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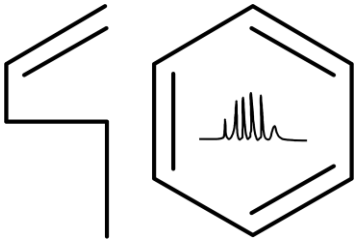

THE 40th SYMPOSIUM

CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS

MAY 23rd - 26th, 2017

KATOWICE – SZCZYRK, POLAND

PROGRAM

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TUESDAY, MAY 23rd, 2017

2.30 pm LUNCH

3.30 – 5.30 pm WORKSHOP

"(HP)TLC combined with bioactivity detection"

Appointed instructor: Dr. Agnes MORICZ

Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

7.00 pm DINNER

SESSION I WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS: Danica Agbaba and Anđelija Malenović

8.55 – 9.00 am OPENING ADDRESS

9.00 – 9.30 am

1. Possibilities of instrumental planar chromatography in drug analysis

Slavica Oljačić, Katarina Nikolić, Marija Čarapić, Darija Obradović, Danica Agbaba

9.30 – 10.00 am

2. Novel insights into the pH-dependent retention behavior of analytes in chaotropic chromatography

Anđelija Malenović, Jelena Čolović

10.00 -10.30 am

3. Applications of NIR spectroscopy for qualitative evaluation of substances

Erika Bojnanska, Josef Jampílek

10.30 – 11.00 am COFFEE BREAK

11.00-11.30 am

4. Bioanalytically-supported precision medicine (personalized pharmacotherapy)

Roman Kaliszan

11.30-12.00 am

5. Analysis of basic drugs in pharmaceutical formulations, biological fluids and tissues by HPLC method

Monika Waksmundzka-Hajnos, Anna Petruczynik, Karol Wróblewski

12.00-12.30 am

6. Quantitative structure-retention relationship models based on different computational techniques in micellar liquid chromatography of antipsychotic drugs

Biljana Otašević, Jovana Krmar, Milan Vukićević, Ana Protić, Jelena Golubović, Mira Zečević

1.00 pm LUNCH

SESSION II WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS: Monika Waksmundzka-Hajnos and Roman Kaliszan

2.30 – 3.00 pm

7. Illustration of the new hybrid optimization method for preparative chromatography column separation using enantiomeric mixtures as a model

Marcin Chutkowski, Jörgen Samuelsson, Marek Leśko, Martin Enmark, Krzysztof Kaczmarski, Erik Forss, Joakim Högblom, Torgny Fornstedt

3.00 – 3.30 pm

8. Towards 'sweet spot' approach: *In silico* estimation of lipophilicity profile for a set of drug transporters

Andrzej Bak, Violetta Kozik, Paulina Dybał, Adam Smoliński, Joseph Jampilek

3.30 – 4.00 pm

9. Whole body autoradiography combined with HPLC

Huba Kalász, Kornélia Tekes

POSTER SESSION I WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS: Marcin Chutkowski and Grzegorz Jóźwiak

4.00 – 5.30 pm (COFFEE BREAK)

6.30 pm BONFIRE

SESSION III THURSDAY, MAY 25th, 2017

CHAIRPERSONS: Irena Vovk and Danilo Corradini

9.00 – 9.30 am

10. Chromatographic and hyphenated techniques in the development of functional food enriched with xanthophylls

Irena Vovk, Breda Simonovska, Alen Albreht, Eva Kranjc, Lučka Brulc, Vesna Glavnik, Nataša Ferant, Matjaž Červek

9.30 – 10.00 am

11. Capillary electrophoresis and HPLC of biomolecules in agro-food matrices

Danilo Corradini

10.00 – 10.30 am

12. New analytical technologies for natural product research

Günther K. Bonn, Rania Bakry

10.30-11.00 am COFFEE BREAK

11.00 – 11.30 am

13. Identification of bioactive peptides in food matrices by multidimensional liquid chromatography and bioinformatics

Riccardo Zenezini Chiozzi, Francesca Ferraris, Giorgia La Barbera, Carmela Maria Montone, Aldo Laganà

11.30 – 12.00 am

14. How to determine aromatic hydrocarbons in bees and bee products using chromatographic techniques?

Katarzyna Zięba, Agnieszka Moos, Anna Król

12.00 - 12.30 am

15. Being uncertain in thin layer chromatography – some thoughts about latent details in the retention estimation

Łukasz Komsta

1.00 pm LUNCH

SESSION IV THURSDAY, MAY 25th, 2017

CHAIRPERSONS: Agnes Moricz and Gertrud Morlock

2.30 - 3.00 pm

16. Liquid chromatography coupled with bioactivity tests. HPLC or HPTLC?

Ágnes M. Móricz

3.00 – 3.30 pm

17. Latest research in hyphenated HPTLC

Gertrud Morlock

3.30 – 4.00 pm

18. Thin-layer chromatography-direct bioautography as a semi-quantitative method

Irena Choma

POSTER SESSION II THURSDAY, MAY 25th, 2017

CHAIRPERSONS: Kamilla Acs and Biljana Otašević

4.00 – 5.30 pm (COFFEE BREAK)

7.00 pm GALA DINNER

SESSION V FRIDAY, MAY 26th, 2017

CHAIRPERSONS: Teresa Kowalska and Andrzej Bąk

10.00 – 10.20 am

19. TLC-bioautography: An appropriate test for detection of antibacterial activity of essential oils

Kamilla Ács, Viktória L. Balázs, Béla Kocsis, Andrea Böszörményi, György Schneider, Ágnes Móricz, Péter G. Ott, Györgyi Horváth

10.20 – 10.40 am

20. Biotreatment of styrene, ethanol and dimethyl sulfide mixture in the contaminated airstream using the compact trickle bed bioreactor

Paulina Dybał, Andrzej Bąk, Violetta Kozik, Sławomir Kuś

10.40 – 11.00 am

21. Fatal case of poisoning with a new cathinone derivative, α-propylaminopentiophenone chromatographic and spectroscopic analysis of *postmortem* material

Milena Majchrzak, Rafał Celiński, Mieczysław Sajewicz

11.00 am CLOSING REMARKS

12.00 am LUNCH

POSTER SESSION I

1.

Thin-layer chromatographic identification and quantification of anthocyanins in food products

Agnieszka Fulczyk, Eliza Łata, Mieczysław Sajewicz

2.

Thin-layer chromatographic identification of amino acids in spider web

Magda Michalik, Maja Surmacka, Monika Stalmach, Grażyna Wilczek, Teresa Kowalska, Mieczysław Sajewicz

3.

Identification and quantification of polyphenol compounds in lavender and thyme honeys using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry

Kinga Gyergyák, Éva Horváth, Ágnes Farkas, Borbála Boros

4.

Chromatographic comparison of biological activity of essential oil and two *Acorus calamus* rhizome extracts using TLC with direct bioautography as detection method

Grzegorz Jóźwiak, Monika Waksmundzka-Hajnos, Barbara Majer-Dziedzic, Aneta Rojek

5.

Lipophilicity of selected terephthalamides

Violetta Kozik, Andrzej Bąk, Krystyna Jarzembek, Paulina Dybał, Danuta Pentak, Barbara Hachuła, Marcin Rojkiewicz, Aleksandra Świetlicka, Dominika Bożek, Joanna Kozłowska, Piotr Kuś

6.

Determination of selected pollutants in fuel samples

Violetta Kozik, Andrzej Bąk, Krystyna Jarzembek, Paulina Dybał, Agata Nobis, Janusz Klecki, Danuta Pentak, Katarzyna Sikora, Klaudia Haśnik

7.

Determination of the lipophilicity of selected furanosteroids

Małgorzata Dołowy, Alina Pyka-Pająk, Marcin Pacholczyk

8.

Synthesis of crown thiaethers – potential complexing agents for transition metals

Hubert Hellwig, Piotr Kuś

9.

Voltammetric Determination of Heavy Metals in Green and Black Tea

Alexandra Planková, Ľubomír Švorc, Josef Jampílek, Peter Mikuš

10.

Thin-layer chromatographic analysis of stanozolol

Małgorzata Dołowy, Karolina Chrystow-Chrystow, Alina Pyka-Pająk, Krzysztof Marciniec

11.

Identification of instability products of the cathinone derivatives

Jakub Wantulok, Krzysztof Byrdy, Tadeusz Paździorek, Jacek E. Nycz

12.

Separation and quantitation of selected water-soluble vitamins using different HILIC stationary phases

Justyna Kamińska, Lidia Zapała, Marcin Chutkowski, Piotr Ziobrowski, Wojciech Zapała

13.

Comparison of isocratic retention models for hydrophilic interaction liquid chromatography

Justyna Kamińska, Lidia Zapała, Marcin Chutkowski, Piotr Ziobrowski, Wojciech Zapała

14.

Multivariate analysis of variance of designed chromatographic data

Danuta Liberda, Jade Tobin, Dalene de Beer, Beata Walczak

15.

Simple and rapid screening procedure for 66 synthetic cannabinoids by liquid chromatography-tandem mass spectrometry

Katarzyna Ambroziak, Piotr Adamowicz

POSTER SESSION II

1.

Chromatographic determination of phenolic compounds in commercial samples of *Cistus incanus* L

Magdalena Knaś, Dariusz Szeremeta, Alicja Król, Karolina Męcik, Ewa Długosz, Paweł Olczyk, Teresa Kowalska, Mieczysław Sajewicz

2.

Development and validation of RP HPLC method for determination of cyanocobalamin and phenol in pharmaceutical dosage form

Branka Ivković, Jasmina Brborić, Olivera Čudina

3.

Analysis of retention behavior of selected antipsychotics and their impurities by thin layer chromatography

Slavica Oljačić, Anđela Arsić, Darija Obradović, Katarina Nikolić, Danica Agbaba

4.

Characterization of unknown impurity of ziprasidone with new UPLC MS/MS method

Marija Čarapić, Katarina Nikolic, Bojan Marković, Danica Agbaba

5.

Metabolite profiling of MAO-A inhibitor – moclobemide with the use of human liver microsomes and LC-MS method

Maciej Gawlik, Robert Skibiński

6.

Streamlined workflow to discover antibiotics

Gertrud Morlock, M. Jamshidi-Aidji

7.

Validation of RP HPLC method for quantitative analysis of antiretrovirals drugs in human plasma samples

Nemanja Turković, Branka Ivković, Božana Dimitrijević, Ivan Kovačević, Radmila Novaković, Gordana Dragović Lukić, Zorica Vujić

8.

Application of a polynomial modified Gaussian model and a half-width plot approach to describe chromatographic peaks and reveal column performance in chaotropic chromatography

Jelena Čolović, Anđelija Malenović

9.

Robustness testing of chaotropic chromatpgraphy method for the determination of olanzapin and its two impurities

Milena Rmandić, Jelena Čolović, Anđelija Malenović

10.

Influence of selected mobile phase properties on the TLC retention behavior of ziprasidone and its impurities

Darija Obradović, Slavica Oljačić, Katarina Nikolić, Danica Agbaba

11.

TLC-Bioautography as an appropriate technique for screening anti-*haemophilus* activity of essential oils

Viktória Lilla Balázs, Béla Kocsis, Judit Krisch, Györgyi Horváth

12.

Comparative analysis between chromatographically and computationally estimated lipophilicity descriptors of synthetic rhamnolipids

Jovana Krmar, Biljana Otašević, Ana Protić, Jelena Golubović, Mira Zečević, Nevena Maljurić

13.

Lipophilicity of some quinothiadiazine derivatives

Maria J. Maślankiewicz, E. Chrobak, Danuta Pentak, Danuta Kwapulińska

14.

Determination of 1,2-dichloroethane as relevant impurity of ethephon using HS-GC-MS technique

M. Płonka, M. Miszczyk, D. Kronenbach-Dylong, Patrycja Marczewska, Mieczysław Sajewicz

15.

Office Chromatography - do it yourself!

G. Morlock, D. Fichou

SESSION I

WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS:

Danica Agbaba

and Anđelija Malenović

1.

Possibilities of instrumental planar chromatography in drug analysis

Slavica Oljačić, Katarina Nikolić, Marija Čarapić, Darija Obradović, **Danica Agbaba**

Department of Pharmaceutical Chemistry, University of Belgrade, Vojvode Stepe 450, Belgrade, Serbia

Demands to ensure safe and secure medicinal products for protection and treatment of human health and society led to the development and implementation of numerous high performance instrumental techniques for the estimation of their quality. The classical chemical methods of drug analysis have been replaced over the time with the so-called instrumental methods of analysis. The relevant regulatory authorities, EDQM, FDA and the National Drug Agencies following contemporary scientific investigations in drug research and development continuously implemented them as mandatory in the routine drug analysis methodology. In this presentation, different chromatographic methods/systems for the assessment of purity of ziprasidone and moxonidine will be discussed.

2.

Novel insights into the pH-dependent retention behavior of analytes in chaotropic chromatography

Anđelija Malenović, Jelena Čolović

University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis, Vojvode Stepe 450, Belgrade, Serbia

In chaotropic chromatography quite complex mechanisms underlay the solute retention. The chromatographic behavior of analytes in these systems can be affected by type and concentration of chaotropic ions and organic modifiers, mobile phase ionic strength and stationary phase hydrophobicity. Furthermore, we have recently observed an increase in the retention times with increasing pH in the absence of any changes in the ionization state of the solutes. This result was rationalized by the increasing magnitude of surface potential that occurs due to the increasing surface excess of the chaotropic agent. Since the increase in the surface potential can be influenced by ionic strength, in this study we tested whether the observed retention behavior of completely protonated solutes and chaotropic ion adsorption were caused by a mobile phase pH variance or ionic strength effects. To that aim, two sets of experiments were performed and in the first set, the ionic strength (*I*) was varied with the concentration of $NaPF_6$ and additives that adjusted the mobile phase pH, while in the second set, *I* was kept constant by adding the appropriate amount of NaCl. In each set, the retention behavior of 13 analytes was qualitatively examined in 21 chromatographic systems, which were defined by the $NaPF_6$ concentration in their aqueous phases (1–50 mM) and the pH of their mobile phases (2, 3 or 4); the acetonitrile content was fixed at 40%.

The excess of Na^+ ions affected PF_6^- ions adsorption to the stationary phase and the magnitude of the consequential development of the surface potential significantly reducing the differences among retention factors at studied pH. An extended thermodynamic approach was used for a quantitative description of the observed phenomenon. In the set with varying *I* the contribution of ion-pair formation in the stationary phase to the retention of the solutes was confirmed at all the studied pH. On the other hand, in the systems with a constant *I*, the shielding effect of the Na^+ ions on the surface charge lowered the attractive surface potential and diminished the aforementioned interactions and consequently the effect of the mobile phase pH on solute retention.

3.

Applications of NIR Spectroscopy for Qualitative Evaluation of Substances

Erika Bojnanska[1], Josef Jampílek[2]

[1]Saneca Pharmaceuticals a.s., Nitrianska 100, 920 27 Hlohovec, Slovakia

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojarov 10, 832 32 Bratislava, Slovakia; e-mail: josef.jampilek@gmail.com

Near infrared (NIR) spectroscopy is a fast, non-destructive and cheap method of spectral analysis that gains its importance. Nowadays, the applications of this analytical method are very broad: it is useful as a powerful tool in pharmaceutical industry, food industry as well as in the field of law enforcement, i.e. in identification of counterfeit medicines or quick identification of drugs of abuse, forensic medicine and many more. NIR spectroscopy was recognized as a leading analytical method of process analytical technology (PAT) applied within innovative approaches to the pharmaceutical quality assurance systems. This contribution is focused on qualitative applications of NIR spectroscopy in pharmaceutical analysis, for example, for evaluation of identity, solid forms, particle size, etc., of active pharmaceutical ingredients as well as excipients.

4.

Bioanalytically-Supported Precision Medicine (Personalized Pharmacotherapy)

Roman Kaliszan

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland; e-mail: roman.kaliszan@gumed.edu.pl

Pharmaceutics emerged as a complex of scientific disciplines, dominated by chemistry. Recently: mostly by bioanalytical chemistry. That is due to the down of precision medicine and personalized pharmacotherapy. New approaches to designing single-agent and combination medicine regimens for patient subpopulations and for individual subjects are becoming feasible owing to detailed patient information more and more available from genomic, proteomic and metabolomics platforms, along with molecular imaging and other diagnostic capabilities – all usually subjected to the advanced bioinformatics data processing. Symptomatically enough, President Barrack Obama in 2016 announced the era of precision medicine to prevail in the next decade.

The reason for the change of basic pharmacotherapeutic paradigm from "each drug fits all" to "specific drug for individual patient at optimum dose", has been unsatisfactory progress in the treatment of some diseases, cancer in particular, in spite of the dramatically increasing costs of health care. With the presently available analytical tools one can identify and quantitatively determine any substance present in diverse matrices at minute levels. By means of nanosensors specific disease biomarkers can be determined in various biological material from patients. Genomic analysis forms the basis for subpopulation classifications of patients from the point of view of drug responsiveness or resistance towards them. Large matrices of data on multitude of metabolites, determined by advanced separation techniques, usually combined with mass-spectrometric detection, can be processed chemometrically to detect metabolite profiles of a specific disease-diagnostics potency. There a term emerged: "**Theranostics**", implicating the use of a combination of a given drug (**thera**peutics") with a proper bioanalytical test ("diag**nostics**"): the so-called "companion diagnostics". The sets of drugs, for which the U.S. Food and Drug Administration requires, recommends or just offers biomolecular ("omics") tests before application are systematically increasing..

Modern bioanalytical approach has been expected to determine progress of the Evidence-Based Medicine.

5.

Analysis of basic drugs in pharmaceutical formulations, biological fluids and tissues by HPLC method

Monika Waksmundzka-Hajnos, Anna Petruczynik, Karol Wróblewski

Department of Inorganic Chemistry, Medical University of Lublin, Lublin, Poland

Sorbents used in chromatographic analysis of active compounds are usually silica-based materials. They ensured high porosity and large surface areas as well as satisfactory pressure stability of materials and possibility to achieving sorbent particles of proper shape. However, the problem is sometimes connected with surface silanols. In chemically modified chromatographic sorbents instead of stationary phase ligands of different origin residual silanols play also role in mechanisms of analytes' separation. It often causes analytical problems because generated 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 tissues. 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 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. Optimization of separation conditions was also performed for antiepileptic drugs in mouse brain tissues. Often determination of active metabolites was also necessary. Methods of simultaneous determination of acidic and basic components in pharmaceutical formulations were developed.

6.

Quantitative structure-retention relationship models based on different computational techniques in micellar liquid chromatography of antipsychotic drugs

Biljana Otašević*[1], Jovana Krmar[1], Milan Vukićević[2], Ana Protić[1], Jelena Golubović[1], Mira Zečević[1]

[1] *Department of Drug Analysis, University of Belgrade – Faculty of pharmacy, 450 Vojvode Stepe, 11221 Belgrade, Serbia*

[2] *Center for Business Decision Making, University of Belgrade – Faculty of organizational sciences, 154 Jove Ilića, 11000 Belgrade, Serbia*

** Presenting and author for correspondence: E-mail: biljana.otasevic@pharmacy.bg.ac.rs*

In QSRR studies, the retention of a compound in a chromatographic system is modeled as a function of molecular descriptors, the numerical quantities used to characterize certain chemical information. The purpose of this approach is to create a mathematical correlation model, using experimental data, which can be used to predict retention, or some other physicochemical property, of a new compound without need for additional experimentation. QSRR models are commonly built using regression-based approaches like multiple linear regression (MLR) and partial least squares (PLS). In addition to these conventional methods, various machine learning tools, such as artificial neural networks (ANN) and Support Vector Machine (SVM), are recently found to be very useful when underlying mechanisms are unknown and when complex chemical information is bearing a nonlinear relationship with response. Therefore, the aim of the present study was to put on different computational techniques and describe appropriate QSRR models with usable predictive capability for series of structurally related compounds. Presented study could also contribute to understanding of retention mechanisms in micellar liquid chromatography based on the physicochemical meaning of the molecular descriptors used for building QSRR models. The importance of this additional achievement lies in well-known fact that retention behavior of a compound in this system is very complex due to multiple chemical interactions (micelle-compound, micelle-stationary phase and compound-stationary phase).

For all these reasons, a set of compounds consisting of atypical antipsychotic drug aripiprazole and its process-related organic impurities was used. The data table for model building was composed of independent variables represented by molecular descriptors and varied chromatographic conditions. The experimental design methodology based on fractional factorial design, was used in screening the most influential instrumental parameters in the observed chromatographic system. Afterwards, the plan of experiments, according to the Box-Behnken response surface design, was used for exploring the experimental region for statistically significant parameters: the concentration of non-ionic surfactant *Brij L23* and the pH value of the water phase, as well as the percentage of organic modifier acetonitrile in the mobile phase. For every experimental point, structures of investigated compounds in their

dominant ionic and/or non-ionic form were subjected to energy minimization by the semi-empirical MOPAC/AM_1 method and used for calculation of molecular descriptors that encompass all major groups of descriptors (physicochemical, quantum-chemical, topological and spatial structural descriptors). Among large number of descriptors, the selection of ones to be included in model was done taking into consideration the intercorrelation between each pair of descriptors.

Recently developed supervised learning machine techniques were in the focus of the presented study. A computational simulation of biological networks, multi-layer perceptron artificial neural network with back propagation training algorithm, was firstly applied. The input layer in the network architecture was formed by a number of neurons equal to the total number of molecular descriptors and varied chromatographic conditions. Number of nodes in the hidden layer, number of epochs, momentum and the learning rate were optimized through the process of network training. The output layer had one node which corresponded to the retention factor of the compound. Another specific class of learning algorithms that can be used for both classification and regression analysis is represented by Support Vector Machines. They are characterized by usage of kernel functions which operate by constructing hyperplanes in a multidimensional feature space. The adoption of concept known as the structure risk minimization principle which is considered as superior to the traditional empirical risk minimization principle (employed by neural networks) is their main advantage. Additionally, gradient boosted trees and random forests were evaluated. These algorithms are based on ensembles of multiple algorithms and recently showed cutting edge results in many application areas. Finally, traditional linear regression model is evaluated as a benchmark for advanced algorithms.

Data fitting was followed with the model validation in order to ensure good predictability and estimate performance on new data. Internal validation metrics within leave-one out and 10-fold cross-validation methods were applied. Predictive performance of used modeling strategies was evaluated by the statistical significance of the model (squared correlation coefficient for model fitting), root mean square errors, absolute errors and correlation coefficients for the comparison between values predicted by the model and experimentally observed values. Additionally, stability of each model is assessed by analyses of standard deviations over each performance metric.

References

1. Kaliszan R. Chem Rev 2007; 107: 3212-3246.
2. Put R. and Vander Heyden Y. Anal Chim Acta 2007; 602: 164-172.
3. Bodzioch K. et al. Talanta 2010; 81: 1711-1718.
4. Weiping M. et al. J Chromatogr A 2006; 1113: 140-147.

SESSION II

WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS:

Monika Waksmundzka-Hajnos

and Roman Kaliszan

7.

Illustration of the new hybrid optimization method for preparative chromatography column separation using enantiomeric mixtures as a model

Marcin Chutkowski[b], Jörgen Samuelsson[a], Marek Leśko[b], Martin Enmark[a], Krzysztof Kaczmarski[b], Erik Forss[a], Joakim Högblom[c] ,Torgny Fornstedt[a,]

[a] *Department of Engineering and Chemical Sciences, Karlstad University, SE-651 88 Karlstad, Sweden*

[b] *Department of Chemical Engineering, Rzeszow University of Technology, PL-35 959 Rzeszów, Poland*

[c] *Akzo Nobel Pulp and Performance Chemicals AB, SE-445 80 Bohus, Sweden*

In this study a robust method of scale up chromatography column separation on the basis of few experiments in analytical and preparative scale has been presented involving test system with mixture of two enantiomeric compounds: omeprazole and etiracetam. The processes have been numerically optimized applying the general rate model and utilizing a hybrid optimization method where global simulated annealing procedure was combined with local simplex algorithm.

The objective function was productivity and the decision parameters were: adsorbent particle diameter, column length and injection time with maximum flow rate assumed. The results have been experimentally verified in both analytical and pilot-scale at maximum allowed backpressures of 80 and 200 bar, respectively, representing contemporary standard equipment.

In this study we have shown that at least for our cases the shorter columns are more suitable utilizing packing materials with smaller particle sizes. In both investigated process scales a column length of 10 cm was found to be optimal. We also have shown that increasing the allowed maximum pressure 2.5 times resulted in around 1.5 times higher productivity. Another benefit of operating under elevated pressure levels was 40% solvent consumption reduction.

This work was supported by two grants: (i) Grant (Nr 2015/18/M/ST8/00349) from National Science Centre and (ii) Grant KK HÖG 15 (Nr 20150233) from the Swedish Knowledge Foundation (KK)

8.

Towards 'sweet spot' approach: *In silico* estimation of lipophilicity profile for a set of drug transporters

Andrzej Bak[1], Violetta Kozik[2], Paulina Dybał[1], Adam Smolinski[3] and Joseph Jampilek[4]

[1]*Department of Organic Chemistry, Institute of Chemistry, University of Silesia, Katowice, Poland;*

[2]*Department of Synthesis Chemistry, Institute of Chemistry, University of Silesia, Katowice, Poland;*

[3]*Department of Energy Saving and Air Protection, Central Mining Institute, Katowice, Poland;*

[4]*Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic;*

e-mail: Andrzej.Bak@us.edu.pl

Finding a balance between desired drug potency and its physicochemical properties called 'sweet spot', important for creating a molecule's pharmacokinetic or pharmacodynamics profile is still a challenging issue in rational drug discovery. The *a priori* calculation of the molecular descriptors, crucial for the compound bioavailability and hence critical for the prospective drug candidate is necessary to make predictions of chosen property profiles. Lipophilicity is generally regarded as a first-rate physicochemical parameter increasingly relevant to characterization of both the pharmacokinetic (ADMET) and pharmacodynamic aspects of drug-receptor/enzyme interactions which often correlates well with bioactivity of chemicals. Quantitative assessment of the lipophilic characteristics of potential drug molecules is indispensable for efficient development of ADMET-tailored structure-activity models; therefore reliable procedures for deriving logP from molecular structure are desirable.

A number of modern drugs are not available to the patients due to their poor aqueous solubility and permeability. Generally, modification/optimization of poor permeability through membranes can be solved by selecting appropriate excipients to function as transporting components of a dosage form. Numerous compounds of different chemical structures were evaluated/applied as absorption promoters - cholic acid is one of the most important human bile acids as a relevant class of compounds with a range of pharmacological activities.

A range of various software logP predictors for estimation of the numerical lipophilic values for a set of cholic acid derivatives have been employed and subsequently crossed-compared with the experimental parameter. Thus, the empirical lipophilicity (R_M) was compared with the corresponding logP characteristics calculated using alternative methods for deducing the lipophilic features. The mean values of the selected molecular descriptors that average over the chosen calculation methods (*consensus* clogP) were subsequently correlated with R_M parameter. As an additional experiment, the IVE-PLS methodology for an ensemble of descriptors retrieved from DRAGON 6.0 software have been applied for a set of drug transporters. To investigate the variations within the ensemble of cholic acid derivatives PCA and SOM procedures were employed to visualize the major differences in the performance of drug promoters with respect to their lipophilic profile.

In the current study a range of calculation methods was employed to analyze the experimental and *in silico* data, but one should be aware that '*statistical unicorns beasts exist on paper not in reality*', therefore we should not blindly follow theoretical estimators and sometimes do the experiments.

9.

Whole Body Autoradiography combined with HPLC

Huba Kalász and Kornélia Tekes

Semmelweis University, Budapest, Hungary

Autoradiography is the method to discover and detect radioactivity Whole body autoradiography (WBA) became a useful tool in pharmacology in time to follow distribution of the radiolabelled compounds in the body.

Toguzov and Tikhonov considered human and animal body as a complex chromatographic system. In living organisms various pseudo-chromatographic processes take place and retardation of the compounds studied is based on their size and lipophilicity. Moreover, it is based on affinity binding of compounds to receptors and to other binding sites of the body (R.T. Toguzov and Yu.V. Tikhonov: Natural chromatographic systems in biological objects. In "Chromatography, the State of the Art", H. Kalász and L.S. Ettre, Eds., Akadémiai Kiadó, Budapest, 1985, pp. 153-160).

WBA serves to scout radiolabelled compounds and certain segments of the drug, while HPLC determines both parent compound and its metabolites in the body compartments. Selegiline (an antiparkinsonian drug) was radiolabelled, and its fate in male Wistar rats was analyzed. WBA made possible to illustrate the progress of distribution of selegiline. Novel binding sites could be found for selegiline, and significance of these binding sites has been analyzed for its non-labeled analogues.

This project was financially supported by the grant of OTKA 100155. Advice and technical assistance of our colleagues (Drs. L. Balogh, Z. Demeter, E.B. Faigl, J. Horváth, Z. Pöstényi, A. Polyák, G. Trencsényi and G. Guth) are appreciated.

SESSION III

THURSDAY, MAY 25th, 2017

CHAIRPERSONS:

Irena Vovk and Danilo Corradini

10.

Chromatographic and hyphenated techniques in the development of functional food enriched with xanthophylls

Irena Vovk[1], Breda Simonovska[1], Alen Albreht[1], Eva Kranjc[1], Lučka Brulc[1], Vesna Glavnik[1], Nataša Ferant[2], Matjaž Červek[3]

[1]National Institute of Chemistry, Department of Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia; irena.vovk@ki.si
[2]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, SI-3310 Žalec, Slovenia
[3]Emona RCP, Kavčičeva 72, SI-1000 Ljubljana, Slovenia

Current trend in food industry is to develop new functional foods and functional food ingredients. This can be achieved by addition of health promoting ingredients during food processing or by enrichment during farming (breeding). Chromatographic and hyphenated techniques are indispensable in the process of development of functional food ingredients and functional food products. The most critical steps are selection of raw materials rich in bioactive ingredients, optimisation of extraction procedures, quality control of the final products, etc.

We will present the role chromatographic and hyphenated techniques in development of functional foods enriched with xanthophylls zeaxanthin and β-cryptoxanthin (provitamin A). Zeaxanthin and its isomer lutein are both equally important and essential micronutrients in prevention of age related diseases such as macular degeneration. Due to the lack of dietary sources (including rare food supplements) of zeaxanthin, and due to its low bioavailability from available sources, we designed a new functional food - zeaxanthin and β-cryptoxanthin enriched egg by feeding 30 laying hens with zeaxanthin and β-cryptoxanthin enriched feed. For this purpose we developed and validated HPLC methods for the determination of carotenoids in plant materials, in feed and in egg yolks (8 permitted in animal feed in the EU). For identification of other bioactive ingredients (e.g. flavonoids) in the used plant materials we developed several methods based on HPTLC-densitometry, HPTLC–MS/MS, HPLC–UV and HPLC–UV–MS^n.

11.

Capillary electrophoresis and HPLC of biomolecules in agro-food matrices

Danilo Corradini

National Research Council, Institute of Chemical Methodologies, Area della Ricerca di Roma 1 00015 Montelibretti, Rome, Italy; danilo.corradini@cnr.it

The plethora of natural organic compounds produced in plants by secondary metabolism comprise food ingredients conferring specific sensorial characteristics and/or beneficial effects on human health. The identification and quantification of these target compounds in plants and agro-food matrices is a challenging task, continuously requesting the development of more robust, efficient and sensitive instrumental analytical techniques. This communication discusses fundamental and practical aspects of both reversed phase high performance liquid chromatography (RP-HPLC) and capillary zone electrophoresis (CZE) employed for the analysis of plant secondary metabolites occurring in food matrices. The two analytical separation techniques might display complementary capability in separating secondary metabolites, as it is discussed for the analysis of phenolic compounds in plant extracts. The different selectivity exhibited by RP-HPLC and CZE in separating phenolic compounds has been ascribed to the concomitant presence of hydrophilic, hydrophobic and ionogenic groups displayed by most of these compounds, which is expected to influence to different extents the separation mechanisms operating in CZE and in RP-HPLC of molecules bearing multifunctional moieties. The presentation evaluates the influence of various operational parameters and experimental conditions employed in CZE and in RP-HPLC on the separation performance of phenolic compounds, which are widely distributed in the plant kingdom, form an integral part of human diet, and have a remarkable position as active components in functional foods and food supplements. Also discussed is the practical application of either CZE or RP-HPLC to the study secondary metabolites in transgenic food of plant origin as well as the determination of phenolic compounds in agro-food matrices during the transformation of raw ingredients into food and in the production of foods that have a potentially positive effect on health and disease prevention beyond basic nutrition.

12.

New analytical technologies for natural product research

Günther K. Bonn[1,2] and Rania Bakry[1]

[1] *Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria; Guenther.Bonn@uibk.ac.at*
[2] *ADSI - Austrian Drug Screening Institute, Innrain 66a, 6020 Innsbruck, Austria*

The progress in natural product research has been very fruitful for many decades for various areas of modern life, including medicine, cosmetics and nutrition. However the development of reliable analytical methods for the monitoring of the active constituents, as well as for the detection of low concentrations of compounds, which could be important for e.g. the identification of allergenic effects, are necessary.

Advances in analytical technologies play an important role. Especially sample preparation including enrichment and separation, have greatly facilitated the state-of-art research. Although numerous methods have been developed, still many challenges remain because of the complexity of the sample matrix and the diversity of the analysed products. In general the development of complete analytical methods for a natural product includes sampling, sample preparation, separation, detection and data evaluation.

We are focusing on the development of novel analytical methods for phytopharmacy, phytocosmetics and phytonutrition. New isolation, enrichment and purification tools based on solid phase extraction technologies were developed in order to reduce the complexity of the plant sample, remove interfering components and detect traces of analytes. The developped methods improve the speed and accuracy, allowing high selectivity, high throughput, robustness and automation. Great attention is paid to the development of macroporous, monolithic separation columns with tailored properties such as surface area, surface chemistry and porosity, to achieve high efficiency and selectivity.

Furthermore, online sample preparation and LC-MS methods play an important role in the analysis of natural products. They permit the identification and quantification of the target analytes. Apart of MS, other spectroscopic technologies such as NIR are studied.

On the other hand, the Austrian Drug Screening Institute (ADSI) as a translational research institute, is a research enterprise of the University of Innsbruck, which offers screening technologies for phyto-relevant companies and academic research institutions.

13.

Identification of bioactive peptides in food matrices by multidimensional liquid chromatography and bioinformatics

Riccardo Zenezini Chiozzi, Francesca Ferraris, Giorgia La Barbera, Carmela Maria Montone and Aldo Laganà

Dipartimento di Chimica - Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Rome, Italy;
riccardo.zenezini@uniroma1.it

Bioactive peptides are amino acids chain generated from the hydrolysis of proteins that exert in the body a biological activity, while being completely inert in the parent protein. Bioactive peptides can be generated mainly in three different ways: directly in the food during processing, *in vivo* during digestion and *in vitro* selected a specific enzyme. In the literature, more than 40 activities have been studied [1], such as antioxidative, antimicrobial, anticancer.

Bioactive peptides can have a wide range of sizes and physicochemical properties giving a very complex matrix. In fact, while trypsin generates peptides that share similar properties (e.g. charge state or length) other enzymes, even when used alone, have semi-specific cleavage site, like pepsin or chymotrypsin or completely unspecific (e.g. Alcalase, Protamex or Flavorzyme).

Nowadays, multidimensional LC is the most effective analytical tecnique in simplifing the complexity of the peptide matrices. The sequential use of different HPLC columns (mainly with orthogonal mechanism [2]) led to obtain higher resolution and higher peak capacity that is useful for this kind of analysis.

In a typical peptidomic protocol, each collected fraction, obtained from the chromatographic dimension, is usually assayed and the most asctive ones are further analysed by nanoRPLC coupled with an Orbitrap mass spectrometry for peptide sequencing [3]. After database search, the identified peptides are further mined by *in silico* analysis using bioinformatic softwares, which provided a bioactivity score later used to select candidates for chemical synthesis. Otherwise, scaling up the chromatographic system, using preparative columns, is possible to isolate and purify the most active peptides. This could simplify the entire procedure making the protocol less time consuming and expensive since no bioinformatic and chemical synthesis would be required.

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14.

How to determine aromatic hydrocarbons in bees and bee products using chromatographic techniques?

K. Zięba, A. Moos, A. Król

Among the many factors affecting bee survival and the quality of bee products, one of the most important are aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes) and polycyclic aromatic hydrocarbons (for example naphthalene or benzo(a)pyrene). Aromatic hydrocarbons in environment originate from industry, transport and other combustion processes.

Determination of aromatic hydrocarbons in bees and bee products using chromatographic techniques involves the preparation of a sample (isolation of analytes from the matrix) and chromatographic analysis.

The studies covered by this presentation are subject to extraction of monocyclic and polycyclic hydrocarbons. Extraction of monocyclic compounds was carried out using headspace technique and solid-liquid extraction by shaking with organic solvents (hexane and acetone). Extraction of polycyclic hydrocarbons was carried out in a traditional solid-liquid system by shaking and in the Soxhlet apparatus. For bee samples artificially contaminated with hydrocarbons' standards, extraction efficiencies were determined. The analyses were performed using a gas chromatography technique coupled to a mass spectrometer.

In addition, the degree of accumulation of polycyclic aromatic hydrocarbons in bee products exposed to traffic pollutants was checked. The content of polycyclic aromatic hydrocarbons (naphthalene, acenaphthylene, fluorene, phenanthrene and fluoranthene) in honey and propolis samples exposed for 30 days near Polish road no. 7 (near Myślenice) was much higher than the contents of these compounds in samples kept at the same time far away from communication lines (in a forested area situated about 4 km from road no. 7). A particularly large difference (44-fold) in the total hydrocarbon content of the tested hydrocarbons was observed for the multiflorous honey.

The presented results compare the different methods of preparation of and bee and bee products samples for analysis of monocyclic and polycyclic aromatic hydrocarbons using gas chromatography technique.

15.

Being uncertain in thin layer chromatography – some thoughts about latent details in the retention estimation

Łukasz Komsta

Department of Medicinal Chemistry, Faculty of Pharmacy
Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

During the investigation of thin-layer chromatography retention behavior, the most frequently used approach is to note R_F values up to two decimal places and R_M values up to three. Every parameter derived from the retention (for example ΔR_M or lipophilicity) is often presented in similar accuracy to give a reference value. Most of TLC chromatographers are aware that the real uncertainty of these results is higher, but the problem is complex and studying the mathematical dependences in error propagation often leads to surprising results. The presentation would go as deep as possible into basic details of TLC retention uncertainty, giving answers to several legitimate, but difficult (and uncovered directly in the literature) questions, such as:

1. What is the real uncertainty of R_M value?
2. Which R_F range correspond to low absolute, and which to low relative uncertainty?
3. When computing ΔR_M, what is the role of R_M covariance in the error value?
4. What is the practical importance of pure error and lack-of-fit error in lipophilicity estimation?
5. Can we estimate the intercept of extrapolated retention uncertainty without taking into account the slope uncertainty?
6. What is the dependence between uncertainty and the regression type?
7. How many accurate significant digits can we obtain during typical retention experiments?

Depending on the problem complexity, various solutions can be considered. In most complex problems, the best solution is the Monte Carlo simulation or bootstrapping the results.

SESSION IV

THURSDAY, MAY 25th, 2017

CHAIRPERSONS:

Ágnes Móricz and Gertrud Morlock

16.

Liquid chromatography coupled with bioactivity tests. HPLC or HPTLC?

Ágnes M. Móricz

Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman O. Str. 15, H-1022 Budapest, Hungary

There is a great demand for new, easy-to-obtain, bioactive agents in the fight against various human, animal and plant diseases. This problem necessitates the interminable production and isolation/detection of new, effective chemicals in medicine, like antimicrobials against pathogens, antioxidants to treat cardiovascular diseases, estrogens to balance the hormonal and homeostatic systems and enzyme inhibitors or inducers to prevent e.g. Alzheimer's disease or diabetes. As the plant kingdom is rich in unexplored compounds having the most diverse chemical structures it could be expected that drug industry effuses a lot of new drugs sourced from plants. Chemical analyses of plant constituents is strongly linked to the use of liquid chromatographic systems with column or planar arrangements, which enable the efficient separation and isolation of plant ingredients.

Effect-directed analysis gave a new impetus for the discovery of new potential drug compounds from natural sources. Liquid chromatographic hyphenations were introduced and generally used for simultaneous or parallel detection and characterization of separated bioactive substances. The open planar layer chromatographic systems provide implicitly the performance of direct assays in situ in the adsorbent bed after development and drying of the chromatoplate. Column liquid chromatography generally gives more efficient separation, however its compatibility with the biological systems is often problematic.

In this lecture I would like to summarize and compare the opportunities provided by planar layer (TLC/HPTLC) and column liquid (HPLC) chromatographic systems in the effect-directed discovery of bioactive compounds.

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17.

Latest research in hyphenated HPTLC

Gertrud Morlock
Chair of Food Sciences, Justus Liebig University Giessen, Germany,
gertrud.morlock@uni-giessen.de

Latest progress is reported in the combination of HPTLC with bioassays [1-3], open source-based multivariate data analysis [4], mass spectrometry and structure elucidating techniques [5, 6]. Complementary to commonly used target analysis that focuses on known bioactive compounds, a planar chromatographic bioprofiling can provide comprehensive effect-directed answers and can easily discover unknown active compounds in complex samples. For example, estrogen-effective compounds are discovered in wine, beer, spices, nutraceuticals, river water and waste water. A similar outcome is shown for antibiotics; especially, the discovery of natural antibiotics, their quantitation as well as equivalency calculation is outlined. Mostly, these discovered, active compounds are unknown; hence, an option for a fast structure elucidation is demonstrated with the planar separation as a start.
Some advantages of this strategy: Only effective compounds are focused and characterized in a complex sample that might consist of up to 4000 different single compounds. At one go, 20 samples on a plate are analyzed in parallel, which makes effect-directed analysis (EDA) efficient, taking between 5 and 15 min per sample depending on the bioassay. This way, attention is drawn to important active components in complex samples in a highly streamlined workflow, making HPTLC-EDA an important cost-efficient tool in the analytical toolbox of experts.

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18.

Thin-layer chromatography-direct bioautography as a semi-quantitative method

Irena Choma

Chromatographic Method Department, M. Curie-Skłodowska University, Lublin, Poland

Thin-layer chromatography–direct bioautography (TLC-DB) is a hyphenation of TLC with a bioassay which is performed directly on a developed and dried (HP)TLC plate. TLC-DB, followed by analytical and/or spectroscopic methods, belongs to the effect directed analysis (EDA) which enables detection and identification of compounds responsible for biological effect in a given sample [1,2]. Planar chromatography is a very convenient separation technique to be used in EDA because of limited purification steps, possibility of analyzing many samples in parallel and evaporation of mobile phase that could influence test organisms used in bioassays. In case of TLC-DB, mostly antimicrobial properties of separated compounds are measured. The developed and dried plate is dipped in a suspension of microorganisms growing in a proper broth and then incubated under optimized conditions. Microorganisms grow directly on a plate surface excluding places where antimicrobial agents are located. Also other effects can be measured using TLC-DB, e.g. antioxidant, antimutagenic, enzyme inhibition or estrogenic activities. Zones of inhibition/activity can be visible directly or after treating with specific detection reagents.

TLC-DB is a perfect method for biological screening. Biological fingerprints compared with UV chromatograms and those obtained after chemical derivatization deliver qualitative information. However, there are few examples for using this method for quantitative (or at least semi-quantitative) measurements [2,3]. This problem will be discussed in detail basing on our own results as well as those described in the literature. The focus will be done on TLC-DB with microbiological detection but also other tests (DPPH, planar yeast estrogen screen (p-YES), enzymatic) will be discussed. Various types of calibration curves (linear, exponential, sigmoidal) will be presented.

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SESSION V

FRIDAY, MAY 26th, 2017

CHAIRPERSONS:

Teresa Kowalska and Andrzej Bąk

19.

TLC-bioautography: an appropriate test for detection of antibacterial activity of essential oils

Kamilla Ács[1*], Viktória L. Balázs[1], Béla Kocsis[2], Andrea Böszörményi[3], György Schneider[2], Ágnes Móricz[4], Péter G. Ott[4], Györgyi Horváth[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, H-7624, Pécs, Rókus str. 2., Hungary*
[2]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, H-7624, Pécs, Szigeti str. 12., Hungary*
[3]*Semmelweis University, Faculty of Pharmacy, Institute of Pharmacognosy, H-1085, Budapest, Üllői str. 26., Hungary*
[4]*Plant Protection Institute, Centre of Agricultural Research, Hungarian Academy of Sciences, H-1022, Budapest, Herman Ottó str. 15., Hungary*

**Corresponding author. E-mail: kamilla.acs@aok.pte.hu, gyorgyi.horvath@aok.pte.hu*

The discovery of new alternative treatments against antibiotic-resistance which could support the medical therapy is an urgent challenge nowadays. Essential oils (EOs) are complex, non-water soluble, volatile herbal substances with different biological activities, e.g. antifungal, antiviral, and anti-inflammatory properties. Although the antimicrobial activity of EOs has already been studied by several *in vitro* techniques, but the standardized assays are still not available. Due to the lipophilic character of EOs the classic microbiological tests (e.g. disc diffusion, agar absorption, agar dilution) provide inappropriate results. TLC-direct bioautography (TLC-DB) is an effect-directed method, which connects bioassay with separation techniques. The procedure can be performed without or after TLC separation as well, thus the biological activity of an extract or a unique component could also be directly determined.

The aim of our research group was to detect bioactive compounds in EOs with TLC-DB, which could be promising and alternative method for finding new antibacterial agents.

The EOs (cinnamon bark, eucalyptus, tea tree, scots pine, clove, peppermint, spearmint, citronella, thyme, lavender, and rosemary) were obtained from Aromax Ltd. or were isolated by water-steam distillation (*Artemisia adamsii* Besser). The chemical and percentage compositions of EOs were determined by gas chromatography-mass spectrometry (GC-MS). The microbiological tests were performed on human pathogenic bacteria including anaerobic and microaerophilic, and antibiotic-resistant strains as well. The EOs were diluted in absolute ethanol. An aqueous solution of MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma-Aldrich Ltd.) was used for the visualization of inhibition zones (expressed in mm).

According to the results, the EOs of cinnamon bark, clove, and thyme showed the most significant antibacterial effect. Furthermore, clove and thyme were also active against *Clostridium perfringens* and *Campylobacter jejuni* in modified test systems. The EO of the Mongolian plant, *Artemisia adamsii*, showed activity against different *Staphylococcus* strains. Eucalyptus and scots pine produced moderate activity against resistant pathogens.

We suggest that TLC combined with an *in situ* bioassay allows a rapid and cost-effective identification of the active compound in a complex mixture, e.g. EOs. In most cases antibacterial activity of EOs is related to their main component, but further experiments are needed to confirm this hypothesis. In our further studies, we are planning to determine the mode of action of the effective oils in *in vivo* models.

20.

Biotreatment of Styrene, Ethanol and Dimethyl Sulfide Mixture in the Contaminated Airstream using the Compact Trickle Bed Bioreactor

Paulina Dybał[1,2], Andrzej Bąk[2], Violetta Kozik[1], Sławomir Kuś

[1]*University of Silesia, Institute of Chemistry, Department of Synthesis Chemistry, 9 Szkolna St., Katowice, Poland*
[2]*University of Silesia, Institute of Chemistry, Department of Organic Chemistry, 9 Szkolna St., Katowice, Poland*

Modern civilization is confronted with a worldwide rise of atmospheric pollution due to the expansion of industrial and agricultural areas as well as urban settlements. Volatile organic and inorganic compounds (VOC's & VIC) compose the class of the most hazardous atmospheric contaminants. This class of compounds is composed of isoprenoids, alkanes, alkenes, aromatics, carbonyls, alcohols, esters, ethers, organic acids and others. Some VOCs like styrene and its metabolites are known to have potentially serious detrimental impact on human health revealing toxic and carcinogenic properties.

The increasing public awareness of necessity for environmental protection with nuisance-free, breathable air was the main driving force for the increasingly stringent regulations governing release of hazardous air pollutants (HAPs) and reduced sulfur compounds (RSCs). Environmental legislations are constantly pushing industry for reduction in emission of poisonous low-molecular weight gases and developing/optimizing of cost-effective 'green' manufacturing technologies that impose less burden on the ecosystem. The VOC's biotreatment carried out in the Compact Trickle Bed Bioreactor (CTBB) has become an attractive alternative for many physicochemical methods of air purification. The main advantages include low pressure and low temperature of the biodegradation process, friendliness to human beings and surrounding environment, lack of secondary waste and low operating costs.

The principal objective of this study was to specify operating boundaries of parameters at which the sampled microorganisms were most effective in the biodegradation of gaseous streams containing styrene, ethanol and dimethyl sulfide mixture at dynamic variations of pollutant load.

The average conversion factor for the 3-component VOCs mixture was higher than 95% at lower range of the individual pollutant load and basically fell to 80% at middle range vs. 55% at the higher contaminant loads; however, the effectiveness of ethanol biodegradation is stable at the entire investigated range of the mass load. The consequences of an unexpected pollutant overload (media clogging) and the time necessary for the subsequent regeneration of the microbial community and restoring the process stability were investigated as well.

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21.

Fatal case of poisoning with a new cathinone derivative, α-propylaminopentiophenone chromatographic and spectroscopic analysis of postmortem material

Milena Majchrzak [1,2)], Rafał Celiński [2)], Mieczysław Sajewicz[1)]

[1]*Department of General Chemistry and Chromatography, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland*

[2]*Toxicology Laboratory ToxLab, 6 Kossutha Street, 40-844 Katowice, Poland*

Compounds known as new psychoactive substances (NPS) are a group of biologically active substances which affect human central nervous system in a way similar to narcotics. These substances are also colloquially named as designer drugs and their legality makes them appear increasingly more frequently on the global narcotics market. Nowadays, the designer drugs are sold on a large scale in the Internet and the stationary shops under the funny names like "Aromas and others", "Magic Liquid", "Funny Shop" etc. This easy access to new psychoactive substances is a reason of an increasingly frequent experimentation with their use by consumers. Currently, one of the most numerous groups, next to synthetic cannabinoids, are synthetic derivatives of cathinone, a biologically active alkaloid derived from the plant known as khat (*Catha edulis*). Generally, an unknown composition of these commercial products and an unknown mechanism of action of the designer drugs are the reason of very large amounts of poisoning, including mortal cases.

The aim of this study is to discuss identification and then quantification of a new psychoactive substance in the post-mortem material derived from a young woman with use of liquid chromatography coupled with mass spectrometry (LC-MS).

As a result, a new psychoactive substance, α-propylaminopentiophenone, was identified and quantified in the postmortem material. In combination with the autopsy, these results allowed defining the death cause as poisoning with the new cathinone derivative.

POSTER SESSION I

WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS:

Marcin Chutkowski

and Grzegorz Jóźwiak

1.

Thin-layer chromatographic identification and quantification of anthocyanins in food products

Agnieszka Fulczyk, Eliza Łata, Mieczysław Sajewicz

Department of General Chemistry and Chromatography, Institute of Chemistry

University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

Anthocyanins constitute a group of flavonoids which is widely distributed in vascular plants. Anthocyanin dyes are classified as so-called natural non-food vegetable substances soluble in water. They give flowers and fruits intense colours, from orange through various shades of red and violet to black. Up to now, hundreds of natural anthocyanin dyes have been discovered and many of them are synthetically produced [1].

Anthocyanins have an antioxidant effects, they have the ability to catch free radicals. They counteract the fragility of blood vessels, mainly the capillaries and stimulate the production of rhodopsin - a substance important in the process of vision. Moreover, they lower the rate of oxidation reaction of LDL cholesterol, which is a component of atherosclerotic plaques [2]. The intense colour and health benefits of anthocyanins make them very popular in the food and cosmetics industries. In the food industry, anthocyanins are used to color beverages, juices, yoghurts, jams, sweets or wines [1, 3]. They are labeled as E163 on the list of food additives [4]. Anthocyanin dyes are used as indicators for assessment the quality of colour food. They also protect food products from spoilage, which is related to the antagonistic activity of these dyes against certain bacteria, viruses and fungi [1].

The purpose the studies was to develop a method for the identification and quantification of selected anthocyanins in food products. The standards were cyanin chloride, keracyanin chloride, pelargonidin chloride and delphinidin chloride. The presence of the above compounds was studied in juices (the commercial and home-made), syrups, nectars and non-carbonated drinks. The studies were conducted by thin - layer chromatography. The results of the TLC were densitometrically evaluated with use of the CD-60 model scanning densitometer. Spectrophotometric measurements were performed with use of a Varian Cary Eclipse Fluorescence Spectrophotometer.

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2.

Thin-layer chromatographic identification of amino acids in spider web

Magda Michalik[1], Maja Surmacka[1], Monika Stalmach[2], Grażyna Wilczek[2], Teresa Kowalska[1], Mieczysław Sajewicz[1]

[1]*Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland*

[2]*Department of Animal Physiology and Ecotoxicology, Faculty of Biology and Environmental Protection, University of Silesia, 9 Bankowa Street, 40-007 Katowice, Poland*

Spider web is among the strongest biological materials and it is noteworthy that in proportion to the spider's weight, it is more durable than steel or Kevlar. The purpose of our study was the amino acid analysis of *Steatoda grossa* (Theridiidae) spider silk. Natural spider silk has unique properties such, as stretchability, durability or biocompatibility [1]. The main components of the thread are proteins, so it is important to know an exact chemical composition to better understand the properties of the thread. Moreover, due to its mechanical properties, spider silk seems a promising candidate material in technology and medicine. Knowledge of composition and structure of the spider web could help devise biocompatible coatings or microcapsules in which active substances of drugs could be contained.

Spider webs make an interesting research subject, also in the ecotoxicological context. Until now, it has not been specified whether and to what extent metals ingested with food alter the processes proceeding in the silk glands and if such changes could consequently influence chemical properties of the spun web threads. In this study, a simple food chain model: medium with cadmium → Drosophila hydei flies → females of the synanthropic *Steatoda grossa* spider, was used to investigate whether and to what extent metal, ingested with food, alters the amino acid composition of the spun web threads produced by the examined species. Three experimental groups were distinguished: control (C), spiders exposed to cadmium for four weeks (4-Cd), and spiders exposed to metal for one year (L-Cd).

In order to obtain the monomeric amino acids, the spider silk was preliminarily subjected to the acidic hydrolysis by means of hydrochloric acid at 110℃ for the period lasting 20 hours. The thin-layer chromatographic separation of amino acids was carried out on the silica gel pre-coated chromatographic plates, using the acetone + butanol + acetic acid + water mixture, 7: 7: 2: 4 (v/v/v/v). The development of the chromatograms lasted 4 h and the plates were visualized with the 0.5% ninhydrin solution.

The research was aimed at qualitative and quantitative determination of individual amino acids in spider web, depending on the presence (or otherwise) of Cd in the spider diet. The following amino acids were identified: alanine, glycine, glutamic acid, serine, cysteine, methionine, proline, threonine, isoleucine, arginine, leucine, phenylalanine, histidine and aspartic acid. Quantification of amino acid was performed with use of densitometry and the adequate results were presented.

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3.

Identification and quantification of polyphenol compounds in lavender and thyme honeys using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry

Kinga Gyergyák[1], Éva Horváth[2], Ágnes Farkas[1], Borbála Boros[2]

[1]*Institute of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Rókus u. 2., H-7624, Pécs, Hungary*
[2]*Department of Analytical and Environmental Chemistry, Faculty of Science, University of Pécs, Ifjúság útja 6., H-7624, Pécs, Hungary*

Honey, a natural product prepared from the nectar of various plants, is gathered, modified and stored in honey combs by honeybees (*Apis mellifera* L.). Honey is official in several pharmacopoeias (e.g. *Mel*, Ph. Eur. 5.1), valued for its antimicrobial and antioxidant activity, which can be largely attributed to its polyphenol content. Additional beneficial properties may characterize unifloral honeys that contain further specific compounds beside the usual sugar components and phenolic substances. Some compounds are unique to a certain type of unifloral honey, and may serve as markers that can be used for identification purposes and preventing honey adulteration.

The current study aimed at determining the major polyphenolic compounds in lavender and thyme honeys produced in Hungary, Croatia, France and Spain.

A sensitive method coupling high-performance liquid chromatography with diode-array detector and electrospray ionization mass spectrometry was optimized for the separation and identification of polyphenolic compounds. The novel method was successfully applied to quantify the polyphenols in lavender and thyme honeys after a simple sample preparation step. Separation was performed on a new generation of core-shell particle packed column (Sunshell C18 column; 30×2.1 mm, 2.6 μm, ChromaNik Technologies Inc, Japan). Fragmentation behavior of polyphenolic compounds was investigated using ion trap mass spectrometry in negative electrospray ionization. The MS, MS^n and UV data together with HPLC retention time of polyphenols allowed structural characterization of these compounds. Several validation parameters (repeatability and intermediate precision, LOD, LOQ, calibration range, and recovery) have been calculated in the developed method.

Our investigations confirmed that the above chromatographic techniques can be applied successfully for detecting marker compounds in honey samples and can be useful tools in checking the botanical origin of unifloral honeys.

4.

Chromatographic comparison of biological activity of essential oil and two *Acorus calamus* rhizome extracts using TLC with direct bioautography as detection method

Grzegorz Jóźwiak[1], Monika Waksmundzka-Hajnos[1], Barbara Majer-Dziedzic[2], Aneta Rojek[1]

[1] *Medical University of Lublin, Department of Inorganic Chemistry, Chodźki 4A, 20-093 Lublin, Poland*

[2] *University of Life Sciences, Department of Veterinary Microbiology, Akademicka 13, 20-950 Lublin Poland*

Acorus calamus, also called as sweet flag or calamus (in Poland - ajer or tatar herb) is a perennial, monocotyledon of the Acoraceae family in the genus Acorus. Herb grows in wetlands and marshlands - edges of small lakes, ponds and rivers, is native to India, central Asia, southern Russia and Siberia, and in Eastern Europe. Calamus was introduced into Europe and North America for its medicinal purposes, dried and shredded rhizoma is used as medical raw material. Biological properties of calamus rhizome extracts and essential oil: digestive - bitter substances (acorin), slightly sedative (azarone) are often reported. Preparations for external use has an antifungal and antimicrobal properties and more minor meaning activities.

Isolation of biological active compounds or groups of compounds from natural extracts allows using concentrated preparations, focused on their specific properties. Preparative TLC seems to be a good technique for isolation small quantities of interesting metabolites (groups of metabolites) from raw material as herbal extract.

Various biological methods of detection allow to fish out compounds of defined activity to find pharmacologically active drug candidates from its mixture in herbal extracts. The separation of active compounds (fractions) from *Acorus calamus* rhizoma is especially important because of content of acorin in the material, substance which is toxic for human and animals.

In our work aqueous and methanolic extracts and distilled essential oil from the plant were examined. Aqueous extract was obtained from shredded and dried rhizome by use of percolation method after prior ultrasonic accelerated maceration. To obtain methanolic extract the raw material was continuously extracted in Soxhlet apparatus. Essential oil was distilled with steam in Deryng apparatus. The TLC systems with silica layer and multicomponent eluent composed of organic solvents were used for separation of extracts and oil. Chromatographic systems were found in literature and used after small modification of solvent content and developing method.

The direct bioautography method were used for preliminary evaluation of antimicrobial properties of extract fractions. Separated extracts and oil were examined with *Bacillus subtilis* colony test. Test results will be used to TLC preparative isolation of fractions and for identification of compounds (groups of compounds) which will be responsible for bactericidal properties.

5.

Lipophilicity of selected terephthalamides

Violetta Kozik, Andrzej Bąk, Krystyna Jarzembek, Paulina Dybał, Danuta Pentak, Barbara Hachuła, Marcin Rojkiewicz, Aleksandra Świetlicka, Dominika Bożek, Joanna Kozłowska, Piotr Kuś

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

The chromatographic data were determined for the investigated set of new amides derivatives by RP-TLC method and related with theoretical partition coefficient calculated by means of *in-silico* procedures. Statistically, significant correlation was found between experimental R_{MO} values and the quantitative descriptor of lipophilicity (logP) specified by OSIRIS and Sybyl predictors. The impact of the calculated physicochemical and structural descriptors on the retention parameters was elucidated by variable elimination procedure IVE-PLS, indicating the involvement of various factors on hydrophobic forces.

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6.

Determination of selected pollutants in fuel samples.

Violetta Kozik[1], Andrzej Bąk[1], Krystyna Jarzembek[1], Paulina Dybał[1], Agata Nobis[2,3], Janusz Klecki[2,3], Danuta Pentak[1], Katarzyna Sikora[1], Klaudia Haśnik[1]

[1]*Institute of Chemistry, University of Silesia in Katowice*

[2]*i-Petrol Sp z o.o., Katowice*

[3]*Odczynniki Sp z o.o., Sp. k., Katowice*

The presence of sulfur in the fuel exerts an adverse effect on the physical state of the combustion engine, but is also undesirable for ecological reasons. Running the engine emits exhaust gases into the environment. In case of high sulfur content in the fuel, more toxic sulfur (IV) oxides are emitted into the atmosphere. Oxides contribute to smog, threatening the environment, irritating the respiratory tract. It can cause not only allergic reactions but also endangering human life and health. Due to serious environmental risks, the monitoring of sulfur content in fuels is very important. Determination of the sulfur content of liquid fuels for vehicles has been carried out in accordance with the applicable national standard, ISO / IEC 20846: 2011 based on international standard ISO 20846. In addition, the content of solid impurities in fuels was determined. More than 200 fuel samples were tested.

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7.

Determination of the lipophilicity of selected furanosteroids

Małgorzata Dołowy[1], Alina Pyka-Pająk[1] and Marcin Pacholczyk[2]

[1]*Department of Analytical Chemistry, School of Pharmacy with Division of Laboratory Medicine, Medical University of Silesia in Katowice, 4 Jagiellońska St., 41-200 Sosnowiec, Poland*

[2]*Faculty of Automatic Control, Electronics and Computer Science, Institute of Automatic Control, Silesian University of Technology, 16 Akademicka St., 44-100 Gliwice*

Viridin and wortmannin belong to the small group of naturally occurring furanosteroids. The compounds have been widely known as potent inhibitors of the lipid kinase PI-3K. They show also anti-proliferative effects against a breast cancer line [1,2].
Different physicochemical parameters, such as lipophilicity are useful in the prediction of action, toxicity, metabolism, transport, pharmacokinetic and protein binding of various drug substances including described furanosteroids [3]. Because there is a lack in available literature the experimental value of lipophilicity parameter of both furanosteroids, the main aim of this work was to use thin-layer chromatographic technique in reversed phase system (RP-TLC) to predict the experimental value of lipophilicity parameter for viridin and wortmannin. Chromatographic lipophilicity parameter (R_{MW}) of examined furanosteroids have been studied under different conditions; various chromatographic plates for RP-TLC and different mobile phases like methanol-water, dioxane-water and acetonitrile-water. All chromatographic lipophilicity parameters (R_{MW}) obtained for two studied compounds accordance with Soczewiński-Wachtmeister equation were compared with the theoretical partition coefficients calculated by different computing programs: AlogPs, AClogP, AlogP, MlogP, xlogP2 and xlogP3 [4]. In addition to this, R_{MW} values determined for these drug substances were correlated with their binding energy with human proteins (i.e. human serum albumin HAS and also with sex hormone-binding globulin SHGB) obtained using docking software.

Our study confirms the usefulness of thin-layer chromatographic technique and calculation software in the predication of lipophilicity and binding energy with human proteins of examined furanosteroids.

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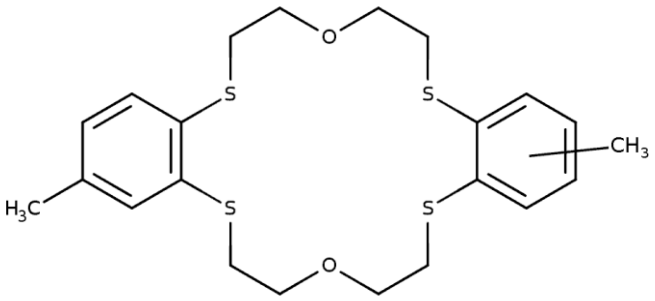
8.

Synthesis of crown thiaethers – potential complexing agents for transition metals

Hubert Hellwig, Piotr Kuś

Department of Organic Synthesis, Institute of Chemistry, University of Silesia in Katowice

Thiacrown ethers are macrocylic compounds containing sulfur atoms. In opposite to standard crown ethers (which contains only oxygen atoms), thiacrown ethers are capable of forming complexes with transition metal cations, for example Co^{2+}, Ni^{2+}, Fe^{2+}, Rh^{3+}, Pd^{2+}, Ag^{+}, Cu^{2+}, Hg^{2+}, Cd^{2+} and many others [1]. Modification of macrocyclic ring structure, symmetry and positions of sulfur atoms leads to changes the metal complexation selectivity of crown thiaethers [2]. According to these properties they can be used in separation techniques as solid or liquid phase modifiers in chromatography (in stationary phase the macrocyclic ethers are chemically linked to polymer). Crown thiaethers can also be applied in waste water purification from heavy metals [3]. 2,3,11,12-Bis(4',4"(5")-methylbenzo)-1,4,10,13-tetrathia-7,16-dioxacyclo-octadecane was synthesized and isolated in pure form by column chromatography. Its structure was confirmed by ^{1}H, ^{13}C NMR spectroscopy and ESI-MS spectrometry. Further research are under way.



Structure of 2,3,11,12-bis(4',4''(5'')-methylbenzo)-1,4,10,13-tetrathia-7,16-dioxacyclooctadecane

Literature:

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9.

Voltammetric Determination of Heavy Metals in Green and Black Tea

Alexandra Planková[1], Ľubomír Švorc[2], Josef Jampílek[3], Peter Mikuš[1]

[1]*Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia;*
e-mail: plankova@fpharm.uniba.sk
[2]*Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia*
[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia*

Tea is one of the most popular beverages that is important source of bioactive compounds that influence human health, especially acts as antioxidant in cancer diseases, cardiovascular diseases or in diabetes. On the other hand, the habitual tea drinking worldwide and its social and cultural roles, essential and non-essential metals present in black and green tea beverages can have health impacts or impose hazard to consumers and decline their well-being. Heavy metal contaminants might accumulate during tea growth, transportation, packaging or processing, and are harmful to human health.

Differential pulse techniques are extensively employed in electroanalysis due to their high sensitivity, good definition of signals and reduction of double layer and background currents. These advantageous properties arise from the subtractive nature of the signal and the rapid decay of the charging current in a constant potential pulse, which gives rise to well-defined peak-shaped responses. Differential pulse voltammetry was used for the direct simultaneous determination of Cd, Pb, Cu, Sb and Bi in 0.1 M HCl solution (pH 1) containing 1 M KCl. Zn was subsequently determined after raising the pH of the same solution to pH 4. Next, the pH of the medium was raised to pH 8.5 by adding NH_3/NH_4Cl buffer for the determination of Mn. Finally, Ni and Co were determined in the same solution after adding dimethylglyoxime.

The information on the total content of trace metals can be a criterion that makes the tea products admissible for consumption, hence, it is an important part of the quality control that assures purity, safety of black and green teas and evaluates the eventual intoxication risks.

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10.

Thin-layer chromatographic analysis of stanozolol

Małgorzata Dołowy[1], Karolina Chrystow-Chrystow[2], Alina Pyka-Pająk[1] and Krzysztof Marciniec[3]

[1]*Department of Analytical Chemistry, School of Pharmacy with Division of Laboratory Medicine, Medical University of Silesia in Katowice, 4 Jagiellońska St., 41-200 Sosnowiec, Poland*

[2]*Student Research Group at the Department of Analytical Chemistry, School of Pharmacy with Division of Laboratory Medicine, Medical University of Silesia in Katowice, 4 Jagiellońska St., 41-200 Sosnowiec, Poland*

[3]*Department of Organic Chemistry, School of Pharmacy with Division of Laboratory Medicine, Medical University of Silesia in Katowice, 4 Jagiellońska St., 41-200 Sosnowiec, Poland*

Stanozolol is an synthetic steroid compound which has been approved for humane by FDA (Food and Drug Administration) in 1962. It will be used orally or intramuscularly in treating anaemia and hereditary angioedema [1]. Moreover, stanozolol like other anabolic steroids is commonly used in many different kinds sports by men and women to attain a competitive edge. For this reason it is classified as a controlled substance by WADA (World Anti-Doping Agency) [2]. The development of simple to use and economical thin-layer chromatographic method can be useful in the determination of stanozolol in counterfeit pharmaceutical products of anabolics as well as illegal drugs of anabolics available by black market. In this work a new, simple in use and economical TLC-densitometric method in normal phase system (NP-TLC) has been developed for the identification and quantitative determination of stanozolol in the samples containing stanozolol and its related compounds (impurities A and B). Different chromatographic conditions have been tested in this study. Of all applied chromatographic conditions, the mobile phase consisted of toluene-2-propanol in volume composition 45:2 or 43:5 and chromatographic plates precoated with the mixture of silica gel 60 and kieselguhr F_{254} (Art. 1.05567, E. Merck, Germany) are the best. Densitometric analysis was carried out at λ=228 nm. The proposed NP-TLC densitometric method was verified in terms of its specificity, linearity, precision, accuracy, robustness and sensitivity.

Developed TLC-densitometric method can be successfully used in routine quality control of stanozolol in bulk material as well as in available pharmaceutical formulations.

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11.

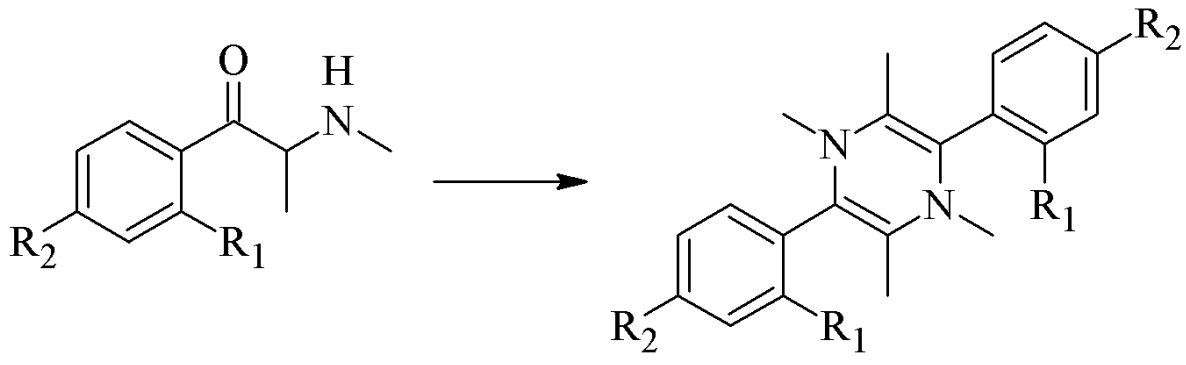
Identification of instability products of the cathinone derivatives

Jakub Wantulok[1], Krzysztof Byrdy[1], Tadeusz Paździorek[2], Jacek E. Nycz[1]

[1] *Institute of Chemistry, University of Silesia in Katowice, ul. Szkolna 9; PL-40007 Katowice, Poland.*

[2] *Department of Chemistry, Forensic Laboratory, The Regional Headquarters Katowice, Poland.*

In this poster we presented the identified by GC–MS techniques instability products of 2-(methylamino)-1-(2-methylphenyl)-1-propanone (**1a**) (2-MMC) and 1-(4-chlorophenyl)-2-(methylamino)propan-1-one (**1b**) (4-CMC) in common organic solvent, chloroform. These products were formed by intramolecular condensation leading to appropriate cyclic dimers or trimers. Additionally the use of amination reaction with 2-(pyrrolidin-1-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)pentan-1-one (TH-PVP) for identification of instability product will be presented. The presented findings can be used in future to identify and separate new cathinone derivatives and their decomposition products or metabolites.



1a	**1b**
$R_1 = CH_3$	$R_1 = H$
$R_2 = H$	$R_2 = Cl$

Scheme 1. Dimerization of **1a** and **1b**

12.

Separation and quantitation of selected water-soluble vitamins using different HILIC stationary phases

Justyna Kamińska[1)], Lidia Zapała[2)], Marcin Chutkowski[1)], Piotr Ziobrowski[1)], Wojciech Zapała[1)]

[1)] Department of Chemical and Process Engineering, Chemical Faculty, Rzeszów University of Technology, Powstańców Warszawy 6, 35-959 Rzeszów, Poland
[2)] Department of Inorganic and Analytical Chemistry, Chemical Faculty, Rzeszów University of Technology, Powstańców Warszawy 6, 35-959 Rzeszów, Poland

Hydrophilic interaction chromatography (HILIC) was first described by Alpert who used the method for separation of proteins, peptides, amino acids, oligonucleotides and carbohydrates. The HILIC is based on polar stationary phases combined with partly aqueous eluents (around 2–40% water) containing acetonitrile or other solvents, e.g., alcohols. Hydrophilic interaction chromatography has been proved to be a useful technique for the retention and separation of polar compounds offering selectivity complementary to RP chromatography.

The group of water-soluble vitamins consists of vitamin C (L-ascorbic acid) and eight vitamins of group B including thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9) and cyanocobalamin (B12). These substances are a group of compounds with hydrophilic character. This suggests they should be retained under HILIC conditions. However, B vitamins are characterized by structural complexity and have diverse chemical and physicochemical properties. For these reasons their chromatographic separation is rather challenging task.

The objective of this study is to develop and validate a HILIC method for the separation of selected water-soluble vitamins with a variety of functional groups, on a diol (Acclaim Mixed-Mode HILIC-1) and zwitterionic (Kanuer Eurospher II 100-5 HILIC) columns. Chromatographic conditions including type and percentage of organic solvent in the mobile phase, pH and concentration of buffer salt have been investigated.

It has been found that the mobile phase composition plays an important role in the separation of B vitamins in each of the columns. Besides, both pH and concentration of applied buffer salt effects on the separation selectivity of the analyzed compounds have been discovered. Finally, the optimal conditions for selective separation of B-group vitamins mixture were proposed.

13.

Comparison of isocratic retention models for hydrophilic interaction liquid chromatography

Justyna Kamińska[1)], Lidia Zapała[2)], Marcin Chutkowski[1)], Piotr Ziobrowski[1)], Wojciech Zapała[1)]

[1)] *Department of Chemical and Process Engineering, Chemical Faculty, Rzeszów University of Technology, Powstańców Warszawy 6, 35-959 Rzeszów, Poland*

[2)] *Department of Inorganic and Analytical Chemistry, Chemical Faculty, Rzeszów University of Technology, Powstańców Warszawy 6, 35-959 Rzeszów, Poland*

Quantitative retention versus eluent composition relationships are of fundamental importance for method development in chromatography. Therefore, in this work the applicability and accuracy of six different retention models used for retention prediction in hydrophilic interaction chromatography (HILIC) have been studied.

Four of the tested models assume additivity of the reversed phase liquid chromatography (RPLC) and of the HILIC contributions to the energy of retention in chromatographic systems, i.e. the mixed mode retention mechanism. The two other non-linear models were adopted from RPLC.

The models have been compared and verified on the basis of various and numerous experimental data exhibiting strongly nonlinear and/or U-shaped retention dependencies versus mobile phase composition. The accuracy of all the models has been statistically verified by means of different statistical criteria and the optimal solution has been proposed.

14.

Multivariate analysis of variance of designed chromatographic data

Danuta Liberda[1], Jade Tobin[2], Dalene de Beer[2,3], Beata Walczak[1]

[1]*University of Silesia, Institute of Chemistry, Katowice, Poland*

[2]*Plant Bioactives Group, Post-Harvest and Wine Technology Division, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Stellenbosch, South Africa*

[3]*Department of Food Science, Stellenbosch University, Stellenbosch, South Africa*

The main goal of our project is a study of the kinetics of the rooibos tea fermentation. Realization of this goal requires, among others, identification of the main components of the tea extracts involved in the fermentation process. In order to estimate an influence of the semi-fermentation and fermentation process on the concentrations of these components, ten plants were studied, and three subsamples of each plant underwent three different treatments (i.e., extracts of the raw, semi-fermented and fermented materials were investigated). The resulting chromatographic data has the dimensionality of 120 samples x 56 compounds. It is the multivariate data which requires application of the multivariate analysis methods. At this stage of our study, we would like to estimate a significance of the treatment effect and to identify these compounds, which significantly contribute to it. As a well-suited method of analysis of variance of multivariate correlated data, the ASCA method was applied for the log transformed data. However, the results of ASCA were inconsistent with the assumption of the PQN normalization method. To solve the normalization problem, we propose application of the pair-wise log ratios.

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15.

Simple and rapid screening procedure for 66 synthetic cannabinoids by liquid chromatography-tandem mass spectrometry

Katarzyna Ambroziak [1] , Piotr Adamowicz [2]
[1] *University of Silesia, Faculty of Chemistry, Szkolna 9, 40-006 Katowice*
[2] *Institute of Forensic Research, Westerplatte 9, 31-033 Krakow*

In recent years many synthetic cannabinoids (SC) have appeared on the drug market. These substances sold as 'herbal highs' or 'research chemicals' belong to different chemical classes. According to reports of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), more than 160 SC were introduced to the European market up to 2015. Despite the increasing number of SC, there are few comprehensive screening methods for their detection in biological specimens. The variety of SC, their low active doses, low concentrations in biosamples, as well as rapid and numerous metabolic changes create great analytical problems. The analytical method should be sensitive, selective and screening procedures should be open, i.e. ensuring the possibility of continuous inclusion of new compounds.

The purpose of this study was to develop a fast and simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening procedure for detection and identification of 66 SC in blood.

Blood samples (0.2 mL) were precipitated with acetonitrile (0.6 mL). Analyses were performed on an Agilent Technologies 1200 series liquid chromatograph connected to a 6460 Triple Quad mass spectrometer. The separation was achieved a Kinetex C18 2.6u 100Å (100×4.6 mm) column (Phenomenex). The mobile phase consisted of a mixture of 0.1% formic acid in acetonitrile (v/v) and 0.1% formic acid in water (v/v) was delivered under the following flow rate conditions: 0 min – 0.5 mL/min, 1 min – 0.5 mL/min, 3.5 min – 0.8 mL/min, 10 min – 0.8 mL/min, 10.5 min – 0.5 mL/min, 16 min – 0.5 mL/min, and the following mobile phase gradient conditions (shown in relation to acetonitrile content): 0 min – 40%, 1 min – 40%, 3.5 min – 60%, 4.5 min – 90%, 10 min – 90%, 10.5 min – 40%, 16 min – 40%. Dynamic multiple reaction monitoring (dMRM) with positive ion detection was applied (retention time window was set at 1 min). The total number of transitions monitored was 199, and the total analytical run time was 16 min.

Despite differences in chemical structures, the method allowed the simultaneous detection and identification of 66 SC from different groups (naphtoylindoles, phenylacetylindoles, naphtoylindazoles, naphtoylpyrroles, dibenzopyranes and other). The application of the gradient flow rate and gradient mobile phase conditions made that all of the compounds were well differentiated by their retention times and/or transitions. The retention times of compounds were from 2.53 to 9.15 min. Prepared blood calibration curves (number of replicates for each level, n = 3) were linear in the range of from 0.1-5 to 100 ng/mL with correlation r in the range of 0.9958-0.9993. The limits of detection (LODs) established for 49 compounds (for the signal-to-noise ratio equalling 3 (S/N=3) for the transition with the lowest intensity) were in the range 0.001-0.48 ng/mL making this assay suitable for the analysis of biological material

We developed a sensitive LC-MS/MS method for simultaneous identification of 66 SC in blood. The developed procedure allows performing rapid screening analysis and requires only 0.2 mL of blood. The procedure can be easily expanded for more substances. Such

methods are needed for forensic and clinical laboratories due to the ever-increasing spectrum of new SC.

Keywords: LC-MS/MS, synthetic cannabinoids, blood screening analysis

POSTER SESSION II

THURSDAY, MAY 25th, 2017

CHAIRPERSONS:

Kamilla Acs and Biljana Otašević

1.

Chromatographic determination of phenolic compounds in comercial samples of *Cistus incanus* L

Magdalena Knaś[1], Dariusz Szeremeta[1], Alicja Król[1], Karolina Męcik[1], Ewa Długosz[2], Paweł Olczyk[2], Teresa Kowalska[1], Mieczysław Sajewicz[1]

[1] *Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice, Poland*

[2] *School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Community Pharmacy, Medical University of Silesia in Katowice, Sosnowiec, Poland*

Phytotherapy is becoming very popular and increasingly common [1] and among the herbs, *Cistus* species has gained great popularity because of a wide spectrum of activity (e.g. antibacterial, antioxidant, antiviral and antifungal activity) [2,3]. Among the chemical compounds which determine the pharmacological properties of *Cistus incanus L.*, polyphenol compounds are the most significant.

The aim of this study is comparison of the phenolic fraction of tested comercial samples within one species of the herb originating from different manufacturers. Samples of each plant were macerated and subjected to exhaustive extraction in the Soxhlet apparatus. The herb extracts prepared in that way were subjected to a multistage extraction allowing for the isolation of individual fractions of phenolic compounds, including flavonoid aglycones, free phenolic acids and flavonoid glycosides. Each fraction was then analyzed by means of TLC chromatography.

Tested in our research commercially available samples showed significant differences in the composition of the phenolic fraction. The obvious conclusion is that the composition of herbally based preparations can be quite variable. Various samples of *Cistus incanus* L. have a different polyphenol profile which may involve their different health benefits. Thus, proper standardization the chemial composition and quality control of raw materials and the herbal products should be carried out.

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2.

Development and validation of RP HPLC method for determination of cyanocobalamin and phenole in pharmaceutical dosage form

Ivković Branka, Brborić Jasmina, Čudina Olivera

Department of Pharmaceutical Chemistry, University of Belgrade – Faculty of Pharmacy, 11000 Belgrade, Serbia

In this paper, development and validation of RP HPLC method for determination of cyanocobalamin (B12), water-soluble vitamin and antioxidant phenol is presented. The analysis was performed using column C8 250 mm x 4.6 mm, 5 μm particle size. Mobile phase was mixture of water and methanole (75 : 25 v/v). Column temperature was 30°C, mobile phase flow rate 1.5 ml/min and detection wavelength 361 nm for vitamin and 270 nm for phenol. The method was validated according to ICH Q2(R1) requirements. It was proved that this method is selective for determination of B12 and phenol. Linearity was confirmed with r values (r = 0,9994 for B12; r = 0,9992 for phenol). Accuracy was tested at three concentrations levels (80%, 100% and 120%) and confirmed by calculated recovery values (98.56 – 99.72% for B12; 99.07 – 101.39% for phenol). Precision was tested at two levels: intra-assay precision and intermediate precision. Calculated relative standard deviations were 0.92% and 0.18%, respectively. Small variations of mobile phase composition (organic solvent), column temperature and flow rate did not affect qualitative and quantitative system responses significantly, which proved method's robustness. Applicability of the method in routine was confirmed by analysis of commercialy available injecton for veterinary use.

Keywords: cyanocobalamin, phenol, RP-HPLC, ICH

3.

Analysis of retention behavior of selected antipsychotics and their impurities by thin layer chromatography

Slavica Oljačić, Anđela Arsić, Darija Obradović, Katarina Nikolić, Danica Agbaba

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Retention behavior and lipophilicity of aripiprazole and its nine impurities as well as ziprasidone and its five impurities have been examined by thin layer chromatography using RP-18 stationary phase and different mixtures of methanol, water and ammonia; and ethanol, water and ammonia as mobile phases. In both examined chromatographic systems linear relationships were established between retention parameters and the volume fraction of the methanol/ethanol in mobile phase ($r>0.948$ for methanol and $r>0.971$ for ethanol). Something higher correlation between determined hydrophobic parameters R_M^0 and calculated logP values was observed for methanol-water-ammonia/RP-18 ($r= 0.939$) compared to the ethanol-water-ammonia/RP-18 ($r=0.913$) chromatographic system. Also, retention parameters obtained when methanol was used as organic modifier showed higher values compared to the ethanol as organic modifier in the mobile phase. Experimentally obtained R_M^0 values and computed molecular parameters of the examined compounds were further used for the quantitative structure–retention relationship (QSRR) study in order to determine the most important properties governing retention. The QSRR modeling was performed with use of the partial least squares regression, and predictive performances of the developed QSRR models were tested by use of the cross-validation and external test set prediction. The obtained results revealed that apart from lipophilicity, topological descriptors and molecular weight of the tested compounds has the strongest influence on the retention behavior of examined antipsychotics and their impurities in the reverse phase thin layer chromatography. The predictive performance of the created QSRR model suggests its applicability for a reliable prediction of the retention behavior of the congeners.

4.

Characterization of unknown impurity of ziprasidone with new UPLC MS/MS method

Marija Čarapić[2], Katarina Nikolic[1], Bojan Marković[1], and Danica Agbaba*[1]

[1] *Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, PO Box 146, 11000 Belgrade, Serbia.*

E-Mail: danica@pharmacy.bg.ac.rs

[2] *Medicines and Medical Devices Agency of Serbia, Vojvode Stepe 458, 11000, Belgrade, Serbia*

Ziprasidone is chloro-indolone class of an atypical or second-generation antipsychotic drug effective in the treatment of positive, negative and affective symptoms of schizophrenia showing a low propensity for extrapyramidal symptoms side effects, cognitive deficits and almost no effect on weight, glucose, lipid and prolactin levels [1].
With previously developed HPLC method for analysis of ziprasidone and its five main impurities (I-V) by our research group [2] was detected and successfully separated one novel unknown impurity (t_R 11.270 min) in the test solution, after 24 hours stored at the room temperature [3]. Chemical characterization of the unknown impurity of ziprasidone is essential for defining genotoxic potential of the compound and consequently establishes the quality, safety and efficacy of the drug substance [4]. Thus, a new highly sensitive and rapid UHPLC-MS/MS method was developed for qualitative and quantitative assay of the ziprasidone and its six impurities in raw material and pharmaceuticals and at the same time with possibility for characterization of unknown impurities. All seven analytes were eluted within the 7 min run time. The method was used for the detection and characterization of a new impurity of an unknown structure. The best separation was obtained on the Acquity UPLC BEH C18 (50 mm x 2.1 mm x 1.7 μm) column with mobile phase consisted of 10 mM ammonium-formate aqueous solution, pH = 4,7 adjusted with formic acid and acetonitrile, with a timed gradient mode and the flow rate of 0.3 mL/min and at the column temperature of 30°C. UHPLC–MS/MS analyses were carried out on a UHPLC–MS/MS system coupled to a triple quad Mass spectrometer with a heated electrospray ionization (HESI) interface in a positive-ion, except for the impurity IV in a negative-ion mode. The MS/MS fragmentation conditions were optimized individually for each compound in order to obtain both specific fragments and high signal intensity. Both calibration and sample data were obtained by selective reaction monitoring acquisition. Collision-induced dissociation mass spectra of known compounds, ziprasidone and impurities I-V were obtained by flow injection analysis (FIA). All compounds were identified and their masses were measured. The mechanisms of fragmentation of ziprasidone, impurities I-V and unknown impurity were proposed. Thus, with regard to the peak at *m/z* 823, HESI-spectrum, and the proposed fragmentation mechanism, the most probable structure of unknown impurity was presented.

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5.

Metabolite profiling of MAO-A inhibitor – moclobemide 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

Moclobemide (4-chloro-N-(2-morpholin-4-ylethyl)benzamide) is a reversible and highly selective inhibitor of monoamine oxidase A (MAO-A). Moclobemide increases the content of serotonin, noradrenaline and dopamine in the brain and decreases the level of their metabolites. Due to its properties, moclobemide has a broad spectrum of antidepressant activity.

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.

Ultra high performance liquid chromatography (UHPLC) coupled with accurate quadrupole-time-of-flight (Q-TOF) mass spectrometry was used to the metabolite profiling of moclobemide after the incubation with HLMs. The separation was performed on Kinetex C18 (dp=1.7 μm) column and gradient elution with a mixture of acetonitrile and 0.1% solution of formic acid in water was used. Mass spectrometry was performed in auto MS/MS mode in order to collect MS as well as all the fragmentation spectra of moclobemide and its metabolites. After two hours of *in vitro* incubation of moclobemide with HLMs six metabolites were found and structurally characterized. N-oxide derivative of moclobemide was identified to be the main metabolite of the analyzed drug. UHPLC coupled 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.

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6.

Streamlined workflow to discover antibiotics

G. Morlock, M. Jamshidi-Aidji
Chair of Food Sciences, Justus Liebig University Giessen, Germany,
gertrud.morlock@uni-giessen.de

Hyphenated planar chromatography (HPTLC-UV/Vis/FLD-EDA-HRMS) proved to be well-suited as a high-throughput bioanalytical tool [1] that can contribute in the discovery of new antibiotics. The *Bacillus subtilis* bioassay was directly applied in the chromatogram to demonstrate the streamlined strategy from screening, characterization and identification to bioquantification of natural antibiotics in root extracts of *Salvia miltiorrhiza* [2]. The sample preparation was kept simple to let the sample extract as native as possible. The bioassay applied in the chromatogram (bioautogram) eased the direct correlation of separated zones and effective zones. An inverse densitometric measurement was employed for bioquantification. The importance of two unknown antibiotics was specified via bioequivalency calculation. As a reference, cryptotanshinone was used. The overall antimicrobial result obtained was referred to the activity of two synthetic antibiotics, ciprofloxacin and marbofloxacin. These calculations were performed in a single run on the same plate. This strategy can be installed in every analytical laboratory without much microbiological effort. Any type of bacteria can be selected, depending on the effect of interest, and applied on the plate. Especially the linkage to microbiological assays with pathogenic bacteria will be of high relevance, in combination with HRMS/NMR/IR and bioquantification. A planar chromatographic approach for streamlined structure elucidation was recently reported [3]. The demonstrated potential of this bioprofiling can contribute to discovery of new antibiotics from natural sources.

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7.

Validation of RP HPLC method for quantitative analysis of antiretrovirals drugs in human plasma samples

Nemanja Turković[1], Branka Ivković[2], Božana Dimitrijević[3], Ivan Kovačević[4], Radmila Novaković[5], Gordana Dragović Lukić[5], Zorica Vujić[2]

[1]Agency for Medicines and Medical Devices of Montenegro, Podgorica, Montenegro
[2]Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Belgrade, Serbia,
[3]Institute for Biomedical Statistics, School of Medicine, University of Belgrade, Belgrade, Serbia
[4]Country Medical Director, GlaxoSmithKlineExport Ltd, representative office, Serbia
[5]Institute of Pharmacology, Clinical Pharmacology and Toxicology
Medical faculty, University of Belgrade

Therapeutic drug monitoring (TDM) of antiretrovirals requires accurate and precise analysis of plasma drug concentrations. This work describes a simple and sensitive UV HPLC method for determination of the commonly used protease inhibitors such as darunavir, lopinavir, ritonavir, fosamprenavir, tenofovir a nucleoside reverse transcriptase inhibitor (NRTI), the non-NRTI such as efavirenz, and *Dolutegravir* an integrase inhibitor . The diazepame internal standard was added to plasma aliquots prior to protein precipitation with methanol and acetonitrile. This method employed highperformance liquid chromatography with PDA detector. All compounds eluted within 30-min run time. Calibration curves were validated, with correlation coefficients (r) higher than 0.998, for analysis of therapeutic concentrations reported in the literature. Inter- and intra-assay variations were <15%. Evaluation of accuracy shows a deviation <15% from target concentration at each quality control level. No significant matrix effect was observed for any of the antiretroviral studied. This new validated method fulfills all criteria for TDM of antiretrovirals and was successfully applied in routine TDM of antiretrovirals.

8.

Application of a polynomial modified Gaussian model and a half-width plot approach to describe chromatographic peaks and reveal column performance in chaotropic chromatography

Jelena Čolović, Andjelija Malenović

University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis, VojvodeStepe 450, Belgrade, Serbia

Theoretical understanding and accurate prediction of analytes chromatographic behavior is very important for the development of an efficient separation method. We have proposed the three−step procedure of polynomial modified Gaussian model (PMG) and successfully used it to predict amlodipine and its impurity A retention behavior in chaotropic chromatography. In this study, we extended the applicability of three−step PMG procedure and tested its capability to relate the shifts in retention behavior of risperidone and its three impurities with the change of quantitative and qualitative parameters in chaotropic chromatographic systems. The experimental plan was defined by D−optimal experimental design due to its capability to assess both quantitative and qualitative parameters. The studied quantitative parameters were: acetonitrile content in the mobile phase (20 %, 30 %), the pH of the aqueous phase (3, 5) and the content of chaotropic agents in the aqueous phase (10 mM, 100 mM); while the qualitative parameters were: type of chaotropic agent ($NaClO_4$, NaTFA) and column type (Zorbax Extend, Zorbax Eclipse). Firstly, the appropriate models for retention factors of peak beginning (k_B), peak apex (k_A), peak ending (k_E), as well as for peak heights (H_0) were defined. Subsequently, indirect modeling of the following parameters: peak width at 10% of peak height ($W_{0.1}$), individual values of left half−width (A) and right half−width (B), number of theoretical plates (N) and tailing factor (Tf) was performed. Afterwards, the investigated experimental domain was divided by discretization to acquire a grid of appropriate density. On the basis of the predicted results for Tf and N and the defined criteria for the furthersimulation, the optimal region for solutes' separation was selected for every combination of qualitative parameters. The appropriate agreement between the predicted and experimental values verified the ability of three−step PMG procedure to successfully simulate the influence of quantitative and qualitative parameters on the solutes retention behavior in chaotropic chromatography.

Apart from unacceptably low retention, protonated form of basic analytes in reversed−phase HPLC (RP−HPLC) elute as highly deteriorated peaks due to secondary interactions with free silanol groups of the stationary phase. A half-with plots approach represents valuable and simple tool for predicting peak shape, but also column performance and its kinetics. Therein, this approach was used in the order to characterize performance of stationary phases with different end-capping in pH range close to pKa of silanol groups. The half−width plots were constructed using indirectly modeled values of left half−width (A) and right half−width (B). According to the peak broadening rate (r_{PB}), Zorbax Eclipse column, with tremethylsilyl end-capping, expressed better characteristics than Zorbax Extend column where bidentate bonding was used to block free silanol groups.

9.

Robustness testing of chaptropic chromatpgraphy method for the determination of olanzapin and its two impurities

Milena Rmandić, Jelena Čolović, Andjelija Malenović

University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis, VojvodeStepe 450, Belgrade, Serbia

According to ICH Q2 robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal application. The aim of robustness testing is the identification of the factors with significant influence on the metod qualitative or quantitative performances. In this study, robustness testing of a chaotropic chromatographic method for the determination of olanzapine and its two impurities (impurity B and C) is performed. Plackett–Burman design was applied to discern the significant/influential factors. The factors to be investigated in a robustness testing are selected based on the conclusions from method development and optimization. Six quantitative factors (acetonitrile content in the mobile phase, sodium perchlorate concentration in water phase, pH of the water phase, column temperature, flow rate, detection wavelength), two qualitative factors (mode of mixing mobile phase constituents and stationary phase type), and three dummy factors were included in experimental plan. Peak areas of olanzapine and its impurities were selected as quantitative responses (*area O, area B* and *area C*).

The traditional approach of examining the results obtained by Plackett–Burman design assumed that the factor interactions do not affect the model significantly, so they can be neglected. If the model defined only with main factors has unsatisfactory statistical parameters, it is completely useless and a reevaluation of the model, including analysis of interactions contribution, should be carried out. In order to improve the accuracy and reliability of our initial models *demasking large dummy effects* (DDE) approach was used. In the first step the factor effect estimates were ranked and the main and dummy factors with the greatest effects were selected. Further on, the impact of every factor interaction was evaluated with the aid of alias matrix and used for the identification of important factor interactions. New models were defined comprising main factors with greatest effect and identified important interactions. These models showed significant improvement of statistical parameters (coefficient of determination, R^2 and adjusted coefficient of determination, *adj.* R^2) compared to initially proposed models with low and slightly low determination coefficient. The model improvement is achieved by adding three (*area B* and *area C*) and four interaction terms (*area O*) which makes model simple for real interpretation.

10.

Influence of selected mobile phase properties on the TLC retention behavior of ziprasidone and its impurities

Darija Obradović, Slavica Oljačić, Katarina Nikolić, and Danica Agbaba

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Properties of solvents used for chromatography significantly influence retention behavior and separation of the analytes. Selection of an appropriate mobile phase mixture is based mainly on interactions of solvents with the analytes and stationary phase. In this study influence of nine different solvents alone or in a mixture, a total of 19 mobile phases, on the retention behaviour of ziprasidone and its five impurities was examined by normal phase thin-layer chromatography in order to find the best mobile phase composition for separation of ziprasidone and its five impurities. Migration distances (MD) of the examined compounds obtained under the examined chromatographic conditions were correlated with calculated mobile phase properties, such as Snyder polarity and Hansen solubility. Both, linear and polynomial relationships were evaluated and equations with the best statistical parameters were selected. High correlation coefficients ($r > 0.706$) and satisfactory statistical parameters were obtained for mathematical relationships between the Snyder polarity or Hansen solubility parameters of mobile phases on the one hand and experimentally obtained migration distances of impurities I, II, V, and ziprasidone on the other. None of the mobile phase properties can be correlated with the retention behavior of impurities III and IV, but their retention behavior can be reliable predicted from the respective differences between the MD values, ΔMD(I–III) and ΔMD(IV-V). The obtained results indicate that for the selected polarity of mobile phase, migration distance of the impurity can be easily calculated.

11.

TLC-bioautography as an appropriate technique for screening anti-*haemophilus* activity of essential oils

Viktória Lilla Balázs[1], Béla Kocsis[2], Judit Krisch[3], Györgyi Horváth[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Pécs, Hungary*
[2]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Hungary*
[3]*Department of Food Engineering, Faculty of Engineering, University of Szeged, Hungary*

Essential oils (EOs) have been widely used for antimicrobial, medicinal and cosmetic purposes. EOs and their components are becoming increasingly popular as naturally occurring antimicrobial agents, however, the reliability of the common antimicrobial assays used for EOs is questionable. The aim of this study was the evaluation of antimicrobial properties of cinnamon (*Cinnamomum verum* J. Presl.), clove (*Syzygium aromaticum* (L.) Merr. and Perry), peppermint (*Mentha x piperita* L.), thyme (*Thymus vulgaris* L.) EOs and their main components against the Gram-negative bacteria, *Haemophilus influenzae* (DSM 4690) and *H. parainfluenzae* (DSM 8978).

The chemical composition of the EOs was measured with gas chromatography – mass spectrometry (GC-MS). Thin-layer chromatography – direct bioautography (TLC-DB) was used for the detection of the antibacterial activity of EOs, which is an appropriate method for investigation of complex extracts. EOs (100 µL) were diluted in 500 µL of absolute ethanol. From this solution, 0.5 µL and 1 µL were applied to the TLC plates (aluminium foil-backed silica gel TLC plates, Merck, Germany). An aqueous solution of MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma-Aldrich Ltd.) was used for the visualization of inhibition zones (expressed in the diameter, mm). Standards of eugenol, cinnamaldehyde, menthol and thymol (Sigma-Aldrich Ltd.) were also involved in TLC-DB experiment.

Both bacteria were the most sensitive to cinnamon EOs (19,5 mm) and cinnamaldehyde (23 mm). The EOs of thyme and clove were also effective with 11 mm and 12,5 mm diameters of inhibition zones, respectively. Peppermint oil showed weaker activity (8 mm). To the best of our knowledge, we performed TLC-DB with *Haemophilus* species firstly.

12.

Comparative analysis between chromatographically and computationally estimated lipophilicity descriptors of synthetic rhamnolipids

Jovana Krmar*, Biljana Otašević, Ana Protić, Jelena Golubović, Mira Zečević, Nevena Maljurić

Department of Drug Analysis, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia
E-mail of corresponding author: jovanak@pharmacy.bg.ac.rs

Rhamnolipids are glycolipids of biological origin with an amphiphilic character. Mainly owing to their significant surface-active property, rhamnolipids have attracted much attention in recent years and, therefore, many new applications of these biomolecules have been suggested. Very intriguing therapeutic potential has been recognized in the light of the fact that rhamanolipds can inhibit biofilm formation. Bacterial biofilms are surface-adherent, multicellular communities which show increased antibiotic resistance and tolerance compared to free-living planktonic forms. Therefore, in the context of drug discovery which is, at target identification phase, consisted of high-throughput screening of numerous rationally proposed compounds, rhamnolipids manifestly represent good starting structures for design pathway of antibiofilm drugs. Aiming to deliberate mentioned concept, five rhaminolipids have been synthesized and characterized.

Since it has been noticed in the past that one of the main reasons for high attrition rates for compounds entering clinical trials is their poor pharmacokinetics and bioavailability, drug development in its early phases and parallel with structure−activity relationship (SAR) studies includes knowledge of absorption, distribution, metabolism, excretion, and toxicity (ADMET) of a drug candidate. Within current strategy, lipophilicity, log*P* has been distinguished as a key property that directs clinical success of drug candidate. The importance of this physico-chemical attribute is a result of its ability to determine solubility of a compound, compound's passive diffusion through biological membranes and the affinity for the target, as well as, how long compound will remain active in the body. Hence, determination of lipophilicity represents an imperative and noteworthy task in terms of prediction of the biological activity of investigated compounds.

From the analytical point of view, widely accepted reference system for measuring log*P* values is 1-octanol–water system. However, some significant disadvantages which have been noticed so far and which are connected to this traditional shake-flask method, implied strong need for finding simpler, yet more accurate experimental method for the determination of lipophilicity. As a consequence, measuring lipophilicity by means of chromatographic and computational techniques lately has gained popularity and significant base of new lipophilicity parameters has been generated.

The aim of the present study was to determine measures of lipophilicity using chromatographic techniques and to compare derived indices with computationally estimated lipophilicity scales. In order to find the most appropriate experimental method or computational approach for the determination of lipophilicity of proposed substances, comparative analysis was performed using novel ranking method based on the sum of ranking differences, SRD that had been developed by *Hébergerb K.*

Finally, based on the obtained results, a good perspective for extension of research in area of modeling biological activity of tested rhamnolipids (QSAR) regarding their lipophilicity is provided. Consideration of rhamnolipids of the most promising pharmacokinetic profile among the studied series, would be an ultimate goal related to further development of antibiofilm drug.

13.

Lipophilicity of some quinothiadiazine derivatives

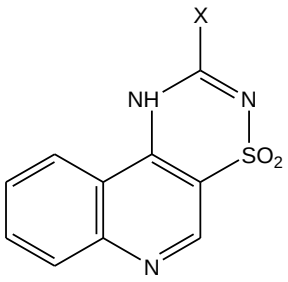
M.J. Maślankiewicz[1], E. Chrobak[2], D. Pentak[1], D. Kwapulińska[1]

[1]*Institute of Chemistry, University of Silesia, Katowice, Poland*

[2]*Department of Organic Chemistry, Medical School of Silesia, Sosnowiec, Poland*

Compounds containing azine moiety are of interest due to their biological activity. Several of them are potential antitumor agents.

Lipophilicity is an important factor in determinating the biological activity of drugs. RP TLC is a very useful method for determination of lipophilicity. We used this method in our study of selected quinothiadiazines:



X = Me, OMe, $N(Me)_2$, NH(n-Bu)

and its N-methyl derivatives.

TLC was performed on silica gel RP-18 F_{254} plates (Merck #115389), activated by heating at 100 °C for 1 h. Mixtures of methanol-water solutions were used as mobile phases. Relationship between R_M values obtained from chromatography and partition coefficients is described by the equation $R_M = a \log P + b$, where a and b are constants for a particular system and P is the partition coefficient.

Coefficients a and b were found in experiments for compounds with known log P values. Log P values obtained for the investigated compounds were correlated with values calculated by several theoretical methods. Lipophilicity of investigated quinotiadiazines was compared with lipophilicity of antitumor agents: irinotecan and hesperidin.

14.

Determination of 1,2-dichloroethane as relevant impurity of ethephon using HS-GC-MS technique

M. Płonka[1], M. Miszczyk[1], D. Kronenbach-Dylong[1], P. Marczewska[2], M. Sajewicz[2]

[1]Pesticide Quality Testing Laboratory, Institute of Plant Protection National Research Institute Sośnicowice Branch
[2] Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia

Plant protection products (PPPs) placed on the market should have sufficient quality, i.e. they should meet the technical requirements established for them in the process of their registration. It is especially important that the permitted concentrations of so-called relevant impurities are not exceeded. According to SANCO/3030/99 rev.4 document, relevant impurities are defined as "impurities of toxicological and/or ecotoxicological or environmental concern which are known, or can be expected, to occur in the active substance as manufactured."

Recent years a significant increase in the number of plant protection products marketed in Poland containing ethephon as the active substance was observed. According to the FAO specification, 1,2-dichloroethane is a relevant impurity of ethephon. The maximum permissible concentration of 1,2-dichloroethane in the preparation should not exceed 0.5 g/kg of ethephon, i.e. 0.05% The aim of this work was to develop a method for determination of the relevant impurity of ethephon in plant protection products in form water soluble concentrate (SL) using surface-to-surface analysis combined with mass spectrometry (HS-GC-MS).

15.

Office Chromatography - do it yourself!

G. Morlock, D. Fichou

Chair of Food Sciences, Justus Liebig University Giessen, Germany,
gertrud.morlock@uni-giessen.de

The Office Chromatography concept combines all relevant steps for online miniaturized planar chromatography by a single device [1, 2]. 3D printing of silica gel layers was recently demonstrated to be integrable into this concept [3]. This success outlined the potential of a 3D printing environment in planar chromatography and opened new avenues and new perspectives for tailor-made plates. Inspired by the do-it-yourself maker society, the 3D printing of thin silica gel layers was realized using open-source packages to encourage re-use and improvements and to stimulate the users to contribute to this emerging technology. All modifications of hard- and software for 3D-print of planar separation media were released open-source. A self-designed slurry doser replaced the plastic extruder of an open-source self-mounted Prusa i3 printer. Investment costs for the modified hardware were 630 Euro. After investigation of the optimal parameters for layer print, planar chromatographic separations were successfully demonstrated on these printed layers. Printing a 0.2-mm layer on a 10 × 10 cm format took less than 5 min, at running costs less than 0.25 Euro. Printed plane layers were compared with printed channeled layers. Therefore, 40 channels were printed on a 10 × 10 cm format for the separation of 40 samples in parallel, at running costs below 0.04 Euro. The printing process of such a channeled plate took only 2 min. New perspectives for tailor-made plates were opened with regard to layer materials, their combinations, gradient plates, different layer shapes and patterns. Streamlined open-source-based software for image evaluation of planar chromatograms, termed rTLC, was recently developed [4]. The integration of printing of sample solutions and mobile phase is in progress. Its combination with mass spectrometry (MS) [5, 6] and bioassays [7-10] in the near future will proof its potential for high-throughput microscale effect-directed analyses (EDA). Office Chromatography might be the next important tool in the analytical toolbox of experts!

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