and his peace. It is hard to say goodbye, because in the past 60 years Dieter Jänchen has vastly influenced CAMAG and the company's culture. Unfortunately, his health denied him participation in the festivity of the 60th anniversary of the company on December 14, 2018.

We keep Dieter Jänchen in our memories. He was a figure of respect and, though not always easy to deal with, a highly esteemed, caring and well-respected company boss. He was no friend of quick decisions. He reflected, discussed with others, analyzed the pros and cons, and thereafter, whatever he did, he led straightforward in the desired direction. Well-justified arguments convinced him to continue in the chosen direction.

Dieter Jänchen was born in Berlin where he studied Chemistry at the Technical University. In 1954 after a promotion and on recommendation of his supervisor, he found a job as a young chemist in Switzerland. As a result of favorable circumstances, he was able to start his own business and in December 1958, he founded the company CAMAG.

He managed the development of this highly specialized company and succeeded in advancing the initially small company into a well-known brand. Through his persistent commitment, he led the company to international acceptance. The company group consists of the headquarters in Muttenz and daughter companies in Germany and the USA as well as an established network of distributors around the world. Today CAMAG stands for a solid high-tech enterprise with highly qualified and highly motivated employees.

In time, Dieter Jänchen saw the logic of connecting the sale of instrument systems with application methods. For this, he founded the application laboratory and the scientific literature service. His CAMAG Bibliography Service, named CBS, and the cumulative CBS database (CCBS) are highly recognized among experts. Over the decades, the literature service resulted in an extensive collection of TLC/HPTLC methods and literature abstracts, which are a valuable, cost-free source of information for the user.

Dieter Jänchen dedicated his life's work to the building of CAMAG. He literally lived CAMAG. CAMAG is Dr. Dieter Jänchen. He remains unforgettable.

He lived modestly for his means. Besides the company, he had one sole passion – flying. In the 60ies, he joined the Luftsportgemeinschaft Hotzenwald, a local air sports community. His hobby came clearly second after the company, even though he was intensely devoted to this hobby. He practiced first glider and later motor flying. As a motivated flying instructor and local division trainer, he shared his experiences as a top pilot with his pilot trainees. He was very influential to the Luftsportgemeinschaft Hotzenwald, serving in many capacities and was a mentor to many.

10 years ago, Dieter Jänchen decided to gradually step back. To ensure the continuity of his life's work CAMAG, the CAMAG foundation was created, which is inalienable and thus controls the fate and the future of CAMAG. The slow, irresistible aging process shaped Dieter Jänchen's last years. Except for the last days, he was able to spend the end of his long life at home, as was his wish.

We are very grateful for his exemplary work. We will keep Dieter Jänchen in a very good memory and will think of him with heartfelt respect.

Prof. Dr. Gertrud Morlock
Editor CBS

Hans Reichenbach
Member of the CAMAG foundation

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Printed on FSC paper

Validated method for fast quantification of glycine in cosmetics

Caroline Petitti

The main activity of the analytical laboratory at the Development Center of Bayer Healthcare, Gaillard, France, is to develop, optimize and validate methods for analysis of new Over The Counter products in the field of nutritionals and skin care brands, such as Berocca® and Bepanthen®. Caroline Petitti has previously reported on the quantification of amino-propanol in dermatological products (CBS 98, 2007, 2–4). These methods are transferred to the quality control laboratories at production sites all over the world, as has the following HPTLC method to a German production site. For quantification, HPLC is the most used technique; however, HPTLC is the top choice for QC laboratories already equipped with HPTLC.

Introduction

The goal of this study was to develop a new method for the quantification of glycine at a low quantity (<1% assay) in a gel in oil formula. As an amino acid, glycine is a small polar molecule, which makes its analysis difficult for RP-HPLC due to low sensitivity and retention.

Hence, HPTLC had been considered due to its decisive advantages. HPTLC is accurate, precise and reproducible, as proven by the method validation. The sample preparation is simple and the development time is short (4 min). The derivatization agent is simply included in the mobile phase (advantageous for safety), and the costs of analysis are low due to low solvent consumption and parallel analysis of 12 samples on one HPTLC plate.

Standard solution

Aqueous glycine solution (0.25 mg/mL)

Sample preparation

Liquid-liquid extraction of the sample (600 mg) with dichloromethane – water 1:2.5 (35 mL); aqueous phase was taken

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm, prewashed with methanol, followed by drying on the TLC Plate Heater at 80 °C for 15 min

Sample application

Automatic TLC Sampler (ATS 4), bandwise application, band length 8.0 mm, delivery speed 50 nL/s, application volumes 2.0 µL for sample solutions and 1.0 to 2.0 µL for standard solution (250 to 500 ng/band; note that this narrow calibration range is justified due to the targeted known sample content)

Chromatography

In the Twin Trough Chamber (saturated with mobile phase) with 0.5% (m/V) ninhydrin (derivatization reagent added to the mobile phase) in ethanol – water – glacial acetic acid 14:5:1 up to 2 cm

Postchromatographic plate treatment for derivatization

Drying on the TLC Plate Heater at 100 °C for 2 min until orange-violet zones appear at hR_F 50

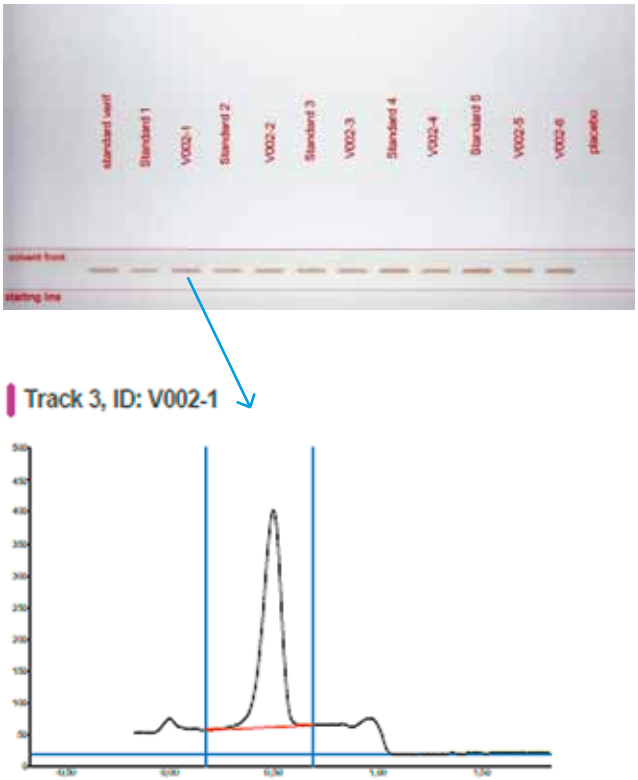
Densitometry

Absorbance measurement at 386 nm with TLC Scanner 3 and winCATS

Results and discussion

The developed method is fast and specific. It takes only 4 min for separation of up to 12 samples in parallel. The method's specificity was verified by applying 2.0 µL of a prepared placebo sample (last track). As no colored zone appeared, the method was proven to be specific (specific derivatization).

The repeatability of the method was checked by applying 6 preparations of the same sample, resulting in a relative standard deviation of 1.5%, proving a good precision. The analytical response was demonstrated by applying 5 different levels of the glycine solution (50 to 150% of the targeted sample content), whereby each level was prepared twice. For the resulting linear regression, the coefficient of correlation was ≥0.990 (sdv 3%) and the axial intercept was close to 0 (-1.6%), which proved that the sample did not degrade. A Michaelis Menten 1 function showed the best performance as calibration curve (sdv 0.6%). The mean recovery



1

HPTLC-Vis chromatogram of cosmetic samples, standards and placebo as well as densitogram at 386 nm as example

was 100% showing the high accuracy of the method. The intermediate precision was verified by analyzing the same batch on 2 different days by 2 different analysts with different reagents and plate batches. Each analyst prepared the sample 6 times. The resulting intermediate precision (*%RSD*) was 2.5%. As the relative standard deviation of each series corresponded to the acceptance criteria, the method was considered to be precise.

Further information is available on request from the author.

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HPTLC-UV fingerprints of *Gelsemium elegans* and koumine contents determined by densitometry compared to UPLC-MS/MS

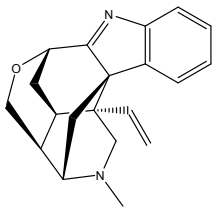
From left: Zhao Qin Yeap, Dr. Mun Fei Yam, Chiew Hoong Ng, Dr. Chu Shan Tan

Dr. Yam won the Xie Peishan Award for the Young Researcher at HPTLC 2018 Bangkok for his excellent lecture held. He is senior lecturer and researcher at the School of Pharmaceutical Sciences, University of Science, Penang, Malaysia, where he works with his team on hypertension bioassays and chemical fingerprinting. After exploring the robustness of HPTLC in chemical fingerprinting in previous projects, he is now testing the method against UPLC-MS/MS to find out if HPTLC is already good enough for the fingerprinting of herbs. The need for a cheap, robust, accurate and easy to implement technique for solving quality control issues in traditional medicine via chemical fingerprinting is exactly why HPTLC is the preferred method.

Introduction

Gelsemium elegans, also known as "heartbreak grass", is a flowering plant genus of the *Gelsemiaceae* family, found in China and Southeast Asia. There are drawbacks in consuming this plant for long periods of time. Since there are different amounts of alkaloids present in different parts of the plant, it is important to chemically distinguish these parts to both assess the pharmaceutical properties of the herb and to avoid over-consumption. The content of the alkaloid koumine present in different plant parts (stem, root and leaf) was determined by HPTLC. UPLC-MS/MS was used for comparison.

HPTLC is a straightforward versatile technique applied in pharmaceutical research for both qualitative and quantitative assessment of chemical constituents. HPTLC is the only chromatographic technique that presents the outcome as an image, *e.g.*, the simple presentation of separated components by UV light. The visible outcome and simplicity of the HPTLC technique allow inexperienced analysts to easily run the chromatographic procedure. Analysis time is relatively short, and numerous samples can easily be analyzed side by side on the plate, making HPTLC the method of choice for simple and rapid evaluation.



Structural formula of koumine

Chromatogram layer

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

Standard solutions

Methanolic solutions of koumine (125, 250, 500, 1000 and 2000 µg/mL)

Sample preparation

100 mg of dried, powdered *Gelsemium elegans* were mixed with 1 mL of ethanol – water 7:3 and sonicated for 10 min. The mixture was centrifuged for 5 min and the supernatant was used for analysis. The samples were spiked by adding a known amount of koumine (250 µg/mL for stem or leaf, and 500 µg/mL for root) at the ratio of 1:1 (100 µL:100 µL).

Sample application

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

Chromatography

In the Automatic Developing Chamber (ADC 2) with chamber saturation (with filter paper) for 20 min and conditioning of the plate at 33% relative humidity for 10 min (using a saturated solution of magnesium chloride), development with chloroform – methanol – water 30:10:1, migration distance 70 mm from lower plate edge, drying for 5 min

Documentation

With TLC Visualizer under UV 254 nm and UV 366 nm

Densitometry

TLC Scanner 4 with *visionCATS* software, spectra recording from 200 to 400 nm, absorption measurement at 220 nm, slit dimension 5 mm x 0.20 mm, scanning speed 20 mm/s, polynomial regression, evaluation by peak area

UPLC-MS/MS

Separation on a ACQUITY UPLC BEH C18 column (100 mm x 2.1 mm, particle size 1.7 µm) with a gradient based on acetonitrile and 1% formic acid in water using a Waters ACQUITY UPLC I-Class

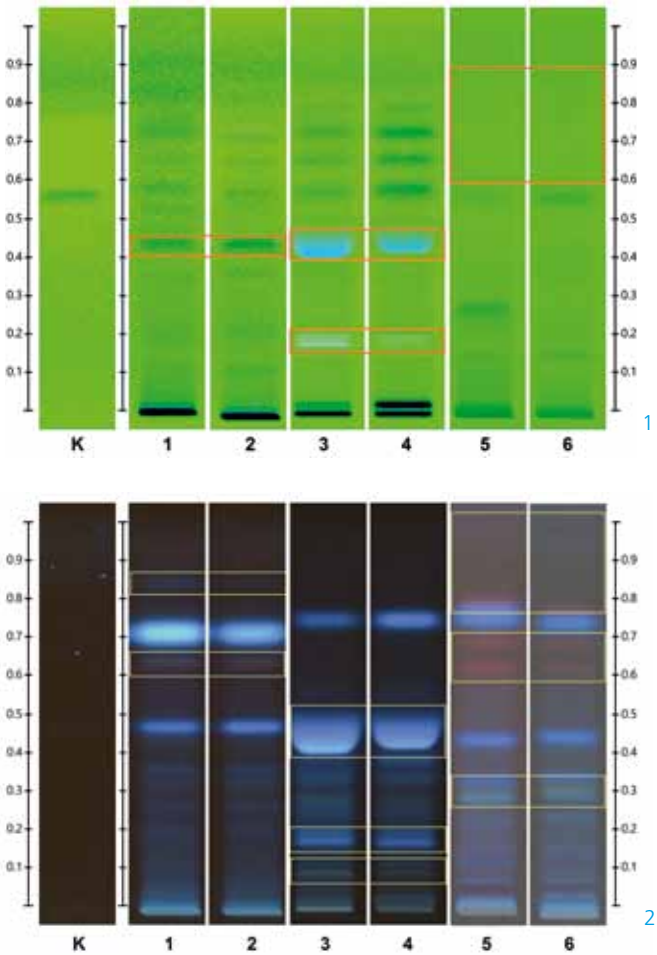
Results and discussion

Samples spiked with koumine showed the same hR_F value as the koumine standard zone, well separated from matrix components. At UV 254 nm, the chromatograms of samples exhibited a zone similar in hR_F (55 to 58) and color to the koumine standard zone, which proper assignment was underlined by the recorded HPTLC-UV spectra of the respective sample zones compared to the koumine standard zone. The optimum wavelength at 220 nm for densitometric evaluation was confirmed by the UV spectrum. Polynomial calibrations in the working range of 125–2000 µg/mL led to determination coefficients $R^2 > 0.9995$.

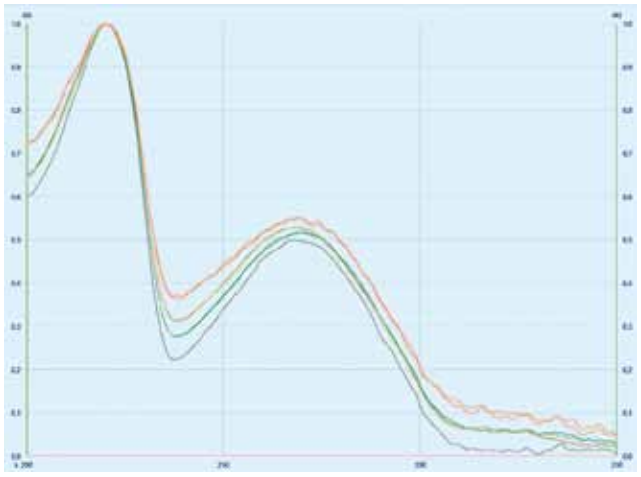
On the one hand, the stem fingerprints showed a dark zone (hR_F 40) at UV 254 nm as well as light pink (hR_F 62) and light blue fluorescent zones (hR_F 82) at UV 366 nm. These bands can only be seen in the fingerprints of the stem, but not in those of the root and leaf. Hence, these zones can be used as markers to differentiate the stem extract from others.

On the other hand, the root fingerprints showed blue zones (hR_F 18 and 40) at UV 254 nm, whereas a yellow (hR_F 10), two blue (hR_F 15 to 20) and a broad blue fluorescent zone(s) (hR_F 40 to 50) were detected at UV 366 nm, which featured a characteristic fingerprint for the differentiation of the root extract from others.

Besides the fact that the leaf fingerprint at UV 254 nm did not show any zones at hR_F 60 to 90, which are observed for the stem and root extracts (at least four zones), the absence of these bands can be used as a discrimination of the leaf extract. At UV 366 nm, two red band patches were exclusively observed from hR_F 58 to 70 and hR_F 78 to 100 for leaf extract as well as a faint yellow zone at hR_F 32 (not in stem and root fingerprints). These markers additionally can differentiate the leaf extract from others.



Chromatograms at UV 254 and 366 nm of Gelsemium elegans extracts (tracks 1/3/5: stem/root/leaf from Fu Jian province versus 2/4/6: stem/root/leaf from Guang Xi province) compared to koumine (K)



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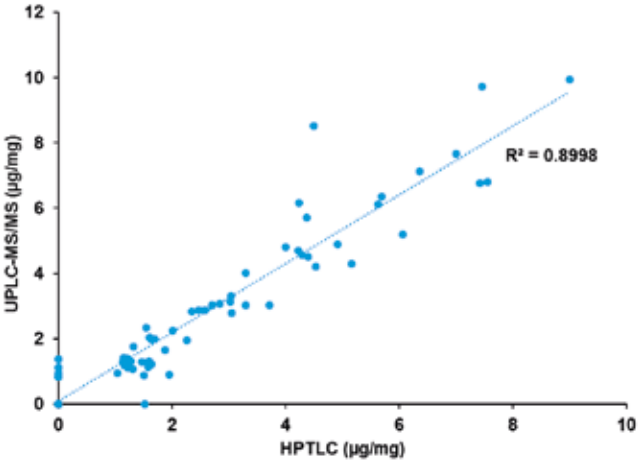
HPTLC-UV spectra of koumine standard compared to the 6 sample zones

The quantity of koumine present in the plant was determined by HPTLC-UV and UPLC-MS/MS. The validation of both methods showed good results in terms of precision (2.7% for HPTLC and 2.4% for UPLC-MS/MS), reproducibility (2.6% for HPTLC and 3.1% for UPLC-MS/MS) and recovery (101.3% for HPTLC and 104.4% for UPLC-MS/MS). The koumine results correlated with a determination coefficient R^2 of 0.8998, proving that the results from both methods can be cross checked. The actual amounts differed due to the orthogonality of the methods, *e. g.*, different solvent systems, stationary phases and detection principles.



4

Correlation of the koumine contents determined by UPLC-MS/MS versus HPTLC-UV

Contact: Dr. Yam Mun Fei, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Minden, Penang, Malaysia, yammunfei@usm.my

5

CAMAG Derivatizer

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.

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

CAMAG celebrated its 60th anniversary in 2018

The entire CAMAG Berlin team, including all employees as well as retirees, accepted the invitation to travel to Switzerland for the anniversary celebration on December 14, 2018 and experienced a splendid evening in a cozy yet stylish atmosphere.

Unfortunately, the company's founder, Dr. Dieter Jänchen, was unable to attend for health reasons, which was disappointing for everyone present. Dr. Jaenchen sent his sincere regards, accompanied by the message to ring in the next 60 years of CAMAG with this special anniversary celebration.

Dr. Markus Wyss (CEO) and Mr. Hans Reichenbach, member of the board and long-time companion of the company's founder hosted this special anniversary celebration and delivered informative insights into the 60-year company history, backed by pictures, short film sequences and some anecdote.

From his own experience, Mr. Reichenbach was able to tell about the first steps of the company's founding in December 1958. At that time, probably no one suspected that the company would in time become the world's Number One in the field Planar Chromatography.

The founding of CAMAG coincided with the rapid spread of Thin-Layer Chromatography. CAMAG advocated and nurtured the new method and by 1962, the company already had an extensive product range for all steps of the process used at that time: self-preparation of plates, sample application, chromatogram development, derivatization and UV inspection. Since then, a great deal has happened in terms of technological development at CAMAG.

Today's extensive range is state-of-the-art, both hardware and software. Practical functionality and sustainable high quality have made the name CAMAG synonymous with the Planar Chromatography/ HPTLC worldwide, due in some measure to the farsighted release of the Bibliography Service CBS, which began almost simultaneously with the company founding. For decades, CBS has provided interested readers with short presentations and standardized examples, covering a broad range of applications in Thin-Layer Chromatography.

Without the long-standing close cooperations with opinion leaders from universities and without the constant dialogue with customers worldwide about analytical challenges and associated solutions in daily laboratory routine, the continued high acceptance of CAMAG systems would be inconceivable.

It is also a tradition for CAMAG to actively participate in scientific events and exhibitions/trade fairs. Special highlights in the anniversary year were the HPTLC Symposium in Bangkok (28–30 November), the ANALYTICA 2018 in Munich (10–13 April) and the Summer Meeting of CAMAG managers in Muttenz (21–24 August).

Dr. Konstantinos Natsias
President of the Board

CBS 122

CAMAG BIBLIOGRAPHY SERVICE

MARCH 2019

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

With this CBS issue, 90 abstracts were added to the CCBS database, reporting on relevant TLC/HPTLC information from the latest scientific publications in our field. Several publications dealt with matrix-assisted laser desorption/ionization mass-spectrometric detection of compound zones. Another publication showed that the presence of the green fluorescence indicator F_{254}, a manganese-activated zinc silicate, changed the TLC migration properties of selected phospholipids. In most cases, it does not matter whether a plate with or without fluorescent indicator is used, however, there are exceptions. The latest biennial review of planar chromatography summarized the many publications in the years 2015–2017, and thus serves as an excellent survey.

Remembering the proper selection of the working range and calibration function, addressed in CBS 121, such aspects also came up during editing the current 90 abstracts. This time, the attention was laid on the mobile phase composition. During method development, the optimization of the solvent system may mathematically end up in complex solvent ratios, *e. g.*, ethyl acetate – ethanol – acetone – ammonia 2239:370:250:75, which are inconvenient to prepare. For such cases, I always wondered whether more simplified solvent ratios, here 30:5:3:1, could also lead to satisfying separations? Why not simplifying the solvent ratios at the final stage to have easily readable integers? Low integer milliliter volumes can be read and measured easily, and thus the solvent system can be prepared faster.

Dear friends

This CBS issue is dedicated to Dr. Dieter Jänchen, the founder of CAMAG. He deceased on December 22, 2018, few days after the 60th CAMAG jubilee. An obituary on page 2 acknowledges his impressive lifework.

2018 was also the year of the 80th anniversary of planar chromatography. By chance in 1938, the planar chromatography was discovered. It started as a circular separation – a laboratory mishap, which generated a huge follow-up interest. In this dynamic, early stage of planar chromatography, the young chemist Dr. Jänchen founded CAMAG. Planar chromatography has attracted scientists' attention for 8 decades, and still has the power to impress us by awesome fingerprints or straightforward solutions, which makes the fan community grow. The technique is flexible and challenges our creativity to achieve solutions that seemed unimaginable before. Every day, it inspires us to solve an analytical task in an even more efficient way.

In 2018 for the first time the International Symposium on HPTLC took place in Asia. A resume on the HPTLC 2018 conference in Bangkok, 28.–30. November (www.hptlc.com) is presented on the fourth inner page in this issue.

Kind regards

Gertrud Morlock
cbs@camag.com

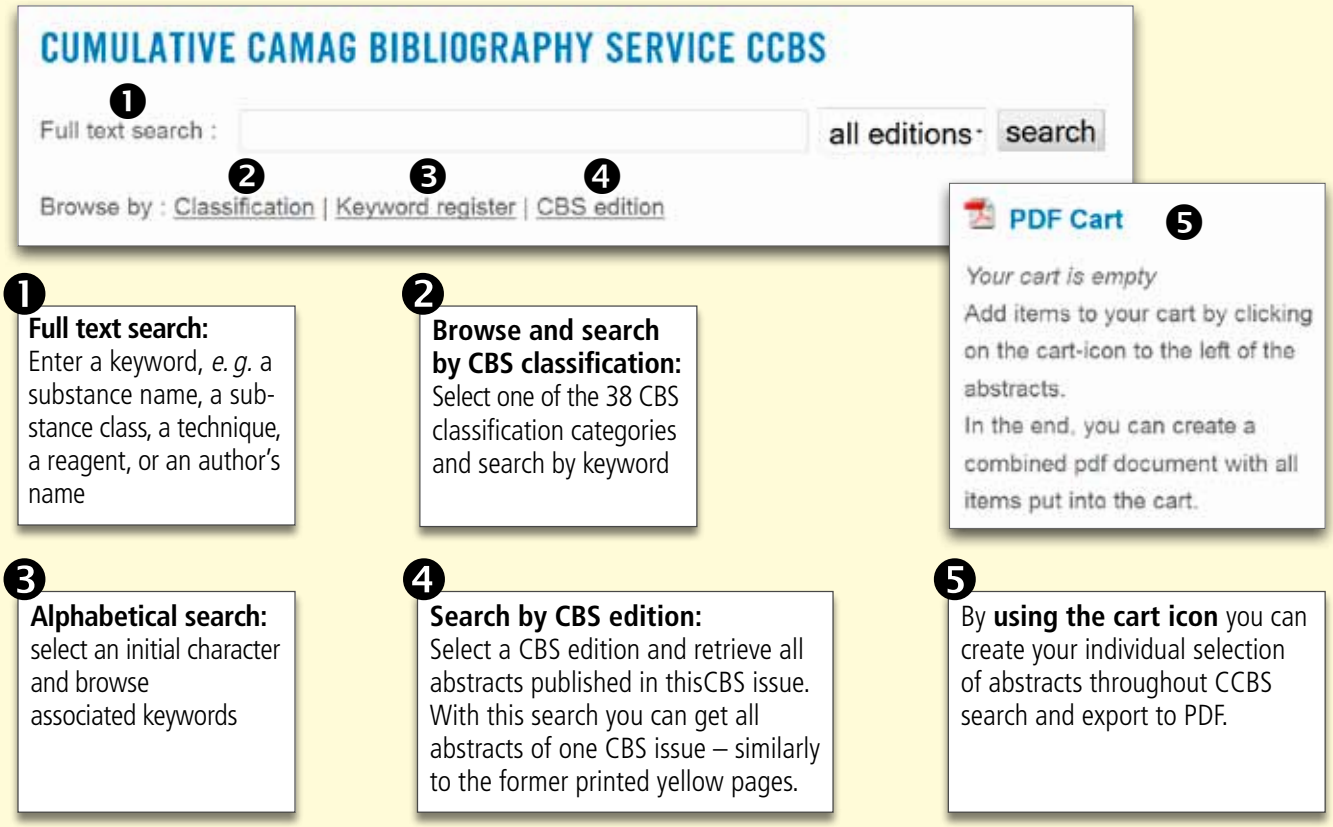
CAMAG®

CAMAG LITERATURDIENST
CAMAG BIBLIOGRAPHY SERVICE
PLANAR CHROMATOGRAPHY

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.

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 HPTLC 2018 Bangkok November 27–30

With the aspirations of involving more researchers from Asia and expanding the HPTLC network, the 24th international symposium HPTLC 2018 was organized in Bangkok, Thailand.

The year 2018 was very significant since it was for the first time in Asia and the year of the 70th anniversary of Planar Chromatography! Sharing ideas and innovations from all participants, the current advancements as well as challenges in a broad range of HPTLC applications were discussed. As expected a considerable contribution from the Asian researchers was featured. More than 50% of oral lectures and 75% of poster presentations were from Asia. The short courses, lectures and presentations covered almost all aspects of HPTLC applications.

During these four days of interactive discussions, the up-to-date methodologies in botanical analysis, effect-directed analysis, and compounds characterization were thoroughly described. New (bio)detectabilities and interesting hyphenations were also presented. By the end, the outcome of the conference and the proposed initiatives to continually improve the HPTLC field were highlighted.

Apart from the scientific event, the post-conference social program was delightful. After the fruitful scientific talks, a nice tour of the charming city of Bangkok was enjoyed.

Great thanks is owed to Prof. Dr. Wanchai De-Eknamkul, the Chairman of the Organizing Committee. His group produced an extraordinary masterpiece! All committee members and presenters contributed greatly to the success of this Asian event. Out of the many excellent young presenters, the following were awarded. The Scientific Committee thanks all participants for the many fruitful and lively discussions!

Dr. Mun Fei Yam, Malaysia (Xie Pheisan Award for the Young Researcher) as well as Poster Prize Awardees Yugandhara Patil, India, Laksana Charoenchai, Thailand and Alan Bergmann, Switzerland (in between Prof. Dr. Morlock and Mr. Bernard-Savary)

Screening of steroids as adulterants in food supplements

From left: Dr. Simone Biella, Dr. Francesca Colombo, Dr. Chiara Di Lorenzo and Prof. Dr. Patrizia Restani

The group of Professor Dr. Patrizia Restani, Food Chemistry and Toxicology, Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy, is involved in different research projects in the field of food safety, dietetic products and risk and benefit assessment of food supplements. Among the analytical techniques employed, chromatography is used for the characterization of food (supplements) and the identification of adulterants present in these products. HPTLC is often used in the laboratory thanks to its flexibility, reproducibility and time saving. The following method was developed during the PhD study of Francesca Colombo, guided by Prof. Dr. Restani, and with the collaboration of Dr. Biella and Dr. Di Lorenzo.

Introduction

Adulteration and fraud are quite common problems in commercial food supplements (FS). Among the illicit compounds present in FS, steroid hormones (*e.g.*, androstenedione, nandrolone, stanozolol, testosterone and testosterone enanthate) are often added to supplements for athletes to enhance their physical performances. The quality and safety control of these products cannot be easily carried out due to the large number of FS on the market and especially considering that these molecules are masked by the complex matrix. Thus, HPTLC is usually applied for the preliminary identification of adulterations of FS with conventional drugs. [1]

HPTLC is often the best analytical technique for screening a large number of samples characterized by a complex matrix, as in the case of adulteration and fraud. HPTLC is simple, flexible, relatively inexpensive and a useful analytical tool for laboratories involved in food control to screen several classes of adulterants. The use of more expensive and sensitive techniques, such as HPLC coupled to different detectors, can be limited only to the positive samples, allowing a wider control of the market.

Standard solutions

Methanolic solutions of testosterone (T), androstenedione (A), dehydroepiandrosterone (D), methandrostenolone (M), nandrolone (N), stanozolol (S) and testosterone enanthate (TE) of 1 mg/mL each

Sample preparation

A representative quantity of the FS was ground and mixed, and 0.2 g of the resulting powder was added to 10 mL methanol. The solution was stirred with a magnetic bar for 15 min, filtered (0.45 µm) and concentrated to 1 mL under a stream of nitrogen.

Chromatogram layer

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

Sample application

Linomat 5, 15 tracks, band length 8.0 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volume 5 µL for sample solutions and 15 µL for standard solutions

Chromatography

In the Twin Trough Chamber 20 × 10 cm with chamber saturation (with filter paper) for 20 min, with chloroform – acetone 17:3 to the migration distance of 80 mm (from the lower edge), followed by drying for 15 min

Documentation

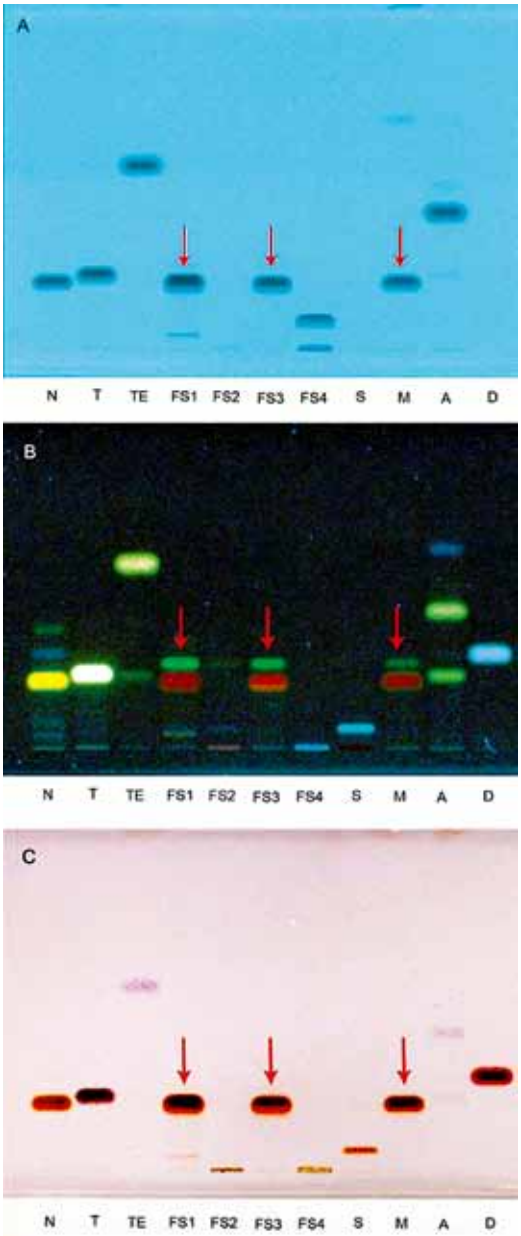
With the TLC Visualizer at UV 254 nm before derivatization as well as at UV 366 nm and white light illumination after derivatization

Post-chromatographic derivatization

By spraying with 5% ethanolic sulphuric acid, drying and heating at 110 °C until colored zones appear

Results and discussion

A representative screening quickly determined the presence or absence of forbidden hormones in four different FS under control. The samples FS1 and FS3 showed a zone at hR_F 34. This hR_F correspond to nandrolone and methandrostenolone standards; however, the band in samples characterized by hR_F 34 was tentatively assigned to methandrostenolone, thanks to its characteristic red coloration at 366 nm (the green band along with the reference standard could be a methandrostenolone-derived compound). This compound is included in the list of doping substances and is forbidden in FS. Only the positive samples were confirmed by subsequent HPLC-MS. Although it is difficult to estimate the share of adulterated samples present on the market, in the seized samples analyzed in this study, about 20% resulted positive for steroid hormones. The repeatability was evaluated using the same standard solutions on three different days and comparing the hR_F values obtained. On plates from three days, the deviation of hR_F values was ±1.2% and confirmed the repeatability of the separation. The limit of detection (LOD) was evaluated applying decreasing volumes of the hormone standard solutions on the plate. Evaluation at UV 366 nm showed the best detectability for visual inspection. Resulting LODs were in the range of 6 to 9 ng/band or µg/g. This simple HPTLC method showed a good performance suited for fast detection of steroid hormones as adulterants in FS.



HPTLC chromatograms at UV 254 nm (A), after derivatization at UV 366 nm (B) and white light illumination (C) of hormone standards and four FS (zones at hR_F 34 marked)

[1] Rocha T. *et al.* Compr Rev Food Sci Food Saf 15 (2016) 43–62

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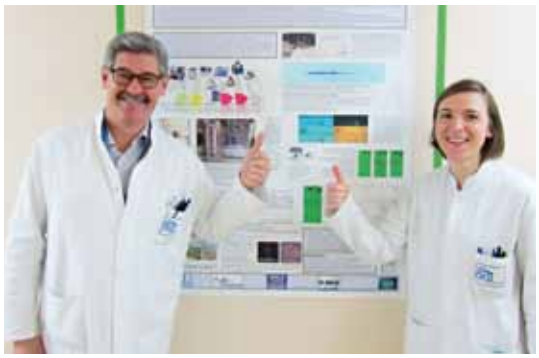
TLC and HPTLC-MS in the manufacturing of clinical API batches



Daniel Dron and Amélie Havard

Amélie Havard and Daniel Dron work in the R&D department Analytical Innovative Technologies at the Industrial Research Centre at Oril Industrie, in Bolbec, France. The R&D team specializes in purification processes of intermediates and active pharmaceutical ingredients (APIs) for toxicological, galenical or clinical studies. Part of their work is also the isolation of impurities and production of APIs or impurity reference batches. In 2018, the team launched their preparative chromatography service InnoPrep™, dedicated to small- and large-scaled purifications. APIs, intermediates and impurities are characterized by MS and NMR. A quantitative 1D and 2D NMR method is currently being developed.

Introduction

Efficient purifications by a selective preparative chromatography are widely used in the manufacturing of API batches. The described procedure helps in the understanding of target molecules and related impurities. Impurities, present at very low levels can be isolated at a high purity, which eases identification and finally improves the process. By providing higher purity products in less time, significant financial gain can be achieved.

The required conditions for an efficient purification (ca. 75% use silica gel at Oril Industrie) are determined by TLC. Then, the purification progress is checked by preparative column chromatography via HPTLC. Twenty fractions were analyzable within 1 hour. TLC/HPTLC is the method of choice due to its simplicity, rapidness and the successful scale up from TLC to preparative separations. HPTLC-MS helped to quickly resolve the composition of a mixture.

Sample preparation

Crude product (0.05 g) dissolved in 5 mL ethyl acetate

Chromatogram layer

TLC plate silica gel 60 F_{254} (Merck), 20 x 5 cm
HPTLC plate silica gel 60 F_{254s} (Merck), 20 x 10 cm

Sample application

Automatic TLC Sampler (ATS 4), bandwise application, up to 20 tracks, band length 8.0 mm, sample volumes of 1–15 µL

Chromatography

In the Twin Trough Chamber 20 x 20 cm (TLC) or 20 x 10 cm (HPTLC) with chamber saturation (with filter paper) for 20 min with different solvents to the migration distance of 100 mm for TLC and 50 mm for HPTLC (both from the lower edge), drying in a stream of cold air for 5 min

Documentation

TLC Visualizer at UV 254 nm

Mass spectrometry

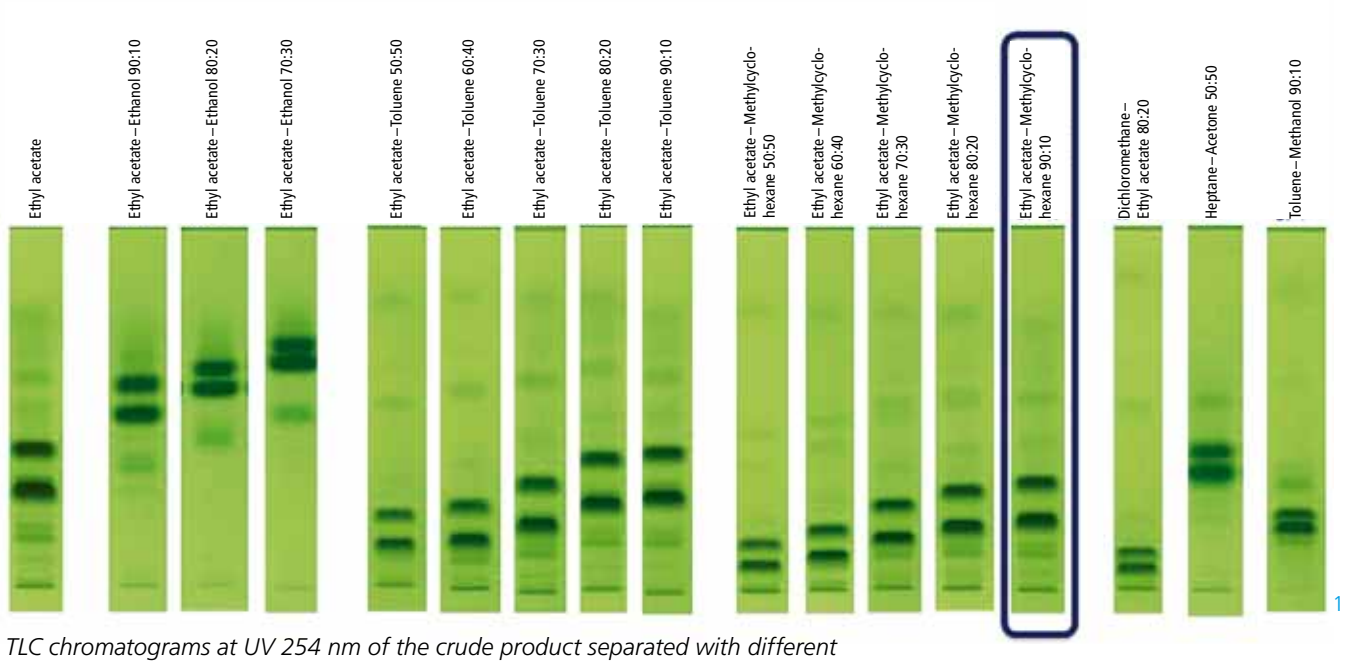
Elution of zones with the TLC-MS Interface (oval elution head) at a flow rate of 0.2 mL/min with methanol – water 1:1 into a Q-TOF-MS (Xevo® G2-XS QTof, Waters), operating in the positive ionization mode (*m/z* 50–1200)

NMR

Elution of zones with the TLC-MS Interface (oval elution head) at a flow rate of 0.2 mL/min with methanol into a vial, followed by evaporation to dryness, dissolution of the residue in deuterated chloroform (with one drop of sodium deuteroxide solution) and ^{1}H NMR recording (400 MHz, Bruker)

Results and discussion

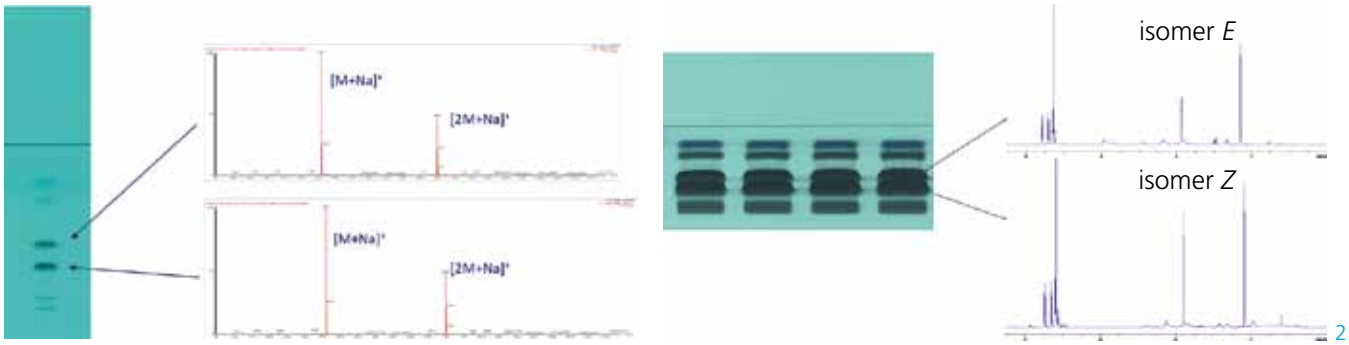
The aim of this study was to obtain a batch with a purity >99% of the isomer *Z*, containing <1% of isomer *E* and <0.15% of other impurities. By RP-HPLC the isomers were not separated satisfyingly, and TLC was selected for method development to separate the two isomers. Ethyl acetate – methylcyclohexane 9:1 was the best option to separate all compounds at reasonable hR_F values allowing a fast purification.



1

TLC chromatograms at UV 254 nm of the crude product separated with different mobile phases

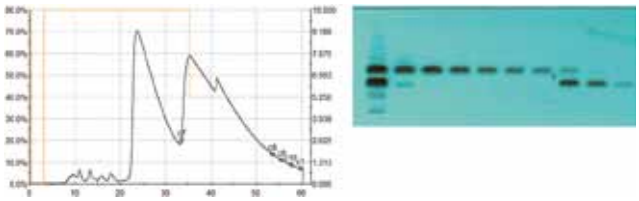
Mass spectra were recorded to characterize the different compounds. The same sodium adduct $[M+Na]^+$ and respective dimer $[2M+Na]^+$ were obtained for both *Z/E* isomeres. For NMR, the crude product solution was concentrated by a factor of 10, applied (15 µL) on the HPTLC plate, separated, and four zones of each target zone were eluted and combined.



2

HPTLC chromatograms at UV 254 nm of the crude product (10 g/L, 1 µL versus 100 g/L,15 µL) and mass spectra (left) versus ^1H NMR spectra of isomeres (right)

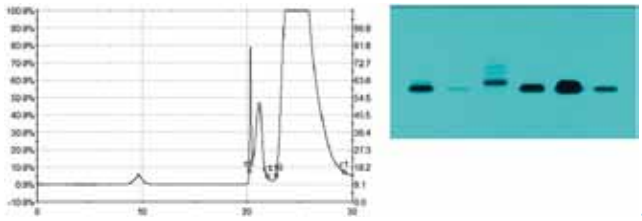
The purification of the crude product (2 kg dissolved in toluene) on a 45-cm column (packed at 40 bars with 40 kg silica gel 60, 15–40 µm, Merck) at a flow rate of 10 L/min with ethyl acetate – methylcyclohexane 9:1 led to a productivity of 20 kg per day. The elution process was monitored online by UV 254 nm detection and in parallel offline by HPTLC.



3

Online monitoring of the purification process by LC-UV (254 nm, left) versus offline by HPTLC-UV (individual fractions at 254 nm, right)

The different fractions of the target isomer *Z* were collected and the combined fractions analyzed by NMR. The purity was not sufficient, as the NMR spectrum showed several impurities. Thus, the purification was optimized. The new eluent of dichloromethane – ethanol 19:1 together with a crude product load of 0.5 kg also led to a productivity of 20 kg per day. The purity obtained for the *Z* isomer was 99.8% with a yield of 88%.



4

Optimized purification process (as mentioned)

TLC is the best method for development and optimization of purification processes using silica gel. It is simple and allows a rapid upscaling to preparative columns. HPTLC is an efficient tool for offline monitoring of the eluted fractions. Up to 20 fractions can be analyzed in parallel and compared in the HPTLC chromatogram at UV 254 nm, achieving a good overview on the purity and amount of the target compound per fraction.

Further information on request from the authors.

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5

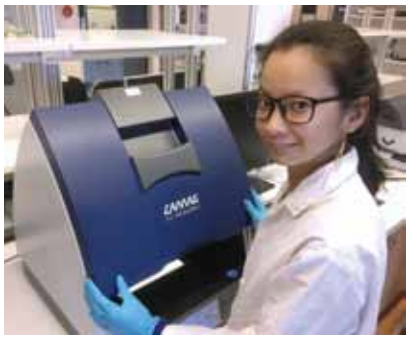
CAMAG TLC-MS Interface 2

The CAMAG TLC-MS Interface 2 is the second generation for the pioneering concept of hyphenating HPTLC with mass spectrometry. Plate positioning is simplified. The elution head has been modified and an easily accessible, exchangeable filter has been arranged in front of the valve.

Cleaning is facilitated as compared to the previous version, making it highly efficient. By pushing a button, the elution path is cleaned of matrix particles with compressed air, increasing the lifetime of the filter and preventing the system from becoming clogged. Filters are separately available and can be easily replaced without any modification to the elution head.

The CAMAG TLC-MS Interface 2 allows for rapid and contamination-free elution of TLC/HPTLC zones with online transfer to a mass spectrometer. The interface can be installed plug & play in any LC-MS system without adjustments or mass spectrometer modifications. Depending on the MS system, the presence of a substance can be confirmed via its mass spectrum, or for an unknown substance, the respective molecular formula is obtained within a minute.

Rapid separation of explosives by HPTLC



Tiên Do, CAMAG

Lisa Dunn and Hamda Ali Sultan Al Obaidly, General Department of Forensic Science and Criminology, Dubai Police HQ

Alexandre Sarbach and Marc Stulz, Swiss Federal Department of Defense, Civil Protection and Sport, Armasuisse Science and Technology

Introduction

The forensic identification of explosives is a major challenge in fields such as crime detection and investigative trace analysis. Polynitroarylenes, nitramines, and nitrate esters are major components of the explosives employed for filling warheads and shells.

This study includes hexogen (cyclotrimethylenetrinitramine, RDX), tetryl (*N*-methyl-N,2,4,6-tetranitroaniline), TNT (2-methyl-1,3,5-trinitrobenzene, trinitrotoluene), NG (nitroglycerin), PETN (pentaerythritol tetranitrate, nitropenta), HMTD (hexamethylene triperoxide diamine) und TATP (triacetone triperoxide). Conventional thin layer chromatography (TLC) has been reported several times for the separation and the detection of common explosives, however the practical use of these methods was limited because the results were not reproducible. The aim of this work was the development of a rapid, reproducible HPTLC method on silica gel plates for the simultaneous detection of seven organic and peroxide explosives with high sensitivity in field samples.

Converting and combining conventional TLC methods into a single HPTLC method significantly improved the resolution and repeatability of the identification. Moreover, HPTLC is almost 4 times faster than TLC (40 *vs* 150 min) and consumes less solvent (35 *vs* 100 mL). The analysis of real samples showed that with HPTLC the explosives can be detected even in the presence of complicated matrices/interferences. The sensitivity was improved to detect 20 ng/zone for RDX, tetryl and TNT, and 8 ng/zone for PETN and NG.

Standard solutions

RDX, tetryl, TNT at 50 µg/mL in acetonitrile – methanol 1:1, NG at 20 µg/mL in ethanol – methanol 97:3, PETN at 20 µg/mL in methanol, HMTD and TATP at 100 µg/mL in acetonitrile

Sample preparation

Cotton swabs of post-explosion samples were collected from different metal surfaces using acetone. The swabs were extracted with max. 10 mL of acetone and reduced to 200 µL under nitrogen.

Chromatogram layer

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

Sample application

Bandwise application with ATS 4, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2 µL

Note: The samples were applied in duplicate (single application on each half of the plate)

Chromatography

In the ADC 2 with 20 min chamber saturation (with filter paper) and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride, development with toluene – chloroform – ethyl acetate – heptane 9:1:1:2 to a developing distance of 70 mm (from lower plate edge), drying for 5 min

Post-chromatographic derivatization

For organic explosives (first half of plate):

Reagent 1: 5% potassium hydroxide solution in ethanol

Reagent 2 (Griess reagent): mixture of solutions A and B 1:1

Solution A: 0.05 g of α-napthylamine dissolved

in 100 mL of acetic acid 30%, solution B: 0.8 g of sulfanilic acid dissolved in 250 mL of acetic acid 30%; immersion of the plate into reagent 1, heating at 100 °C for 2 min; after image documentation, heating at 100 °C for 10 min, immersion into reagent 2, drying for 5 min in a flow of cold air

For peroxide explosives (second half of the plate):

Reagent 3: Spraying the plate (manually) with diphenylamine reagent (1 g of diphenylamine in 100 mL of concentrated sulfuric acid), drying for 5 min in a flow of cold air.

Documentation

RDX, tetryl, and TNT: under UV 254 nm prior to derivatization, and under white light after derivatization with reagents 1 and 2

PETN and NG: under white light after derivatization with reagents 1 and 2

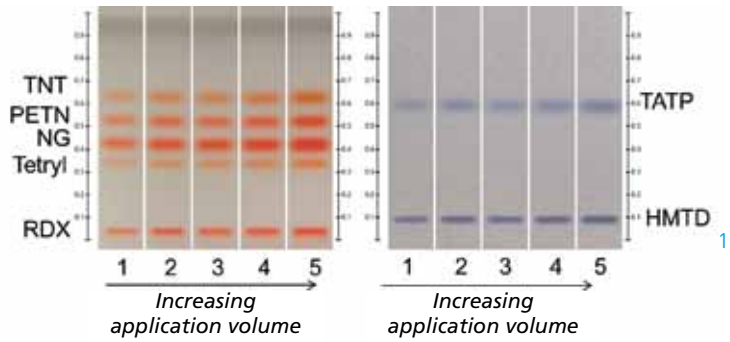
HMTD and TATP: under white light after derivatization with reagent 3

Densitometry

TLC Scanner 4 and *visionCATS*, absorbance measurement at 240 nm (for RDX, tetryl and TNT) prior to derivatization and at 480 nm (for PETN, NG, HMTD and TATP) after derivatization, slit dimension: 5 mm x 0.2 mm, scanning speed: 20 mm/s; data resolution: 50 µm/step, spectra are recorded from 190 to 450 nm

Results and discussion

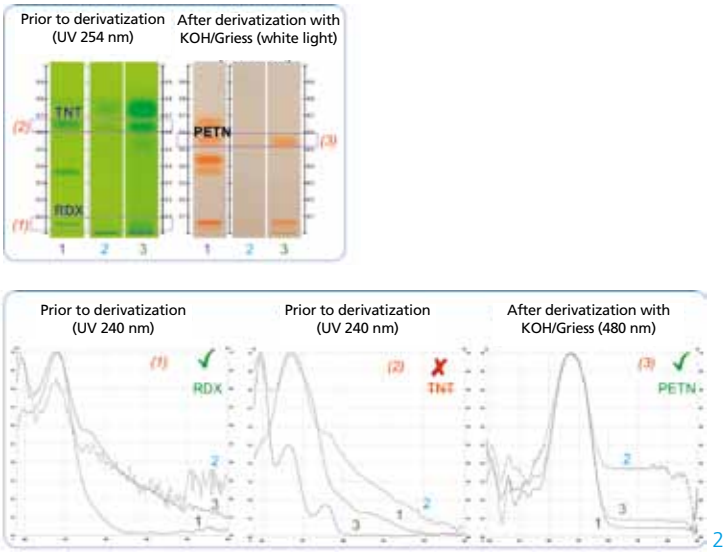
A screening methodology using complementary detection on the same plate after cutting it into halves with the SmartCut, enables the simultaneous determination of the presence or absence of organic and peroxide explosives. The proposed mobile phase provides a good separation of RDX (hR_F 0.07), HMTD (hR_F 11), tetryl (hR_F 37), NG (hR_F 45), PETN (hR_F 57), TATP (hR_F 60), and TNT (hR_F 67).



1

HPTLC chromatograms under white light after derivatization with KOH/Griess (left), and after derivatization with diphenylamine (right)

The specificity of the method was shown by analyzing standards and real samples. The identity of the target zones in the samples is confirmed by comparing R_F values and UV spectra prior to and after derivatization to standard zones.



2

Chromatograms of standards (track 1: RDX, tetryl, NG PETN, and TNT, with increasing R_F) and two post-explosion samples (tracks 2–3) and UV-spectra comparison of three selected sample zones

The presented HPTLC method allows the precise analysis and efficient evaluation of six organic and two peroxide-based explosives in several samples on the same plate as well as the densitometric quantification of RDX, tetryl, NG, PETN and TNT.

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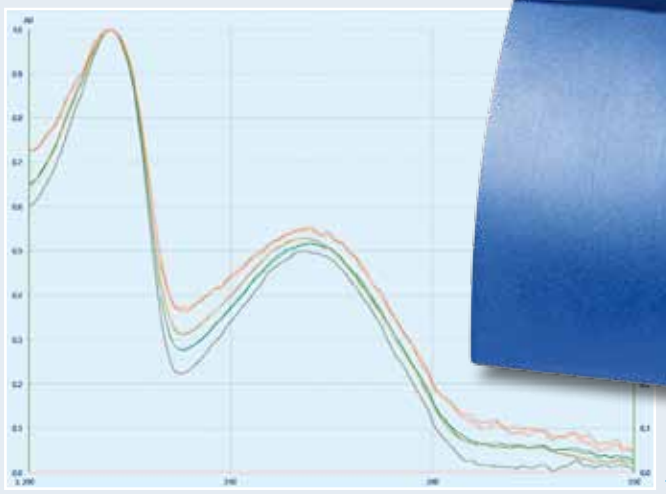
CAMAG TLC Scanner 4

Controlled by *visionCATS* HPTLC software, the CAMAG TLC Scanner 4 facilitates the densitometric evaluation of planar chromatograms.

The state-of-the-art software controls all functions of the TLC Scanner 4 and enables optimal evaluation of measured data.



1

2

HPTLC-UV spectra of koumine: overlay of sample and reference spectra (CBS 122 pp. 5–7)

Technical specifications

- Measurement of reflection, either in absorption or fluorescence mode
- Object formats up to 200 x 200 mm
- Spectral range 190–900 nm
- Automatic start of all lamps: deuterium, halogen-tungsten, and high pressure mercury lamp
- Data step resolution 25–200 µm
- Scanning speed 1–100 mm/s

visionCATS supports the following functions

- Single-wavelength scan
- Multi-wavelength scan for quantitative evaluation of differently absorbing substances in one single measuring run
- Wavelength subtraction for baseline correction
- Spectra recording for checking identity
- Scanner selftest and IQ/OQ
- 21 CFR Part 11

Further information:
www.camag.com/tlcscanner

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CBS 122 E 996.2270-25