CBS 114 • MARCH 2015

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MARCH 2015

visionCATS 2.0 – Our new software for qualitative and quantitative HPTLC analysis

Other topics of this issue: Determination of monoacylglycerides in biodiesel – Screening for PDE5-Inhibitors – Cleaning validation at API production units – New screening concept for pesticide residue analysis

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No. 114, MARCH 2015

CAMAG Bibliography Service
Planar Chromatography
Edited by Gertrud Morlock
cbs@camag.com
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Procedures, applications

Products featured in this issue

Column: Know CAMAG

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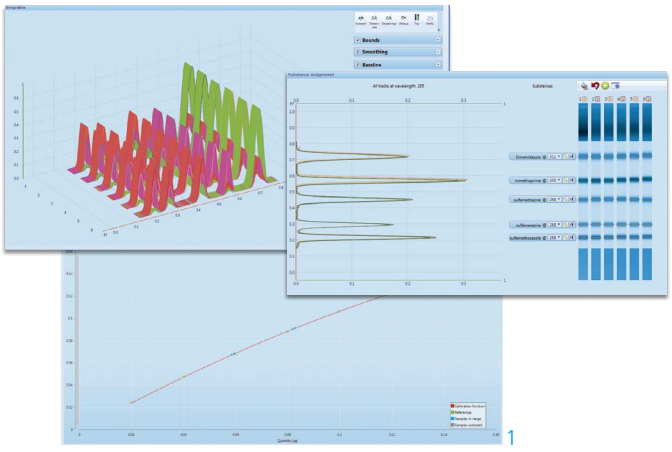
visionCATS 2.0 – Our new software for qualitative and quantitative HPTLC analysis

With the release of version 2.0 visionCATS is now complete with full capability for quantitative, densitometric, chromatogram evaluation with the TLC Scanner, thus becoming our primary software*. Only spectra recording is still under development and is expected to be released in September 2015. visionCATS's unique features for highly reproducible, sophisticated, qualitative HPTLC analysis were already presented in CBS 113.

By design visionCATS can be intuitively used, which makes any routine analysis an easy task. The software guides the user through all steps of the HPTLC analysis. Researchers who want to unlock its extensive flexibility are still free to change any parameter.

Scanning densitometry

The entire spectral range of light from 190 to 900 nm is available for selecting single wavelengths for scanning densitometry. Detection can thus be fine-tuned to match the spectral properties of the analyte to its optimized specificity and sensitivity of the detection. For almost unlimited flexibility, several scanning steps, *e.g.* before and after derivatization, can be selected. Each scanning step may also include up to 31 individual wavelengths using different light sources (deuterium lamp, mercury lamp, tungsten lamp). The detection modes "absorbance" or "fluorescence" can be combined. The generated data can then be evaluated. Integration of peaks and assignment to separated substances is a matter of a few mouse-clicks. The intuitive user interface of visionCATS provides clear guidance. Quantitation of substances can be performed via peak height or peak area. The best fitting calibration model with single-level calibration or multi-level calibration via linear, polynomial or Michaelis-Menten regression is selected. As an additional feature visionCATS 2.0 can also generate densitograms from images obtained with the TLC Visualizer.



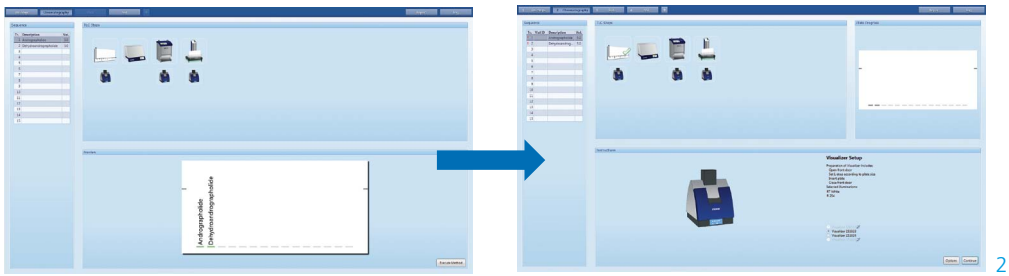
1

Scanning densitometry: 3D view and peak integration settings (top), peak assignment (right), polynomial calibration curve (bottom)

*We will continue to support winCATS for the benefit of customers using this generation of TLC Software.

HPTLC analysis – made easy

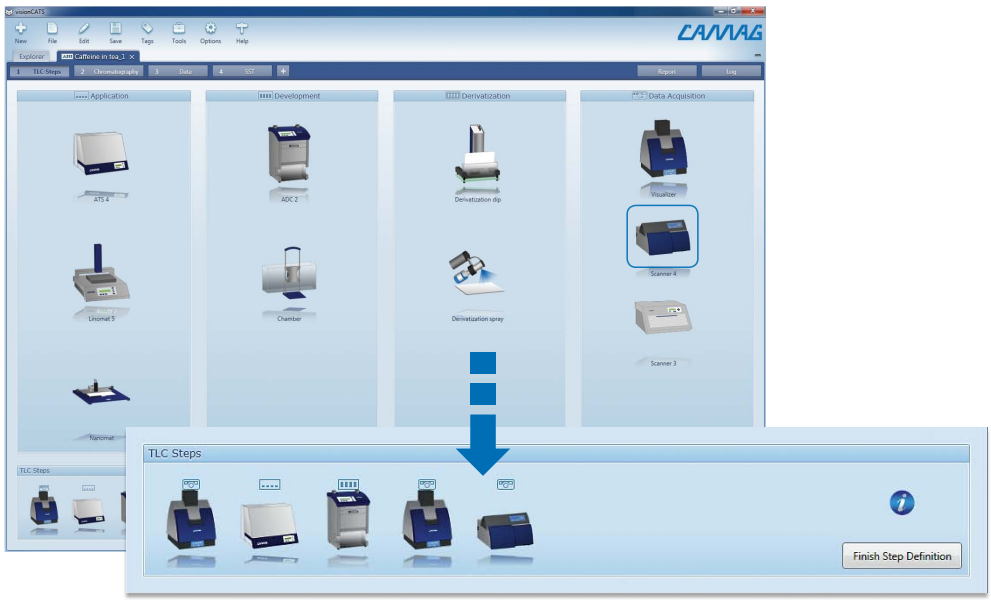
visionCATS organizes the workflow of HPTLC and controls all involved CAMAG instruments. The most convenient approach is to select one of the default methods and start working. Just fill the sequence table, select a mobile phase and the derivatization reagent. Modify detection parameters if necessary. Then visionCATS will guide you. Building your own method is easy as well by selecting the desired steps.



2

Select a method and visionCATS will guide you

The default parameters for each instrument are optimized and in compliance with the definitions of HPTLC as provided by the USP Chapter <203> and PhEur Chapter 2.8.25. Of course, the user has the choice of modifying all parameters, however, this requires additional clicks.



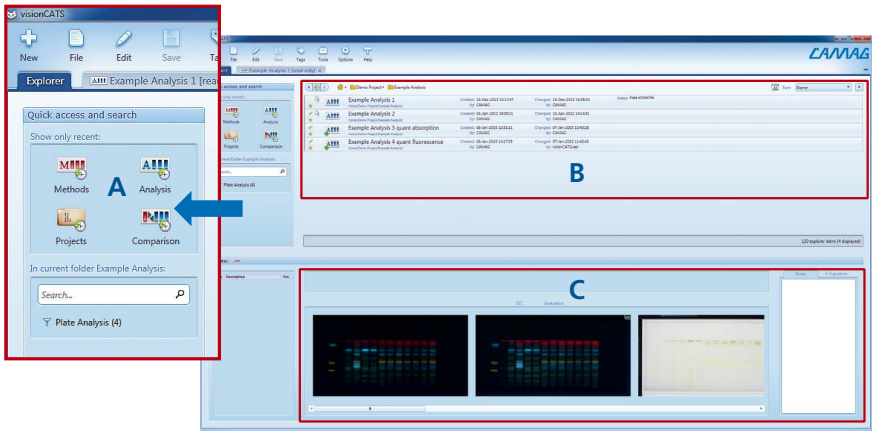
3

Create your own method with a few mouse-clicks

visionCATS offers support for routine analysis in the identification of botanicals and herbal drugs. Further information is available in the CBS 113 and in the supporting information at: **www.camag.com/cbs**

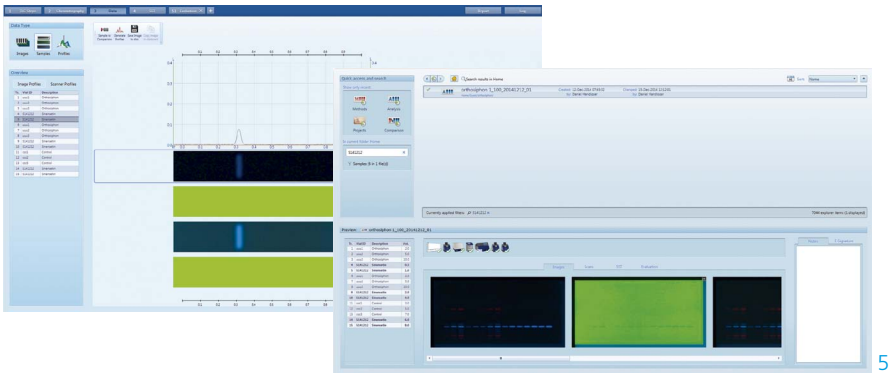
Information about any sample ever analyzed is easily retrieved

A powerful search routine located in the Explorer Tab of visionCATS finds any previous sample by its ID or name.



4

visionCATS Explorer: search entry for name, ID or keyword (A); view of file results (B); file preview of a selected analysis with the thumbnail of the captured images



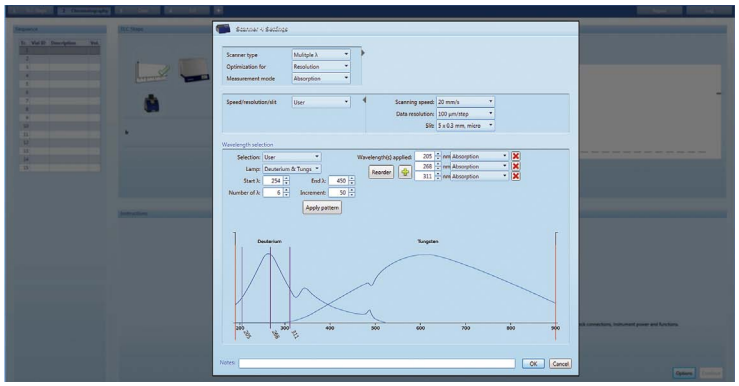
5

Sample view (left) and plate preview (right)

By simply selecting one of the previous analyses the software displays all available information for that sample. That includes images and profiles. The plate view shows the samples in the context of the entire plate.

Supporting your research

Analysts who want to utilize the full flexibility of the planar chromatographic process have access to all parameters of the HPTLC system. When all steps of the analysis are defined a right mouse click on the individual icons opens so-called expert windows.

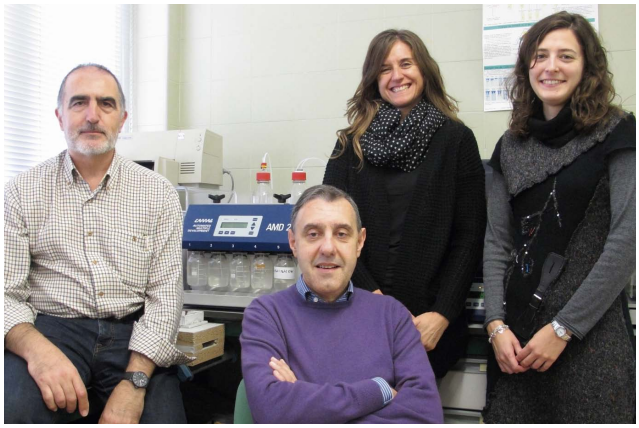


6

Expert mode allows the change of all default settings for each step and instrument , e.g. for the TLC Scanner 4

Download a fully functional version for a 60 day trial at:
www.camag.com/visionCATS

Determination of monoacylglycerides in biodiesel



From left: Dr. Luis Membrado, Dr. Vicente L. Cebolla, Dr. Carmen Jarne, María P. Lapieza

The activity of the Separation and Detection Technology group of the Instituto de Carboquímica (CSIC) in Zaragoza, Spain, has been focused on the development of original analytical techniques for characterizing lipid and/or hydrocarbon-related complex mixtures. HPTLC has rarely been used for petrochemical and bio-fuel analysis, although it is a suitable technique for these types of samples, especially when compound classes rather than individual species are to be determined. As all compounds in a sample are stored on the plate after development, a quantitative analysis is possible. This is an advantage over column chromatography, in which polar and/or high molecular weight compounds in fuels may be irreversibly adsorbed on the stationary phase.

Introduction

Biodiesel is a lipid-based alternative fuel mostly composed of methyl esters of fatty acids. It is used as a total or partial substitute for petroleum-derived diesel fuel, either in its pure form (B100), or blended with diesel fuel in different proportions (BX, with X being the volume percent of B100 in the mixture) without requiring any essential modification in the ignition engines. A fatty-acid methyl ester content lower than 98 wt% indicates inappropriate reaction conditions for biodiesel production, and therefore, the presence of impurities in the final product. One impurity class in biodiesel are monoacylglycerides which can produce obstruction in fuel filters. The European standard UNE EN 14214:2013 establishes that their maximum concentration in BX has to be 0.8 wt% [1].

As a modular and flexible technique, HPTLC-based hyphenated systems can be designed by combining different approaches concerning chromatographic development systems and detectors in a sequential mode. A hyphenated procedure is reported that provides quantitative determination of the monoacylglycerides as a compound class in the BX sample as well as the BX composition profile, if so desired all obtained from a single plate.

Layer

HPTLC plate silica gel 60, 20 x 10 cm (Merck) prewashed by development with tetrahydrofuran up to 90 mm

Standard and sample application

Bandwise application of the 1 mg/mL monoacylglyceride standard (0.1–2.5 µL) and 100 mg/mL BX sample (25 µL) using the Automatic TLC Sampler (ATS 4)

Chromatography

3-Step development with an AMD 2 system, starting with 100 % *t*-butyl methyl ether (up to 40 mm), followed by dichloromethane – *n*-heptane 4:1 up to 60 mm and then 3:2 up to 90 mm as final migration distance

Post-chromatographic derivatization

The plate was immersed into a 0.02 % methanolic solution of primuline using the Chromatogram Immersion Device, immersion time 2 s and immersion speed 5 cm/s.

Densitometry

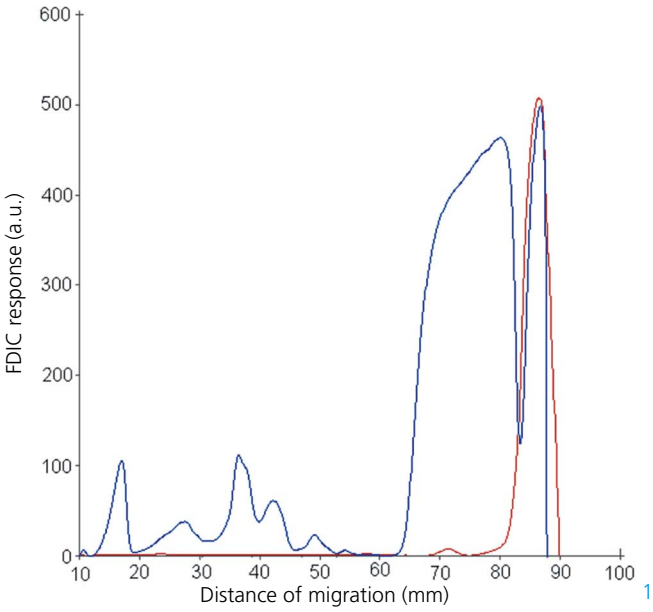
Fluorescence measurement at 366/>400 nm using the TLC Scanner 3

Mass spectrometry

Zones were eluted with methanol at a flow of 0.2 mL/min via the TLC-MS Interface (oval elution head 4 x 2 mm) into an ion-trap MS (Bruker Esquire 3000 Plus) operating in positive ESI mode (ESI^+). Blanks of the plate background were recorded as well.

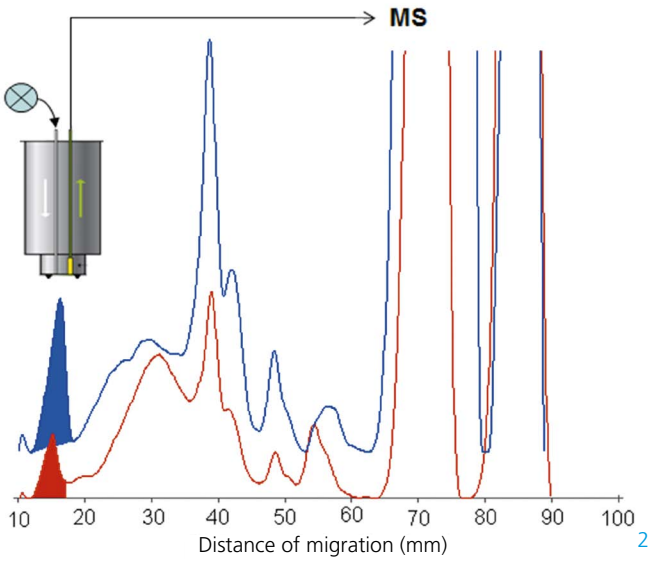
Results and discussion

The AMD 2 densitogram showed the monoacylglyceride peak of BX at the migration distance of 15–20 mm. Other lipids detected were diacylglycerides, triacylglycerides, fatty acids (20–60 mm) and fatty acid methyl esters (60–83 mm), the preponderant chemical class. The sample peak at 86 mm migrated in the same way as pure diesel, corresponding to the diesel component in the blend.



1

Densitogram comparison of B50 (5000 µg/band, blue) and pure diesel (2500 µg/band, red)

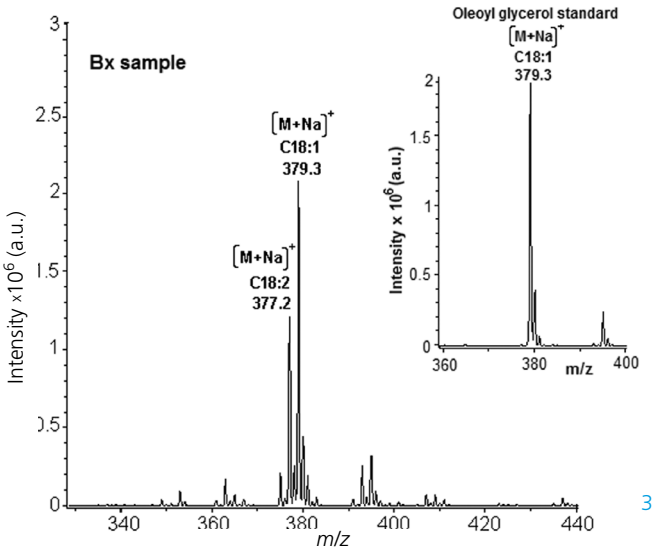


2

Densitogram comparison of B5 (red) and B20 (blue), both 5000 µg/band: monoacylglyceride bands at 16 mm were eluted via the TLC-MS Interface into the ESI-MS

The sum peak area of the monoacylglycerides increased with the proportion of B100 in BX, as visible in the densitogram of B5 and B20.

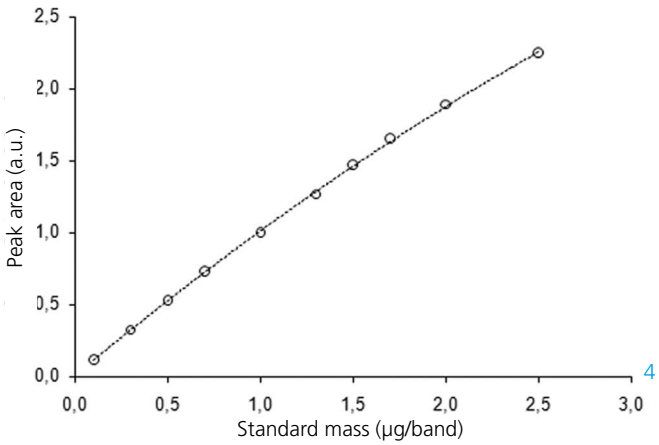
The post-chromatographic derivatization with the primuline reagent (termed fluorescence detection by intensity changes, FDIC [2]) is a non-covalent interaction, generating an increased fluorescence of molecules with long hydrocarbon chains (e.g., lipids and saturated hydrocarbons). This adsorption did not impair subsequent MS recordings. After derivatization, the monoacylglyceride band (16–20 mm) of the B100 sample was transferred to ESI-MS using the TLC-MS Interface and compared with the corresponding monoacylglyceride standard. The mass signal at *m/z* 379.3, which corresponded to 1-oleoyl glycerol, was the most abundant signal in the analyzed sample. The HPTLC-ESI spectrum displayed also other ions corresponding to further monoacylglycerides in the sample. Thus, the fatty acid profiles of the corresponding monoacylglycerides in BX can be obtained, helpful for identifying the origin of the lipids in the biodiesel (*i.e.* vegetal, animal, waste cooking oil).



3

HPTLC-ESI-MS spectra of the monoacylglyceride band in the B100 (16 mm) sample and of the oleoyl glycerol standard

Quantitation of monoacylglycerides was performed via the 1-oleoyl glycerol standard. The proposed method was suited for determination of monoacylglycerides in BX (X≥5) at low concentrations according to current standards.



4

Polynomial calibration curve of the oleoyl glycerol standard ($y = -0.070\ x^2 + 1.077\ x + 0.006$; $r^2 = 0.999$)

References

[1] Liquid petroleum products – fatty acid Methyl esters (FAME) for use in diesel engines and heating applications – Requirements and test methods. UNE-EN 14214:2013

[2] V.L. Cebolla *et al.* ChemPhysChem 13 (2012) 291

Further information is available on request from the author.

Contact: Dr. Vicente L. Cebolla, CSIC, Instituto de Carbochímiqa, c/Miquel Luesma, 4, 50018 Zaragoza, Spain, vcebolla@icb.csic.es

Acknowledgment

Thanks to the Spanish Ministerio de Economia y Competitividad (MINECO) and FEDER (UE) (Plan Nacional de I+D+I, project CTQ2012-035535) and to DGA-ESF for financial support. MPL thanks MINECO for the grant BES-2013-063673.

5

CAMAG Linomat 5

Precision of the applied volume, exact positioning and compactness of the application zones are decisive for the quality of the analysis.

With the Linomat samples are sprayed onto TLC/HPTLC plates in the form of bands with nitrogen or compressed air. This permits the application of larger sample volumes than is possible with contact sample transfer, since the solvent almost completely evaporates during the process. Even when strongly polar solvents are used, the application zones remain compact and narrow, ensuring the highest resolution attainable.

Sample application is automatic, only changing the sample (filling, inserting and rinsing the syringe) is manual. The self-adjusting plate support allows the use of layers differing in thickness without re-adjusting the spray nozzle.

New face in Scientific Business Development

Dr. Melanie Broszat (34)

I have been working at CAMAG as Scientific Business Development Manager since April 2014. After studying Process Engineering at the University of Applied Sciences in Offenburg, Germany, I worked as an engineer in different research fields together with Prof. Dr. Bernd Spangenberg on the development of HPTLC methods in the food and environmental application fields. Subsequently, I did my Ph.D. in molecular biology and environmental microbiology under the supervision of Prof. Dr. Elisabeth Grohmann at the Albert-Ludwigs-University in Freiburg, Germany, which I successfully finalized at the end of 2013.

In my current position I am trying to understand the different needs of HPTLC users worldwide in various application fields from a scientific point of view. This new knowledge will be important in the future development of applications and new instruments. Thus, I see myself as a link between our internal departments (Development, Laboratory, Sales and Marketing) and our distributors and the HPTLC experts from Research and Industry. The scientific evaluation of our markets, the build-up of an HPTLC-expert-network, the training of our distributors and the organization of seminars and symposia for our customers worldwide are other goals that I foresee for my position.

First results of my work at CAMAG can be seen in the recently uploaded HPTLC Case Studies located on our website under "Application Fields". Here, typical analytical tasks are addressed, which can be performed in a faster, easier, cheaper and/or better way by HPTLC. Application videos are provided for practical support. Additionally, I was assigned the exciting job to coordinate the CBS. Therefore, I am always searching for interesting applications and I am also looking forward to all requests about suspenseful topics.

Stay curious and let yourself continually be inspired by the versatility of Planar Chromatography!

Dr. Melanie Broszat
Scientific Business Development Manager

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MARCH 2015

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

The abstracts of 108 papers have been added from this CBS to the CCBS database. Diverse compound groups were investigated and reported, although botanicals seem to be a leading group. The mode of application of the method is also diverse and the effectiveness of the TLC/HPTLC method presented has to be reflected carefully. For example, papers can still be found that scrape off spots from the plates, *e.g.* for photometric quantification.

On the other hand densitometers are increasingly used, and validation details are reported in the papers. Nevertheless, there seems to be a need for information on quantitation details. For example, LOD and LOQ values reported may likely not be 33 ng/zone and 36 ng/zone, respectively. Or every once a while, absorbance measurement at UV 254 nm is reported for compounds not having an absorbance maximum at 254 nm due to the non-reflected transfer of the wavelength from a prior image evaluation at UV 254 nm. In contrast, having the maximum wavelength of each compound ensures the most robust and most sensitive quantitative determinations and is definitely the best option. Test it to see the difference and avoid future user mistakes with regard to quantitation!

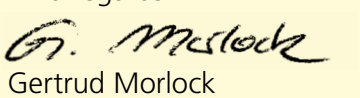
There is a need for quantitative method comparisons in which HPTLC is adequately and correctly applied. In this context it is valuable to point out the good accuracy of the HPTLC method, which has been determined in a paper on stachydrine quantitation in *Leonurus* species. Quantitative NMR (qNMR) is a technique of utmost reliability and was employed for this comparison with instrumental HPTLC. The HPTLC results for the stachydrine content in the samples were found to be in high accordance with independently performed 1H-qNMR measurements. *Quod erat demonstrandum!*

Dear friends

Since the introduction of the Cumulative CAMAG Bibliography Service (CCBS) online search in September 2014, the access rate to the database has been three times higher when compared to downloads of the previous CCBS database file. Hence, we may conclude that the transfer to an online database with open access to the abstracts was a fine step forward. Details for the online search, you will find on Yellow Page 3. Remarks on the abstracts newly added to the CCBS database, can be found on this left side.

On the last Yellow Page, we have compiled the latest books in the field. Often the discussion occurs whether it is a TLC or an HPTLC method and the differentiation seems to be at one's own discretion. For instance, authors use HPTLC as a synonym for instrumental TLC. However for HPTLC papers, an advanced coating material (*e.g.*, lower particle size and narrower particle size distribution) combined with advanced instrumentation for most of the steps are precondition. This agreement was approved during a meeting of the leading scientists in that time*. Whereas the main category "planar chromatography" is defined as a "separation technique in which the stationary phase is present as or on a plane" according to the International Union of Pure and Applied Chemistry (IUPAC). Note that methods dealing with non-separated samples, only applied on a TLC or HPTLC plate (used as a carrier for bioassays or MS recordings), may not be termed as TLC/HPTLC methods. In any case we are proud to have highly engaged scientists, and other books are in process. We will keep you informed!

Kind regards



Gertrud Morlock
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*R. Kaiser, A. Zlatkis (Eds.), High Performance Thin-Layer Chromatography, Elsevier Scientific, Amsterdam, The Netherlands, 1977, pp. 9–13

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
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    a) Synthetic dyes
    b) Chloroplasts and other natural pigments
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    c) Drug monitoring
    d) Toxicological applications
    e) Plant extracts, herbal and traditional medicines
    f) Clinico-chemical applications and profiling body fluids
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    b) Anions
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35. **Other technical products and complex mixtures**
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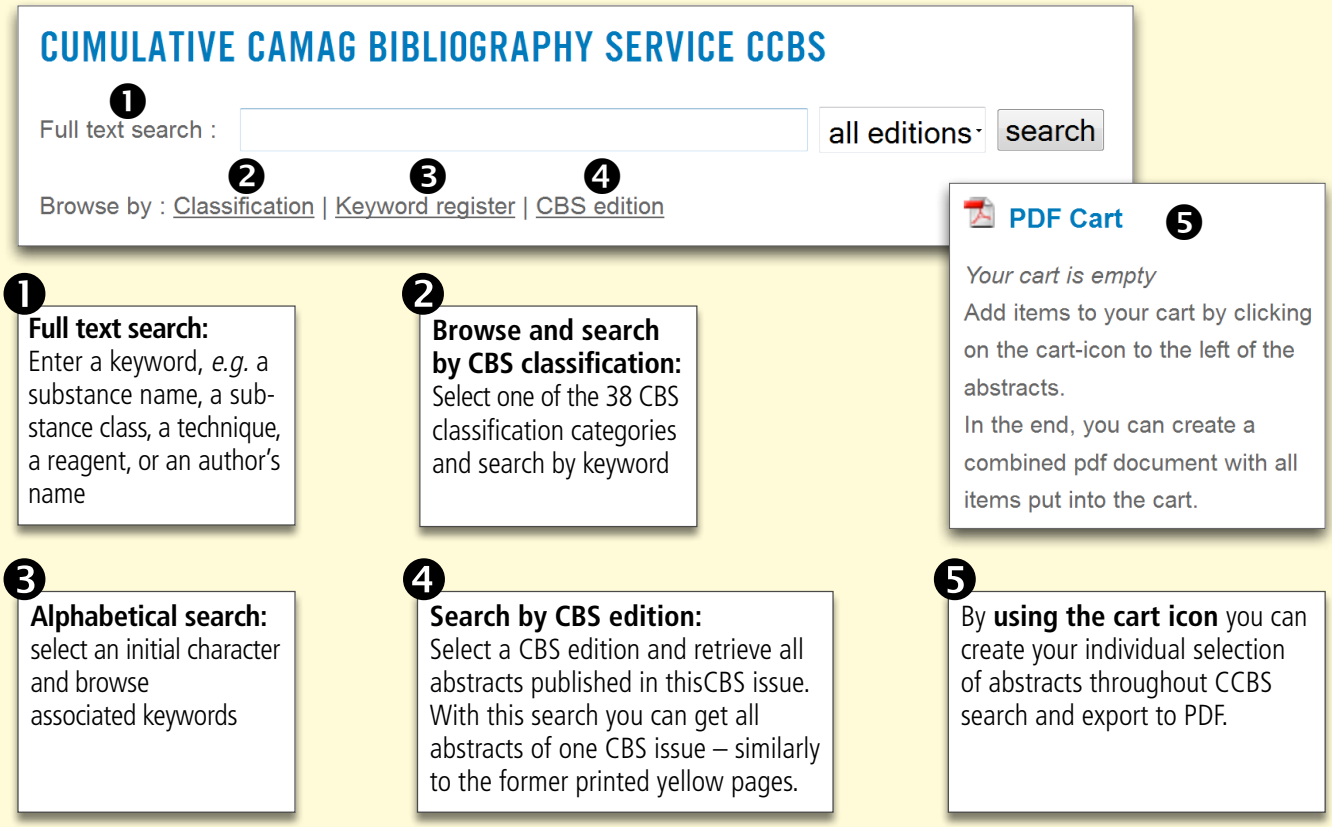
    b) Antioxidants and preservatives
    c) Various specific technical products
    d) Complex mixtures and non-identified compounds
36. **Thin-layer electrophoresis**
37. **Environmental analysis**
    a) General papers
    b) Air pollution
    c) Water pollution
    d) Soil pollution
38. **Chiral separations**

XX. (abstract number underlined) refers to HPTLC related publication or application using HPTLC materials

Cumulative CAMAG Bibliography Service (CCBS) Online Search



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

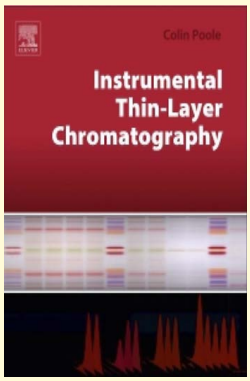
The database covers most relevant scientific journals in the field of Planar Chromatography including also various non-English publications in German, French, Spanish, Portuguese and Chinese. The CCBS features additional practical information for the analyst in the lab, for example details on the mobile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

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

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

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

Recommended books



Colin Poole:
Instrumental Thin-Layer Chromatography (2014)
Published by Elsevier
ISBN: 9780124172234

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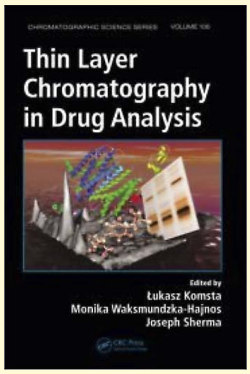

Lukasz Komsta,
Monika Waksmundzka-Hajnos,
Joseph Sherma:
Thin Layer Chromatography in Drug Analysis (2013)
Published by CRC Press
ISBN 9781466507159

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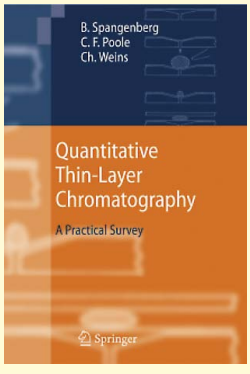

Bernd Spangenberg,
Colin F. Poole, Christel Weins:
Quantitative Thin-Layer Chromatography
A Practical Survey (2011)
Published by Springer
ISBN 9783642107276

A German version was published by B. Spangenberg in 2014,
ISBN 9783642551017

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Screening of three PDE5-Inhibitors and eight of their analogs in lifestyle products

CAMAG Laboratory team (from left): Tiên Do, Dr. Eike Reich, Valeria Widmer, Eliezer Ceniviva, Debora Frommenwiler, Ilona Trettin, Dr. Anita Ankli, Daniel Handloser

Introduction

The sale of "Natural Aphrodisiacs" seems to be a prospering business. Frequently these products are adulterated with synthetic phosphodiesterase type 5 inhibitors (PDE5-Is), including the active principles of Viagra (sildenafil), Levitra (vardenafil), and Cialis (tadalafil). Not only are these regular pharmaceuticals found in lifestyle products, but also their unapproved analogs such as hydroxyacetildenafil, homosildenafil, thiohomosildenafil, acetildenafil, acetaminotadalafil, propoxyphenyl hydroxyhomosildenafil, hydroxyhomosildenafil, and hydroxythiohomosildenafil [1]. Because of their significant potential of causing serious health risks, PDE5-Is has got the attention of the US Food and Drug Administration (FDA) and other authorities [2]. The United States Pharmacopoeia has decided to draft a general chapter <2251> "Adulteration of Dietary Supplements with Drugs and Drug Analogs" and has called for suitable methods [3].

In response to the call, we have developed a comprehensive, yet straightforward, HPTLC approach including several levels of confirmation. HPTLC can be used for rapid screening of commercial products for adulteration with eleven known PDE5-Inhibitors. It is applicable to a variety of finished products without interference from matrix and excipients. The adulterants may be confirmed by hR_F values and UV spectra compared to those obtained with reference substances. The use of the TLC-MS Interface can further confirm the identity of known adulterants even if no reference substances are available.

Chromatogram layer

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

Standard solutions

Methanolic solution of reference substances (0.2 mg/mL)

Sample preparation

One unit of each lifestyle products (*e.g.*, powdered pill, content of a capsule, chewing gum, chocolate) was crushed in a mortar, then extracted in an ultrasonic bath with 10 mL of methanol for 30 min, and centrifuged. The supernatants were used.

Sample application

Bandwise with Automatic TLC Sampler 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 3 µL

Chromatography

In the ADC 2 with chamber saturation (with filter paper) for 20 min and after plate conditioning at 47 % relative humidity for 10 min using a saturated solution of potassium thiocyanate, development with *t*-butyl methyl ether – methanol – ammonia (28 %) 20:2:1 up to the migration distance of 70 mm (from lower plate edge), drying for 5 min.

Documentation

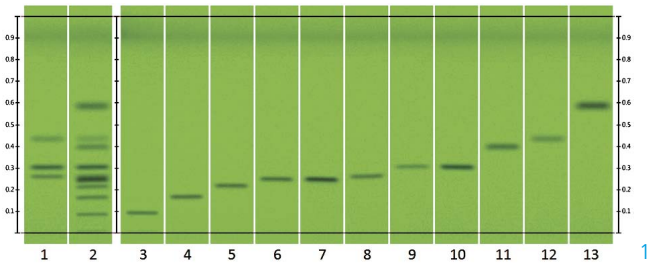
With the TLC Visualizer under UV 254 nm and UV 366 nm

Densitometry

TLC Scanner 4 and winCATS, absorption measurement at 232 nm, slit dimension 5.00 × 0.30 mm, scan speed 20 mm/s, spectra recording from 190 to 550 nm

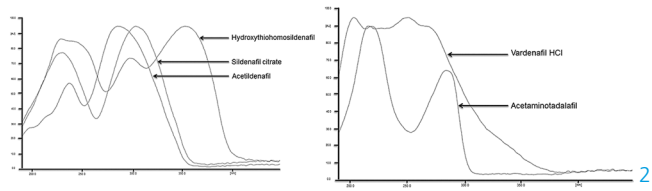
Results and discussion

The recently developed mobile phase was successfully applied to the separation of eleven available PDE5-Is [4]. Only two sets of substances with critical hR_F values were found: acetildenafil (track 6), hydroxythiohomosildenafil (track 7), and sildenafil (track 8) as well as acetaminotadalafil (track 9) and vardenafil (track 10) had similar hR_F values and were not separated. However, their UV spectra were distinctly different.



1

Chromatogram of SST (track 1: sildenafil, vardenafil, tadalafil), reference mixture (track 2) and reference substances under UV 254 nm)

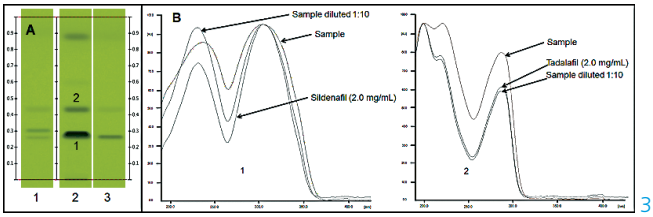


2

Overlaid UV spectra of the two sets with critical hR_F values

For the qualification of the chromatographic results obtained in the screening of lifestyle products, each plate was submitted to a system suitability test (SST). Each sample was visually evaluated for the presence of zones matching in hR_F value with those of the previously analyzed reference substances. UV spectra of suspect zones were recorded and compared with those of the reference substances.

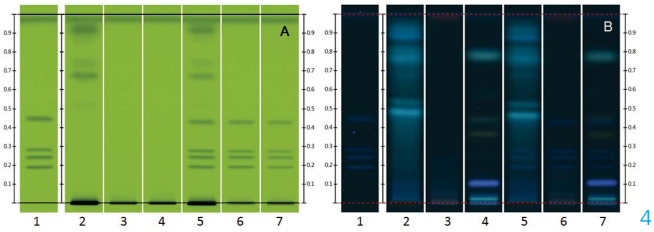
One of the samples was found to contain two zones matching the hR_F values of (1) sildenafil and (2) tadalafil. The UV spectra of the zones did not match, but it was suspected that this was caused by the high concentration of the target zones. Spectra obtained after chromatography of a 1:10 diluted solution of the sample gave a better match and confirmed the presence of the suspected drugs. The identity of the two substances was further confirmed by mass spectrometry using a TLC-MS Interface and Advion Expression CMS with electrospray ionization.



3

(A) Chromatogram of a Triple Miracle Zen Platinum capsule (track 1: SST; track 2: sample (1 capsule/10 mL); track 3: 1:10 diluted sample, (B) UV spectra of zones (1) and (2) at different concentrations and standards

Some plants, such as *Eurycoma longifolia, Tribulus terrestris*, and *Pausinystalia johimbe* have an established traditional medical use in the treatment of erectile dysfunction. To verify the specificity of the proposed method, plant extracts were spiked with PDE5-Inhibitor substances. Polar plant components do not migrate under the proposed conditions. All PDE5-Is are sufficiently resolved from excipients and plant matrix.



4

Chromatogram under (A) UV 254 nm and (B) UV 366 nm, track 1: sildenafil, propoxyphenyl hydroxyhomosildenafil, homosildenafil, hydroxyhomosildenafil with increasing hR_F, tracks 2–4: plants extracts, and tracks 5–7: spiked plant extracts

Twenty-four commercial products, provided by USP and suspected to contain PDE5-Is, were tested together with 21 additional commercial products purchased in local markets. Sildenafil, tadalafil, propoxyphenyl hydroxyhomosildenafil, and dimethylsildenafil (aildenafil) were found in 31 of those products without being declared on the product label.

Further information is available from the authors.

[1] Singh *et al.*, Tr Anal Chem 28 (2009) 13–28

[2] Nickum and Flurer, J Chromatogr Sci 24 (2014) 38–46

[3] Cohen *et al.* J Am Med Assn 312 (2014) 1691–1693

[4] Caprez, Development of methods for analysis of synthetic adulterants in herbal medicines by HPTLC, Diploma thesis 2005

[5] Do *et al.*, J. AOAC, in submission

Contact: Tiên Do, CAMAG, Sonnenmattstrasse 11, 4132 Muttenz, Switzerland, tien.do@camag.com

Cleaning validation at API production units

Lionel Briffa

At the Sanofi-Chemistry production site at Aramon, France about 60 active pharmaceutical ingredients (APIs) are produced by organic synthesis, plant extraction and biotechnology. These APIs are used in pharmaceutical formulations for the treatment of various diseases such as metabolic disease, cardiovascular disease, cancer, and disorders of the central neurologic system.

Introduction

At industrial sites when production cycles change for different ingredients, cleaning becomes essential to ensure the quality of the products and the elimination of the risk of cross-contamination. During regulatory inspection of facilities where industrial cleaning is so critical, authorities are increasing requirements, in particular on the concept of cleaning validation. Cleaning validation provides evidence that the cleaning process of the industrial equipment was successful and that all residue amounts from the previous production process have met maximum specifications for the specific API.

HPTLC is ideal for cleaning validation because it is inexpensive and time-saving. All samples can be simultaneously determined in less than one hour. The HPTLC methods established at our laboratories are simple, rapid, robust, accurate, sensitive and precise.

Chromatogram layer

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

Standards

Usually aqueous solutions with a concentration of 100 µg/mL

Sample preparation

Industrial installations were cleaned by successive fillings with cleaning solvent. The cleaning was completed by a mechanical action, reflux distillation and wash nozzle. The sample (250 mL) was directly levied at the time of the emptying of the equipment and used for HPTLC without any sample preparation. The specification (maximum acceptable concentration) was calculated on the basis of the maximum allowable carryover (MACO) and the volume of the equipment (Supporting Information).

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 16 to 20 tracks, band length 6 mm, track distance 10 mm, distance from the side 20 mm, distance from lower edge 8 mm, usual application volumes between 0.5 and 2 µL for standard solutions and between 0.5 and 100 µL for samples solutions according to the respective specification (Supporting information).

Chromatography

In the ADC 2 with chamber saturation (usually for 3 min), migration distance 50 mm from lower plate edge. Prior to development, plates were conditioned at 50 % relative humidity for 5 min using a saturated solution of potassium thiocyanate. The mobile phase depended on the method selected due to the different polarities of the compounds.

Densitometry

TLC Scanner 3 with winCATS software, spectra recording from 200 to 700 nm for identification (Supporting information), quantification by absorption measurement at the specific wavelength for each target analyte, slit dimension 5.00 x 0.45 mm, scanning speed 20 mm/s, polynomial calibration by peak height

Results and discussion

The conformity of a cleaning was assessed on two criteria:

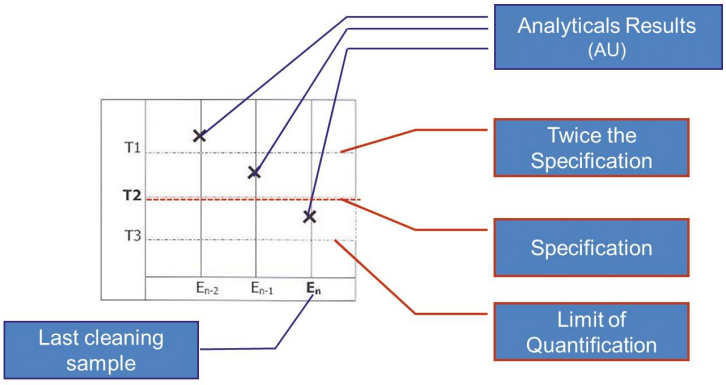
1) The last 3 rinses were controlled to verify the effectiveness of the cleaning. This efficiency was assessed and evident by the gradual decrease of the residual product concentration in the different samples.
2) In the last rinse, the residual product concentration in the sample had to be below the respective specification.

Each sequence consisted of three samples per cleaning, one sample of solvent of "white" rinse (the solvent blank used for the last rinsing) and standards. Three standard levels were used to facilitate the assessment, *i.e.* the identification of the target substances (the APIs) and evaluation of compliance: one standard level corresponding to

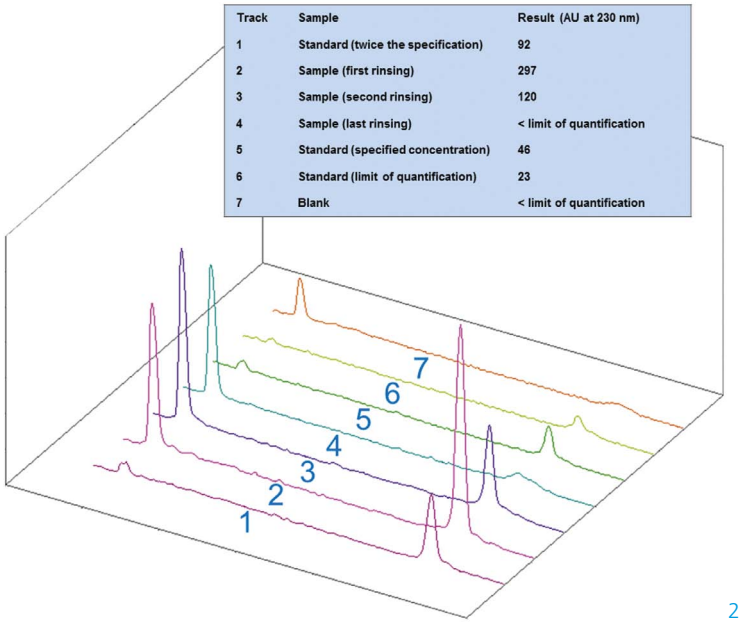
- the limit of quantification (T3)
- the specification (T2)
- twice the specification (T1)

Conclusions

HPTLC as routinely used at our site meets our expectations for validation and control of industrial cleanings. The method was simple and quick. Results obtained by densitometry were precise. Our developed methods for cleaning validation allow the detection of product traces down to ≤ 0.03 µg/mL without prior concentration of the sample. The specificity was identical to that of an HPLC method.



1



2

Example of a densitogram at 230 nm for cleaning validation

Further information is available from the author on request

Contact: Lionel Briffa, Contrôle Qualité, Sanofi-Chimie France, Usine d'Aramon, Route d'Avignon, 30390 Aramon, France, lionel.briffa@sanofi.com

Supporting information at: www.camag.com/cbs

New screening concept for pesticide residue analysis in fruit and vegetables – HTpSPE-HRMS

Dr. Claudia Oellig, Prof. Dr. Wolfgang Schwack

The major research topic of Professor Schwack, University of Hohenheim, Stuttgart, deals with method development in multi-residue analysis of pesticides. Automatization of the extraction process, optimization of methods for the special class of dithiocarbamate fungicides, development of new clean-up strategies and screening methods for pesticide residues are considerations in his research.

Introduction

Non-target substances can be analyzed with high resolution mass spectrometry, although not with the commonly used "target" MS/MS methods. An effective and efficient clean-up thereby is undoubtedly the best way to prevent matrix effects, mostly occurring as signal suppression in mass spectrometry (MS), thus providing reliable results in pesticide residue analysis [1]. A high-throughput planar solid phase extraction (HTpSPE) clean-up for extracts of fruit and vegetables [2] and tea [3] was used for a rapid screening in the analysis of pesticide residues. For the simple target and non-target screening, HTpSPE extracts were analyzed with a time-of-flight MS (TOFMS) without a chromatographic separation.

The new HTpSPE–TOFMS screening was tested with a pesticide mixture of different substance classes in several plant matrices. All spiked pesticides were correctly identified by a target oriented database search and recovered close to 100%. Compared to dispersive solid phase extraction (dSPE) clean-up, false-positives or false-negatives were rare and matrix effects were almost completely eliminated. Additionally, a non-target search provided a mass list of all unknown substances of the sample. Finally, the method performance of the new screening could be proven with several real samples, when the identified pesticides were identical to those determined by LC–MS/MS analysis [4].

Sample extracts

Organic fruit and vegetable samples (10 g) were extracted with acetonitrile (10 mL) according to the QuEChERS method [5]. For phase separation, a buffer salt mixture of 4 g magnesium sulphate (anhydrous), 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate, and 0.5 g di-sodium hydrogen citrate 1.5-hydrate was used.

Pesticides and internal standards

Raw sample extracts were spiked at a level of 0.5, 1.0 or 2.0 mg/kg with a mixture of seven representative pesticides (acetamiprid, azoxystrobin, chlorpyrifos, fenarimol, mepanipyrim, penconazole, and pirimicarb). As internal standard, tris(1,3-dichloroisopropyl)phosphate (TDCPP) was used for quantitation and Sudan II as visible marker for the pesticide zone.

Chromatogram layer

TLC aluminum foils silica gel 60 NH_2 F_{254}s (Merck), 20 × 10 cm, two times prewashed by chromatography with acetonitrile (19.5 cm), each time dried for 10 min in a fume hood, then cut at 10 cm in the prewash-direction

Layer pre-treatment

Dipping the TLC aluminum foils (20 × 10 cm) in a 2 % formic acid solution in acetonitrile with the TLC Immersion Device, immersion depth 2 cm, immersion speed 2 cm/s, immersion time 0 s, drying for 10 min

Sample application

Rectangular application (length 3.0 mm, height 4.0 mm, 10 areas) with Automatic TLC Sampler 4 (ATS4), track distance 14 mm, distance from the left side 23 mm, distance from lower edge12 mm, application volume 50 µL

HTpSPE

In the Automatic Developing Chamber (ADC2) with 10 mL acetonitrile, migration distance 75 mm (migration time 10 min), drying 5 min; 2nd development in the backwards direction with 10 mL acetone, migration distance 45 mm (migration time 3 min), drying 3 min. The plate activity was equilibrated for 5 min before each development with $MgCl_2$ (33% relative humidity).

Documentation

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

HTpSPE and µL-flow injection analysis (µL-FIA)–TOFMS

Elution of the target analyte zone with TLC–MS Interface into autosampler vials with acetonitrile – 10 mM ammonium formate 1:1, flow rate 0.2 mL/min, elution time 60 s. A HPLC system (Agilent) with a capillary pump (flow rate 2 µL/min) and a micro well-plate autosampler (injection volume 2 µL) were coupled via a PEEK capillary and a nano-electrospray ionization interface (nano-ESI) to a TOFMS (LECO) without an analytical column.

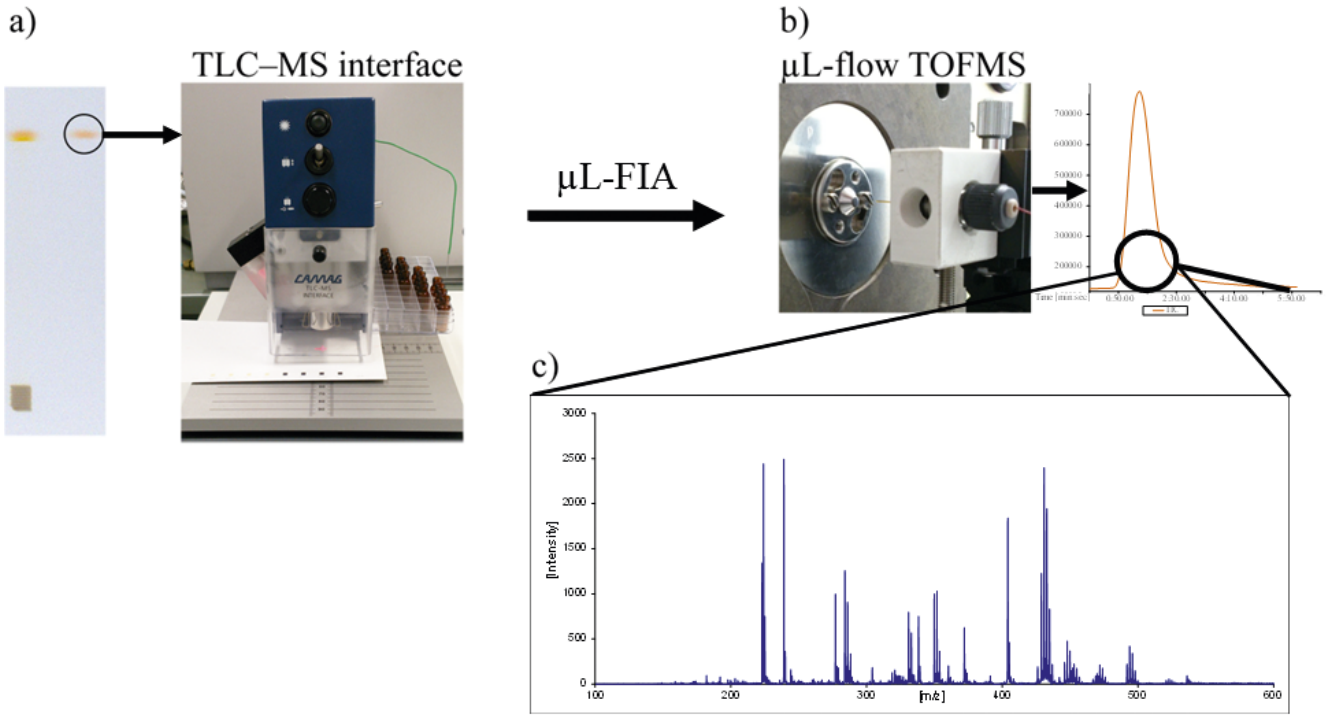
Results and discussion

HTpSPE was performed on TLC amino foils to separate the matrix from pesticides and focus them into a sharp zone (same hR_F-value). The target zone (pesticides, visible by the addition of Sudan II) was eluted by the TLC–MS Interface into vials and analyzed by µL-FIA without a chromatographic separation using a TOFMS. This strategy resulted in a single sample peak in the full scan FIA chronogram (showing subsequent MS recordings) with a single mass spectrum for each sample, revealing all pesticides as well as all further substances present in the extract. Thereby all pesticides are simultaneously detected and unequivocally identified by their exact masses.

The new µL-FIA–TOFMS approach was developed for a simple and rapid screening in pesticide residue analysis, when matrix free extracts were strongly required. In this respect, the efficient and fast HTpSPE clean-up [2, 3] is the superior alternative to the currently applied (d)SPE methods in pesticide residue analysis [1, 5]. Thus, the new screening concept was successfully introduced for several pesticides of different substance classes in different plant matrices. After HTpSPE, there was nearly no difference in both, the amount and the intensity of the mass signals of spiked cucumber extracts for example (spectrum b) and the standard mixture solution (spectrum d). The matrix load of spiked cucumber extracts was significantly lower after HTpSPE, than after dSPE (spectrum a); only the mass signals of the spiked pesticides were present. Regarding the clean-up efficiency for tomato, apple and grape extracts, identical results were generally obtained.

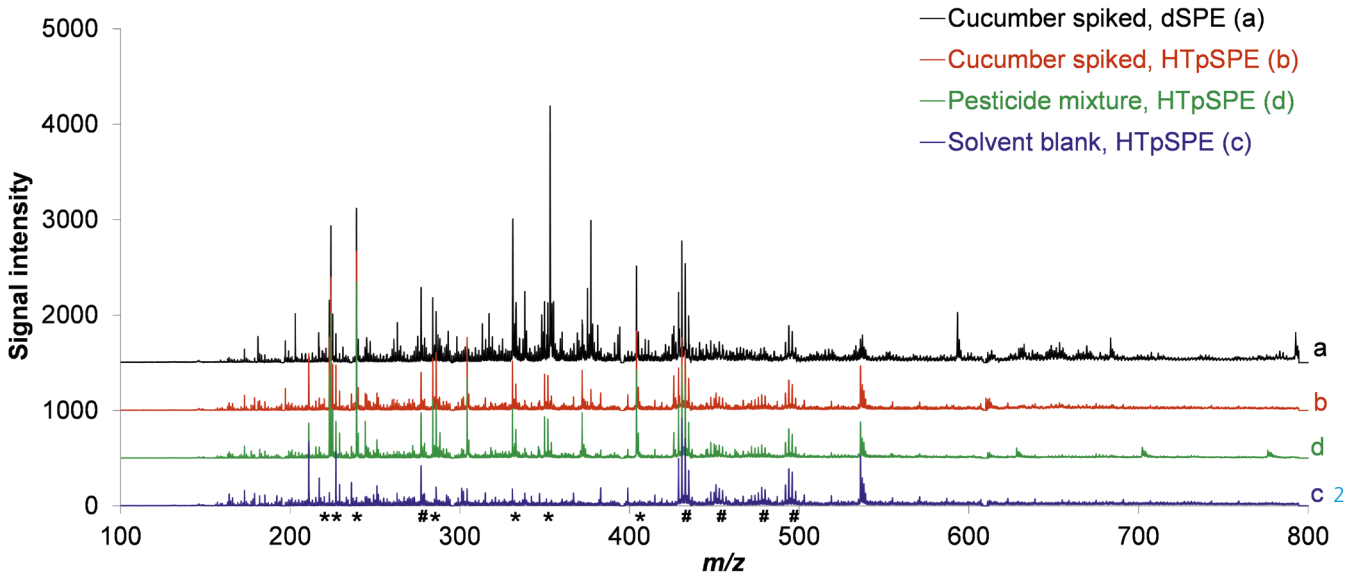
The method performance was assessed by recovery experiments with a pesticide mixture spiked into fruit and vegetable extracts. Mean recoveries between 86 % and 116 % with %*RSDs* of 1.5–10 % ($n = 5$), calculated via the standard mixture solution, clearly demonstrate the new screening as reproducible without the loss of pesticides. With the help of a mass database searching tool, all spiked pesticides were correctly identified. In blank and spiked HTpSPE extracts, only very low numbers of false-positive or false-negative findings occurred. An additionally implemented non-target screening approach offered a mass list of all charged substances, which were present in the sample extracts, but not included in the mass database (unknown substances). This option is only possible, because the whole mass information of a sample is provided by a single mass spectrum.

Finally, the new screening was successfully applied to several real samples, when the results (identified pesticides) were quite identical to those of LC–MS/MS analyses (after dSPE). The method performance of the new screening was thereby additionally confirmed.



1

HTpSPE combined with µL-FIA–TOFMS via TLC–MS Interface: elution of a target zone (a); nano-ESI and µL-FIA–TOFMS elution peak of a cucumber extract spiked with a pesticide mixture (b) and respective mass spectrum showing all pesticides (c).



2

µL-FIA–TOF mass spectra of a spiked cucumber extract after dSPE (a) and HTpSPE (b), and of a standard mixture solution after HTpSPE (d), showing the mass signals of all pesticides (*) and internal standards (#); for comparison a solvent blank after HTpSPE (c)

[1] C. Hemmerling, *et al.*, DLR 105 (2009) 633–644
[2] C. Oellig, W. Schwack, J. Chromatogr. A 1218 (2011) 6540–6547
[3] C. Oellig, W. Schwack, J. Chromatogr. A 1260 (2012) 42–53
[4] C. Oellig, W. Schwack, J. Chromatogr. A 1351 (2014) 1–11
[5] www.quechers.com

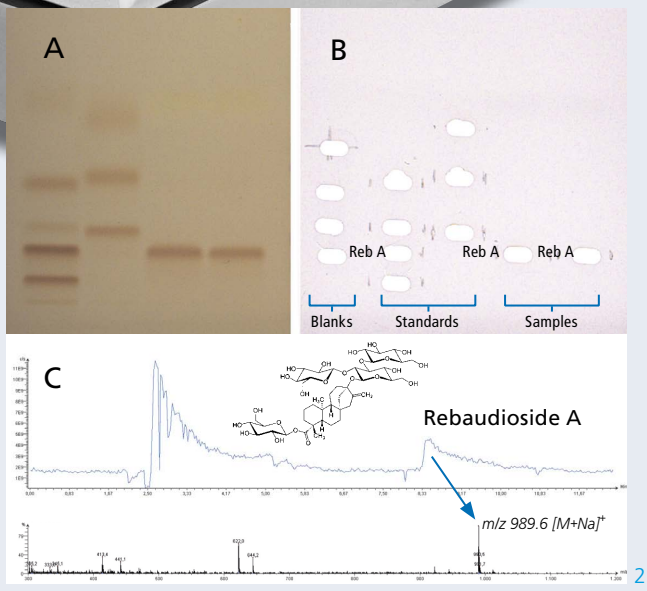
Contact: Dr. Claudia Oellig, Institute of food chemistry, University of Hohenheim, 70599 Stuttgart, Claudia.Oellig@uni-hohenheim.de

CAMAG TLC-MS Interface

In combination with mass spectrometry used worldwide for identification and confirmation of (un)known substances in research and routine analysis

1

Hyphenating planar chromatography with mass spectrometry opens new possibilities for reliable characterization of chromatographic fractions. This interface allows rapid and contamination-free elution of TLC/HPTLC zones with online transfer to the respective spectrometer. The advantage is its plug & play integration in any given HPLC/MS system without modification. Depending on the MS system selected, a substance can be identified within a minute via its mass spectrum, or for an unknown substance zone, the respective sum formula can be obtained. Furthermore, interesting zones can be eluted into vials for profound investigations with, *e. g.*, NMR and (ATR-)FTIR.



2

Characterization of separated compounds by mass spectrometry (Steviol glycosides in Stevia formulations)*

A: Chromatogram for localizing the zones (derivatized with ß-naphtol reagent)

B: HPTLC plate after elution of zones with the TLC-MS Interface

C: HPTLC-ESI-MS spectra of Rebaudioside A, m/z 989.6 $[M+Na]^+$

**Morlock et al., Journal of Chromatography A, 1350 (2014) 102–111 and CBS 109, pp.10–12*

Further information: **www.camag.com/tlc-ms**

CAMAG WORLD LEADER IN PLANAR CHROMATOGRAPHY

CBS 114 E 996.2270-17