Title: The 42nd Symposium Chromatographic Methods of Investigating Organic Compounds : Book of abstracts

Author:

Citation style: The 42nd Symposium Chromatographic Methods of Investigating Organic Compounds : Book of abstracts. (2019). Katowice : Institute of Chemistry University of Silesia in Katowice.

Institute of Chemistry
University of Silesia in Katowice
Katowice, Poland

The 42nd Symposium Chromatographic Methods of Investigating Organic Compounds

Book of abstracts

June, 4th – 7nd, 2019
Szczyrk, Poland

ISBN 978-83-226-3726-5

Lectures

Session 1: ***Practical applications of hyphenated TLC/HPTLC***

Chairpersons: Prof. Jerzy Silberring and Prof. Piotr Wieczorek

9:00 - 9:30 G. Morlock,
Office chromatography and hyphenations

9:30 - 10:00 I. Vovk, V. Glavnik, U. Jug, V. Metličar, A. Albreht, K. Naumoska,
Investigation of the potential of invasive Japanese knotweed with chromatographic and hyphenated techniques

10:00 - 10:30 E. Łata, A. Fulczyk, M. Sajewicz, T. Kowalska,
Spectacular separation effects in TLC/HPTLC, their possible origin and certain benefits

10:30 - 11:00 T. Tozar, M. Boni, A.-M. Udrea, S.S. Costa, A. Staicu, I.L. Couto, M. Viveiros, M.L. Pascu,
Laser-induced fluorescence coupled with high-performance thin layer chromatography for developing new antimicrobials agents

Office Chromatography and hyphenations

Gertrud Morlock*

Chair of Food Science, Justus Liebig University Giessen, Giessen, Germany,
**email: gertrud.morlock@uni-giessen.de*

Abstract

Already learned at school, planar chromatography summarizes sample results as illustrative image (fingerprints) understood intuitively. The list of globally urgent analyses is long to check the quality of especially food, dietary supplements, nutraceuticals, pharmaceuticals, environmental samples etc. An analytical public domain open source tool could substantially contribute to improve the product quality, if everybody could perform a fast product check. Though also spectral techniques are powerful, there has not been a panacea tool so far. Additionally, the exploitation of especially open-source technologies is crucial to spread knowledge.

The potential of open-source hardware and software as well as 3D printing is exploited for Office Chromatography, a concept combining all steps of miniaturized planar chromatography in the same device [1]. 3D printing consists in the additive application of a layer of materials on a plane surface. Per se, planar chromatography has such a plane format, but instead of material layers for manufacturing, chemicals were applied on glass. The fabrication of thin silica gel layers for planar chromatography using a modified 3D printer has been demonstrated recently [2], opening new perspectives for layer materials and patterns.

Instead of ink printing on papers, chemical solutions were printed on layers. Sample application, development and derivatization were managed by an inkjet print head used for commercial printers. Empty ink cartridges were filled with a chemical solution and printed, though chemical resistance is still limited. Open source soft- and hardware allowed full control on the inkjet technology with a drop-volume of 100 pL. The instrument was designed in OpenSCAD and most of its parts were 3D printed. Control software was developed to create GCODE files to be send to the printer. The resulting apparatus was affordable (810 Euro) and compact (26 x 31 x 26 cm3) [3]. Common hyphenations used in TLC/HPTLC are demonstrated for a miniaturized plate format.

Open-source developments including sophisticated image evaluation by artificial neural network are similar to radical chain reactions, exponential in progress and highly dynamic. It is in many aspects an interesting experiment worth to contribute!

References

[1] G.E. Morlock, Miniaturized planar chromatography using office peripherals - Office chromatography, J. Chromatogr. A, 1382 (2015) 87-96

[2] D. Fichou, G.E. Morlock, Open-source-based 3D printing of thin silica gel layers in planar chromatography, Anal. Chem. 89 (2017) 2116-2122

[3] D. Fichou, G.E. Morlock, Office Chromatography: Miniaturized all-in-one open-source system for planar chromatography, Anal. Chem. 90 (2018) 12647-12654

Investigation of the potential of invasive Japanese knotweed with chromatographic and hyphenated techniques

Irena Vovk*, Vesna Glavnik, Urška Jug, Valentina Metličar, Alen Albreht, Katerina Naumoska

National Institute of Chemistry, Department of Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
**e-mail: irena.vovk@ki.si*

Abstract

Invasive alien plants species pose huge ecological and economic problems with serious impact on biological diversity and/or damage the infrastructure in Europe and North America. Most of these plants originate from Asia, where many of them have been used for centuries in traditional medicine for treatment of different diseases. Additionally, some parts (e.g. rhizomes, flowers) of these plant species are used as raw materials for different food and health beneficial products. One of such examples is Japanese knotweed (*Fallopia japonica* Houtt.), rhizomes of which are sold in the form of a powder, extracts and herbal infusions in Asian market, while only food supplements containing resveratrol from rhizomes of Japanese knotweed can be found in Europe. Japanese knotweed is on the list of the "100 World's Worst Invasive Alien Species".

In this lecture the potential of one- and multi-dimensional HPTLC combined with different detections (UV, Vis, FLD, MS/(MS)) before and after derivatization for targeted and non-targeted analyses of Japanese knotweed extracts will be presented. Challenges in the analyses of different groups of compounds (e.g. proanthocyanidins, anthraquinones, carotenoids) using different stationary phases (silica gel, C18, diol, cellulose) will be discussed. Influence of the sorbent, pre-developing and developing solvents on stability of the analytes (before and after development) and ion suppression in HPTLC-MS and HPTLC-MS/MS analyses will be shown. Examples of peculiar phenomenon of unexpected rising of densitometrically determined peak areas and peak heights of some compounds will also be given.

Acknowledgement

The authors acknowledge the financial support from the Slovenian Research Agency (research core funding No. P1-0005), the generous support of Merck KGaA, Germany (providing HPTLC plates) and the contributions of Andreja Starc (National Institute of Chemistry) during the experimental work.

Spectacular separation effects in TLC/HPTLC, their possible origin and certain benefits

Eliza Łata, Agnieszka Fulczyk, Mieczysław Sajewicz, Teresa Kowalska*

Department of General Chemistry and Chromatography, University of Silesia, Katowice, Poland
**e-mail: teresa.kowalska@us.edu.pl*

Abstract

Effective diffusion is one of these phenomena which on an everyday basis take place in chromatographic systems, yet usually tend to be overlooked in an everyday analytical practice. In most instances, effective diffusion is a negative phenomenon which lowers the height equivalent to one theoretical plate (H) and hence, it diminishes resolution power of a given chromatographic system. Unlike in column chromatography, in the planar chromatography systems effective diffusion is two-dimensional, i.e., taking place both in the vertical and horizontal direction [1].

In our earlier studies, it was shown with use of spectroscopy of circular dichroism (CD) that precipitation of silica gel for chromatography results in certain enantiomeric excess of one form of the asymmetric silicon dioxide microcrystals over the other [2], which in combination with horizontal effective diffusion can result in spectacular yet rare horizontal separations [3]. Moreover, it was shown that horizontal separations in the TLC/HPTLC systems most likely occur in the case of the rod-like shaped analyte molecules and further it was assumed that they might relocate along the chromatographic layer with a propeller-like motion either auto-driven, or moved by the Magnus forces [3].

Horizontal separations in the TLC/HPTLC systems are rare enough phenomena and therefore in this study the aforementioned effect is going to be discussed upon the rod-shaped red betacyanin pigments derived from the red beet (*Beta vulgaris* L.) juice. Further, it will be shown that horizontal separation of the betacyanin pigments rather than their sensitivity toward the external conditions (e.g., light, temperature and pH) makes their quantification in planar chromatography systems virtually impossible. Moreover, it will be demonstrated that betanin as the main representative of the betacyanin pigments which is commercially distributed as a phytochemical standard represents a mixture of an unknown number of unknown betacyanins. Last not least, horizontal separation of betacyanin pigments can prove beneficial for their detection in certain alimentary products colored with the red beetroot juice and for the authentication (or otherwise) thereof.

References

[1] T. Kowalska, K. Kaczmarski, W. Prus, Theory and mechanism of thin-layer chromatography. Chapter II in Handbook of Thin-Layer Chromatography, 3rd Ed. Revised and Expanded, Eds: J.Sherma and B.Fried, Marcel Dekker, Inc., New York, 2003; pp. 47-80

[2] M. Sajewicz, H.-E. Hauck, G. Drabik, E. Namysło, B. Głód, T. Kowalska, Tracing possible structural asymmetry of silica gel used for precoating thin-layer chromatographic plates, J. Planar Chromatogr. - Modern TLC, 19 (2006) 278-281

[3] J. Polański, M. Sajewicz, M. Knaś, A. Żywociński, M. Weloe, T. Kowalska, Chiral rotors and an evidence of their action in planar chromatography, Chemistry Today, Monographic Supplement Series "Chiral Technologies", 30 (2012) 18-21

Laser-induced fluorescence coupled with high performance thin layer chromatography for developing new antimicrobials agents

Tatiana Tozar*(1), Mihai Boni (1), Ana-Maria Udrea (1), Sofia Santos Costa (2), Angela Staicu (1), Isabel Leitão Couto (2), Miguel Viveiros (2), Mihail Lucian Pascu (1)

(1) National Institute for Laser, Plasma and Radiation Physics, Bucharest, Romania
(2) Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisbon, Portugal
**e-mail: tatiana.alexandru@inflpr.ro*

Abstract
One of the most important health-related problems is the fight against multiple drug-resistant bacterial pathogens. The number of drug-resistant bacteria and their proved resistance are increasing, and the possibilities for successful treatment are narrow due to the lack of solutions in eradicating them with current treatments. Large efforts in drug development are directed towards fast and efficient ways to combat multidrug resistance in bacterial pathogens. In this respect, UV laser exposed thioridazine solutions proved to be suitable candidates in fighting antimicrobial resistance in gram-positive bacteria. The antimicrobial activity of the irradiated medicine solutions was improved several times and the toxicity decreased if compared with the unirradiated solutions.
Another important issue is drug analysis in all stages of drug discovery and development process, which is performed mostly using HPLC systems. A fast and cheap approach during drug development is needed in order to identify and characterize the drugs. A good solution is the high-performance thin layer chromatography (HPTLC), an offline method that is superior to other analytical techniques in terms of total cost and time for analysis. HPTLC plates were investigated using a single-track scanner that employed a monochromatic light generated by a picosecond laser; an optical fiber was used for the collection of reflected light in conjunction with a spectrograph (fluorescence) and oscilloscope (fluorescence lifetime). This approach simplified data acquisition and allowed facile application of modern approaches for separation and optical characterization of mixtures with antimicrobial properties. Also, it improved the quality of available data from thin-layer separations and offered additional information about the photoproducts, like fluorescence lifetime.
The investigated solutions were aqueous thioridazine solutions exposed to 266 nm laser radiation for various irradiation intervals. The susceptibility assays performed on gram-positive bacteria was used to select those irradiated thioridazine solutions that possessed the best antimicrobial activity. After separation of the photoproducts on HPTLC plates, the photoproducts were characterized by steady-state and time-resolved fluorescence spectroscopy.
In conclusion, these techniques proved to be suitable analytical means to study the photoproducts formed during laser irradiation and provided information about the transformations that occur in the molecular structure during irradiation.

Acknowledgments
This work was supported by grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, project number PN-III-P1-1.1-PD-2016-1072, within PNCDI III.

Session 2: ***Method development***

Chairpersons: Prof. Gertrude Morlock and Prof. Teresa Kowalska

12:00 - 12:30 D. Obradović, S. Oljačić, K. Nikolić, D. Agbaba,
Investigation and prediction of retention characteristics for the selected set of the central nervous system active compounds on the mixed mode diol stationary phase

12:30 - 13:00 M. Waksmundzka-Hajnos, A. Petruczynik, K. Wróblewski,
Selected problems in determination of basic drugs in biological fluids by HPLC

13:00 - 13:30 P.P. Wieczorek,
Solid phase extraction and supported liquid membranes as useful sample preparation methods

13:30 - 14:00 A. Poliwoda,
Capillary electrophoresis: is it better analytical technique than liquid chromatography?

Investigation and prediction of retention characteristics of selected set of central nervous system active compounds on mixed-mode diol stationary phase

Darija Obradović, Slavica Oljačić, Katarina Nikolić, Danica Agbaba*
Department of Pharmaceutical Chemistry, University of Belgrade-Faculty of Pharmacy, Vojvode Stepe 450, Belgrade 11000, Serbia.
**e-mail: danica@ pharmacy.bg.ac.rs*

Abstract
Investigation of the retention behavior of a wide range of analytes (43 nitrogen containing heterocyclic and guanidine derivatives such, as imidazoline and serotonin receptor ligands, or their related compounds) was performed on the mixed-mode stationary phase in the combined reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) modes. The imidazoline receptor ligands are the imidazoline or guanidine analogues with numerous therapeutic applications (such, as antihypertensives, diuretics, antiallergenics, antidiabetics, and antipsychotics), while the serotonin receptor ligands are the piperazine derivatives which exert an effect on positive and negative symptoms of schizophrenia, mania and mixed states of bipolar disorder.
On the mixed-mode stationary phase, the retention behaviour of investigated compounds was described as a function of the aqueous eluent volume fractions, φ(aq), and total polarity of mobile phase (P_{tot}). The turning point was discussed based on different mobile phase characteristics representing the shift between the HILIC and the RP chromatographic mode. The influence of molecular properties on the main retention characteristics (turining point and the extrapolated retention parameters) is going to be discussed.

References
[1] P. Jandera, T. Hajek, Mobile phase effects on the retention on polar columns with special attention to the dual hydrophilic interaction-reversed-phase liquid chromatography mechanism, J. Sep. Sci. 41 (2017) 145-162
[2] D. Obradović, S. Oljačić, K. Nikolić, D. Agbaba, Investigation and prediction of retention charcteristics of imidazoline and serotonin receptor ligands and their related compounds on mixed-mode stationary phase, J. Chromatogr. A, 1585 (2019) 92-104

Selected problems in determination of basic drugs in biological fluids and extracts by HPLC

Monika Waksmundzka-Hajnos*(1), Anna Petruczynik (1), Karol Wróblewski (2)

(1) Department of Inorganic Chemistry, Medical University of Lublin, 20-923 Lublin, Poland
(2) Department of Experimental and Clinical Pharmacology, University of Rzeszów, 35-959 Rzeszów, Poland
**e-mail: monika.hajnos@umlub.pl*

Abstract

Sorbents used in chromatographic analysis of active compounds are usually silica-based materials with ligands of different origin. Unfortunately residual silanols play also role in mechanisms of analytes' separation. It often causes analytical problems because generates incorrect peak shapes and poor system efficiency and influences negatively selectivity of separation.

There are several methods to reduce effect of residual silanols: the use of buffered mobile phases, the use of eluent additives such as amines, acids, ion-pairing reagents and ionic-liquids. Specially synthesized stationary phases with endcaped residual silanols or embedded ligands, are often applied. Recently sorbents possessing moieties enabling π-π interactions are commercially available.

In our investigations various methods were optimized to elaborate procedures for determination of basic drugs and their metabolites in biological fluids and extracts. Often systems with double protection were applied. It means that special stationary phases synthesized for separation of basic analytes and the use of mobile phase additives blocking residual silanols were necessary for achievement of satisfactory results.

Analysis of group of psychotropic drugs in human serum and saliva were elaborated to the use for control of their level in the serum of psychiatric patients. Often determination of active metabolites was also necessary. The method was applied in therapeutic drug monitoring. The relationships between dose, serum concentration of the drug and the therapeutic effect were also determined. The therapeutic effect was assessed with the Positive and Negative Syndrome Scale (PANSS).

The double protection methods can be applied in analysis of alkaloids in plant extracts and biological fluids.

In some analytical cases the alternative for RP-HPLC is the use of IEC systems with ion-exchange columns. The method gives more satisfactory results especially for determination of most hydrophilic analytes.

Solid phase extraction and supported liquid membranes as useful sample preparation methods

Piotr P. Wieczorek*

Department of Analytical and Ecological Chemistry, Faculty of Chemistry, University of Opole, Opole, Poland
**e-mail: piotr.wieczorek@uni.opole.pl*

Abstract
Taking into account the literature information, it might be noticed a growing trend in analytical chemistry in the field of designing and developing a new types of methodical and apparatus/instrumental solutions, which significantly increase the efficiency and selectivity of the analytes isolation or/and preconcentration process. As essential step in the development of many analytical method is sample preparation. Many techniques have been used for sample preparation including liquid-liquid extraction (LLE), solid phase extraction (SPE), and also membrane techniques. From membrane techniques supported liquid membranes (SLM) extraction demonstrate as a very efficient and selective separation method. Therefore this method is applied for sample cleanup, and enrichment of various types of chemical substances such as metal ions, organic acids, amines, amino acids, phenolic compounds, peptides, pesticides and drugs [1]. SPE extraction, compare to SLM, is not selective enough. One of the most important solution of this problem of SPE are selective sorption materials, commonly defined as molecularly imprinted polymers (MIPs). Due to their simple preparation protocol, thermal and chemical stability and selectivity, MIPs have found application as a stationary phases in advanced separation techniques, in electrochemical sensors or as a sorbents in the solid phase extraction technique (SPE) [2].

References
[1] P. Dżygiel, P.P. Wieczorek, Supported liquid membranes and their modifications: definition, theory, stability, application and perspectives, in V. S. Kislik, Ed.: Liquid Membranes, Elsevier, 2010, pp. 73-140
[2] M. Marć, T. Kupka, P.P. Wieczorek, J. Namieśnik, Computational modeling of molecular imprinted polymers as a green approach in the field of developing new analytical sorbents, Trends in Analytical Chemistry, 98 (2018) 64-78

Capillary electrophoresis: Is it better analytical technique than liquid chromatography?

Anna Poliwoda*

Department of Analytical and Ecological Chemistry , Faculty of Chemistry, Opole University, Poland
**e-mail: Anna.Poliwoda@uni.opole.pl*

Abstract
Liquid chromatography (LC) is considered as a preferred technique for the determination of numerous chemicals due to its selectivity, accuracy and specificity. The possibility of employing various strategies during method development that include the use of different types of stationary phase, various compositions of mobile phase, and a wide range of selective detectors, make it all the more suitable for the analysis of analytes with diverse structure and polarity. However, the use of LC for some analysis sometimes has also drawbacks, such as complex sample pretreatment, high solvent consumption, limited resolution, and short column lifetime owing to easy contamination with numerous accompanying substances. In this case, capillary electrophoresis (CE) is increasingly recognized as an important analytical separation technique because of its speed, efficiency, reproducibility, ultrasmall sample volume, low consumption of solvent, and easy removal of contaminants. These advantages make CE an ideal technique for the determination of complex mixtures and complementary to HPLC. In this review the basic principles of CE, general strategies for method development are described to achieve selective, efficient, precise, fast, sensitive, and validated methods. Sample pre-treatment requirements would be also discussed. Standard buffer recipes, surfactants used in micellar electrokinetic chromatography (MEKC), chiral selectors, useful buffer additives, actions to deal with complex matrices have been collected. Finally, the aspects and the selected examples in which the use of CE is more effective or possibly ineffective compared to LC will be indicated.

Session 3: ***Chemometrics in separation sciences***

Chairpersons: Dr.Sc. Ivana Stanimirova and Prof. Yvan vander Heyden

15:00 - 15:40 O.M. Kvalheim, J.J. Kellogg, L. Caesar, N.B. Cech,
The use of chromatography and hyphenated chromatography in combination with chemometrics for detecting bioactive components in very complex extracts of natural products

15:40 - 16:20 B. Khakimov, S.B. Engelsen,
Processing of complex chromatographic datasets using multivariate data analysis: applications in food science

16:20 - 17:10 L.C. Klein-Júnior, J. Viaene, A. Slosse, M. Esteki, Y. Vander Heyden,
Metabolite profiling and data-handling in natural product research

17:10 - 17:30 Ł. Pieszczek, M. Daszykowski,
The start-to-end processing of TLC multi-wavelength densitograms

Using chromatography and chemometrics for detecting bioactive components in very complex extracts of natural products

Olav M. Kvalheim*(1), Josh J. Kellogg (2), Lindsay Caesar (2), Nadja B. Cech (2)

(1) Dept. of Chemistry, University of Bergen, Bergen, Norway
(2) Dept. of Chemistry & Biochemistry, University of North Carolina Greensboro, Greensboro, North Carolina, United States
**e-mail: olav.kvalheim@uib.no*

Abstract
Botanic extracts represent a rich source of compounds for medicinal use, but the detection of bioactive compounds in a mixture of hundreds of compounds may be comparable to looking for a needle in a haystack. It is an extremely laborious task using common approaches requiring several fractionation and isolation steps and it is easy to loose potential candidates in the process. Chemometrics provides tools to reduce the experimental burden and to reduce the risk of losing bioactive candidates. Chromatographic fingerprinting followed by regression modelling and variable selection has previously been shown to be able to reveal bioactive compounds [1,2]. This approach has recently been extended and developed into a scheme for rapid detection of bioactive fractions and individual compounds [3-6]. In this lecture, we provide a flow chart built on chromatographic fingerprinting and chemometrics for detection of bioactive compounds. Strength and limitations of our approach is discussed and illustrated with applications.

References
[1] F.-T. Chau, H.-Y. Chan, C.-Y. Cheung, C.-J. Xu, Y. Liang, O.M. Kvalheim, Recipe for uncovering the bioactive components in herbal medicine, Anal. Chem. 81 (2009) 7217-7225
[2] O.M. Kvalheim, et al., Chromatographic profiling and multivariate analysis for revealing and quantifying the contributions from individual components to the bioactive signature in natural products, Chemometr. Intell. Lab. 107 (2011) 98-105
[3] J.J. Kellogg, et al., Biochemometrics for Natural Products Research: Comparison of data analysis approaches and application to identification of bioactive compounds, J. Nat. Prod. 79 (2016) 376−386
[4] J.J. Kellogg, T.N. Graf, O.M. Kvalheim, N.H. Oberlies, N.B. Cech, Comparison of chemometric approaches for pre-clinical selection of complex dietary supplements: green tea as a case study, J. Nat. Prod. 80 (2017) 1457-1466
[5] E. Britton, J.J. Kellogg, O.M. Kvalheim, N.B. Cech, Biochemometrics to identify synergists and additives from botanical medicines: a case study with hydrastis canadensis, J. Nat. Prod. 81 (2018) 484-493
[6] L.K Caesar, J.J. Kellogg, O.M. Kvalheim, N.B. Cech, Opportunities and limitations for untargeted mass spectrometry metabolomics to identify biologically active constituents in complex natural product mixtures, J. Nat. Prod. (2019) in press

Processing of complex chromatographic datasets using multivariate data analysis: applications in food science

Bekzod Khakimov*, Søren Balling Engelsen

Chemometrics and Analytical Technology, Department of Food Science, University of Copenhagen
**e-mail: bzo@food.ku.dk, www.models.life.ku.dk*

Abstract

Hyphenation of chromatography with multi-channel detectors such as mass spectrometer generates megavariate and complex data from biological samples containing rich metabolomics information. To date, thausands of studies have been conducted applying chromatography to map metabolomes and foodomes from various sample matrices [1-3]. However, extraction of these metabolite information from complex chromatographic datasets remain as the main bottleneck of the field.
In Gas Chromatography-Mass Spectrometry (GC-MS) this complexity attributed by overlapping of peaks, retention time shift of peaks across samples, baseline drifts and low S/N. When analysing e.g., few hundreds F1 and F2 populations of *Barbarea vulgaris* plant leaf extracts using GC-MS over several batch sequence runs, a generated dataset will be complex chromatograms where each chromatogram comtains few hundreds peaks that might be partually or fully overlapped with other peaks. This data will look even more complex when all samples are superimposed. Extracting metabolite information form such a complex dataset, meaning generating a metabolite table where each peak is identified and quantified, can be a cumbersome task. Despite, several methods exist to deal with these datasets most of them are not able to solve above mentioned complications present in GC-MS data and on top of that they require multiple parameter to be set by the user e.g. noise level, RT shift allowance, etc., which makes data processing bias, lab specific and not compilable with other datasets.
In the last decade, several multivariate data analysis algorithms have been developed to assist solving chromatographic problems inclusing two- and three-dimentional chromatographic data alignemnt using icoshift [4,5], deconvolution of elusive peaks from LC-MS data [6], and PARAFAC2 based Deconvolution and Identification system (PARADISe) for GC-MS data [7]. These methods are open access and can be downloaded from http://models.life.ku.dk/.

References

[1] B. Khakimov, S. Bak, S.B. Engelsen, High-throughput cereal metabolomics: Current analytical technologies, challenges and perspectives, J. Cereal Sci. 59 (2014) 393-418
[2] B. Khakimov, et al., New nordic diet versus average Danish diet: A randomized controlled trial revealed healthy long-term effects of the new nordic diet by GC-MS blood plasma metabolomics, J. Proteome Res. 15 (2016) 1939-1954
[3] B. Khakimov, et al., From metabolome to phenotype: GC-MS metabolomics of developing mutant barley seeds reveals effects of growth, temperature and genotype, Sci. Rep. 7 (2017) 8195
[4] F. Savorani, G. Tomasi, S.B. Engelsen, icoshift: A versatile tool for the rapid alignment of 1D NMR spectra, J. Magn. Res. 202 (2010) 190-202
[5] G. Tomasi, F. Savorani, S.B. Engelsen, icoshift: An effective tool for the alignment of chromatographic data, J. Chromatogr. A, 1218 (2011) 7832-7840
[6] B. Khakimov, J.M. Amigo, S. Bak, S.B. Engelsen, Plant metabolomics: Resolution and quantification of elusive peaks in liquid chromatography-mass spectrometry profiles of complex plant extracts using multi-way decomposition methods, J. Chromatogr. A, 1266 (2012) 84-94
[7] L.G. Johnsen, P.B. Skou, B. Khakimov, R. Bro, Gas chromatography - mass spectrometry data processing made easy, J. Chromatogr. A, 1503 (2017) 57-64

Metabolite profiling and data handling in natural product research

Luiz Carlos Klein-Júnior (1), Johan Viaene (2), Amorn Slosse (2), Mahnaz Esteki (3), Yvan Vander Heyden*(2)

(1) Chemical-Pharmaceutical Investigation Nucleus, University do Vale do Itajaí - UNIVALI, Itajaí/SC, Brazil
(2) Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Vrije Universiteit Brussel – VUB, Laarbeeklaan 103, Brussels, Belgium
(3) Department of Chemistry, University of Zanjan, Zanjan, Iran
**e-mail: Yvan.Vander.Heyden@vub.be*

Abstract
Although medicine has undergone massive evolutions, challenges are still present. Technological evolutions have had a major influence on the drug discovery process. This process nowadays has become much more rationalized. However, in the end, substances are still screened for their ability to interact with patho-physiological processes in a trial-and-error approach. With chemical synthesis, chemicals with various related structures can be created, which are then subjected to screening tests. In some cases, active compounds are found, which then go through a lengthy process of characterizing their properties and turning them into drugs. However, many synthesized compounds do not make it to a final drug substance.
To rationalize the drug discovery process, researchers often rely on earlier knowledge. One strategy is to explore the active substances in natural products with known applications in traditional medicine, hoping that the active compounds are different from those currently used in the treatment of a given pathology, or allow treatments of health issues that could not be treated effectively before. Because of the complexity of natural products, high requirements are set on the sample preparation and chemical separation techniques to create a metabolite profile (or fingerprint), which is then processed chemometrically to find potentially active compounds.
Secondly, the increase of food falsification, inducing large financial losses as well as the confidence of consumers, is another domain where fingerprints, in combination with chemometrics, are usually considered as the main references.

The start-to-end processing of HPTLC multi-wavelength densitograms

Łukasz Pieszczek*, Michał Daszykowski

Institute of Chemistry, the University of Silesia in Katowice, Katowice, Poland
**e-mail: pieszczek.lukasz@gmail.com*

Abstract

Recently, the high-performance thin-layer chromatography (HPTLC) regain attention. It enables the development of several samples in a single run, offers a good resolution, relatively fast separations and requires a small amount of mobile phase. Moreover, TLC can be hyphenated with advanced and comprehensive detection methods [1], including mass spectrometry, densitometry, and hyperspectral imaging. In-depth analysis of a TLC plate is often carried out using densitometry that describes chromatographic bands by reflectance values observed at given locations and registered either at one or many wavelengths. The multi-wavelength provides information about the purity of chromatographic bands. On the other hand, such detection mode leads to large and complex data that require for their efficient processing a specific strategy.

In this study, we examined a few chemometric methods well-suited for processing muti-wavelength densitograms. Significant spectroscopic interferences, usually affecting densitometric measurements, were identified. Automated data pretreatment strategy, including data trimming and denoising, interpolating and subtracting background, and smoothing of the spectroscopic signals, was proposed and carefully tested. The baseline of each signal was estimated in an automated manner using the asymmetric least squares smoothing and the non-quadratic asymmetric function also considered to remove background from FTIR and Raman spectra [2,3]. Commonly applied the Savitzky-Golay smoother was replaced with a more appropriate Whittaker's algorithm [4]. The multi-wavelength densitograms can be reduced and visualized. For instance, one can combine data from different imaging methods and use discreet information from the HPTLC plates to score similarities among a set of samples. The usefulness of the proposed strategy was tested using a set of densitograms describing 30 samples of water-based inks (10 different brands in triplicate) extracted from paper sheets with 0.5 mL acetone and water 1:1 (V/V). Samples were developed on the HPTLC silica gel plate using as mobile phase butanol, ethanol and water 50:10:15 (V/V/V). Each sample was characterized by multi-wavelength densitograms recorded using Desaga CD60 within 200-800 nm every 20 nm.

Acknowledgment

This research was supported by the National Science Centre, Poland (research grant no. 2018/29/N/ST40/01547)

References

[1] S. Kirchert, R.E. Kaiser, G.E. Morlock, In-process quality control of wine by planar chromatography versus micro planar chromatography, J. Chromatogr. A, 1588 (2019) 137-149

[2] J. Peng, S. Peng, A. Jiang, J. Wei, C. Li, J. Tan, Asymmetric least squares for multiple spectra baseline correction, Anal. Chim. Acta, 683 (2010) 63-68

[3] V. Mazet, C. Carteret, D. Brie, J. Idier, B. Humbert, Background removal from spectra by designing and minimising a non-quadratic cost function, Chemometr. Intell. Lab. 76 (2005) 121-133

[4] P.H.C. Eilers, A perfect smoother, Anal. Chem. 75 (2003) 3631-3636

Session 4: ***Metabolomics - part 1***

Chairpersons: Prof. Frederik-Jand van Schooten and Dr. Agnieszka Smolińska

9:00 - 9:40 T. Cajka, P. Zednikova, O. Kuda, J. Hricko, M. Novakova, M. Paucova,
Metabolomics 2.0: tools for improving and streamlining the biomedical research workflow

9:40 - 10:20 A. Checa,
Liquid chromatography tandem mass spectrometry determination of lipid mediators and their application to biomedical research

10:20 - 11:00 A. Drabik, J. Ner-Kluza, K. Piechura, A. Bodzoń-Kułakowska, P. Mielczarek, P. Suder, J. Silberring,
How to identify a compound? Problems and pitfalls

Metabolomics 2.0: Tools for improving and streamlining the biomedical research workflow

Tomas Cajka*(1,2), Petra Zednikova (1), Ondrej Kuda (3), Jiri Hricko (1), Michaela Novakova (1), Michaela Paucova (1)

(1) Department of Metabolomics, Institute of Physiology CAS, Prague, Czech Republic
(2) Department of Translational Metabolism, Institute of Physiology CAS, Prague, Czech Republic
(3) Department of Metabolism of Bioactive Lipids, Institute of Physiology CAS, Prague, Czech Republic
**e-mail: tomas.cajka@fgu.cas.cz*

Abstract

Over the last decade, mass spectrometry-based metabolomics and lipidomics have become established as the key platforms for comprehensive profiling of low-molecular-weight compounds in complex biological systems. Coupling liquid chromatography to mass spectrometry is the preferred technique in metabolomics and lipidomics permitting effective compound separations and detection. Howerver, the true breadth of a metabolome cannot be captured by a single extraction method or instrumental platform. Human biospecimens, such as blood, can contain metabolites covering ~40 orders of magnitude on the octanol/water coefficient scale. Hence, the main task is to cover polar metabolites, lipids, and exposome compounds (e.g., drugs, food components) using as few platforms as possible while maintaining the requisite precision and accuracy for the metabolite classes detected by the chosen platforms [1]. On top of that, processing of metabolomics/lipidomics data such as feature detection, peak alignment, exclusion of false-positive peaks, and automated annotation of peaks based on MS/MS library search is still challenging. Also, it is estimated that in untargeted metabolomics only 20% of detected molecular features can be identified based on mass spectra library matches.

In this lecture, I would like to address these challenges and introduce an LC–MS workflow (LIMeX) for simultaneous extraction of complex lipids, polar metabolites, and exposome compounds that combines targeted and untargeted analysis. The sub-groups of compounds are isolated using an 'all-in-one' extraction with a methanol/methyl tert-butyl ether mixture and water. These extraction solvents contain over 60 internals standards covering main lipid classes, selected polar metabolites, and exposome compounds. Separate analysis of nonpolar fraction (lipids) is conducted using reversed-phase LC, and polar fraction (polar metabolite and exposome compounds) using hydrophilic interaction chromatography. Simultaneous acquisition of MS1 and MS/MS spectra in data-dependent mode is used for each platform. The acquired raw data sets are processed using user-friendly MS-DIAL [2] software including a newly implemented algorithm for peak alignment employing internal standards. This software also permits automated annotation of lipids and polar metabolites/exposome compounds using MS/MS library search. The exported data are curated by our newly developed software MetaboViewer prior to statistical analysis. For unknown spectra MS-FINDER [3] software provides solution for formula predictions, fragment annotations, and structure elucidations. Our aim was to look at each step of metabolomics/lipidomics workflow and streamline where possible to ultimately developa simple, fast, and robust protocol that can be applied in large cohort studies for diverse sample types.

References

[1] T. Cajka, O. Fiehn, Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics, Anal. Chem. 88 (2016) 524-545

[2] H. Tsugawa, et al., MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis, Nat. Methods, 12 (2015) 523-526

[3] H. Tsugawa, et al., Hydrogen rearrangement rules: Computational MS/MS fragmentation and structure elucidation using MS-FINDER software, Anal. Chem. 88 (2016) 7946-7958

Liquid chromatography tandem mass spectrometry detemination of lipid mediators and their application to biomedical research

Antonio Checa*

Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
**e-mail: antonio.checa@ki.se*

Abstract
In addition to acting as structural blocks and energy stores, lipids play key roles as part of the innate immunity defense and take part in the remodeling of tissues with the aim of restoring homeostasis and functionality [1]. Due to their high sensitivity and specificity, LC-MS based analytical methodologies have become fundamental tools in decyphering the functions that many of these lipids play in many diseases. Despite of the high specificity that both selected reaction monitoring and high resolution approaches can provide in mass spectrometry, chromatography still plays an indispensable part to handle the problems derived from the presence of isobaric species, in-source fragmentation or ion supression caused by co-elunting compounds. Here, examples of selected LC-MS/MS lipid mediator targeted platforms from the sphingolipid, eicosanoid and endocannabinoid families together with their application in biomedical research will be presented [2,3]. The importance of sample preparation and chromatography in these quantitative methods will be highlighted.

References
[1] D. Balgoma, A. Checa, D. Garcia-Sar, S. Snowden, C.E. Wheelock, Quantitative metabolic profiling of lipid mediators, Mol. Nutr. Food Res. 57 (2013) 1359-1377
[2] A. Checa, et al., Dysregulations in circulating sphingolipids associate with disease activity indices in female patients with systemic lupus erythematosus: a cross-sectional study, Lupus, 26 (2017) 1023-1033
[3] M. Sjödin, et al., Soluble epoxide hydrolase derived lipid mediators are elevated in bronchoalveolar lavage fluid from patients with sarcoidosis: a cross-sectional study, Respir. Res. 19 (2018) 236

How to identify a compound? Problems and pitfalls

Anna Drabik (1), Joanna Ner-Kluza (1), Kinga Piechura (1), Anna Bodzoń-Kułakowska (1), Przemysław Mielczarek (1), Piotr Suder (1), Jerzy Silberring*(1,2)

(1) Department of Biochemistry and Neurobiology, AGH University of Science and Technology, Kraków, Poland
(2) Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland
**e-mail: jerzy.silberring@agh.edu.pl*

Abstract
Identification of endogenous molecules is a serious challenge, in particular in the "omics" era. Those high-throughput strategies identify molecules, such as proteins, based on the partial information. Typically, one or two tryptic peptide fragments are sequenced. Such identification does not provide a complete insight into the molecular identity of a given protein and requires further characterization.

This talk is focused on several cases where several examples of more specific identification of endogenous molecules has been performed. In addition, more specific studies will be described to show a strategy for localization of sugar moieties attached to a given protein and their changes upon interactions with aptamers. Another approach concerns a difference between enzymatic activity and enzyme quantity, which is often misunderstood in the "omics" approach. Moreover, selected problems and pitfalls associated with high throughput and sample preparation will be discussed. This latter step is a key factor determining further success of the analysis, and therefore is of particular attention in the laboratory practice. On the other hand, sample heterogeneity, even this withdrawn from one tissue, may influence final results of such analysis.
Thus, detailed knowledge on the sample nature and its origin should be carefully considered, well before the experiment. Naturally, application of various types of separations in the above work will be discussed.

Acknowledgments
This work was supported by the grant EuroNanoMed "META" No. 05/EuroNanoMed/2012 and the grant HEMO for the Polish-Taiwanese collaboration, No. PL-TW/V/2017/17.

References
[1] A. Drabik, J. Ner-Kluza, A. Bodzon-Kulakowska, P. Suder. MYTHBUSTERS: a universal procedure for sample preparation for mass spectrometry, Eur. J. Mass Spectrom. 22 (2016) 269-273
[2] A. Drabik, J. Ner-Kluza, P. Mielczarek, L. Civit, G. Mayer, J. Silberring. Advances in the study of aptamer-protein target identification using the chromatographic approach, J. Proteome Res. 17 (2018) 2174-2181

Session 5: ***Exhaled breath analysis***

Chairpersons: Prof. Michał Markuszewski and Dr.Sc. Piotr Młynarz, Assoc. Prof.

12:00 - 12:40 F.-J. van Schooten, A. Smolińska,
Exhaled breath analysis in health and disease

12:40 - 13:10 A. Smolińska, F.-J. van Schooten,
Investigating colorectal cancer and primary sclerosing cholangitis by exhaled breath analysis

13:10 - 13:40 F. Monedeiro, M. Milanowski, T. Ligor, B. Buszewski,
Studies on volatile organic compounds as colorectal cancer biomarkers

13:40 - 14:00 M. Milanowski, F. Monedeiro, T. Ligor, B. Buszewski,
Analysis of target VOCs in breath samples directed towards the diagnosis of respiratory diseases

Exhaled breath analysis in health and disease

Frederik-Jan van Schooten, Agnieszka Smolinska

Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Research Institute NUTRIM, Maastricht University, The Netherlands
**e-mail: f.vanschooten@maastrichtuniversity.nl*

Abstract
Volatile metabolites (volatile organic compounds; VOCs) are produced in different organs that are transported by blood to the lungs where they are released. Thousands of VOCs are detected in breath and it has been shown that certain VOCs are associated with inflammatory, oxidative, microbial and neoplastic processes in different organs including lung, bowel and liver. This has promoted the development and clinical application of non-invasive metabolomics in exhaled air (breathomics). We use Gas Chromatography-Mass Spectrometry to measure all VOCs (volatome) in exhaled breath combined with chemometric analysis that allows the selecting of compounds with maximal diagnostic information. Previously, we identified profiles of compounds associated with inflammatory related conditions concerning the pulmonary tract (Asthma and COPD), intestinal tract (IBD) and liver (NASH), demonstrating moderate to good diagnostic accuracies. Furthermore studies have been performed to examine the effects of dietary interventions (gluten free) and environmental exposures (diesel) on exhaled VOCs. After these proofs of concept studies, the latest efforts in the breathomics field are focused on standardization of sampling and detection technologies, optimization of analytical algorithms and independent validation of clinical classification and prediction. Also revealing the underlying pathophysiological pathways, in for instance in in vitro and animal models, is of importance to enhance the value of putative diagnostic VOCs. The results of these efforts are needed before breathomics can be used as a reliable indicator of disease as well as an indicator of effects induced by dietary or inhalatory exposures.

Combining exhaled breath and microbiome data in Crohn's disease

Agnieszka Smolinska, Frederik-Jan van Schooten

Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Research Institute NUTRIM, Maastricht University, The Netherlands
**e-mail: a.smolinska@maastrichtuniversity.nl*

Abstract

The occurrence of Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), has been increasing in various industrialized countries. Mucosal inflammation in the colon is characteristic for UC while CD is rather transmural and discontinuous and can affect any part of the gastrointestinal tract. Exacerbations of symptoms such as abdominal pain and (bloody) diarrhea (with active inflammation) exchange with the periods of remission. Gut microbiota has an essential role in the development and disease progression since it has enormous influence on host-metabolism by e.g. direct production of various metabolites and volatile metabolites that might be excreted into feces but also enter the systemic circulation where they can be further modified by the host. 194 fecal, and breath samples were collected from CD patients visiting an outpatient clinic. Patients were assigned into two groups: active disease (n=97) and inactive disease (n=97) using combination of biochemical markers and Harvey Bradshaw index. The microbial composition of fecal samples was analysed by 454 pyrosequencing (16S rRNA).

Using CD patients as case study we will present a novel approach for data fusion strategy based on combined analysis of multiple kernel learning (MKL) and Random Forest (RF). MKL is an extension of single kernel to integrate multiple kernels in Support Vector Machine or Partial Least Square Discriminant Analysis, and has been successfully applied for metabolomics data. RF is an embedded technique capable of finding linear and non-linear relation in the data. The presented data fusion strategy can be implemented in the supervised as well unsupervised RF model. Combining MKL and RF enabled obtaining the improved prediction of disease activity. Moreover, it allowed for the discovery of significant relation between volatile metabolites and gut microbiome with respect to the disease activity. In addition, the improved prediction of disease activity can be achieved.

Studies on volatile organic compounds as colorectal cancer biomarkers

Fernanda Monedeiro (1,2,3), Maciej Milanowski*(1,2), Tomasz Ligor (1,2), Bogusław Buszewski (1,2)

(1) Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland
(2) Interdisciplinary Centre of Modern Technologies, Nicolaus Copernicus University, Toruń, Poland
(3) Department of Chemistry, Faculty of Philosophy, Science and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
**e-mail: milanowski.maciej@gmail.com*

Abstract
Colorectal cancer is one of the most recurrent type of cancer in the world, associated to high mortality rates [1]. In this sense, studies regarding the investigation of volatiles profiles as biomarkers of cancer have been carried out, with the intention to develop a preliminary screening method for early diagnosis of the disease. Between the selected biological matrices for such examinations, attending to a non-invasive approach, breath and faeces can be studied. Different methods of analysis have been employed in the assessment of volatiles related to colorectal cancer, both online and offline. Techniques such as solid phase microextraction (SPME) and thermal desorption (TD) sorbent cartridges have been employed for pre-concentration of such compounds. The suggested volatile biomarkers of colorectal cancer in faeces presented to be compounds with more polar characteristics and also can be addressed as bacterial metabolites, underlying the important role of varied gut microbiota in the disease development and risk. The referred matrix is complex, and the data processing can be impaired by many factors, in relation to this, modelling computational methods can be helpful. In general, sample preparation is simple and avoid sample handling, but divergent amounts of samples were used for analysis of profiles. Designed test with sensitivities and specificities above 90% can represent notorious progress in the current scenario. In contrast to the findings related to the faeces samples, the main potential biomarkers of colorectal cancer in breath are compounds with apolar nature and highly volatile. A model panel containing a larger number of metabolites may be necessary to provide high sensitives in the diagnostic trial. As conclusions, the analysis based in volatile biomarkers has potential to be applied in the clinical practice as a screening method, prior to the recruitment to invasive exams.

Acknowledgments
This work was financed by The National Centre for Research and Development (Warsaw, Poland), project "Airborne Biomarkers for Colorectal Cancer", grant ERA-NET TRANSCAN/023/2018.

References
[1] H.J. Kim, M.H. Yu, H. Kim, J. Byun, C. Lee, Noninvasive molecular biomarkers for the detection of colorectal cancer, BMB Rep. 41 (2008) 685-692

Analysis of target VOCs in breath samples directed towards diagnosis of respiratory diseases

Maciej Milanowski (1,2), Fernanda Monedeiro*(1,2,3), Tomasz Ligor (1,2), Bogusław Buszewski (1,2)

(1) Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland
(2) Interdisciplinary Centre of Modern Technologies, Nicolaus Copernicus University, Toruń, Poland
(3) Department of Chemistry, Faculty of Philosophy, Science and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
**e-mail: fmonedeiro@umk.pl*

Abstract
Metabolic processes in the organism lead to the formation of volatile organic compounds (VOCs), therefore, in a pathological condition, differentiated processes occur in the cells, possibly generating a different set of these metabolites. A promising approach concerning the assessment of biochemical changes in organism is to study samples that can be obtained by non-invasive collection [1]. Breath is a biological specimen that shows correspondence with respiratory system, presenting less complex composition and simple collection. Breath samples were collected using Tedlar bags, from healthy individuals (including smokers and non-smokers) and from volunteers with diagnosed respiratory diseases, recruited in local hospital dependencies. This work aimed to quantify 29 target VOCs previously reported as biomarkers of pulmonary clinical conditions and, additionally, to investigate volatile global profiles in breath samples, in order to investigate variations in compounds distribution along the studied groups of subjects. Dynamic solid phase microextraction (Dynamic SPME) and Needle trap device (NTD) were employed in VOCs preconcentration, analysis was carried out by gas chromatography coupled to mass spectrometry (GC/MS). Limits of quantitation ranged from 0.55 to 4.44 ppbv for Dynamic SPME and from 0.59 to 18.08 ppbv for NTD, relative standard deviation (RSD) did not exceed 10%, in both methods. The obtained data was assessed using statistical approaches (Mann-Whitney U test, principal components analysis and hierarchical cluster) and results revealed that at least 6 targets presented to be differentially distributed in samples from healthy and diseased individuals. In comprehensive analysis, over 80 compounds could be detected by the applied methodologies, from these, at least 11 presented discriminating features. The present study shows preliminary data to support the employment of breath samples in clinical setting in more detailed investigations.

Acknowledgments
This work was financed by The National Centre for Research and Development (Warsaw, Poland) in frame of Polish-Turkish bilateral project "A comparative study of volatile organic compound biomarkers in breath and urine samples collected from Polish and Turkish communities for monitoring of various respiratory diseases" (POLTUR2/4/2018).

References
[1] M. Hakim, Y.Y. Broza, O. Barash, N. Peled, M. Phillips, A. Amann, H. Haick, Volatile organic compounds of lung cancer and possible biochemical pathways, Chem. Rev. 112 (2012) 5949-5966

Session 6: ***Metabolomics - part 2***

Chairperson: Assoc. Prof. Tomas Cajka and Assist. Prof. Antonio Checa

15:00 - 15:20 G. Stavropoulos, J. Hill, F.-J. van Schooten, A. Smolińska,
Enhanced prediction accuracy of disease activity in Crohn's disease by fusing metabolomic, volatilomic, and microbiome data

15:20 - 15:50 T. Lundstedt, K. Lundstedt-Enkel, K. Bennett, C. Russell, R. Martín-Jiménez, M. Campanella, S. Mole, J. Petschnigg, T. Moritz, J. Trygg,
Endogenous metabolic profiling as a fundament for personalised medicine

15:50 - 16:20 T. Wieczorek, B. De Lacy Costello, N. Drabinska, P. Jones, O. Gould, N. Ratcliffe,
Can we predict volatile organic compounds (VOCs) associated with disease - the case of bladder cancer?

16:20 - 16:50 P. Młynarz, W. Wojtowicz, A. Ząbek, P. Juszczyński, E. Białotropowicz, T. Rzymski, A. Wróbel, A. Szyba, A. Chachaj,
Metabolomics studies on oncological diseases

16:50 - 17:20 M.M. Markuszewski,
Metabolomics in urogenital cancer by use of complementary analytical techniques

17:20 - 17:30 Intertech Poland
J. Grodowski,
New real time pptv level VOC measurement by PTR MS

17:30 - 17:40 Ertec Poland
E. Reszke, G. Schroeder, M. Cegłowski, J. Silberring, M. Smoluch,
Construction and analytical applications of the ionization sources of FAPA - type energized

Enhanced prediction accuracy of disease activity in Crohn's Disease by fusing metabolomic, volatilomic, and microbiome data

George Stavropoulos*(1), Jane Hill (2), Frederik-Jan van Schooten (1), Agnieszka Smolinska (1)

(1) Department of Pharmacology and Toxicology, NUTRIM School of Nutrition and Translational Research, Maastricht University, Maastricht, The Netherlands
(2) Thayer School of Engineering, Dartmouth College, Hanover, USA
**email:g.stavropoulos@maastrichtuniversity.nl*

Abstract

Introduction: Crohn's Disease (CD) is a form of Inflammatory Bowel Disease that affects any part of the gastrointestinal tract, from the mouth to the anus. CD may lead to debilitating or even life-threating complications, and it usually requires heavy medication. CD patients undergo periods of non-active and active stages of the disease. Symptoms during the active phase of the disease include severe abdominal pain, bloody diarrhoea, as well as high fever. The inflammatory stage of the disease is highly related to changes in human metabolome, volatilome, and the gut microbiome. Affected and inflamed organs in CD patients produce, and therefore, release Volatile Organic Compounds (VOCs) in blood and exhaled breath, as well as specific metabolites in plasma. At the same time, gut microbiome dysbiosis has always been observed. Previously, analyses of VOCs in breath and gut microbiome, individually, have successfully differentiated CD patients in the active stage of the disease from remission CD patients with prediction accuracies of 80% and 82%, respectively. The present study aims to fuse data from four-omics platforms (i.e. VOCs in breath, VOCs in blood headspace, metabolites in blood, and microbial Operational Taxonomic Units (OTUs) in the gut) to enhance the prediction accuracy of the disease activity in CD patients.

Approach: In the present study, 130 breath, faecal, and blood samples were collected from CD patients while visiting an outpatient clinic, and they were classified into active (n = 65) and remission (n = 65) cases by using a combination of biochemical biomarkers and the Harvey Bradshaw index. VOCs in breath were measured by Gas Chromatography time-of-flight Mass Spectrometry (GC-tof-MS), while VOCs in blood headspace were measured by GC/GC-tof-MS. Metabolites in blood were analysed by Nuclear Magnetic Resonance, whereas OTUs in faeces were assessed by 16S rRNA pyrosequencing. To fuse the data, several data fusion strategies were applied, compared, and evaluated: mid-level, high-level, as well as Multiple Kernel Learning (MKL), a specific case of fusion approach.

Results: Low-level fusion attempts were not successful due to the enormous increase in data dimensionality. Random Forest (RF) and Gradient Boosting Trees (GBT) succeeded in finding discriminatory features to be concatenated in the mid-level fusion case and to get predictions to be fused in the high-level fusion case. RF and GBT were also implemented in the MKL fusion attempt. All fusion strategies were evaluated based on their sensitivity and specificity to detect disease activity in CD patients.

Conclusion: The fusion strategies, as mentioned above, demonstrated a comparable or improved prediction accuracy, and at the same time, correlations among all these data platform variables were found.

Endogenous metabolic profiling as a fundament in personalized medicine

Torbjörn Lundstedt*(1,2,7), Katrin Lundstedt-Enkel (1,3), Kate Bennett (1), Claire Russell (4), Rebeca Martín-Jiménez (4), Michelangelo Campanella (4), Sara Mole (5), Julia Petschnigg (5), Thomas Moritz (1,6), Johan Trygg (1,7)

(1) AcureOmics AB, Umeå
(2) Department of Pharmaceutical Chemistry, BMC, Uppsala University, Sweden
(3) Department of Environmental Toxicology, EBC, Uppsala University, Sweden.
(4) Department of Comparative Biomedical Sciences, Royal Veterinary College, UK
(5) MRC Laboratory for Molecular Biology, University College London, UK
(6) Umeå Plant Science Centre, Swedish University of Agricultural Science, Umeå, Sweden.
(7) Umeå Research group for Chemometrics, Institute of Chemistry, Umeå University, Sweden.
**e.mail: torbjorn.lundstedt@acureomics.com, www.acureomics.com*

Abstract
Metabolomics has grown into an established tool in research for;

A. Diagnosis, i.e. classification
B. Identification of biomarkers in relation to e.g. diseases
C. Dynamic studies when identifying effects from, for instance, medical treatment, changes in life style, environmental or genetical changes.

In this presentation the use of metabolomics as a tool in drug discovery and diagnostics will be highlighted. In the first part the differences in biochemical profiles between healthy volunteers and persons with the diagnosis rheumatoid arthritis (RA) are discussed and identification of novel biochemical pathways for understanding the underlying factors of the disease are presented. In addition to this two novel methods to restore a disturbed metabolic profil caused by a mutation in Cln3 will be discussed.
In the next part a comparison to different animal models is made, in order to identify the most relevant animal model for describing the disease in humans. The animal models are used for evaluation of novel treatments.
In the last part, an example from the BATCure project will be presented for a CLN3 disease yeast model, comparing the btn-1 mutant vs. wild type. In addition, from the zebrafish (Danio rerio) CLN2 disease model, we compared tpp1-/- with the metabolic profile of wild type. Results will be presented and discussed in relation to metabolic profiles and biochemical pathways and how these findings can help us to identify novel methods of treatments.

Can we predict Volatile Organic Compounds (VOCs) associated with disease-the case of bladder cancer?

Tom Wieczorek, Ben De Lacy Costello, Natalia Drabinska, Peter Jones, Oliver Gould, Norman Ratcliffe*

Institute of Biosensor Technology, University of the West of England, Coldharbour Lane, Frenchay, Bristol, BS16 1 QY
**e-mail: norman.ratcliffe@uwe.ac.uk*

Abstract

Efforts to use VOCs signatures to try to diagnose disease are increasing. These VOCs may arise from breath, urine, stool etc and are being analysed by a plethora of methods. In many cases there is little biochemical evidence to justify correlation of the VOCs with the disease state. We have attempted to predict the VOCs from one particular disease based on a biochemical rationale, with the intention of looking for these VOC compounds in urine from controls and urine from patients with bladder cancer.

We have made the assumption that oxidative stress is associated with cancer, as this has been postulated in the literature. As oxidative stress causes radical based chain reactions many different compounds would be expected to build up in concentration as a result. The compounds would be expected to remain localised in bladder cancer and concentrate in urine. Oxidative stress compounds arise from oxidation of unsaturated fatty acids (UFAs), and these compounds vary in composition depending on the acid. UFAs can also vary in the cancerous state e.g. it has been reported that the concentration of oleic acid increased and arichidonic acid decreased In bladder cancer. Examination of non-enzymatic oxidation of these two acids gives many possible compounds, which might make their way into urine and be used as putative indicators of bladder cancer. However certain compounds might be expected to be more significant as it has been reported that enzyme activity can accelerate formation of certain metabolites; in particular, 9- and 12-lipoxygenases have been reported to be up-regulated and 15-lipoxygenases down-regulated in bladder cancer. Phospholipase enzyme has also been reported to increase in bladder cancer, which would be expected to increase the release of unsaturated fatty acids from lipids with an increase in peroxidation fatty acid products in the urine. Another factor to consider is that polyunsaturated fatty acids (PUFAs) are much more susceptible to peroxidation with increase in the number of double bonds.

Summing this up and assuming the decrease in concentration of UFAs is due principally to lipoxidation, then a relative increase in concentration of metabolites due to 9- and 12-lipoxygenases acting on arachidonic acid would be expected and likewise a decrease in concentration of certain metabolites due to 15-lipoxygenase. Also peroxidation of oleic acid should result in a change in products, as oleic acid has been reported to be reduced in bladder cancer.

Examples of compounds predicted to change in concentration are some fatty acids, hexanal and nonanal, $CH_3(CH_2)_4CH{:}CHCH_2OH$, $CH_3(CH_2)_4(CH{:}CH.CH_2)_3.CHO$, $CHO(CH_2)_3CO_2H$, $CHO(CH_2)_6CO_2H$*, $CH_2OH(CH_2)_2CO_2H$, $CHOCH_2CH{:}CH(CH_2)_4CH_3$, $CH_2{:}CH(CH_2)_6CH_3$*, $CH_3(CH_2)_3CH_2OH$ and $CH_3(CH_2)_6CH_2OH$*. (* from oleic acid, others from PUFAs).

Some predicted compounds are not in the NIST library and don't have CAS numbers, hence it's unlikely they will have been previously discussed in the literature.There can be differences in the unsaturated fatty acids in different organs and peroxidation of these should be able to give a diagnostic organ specific VOC fingerprint, especially if there are changes in fatty acid composition between cancer and non-cancer states. Furthermore, there is evidence of up and down regulation of lipoxygenases in cancers and monitoring of these is difficult as an invasive biopsy would be needed. However the action of these up and down regulated enzymes would be expected to produce characteristic VOC concentration changes, monitorable non-invasively by analysing breath and bodily fluids.

Metabolomics studies on oncological diseases

Piotr Młynarz*(1), Wojciech Wojtowicz (1), Adam Ząbek (1), Przemysław Juszczyński (2), E. Białotropowicz (2), Tomasz Rzymski (3), Anna Wróbel (3), A. Szyba (4), Angelika Chachaj (4)
(1) Wroclaw University of Science and Technology, Department of Bioorganic Chemistry, Wroclaw, Poland
(2) I*nstitute of Hematology and Transfusion Medicine, Warsaw, Poland*
(3) Selvita S.A., Krakow, Poland
(4) Wroclaw Medical University, Department of Angiology, Wroclaw, Poland
**e-mail: piotr.mlynarz@pwr.edu.pl*

Abstract
The investigations of cancer cells metabolism together with serum as a biofluid, by analytical chemistry methods (NMR and MS), allow monitoring metabolite levels and thus gives insight into cellular metabolism and its perturbations caused by external factors as (eg. gen knockout, pathogenic occurring processes or treatment). The metabolomics studies with employed both mentioned methods allowed to delineate the perturbed biochemical pathways giving an answer about potential molecular targets (particular enzyme) as well overthrow hypothesis about their role in oncological diseases. The most used way for tracking metabolic pathways, are based on metabolic flux analysis (MFA). This method uses the ^{13}C isotopically enriched substrates (metabolites), which are present in medium for cancer cells culturing, introduced intravenously and/or via oral route, for animal xenograft models.
In this presentation, the capabilities of nuclear magnetic resonance NMR and mass spectrometry MS in MFA will be given basing on cell cultures and mouse cancer xenografts extracts. Besides the model based on simply comparison between healthy control and cancer suffering patients (breast cancer, haematological cancers and thyroid cancer) will be given to delineate biochemical pathways as potential targets for designing of therapeutic agents.

Acknowledgements
This study was financed by the project EPTHERON (STRATEGMED 1/233574 /15/ NCBR/ 2015) supported by The National Centre for Research and Development. Wroclaw University of Technology S40531/Z0303 together with WCB KNOW 2014-2018 grant.

Metabolomics in urogenital cancer by use of complementary analytical techniques

Michał J. Markuszewski

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gdańsk, Poland
**e-mail: markusz@gumed.edu.pl*

Abstract
Metabolomics approach was proposed for the assessment of changes in metabolites composition which can reflect currently present pathological processes in a living organism and constitute a basis for diagnosis and treatment improvements. Thus, the multiplatform metabolomics approach was applied for the investigation of molecular mechanisms of chronic kidney disease (CKD) [1] progression and bladder cancer (BCa) [2]. The high-performance liquid chromatography coupled with time-of-flight mass spectrometry (HPLC-TOF-MS) and gas chromatography coupled with triple quadrupole mass spectrometry (GC-QqQ/MS) serum and urine metabolic fingerprinting followed by uni- and multivariate statistical analysis was carried out to determine metabolic pattern differentiating patients and healthy controls. The datasets obtained were submitted to univariate and multivariate statistical analyses. Specific metabolites were found to significantly discriminate serum and urinary profiles from healthy volunteers and both CKD and BCa patients. They are mainly involved in amino acid metabolism, pyrimidine and purine metabolism, as well as energy metabolism and might play a crucial role in the pathogenesis of diseases.

References
[1] M. Kordalewska, S. Macioszek, R. Wawrzyniak, et al., *J. Chromatogr. B*, 1117 (2019) 49-57
[2] J. Jacyna, R. Wawrzyniak, S. Balayssac, et al. *Talanta*, (2019) doi. 10.1016/j.talanta.2019.05.039

New real time pptv level VOC measurement by PTR MS.

Jarosław Grodowski*

INTERTECH POLAND, Warsaw Poland
**e-mail: jgrodowski@intertechpoland.pl*

Abstract
New design of IONICON PTR MS spectrometers ensures:

* high sensitivity - LOD <1 pptv,
* time resolution <100 ms,
* measurement without sample preparation,
* full mass range registered by TOF in a fraction of a second.
* does not require a carrier gas,
* linearity > 6 orders of magnitude (ppt ... ppm)
* precursor ions - SRI: H3O +, NO +, O2 +.

Novel atmospheric pressure (API) sample introduction options will be announced.

Construction and analytical applications of the ionization sources of FAPA - type energized with high tension electric fields and microwaves.

Edward Reszke* (1), Grzegorz Schroeder, Michał Cegłowski (2), Jerzy Silberring, Marek Smoluch (3)

(1) ERTEC, Poland
(2) UAM, Poznań, Poland
(3) AGH, Kraków, Poland
**e-mail: ertec@wp.pl*

Abstract
Selected designs of Flowing Ambient Plasma Afterglow (FAPA) devices will be presented based upon published papers and those being subject of the current research performed in both AGH - Academy in Krakow and UAM - University in Poznan.

Perspectives of future development shall be discussed herewith.

Session 7: ***Analysis of complex samples - part 1***

Chairpersons: Prof. Olav Kvalheim and Prof. Bekzod Kahkimov

9:00 - 9:25 M. Friedrich, F. Latzke, B. Gawlik, A. Antoniewicz,
Efficiency control of dynamic olfactometer by usage of gas chromatography

9:25 - 9:50 N. Drabińska, R. Persad, P. Jones, B. de Lacy Costello, N. Ratcliffe,
An old method for modern needs - the application of liquid-liquid extraction to look for putative urinary volatile biomarkers of bladder cancer

9:50 - 10:15 M. Kordalewska, R. Wawrzyniak, J. Godzień, J. Jacyna, J. Raczak-Gutknecht, Á.L. Gonzálves, M. Markuszewski, M. Matuszewski, C. Barbas, M.J. Markuszewski,
Alterations in biochemical pathways potentially related to renal cell carcinoma

10:15 - 10:30 K. Świderski, K. Gręda, P. Pohl, P. Jamróz,
APGD-OES as a new detection system for various chromatographic separation techniques

Efficiency control of dynamic olfactometer by usage of gas chromatography

Małgorzata Friedrich*, Filip Latzke, Bartosz Gawlik, Aleksandra Antoniewicz

Laboratory for Odour Quality of the Air, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology, Szczecin, Poland
**e-mail: malgorzata.friedrich@zut.edu.pl*

Abstract

Quantities such as mass concentration corresponding to odour detection threshold of chemical compounds, odour detection threshold of a mixture of substances, odour concentration, odour emission or odour abatement efficiency is estimated - according to EN 13725:2003 (PN-EN 13725:2007) - by the method of dynamic olfactometry. Measurements of these quantities come down to estimate the dilution ratio, where probability of odour detection in specific conditions of measurement are equal to 50%.

During the measurements of the odour gas sample, the sample is dynamically diluted with inert gases in specific proportion with special instrument, called the dynamic olfactometer, and presented to the odour panel, which fulfils a role of the measuring sensor. The task given to the panel is to state if they are able to detect odour of the sample in every presented dilution.

The quality of obtained results is dependent on both of the olfactory sensitivity of panel members and precision of dilutions obtained through dynamic olfactometer. PN-EN 13725:2007 precisely describes the requirements of panel members selection and control of panel members ability to participate in the olfactory measurements. However, guidelines to calibrating dynamic olfactometer are vague. Adhering to the norm, the olfactometer shall be calibrated once per year. To calibrate the olfactometer there is need for high class and high precision analytic instruments. Due to these circumstances, olfactometric laboratories outsource the calibration to the third party firms and due to the high cost of such action calibration is most often performed only once per year.

The purpose of this research was to develop efficiency control of dynamic olfactometer procedure, which would be relatively easy, low cost and available for everyday use in every laboratory with the access to gas chromatograph. In order to develop such procedure, a considerable amount of analyses was performed to select the best possible methods of obtaining samples, the number of observations and conditions of the measurement area. The developed result is a procedure with unusual usage of gas chromatography which allows to control the level of dynamic olfactometer dilution. This allows improve the quality of results obtained with the dynamic olfactometry method.

An old method for modern needs - the application of liquid-liquid extraction to look for putative urinary volatile biomarkers of bladder cancer

Natalia Drabińska*(1,2), Raj Persad (3), Peter Jones (4), Ben de Lacy Costello (1), Norman Ratcliffe (1)

(1) Institute of Biosensor Technology, the University of the West of England, Bristol, United Kingdom
(2) Department of Biodynamics of Food, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland
(3) Bristol Urological Institute, Southmead Hospital, Bristol, United Kingdom
(4) Indigo Science Ltd., Bristol, United Kingdom
**e-mail: n.drabinska@pan.olsztyn.pl*

Abstract
Urothelial bladder cancer (UBC) is the 5th most common cancer in Western societies, accounting for 260 000 new cases per year in the UK, USA and EU, and with a rising global incidence (ca. 2.1 million worldwide). The diagnosis of this disease is very expensive and very often the disease is diagnosed too late. Therefore, there is a strong need to find easier and faster diagnostic methods. In recent years, there have been many reports on dogs sniffing out tumours in their owners [1]. This suggests that changes in metabolism by malignant cells result in the formation of specific volatile organic compounds (VOCs). VOCs are analysed using different analytical approaches, such as solid-phase microextraction fibres and thermal desorption tubes, obtaining a limited number of data. For example, the study of VOCs in urine using solid-phase extraction identified 103 compounds in the headspace above the urine [2]. In the present study, the application of the conventional approach, liquid-liquid extraction, has been applied to analyse the VOCs in urine from UBC patients. The main goal was to obtain the maximum number of compounds, in order to find the differences between UBC patients and healthy controls. 50 urine samples (25 from UBC and healthy people each) were collected. The extraction conditions were optimized and the analysis was performed with 1 M sulphuric acid, sodium sulphate and methylene chloride. The VOCs in the extracts were analysed using gas chromatography coupled with mass spectrometry. Data were analysed using XCMS software. Promising results have been obtained to show that the "old school" approach of analysis is perfectly sufficient to obtain satisfactory results for VOCs analysis.

Acknowledgements
The study was supported by a grant from the Above and Beyond Charity in Bristol.

References
[1] O. Gould, A. Smart, N. Ratcliffe, B. de Lacy Costello, Canine Olfactory Differentiation of Cancer: A Review of the Literature, (2015) arXiv:1510.05495
[2] G. A. Mills, V. Walker. Headspace solid-phase microextraction profiling of volatile compounds in urine: application to metabolic investigations, J. Chromatogr. B Biomed. Sci. Appl., 753 (2001) 259-268

Alterations in biochemical pathways potentially related to renal cell carcinoma

Marta Kordalewska*(1), Renata Wawrzyniak (1), Joanna Godzień (2), Julia Jacyna (1), Joanna Raczak-Gutknecht (1), Ángeles López Gonzálves (2), Marcin Markuszewski (3), Marcin Matuszewski (3), Coral Barbas (2), Michał J. Markuszewski (1)

(1) Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gdańsk, Poland
(2) CEMBIO, Centre of Metabolomics and Bioanalysis, San Pablo CEU University, Madrid, Spain
(3) Department of Urology, Medical University of Gdańsk, Gdańsk, Poland
**e-mail address: marta.kordalewska@gumed.edu.pl*

Abstract

Metabolites, as end products of biological processes, are the best reflection of current organisms' state. Changed metabolome composition could be a result of changed metabolism related to disease development and progression. Nowadays, the metabolomics approaches are successfully applied for searching of such alterations in biochemical pathways. Therefore, urine metabolic fingerprinting was applied to explain biochemical changes related to RCC development.

In the presented study, two groups of patients were selected: one with diagnosed RCC (n=30) and second consisting of healthy volunteers (n=29). Urine metabolic fingerprinting was performed using gas chromatography coupled with triple quadrupole mass spectrometry (GC-QqQ/MS), liquid chromatography and capillary electrophoresis coupled with time of flight mass spectrometry (LC-TOF-MS, CE-TOF-MS). Obtained datasets were pretreated with extraction, deconvolution, alignment, filtration and normalization steps. Afterwards, different feature selection methods, such as: Selectivity Ratio (SR), Variance Importance into Projection (VIP), univariate statistical analysis with *p*-value corrected using Benjamini-Hochberg procedure were applied. The identification of selected metabolites using NIST11 spectra library, METLIN database, as well as HMDB and KEGG by means of CEU Mass Mediator allowed for creation of a list of putative indicators and related biochemical pathways which they are involved in. Furthermore, the obtained preliminary results were validated by the analysis of urine samples collected form newly selected, external group of RCC patients (n=38) and healthy volunteers (n=37). The analyses were performed with the use of LC-TOF-MS and GC-QqQ/MS techniques. All the analytical, data pretreatment and statistical procedures were the same as those applied for the previously studied groups.

The comparison of the results obtained for both studied groups provided a list of metabolites that can be considered as a potential metabolic indicators of RCC. The disturbances in amino acid, carbohydrate and purine metabolism, as well as TCA cycle were observed. This preliminary study constitutes a proof of concept regarding application of metabolomics approach in evaluation of RCC metabolic phenotype. However, it should be underlined that the results derived from this pilot study require validation on larger set of samples, with the use of the targeted metabolomics approach, to confirm their potential value in evaluation of RCC metabolic signature.

Acknowledgements

The project was supported by the National Science Centre grant no. 2015/19/N/NZ7/03397 and the Ministry of Science and Higher Education of the Republic of Poland, from the quality-promoting subsidy under the Leading National Research Centre (KNOW) programme for the years 2012-2017.

APGD-OES as a new detection system for various chromatographic separation techniques

Krzysztof Świderski*, Krzysztof Gręda, Paweł Pohl, Piotr Jamróz

Division of Analytical Chemistry and Chemical Metallurgy, Faculty of Chemistry, Wrocław University of Science and Technology
**e-mail: krzysztof.swiderski@pwr.edu.pl*

Abstract
The chromatographic techniques require a proper detection method, which allow to qualitatively and quantitatively determination of the separated species. Most of commonly used versatile method of detection, such as mass spectrometry (MS), is very efficient, but their cost of purchase is expensive and operation cost is very high. The chance to reduce the cost of apparatuses and analysis is to use discharge plasma source. Among the various plasma sources, the greatest interest has arisen in the miniaturized atmospheric pressure glow discharge (APGD) generated in contact with the flowing liquid electrode and coupled with optical emission spectrometry (OES).
In APGD system generated in air (or other gas atmosphere), the various diatomic molecules as well as simple atomic species were excited. By detection of that band there can be determine the presence of various molecules (or product of their reaction) in analysed sample. To quantitatively determine the organic compounds it can be previously transform into its metalloorganic derivative and the analysis can be done by analyse the emission of metallic species used to obtain metalloorganic molecule.
In my poster, I will present the latest achievements in APGD-OES systems used as detection methods in various types of chromatography.

References
[1] Ch.G. Decker, M.R. Webb, Measurement of sample and plasma properties in solution-cathode glow discharge and effects of organic additives on these properties, J. Anal. Atom. Spectrom. 31 (2016) 311-318
[2] S. Shekhar, D. Karunasagar, K. Dash, M. Ranjit, Determination of mercury in hepatitis-B vaccine by electrolyte cathode glow discharge atomic emission spectrometry (ELCAD-AES), J. Anal. Atom. Spectrom. 25 (2010) 875-879

Session 8: ***Analysis of complex samples - part 2***

Chairpersons: Prof. Monika Waksmundzka-Hajnos and Dr. Irena Vovk

11:00 - 11:30 M. Chrubasik, R. Muzyka, M. Pogoda, J. Tarnowska, M. Sajdak,
Did only fuel type has an effect on the polycyclic aromatic hydrocarbons concentration in the exhaust gases from a central heating furnace - case of study

11:30 - 12:00 K. Zięba, E. Szostak, H. Szentgyörgyi, A. Moos-Matysik,
Bees and their environment - the use of gas chromatography technique with MS detection for determination of volatile aromatic hydrocarbons

12:00 - 12:20 E. Makowicz, I. Jasicka-Misiak, P. Kafarski,
Chromatographic methods of determination botanical origin of Polish honeys of different botanical origin

12:20 - 12:40 A. Dębczak, P. Hodurek, K. Tyśkiewicz, E. Rój,
Application of centrifugal partition chromatography in the separation of bioactive compounds from plant oils

12:40 - 13:00 K. Tyśkiewicz, M. Konkol, E. Rój, K. Skalicka-Woźniak,
The separation of biologically active compounds from materials rich in omega-3 fatty acids

Did only fuel type has an effect on the polycyclic aromatic hydrocarbons concentration in the exhaust gases from a central heating furnace - case of study

Maciej Chrubasik, Roksana Muzyka, Małgorzata Pogoda, Justyna Tarnowska, Marcin Sajdak*

Institute for Chemical Processing of Coal, Zabrze, Poland
**e-mail: msajdak@ichpw.pl*

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are one of the environmental pollutants which are primarily generated during the incomplete combustion of organic materials, e.g. different kind of solid fuels. In Polish condition, the significant anthropogenic sources of PAHs, excluding industrial processes, are the combustion process of solid fuels in central heating furnaces. Many PAHs hurt human health, are toxic, mutagenic and have carcinogenic properties [1]. In this reason, constant control of this pollutants and searching for effective solutions aimed at minimising PAH emissions. One of the potential methods for minimalisation of PAHs emissions could be using suitable central heating furnaces or a fuel characterised by lower emissivity.

In the present study, we have investigated the influence of the various types of central heating furnaces and different variety of solid fuels including hard coal, coal flotation concentrate, wood, thermal treated coal on the PAHs content in exhaust gases. In additions, the correlations between fuel parameters and chemical properties of exhaust gases were proposed. All samples were collected during the combustion process in central heating furnaces in real conditions using filters, which were then subjected to extraction and then analysed by GC-MS. Sixteen polycyclic aromatic hydrocarbons were analysed qualitatively and quantitatively: naphthene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(a)pyrene, benzo(e)pyrene, benzo(b,k)fluoranthene, dibenzo(a,h)anthracene indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene, perylene.

To complex data analysis, the chemometric multivariate analysis such as clustering analysis, principal component analysis, multivariate analysis of variance was applied. It allowed to described which parameters of solid fuels, types of fuel and central heating furnaces affects the chemical properties of exhaust gases.

References

[1] B.G. Armstrong, E. Hutchinson, J. Unwin, T. Fletcher, Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: A review and meta-analysis, Environ. Health Perspect. 112 (9) (2004) 970-978

Bees and their environment - the use of headspace gas chromatography technique with MS detection for determination of volatile aromatic hydrocarbons.

Katarzyna Zięba*(1), Elżbieta Szostak (1), Hajnalka Szentgyörgyi (2), Agnieszka Moos-Matysik (1)

(1) Faculty of Chemistry, Jagiellonian University, Kraków, Poland
(2) Faculty of Biotechnology and Horticulture, University of Agriculture, Kraków , Poland
**e-mail: kzieba@chemia.uj.edu.pl*

Abstract

The headspace gas chromatography technique with MS detection was used for determination of volatile aromatic hydrocarbons in bee organisms and bee pollen. Samples of bees and bee pollen were collected at two points in the city of Kraków and at two points near Olkusz. At each point bee larvae and bee pollen were collected from three hives (nest 1, nest 2 and nest 3) from local beekeepers. Two pieces of honeycomb of a surface area of a few hundred square cm were cut from each nest - one containing stored bee pollen and the other - closed larvae. The studies were conducted for two years (in 2017 and 2018) in the spring and summer season.

The larvae and the bee pollen were removed from the combs and immediately after being removed they were stored in tightly closed polyethylene bags. Then they were stored in the freezer until they were analyzed. Prior to the analysis of volatile organic hydrocarbons, the samples were defrosted and homogenized to obtain the most homogenous mass, and then weighed. BTEX hydrocarbons (benzene, toluene, ethylbenzene and p-xylene) were analyzed using the GC/MS technique with headspace injector and n-buthylbenzene as an internal standard.

The results show that pollution of environment (bee pollen) with mononuclear hydrocarbons in Kraków was almost 10 times higher in 2017 than in 2018. In addition, the concentration of mononuclear hydrocarbons in samples collected in spring 2017, was higher than in samples collected in the summer of the same year. However, in the samples collected in 2018 hydrocarbon concentrations are comparable regardless of the season.

Environmental pollution in the vicinity of Olkusz in 2018 is comparable to environmental pollution in Kraków in the same year, while in 2017 it is 2 times higher than in Olkusz. The results of the research from 2017 also indicate that exceeding some level of bee pollen pollution, respectively 320 and 500 ng/g does not translate into an increase in BTEX concentration in bee. The results obtained in 2017 and 2018 indicate differences in the accumulation of pollutants between individual bee families (beehives). This may be due to the fact that these bee families feed on different areas.

It can also be stated that the high concentration of hydrocarbons in the environment has a negative impact on bee populations, which is particularly evident in the area of Olkusz. The proportion of individual hydrocarbons in bees and bee pollen samples was also analyzed.

Acknowledgments

This study was supported by the Polish National Science Center (NCN) grant number UMO-2016/21/B/NZ9/01163.

GC-MS and HPTLC techniques for authentication of botanical origin of honeys

Ewa Makowicz*(1), Izabela Jasicka-Misiak (1), Paweł Kafarski (2)

(1) Department of Analytical and Ecological Chemistry, University of Opole, Poland
(2) Department of Bioorganic Chemistry, Wroclaw University of Science and Technology, Poland
**e-mail: emakowicz@uni.opole.pl*

Abstract

Honey is a natural, sweet, aromatic and complex food product produced by honey bees (Apis melifera). Valuable nutritional and therapeutic properties of honeys are resulting from presence of rich cocktail of organic compounds derived from flower nectar or honeydew. Consumer interest in this product is constantly increasing. In the market it is a wide range of diverse types of honeys of different botanical and geographical origin. Each of them is characterized by unique flavor, nutritional values and therapeutic effects. The chemical composition of honey is variable and strongly depend on huge number of varied factors, such as bee species, climate, soil characteristic, botanical and geographical origin, age of honey, storage methods and honey processing (harvest technology and condition). Unfortunately, it is very disturbing that honey is one of the most common falsified food products, what leads to a significant decrease of the unique qualities of honey; moreover, methods commonly used to control its quality are not sufficient.

The aim of presented study was evaluation of volatiles chemical content of Polish honeys of different botanical origin, which are characterized by high flavour and nutritional values (e.g. goldenrod, phacelia, dandelion, heather or buckwheat honeys) and construction of chemical profiles based on the different extraction methods and GC-MS analysis. Moreover, for analysis of whole lipophilic extracts of honeys HPTLC method was apply. This method allows us to construct something like cod-bars useful to differentiate of Polish honeys of different botanical and geographic origin as well as to determine their quality.

Additionally, based on the obtained results we could determine and identify the markers, for different unifloral honeys and show that combination of GC-MS and HPTLC analysis create a specific fingerprint of honeys with different botanical origin and that those methods are powerful techniques for differentiation origin of honeys.

Acknowledgements

This study was carried out with financial support from the National Science Centre in Poland project No. 2017/25/N/NZ9/00623 “Fluorescence compounds as a specific markers of Polish honeys with different botanical origin” and project No. 2014/15/B/NZ9/02182 “Chemical markers of unifloral honeys”.

Application of Centrifugal Partition Chromatography in the separation of bioactive compounds from plant oils

Agnieszka Dębczak*(1), Paweł Hodurek (2), Katarzyna Tyśkiewicz (1), Edward Rój (1)

(1) Department of Supercritical Extraction, New Chemical Syntheses Institute, Puławy, Poland
(2) Department of Medical Biochemistry, Wroclaw Medical University, Faculty of Medicine, Wrocław, Poland
**e-mail: agnieszka.debczak@ins.pulawy.pl*

Abstract
Centrifugal Partition Chromatography (CPC) with Gilson (Armen) CPC apparatus has been applied to perform separations of non-glyceride components, including free fatty acids (FFA) and minor components such as bioactive constituents of vitamin E; tocopherols and tocotrienols. This study concerns isolation of the pure compounds from crude plant oils i.e. wheat germ oil and pumpkin seed oil, which have been well documented for their strong antioxidant activity and high concentrations of lipophilic bioactive minor components accompanying acylglycerols. Regarding vitamin E components partitioning tests were performed first with apolar solvent systems such as n-heptane/methanol and n-heptane/acetonitrile. The APCI in positive ion mode was chosen for development of LC-MS method for simultaneous determination of eight naturally occurring forms of tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3, δ-T3) using a PFP (pentafluorophenyl) column. The solvent systems yielded a KD for alpha-, beta-, gamma- and delta-tocopherol as follows 1.01±0.03, 1.07±0.02, 1.15±0.12, 1.45±0.02, as determined by LC-MS (n=3). Separations were performed in dual-mode. Natural mixed tocopherol Covi-ox T70 (BASF) containing 70% of total tocopherols with predominant gamma-homologue was applied to determine distribution coefficients KD.
An overview of literature describing the use of countercurrent chromatography in the separation of nonpolar lipid compounds revealed that biphasic non-aqueous solvent systems in which these compounds spread evenly are very difficult to obtain in the nonpolar range [1,2]. To our best knowledge later works of Vetter at al. identified the opportunities of CCC in separation of lipophilic compounds i.e. conjugated linolenic acids, tocochromanol homologues, phytosterols and carotenoids with the simple binary n-hexane/acetonitrile system and ternary system with novel modifier (trifluoromethyl)benzene BFT from selected plant oils (pomegranate, rapeseed), supplementary capsules made from palm oil and crude carrot extract [3,4].

References
[1] J.B. Friesen, J.B. McAlpine, S.-N. Chen, G.F. Pauli, Countercurrent separation of natural products: An update, J. Nat. Prod. 78 (2015) 1765-1796
[2] K. Skalicka-Woźniak, I. Garrard, A comprehensive classification of solvent systems used for natural product purification in countercurrent and centrifugal partition chromatography, Nat. Prod. Rep. 32 (2015) 1556-1561
[3] W. Vetter, S. Hamman, M. Muller, M. Englert, Y. Huang, The use of countercurrent chromatography in the separation on non-polar lipid compounds, J. Chromatogr. A., 1501 (2017) 51-60
[4] M. Englert, S. Hamman, W. Vetter, Isolation of β-carotene, α-carotene and lutein from carrots by countercurrent chromatography with the solvent system modifier benzotrifluoride, J. Chromatogr. A, 1388 (2015) 119-125

The separation of biologically active compounds from materials rich in omega-3 fatty acids

Katarzyna Tyśkiewicz*(1), Marcin Konkol (1), Edward Rój (1), Krystyna Skalicka-Woźniak (2)

(1) Instytut Nowych Syntez Chemicznych, Zakład Ekstrakcji Nadkrytycznej, Puławy, Poland
(2) Uniwersytet Medyczny, Katedra i Zakład Farmakognozji z Pracownią Roślin Leczniczych, Lublin, Poland
**e-mail: katarzyna.tyskiewicz@ins.pulawy.pl*

Abstract
One of the materials rich in omega-3 fatty acids is waste fish oil. The waste fish oil is an oil that may be produced from the fish head, flesh, frames, tail, fin, skin and guts, which are the by-products of fish processing and treatment performed by the industries for food and pharmaceutical purposes. Simultaneously, such waste fish management as mentioned above affects the reduction of environmental pollution. Another promising use of waste fish oil is to produce biodiesel which possesses similar properties as biodiesel produced from crops, as was reported by Kara et al. [1].
There is a strong need to provide further efficient management of waste fish oil for cosmetic and pharmaceutical purposes. Numerous studies have established that waste fish oil is a source of biologically active compounds, including fat-soluble vitamins [2,3], phospholipids as well as fatty acids [4]. According to Sudeepa and Balasarvanan [5], the waste fish oil is also a source of biomolecules, such as squalene, amino acids, proteins, collagen as well as gelatin and enzymes. Concerning phospholipids, they form liposomes in the size range of 10 nm to 80 nm. Due to both polar and non-polar nature of phospholipids, such liposomes are appropriate for drug-based formulations with an extended release time [6]. Furthermore, egg yolk and soybean phospholipids have been evaluated the most in terms of liposomes so far [7]. This opens up new possibilities for the isolation of phospholipids from the waste fish oil and the production of both liposomes and immunoliposomes that might be used in cosmetics and pharmaceutical products, respectively.

References
[1] K. Kara, F. Ouanji, E.M. Lofti, M.E. Mahi, M. Kacimi, M. Ziyad, Biodiesel production from waste fish oil with high free fatty acid content from Moroccan fish-processing industries, Egypt. J. Pet. 27 (2018) 249-255
[2] K. Tyśkiewicz, A. Dębczak, R. Gieysztor, T. Szymczak, R. Rój, Determination of fat- and water-soluble vitamins and its isomers by supercritical fluid chromatography: A Review, J. Sep. Sci. 41 (2018) 336-350
[3] K. Tyśkiewicz, R. Gieysztor, I. Maziarczyk, P. Hodurek, E. Rój, K. Skalicka-Woźniak, Supercritical fluid chromatography with photodiode array detection in the determination of fat-soluble vitamins in hemp seed oil and waste fish oil, Molecules, 23 (2018) 1131
[4] J.P. Kerry, S.C. Murphy, Physical and chemical properties of lipid by-products from seafood waste, Chapter 2 [in:] Maximising the Value of Marine By-Products, Woodhead Publishing Series in Food Science, Technology and Nurition, (2007) 22-46
[5] E.S. Sudeepa, T. Balasarvanan, Commercialization of fish processing wastes on various ways, Biotechnol. Res. 4 (2018) 1-10
[6] A. Garud, N. Garud, V. Tomar, M. Maheshwari, Topical liposomal gel with aceclofenac: characterization and in vitro evaluation, The Pharmacist, 2 (2007) 41-44
[7] J. Li, X. Wang, T. Zhang, C. Wang, Z. Huang, X. Luo, Y. Deng, A review on phospholipids and their main applications in drug delivery systems, Asian J. Pharm. Sci. 2 (2015) 81-89

Poster session

The 42nd Symposium
Chromatographic Methods of Investigating Organic Compounds
June 4-7, 2019, Szczyrk, Poland
www: www.chromatographia.us.edu.pl
fb: www.facebook.com/chromatographia/

Development and optimization of the RP-HPLC method for efficient separation of ivabradine and its eleven impurities

Jovana Tomić, Branka Ivković, Slavica Oljačić, Katarina Nikolić, Ana Protić, Danica Agbaba*

University of Belgrade - Faculty of Pharmacy, Belgrade, Serbia
**e-mail: danica@ pharmacy.bg.ac.rs*

Abstract

The aim of this study was to develop novel RP-HPLC method for efficient separation of ivabradine and its eleven impurities. Since sample mixture contained impurities of very similar polarity, the method optimization was achieved only when different chemometrical tools, such as Principal Component Analysis (PCA), Box- Behnken Design of Experiments (BB DoE) and Face Centered Central Composite Design (FC-CCD), were employed. Chromatographic retention parameters obtained with the mobile phase composition of 30 mM phosphate buffer and acetonitrile (80:20, v/v) on four different RP-LC columns and at three different pH values (3.0, 4.0, and 5.0) were subjected to PCA analysis in order to select the column with the most suitable selectivity. Afterwards, column temperature, pH value of the water phase, phosphate buffer molarity and ratio of organic solvent in the mobile phase were examined by use of BB DoE. Since this statistical tool did not propose chromatographic conditions that enabled separation of three positional isomers of ivabradin, it was decided to use mixture of acetonitrile and methanol as organic phase and to optimize its ratio as well as the content of organic phase using FC-CCD. Finally, the developed RP-HPLC method for efficient separation of ivabradine and its eleven impurities was achieved on Zorbax Eclipse Plus C18 chromatographic column (100 × 4.6 mm, 3.5 µm particle size; Agilent Tehnologies, USA), with column temperature set at 35°C, flow rate of the mobile phase of 1.6 mL min^{-1}, and UV detection at 220 nm. Mobile phase composition included water phase A: 30 mmol phosphate buffer pH 6.0 and organic phase B: acetonitrile/methanol (41:59, v/v) in the A : B ratio 81.7:18.3, v/v.

What happens with Solvent Red 19 and 164 over time under the influence of UV-A irradiation, and temperature?

Michał Daszykowski*(1), Joanna Orzeł (1), Ivana Stanimirova (1), Anna Poliwoda (2), Piotr Młynarz (3), Dorota Prukała (4)

(1) Institute of Chemistry, The University of Silesia, Katowice, Poland
(2) Faculty of Chemistry, Opole University, Opole, Poland
(3) Department of Chemistry, Wroclaw University of Technology, Wroclaw, Poland
(4) Faculty of Chemistry, Adam Mickiewicz University in Poznań, Poznań, Poland
**e-mail: michal.daszykowski@us.edu.pl*

Abstract
Fiscal markers are chemical compounds with unique properties added to diesel oil to label the intended purpose of its usage and indicate expected tax level [1]. In this study, we analyzed chemical changes in model systems with one of two red disazo dyes, the so-called Solvent Red 19 and 164 (concentration of dye equal to 4.6 mg·dm^{-3}), dissolved in heptane (HPLC grade).
Both dyes are used in Poland interchangeably as fiscal fuel additives [2]. The amount of a dye in diesel oil is specified by law and controlled. Therefore, dye concentration found below expected level is an indication of fuel counterfeiting. The two red dyes are deemed to be chemically stable under normal exploitation conditions. However, as proven by results obtained in our study, the two dyes are unstable when they are exposed to different external conditions especially the UV-A irradiation and temperature. Changes in the chemical composition of model systems most likely can be explained by the mechanism of photoinduced degradation [3] in which two disazo bonds present in chemical structures of both dyes play a crucial role. Photoinduced degradation, observed over a more extended period, results in spectacular fading of a sample color and a substantial decrease of a dye concentration.
Model samples were sealed quartz flasks and stored in climatic chamber MK 240 (Binder) for 64 days. During the experiment, the temperature in the climatic chamber was kept at a constant level (30°C), and samples were exposed to the UV-A irradiation (5 × 15W, Actinic BL TL-D, Philips). Based on the design of experiments approach [4], these two factors had the most significant influence on the decrease of sample color that was evaluated using a spectrophotometer (Thermo Scientific Nicolet Evolution 220 LC coupled with the Peltier system). Samples were analyzed after 17 and 64 days. Changes in the content of chemical components were monitored using UV spectra, the fluorescence excitation-emission spectra as well as high-performance liquid chromatography analysis using UV and mass spectrometry detection (UHPLC-UV-ESI-MS) and were compared to the content of reference samples.

Acknowledgment
MD, JO, IS, AP, PM, and DP acknowledge the support of the National Science Centre, Poland (research grant no. 2014/13/B/ST4/05007).

References
[1] 2003/900/EC: Commission Decision of 17.12.2003 amending Decision 2001/574/EC establishing a common fiscal marker for gas oils and kerosene
[2] Disposition of Polish Minister of Finance concerning marking and dying of energy products, (Dz. U. 2010, No. 157, item 1054)
[3] R. Podsiadły, J. Sokołowska, Photostability of a range of azobenzene dyes and their benzothiazolyl analogs in the presence of air, Coloration Technology, 119 (2003) 341-344
[4] D.C. Montgomery, Design and analysis of experiments, Wiley, 2012

Metabolite profiling of calcium channel blocker - flunarizine with the use of human liver microsomes and LC-MS method

Maciej Gawlik*, Robert Skibiński

Department of Medicinal Chemistry, Faculty of Pharmacy Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland
**e-mail: maciej.gawlik@umlub.pl*

Abstract
Flunarizine (1-[bis(4-fluorophenyl)methyl]-4-[(E)-3-phenylprop-2-enyl]piperazine) is a selective calcium channel blocker most commonly used as a migraine prevention agent, in treatment of vestibular vertigo and as an adjuvant in the therapy of epilepsy. The pharmacological activity of this drug is not devoid of side effects, which are described as a potential effects of the accumulation of metabolic products. It has been already proven that flunarizine undergoes hepatic metabolism pathway.
The selected metabolism test method assumes the use of human liver microsomes (HLM) with NADPH as a cofactor and thermal incubation. This method is cheap and fast, which is the reason of its frequent use in context of newly introduced drugs. It can be also equally effectively applied to the commonly prescribed medicine.
Ultra high performance liquid chromatography (UHPLC) coupled with accurate quadrupole-time-of-flight (Q-TOF) mass spectrometry was used to the metabolite profiling of flunarizine after the incubation with HLM fraction. The separation was performed on Kinetex C18 (dp = 1.7 µm) column and gradient elution with a mixture of water containing 5% addition of acetonitrile and acetonitrile with addition of 0.1% solution of formic acid in both media was used as a mobile phase. Mass spectrometry was performed in auto MS/MS mode in order to collect MS as well as all the fragmentation spectra of flunarizine and its metabolites. After four hours of *in vitro* incubation of flunarizine with HLM fraction six metabolites were found and structurally characterized.
The 4-hydroxy flunarizine was identified to be the main metabolite of the analyzed drug. Moreover, two new and not described yet metabolites of flunarizine were observed and structurally characterized during this study. UHPLC combined with Q-TOF high resolution MS method was found to be a powerful tool for the metabolite profiling of drugs after *in vitro* incubation with human liver microsomes.

Carbon-based nano-adsorbents for the preconcentration and determination of lanthanides and actinides ions

Karina Kocot*, Michalina Kuban, Daria Jeruszka, Rafał Sitko

Department of Analytical Chemistry, University of Silesia, Katowice, Poland
**e-mail: karina.kocot@us.edu.pl*

Abstract

Efficient determination of lanthanides and actinides has received considerable attention of researchers due to two pressing reasons: (i) the growing demand for f-elements which are essential to civilian and military technologies, advanced defense systems and 21-century global economy, and (ii) the complexity of f-elements effective extraction/isolation and nuclear waste management. An increasing number of lanthanides and actinides applications in industrial and agricultural fields justify the need for the development of precise, rapid and accurate procedures for their determination [1]. Nevertheless, the direct determination of f-elements with the use of commonly applied spectroscopic techniques is usually hampered due to low concentrations of analytes or too complicated matrix. For that matter an application of appropriate preconcentration/separation step before the actual measurement is necessary.On the account of abovementioned reasoning, the aim of the research consists in the development of analytical procedures based on dispersive micro-solid phase extraction (DMSPE) with graphene oxide (GO) and oxidized multi-walled carbon nanotubes modified with(ox-MWCNTs): β-cyclodextrins (O-donor ligands) for bulk extraction of U and Eu ions from complicated matrix (e.g. saline water samples) [2].

In the series of batch experiments the sorption of U and Eu ions was examined. In the first stage of our procedure an appropriate amount of adsorbent phase (in the µg range) was added to the analyzed solution and dispersed by sonication. Subsequently, the sample is stirred or vigorously shaken and in the last step the suspension is passed through a cellulose filter using a filtration assembly. The concentration of elements in solution is determined by ICP-OES, whereas graphene or MWCNTs with adsorbed metals collected onto the filter are dried under an IR heater and measured with the use of EDXRF spectrometry. The strategy consisting in the use of DMSPE promotes immediate interaction between analytes and sorbent molecules and thus shortness time of sample preparation.

References

[1] K. Pyrzyńska, A. Kubiak, I. Wysocka, Application of solid phase extraction procedures for rare earth elements determination in environmental samples, Talanta, 154 (2016) 15-22

[2] Q. Fu, L. Yang, Q. Wang, On-line preconcentration with a novel alkyl phosphinic acid extraction resin coupled with inductively coupled plasma mass spectrometry for determination of trace rare earth elements in seawater, Talanta, 72 (2007) 1248-1254

Chemometrics meets TLC solvent strength

Łukasz Komsta*, Robert Skibiński, Joanna Wróbel, Sebastian Gadowski, Karolina Skrzypiec, Jakub Trawiński, Maciej Gawlik

Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland
**e-mail: lukasz.komsta@umlub.pl*

Abstract
The elution strength of a particular solvent can vary on the chemical structure of chromatographed substance. However, it is widely accepted to present this property as one value, perceived like an average (mean) elution force, useful in design of chromatographic systems. Most of known solvent strength values are derived from physicochemical properties of solvents.
The lack of truly experimental data about solvent strength is caused mainly by the difficulty to suppress the bias of other solvents used in an experiment. The extrapolation of binary mixture series gives us the retention data for pure solvent, but the result depends in practice on the second solvent chosen for the experiment. Averaging the results from many binary mixtures requires enormous number of mobile phases to investigate, still carrying some uncertainty about the result bias. This problem can be solved with an appropriate mixture experimental design. In this case, the strength of each solvent can be extracted by a regression approach from an acceptable number of experiments, done with pure solvents and multicomponent mixtures. As one can use a mixture screening approach for such a task, the experiment number can be reduced to 3n+1, where n is the number of investigated solvents. The above approach gives the solvent strength for one particular solute. In our recent research we performed this approach on 35 model substances to derive averaged solvent strengths for most popular solvents and adsorbents. The estimation uncertainty of the strength was additionally validated by the bootstrap approach.
The greatest advantage of the obtained results is the possibility of their interpretation as RM value differences, which simplifies the design and optimization of TLC systems.

References
[1] Ł. Komsta, B. Stępkowska, R. Skibiński, The experimental design approach to eluotropic strength of 20 solvents in thin-layer chromatography on silica gel, J. Chromatogr. A, 1483 (2017) 138-141
[2] Ł. Komsta, U. Dunin, R. Skibiński, Can RP-8 plates be used in normal-phase systems? a chemometric approach to estimate the elution strength of popular solvents, JPC - J. Planar Chromat. 30 (2017) 401-404
[3] Ł. Komsta, R. Skibiński, S. Gadowski, J. Wróbel, K. Skrzypiec, Experimental evaluation of solvent elution strength in reversed-phase thin-layer chromatography on RP2 and RP8 plates. JPC - J. Planar Chromat. 31 (2018) 351-354
[4] Ł. Komsta, R. Skibiński, J. Wróbel, S. Gadowski, K. Skrzypiec. Experimental evaluation of solvent elution strength in reversed-phase TLC on medium polarity phases, J. Liq. Chromatogr. R. T. (Online first). DOI 10.1080/10826076.2019.1585610

Design of Experiments approach as a way to simplify sample preparation procedure in GC-MS/MS metabolomics

Julia Jacyna*, Marta Kordalewska, Joanna Raczak-Gutknecht, Michał J. Markuszewski

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Poland
**e-mail: julia.jacyna@gumed.edu.pl*

Abstract

Experimental design (Design of Experiments, DoE) consists of making specific and controlled modifications in a studied system in order to create a mathematical model that will allow to predict how monitored responses are affected by applied modifications [1]. In other words, the use of DoE approach allows to screen the most important factors, predict relationships between them and generate the most optimal settings in order to get the most favorable response while saving the time spent on the optimization and reducing the cost of analysis. Its main advantage is the ability to provide optimal parameters' settings by performing a minimal number of experiments. In general, experimental design workflow consists of 6 stages, namely: selection of system's factors, selection of system's responses, construction of experimental design strategy, performance of all the experiments, statistical analysis of the obtained data, conclusions and recommendation and subsequent application of the optimized settings [2].

The objective of the study was to develop and optimize a simple method for preparation of human urine samples for determination of concentration of previously selected metabolites by means of GC-MS/MS analysis. A rapid, simple and reliable method is necessary for targeted metabolomics analysis. Moreover, since sample preparation step for GC-MS/MS is usually very complicated, time-consuming and requires the use of toxic reagents, implementation of Design of Experiments was reasonable.

Urine sample preparation procedure was developed using Fractional Factorial Design plan based on the evaluation of several parameters, such as: amount of urease, volume of methanol, incubation time, volume of pyridine solution of methoxyamine, time of subsequent incubation, volume of mixture of TMCS and BSTFA and time of vortex-mixing. Fractional Factorial Plan was implemented as a screening procedure in order to evaluate the significance of the variables and to limit the amount of organic reagents used.

Acknowledgments

This project was funded by the National Science Centre allocated on the basis of the decision number 2016/23/N/NZ7/02875.

References

[1] L. Eriksson, Design of Experiments: Principles and Applications, Umetrics, 2008

[2] A. Tebani, I. Schmitz-Afonso, D.N. Rutledge, B.J. Gonzalez, S. Bekri, C. Afonso, Optimization of a liquid chromatography ion mobility-mass spectrometry method for untargeted metabolomics using experimental design and multivariate data analysis, Anal. Chim. Acta, 93 (2016) 55-62

In silico estimation of structure-activity relationships for novel benzene-based carbamates for AChE/BChE inhibition

Aleksandra Świetlicka (1), Krystyna Jarzembek*(1), Paulina Dybał (1), Violetta Kozik (1), Josef Jampilek (2), Andrzej Bąk (1)

(1) Department of Synthesis Chemistry, Institute of Chemistry, University of Silesia, Katowice, Polan
(2) Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
**e-mail: krystyna.jarzembek@us.edu.pl*

Abstract

A number of clinically implemented drug and pesticide molecules contain amide (- CONH-) and/or carbamate (- OCONH-) groups that can be variously substituted to form privileged structural fragments. Based on the cholinergic hypothesis that assumes the increase in the acetylcholine (ACh) level, carbamate-like drugs can be regarded as one of the mainstays for the contemporary pharmacotherapy for Alzeheimer's disease (AD) by inhibiting cholinesterases (ChEs). Basically, two types of the ChE enzymes that belong to the group of serine hydrolases occur in the human nervous system - acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), respectively. In fact, both enzymes are able to catalyse ACh hydrolysis in the cholinergic synapses, but BChE can hydrolyse other esters as well; therefore, its concentration in the brain seems to be important for those suffering from AD. Inhibiting both enzymes produces an increment of the ACh concentration in the cholinergic synapses that show a symptomatic efficacy in AD treatment; therefore, competitive cholinesterase inhibitors (ChEIs), e.g., galanthamine or rivastigmine as well as non-competitive ChEIs, e.g., tacrine or donepezil are clinically applied to alleviate the neuromuscular symptoms of AD. Research for new ChEIs seems to be required in order to achieve success in the treatment of AD.

A series of new benzene-based derivatives was designed, synthesised and comprehensively characterised. All of the tested compounds were evaluated for their in vitro ability to potentially inhibit the acetyl- and butyrylcholinesterase enzymes. The selectivity index of individual molecules to cholinesterases was also determined. Generally, the inhibitory potency was stronger against butyryl- compared to acetylcholinesterase; however, some of the compounds showed a promising inhibition of both enzymes. In fact, two compounds (benzyl ethyl(1-oxo-1-phenylpropan-2-yl)carbamate and benzyl (1-(3-chlorophenyl)-1-oxopropan-2-yl)(methyl)carbamate) had a very high selectivity index, while the second one reached the lowest IC_{50} value, which corresponds quite well with galanthamine. Moreover, comparative receptor-independent and receptor-dependent structure-activity studies were conducted to explain the observed variations in inhibiting the potential of the investigated carbamate series. The principal objective of the ligand-based study was to comparatively analyse the molecular surface in order to gain insight into the electronic and/or steric factors that govern the ability to inhibit enzyme activities. The spatial distribution of potentially important steric and electrostatic factors was determined using the probability-guided pharmacophore mapping procedure, which is based on the iterative variable elimination method. Additionally, planar and spatial maps of the host-target interactions were created for all of the active compounds and compared with the drug molecules using the docking methodology.

References

[1] A. Bak, V. Kozik, D. Kozakiewicz, K. Gajcy, D Strub, A. Swietlicka, S. Stepankova, A. Imramovsky, J. Polanski, A. Smolinski, J. Jampilek Novel benzene-based carbamates for AChE/BChE inhibition: synthesis and ligand/structure-oriented SAR study, Int. J. Mol. Sci. Res. 20 (2019) 1524.

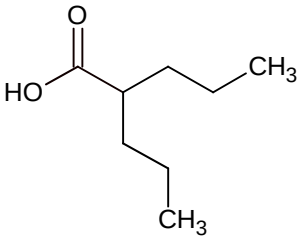
Development and validation of GC-MS method for determination of valproic acid in plasma

Katarzyna Lipska*(1), Anna Gumieniczek (1), Agata Filip (2), Rafał Pietraś (1)

(1) Chair and Department of Medicinal Chemistry, Medical University of Lublin, Poland
(2) Department of Cancer Genetics with Cytogenetics Laboratory, Medical University of Lublin, Poland
**e-mail: katarzyna.lipska@op.pl*

Abstract

Valproic acid (VPA) is a branched short-chain fatty acid with two propyl groups linked to an acetic acid moiety which presents valuable chemical and pharmacodynamic properties. This potent drug with a broad spectrum of anti-seizure effects has been used for the treatment of epilepsy as well as bipolar and migraine diseases for several decades. In recent years, due to strong evidence of its anticancer activity *in vitro*, VPA was proposed as an attractive candidate for therapy of different types of cancer. VPA, by affecting numerous signaling pathways, can modulate different processes in cancer cells and suppress tumors.



We present a new specific, sensitive, and accurate gas chromatography-mass spectrometry method (GC-MS) for determination of VPA in plasma after simple derivatization with methanolic H_2SO_4 followed by extraction with hexane, using benzoic acid (BA) as an internal standard.

The GC separation was achieved on a HP-5MS UI column (30 m×0.25 mm ID, and 0.25 µm film thickness). Analytes were determined using electron impact ionization (EI) in a double quadrupole mass spectrometer. GC/MS was performed in the selected ion monitoring (SIM) mode using target ions at *m*/*z* 159, 116 and 87 for VPA and *m*/*z* 136, 105 and 77 for BA.

The samples of plasma were prepared by protein precipitation with methanol after being spiked with an internal standard (BA).

Standard curves were linear over the range of 0,1 µg/ml-50 µg/ml with a mean correlation coefficient (r) of 0,9982. Intra- and inter-day precision values for VPA in standard solutions were less than 10% and for VPA in plasma less than 15% The analytical recoveries of VPA from plasma were above 90%.

The proposed GC-MS method was proven to be a valid alternative to high-performance liquid chromatography with UV detection, bearing in mind very low absorbance of VPA. It can be also an attractive alternative to liquid chromatography-mass spectrometry (LC-MS).

References

[1] A. Duenas-Gonzalez, M. Candelaria, C. Perez-Plascencia, E. Perez-Cardenas, E. Cruz-Hernandez, L.A. Herrera, Valproic acid as epigenetic cancer drug: preclinical, clinical and trascriptional effects on solid tumors, Cancer Treat Rev. 34 (2008) 206-222

[2] S.A. Brodie, J.C. Brandes, Could valproic acid be an effective anticancer agent? The evidence so far, Expert Rev Anticancer Ther. 14 (2014) 1097-1100

Thin-layer chromatographic investigation of the red beetroot dyes

Eliza Łata*, Agnieszka Fulczyk, Teresa Kowalska, Mieczysław Sajewicz

Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice, Poland
**e-mail: lata.eliza@gmail.com*

Abstract

In the red beetroot (*Beta vulgaris* L.), there are two groups of dyes known as betacyanins (the red dyes) and betaxanthins (the yellow dyes). Our studies focus on the analysis of the red dyes, whose molecules are made of betacyanidins (aglicones) connected to a glucose molecule [1]. There are four main beetroot dyes from the betacyanin group: betanin, isobetanin, neobetanin and prebetanin. The most widespread of all these is betanin, which is the only pigment commercially available. Betanin is designated as E162 on the list of food additives, drawn up by the Directive 2000/13/EC of the European Parliament and the European Council of 20 March 2000 on the approximation of the laws of the Member States relating to labeling, presentation and advertising of foodstuffs [2]. The interest in the analysis of the red beetroot dyes results from their wide application in food industry as natural and safe colorants.

The aim of this study was to develop a new chromatographic separation method for betacyanins. The analysis was carried out for betanin used as a phytochemical standard and for the freshly prepared beetroot juice. The method used in our research was thin layer chromatography (TLC) with densitometric and mass spectroscopic (TLC-MS) detection. The analysis of betacyanins using the TLC method revealed an uncommon horizontal separation of the investigated samples. In the course of chromatographic process, each applied sample separated to two bands, one band deviating to the left and the other one deviating to the right from verticality, and with the same R_F values (the gap between the two separated bands equal to ca. 6 mm). This rare separation might be used as a qualitative fingerprint of the betacyanin dyes in the authentication process of alimentary products.

References

[1] A. Gliszczyńska-Świgło, H. Szymusiak, P. Malinowska, Betanin, the main pigment of red beet: Molecular origin of its exceptionally high free radical-scavenging activity, Food Addit. Contam. 23 (2006) 1079-1087

[2] Directive 2000/13/EC of the European Parliament and of the Council of 20March 2000 on the approximation of the laws of the Member States relating to the labeling, presentation and advertising of foodstuffs

Trimethylsilyl derivatization of saccharides: effectiveness of using BSTFA vs. MSTFA in standard compounds and natural samples.

Justyna Smolarek-Lach (1), Magdalena Goryl (2), Leszek Marynowski*(1),

(1) Faculty of Earth Sciences, University of Silesia, Sosnowiec, Poland
(2) Institute of Geological Sciences, Polish Academy of Sciences, Research Centre in Kraków, Kraków, Poland
**e-mail: leszek.marynowski@us.edu.pl*

Abstract
Saccharides are common compounds in both terrestrial and marine organisms, and can be used as a tool for clarifying sources, and processes of organic materials in natural environments. Difficulties of sugars evaluation and comparison is due to varieties of the techniques used for their isolation and quantification. Because of their high polarity, hydrophilicity and low volatility, saccharides have to be transformed into soluble and stable derivatives [1]. Derivatization is a chemical technique which modifies compounds into products with similar chemical structure, which have better chromatographic properties. The most common reactions for this process are alkylation, acylation and silylation. Silylation is the most frequent derivatization technique. N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-trimethylsilyltrifluoroacetamide (MSTFA) are prevalently used as reagents in the case of saccharides derivatization [2]. In this study we present effectiveness of using BSTFA vs. MSTFA in standard compounds and natural samples. Standard compounds were derivatized with BSTFA reagent. TMS derivatives of standard compounds were stored in two different temperatures. First group of TMS derivatives stored in 4°C and second group of samples stored in a room temperature. The effectiveness of derivatization was significantly higher for the second group of standards. As for monosaccharide like glucose fully TMS derivatized derivatives was ~2% for 4°C, while ~98% for a room temperature. As for disaccharide like trehalose fully TMS derivatized derivatives was ~9% for 4°C, while ~81% for a room temperature. Whereas for sugar alcohol like mannitol fully TMS derivatized derivatives was ~53%, and ~95%, respectively. In the case of natural samples (fungi extracts) there was a significant difference in effectiveness of derivatization with BSTFA and MSTFA. As for monosaccharide like glucose fully TMS derivatized compounds was ~90% for MSTFA, while ~65% for BSTFA. As for disaccharide like trehalose fully TMS derivatized derivatives was ~91% for MSTFA, while ~13% for BSTFA. Whereas for sugar alcohol like mannitol fully TMS derivatized derivatives was ~93% for MSTFA, while ~15% for BSTFA. Natural samples derivatized with BSTFA and standards stored in low temperature after derivatization give indecipherable chromatograms due to multiple peaks, whereas MSTFA help to avoid inconvenience with identification of saccharides in natural samples. Due to the higher effectiveness, we suggest using MSTFA as a reagent for mono- and disaccharides derivatization in natural samples, as well as to store the samples after derivatization in a room temperature.

References
[1] C. Schummer, O. Delhommea, B.M.R. Appenzellerb, R. Wennigb, M. Millet, Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis, Talanta, 77 (2009) 1473-1482
[2] P.M. Medeiros, B.R.T. Simoneit, Analysis of sugars in environmental samples by gas chromatography- mass spectrometry, J. Chromatogr. A, 1141 (2007) 271-278

Metabolomics studies of drug-sensitive and drug-resistant Pseudomonas aeruginosa strains

Karolina A. Mielko*(1), Sławomir Jabłoński (2), Dorota Sands (3), Marcin Łukaszewicz (2), Piotr Młynarz (1)

(1) Bioorganic Chemistry Group, Faculty of Chemistry, Wroclaw University of science and Technology, Wroclaw, Poland
(2) Biotransformation Department, University of Wroclaw, Poland
(3) Mother and Child Institute, Warsaw, Poland
**e-mail: karolina.mielko@pwr.edu.pl*

Abstract
Pseudomonas aeruginosa is the gram-negative bacterium and the most common pathogen, which occurs in the respiratory tract in patients who suffer of cystic fibrosis (CF). In a case of a chronic infection, this microorganism is practically impossible to eradicate (the reason is high resistance for antibiotics treatment). This fact is associated with high morbidity and mortality rate, therefore the detection of early infection can make treatment easier. At this moment, the performed medical intervention usually is conducted too late due to used available diagnostics methods [1].
Metabolome analysis represents a third (with transcriptome and proteome studies) complementary approach to identify the life processes, which occurs in microorganism. Metabolomics involves an analysis of metabolic profiles of microbial extracts (intra and extracellular) using NMR spectroscopy or MS spectrometry methods. However, in recent years, in some research groups, nuclear magnetic resonance (NMR) has become one of leading methods used for metabolomics studies. Metabolomics studies are usually coupled with chemometrics and statistical methods. To the main advantages of NMR method, should be included the quantitative, reproducible and rich in structural information results [2,3].
The main goal of this study was to compare metabolomics profile of two different groups of Pseudomonas aeruginosa - clinical strains isolated from cystic fibrosis patients (which have been considered like drug-resistant) and strains isolated from the environment (which have been considered like drug-sensitive). Principal component analysis (PCA) and partial least squares (PLS) of the obtained data revealed that these two groups can be distinguished on the basis of metabolomic profile with delineation of the perturbed metabolic pathways changed in this bacteria with two types of origin.

References
[1] C. Winstanley et al., Pseudomonas aeruginosa evolutionary adaptation and diversification in cystic fibrosis chronic lung infections, Trends Microbiol. 24 (2016) 327-337
[2] B. B. Aldridge et al., Microbial metabolomics: Innovation, application, insight, Curr. Opin. Microbiol. 19 (2014) 90-96
[3] S. Barnes et al., Training in metabolomics research. II. Processing and statistical analysis of metabolomics data, metabolite identification, pathway analysis, applications of metabolomics and its future, J. Mass Spectrom. 51 (2016) 535-548

Bacterial markers released from infected human tissues and their potential to be used as a diagnostic tool

Ileana-Andreea Ratiu (1,2), Fernanda Monedeiro (1), Tomasz Ligor (1), Jacek Szeliga (3), Marek Jacowski (3), Boguslaw Buszewski*(1,2)

(1) Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Interdisciplinary Centre of Modern Technologies, Nicolaus Copernicus University,7 Gagarina Str., 87-100 Torun, Poland
(2) Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, 11 Arany Janos, RO-400028, Cluj-Napoca, Romania
(3) Dept. of General, Gastroenterologic and Oncologic Surgery Collegium Medicum, Nicolaus Copernicus University, Torun, Poland
**email: bbusz@chem.umk.pl*

Abstract

Tests that have the ability for diagnosis and rapidly distinguishing between infections with different bacteria based on a simple sniff analysis are urgently needed into the community around the whole world. Researcher's collectivity interested in this topic wish for this tests to be cheap, fast, easy applicable, non-invasive, robust, reliable, and sensitive. Analytical instrumentation systems have the ability for real time or within a few tens minutes analysis of volatile organic compounds (VOCs) emitted by bacteria.

Detection of bacterial volatile metabolites produced by human pathogenic bacteria is gaining continuous interest in both scientific and medical fields. Solid-phase microextraction (SPME) is a sampling technique that gained increasing attention in the last years due to its simplicity to implement and sensitivity. Volatile organic compounds released in the headspace over exudates infected with different bacteria were investigated in this work. GC-MS was involved for analysis. The above mentioned VOCs resulted from bacterial metabolism and the afferent processes that occur inside the biological samples. However, the possibility of using volatile metabolites emitted by bacteria as a diagnostic tool is a promising perspective. The potential of discrimination between volatiles emitted by infected and uninfected exudates was proven through this study. Infected samples presented a different cluster modelling, according with the infecting pathogens. A good predictability of diagnosis accuracy, (between 83% and 90%) for infected patients, was achieved when ROC curves were generated. Finally we identified 14 compounds emitted by the infecting pathogens, which can be assumed as bacterial markers.

Nevertheless, additional fast and on-site spectrometry techniques can be used for the detection of biomarker compounds specific to bacteria, once they are known and confirmed by GC-MS. Starting from the key biomarkers detected, instrumentation used for fast screening of bacterial infection from various matrixes can be develop.

Acknowledgments

The financial resources for conducting the scientific research was obtained under the research grant for young researchers from abroad awarded by the Rector of the Nicolaus Copernicus University pursuant to Order no. MNZ/01/1029.

PAHs analysis in the exhaust gases from a central heating furnaces - differences between various type of solid fuels

Małgorzata Pogoda, Justyna Tarnowska, Maciej Chrubasik, Roksana Muzyka, Marcin Sajdak*

Institute for Chemical Processing of Coal, Zabrze, Poland
**e-mail: msajdak@ichpw.pl*

Abstract

Polycyclic aromatic hydrocarbons are a complex group of organic compounds containing two or more condensed aromatic rings. Combustion process in central heating furnaces conducted under reduced oxygen conditions leads to the formation of soot and fine dust particles, carbon monoxide, volatile organic compounds, such as benzene, toluene and polycyclic aromatic hydrocarbons, e.g., benzo(a)pyrene [1]; and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs). Polycyclic aromatic hydrocarbons are formed in high-temperature pyrolysis of fossil fuels, and thus they became indicators of the incomplete combustion process. Moreover, their negative influence on human health has attracted additional attention. The total atmospheric emission of PAHs and PCDDs/PCDFs coming only from combustion processes excluding industrial processes in Poland was estimated at 86.8% and 49.9%, respectively, in 2012 [2]. Therefore, the control and efforts to reduce their concentration are so crucial for the natural environment as well as for human health.

In the present study, we have investigated the correlation between the combustion of various types of solid fuels and PAH content in exhaust gases. Examined samples were obtained by flight ash, separated from exhaust gases and then analysed by GC-MS after extraction with organic solvents. Special attention was paid on sixteen polycyclic aromatic hydrocarbons : naphthene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(a)pyrene, benzo(e)pyrene, benzo(b,k)fluoranthene, dibenzo(a, h)anthracene indeno(1,2,3-cd)pyrene, benzo(g, h, i)perylene, perylene.

The chemometric approach was applied to investigate which of studied parameters have a possible correlation with PAH. Also, this study has clearly shown which of the polycyclic aromatic hydrocarbons are the most correlated with each other how it goes with their concentration in the tested exhaust gases.

References

[1] M. Szewczyńska, E. Dobrzyńska, M. Pośniak, Niekontrolowane spalanie odpadów - zagrożenia dla środowiska i człowieka. CIOP - PIB, Warszawa (in Polish)

[2] KOBIZE - Krajowy bilans emisji SO_2, NO_x, CO, NH_3, NMLZO, pyłów, metali ciężkich i TZO za lata 2011-2012 w układzie klasyfikacji SNAP - Raport syntetyczny. (raport in Polish)

^{1}H NMR analysis of rate serum with endometriosis treatment

Badr Qasem (1), A. Warowicka (1), Anna Goździcka-Józefiak (2), M. Wołuń-Cholewa (1), Piotr Młynarz*(1)

(1) Department of Bioorganic chemistry, Wroclaw University of Science and Technology, 50-370 Wrocław, Poland
(2) Department of Molecular Virology, Adam Mickiewicz University, Faculty of Biology, Umultowska 89, 61-614 Poznań, Poland
(3) Department of Cell Biology, Poznan University of Medical Sciences, 61-701 Poznan, Poland
**e-mail: piotr.mlynarz@pwr.edu.pl*

Abstract

Endometriosis is a disease of women of reproductive age characterized by the presence of endometrial tissue outside the uterine cavity and often is associated with chronic pelvic pain and infertility [1]. Endometriosis affects about 1 in 10 women of childbearing age (eg, usually between the ages of 15 and 49), which is approximately 176 million women in the world [2,3].

Endometriosis is not recognized in a large part of affected women, which results in permanent and progressive symptoms, which are negative health and well-being. Current standards of this disease detection are based primarily on laparoscopy, performed for the purpose of a definitive diagnosis before the start of therapy, therefore often result in prolonged time between onset of symptoms, diagnosis and further treatment. The introduction of rapid and reliable clinical diagnostic techniques can reduce the delay in diagnosis and thus bring relief to suffering patients [4]. Numerous studies have adopted a metabolomics approach to elaborate the diagnostic method (with high specificity and sensitivity) and to discover biochemical mechanisms that can explain the cause and progression of this disease [5]. In this study, we examined serum metabolome after induction of endometriosis and compared with different treatment and vaccination methods, and monitored changes in serum metabolite profile during treatment at different time points.

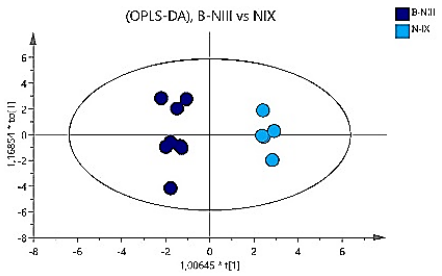


Figure . OPLS-DA model, for metabolites obtained from ^{1}H NMR analysis of serum samples B-NIII from rates after 2 weeks treatment with BBR and serum collection after 3 months to control samples K-IX.

References

[1] P. Parveen, O. Pinar, K.L. Terry, Endometriosis: epidemiology, diagnosis and clinical management, Curr. Obstet. Gynecol. Rep. 6 (2017) 34-41

[2] P.A. Rogers, et al. Priorities for endometriosis research: recommendations from an international consensus workshop, Reprod. Sci. 16 (2009) 35-46

[3]G.D. Adamson, et al., Creating solutions in endometriosis: global collaboration through the World Endometriosis Research Foundation, Journal of Endometriosis, 2 (2010) 3-6

[4] S.K. Agarwal, C. Chapron, L.C. Giudice, M.R. Laufer, N. Leyland, S.A. Missmer, S.S. Singh, H.S. Taylor, Clinical diagnosis of endometriosis: a call to action, Am. J. Obset. Gynecol. 220 (2019) 354

[5] H. Abbiss, G.L. Maker, R.D. Trengove, Metabolomics approaches for the diagnosis and understanding of kidney diseases, Metabolites, 9 (2019) 34

Fluorescent probe to study activity of cysteine proteases

Przemysław Mielczarek*(1,2), Kinga Piechura (1), Jerzy Silberring (1,3)

(1) Department of Biochemistry and Neurobiology, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Krakow, Poland
(2) Laboratory of Proteomics and Mass Spectrometry, Institute of Pharmacology, Polish Academy of Sciences, Krakow
(3) Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland
**e-mail: przemyslaw.mielczarek@agh.edu.pl*

Abstract
The interest of applying fluorescent probes in biological research is constantly increasing due to their versatility, high sensitivity and quantitative capabilities. Such compounds can be attached to a target molecule and act as a marker for biological analysis. For instance, the use of tagged inhibitors provides possibility to localize and identify enzymes, which activity is selectively blocked by these compounds [1-2]. In the present work, novel fluorescent inhibitor of cysteine proteases and its potential application in identifying this class of enzymes, have been described.
To obtain fluorescent inhibitor, the standard Fmoc solid-phase synthesis has been used. The structure of a synthesized compound was confirmed by MALDI-TOF/TOF mass spectrometry. To purify the obtained peptide, the reversed phase HPLC was used. Staphopain c was applied as a model enzyme of cysteine proteases in the enzymatic tests.
It was demonstrated that synthesized inhibitor binds to the staphopain c active site. The optimal pH of binding the inhibitor with enzyme has been determined. Further, the possibility of using the synthesized inhibitor in quantitative approach of the active level of the enzyme has also been confirmed. Several separation methods, including polyacrylamide gel electrophoresis, were applied to verify selective binding of the inhibitor to the enzyme.
Owing to the high interest of our group in proteases that convert neuropeptides, this research is a part of our long-term studies aimed at identification and isolation of a family of endopeptidases that convert dynorphins to shorter, bioactive fragments

Acknowledgements
Authors are grateful to the The National Centre for Research and Development under project No. POWR.03.02.00-00-I004/16.

References
[1] G. T. Hermanson, Fluorescent probes, Bioconjugate Techniques, (2013) 395-463
[2] E.C. Jensen, Use of fluorescent probes: Their effect on cell biology and limitations. The anatomical record: advances in integrative anatomy and evolutionary biology, 295 (2012) 2031-2036

Bioavailability studies by RP-HPLC on the stationary phase with an embedded amide moiety

Anna W. Sobańska*, Elżbieta Brzezińska

Department of Analytical Chemistry, Medical University of Lodz, Muszyńskiego 1, 90-151 Łódź, Poland
**e-mail: anna.sobanska@umed.lodz.pl*

Abstract

The search for new drugs is a very expensive activity with a limited success rate. Especially in vivo studies of new, potential drugs are very labor- and time-consuming which limits their applications. Such studies for large groups of new drugs are difficult. This is the reason why it is so important to search for possibly cost-efficient methods of evaluating the properties of compounds, vital from the point of view of their biological activity (e.g. bioavailability). Such methods, used before an in vivo stage, make it possible to reduce the number of synthetized compounds or even exclude the structures that do not exhibit the expected properties.

Bioavailability of solutes may be predicted in vitro or in silico using their physico-chemical and/or chromatographic parameters. Complex phenomena in a living being can be seldom expressed with a single physico-chemical of chromatographic parameter. The combination of two properties gave rise to the descriptors such as: logP - (N+O), %PSA (PSA and SA). Our earlier research [1,2] was concerned with the application of RP-18 TLC and HPLC chromatography to the prediction of the blood-brain barrier (BBB) permeation. It was discovered that a novel RP-18 TLC parameter (Rf/PSA) makes it possible to distinguish between the compounds that cross and do not cross the BBB. However, a similar HPLC parameter (logk)/PSA does not have a comparable predictive value.

In the course of this study we have examined the possibility of using the HPLC data (logk) obtained on the stationary phase containing an embedded amide moiety in combination with physico-chemical parameters such as PSA (polar surface area), MW (molecular weight) or V (molecular volume) to predict the lipophilicity (logP), blood-brain barrier permeability (logBB), human intestinal absorption (HIA), plasma protein binding (PPB), caco-2 permeability (caco-2) and skin permeability (SP) of 21 compounds. Raw HPLC data were taken from a very inspirational Ref. [3].

Acknowledgements

This work was supported by an internal grant of the Medical University of Lodz no. 503/3-016-03/503-31-001.

References

[1] A.W. Sobańska, K. Wanat, E. Brzezińska, Prediction of the blood-brain barrier permeability using RP-18 thin layer chromatography, Open Chem. 17 (2019) 43-56

[2] A.W. Sobańska, A. Hekner, E. Brzezińska, RP-18 HPLC analysis of drugs' ability to cross the blood-brain barrier, J. Chem. Article ID 5795402 (2019)

[3] O. Farsa, Chromatographic behaviour predicts the ability of potential nootropics to permeate the blood-brain barrier, Sci. Pharm. 81 (2013) 81-91

RP-18 TLC investigations of acid-base equilibria of selected cosmetic raw materials

Anna W. Sobańska*, Mariusz Nowak, Joanna Owczarek, Elżbieta Brzezińska

Department of Analytical Chemistry, Medical University of Lodz, Muszyńskiego 1, 90-151 Łódź, Poland
**e-mail: anna.sobanska@umed.lodz.pl*

Abstract

The pKa value is an important physico-chemical parameter of a molecule that may be helpful in predicting its bioavailability under in vivo conditions. It plays an important role form a purely analytical point of view since the acid-base dissociation equilibria influence e.g. the chromatographic behavior or spectral properties of solutes; it should also be taken into account by a formulation chemist both in the pharmaceutical and in the cosmetic industry.

The most widely used methods of experimental pKa determination are based on potentiometric titration or UV/VIS spectrophotometry but they are not so easily applicable to analytes of poor solubility and require relatively large and pure samples [1]. Chromatographic methods of pKa determination are free of such limitations. pKa of acids and bases may be determined by either isocratic or gradient RP HPLC chromatography. The retention times of the ionized and the unionized form of the compound differ, so changing the pH of the mobile phase throughout the chromatographic experiment (or a series of experiments) makes it possible to determine pKa [2].

Contrary to RP HPLC, RP TLC chromatography has been used to measure the pKa values only by a couple of authors [2-5]; however, the experimental ease, availability and low costs of this technique have encouraged us to investigate the possibility of using it to determine the dissociation constants of some cosmetic raw materials (UV filters and preservatives). RP-18 thin layer chromatography was performed with acetonitrile - buffer (40:60; 50:50; 60:40 and 70:30 v/v) across the buffer pH range 2÷12. It was observed that on the RP-18 support the retardation factors (Rf) are practically independent of pH for all organic modifier concentrations.

Acknowledgements

This work was supported by an internal grant of the Medical University of Lodz no. 503/3-016-03/503-31-001.

References

[1] J. Reijenga, A. van Hoof, A. van Loon, B. Teunissen, Development of methods for the determination of pKa values, Anal. Chem. Insights, 8 (2013) 53-71

[2] B. Palalikit, J.H. Block, Determination of ionization constants by chromatography, Anal. Chem. 52 (1980) 624-630

[3] B. Tyman-Szarman, M. Sajewicz, J. Polanski, Thin-layer chromatographic determination of the pKa values of organic acids and bases, J. Planar Chromatogr. 18 (2005) 323-325

[4] K. Kulig, K. Rybicka, B. Malawska, Application of RP-TLC technique for the determination of dissociation constants of 1-substituted pyrrolidin-2-one derivatives, Biomed. Chromatogr. 22 (2008) 1225-1229

[5] J. Pusz, W. Zapała, B. Papciak, Investigations of selected properties of pharmacologically active compounds by chromatographic and potentiometric methods on the chrysin example, Pol. J. Chem. Technol. 10 (2008) 41-44

Chemometric analysis of the LC-DAD degradation profiles of toloxatone using PARAFAC

Jakub Trawiński*, Robert Skibiński

Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Lublin, Poland
**e-mail: jakub.trawinski@umlub.pl*

Abstract

Degradation study is an indispensible part of a drug testing process. Taking into account that the products of pharmaceutical compounds transformation could possess their own pharmacological properties and toxicity higher that the parent molecule, it is crucial to identify them (1). Furthermore, recognition of the degradation profiles can allow to understand the relationships between the mechanisms of drug degradation under the different conditions.

Toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-1,3-oxazolidin-2-one) is a selective and reversible inhibitor of monoamine oxidase, used in the treatment of major depression (2). In our previous work we identified the structures of its degradation products under the acidic, basic, neutral hydrolytic, oxidative and photolytic (UV-Vis and UVC) conditions. Additionally the degradation profiles, obtained with use LC-HRMS were compared employing PCA (3).

The aim of this study was to perform a comparison of toloxatone forced degradation profiles obtained with the use of two analytical techniques - LC coupled with high resolution MS, and LC coupled with DAD detector. However UV-Vis detection provides significantly less specific information about the compounds structure than HRMS, 3-dimensional data obtained with the use of DAD detector could give results comparable to mass spectrometry. Data exploration was done by the means of two chemometric methods - principal component analysis (PCA) and parallel factor analysis (PARAFAC).

References

[1] R. Skibiński, J. Trawiński, Ł. Komsta, K. Bajda, Characterization of forced degradation products of clozapine by LC-DAD/ESI-Q-TOF, J. Pharm. Biomed. Anal. 131 (2016) 272-280

[2] F. Moureau, J. Wouters, D. Vercauteren, S. Collin, G. Evrard, F. Durant, et al., A reversible monoamine oxidase inhibitor, toloxatone: Structural and electronic properties, Eur. J. Med. Chem. 27 (1992) 939-948

[3] R. Skibiński, J. Trawiński, Ł. Komsta, D. Murzec, Characterization of forced degradation products of toloxatone by LC-ESI-MS/MS, Saudi Pharm. 26 (2018) 467-480

The application of supercritical fluid chromatography (SFC) and liquid chromatography coupled with mass spectrometer (LC-MS/MS) for the determination of polar compounds on the example of phospholipids

Katarzyna Tyśkiewicz*(1), Marcin Konkol (1), Edward Rój (1), Krystyna Skalicka-Woźniak (2)

(1) Instytut Nowych Syntez Chemicznych, Zakład Ekstrakcji Nadkrytycznej, Puławy, Poland
(2) Uniwersytet Medyczny, Katedra i Zakład Farmakognozji z Pracownią Roślin Leczniczych, Lublin, Poland
**e-mail: katarzyna.tyskiewicz@ins.pulawy.pl*

Abstract
Phospholipids belong to a group of compounds being the main components of cell membranes. The characteristic feature of these compounds is their amphiphilic nature. The chemical structure of phospholipids includes hydrophilic "head" and two hydrophobic fatty acids "tails" combined together by a glycerol or alcohol molecule [1]. Both supercritical fluid chromatography (SFC) and liquid chromatography coupled with mass spectrometer (LC-MS/MS) may be applied in phospholipids determination from waste fish oil as well as egg yolk. Moreover, appropriate sample preparation has been applied in order to fractionate phospholipids fraction from triacylglycerols fraction. Polar columns, such as hilic (for LC-MS/MS) and silica modified by fluorophenyl groups (for SFC) resulted in the same interactions of the compounds with polar groups of the stationary phase. However, LC-MS/MS method provides additional information on exact phospholipids forms. On the example of egg yolk, the phosphatidylcholine was analyzed as four forms based on MRM mode in LC-MS/MS method.
Figure 1 presents the chromatogram of phosphatidylethanolamine and phosphatidylcholine in the SFC system.

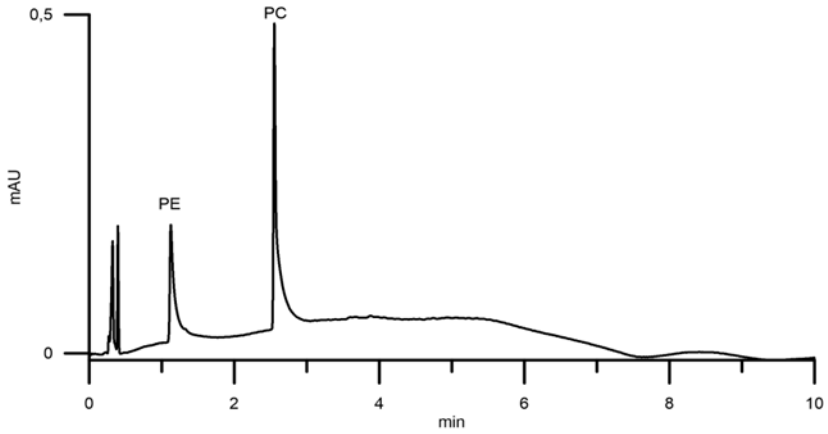


Figure 1. SFC/UV chromatogram of separated phospholipids (PE - phosphatidylethanolamine; PC - phosphatidylcholine)

References

[1] R.P. Singh, H.V. Gangadharappa, K. Mruthunjaya, Phospholipids: Unique carriers for drug delivery systems, J. Drug Deliv. Sci. Technol. 39 (2017) 166-179

Detecting Colorectal Cancer using Breath-omics

Robert van Vorstenbosch*, Hao Ran Cheng, Zlatan Mujagic, Daisy Jonkers, Frederik-Jan van Schooten, Agnieszka Smolinska

Department of Pharmacology and Toxicology, NUTRIM school of Nutrition and Translational Research, Maastricht University, The Netherlands
**e-mail: R.vanVorstenbosch@maastrichtuniversity.nl*

Abstract
Colorectal cancer (CRC) is one of the most prevalent diseases within the EU. The survival rate of CRC patients is dependent on the stage at which the disease is diagnosed. To facilitate early diagnosis, national screening programs are organized. In the Netherlands this consists of a non-invasive iFOBT test and, if tested positive, a follow-up colonoscopy. Unfortunately, the iFOBT test suffers from low sensitivity and a high false positive rate. Therefore, a novel non-invasive diagnostics tool is urgently needed.
The analysis of Volatile Organic Compounds (VOCs) in exhaled breath might be a potential alternative. VOCs relate to host metabolism, gut micro bacteria, oxidative stress, and inflammation, which have previously been shown to correlate to pre-cancerous stages and colorectal cancer. Thus, the aim of this study is to demonstrate the feasibility of exhaled breath analysis to diagnose CRC and to compare its accuracy with the iFOBT test.
In this study, exhaled breath samples (and colonoscopy outcomes) were collected from patients tested positive for the iFOBT test (n=410) by inflating a 3L Tedlar bags and transferring the contents within 1h to stainless thermal desorption tubes where volatile metabolites were trapped. Later, they were analyzed using Gas Chromatography -time of flight- Mass Spectrometry (GC-tof-MS). Before the actual statistical analysis, the data was pre-processed using wavelets and p-splines to diminish effects of noise and baseline. After aligning the chromatograms, they were normalized by probabilistic quotient normalization.
The statistical analysis consisted of several steps. First, data was visualized using PCA, robust-PCA and unsupervised Random Forest. In the consequent step, the data was randomly divided into training (70%) and test set (30%). In order to find the CRC-specific volatile metabolites in exhaled breath, feature extraction was implemented in combination with linear and non-linear classification models including PLS-DA, tree based techniques and SVM. The prediction accuracy of each classification technique was accessed by means of precision-recall curve. This study shows that a specific set of volatile metabolites in breath enables the differentiation among CRC, pre-stages of cancer and healthy patients. Thus, it may prove to be of crucial importance in patients' diagnosis and treatment processes. Exhaled breath analysis can therefore be a potential new non-invasive diagnostic and monitoring tool.

In silico modeling of the drugs' passage into human milk using calculated parameters and NP TLC descriptors

Karolina Wanat*, Elżbieta Brzezińska
Department of Analytical Chemistry, Medical University of Lodz, Lodz, Poland
**e-mail: karolina.wanat@stud.umed.lodz.pl*

Abstract
According to the studies, drug passage into human milk is influenced by various physicochemical features. Simple properties such as: a molecular weight, protein binding, pKa, logP, logD, hydrogen bond donors and acceptors or dipole moment could be used to calculate the M/P ratio (drug's milk and plasma concentration respectively) which is a convenient tool to estimate drug amount in the milk [1][2]. In our studies we have decided to connect a selected physicochemical features, values collected from databases and chromatographic data obtained from NP TLC to develop a M/P prediction model with a set of different drugs. Chromatographic data: Rf values were collected by NP TLC, additionally plates were modified by coating them with 2 mg/mL solution of serum albumin. We obtained several descriptors but only NP2 which is Rf value on albumin-modified plate was used in the equations. Acetonitrile - acetate buffer pH 7.4 - methanol 60:20:20 (v/v/v) were used as a mobile phase.
Two models, which include chromatographic parameter were developed for 28 drugs by the Stepwise Multiple Regression Analysis:

M/P ratio = 2.71(± 0.44) - 1.88(± 0.31) PB - 1.14(± 0.44) NP2 + 0.11(± 0.05) log U/D + 0.37(± 0.23) LactMed - 0.30(± 0.13) eH-eL
R= 0.90; R2 = 0.80 F(4,23) = 23.25 p <0.0000 s = 0.46105 n=28

M/P ratio = 0.18(± 0.13) - 1.99(± 0.31) PB - 1.29(± 0.44) NP2 + 0.07(± 0.05) log U/D + 0.18(± 0.13) LLL H - 0.30(± 0.13) eH-eL
R= 0.91; R2 = 0.83 F(6,21) = 17.112 p <0.0000 s = 0.44844 n=28

where the PB and NP2 descriptors are connected with protein binding, log U/D and eH-eL represent an ionisation state of a compound, LactMed and LLL H are values collected from databases which indicate a drug appearance in milk (LactMed) and its safety during the lactation (LLL H) according to Hale et al. [3]. Both equations describe about 80% of variance of the M/P ratio. The results point out the influence of physicochemical properties on drugs' passage into human milk along with the application of easy-accessible chromatographic parameter.

Acknowledgements
This research was supported by an internal grant of the Medical University of Lodz no. 502-34-106.

References
[1] H.C. Atkinson, E.J. Begg, Prediction of drug distribution into human milk from physicochemical characteristics, Clinical Pharmacokinetics, 18 (1990) 151-167
[2] S. Agatanovic-Kustrin, I.G. Tucker, M. Zecevic, L.J. Zivanovic, Prediction of drug transfer into human milk from theoretically derived descriptors, Anal. Chim. Acta, 418 (2000) 181-195
[3] T.W. Hale et al., Medications and Mothers' Milk, H.E. Rowe, Springer Co. 2016

Relation between salinity stress and fumonisins production by pathogenic fungus *Fusarium proliferatum*

Natalia Witaszak (1), Justyna Lalak-Kańczugowska (1), Agnieszka Waśkiewicz (2), Łukasz Stępień*(1)

(1) Department of Plant Genetics and Pathogen Resistance, Institute of Plant Genetics Polish Academy of Sciences, Poznań, Poland
(2) Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland
**e-mail: lste@igr.poznan.pl*

Abstract
Fusarium is a genus of common plant pathogens which cause economic losses worldwide. One of the most important representatives is *Fusarium proliferatum* - a pathogen of maize, asparagus, garlic and pineapple. This species is known as a producer of broad spectrum of mycotoxins such as fusarin A, fusaproliferin, fusaric acid, beauvericin, moniliformin and fumonisins. Fumonisns are the most harmful mycotoxins from the list but their function for fungal organism is still unknown. Some scientific reports suggest that fumonins may be involved in fungal stress response mechanisms. We investigated the effect of salinity stress on fumonisins B_1, B_2 and B_3 production in *Fusarium proliferatum*.
F. proliferatum strain Asp 42.1 was cultured on liquid medium inducing fumonisins production. Salinity stress was induced after four days of incubation using 0.2 M, 0.7 M and 1.2 M sodium chloride solutions. Samples of medium were collected every two days from the beginning of treatment. Fumonisins B_1, B_2 and B_3 were extracted and analyzed using high pressure liquid chromatography (HPLC) coupled with Multi Fluorescence Detector. Quantification of fumonisins was performed according to external standards.
Fumonisins B_1 and B_2 were observed in all samples in contrast to FB_3 which occurred less often. The amount of fumonisins in samples varied depending on the day after treatment and sodium chloride concentration applied. The content of fumonisins was significantly lower under salinity stress conditions. Interestingly, treated samples and control cultures produced fumonisins increasingly until the eighth day of incubation and then the FBs concentrations decreased. Only at the last day of incubation the level of mycotoxins was higher compared to control.

Acknowledgements
This research was supported by the Polish National Science Centre Project 2015/17/B/NZ9/03577.

References
[1] Ł. Stępień, A. Waśkiewicz, K. Wilman, Host extract modulates metabolism and fumonisin biosynthesis by the plan-pathogenic fungus *Fusarium proliferatu,* Int. J. Food Microbiol. 193 (2015) 74-81
[2] D. Ferrigo, A. Raiola, S. Bogialli, C. Bortolini, A. Tapparo, R. Causin, In vitro production of fumonisins by *Fusarium verticillioides* under oxidative stress conditions, J. Agr. Food Chem. 63 (2015) 4879-4885

Triple negative breast cancer, a differences between in vivo and in vitro metabolome

Wojciech Wojtowicz*(1), Radosław Tarkowski (2), Piotr Młynarz (1)

(1) Wroclaw University of Technology, Department of Bioorganic Chemistry, Wroclaw, Poland
(2) Lower-Silesian Oncology Centre, Wroclaw, Poland
**e-mail: wojciech.wojtowicz@pwr.edu.pl*

Abstract

Triple negative breast cancer (TNBC) is one of the worst prognosis and problematic type of breast cancer. It is characterized by the lack of expression of three receptors main receptors. The TNBC cells do not express the estrogen receptor alpha (ER), the progesterone receptor (PR), and the human epidermal growth factor 2 receptor (HER2) [1]. Understanding the functioning of this subtype of cancer is now one of the main goals in field of breast cancer research.

Metabolomics studies of TNBC could provide an insight of biological information connected the biochemical pathways disturbances in ongoing disease development. The metabolomics studies of cells exometabolome might be treated in some particular cases as a link between in vivo (human serum) and in vitro information [2]. However, there is more common to see the limitation of cell culturing as a model of metabolism [3,4]. It is essential to remember that changes in vitro exometabolome do not fully reflect the cancer and whole organism net interactions and ignores the results of the cross-talk between them. The possible changes in metabolism in vivo and in vitro lead to seeing the limitation of cell culture models. However, differences between them can be valuable information.

A comparative analysis between MDA-MB 468-cell culture media and TNBC patients' serum identified potential systemic response to the carcinogenesis-associated processes. Which could lead to better understanding of organism biochemical reaction for occurrence of TNBC cancer.

References

[1] W.D. Foulkes, I.E. Smith, J.S. Reis-Filho, Triple-negative breast cancer, N. Engl. J. Med. 363 (2010) 1938-1948

[2] M.E. Knott, M. Manzi, N. Zabalegui, M.O. Salazar, L.I. Puricelli, M.E. Monge, Metabolic footprinting of a clear cell renal cell carcinoma in vitro model for human kidney cancer detection, J. Proteome Res. 17 (2018) 3877-3888

[3] J.P. Gillet, S. Varma, M.M. Gottesman, The clinical relevance of cancer cell lines, J. Natl. Cancer. Inst. 105 (2013) 452-458

[4] M.E. Katt, A.L. Placone, A.D. Wong, Z.S. Xu, P.C. Searson, In Vitro tumor models: advantages, disadvantages, variables, and selecting the right platform, Front. Bioeng. Biotechnol. (2016) 4-12

How to prepare the samples of bees and bee products for chromatographic analysis of polycyclic aromatic hydrocarbons?

Katarzyna Zięba*(1), N. Świec (1), Hajnalka Szentgyörgyi (2)

(1) Faculty of Chemistry, Jagiellonian University, Kraków, Poland
(2) Faculty of Biotechnology and Horticulture, University of Agriculture, Kraków , Poland
**e-mail: kzieba@chemia.uj.edu.pl*

Abstract
As a tool for preparation of bees and bee products samples for chromatographic analysis of polycyclic aromatic hydrocarbons the SPE cartridges with molecularly imprinted polymer was used.
Molecularly imprinted polymers are a class of highly cross-linked polymer materials designed to bind one specific target compound or a class of compounds with high selectivity. The molecularly imprinted polymers are sterically and chemically complementary to the target analytes - in this case polycyclic aromatic hydrocarbons. As a result, multiple interactions can take place between the MIP and the analyte.
After extraction polycyclic aromatic hydrocarbons were analyzed using gas chromatograph with mass spectrometry detection and also with high performance liquid chromatography with UV detection.
The results were compared to classical solid-liquid extraction by shaking with hexane and also to extraction with Soxhlet apparatus.
There were extracted 16 polycyclic aromatic hydrocarbons of different number of rings: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene and pyrene.
The efficiency of extraction process was determined by comparing the analytical signal with the signal from standard solution of known concentration wich was not subjected to extraction.

Acknowledgements
This study was supported by the Polish National Science Center (NCN) grant number UMO-2016/21/B/NZ9/01163.

Chemometric analysis of some ß1- and ß2-adrenoceptor agonists and antagonists using chromatographic data and molecular descriptors

Grażyna Żydek*, Wojciech Filipczak, Elżbieta Brzezińska

Department of Analytical Chemistry, Medical University of Lodz, Lodz, Poland
**e-mail: grazyna.zydek@umed.lodz.pl*

Abstract

Chemometric analysis was performed on 22 drugs with ß1- and ß2-adrenoceptor affinity. The physicochemical parameters calculated by HyperChem 7.0 and ACDLabs 8.0 programs and thin layer chromatographic data were applied in (Q)SAR analysis. Chromatography was performed on the glass TLC silica gel 60 F254 plates (20×20 cm, Merck, Darmstadt, Germany) with two variants of the mobile phase: a) acetonitrile:water (80:20, v/v) and b) acetonitrile:0.03 M L-aspartic acid solution (80:20, v/v). Additionally, the stationary phase was modified by impregnation with 0.03 M L-aspartic acid in automatic TLC spray chamber (ChromaJet DS20, Desaga, Germany). Such modified mobile and stationary phases were used as analytical models of ligand-adrenoceptor interactions. The pA2 (antagonist activity) and pKi (receptor binding affinity) values of ß1- and ß2-adrenoceptor ligands were collected from the literature and used to generate the QSAR models. The relationships between chromatographic data, molecular descriptors and biological activity data were found by means of stepwise multiple linear regression (MLR). The good multivariate relationship (R2=0.80) obtained by means of regression analysis can be used to predict the quantitative effect of biological activity of different compounds with ß1- and ß2-receptor affinity. Principal component analysis (PCA) and discriminant function analysis (DFA) were employed to obtain the relationship between the descriptors and receptor binding affinities. The set of ligands was classified into two groups according to their degree of biological activity.

Acknowledgements

This study was supported by Medical University of Lodz, Poland, Research Program No 503/3-016-03/503-31-001.