CECE2019

September 24-26th 2019

Gdańsk, Poland

16th International Interdisciplinary Meeting on Bioanalysis

www.ce-ce2019.pl

Dear Colleagues and Friends!

With great pleasure we are pleased to welcome you at the

16th International Interdisciplinary Meeting on Bioanalysis (CECE'2019)

in Gdańsk, Poland on September 24-26th, 2019

The CECE conferences are organized yearly since 2004 (in Brno, Pecs or Veszprem). At the beginning, the topics of CECE meetings covered issues related to capillary electrophoresis (CE); nowadays they cover the most meaningful topics related to bioanalysis and application of all separation techniques with the emphasize on CE.

In 2019, the CECE conference takes place in Gdańsk, Poland in the Hotel Mercure Gdańsk Stare Miasto. We are sure that the Conference will provide an unique place to meet scientists from variable fields, which could exchange ideas and experience in various analytical expertise. We expect participants not only from Czech Republic, Slovakia, Hungary and Poland, but also from other parts of Europe, South Korea and United States of America.

The detailed topics of the conference is going to cover:

- *sample preparation techniques,*
- *separation techniques applied to pharmaceutical and biomedical researches,*
- *improvements in the equipment applied in analytical chemistry,*
- *miniaturization in analytical techniques,*
- *chemometrics and bioinformatic tools applied in bioanalysis.*

Prof. Dr. Tomasz Bączek

Chairman
of the Scientific and Organizing Committees

International Scientific Committee

Prof. Tomasz Bączek, Chairman of the International Scientific Committee, Medical University of Gdańsk, Gdańsk, Poland

Prof. Bogusław Buszewski, Nicolaus Copernicus University in Toruń, Toruń, Poland

Prof. Doo Soo Chung, Seoul National University, Seoul, Korea

Prof. Frantisek Foret, Institute of Analytical Chemistry, Academy of Sciences, Brno, Czech Republic

Prof. Andras Guttman, University of Debrecen / Research Centre for Molecular Medicine, Debrecen, Hungary

Prof. Hideaki Hisamoto, Osaka Prefecture University, Osaka, Japan

Prof. Milan Hutta, Comenius University in Bratislava, Bratislava, Slovakia

Prof. Vaclav Kasicka, Institute of Organic Chemistry and Biochemistry ASCR, v.v.i., Prague, Czech Republic

Prof. Emmanuelle Lipka, University of Lille, Lille, France

Prof. Michał Markuszewski, Medical University of Gdańsk, Gdańsk, Poland

Prof. Vladimir Patoprsty, Slovak Academy of Sciences, Bratislava, Slovakia

Prof. Cari Sanger, Uppsala University, Uppsala, Sweden

Prof. Michał Szumski, Nicolaus Copernicus University in Toruń, Toruń, Poland

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Dr Szymon Ulenberg, Medical University of Gdańsk, Gdańsk, Poland

Natalia Kossakowska, PhD student, Medical University of Gdańsk, Gdańsk, Poland

Natalia Treder, PhD student, Medical University of Gdańsk, Gdańsk, Poland

Michał Pieckowski, PhD student, Medical University of Gdańsk, Gdańsk, Poland

Paweł Georgiew, Student, Medical University of Gdańsk, Gdańsk, Poland

Confirmed Plenar and Keynote Speakers

Prof. Moran Bercovici
is associate professor in the Faculty of Mechanical Engineering and a head of Technion Microfluidic Technologies Laboratory at the Technion - Israel Institute of Technology, Haifa, Israel. His research currently focuses on electrokinetics, thermocapillary flow, and fluid-structure interaction, with application to bioanalysis, separation, and configurable microfluidic devices.
Topic of the speech during CECE2019: Field-effect electroosmotic flow patterning as a mechanism for diffusion-based separation.

Prof. Doo Soo Chung
is a head of Department of Chemistry, Seoul National University, Korea. His main scientific interest focus on capillary electrophoresis and photophoresis.
Topic of the speech during CECE2019: Synergistic coupling of in-line single drop microextraction and on-line large volume sample stacking for capillary electrophoresis/mass spectrometry.

Dr. Frantisek Foret
is the Director of the Institute of Analytical Chemistry of The Academy of Sciences of the Czech Republic and head of the Department of Bioanalytical Instrumentation therein. He is also a Group Leader at CEITEC, Masaryk University in Brno. His main research interests include capillary separation techniques for bioanalysis, laser induced fluorescence detection, miniaturization and mass spectrometry coupling.
Topic of the speech during CECE2019: Epitachophoresis – new way for large sample volume concentration and separation.

Dr András Guttman

is MTA-Lendulet Professor of Translational Glycomics at the Research Institute for Biomolecular and Chemical Engineering at University of Pannonia, Veszprem, Hungary, also heading of the Horváth Csaba Memorial Laboratory of Bioseparіaton Sciences in University of Debrecen (Hungary), and leading the bioseparation application efforts at Sciex (Brea, CA, USA). His work is mainly focused on capillary electrophoresis, microfluidics and their hyphenation to mass spectrometry for glycomics and glycoproteomics analysis of samples of biomedical and biopharmaceutical interests. Dr Guttman has over 300 scientific publications, 35 book chapters, edited 4 textbooks and holds 23 patents. He is a member of the Hungarian Academy of Sciences, on the board of several international organizations, serves as editorial board member for a dozen scientific journals and has been recognized by numerous international awards.

Topic of the speech during CECE2019: Activation energy associated with the electromigration of proteins in capillary SDS gel electrophoresis.

Prof. Hideaki Hisamoto

is currently working at the Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, Japan. His scientific goal is to "Create new chemistry for the future" through the integration of chemical systems, optical chemical sensing, micro total analysis systems, functional dyes, molecular recognition, microreactors, capillary electrophoresis as well as capillary array-based analytical devices.

Topic of the speech during CECE2019: Some new functional materials and capillary array-based microanalytical devices for single step bioanalysis.

RNDr. Vaclav Kasicka, PhD

is a head of the Laboratory of Electromigration Methods at the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czechia. His research interests comprise theory, instrumentation and methodology of high performance capillary electromigration methods and their applications to separation, analysis and physico-chemical characterization of (bio)molecules. He is chairman of the Chromatography and Electrophoresis Group of the Czech Chemical Society and editor of the Journal of Separation Science.

Topic of the speech during CECE2019: Investigation of (bio)molecular interaction of affinity capillary electrophoresis.

Dr. Emmanuelle Lipka
holds a PhD degree in 2001, in analytical chemistry from the University of Lille, France. She is now an assistant professor at the analytical chemistry lab of the Faculty of Pharmaceutical Sciences (Lille). She has authored 70 journal articles related to chiral separations and 2 chapters of book. Her fields of expertise are separation sciences including High Performance Liquid Chromatography, Supercritical Fluid Chromatography and Capillary Electrophoresis applied to pharmaceutical analysis, for the discovery and development of chiral drugs.
Topic of the speech during CECE2019: Development, evaluation and comparison of capillary electrophoresis, high performance liquid chromatography and supercritical fluid chromatography methods for chiral separation.

Dr. Steven Lock
is a marketing and market development manager EMEAI at SCIEX with a demonstrated history of working in the biotechnology industry. Skilled in Good Laboratory Practice (GLP), Liquid Chromatography-Mass Spectrometry (LC-MS), Protein Chemistry, Spectroscopy, and Laboratory Automation. Strong business development professional with a BSc & PhD focused in Chemistry from the Swansea University, United Kingdom.
Topic of the speech during CECE2019: The application of the CE-MS in biopharma characterization and quantification.

Prof. Michał Markuszewski
is a Dean of Faculty of Pharmacy with the Subfaculty of Laboratory Medicine at Medical University of Gdansk, Poland as well as Professor at the Department of Biopharmacy and Pharmacodynamics, Medical University of Gdansk, Poland. His main research area focus on the optimization of sample preparation techniques and new bioanalytical methods in metabolomics studies.
Topic of the speech during CECE2019: Electromigration techniques in metabolomics studies.

Dr. Cari Sänger - van de Griend

is Associate Professor in the Department of Analytical Pharmaceutical Chemistry at the Uppsala University, Sweden and Adjunct Senior Lecturer at ACROSS School of Chemistry, Hobart, Tasmania, Australia. She is also the founder and managing director of Kantisto BV, a pharmaceutical analytical consultancy (see www.kantisto.nl).

Topic of the speech during CECE2019: Advances and challenges in developing reliable CE methods for biopharmaceuticals.

Prof. Michał Szumski

is associate professor in the Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University in Toruń, Poland. His main interests are connected to analytical techniques miniaturization, electromigration techniques and synthesis of stationary phases for HPLC.

Topic of the speech during CECE2019: Identification of natural dyes in historic objects by miniaturized separation techniques.

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Detailed Program

TUESDAY, 24TH SEPTEMBER, 2019	
9.00 - 10.30 (Group I) **11.00 - 12.30 (Group II)**	**WORKSHOPS** **Detection of a reduced monoclonal antibody (mAb) at low ng/ml concentration in biological samples by CESI-MS** (Venue: *Intercollegiate Faculty of Biotechnology UG & MUG, Abrahama 58 Str., 80-307 Gdańsk*)
9.00 - 12.00	**WORKSHOPS** **Introduction to R for bioanalysis** (Venue: *Hotel Mercure Gdańsk Stare Miasto, Jana Heweliusza 22 Str., 80-890 Gdańsk*)
12.00 - 13.30	**WORKSHOPS** **Sensitive and Selective LC-MS/MS tool for Bioanalytical Quantitation** (Venue: *Hotel Mercure Gdańsk Stare Miasto, Jana Heweliusza 22 Str., 80-890 Gdańsk*)
TUESDAY, 24TH SEPTEMBER, 2019 Venue: *Hotel Mercure Gdańsk Stare Miasto, Jana Heweliusza 22 Str., 80-890 Gdańsk*	
10.00 - 14.00	**Registration**
14.00 - 14.15	**Opening ceremony**
	OPENING SESSION Chairman: Tomasz Bączek - Medical University of Gdańsk, Gdańsk, Poland
14.15 - 14.45 **PL**	**Some new functional materials and capillary array-based microanalytical devices for single step bioanalysis** Hideaki Hisamoto - Osaka Prefecture University, Osaka, Japan
14.45 - 15.15 **PL**	**Advances and challenges in developing reliable CE methods for biopharmaceuticals** Cari Sänger - Uppsala University, Uppsala, Sweden and ACROSS School of Chemistry, Hobart, Tasmania, Australia
15.15 - 15.40 **KN**	**The application of CE-MS in biopharma characterization and quantitation** Stephen Lock – AB SCIEX, Warrington, United Kingdom
15.40 - 16.00	*Coffee break*
	SESSION No. 1 Chairman: András Guttman - University of Pannonia, Veszprem, Hungary
16.00 - 16.25 **KN**	**Epitachophoresis – new way for large sample volume concentration and separation** František Foret - Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic
16.25 - 16.40	**Microfluidic system for evaluation of insulin secretion from a three-dimensional pancreatic islet model** Zbigniew Brzózka - Warsaw University of Technology, Warsaw, Poland

16.40 - 16.55	**Liquid chromatography – triple quadrupole mass spectrometry for top-down quantitative analysis of low abundance intact proteins from biological samples** Kevin Schug - University of Texas Arlington, Arlington, USA
16.55 - 17.15	*Coffee break*
	SESSION No. 2 Chairman: František Foret - Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic
17.15 - 17.40 **KN**	**Activation energy associated with the electromigration of proteins in capillary SDS gel electrophoresis** Prof. András Guttman - University of Pannonia, Veszprem, Hungary
17.40 - 17.55	**What colour is your method? A global evaluation of two capillary electrophoresis-based methods using the RGB additive colour model** Paweł Nowak - Jagiellonian University in Kraków, Kraków, Poland
17.55 - 18.10	**New analytical approaches for monitoring of inflammatory bowel diseases therapy** Katarina Marakova - Comenius University in Bratislava, Bratislava, Slovakia
18.10 - 18.25	**Capillary electrochromatography based novel in vitro tool for the assessment of blood-brain barrier permeability** Barbara Malawska - Jagiellonian University Medical College, Kraków, Poland
18.30	**Welcome reception**
	WEDNESDAY, 25TH SEPTEMBER, 2019 Venue: *Hotel Mercure Gdańsk Stare Miasto, Jana Heweliusza 22 Str., 80-890 Gdańsk*
	SESSION No. 3 Chairman: Vaclav Kašička - Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
9.00 - 9.25 **KN**	**The new Shimadzu LCMS QTOF-9030-uncompromised tool for bioanalysis** Marcin Gawryś – „SHIM-POL A.M. Borzymowski" E.Borzymowska-Reszka, A.Reszka Sp.j., Poland
9.25 - 9.40	**Study of amino acid profiles in patients undergoing stem cell transplantation – chemometric approach** Małgorzata Jaworska - National Medicines Institute, Warsaw, Poland
9.40 - 9.55	**Proteomic and metabolomic characterization of ovarian cancer** Jan Matysiak **-** Poznan University of Medical Sciences, Poznan, Poland
9.55 - 10.10	**Application of the UHPLC-MS/MS method for the interaction study of clopidogrel and statins in patients after coronary angiography/angioplasty** Marta Karaźniewicz-Łada - Poznań University of Medical Sciences, Poznań, Poland
10.10 - 10.25	**Complementary application of analytical methods for the development of medical implants** Thomas Eickner - University Medical Center Rostock, Rostock, Germany
10.25 - 10.45	*Coffee break*
	SESSION No. 4 Chairman: Barbara Malawska - Jagiellonian University Medical College, Kraków, Poland
10.45 - 11.10 **KN**	**Investigation of (bio)molecular interactions by affinity capillary electrophoresis** Prof. Vaclav Kašička - Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

11.10 - 11.25	**Capillary electrophoresis studies of neuroprotection activity of Tat peptides** Piotr Mucha - University of Gdańsk, Gdańsk, Poland
11.25 - 11.40	**The use of the HS-SPME/GC–MS technique to study interactions involving volatile organic compounds at the plant-microbe interface** Małgorzata Waleron - Medical University of Gdańsk, Gdańsk, Poland
11.40 - 11.55	**Bayesian multilevel modeling in chromatography** Paweł Wiczling - Medical University of Gdańsk, Gdańsk, Poland
11.55 - 12.15	*Coffee break*
	SESSION No. 5 Chairman: Jan Matysiak **-** Poznan University of Medical Sciences, Poznan, Poland
12.15 - 12.40 **KN**	**Identification of natural dyes in historic objects by miniaturized separation techniques** Michał Szumski - Nicolaus Copernicus University, Toruń, Poland
12.40 - 12.55	**Application of chromatographic methods to the assessment of food supplements quality and safety** Małgorzata Grembecka - Medical University of Gdańsk, Gdańsk, Poland
12.55 - 13.10	***Cistus incanus* L. commercial products as a good source of polyphenols in human diet** Agnieszka Viapiana - Medical University of Gdańsk, Gdańsk, Poland
13.10 - 14.10	*Lunch break*
14.10 - 15.30	**Poster session**
	YOUNG SCIENTIST SESSION
	ROOM No. 1 Chairman: Doo Soo Chung - Seoul National University, Seoul, Korea
15.30 - 15.55 **KN**	Vladimír Pätoprstý - Slovak Academy of Sciences, Bratislava, Slovakia
15.55 - 16.05	**Capillary electrophoresis based glycan analysis of tryptic digested serum proteins separated by hydrophilic interaction liquid chromatography** Balazs Reider - University of Pannonia, Pannonia, Hungary
16.05 - 16.15	**Environmental threats in preeclampsia - old observations and new conclusions** Katarzyna Gajewska - Medical University of Lublin, Lublin, Poland
16.15 - 16.25	**Multicapillary gel electrophoresis analysis of N-glycans** Beata Borza - University of Debrecen, Debrecen, Hungary and University of Pannonia, Veszprem, Hungary
16.25 - 16.35	**Analytical study of photodegradation and phototoxicity of two antihistaminic drugs, emedastine and ketotifen** Paweł Kozyra - Medical University of Lublin, Lublin, Poland and Medical University of Lublin Student´s Special Interest Groups, Lublin, Poland
	ROOM No. 2 Chairman: Cari Sänger - Uppsala University, Uppsala, Sweden and ACROSS School of Chemistry, Hobart, Tasmania, Australia
15.30 - 15.45	**Application of novel 3D printing techniques and materials in sample preparation** Szymon Ulenberg - Medical University of Gdańsk, Gdańsk, Poland

15.45 - 15.55	**Developing separation gels and methods for the analysis of biotherapeutic proteins via capillary-SDS gel electrophoresis coupled electrospray ionization mass spectrometry** Daniel Sarkozy - University of Debrecen, Debrecen, Hungary
15.55 - 16.05	**Glycomic analysis of human serum from lung cancer, COPD and their comorbidity patients by capillary electrophoresis** Brigitta Meszaros - University of Debrecen, Debrecen, Hungary
16.05 - 16.15	**Phenolic composition, antioxidant activity and acetylcholinesterase inhibitory of *Morus alba L.* commercial samples. A comparative study** Milena Polumackanycz - Medical University of Gdańsk, Gdańsk, Poland
16.15 - 16.25	**Drug delivery systems for medical applications** Małgorzata Borowska - Gdańsk University of Technology, Poland
16.25 - 16.35	**Ionic liquids as amplifiers of extraction efficiency of selected biogenic amines from urine samples before capillary electrophoresis separation** Natalia Kossakowska - Medical University of Gdańsk, Gdańsk, Poland
18.00 - 19.30	**Galleon Cruise to Westerplatte**
20.30 - 23.00	**Conference dinner**

THURSDAY, 26TH SEPTEMBER, 2019

Venue: *Hotel Mercure Gdańsk Stare Miasto, Jana Heweliusza 22 Str., 80-890 Gdańsk*

	SESSION No.6 Chairman: Emmanuelle Lipka - University of Lille, Lille, France
9.00 - 9.25 **KN**	**Electromigration techniques in metabolomics studies** Michał Markuszewski - Medical University of Gdańsk, Gdańsk, Poland
9.25 - 9.40	**A capillary electrophoresis assay for the determination of lactate in human vitreous humor for forensic applications** Anna Bertaso - University of Verona, Verona, Italy
9.40 - 9.55	**Human pharmaceuticals in the environment** Jolanta Kumirska - University of Gdansk, Gdańsk, Poland
9.55 - 10.10	**Saliva and new methods of determining the level of drugs in the body** Ewelina Dziurkowska - Medical University of Gdańsk, Gdańsk, Poland
10.10 - 10.30	*Coffee break*
	SESSION No. 7 Chairman: Michał Markuszewski - Medical University of Gdańsk, Gdańsk, Poland
10.30 - 10.55 **KN**	**Field-effect electroosmotic flow patterning as a mechanism for diffusion-based separation** Moran Bercovici - Israel Institute of Technology, Haifa, Israel
10.55 - 11.10	**A first point of care test for carbohydrate-deficient transferrin (CDT) based on fluorescence resonance energy transfer (FRET)** Giacomo Musile - University of Verona, Verona, Italy
11.10 - 11.25	**Quantitation and purity assessment of extracellular vesicles in isolates from *Pectobacterium carotovorum sp.* culturing media using capillary zone electrophoresis** Szymon Dziomba - Medical University of Gdańsk, Gdańsk, Poland
11.25 - 11.40	*Coffee break*

	CLOSING SESSION Chairman: Tomasz Bączek - Medical University of Gdańsk, Gdańsk, Poland
11.40 - 12.10 **PL**	**Development, evaluation and comparison of capillary electrophoresis, high performance liquid chromatography and supercritical fluid chromatography methods for chiral separation** Emmanuelle Lipka - University of Lille, Lille, France
12.10 - 12.40 **PL**	**Synergistic coupling of in-line single drop microextraction and on-line large volume sample stacking for capillary electrophoresis/mass spectrometry** Doo Soo Chung - Seoul National University, Seoul, Korea
12.40 - 13.00	**Closing Ceremony**
13.00	*Farewell reception*

PL – Plenary Lecture

KN – Keynote

Poster Presentations

PS-1	**Epitachophoresis – new tool for large volume concentration** *Ivona Voráčová, František Foret, Vladimíra Datinská, Jakub Novotný, Pantea Gheibi, Jan Berka, Yann Astier* Czech Academy of Sciences, Institute of Analytical Chemistry, Brno, Czech Republic
PS-2	**Simplify modification mapping at the intact protein level with on-line separation and analysis by CE-MS** *Stephen Lock, Marcin Moczulski* Market Development, Sciex, Warrington, UK
PS-3	**Isolation and fractionation of exopolysaccharide produced by cyanobacterium *Nostoc* sp.** *Iveta Uhliariková, Mária Matulová, Peter Capek, Vladislav Cepák, Jaromír Lukavský* Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Bratislava, Slovakia
PS-4	**Characterization of protein-peptide composition of royal jelly** *Eliza Matuszewska, Zenon J. Kokot, Jan Matysiak* Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland
PS-5	**Capillary electrophoresis and MALDI-TOF MS for rapid and reliable identification of viruses** *Jiří Šalplachta, Marie Horká, Pavel Karásek, Filip Růžička, Michal Roth* Institute of Analytical Chemistry of the CAS, Brno, Czech Republic
PS-6	**Biosensor for determination of cathepsin S based on surface plasmon resonance** *Łukasz Ołdak, Anna Sankiewicz, Zenon Łukaszewski, Ewa Gorodkiewicz* Department of Electrochemistry, University of Białystok, Białystok, Poland
PS-7	**Synthesis of new fluorescent labels to improve glycan analysis** *Richard Čmelík, Jan Křenková, František Foret* Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic
PS-8	**Optimization of pressurized hot water extraction for isolation of allergen proteins from almonds** *Lenka Burdejova, Dana Moravcova, Dana Strouhalova, Pavel Karasek, Michal Roth, Barbora Kudlackova* Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic
PS-9	**The application of SPRI biosensors for determination of fibronectin in natural samples.** *Anna Sankiewicz, Adam Hermanowicz, Julia Gajewska, Ewa Gorodkiewicz* Institute of Chemistry, University of Bialystok, Białystok, Poland
PS-10	**Comparison of oligosaccharide labeling employing various derivatization chemistries** *Jana Krenkova, Richard Cmelik, Jan Prikryl* Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic
PS-11	**Determination of organic acids in honeybee venom using mass spectrometry-based methodology** *Agnieszka Klupczynska, Magdalena Pawlak, Zenon J. Kokot, Jan Matysiak* Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland
PS-12	**Ammonium salts hijack N-glycan separation** *Eszter Anna Váradi, Apolka Domokos, Zsuzsanna Kovács, Márton Szigeti, András Guttman* Horváth Csaba Memorial Institute for Bioanalytical Research, Research Center for Molecular Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

PS-13 **SPE-MEKC method as a tool for simultaneous determination of metanephrines in urine samples**

Ilona Olędzka, Natalia Kossakowska, Małgorzata Przybyłek, Natalia Miękus, Alina Plenis, Piotr Kowalski, Małgorzata Krawczyk, Ewa Bień, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdansk, Poland

PS-14 **Could IgG N-glycome of mother be a potential biomarker for predisposition to obesity and diabetes in offspring?**

Anna Farkas, Andras Guttman, Lilla Siska-Szabó, Sandor G. Vari, Robert Gaspar
Horvath Csaba Laboratory of Bioseparation Sciences, Research Center for Molecular Medicine, Faculty of Medicine, Doctoral School of Molecular Medicine, University of Debrecen, Debrecen, Hungary

PS-15 **Bioanalysis of silicone and polyacrylate transdermal patches – adhesiveness and drug release**

Barbara Mikolaszek, Daria Butkiewicz, Małgorzata Sznitowska
Department of Pharmaceutical Technology, Medical University of Gdansk, Gdansk, Poland

PS-16 **Pre-treatment and elution of amino acids from dried blood spots for direct determination by capillary electrophoresis**

Lenka Ryšavá, Miloš Dvořák, Blanka Miková, Pavel Kubáň
Institute of Food Science and Biotechnology, Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic
Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

PS-17 **A field amplified sample injection (FASI) coupled with hydrophobic interaction electrokinetic chromatography (HIEKC) method for the signal enhancement of selected hydrophobic antibiotics**

Michał Pieckowski, Piotr Kowalski, Ilona Olędzka, Alina Plenis, Natalia Miękus, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-18 **Determination of ketamine by acetonitrle based sample stacking in coated capillaries**

Petr Tůma, Dušan Koval
Department of Hygiene, Praha, Third Faculty of Medicine, Czechia

PS-19 **An on-line preconcentration strategy for the electrophoretic determination of selected preservatives in pharmaceuticals**

Michał Pieckowski, Piotr Kowalski, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-20 **Method development for quantitative analysis of modified deoxynucleosides and nucleosides in biological matrices**

Małgorzata Patejko, Aleksandra Kwiecień, Wiktoria Struck-Lewicka, Danuta Siluk, Marcin Markuszewski, Marcin Matuszewski, Michał J. Markuszewski
Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gdańsk, Poland

PS-21 **A simple and efficient procedure for capillary blood spiking with basic and acidic drugs for subsequent dried blood spot analysis**

Miloš Dvořák, Lenka Ryšavá, Pavel Kubáň
Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

PS-22 **Development of extraction sorbent fabricated by 3D-printing using PETG-carbon nanotubes composite**

Paweł Georgiev, Mariusz Belka, Szymon Ulenberg, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-23 **A new method for epirubicin analysis using high-performance liquid chromatography with fluorescence detector in human plasma and urine and its application to real samples**

Natalia Treder, Olga Maliszewska, Ilona Olędzka, Piotr Kowalski, Natalia Miękus, Tomasz Bączek, Ewa Bień, Małgorzata Krawczyk, Elżbieta Adamkiewicz-Drożyńska, Alina Plenis
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-24 **Determination of polycyclic aromatic hydrocarbons (PAHs) in human breast milk using 3D-printed sorbent for extraction preceding GC-MS analysis.**

Monika Szczutkowska, Paweł Georgiev, Mariusz Belka, Weronika Hewelt-Belka, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-25 **ITP analysis of amine derivatives of adamantane**

Tereza Mudrochová, Přemysl Lubal, Marta Farková
Department of Chemistry, Masaryk University, Brno, Czech Republic

PS-26 **Advances in steroid analysis enabled by 3D-printed sorbents**

Lucyna Konieczna, Mariusz Belka, Szymon Ulenberg, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-27 **Assessment of amino acid levels in non-invasive and invasive samples obtained from bariatric patients using LC-MS/MS method**

Lucyna Konieczna, Anna Krawczyńska, Maria Skrzypkowska, Janusz Siebert, Magdalena Reiwer-Gostomska, Piotr Gutknecht, Łukasz Kaska, Justyna Bigda, Maria Proczko-Stepaniak, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-28 **Amino acids as non-invasive biomarkers in acute respiratory distress syndrome**

Marcin Muża, Lucyna Konieczna, Magdalena Wujtewicz, Radosław Owczuk, Tomasz Bączek
Student Scientific Circle, Department of Pharmaceutical Chemistry, Medical University of Gdansk, Gdańsk, Poland

PS-29 **Decreased level of vitamin D in obesity patients measured by the LC-MS/MS method**

Anna Krawczyńska, Lucyna Konieczna, Maria Skrzypkowska, Janusz Siebert, Magdalena Reiwer-Gostomska, Piotr Gutknecht, Łukasz Kaska, Justyna Bigda, Maria Proczko-Stepaniak, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-30 **The effect of omega-loop gastric bypass surgery on serum amino acids concentration**

Alicja Pakiet, Łukasz Haliński, Maciej Wilczyński, Łukasz Kaska, Monika Proczko-Stepaniak, Patrycja Jabłońska, Adriana Mika
Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

PS-31 **Optimization of analytical conditions for the analysis of selected immunosuppressants in serum samples**

Anna Roszkowska, Alina Plenis, Ilona Olędzka, Piotr Kowalski, Natalia Miękus, Natalia Treder, Tomasz Bączek
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PS-32 **A rapid and simple method for estimation of chemically-induced mitochondrial toxicity in *Chlamydomonas reinhardtii* cells**
Darya Harshkova, Elżbieta Zielińska, Anna Aksmann
Department of Plant Physiology and Biotechnology, University of Gdansk, Gdansk, Poland

PS-33 **The application of LC-FL methods for the quantification of selected anthracycline antibiotics in human plasma and urine samples**
Alina Plenis, Olga Maliszewska, Natalia Treder, Ilona Olędzka, Piotr Kowalski, Natalia Miękus, Ewa Bień, Małgorzata Krawczyk, Elżbieta Adamkiewicz-Drożyńska, Tomasz Bączek
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PS-34 **Postoperative changes in serum free fatty acids concentration after laparoscopic sleeve gastrectomy and omega loop gastric bypass**
Ivan Liakh, Lukasz Kaska, Adriana Mika, Tomasz Śledzinski
Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdańsk, Poland

PS-35 **Direct immersion-single drop microextraction coupled in-line with capillary electrophoresis for the analysis of ibuprofen, naproxen and ketoprofen**
Natalia Miękus, Nader Nciri, Wooyoung Kwon, Tomasz Bączek, and Doo Soo Chung
Medical University of Gdańsk, Department of Pharmaceutical Chemistry, Gdańsk, Poland
University of Gdańsk, Department of Animal and Human Physiology, Gdańsk, Poland

PS-36 **Analyte focusing by micelle collapse for liquid extraction surface analysis coupled with capillary electrophoresis of neural analytes on a solid surface**
Sunkyung Jeong, Farid Shakerian, Doo Soo Chung
Department of Chemistry, Seoul National University, Seoul, Korea

PS-37 **Microfluidic chip for analysis with photon-upconversion nanoparticles**
Jana Křivánková, Antonín Hlaváček, Jan Přikryl, František Foret
Department of Bioanalytical Instrumentation, Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

PS-38 **Development of trimethylsilyl acetate based coatings for bioapplications using plasma of RF capacitively coupled discharge**
Štěpánka Kelarová, Vojtěch Homola, Monika Stupavská, Roman Přibyl, Vilma Buršíková
Department of Physical Electronics, Masaryk University, Brno, Czech Republic

PS-39 **Ethanol sensor based on Eu(iii) ternary complex of DO3A ligand**
Filip Smrčka, Přemysl Lubal
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PS-40 **Free flow isotachophoresis DNA purification in channel from disposable non-woven fabric bed**
Filip Duša, Dana Moravcová, Karel Šlais
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PS-41 **Combined photometric detector utilizing light emitting diodes, 50 nL silica capillary cell, and CCD spectrometer**
Jozef Šesták, Vladislav Kahle
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PS-42 **Temperature effects in capillary sodium dodecyl sulphate gel electrophoresis**
Csenge Filep, Dániel Sárközy, András Guttman
Horváth Csaba Laboratory of Bioseparation Sciences, Research Center for Molecular Medicine, Faculty of Medicine, Doctoral School of Molecular Medicine, University of Debrecen, Debrecen, Hungary

PS-43 **Retention and selectivity of sulfobetaine-stationary phase in methanol- and isopropanol-rich mobile phases**
Dana Moravcová, Josef Planeta
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PS-44 **Buried microfluidic channel for segmented flow analysis**
Evgenia Basova, Zdenka Fohlerová, Tomáš Lednický, Imrich Gablech, Jan Pekárek, Pavel Podešva, Pavel Neužil
Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

PS-45 **Simultaneous detection of peach and apricot DNA by multiplex real-time PCR-HRM with intercalating dye**
Lenka Fialová, Denisa Romanovská, Ivana Márová
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PS-46 **Recent developments of the GUcal application**
Gabor Jarvas, Marton Szigeti, Matthew Campbell, Andras Guttman
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Horváth Csaba Memorial Institute of Bioanalytical Research, University of Debrecen, Debrecen, Hungary

PS-47 **The study of natural honeybee products using targeted metabolomics methods**
Szymon Plewa, Paweł Dereziński, Agnieszka Klupczyńska, Roch Grudzień, Anna Kulawik, Zenon J. Kokot, Jan Matysiak
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PS-48 **Influence of 14-methylpentadecanoic acid on the expression of lipid metabolism-related genes in liver hepatocellular carcinoma cells (HEPG2)**
Paulina Goździk, Tomasz Śledziński, Adriana Mika, Katarzyna Duzowska
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PS-49 **Comparison of the concentrations of metabolic profiles of pterine compounds in urine samples from patients with bladder, renal and prostate cancer**
Piotr Kośliński, Justyna Pawlak, Robert Pluskota, Marcin Gackowski, Katarzyna Mądra-Gackowska, Marcin Koba
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PS-50 **Understanding distribution of metabolomics data : identifying structure and analysis of bimodal metabolomics data**
Emilia Daghir-Wojtkowiak, Marta Kordalewska, Michał J. Markuszewski
Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gdańsk, Poland

PS-51 **Bayesian workflow in statistical analysis of isocratic chromatographic data**
Agnieszka Kamedulska, Łukasz Kubik, Paweł Wiczling
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PS-52 **Influence of sensor structure on selected analytical parameters in technique of surface plasmon resonance – review**
Falkowski Paweł, Gorodkiewicz Ewa
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PS-53 **Application of quantitative structure-activity relationships approach with the use of *ab initio* and semi-empirical modeling methods in analysis of selected antimicrobial sulfonamides**
Piotr Kawczak, Tomasz Bączek
Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

PS-54 **Metabolomics-based elucidation of chronic kidney disease**
Szymon Macioszek, Marta Kordalewska, Renata Wawrzyniak, Adriana Mika, Tomasz Śledziński, Michał Chmielewski, Michał J. Markuszewski
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PS-55 **DOE-based simplification of sample preparation procedure in untargeted GC-MS metabolomics**
Julia Jacyna, Marta Kordalewska, Joanna Raczak-Gutknecht, Michał J. Markuszewski
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PS-56 **Metabolomic studies in search for ethyl alcohol abuse biomarkers in blood and evaluation of the usefulness of the obtained results in forensic medicine**
Dawidowska Joanna, Krzyżanowska Marta, Struck-Lewicka Wiktoria, Jankowski Zbigniew, Kaliszan Michał, Markuszewski Michał J.
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PS-57 **Comperative study on disintegration time of orodispersible films containing incorporated particles**
Katarzyna Centkowska, Martyna Szadkowska, Maria Gąsior, Małgorzata Sznitowska
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PS-58 **Drug release profiles as a marker of changes in lipid microparticles caused by spray drying**
Eliza Wolska, Katarzyna Krzemińska, Maria Ferreira Monteiro, Małgorzata Sznitowska
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PS-59 ***In vitro* dissolution tests for enteric-coated minitablets administered in hydrogels or in capsules**
Maja Szczepańska, Nicola Scanu, Małgorzata Sznitowska
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PS-60 **Fluorescence microscopy in transdermal distribution of hesperidin**
Anna Hering, Justyna Stefanowicz-Hajduk, Krzysztof Cal, J. Renata Ochocka
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PS-61 **Simultanous sequential analysis of 210Po, 210Pb and U (234U, 238U) isotopes in calcium and magnesium supplements**
Dagmara Strumińska-Parulska, Aleksandra Moniakowska, Anna Dzierwanowska
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PS-62 **Slag obtained in the circulating fluidized bed combustion technology as an adsorbent for metal ions recovery**
Tomasz Kalak, Joanna Dudczak, Yu Tachibana, Ryszard Cierpiszewski
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PS-63 **What secrets hides cat's fur (Felis catus)?**
Jarosław Wieczorek, Marcin Kaczor, Mateusz Swoboda, Alicja Boryło
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PS-64 **Melliferous plants as a source of 210Po in honey from central and southern Poland**
Marcin Kaczor, Jarosław Wieczorek, Grzegorz Romańczyk, Alicja Boryło
Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

PS-65 **Acidic and enzymatic hair degradation for mercury speciation analysis**
Inga Petry-Podgórska, Michaela Migašová, Jan Kratzer
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PS-66 **The impact of atmospheric precipitation on the content of 210Po in selected herbs species**
Aleksandra Moniakowska, Dagmara Strumińska-Parulska
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PS-67 **Is cow milk good indicator of environment pollution?**
Alicja Boryło, Grzegorz Romańczyk, Jarosław Wieczorek, Marcin Kaczor, Bogdan Skwarzec
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PS-68 **Capillary electrophoresis as an alternative technique for determination of non-chromophoric polyphosphonates**
Anna Poliwoda, Piotr Wieczorek
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PS-69 **Hydrodistillation parameters affect phthalide content in apium graveolens essential oil**
Adam Kokotkiewicz, Urszula Marzec-Wróblewska, Anna Badura, Żaneta Tabaczyńska, Andżelika Lorenc, Adam Buciński, Maria Łuczkiewicz
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PS-70 **TLC of active compounds from selected populus leaves**
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PS-71 **The differential proteomics of *Salmonella enterica* ssp. *diarizonae* exposed to human serum**

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PS-72 **Plasma free amino acid profiling in leukemia patients by UHPLC-ESI-MS/MS method using dispersive liquid-liquid microextraction for sample preparation**

Anna Czajkowska, Paweł Pasikowski, Michał Surma
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PS-73 **Isolation and identification of genus *Fragaria* DNA in cosmetic products**

Denisa Romanovská, Lenka Fialová, Ivana Márová
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PS-74 **Comparison between plasma and urine metabolic profiles from prostate cancer patients**

Wiktoria Struck-Lewicka, Małgorzata Patejko, Renata Wawrzyniak, Marta Kordalewska, Danuta Siluk, Marcin Markuszewski, Marcin Matuszewski, Michał .J. Markuszewski
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PS-75 **The use of metabolomics in identifying metabolic alterations in patients with polycystic ovary syndrome**

Anna Rajska, Magdalena Buszewska-Forajta, Aleksandra Szybiak, Agnieszka Kowalewska, Dominik Rachoń, Michał J. Markuszewski
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PS-76 **Design, synthesis, radioligand binding studies and chiral separation of potential pyroglutamide-based P2RX7 receptor antagonists**

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PS-77 **Ultra-sensitive Quantification of Monoclonal antibodies and ADCs in Mouse Plasma using Trap-Elute MicroLC-MS/MS Method**

Alexandre Paccou, Lei Xiong, Ji Jiang, Remco van Soest
SCIEX France

Abstracts of Oral Presentations

PL-1 - SOME NEW FUNCTIONAL MATERIALS AND CAPILLARY ARRAY-BASED MICROANALYTICAL DEVICES FOR SINGLE STEP BIOANALYSIS

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ABSTRACT

We have been investigated to develop simple and highly-sensitive microanalytical devices based on the combination of new functional materials and capillary-array for use in bioanalysis. Concerning the capillary-array, capillary-assembled microchip prepared by embedding functionalized capillaries into PDMS channel array [***Anal.Chem.*** 2004, 76, 3222., ***ibid.*** 2007, 79, 908.], and combinable PDMS capillary sensor array prepared by combining two independent PDMS structures immobilizing two different reagents [***Lab Chip*** 2012, 12, 204. ***ibid.*** 2012, 12, 1522.] were developed to demonstrate single step and multiplexed bioanalysis. In all the cases, designing single step chemistries inside capillary or channel played important roles. Here, we focused on the following two topics related with our concept.

1: Reagent-release capillary (RRC) and reagent-release hydrogel (RRG) for use in highly-sensitive enzyme activity assay based on double sweeping [1,2]

2: Graphene/PEG hybrids (GPH) for use in fast and single step immunoassay [3,4]

REFERENCES

[1] R. Sanuki, K.Sueyoshi, T. Endo, H. Hisamoto, Double sweeping: Highly effective sample preconcentration using cationic and anionic micelles and its application to a multiple enzyme activity assay, *Anal. Chem.*, *89* (2017) 6505-6512.

[2] Y. Fujii, K. Sueyoshi, T. Endo, H. Hisamoto, A Simple and Easy-to-Use Capillary Isoelectric Focusing Technique Using Reagent-Release Hydrogels, *Chromatography*, *38* (2017) 79-83.

[3] A. Shirai, K. Nakashima, K. Sueyoshi, T. Endo, H. Hisamoto, Development of a single-step immunoassay microdevice based on a graphene oxide-containing hydrogel possessing fluorescence quenching and size separation functions, *Analyst, 142* (2017) 472-477.

[4] A. Shirai, K. Sueyoshi, T. Endo, H. Hisamoto, Graphene/polyethylene glycol hybrids for single-step immunoassay microdevice, *FlatChem, 13* (2019) 34-39.

PL-2 - ADVANCES AND CHALLENGES IN DEVELOPING RELIABLE CE METHODS FOR BIOPHARMACEUTICALS

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ABSTRACT

The use of CE in the biotech industry is still increasing. Currently almost every Biologics License Application (BLA) contains at least one CE method. Several of these are kit-based chemistries, but an increasing amount of applications is developed within industry. In order to develop fit-for-purpose methods, fundamental understanding is required on both the technique as well as on the requirements on pharmaceutical analytical methods. To support the process, Analytical Quality by Design (AQbD) is applied. In this lecture we will discuss these requirements and some examples of AQbD developed applications that made a significant impact for the production and release of new biopharmaceutical entities.

PL-3 - DEVELOPMENT, EVALUATION AND COMPARISON OF CAPILLARY ELECTROPHORESIS, HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND SUPERCRITICAL FLUID CHROMATOGRAPHY METHODS FOR CHIRAL SEPARATION

Emmanuelle Lipka

The P2X receptors are seven-transmembrane domain G protein-coupled receptors and the 7 subtypes of P2X receptors identified in humans, and named P2X1 to P2X7, are channel receptors whose endogenous ligand is ATP. New antagonists of the P2X7 receptor were developed, since this purinergic receptor was highlighted to be involved in many diseases such as different types of pain, cancer, ischemia, neurodegenerative diseases (including Parkinson's and Alzheimer's diseases) characterized by inflammatory processes.

With the aim of evaluate the impact of chirality on the pharmacological activity of a new P2X7R antagonist, the two enantiomers were separated thanks to a semi-preparative chromatographic method.

Firstly, a capillary electrophoresis method was developed to assess the enantiomeric purity. A dual cyclodextrins system constituted of a SBE-β-CD and a MM-β-CD mixture was found to enhance the signal-to-noise ratio, thus the limits of detection and quantification.

Before carrying out the pharmacological evaluation of each enantiomer, two complementary methodologies, *e.g.* high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC), using an amylose tris (3,5-dimethylphenylcarbamate) chiral stationary phase with mobile phase consisted of carbon dioxide–ethanol (80:20, v/v) for SFC and consisted of *n*-hexane-ethanol (80 :20, v/v) for HPLC, led to the successful separation of the enantiomers in short run time and with good resolution.

Best limit of detection and limit of quantification were obtained with the liquid chromatography method. They were found equal to 0.34 µM and 1.14 µM respectively, for peak 1 and were equal to 0.40 µM and 1.32 µM respectively, for peak 2 at λ = 210 nm. No trace of the other enantiomer was found in the isolated one.

Biological activities of individual enantiomers were then evaluated and revealed no cytotoxicity against cell lines and a significant difference in terms of their IC50 values with respect to the investigated racemate (6.43 µM): 3.49 µM for the (R)-enantiomer and $>10^{-4}$ µM for the (S)-enantiomer, showing that, this antagonist activity is stereospecific.

PL-4 - SYNERGISTIC COUPLING OF IN-LINE SINGLE DROP MICROEXTRACTION AND ON-LINE LARGE VOLUME SAMPLE STACKING FOR CAPILLARY ELECTROPHORESIS/MASS SPECTROMETRY

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ABSTRACT

Single drop microextraction (SDME) and large volume sample stacking using an electroosmotic flow pump (LVSEP) were coupled with capillary electrophoresis/mass spectrometry (CE/MS) for sample clean up and preconcentration. Without filtration or centrifugation of a soil sample containing debris, SDME using a pentanol acceptor drop was directly applied to the sample. After SDME, a large volume of the enriched pentanol extract was injected and further concentrated by LVSEP. For the drop formation in SDME and the sample matrix removal in LVSEP, a run buffer vial was temporarily placed to the electrospray tip, without any physical modification of the CE/MS interface. This method enabled the double preconcentration by SDME and LVSEP, achieving 600~1300-fold enrichments of anionic analytes including pesticide and herbicide compounds to provide limits of detection in the range of 0.4~0.8 ppb in soil.

KN-1 - THE APPLICATION OF CE-MS IN BIOPHARMA CHARACTERIZATION AND QUANTITATION

Stephen Lock

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ABSTRACT

KN-2 - EPITACHOPHORESIS - NEW WAY FOR LARGE SAMPLE VOLUME CONCENTRATION AND SEPARATION

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ABSTRACT

Many clinical and diagnostic applications require high quality nucleic acids for downstream analytical methods. The most common nucleic acids isolation techniques are based on extraction with inherent limitations, with regard to quantitative results. In recent years, there is an increasing interest in sorbent free alternatives. Here, we report on a new instrumental system for processing of large sample volumes by discontinuous electrophoresis with theoretically unlimited concentration factor. The laboratory constructed device was designed in a circular arrangement where sample zones migrated towards a fraction collection well in the center. This allowed focusing of 15 ml sample volumes in a 110 mm device in less than 1 hour. Position of the migrating zone was monitored by laser-induced fluorescence, thermal imaging or surface conductivity detectors. While a discontinuous electrolyte system common for isotachophoresis was used, the selected geometry did not lead to a typical isotachophoretic migration with respect to the linear velocity of the zones. For example, in the constant current mode, most commonly used in capillary isotachophoresis, the speed of the migrating zones increased during the analysis. Thus, we propose the name “epitachophoresis” for this mode of separation [1].

REFERENCES

[1] Foret, F., Datinska, V., Voracova, I., Novotny, J., Gheibi, P., Berka, J., Astier, Y.

Macrofluidic Device for Preparative Concentration Based on Epitachophoresis. *Anal. Chem.* 111 (2019) 7047-7053. DOI:10.1021/acs.analchem.8b05860

KN-3 - ACTIVATION ENERGY ASSOCIATED WITH THE ELECTROMIGRATION OF PROTEINS IN CAPILLARY SDS GEL ELECTROPHORESIS

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ABSTRACT

Sodium dodecyl sulfate capillary gel electrophoresis (SDS-CGE), also known as CE-SDS, is one of the most frequently used electric field mediated separation techniques in the biopharmaceutical industry mostly used for fast purity check and characterization in release and stability studies. The resolution of the method is dependent on the sieving matrix and the separation conditions. In this paper the effect of the separation temperature was investigated for various size new modality biotherapeutics, including nanobodies, multispecific antibodies and fusion proteins with particular attention on resolution. The Arrhenius curves were plotted covering the temperature range of 15 - 60ºC. The activation energy (Ea) values, calculated from the slopes, were exponentially decreasing with the protein size range investigated. As a first approximation we considered this activation energy decrease as the result of distortion in both the polymer fibers and the SDS-protein complexes. Albeit, this sounds contradictory with the general assumption that Ea should increase with the solute size, the polymer chains of the non-crosslinked sieving matrix can distort to allow passage of the highly charged larger molecules under the influence of the high electric field strength. More interestingly, for the different solute types listed above, the activation energy dependent migration characteristics resulted in altered resolution at different temperatures, suggesting a separation optimization opportunity.

KN-4 - THE NEW SHIMADZU LCMS QTOF-9030-UNCOMPROMISED TOOL FOR BIOANALYSIS

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ABSTRACT

Based on our deep understanding of researchers' needs, Shimadzu has engineered the LCMS-9030 with the functionality most important for success. The Shimadzu LCMS-9030 is designed to deliver high resolution accurate mass detection with incredibly fast data acquisition rates allowing you to identify and quantify more compounds with greater confidence. It takes the same engineering DNA from our proven, rugged, high-performance triple quadrupole platform and helps to transform high mass accuracy workflows bringing together high sensitivity, high speed and high-resolution detection.

The mass accuracy of the LCMS-9030 is exceptional over a wide range to allow high-confidence identification of unknowns. More important the mass accuracy stability of theLCMS-9030 ensures that users can achieve the same identification results time after time. Rock stable mass accuracy also means that less frequent calibration is required, making the routine work easy to manage. Because of its high sensitivity, the LCMS-9030 requires only a small sample size for identification of unknowns. Users achieve the quantitation results they need: 100 scans per second for high-sensitivity quantitation of targeted compounds. 100 scans per second also applies to MS/MS acquisition for a truly comprehensive screening of unknowns. The LCMS-9030 not only addresses qualitative and quantitative challenges — it can address both simultaneously.

KN-5 - AFFINITY CAPILLARY ELECTROPHORESIS AND DENSITY FUNCTIONAL THEORY EMPLOYED FOR STUDY OF BIOPEPTIDE INTERACTIONS WITH AMMONIUM AND METAL IONS

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Affinity capillary electrophoresis (ACE) [1] and quantum mechanical density functional theory (DFT) have been applied for quantitative and qualitative characterization of interactions of antamanide (AA), monocyclic decapeptide from fungus *Amanita phaloides* (cycl[-Val(1)-Pro(2)-Pro(3)-Ala(4)-Phe(5)-Phe(6)-Pro(7)-Pro(8)-Phe(9)-Phe(10)-]), and its [Gly^6]-derivative ([Gly^6]-AA) [2], with ammonium and metal ions (Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, and Ca^{2+}) in methanol. The strength of the AA and [Gly^6]-AA complexes with the above ions was quantified by their apparent binding constants, K_b. The K_b values were determined from the dependence of effective mobility of AA and [Gly^6]-AA on the concentration of these ions in methanolic background electrolyte (20 mM chloroacetic acid, 10 mM Tris, 0-40 mM MCl or MCl_2 (M indicates one of the above ions), pH_{MeOH} 7.8) using the non-linear regression analysis. Prior to this analysis, the effective mobilities of [Gly^6]-AA measured at ambient temperature and variable ionic strength of the background electrolyte were corrected to reference temperature 25°C and constant ionic strength 10 mM. Complexes of AA and [Gly^6]-AA with the above metal cations were found to be relatively weak, with K_b in the range 37.8–14.1 L/mol [3, 4]. No interactions were observed between AA and [Gly^6]-AA peptides and lithium and ammonium ions.

Structural details of the AA and [Gly^6]-AA complexes with the above ions, such as position of the ions in the cavity of the AA and [Gly^6]-AA molecules and the interatomic distances within the complexes, were determined by DFT calculations.

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[1] S. Štěpánová, V. Kašička, Capillary electrophoretic methods applied to the investigation of peptide complexes, *J. Sep. Sci.* 38 (2015) 2708-2721.

[2] P. Ruzza, A. Calderan, B. Biondi, M. Carrara, et al., Ion-binding and pharmacological properties of Tyr(6) and Tyr(9) antamanide analogs. *J. Pept. Res.* 53 (1999), 442-452.

[3] S. Pangavhane, S. Böhm, E. Makrlík, P. Ruzza, V. Kašička, Affinity capillary electrophoresis and density functional theory study of noncovalent interactions of cyclic peptide [Gly^6]-antamanide with small cations. *Electrophoresis* 38 (2017) 2025-2033.

[4] S. Pangavhane, E. Makrlík, P. Ruzza, V. Kašička, Affinity capillary electrophoresis employed for determination of stability constants of antamanide complexes with univalent and divalent cations in methanol, *Electrophoresis*, 40 (2019) DOI 10.1002/elps.201900043.

KN-6 - IDENTIFICATION OF NATURAL DYES IN HISTORIC OBJECTS BY MINIATURIZED SEPARATION TECHNIQUES

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ABSTRACT

The knowledge of a chemical composition of samples from historical objects is of paramount importance in the process of their conservation. It allows, for example, to choose a proper method of filling losses in the painting layer or understand visual changes that occurred on the surface of the dyed material throughout centuries. Moreover, the knowledge of old painting techniques might help to prevent or decelerate negative physiochemical changes in painting layers by the proper choice of methodology of conservation and storing of historical objects [1]. It is obvious that a sample taken from the historical work of art must be as small as it is possible, so here miniaturized analytical techniques, particularly separation methods, could be a solution.

Miniaturized separation techniques can be advantageous for couple of reasons:

- they require minute samples;
- mass sensitivity is higher than in classical chromatography;
- nanoLC systems are well suited to mass spectrometry due to low flow rates;
- open tubular systems like capillary electrophoresis or open tubular LC can be employed;
- there is a great reduction of chemicals consumption (e.g. mobile phases) [2].

In this contribution we present the possible applications of miniaturized separation techniques in works of art conservation as well as our non-aqueous capillary electrophoresis (NACE) approach to identification of selected natural dyes is discussed.

REFERENCES

Times New Roman 9 points. The references should be listed in order of appearance.

[5] V. Pauk, P. Barták, K. Lemr, Characterization of natural organic colorants in historical and art objects by high-performance liquid chromatography, *J. Sep. Sci.*, 37 (2014) 3393-3410

[6] M. Szumski, B. Buszewski, State of the art in miniaturized separation techniques, *Crit. Rev. Anal. Chem.*, 32 (2002) 1-46

KN-8 - ELECTROMIGRATION TECHNIQUES IN METABOLOMICS STUDIES

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ABSTRACT

In the recent years, electromigration techniques has joined other analytical separation methods that are widely used in metabolomics. Urinary pterins have been found as potential biomarkers in many pathophysiological conditions including inflammation, viral infections, and cancer. However, pterins determination in biological samples is difficult due to their degradation under exposure to air, light, and heat. Besides, they occur at shallow concentration levels, and thus, standard UV detectors cannot be used without additional sample preconcentration. On the other hand, ultra-sensitive laser-induced fluorescence (LIF) detection can be used since pterins exhibit native fluorescence. The main factor that limits an everyday use of LIF detectors is its high price. Here, an alternative detector, i.e., light-emitted diode induced fluorescence (LEDIF) detector, was evaluated for the determination of pterins in urine samples after capillary electrophoresis (CE) separation. An optimized method was validated in terms of linearity range, limit of detection (LOD), limit of quantification (LOQ), intra- and interday precision and accuracy, sample stability in the autosampler, and sample stability during the freezing/thawing cycle. The obtained LOD (0.1 μM) and LOQ (0.3 μM) values were three-order of magnitude lower compared to UV detector, and two orders of magnitude higher compared to previously reported house-built LIF detector. The applicability of the validated method was demonstrated in the analysis of urine samples from healthy individuals and cancer patients [X].

REFERENCES

[1] W. Grochocki, M. Buszewska-Forajta, S. Macioszek, M.J. Markuszewski, Determination of urinary pterins by capillary electrophoresis coupled with LED-induced fluorescence detector, *Molecules*, 24 (2019) 1166.

KN-9 - DIFFUSION-BASED SEPARATION USING BIDIRECTIONAL ELECTROOSMOTIC FLOW

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ABSTRACT

We present a microscale separation method that leverages bidirectional flow, generated by an array of alternate-current field-effect electrodes, to electroosmotically tune the Taylor-Aris diffusion coefficient of molecules and particles. Under bidirectional flow the relative motion of species due to differences in their molecular diffusivity can be significantly enhanced. The system can be configured such that a lower-diffusivity species experiences a ballistic transport regime and is advected through the chamber, whereas a higher-diffusivity species experiences a diffusion dominated regime with zero average velocity and is retained in the chamber. We experimentally demonstrate the separation of particles, antibodies, and dyes, and present a theoretical analysis of the system, providing engineering guidelines for its optimal design and operation. This method provides means for leveraging molecular diffusivity for analysis and sample preparation applications, particularly for sub-microliter sample volumes that are not compatible with standard separation techniques.

OC-1 - MICROFLUIDIC SYSTEM FOR EVALUATION OF INSULIN SECRETION FROM A THREE-DIMENSIONAL PANCREATIC ISLET MODEL

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ABSTRACT

Diabetes mellitus type 2 is a metabolic disease associated with pancreatic islets β-cells damage [1]. Pancreatic islets are spherical cells aggregates with dimensions from 100 μm to 200 μm and consist of several cell types, such as: α, β, δ, f and ε cells [2]. Currently, the treatment of diabetic mellitus disease begins with a change in diet and lifestyle, and in the most of cases ends with injection of insulin. For this reason, researchers are increasingly looking for new, less invasive therapies [3], [4]. Researches in this field are carried out mainly using standard methods on two-dimensional cell models which do not correspond to the *in vivo* conditions or on tissues isolated from animals.

In this study we present a PDMS/GLASS microfluidic system which consist an elliptical cell culture chamber in which there are 15 round microtraps. Each of the microtraps are built of 6 micropillars, which forces the aggregation of cells by limiting the growth surface. In such a microchip, spherical aggregates with dimensions of about 150 μm and participation of β-cells in the islet core and α-cells on the periphery were obtained. Due to the development of immunostaining protocol using primary and secondary antibody solutions, quantitative and qualitative determination of insulin and glucagon levels were obtained. The cells maintained their morphology, function and high viability for up to 48 hours of culture.

This study presents basic research and in the future, this model can be utilized to simulate diabetes, testing new drugs and therapy in diabetes mellitus treatment.

REFERENCES

[1] J.V. Selby, G.T. Ray, D. Zhang, C.J. Colby, Excess costs of medical care for patients with diabetes in a managed care population. *Diabetes Care*, 120 (1997) 1396–1402.

[2] C. Ionescu-Tirgoviste, P. A. Gagniuc, E. Gubceac, L. Mardare, I. Popescu, S. Dima, M. Militaru , A 3D map of the islet routes throughout the healthy human pancreas *Sci Rep.*, 29 (2005) 5: 14634

[3] H. Guo-Parke, J. T. McCluskey, C. Kelly, M. Hamid, N. H. McClenaghan, P. R. Flatt, Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility. *Journal of Endocrinology* 214 (2012) 3:257–265

[4] A. Chowdhury, V. P. Satagopam, L. Manukyan, K. A. Artemenko, Y. M. Fung, R. Schneider, J. Bergquist, P. Bergsten, Signaling in insulin-secreting MIN6 pseudoislets and monolayer cells., *J Proteome Res*, 121 (2013) 5954–62.

OC-2 - LIQUID CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR TOP-DOWN QUANTITATIVE ANALYSIS OF LOW ABUNDANCE INTACT PROTEINS FROM BIOLOGICAL SAMPLES

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ABSTRACT

Variable protein expression is a crucial marker for numerous diseases, including cancer. Here, we explored the practical opportunities and challenges of a multiple reaction monitoring-based top-down quantitative reversed-phase liquid chromatography – triple quadrupole mass spectrometry method [1, 2] for direct analysis of cancer related intact proteins (growth factors and cytokines) in biological samples. Proteins were analysed using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode or a LCMS-9030 quadrupole time-of-flight. Reversed phase separations were performed on a Restek wide-pore Viva C4 column (2.1×100 mm, 300 Å pore size, 5 µm dp) using variably acidified water and acetonitrile mobile phases under gradient conditions. Optimum MRM transitions for our selected intact proteins (5.5 – 21 kDa) were generated under the moderate CID gas pressure (270 kPa) with low to moderate collision energy. An optimized method performed at 30 °C and using 0.2 mL/min flow rate, 11 %B/mL gradient slope, with 0.05% difluoroacetic acid as a mobile phase modifier provided optimal separation and detection of seven target intact proteins in biological matrices in less than 5 minutes. Limits of detection in biological matrix varied considerably for different proteins and were estimated to be between 2.3 and 58 nM (0.0125 - 1 µg/mL). Matrix effects, as well as a specificity of the method were assessed for variable biological samples and pretreatment methods, since very limited effort has been made to for such a study to date.

REFERENCES

[1] E.H. Wang, P.C. Combe, K.A. Schug, Multiple Reaction Monitoring for Direct Quantitation of Intact Proteins using a Triple Quadrupole Mass Spectrometer. *J. Am. Soc. Mass Spectrom.* 27 (2016) 886-896.
[2] E.H. Wang, Y. Nagarajan, F. Carroll, K.A. Schug, Reversed-Phase Separation Parameters for Intact Proteins using Liquid Chromatography – Triple Quadrupole Mass Spectrometry. *J. Sep. Sci.* 39 (2016) 3716-3727.

OC-3 - WHAT COLOUR IS YOUR METHOD? A GLOBAL EVALUATION OF TWO CAPILLARY ELECTROPHORESIS-BASED METHODS USING THE RGB ADDITIVE COLOUR MODEL

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ABSTRACT

Evaluation of analytical method is a fundamental problem in analytical chemistry, and it is never straightforward. We will point out the perspective for facing this issue with an original tool, the first model allowing one to evaluate any analytical method/procedure in a global sense. It takes into account the three main method attributes: analytical performance, compliance with the "green" chemistry principles [1], and productivity/practical effectiveness. It refers to the RGB additive colour model, and utilizes three primary colours (Red, Green, Blue) and their mixtures, as the symbols displaying qualitatively method competences and potential. White color is an ideal indicating that method is generally complete and broadly applicable. Black method, in contrast, indicates a defective method character. The model provides also an unified quantitative parameter, named as "method brilliance", which integrates all primary colours and treat them with the variable importance, adjusted to the evaluation context and subjective user preferences.

We will demonstrate how to perform compressive method evaluations and comparisons in a simple and transparent manner, and how to interpret the obtained evaluation outcomes. The two different capillary electrophoresis-based methods have been selected for that purpose, developed recently in our laboratory. The former one is devoted to the quantification of food colorants and preservatives in sports drinks, whereas the latter one to the determination of acid dissociation constant of common pH indicators. A special emphasis will be put on the identification and discussion of features/predispositions inherent for capillary electrophoresis as a bioanalytical technique. A future perspective will also be presented.

REFERENCES

[1] M. Tobiszewski, A. Mechlińska, J. Namieśnik, Green analytical chemistry – theory and practice, *Chem. Soc. Rev.* 39 (2010) 2869-2878.

OC-4 - NEW ANALYTICAL APPROACHES FOR MONITORING OF INFLAMMATORY BOWEL DISEASES THERAPY

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ABSTRACT

Inflammatory bowel diseases (IBD) are chronic disorders characterized by the immune-mediated inflammation of gastro-intestinal tract and their incidence has significantly increased in the last years. Thiopurines represent the main group of therapeutics used in the treatment of IBD based on an immune suppressive activity through different active metabolites created throughout complex enzymatic metabolism. These enzymatic reactions can show great inter-individual variations. Approximately one third of IBD patients fail on thiopurine therapy and approximately 10% of IBD patients have to stop the treatment due to the side-effects. Thus, the monitoring of thiopurines, their metabolites as well as other co-medications in biological samples are important for optimizing the IBD therapy. On the other side, a variable expression of endogenous compounds (e.g. biogenic amines) in biological fluids is considered as a relevant biochemical biomarker for various diseases including inflammatory diseases and can help in clinical diagnostics.

For the analyses of multicomponent biological samples, highly sensitive and selective separation methods are required. Nowadays, capillary electrophoresis represents a good alternative to well-established and universal liquid chromatography because of its overall simplicity, low costs, higher efficiency, lower consumption of samples and better matrix tolerance. Moreover, coupled with mass spectrometry it offers enhanced selectivity and sensitivity and more complex information about the samples. In our work we have developed new complementary analytical methods mainly based on capillary electrophoresis that can be implemented in the analysis of biologically active compounds (drugs, their metabolites and endogenous compounds) which can contribute in better understanding, diagnosis and optimization of IBD therapy.

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OC-5 - CAPILLARY ELECTROCHROMATOGRAPHY BASED NOVEL IN VITRO TOOL FOR THE ASSESSMENT OF BLOOD-BRAIN BARRIER PERMEABILITY

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Profiling blood-brain barrier permeability of bioactive molecule at an early drug development stage is a part of the optimization process of a compound's physicochemical properties, and hence pharmacokinetic profile. Presented study was focused on the development of a new *in vitro* method for assessment of compound's brain penetration. The tool is proposed as an alternative to the widely used PAMPA-BBB assay [1] (Parallel Artificial Membrane Permeability Assay for Blood-Brain Barrier) and based on a capillary electrochromatography (CEC) technique. It takes advantage of liposomes as structural substitutes of biological membranes, which are used as a capillary inner wall coating material. Following optimization of analysis conditions, migration times for a set reference drugs (mainly non-ionized in pH 7.4) were examined in a liposome coated capillary. On that basis, the retention factor (log k) was determined for each reference drug. Obtained log k values and experimentally received reference permeability parameters: log BB (*in vivo* data) and log P_e (PAMPA-BBB data) were compared with one another. Correlation coefficients were calculated, giving comparable results for CEC log k/log BB and analogical PAMPA-BBB log P_e/log BB analyses. Approximate ranges of log k for the central nervous system (CNS) permeable (CNS(+)) and non-permeable (CNS(–)) drugs were established. The new method has the potential to work well as a simple tool for early BBB passive permeability assessment of research compounds. It is an innovative, fast and relatively cheap alternative to the PAMPA-BBB technique.

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REFERENCES

[1] L. Di et al. *Eur. J. Med. Chem.* 38.3 (2003) 223-232.

OC-6 - STUDY OF AMINO ACID PROFILES IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION – CHEMOMETRIC APPROACH

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ABSTRACT

The aim of the study was to assess the amino acid (AA) profiles in patients with hematological malignancies in comparison to health control group as well as to follow changes in plasma AA after high-dose chemotherapy preceding haematopoietic stem cell transplantation. Blood plasma samples originated from 40 health volunteers and 50 patients undergoing auto- or allogenic stem cells transplantation suffering from different types of hematopoietic and immune system neoplasms, as well as few germ cell tumors. Evaluation of the AA profile was conducted prior to the administration of chemotherapy, on the day of chemotherapy start (day 0), and then after 7, 10, 14 and 21 days. AA were assayed with HPLC method after post column labelling with AQC according to ref [1].

The obtained data were subjected to statistical analysis using basic as well as chemometric methods (cluster analysis, factor analysis, discriminant analysis). The results indicated significant differences ($p<0,05$) in AA levels between patients and control group (e.g. Cit, Tau, Ala, BCAA, Phe). AA profiles were found also to be characteristic with regard to type of neoplasm diagnosed. Additionally, changes in plasma AA concentrations during chemotherapy were associated with regime scheme. Multivariate models built based on analysed data were able to discriminate samples with sufficient sensitivity and specificity and to reveal factors of potential predictive value.

REFERENCES

[1] Jaworska M, Stańczyk M, Wilk M, Kłaczkow G, Anuszewska E, Barzał J, Rzepecki P. New approach for amino acid profiling in human plasma by selective fluorescence derivatization. Amino Acids. 2012, 43(4), 1653-1661.

OC-7 - PROTEOMIC AND METABOLOMIC CHARACTERIZATION OF OVARIAN CANCER

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ABSTRACT

Ovarian cancer (OC) is one of the most deadly malignancies in the gynecology. So far, the diagnosis is based on the transvaginal ultrasound examination. Other widely used method is measurement of two markers: cancer antigen 125 (CA125) and human epididymis protein 4 (HE4). However, the early diagnostic tools and screening methods still remain insufficient. Therefore, knowledge about pathophysiological processes associated with this cancer should be extended and new studies aiming better OC characterization are needed.

In this studies proteomic and metabolomic approach was proposed for characterization of ovarian cancer.

Serum samples were collected from three groups of patients: with a malignant ovarian tumor, benign ovarian tumor and healthy controls. The study was approved by the Bioethics Committee of the Poznań University of Medical Sciences (Decision No. 165/16).

Different analytical strategies have been used, including both targeted and untargeted analyzes. The research was carried out using: MALDI-TOF, ESI-QqQ mass spectrometers and Luminex technology [1, 2]. The results from all experiments were subjected to advanced statistical analysis, including uni-and multivariate tests and calculation of the discriminatory models, in order to select the most discriminative features.

The conducted study shed light on the complicated pathomechanism associated with ovarian cancer. Proposed strategy have potential as future diagnostic multimarker tool or target for novel drug therapies.

This research was funded by the Polish National Science Centre (grant number: 2014/15/B/NZ7/00964).

REFERENCES

[1] A. Światły, A. Horała, J. Matysiak, J. Hajduk, E. Nowak-Markwitz, Z. J. Kokot, Understanding ovarian cancer: iTRAQ-based proteomics for biomarker discovery, Int. J. Mol. Sci. 19 (2018) 1-15.

[2] S. Plewa, A. Horała, P. Dereziński, A. Klupczyńska, E. Nowak-Markwitz, J. Matysiak, Z. J. Kokot. Usefulness of amino acid profiling in ovarian cancer screening with special emphasis on their role in cancerogenesis. Int. J. Mol. Sci. 18 (2017) 1-11.

OC-8 - APPLICATION OF THE UHPLC-MS/MS METHOD FOR THE INTERACTION STUDY OF CLOPIDOGREL AND STATINS IN PATIENTS AFTER CORONARY ANGIOGRAPHY/ANGIOPLASTY

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ABSTRACT

Resistance to antiplatelet therapy with clopidogrel (CLP) is a common problem in patients after stent implantation [1]. The pivotal role in the inter-patient variability of the CLP pharmacokinetics and pharmacological effect may play interactions with drugs, such as statins, metabolised by the same CYP isoenzymes, which mediate in the formation of the CLP active metabolite [2].

The aim of the study was to apply the validated UHPLC-MS/MS method for the determination of CLP and its metabolites (H3 and H4 isomers of the thiol metabolite and carboxylic acid derivative CLPM) in plasma of patients undergoing coronary angiography/angioplasty.

The chromatographic separation of CLP, CLPM, the derivatized isomers MP-H3 and MP-H4 and deuterated CLP as the internal standard was carried out on a Zorbax Eclipse Plus C18 column and a mixture of 0.1% formic acid in water and acetonitrile, used as a mobile phase. Linearity of the method was confirmed in the concentration ranges of 0.1-5.0 ng/ml for CLP, 0.05-10.0 µg/ml for CLPM and 1-100 ng/ml for MP-H3 and MP-H4. The method was appropriately precise and accurate and fulfilled the validation criteria for bioanalytical methods. It was applied for analysis of plasma concentrations of CLP and its metabolites in 50 patients who underwent coronary angiography/angioplasty and were treated with CLP and atorvastatin or rosuvastatin during the six months after the procedure.

The study confirmed that the systemic exposure to CLP and its active H4 metabolite was not negatively affected by co-administration of atorvastatin as compared with rosuvastatin.

REFERENCES

[1] A. De Miguel, B. Ibanez, J.J. Badimon, Clinical implications of clopidogrel resistance, *Thromb. Haemost.*, 2008 (100) 196–203.

[2] W.C. Lau, L.A. Waskell, P.B. Watkins, C.J. Neer, K. Horowitz, A.S. Hopp, A.R. Tait, D.G.M. Carville, K.E. Guyer, E.R. Bates, Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction, *Circulation*, 107 (2003) 32–37.

OC-9 - COMPLEMENTARY APPLICATION OF ANALYTICAL METHODS FOR THE DEVELOPMENT OF MEDICAL IMPLANTS

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ABSTRACT

Implant development necessitates a highly interdisciplinary approach to the provision of suitable analytical methods in order to ensure biocompatibility and implant function. In particular, challenging methods are being required to assess the properties of polymer/drug coatings for implant-based local drug delivery systems (DDS), which are being used to improve implant-tissue interaction. Here, several properties have to be studied, such as biocompatibility, stability, or effects of sterilization and shelf life. Furthermore, it is necessary to investigate mechanical integrity, degradation processes, as well as physicochemical properties, such as glass transition or crystallinity. Also, drug stability and release kinetics *in vitro* and *in vivo* are major topics with respect to approval and certification of medical device/drug combination products. Often, drug stability in physiological media must be characterized, taking into account pH and composition of the different target environments. In many cases *in vivo* measurements are particularly challenging due to sensitivity and drug stability in different matrix substances, such as enzymes leading to drug degradation. In this context, the immunosuppressant Sirolimus is a challenging compound to describe.

Here we will present analytical methods that have been developed for the assessment of Sirolimus-based DDS for implants. While HPLC is suitable for measuring intact Sirolimus and few degradation products, alternative methods, such as LC-MS and capillary electrophoresis [1], can serve as complementary methods, taking advantage of their higher sensitivity and extended options for detection. Mass spectrometry in particular is being used for derivatives invisible to UV-detection.

REFERENCES

[1] I. Oledzka, P. Kowalski, A. Plenis, N. Miekus, N. Grabow, T. Eickner, T. Baczek, Simultaneous electrokinetic and hydrodynamic injection and sequential stacking featuring sweeping for signal amplification following MEKC during the analysis of rapamycin (sirolimus) in serum samples, *Electrophoresis*, 39 (2018) 2590-2597

OC-10 - CAPILLARY ELECTROPHORESIS STUDIES OF NEUROPROTECTION ACTIVITY OF TAT PEPTIDES

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ABSTRACT

Capillary electrophoresis (CE) has been applied to characterize neuroprotection activity of Tat peptides. Ischemic stroke (IS) is a cerebral insufficiency caused by the brain tissue ischemia and hypoxia. Currently, the only drug approved for the treatment of IS is intravenous recombinant serine protease tissue-type plasminogen activator (rtPA). Previous studies have shown that Tat and other arginine-rich cell penetrating peptides (CPPs) display intrinsic neuroprotective properties [1]. Using of rat neuronal cortical culture and a multifactorial in vitro stroke model consisting of typical neurochemical insults: glucose deprivation, lactic acidosis, oxidative stress induced by sodium azide and excitotoxicity induced by glutamic and kainic acids and NMDA, we have shown that PTD4 peptide (Y^{47}ARAAARQARA^{57}-NH_2) being an Ala-scan analogue of Tat(49-57)-NH_2 peptide (R^{49}KKRRQRRR^{57}-NH_2) showed significant post-injury neuroprotection. The peptides investigated were not neurotoxic up to a concentration of 100 μM. CE showed what Tat and PTD4 peptides could be directly detected in human serum and PTD4 peptide was more stable compare to Tat(49-57)-NH_2. Similar results were obtained studying the peptides behaviour in mouse brain. Using trifluoroethanol as an additive to CE separation buffer it was possible to resolve Tat peptides with different post-translational modifications. Two CPPs investigated, propiolated-Tat(49-57)-NH_2 and TP10 showed significant neurotoxicity at a concentration above 1 μM. The results show that neuroprotective activity of CPPs is not directly correlated with the arginine residues presence in the peptide sequence but rather with its ability to penetrate of cell membrane. CE may be used as a valuable tool in study of peptide behaviour in neuroscience.

REFERENCES

[1] B.P. Meloni, L.M. Brookes, V.W. Clark, J.L. Cross, A.B. Edwards, R.S. Anderton, R.M. Hopkins, K. Hoffmann, N.W. Knuckey, Poly-arginine and arginine-rich peptides are neuroprotective in stroke models, *J. Cereb. Blood Flow Metab*., 35 (2015) 993-1004.

OC-11 - THE USE OF THE HS-SPME/GC–MS TECHNIQUE TO STUDY INTERACTIONS INVOLVING VOLATILE ORGANIC COMPOUNDS AT THE PLANT-MICROBE INTERFACE

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Bacteria and plants, both are able to interact with their environment by emitting volatile organic compounds (VOCs). The ability of bacteria to grow on plants may depend on many factors, also on volatiles emitted by both plants and bacteria. Plants VOCs can have both growth inhibitory properties that prevent colonization of bacteria as well as serve as carbon sources that support the growth of microbes. Different bacterial taxa could occupy strongly diverging niches based on scent emissions of different plants.

The volatiles emitted by different *Pectobacterium* species and by *Arabidopsis* were extracted utilizing head-space solid phase micro extraction and analyzed by gas chromatography mass spectrometry. The effect of *Pectobacterium* volatiles on *Arabidopsis* growth and the effect of plants volatiles on bacterial growth were assessed by cultivating both organisms in a shared atmosphere without any physical contact.

The obtained results show that different VOCs profiles are emitted by different species of *Pectobacterium*. The same bacterial strain produces a different set of VOCs depending on the composition of the growth medium. There are no VOCs newly emitted during the interaction between *Arabidopsis* and *Pectobacterium.* Plants are able to utilize volatiles secreted by bacteria and vice versa. VOCs emitted by plant pathogens inhibit or promote the growth of *Arabidopsis.* VOCs produced by certain *Pectobacterium* strains growing on MRVP medium induce the production of anthocyanins by *Arabidopsis*. *Pectobacterium* produces pheromones attracting insects that act as vectors carrying bacteria between plants.

Our results shed light on interactions between bacterial pathogens, plants and insects.

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OC-12 - BAYESIAN MULTILEVEL MODELING IN CHROMATOGRAPHY

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ABSTRACT

It is relatively easy to collect chromatographic measurements for a large number of analytes, especially using gradient chromatographic methods coupled with mass spectrometry detection. Such data often have hierarchical or clustered structure. For example, analytes with similar hydrophobicity and dissociation constant tend to be more alike in their retention than randomly chosen set of analytes. Multilevel models recognize the existence of such data structures by assigning a model for each parameter with its parameters also estimated from data [1,2]. The multilevel models consist of i) the same deterministic equation describing the relationship between retention time and analyte-specific and instrument-specific parameters, ii) covariance relationships relating various physicochemical properties of analyte to chromatographically-specific parameters through QSRR-based equations, and iii) stochastic components of intra-analyte and inter-analyte variability. Such models can be implemented in the Stan software that provides full Bayesian inference for continuous-variable models through Markov Chain Monte Carlo methods. In this work the general idea and usefulness of multilevel models in chromatography is discussed.

REFERENCES

[1] Wiczling P. Analyzing chromatographic data using multilevel modeling. Anal Bioanal Chem. 2018, 410, 3905-3915.

[2] Kubik Ł, Kaliszan R, Wiczling P, Analysis of Isocratic-Chromatographic-Retention Data using Bayesian Multilevel Modeling, Anal Chem. 2018;90:13670-13679.

OC-13 - APPLICATION OF CHROMATOGRAPHIC METHODS TO THE ASSESSMENT OF FOOD SUPPLEMENTS QUALITY AND SAFETY

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There can be observed a growing popularity of food supplements around the world, which according to the European Union law are classified as food products. According to law requirements they are not subjected to any specific safety assessments prior to commercialisation [1]. Therefore, there is an increased need for more effective control of their production and distribution.

The aim of this work was to assess quality and safety of food supplements, containing green coffee and tea, available on Polish market.

All the experiments were performed using HPLC coupled to Corona CAD and UV/DAD detectors. In total, 37 food supplements were analysed for 12 polyphenols and caffeine content. Moreover, there were tested 14 green tea and coffee products in order to compare their composition with supplements. The newly developed methods of analysis were validated for linearity, precision and accuracy. They were characterized by good accuracy (88.1–120%) and precision (0.26-5.42%). Methods' linearity (regression coefficient $R^2 > 0.999$) and repeatability (RSD <5%) were highly satisfying.

The analyzed products characterized by varied concentrations of individual polyphenols, especially of epigallocatechin gallate, caffeine and chlorogenic acid. What is more, there were found significant (from 9.72% to over 277%) discrepancies in substance levels in relation to the values declared by producers. In case of caffeine, almost all food supplements were found to exceed the values declared by the producer. Such differences might result in misestimating of caffeine and polyphenols consumption in the daily diet, thus, it is important to constantly monitor safety of dietary supplements intake.

REFERENCES

[1] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements

OC-14 - *CISTUS INCANUS* L. COMMERCIAL PRODUCTS AS A GOOD SOURCE OF POLYPHENOLS IN HUMAN DIET

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ABSTRACT

Cistus incanus L. is a typical Mediterranean shrub species distributed along the coastal belt of the Central-Eastern Mediterranean, in Southern Europe, Western Asia and Northern Africa [1]. Among the bioactive compounds to be found in this plant, polyphenols are widely appreciated for their potential beneficial health effects [2]. In the human diet, phenolic compounds are the main antioxidants. Additionally, the antioxidant activity of plants is often connected to their individual phenolic profiles. The aim of this study was to assess the phenolic profile and antioxidant capacity of hydromethanolic and aqueous extracts of commercial *C. incanus* products. Individual phenolic acids and flavonoids were determined using HPLC, while antioxidant capacities were evaluated by use of scavenging assays of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and ferric reducing/antioxidant power (FRAP). Total phenolic, flavonoid, phenolic acid and L(+)-ascorbic acid contents were quantified using UV-Vis spectrometry, whereas *in vitro* antimicrobial activities of aqueous extracts of *C. incanus* were investigated by the broth microdilution technique using 96-well plates. The origin and morthological part were the main factors in differentiating *C. incanus* samples. The results revealed that aqueous extracts of *C. incanus* are richer in phenolic compounds and have stronger antioxidant activities than hydromethanolic extracts. Moreover, aqueous extracts revealed antibacterial activities more effective against Gram-positive bacteria, particularly *S. aureus* (MIC values from 0.5 to 32 mg/mL) and *S. epidermidis* (MIC values from 0.25 to 8 mg/mL) than Gram-negative bacteria. They were also weak inhibitors of *C. albicans* and *C. glabrata* growth (MIC values over 8 mg/mL).

REFERENCES

[1] M. Fernández-Mazuecos, P. Vargas, Ecological rather than geographical isolation dominates Quaternary formation of Mediterranean *Cistus* species, *Mol. Ecol.*, 19(2010) 1381-1395.

[2] D. Andrade, C. Gil, L. Breitenfeld, F. Domingues, A.P. Duarte, Bioactive extracts from *Cistus ladanifer* and *Arbutus unedo* L., *Ind. Crops Prod.*, 30(2009) 165-167.

OC-15 - APPLICATION OF NOVEL 3D PRINTING TECHNIQUES AND MATERIALS IN SAMPLE PREPARATION

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ABSTRACT

For past two years 3D printing techniques have opened a horizon of new possibilities in analytical chemistry. Its value was especially noticed in developing new and customized sample preparation approaches. A basic 3D printing technique, fused deposition modelling (FDM) was used not only to manufacture customized laboratory equipment, but also to test prototype filaments (such as LayFomm) and their potential use in sample preparation [1,2]. Additionally, possibility to create custom shapes enables a repurposing of currently existing laboratory gear to serve a similar purpose, yet customized by the use of certain filament, like the scabbards manufactured using FDM printer [3]. Presented study focuses on two different aspects of using 3D printing in sample preparation. First one is use of prototype filaments and custom made filaments, which open 3D printing to a variety of commercially non-available materials, that can be of great value in sample preparation. Second one showcases the possibility of creating a custom shape with a 3D printer, and how it can help in modern analytic laboratory. The example for such use are 3D printed scabbards, suitable for 96-well plates, which present how FDM technique was used to fabricate sorbents useful in high-throughput analyses.

REFERENCES

[1] M. Belka, S.Ulenberg, T. Bączek, Fused deposition modeling enables the low-cost fabrication of porous, customized-shape sorbents for small-molecule extraction, *Anal. Chem.*, 89 (2017) 4373-4376

[2] L. Konieczna, M. Belka, M. Okońska, M. Pyszka, T. Bączek,. New 3D-printed sorbent for extraction of steroids from human plasma preceding LC-MS analysis, *JCA*, 1545 (2018), 1-11

[3] M. Belka, L. Konieczna, M. Okońska, M. Pyszka, S. Ulenberg, T. Bączek, Application of 3D-printed scabbard-like sorbent for sample preparation in bioanalysis expanded to 96-wellplate high-throughput format, *Anal. Chim. Acta*, in Press.

OC-16 - A CAPILLARY ELECTROPHORESIS ASSAY FOR THE DETERMINATION OF LACTATE IN HUMAN VITREOUS HUMOR FOR FORENSIC APPLICATIONS

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ABSTRACT

The vitreous humour (VH) is currently recognized as an important body fluid in the forensic investigation for its delayed post-mortem changes and easy specimen obtaining without contamination. Therefore, the forensic analytical laboratory is often required to provide data from this peculiar matrix to support the criminal investigation [1,2]. The present research was aimed to study a capillary electrophoresis method for VH lactate assay and to verify its use in assessing the post-mortem interval (PMI). VH specimens were collected from medico-legal autopsies. Capillary electrophoresis (CE) separation was performed at 30 kV in reverse polarity mode with an indirect UV detection. CE was conducted in an uncoated 75 µm fused-silica capillary using hydrodynamic injection (0.5psi x 10s). The background electrolyte was 37 mmol/L TRIS (pH 8.9) containing 4 mmol/L 4-methoxybenzoic acid, for indirect quantification at 254 nm, 1.2 mmol/L alkyl-trimethyl-ammonium bromide as dynamic coating agent. Samples were diluted 1:450 with an I.S. solution (n-butyric acid 0.057 mmol/L final conc.) before injection. The method showed linearity (r^2=0.997) in the concentration range 4-80 mM. The limits of detection and quantification were 2 mM and 4 mM respectively. Intra-day and day-to-day precision was <8% and <15% respectively. Lactate levels of VH specimens (n=40) ranged from 17.2 to 99.9 mM. A preliminary study showed a correlation (r^2=0.531) of VH lactate vs PMI. In conclusion, CE proved to be a valid method for VH lactate quantitative analysis for forensic purposes, and specifically for thanatochemistry studies.

REFERENCES

[1] A. Go, G. Shim, J Park, J. Hwang, M Nam, H Jeong, H. Chung, Analysis of hypoxanthine and lactic acid in vitreous humor for the estimation of post-mortem interval (PMI) using LC-MS/MS, *Forensic Sci Int*, 299 (2019) 135-141.

[2] Z. Mihailovic, T. Atanasijevic, V. Popovic, MB. Milosevic. Could lactates in vitreous humour be used to estimate the time since death? *Med Sci Law*, 51(3) (2011) 156-60.

OC-17 - HUMAN PHARMACEUTICALS IN THE ENVIRONMENT

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ABSTRACT

The human pharmaceutical industry is one of the fastest growing industries the world over. For example, in the European Union, about 3000 different pharmaceuticals, such as analgesics and non-steroidal anti-inflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators or neuroactive compounds are used in human medicine. Pharmaceuticals have been detected in the environment of 71 countries covering all continents. Such disturbance might have significant and long-term effects on the rate and stability of ecosystem functioning. For these reasons, the presence of pharmaceuticals in the environment has attracted attention within the scientific community around the world. There are many investigations performed in terms of determining drug residues in surface and drinking waters around the world. Unfortunately, the knowledge on this topic in Poland is still limited although the level of consumption of drugs in Poland which is one of the highest in Europe. Particular attention should be paid to the monitoring of drugs consumed in very large amounts as well as those that are extremely stable and/or ecotoxic. An important limitation of such studies is the availability of sufficiently sensitive and reliable analytical methods for determining different pharmaceuticals present in trace amounts in such complex matrices. Although great advances have been made in their detection in aquatic matrices, there are limited analytical methodologies for trace analysis of target pharmaceuticals in matrices such as soils, sediments or biota. During this lecture our current researches and review articles in this field will be presented.

OC-18 - SALIVA AND NEW METHODS OF DETERMINING THE LEVEL OF DRUGS IN THE BODY

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ABSTRACT

Blood is the most common biological material used for determination of drugs and their metabolites in the body. Saliva is used relatively rarely due to its different protein profile and low concentration of the determined active substances. However, the use of saliva is a source of many benefits. First of all, its collection is non-invasive, it does not require the presence of trained personnel or special equipment. Thus, saliva can be a valuable diagnostic material in the case of tests performed in children or the elderly, and in the screening tests. Thanks to its unquestionable advantages, saliva is a convenient biological matrix for the determination of both endogenous compounds and xenobiotics.

The aim of these studies were to develop methods for the determination of cortisol, antidepressants and antipsychotics in saliva taken without stimulation either directly or with cotton wool swabs. Both SPE and LLE were used to isolate the compounds. Chromatographic analysis was carried out using HPLC with UV-VIS detection. The mobile phase consisted of acetonitrile:water with the addition of formic acid and triethylamine.

Developed methods were validated for linearity, limits of detection and quantification, precision and recovery. In addition, the stability of the tested compounds was examined during the storage of biological material and samples after extraction. The developed methods were applied to analysis of saliva samples from people treated with the studied drugs. It was found that saliva is suitable for determining the level of drugs.

OC-19 - A FIRST POINT OF CARE TEST FOR CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) BASED ON FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET).

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ABSTRACT

The increase of Carbohydrate-Deficient Transferrin (CDT) as results of an heavy alcohol intake for at least two weeks, is a well-known biochemical modification [1]. Notwithstanding the first commercial kit for the diagnosis of chronic alcohol abuse based on this biomarker was commercially available already thirty years ago, today only complex and expensive analytical methods are available for CDT analysis.

An alternative approach for CDT analysis based on Fluorescence Resonance Energy Transfer (FRET) induced by terbium (III) addition has been developed by our Institute [2]. It leads to a high gain in sensitivity and specificity in comparison to traditional methods based on the UV-Vis light absorption. This allows for a great simplification of the analytical method which meets the needs of the point-of-care tests. The new procedure is based on a cut-off separation of the terbium (III) functionalized CDT in serum. The specimen is loaded and the fluorescence measurement is performed on the collected sample. The entire process requires less than 10 min. The method showed a good correlation (R^2=0.8854) with the reference procedure based on HPLC-Vis using serum samples (n=40). The method is intended for qualitative analysis and has been tested successfully in terms of precision (CV<20%), sensitivity and specificity (AUC=0.9525).

The method, characterized by low-cost and rapidity of analysis, shows high simplicity of operation and requires minimum instrumentation. Therefore, this new approach is suitable for application in alcohol abuse screening contexts in non-strictly regulated environments (e.g. clinical diagnosis) as well as in developing countries or remote areas.

REFERENCES

[1] H. Stibler, C. Allgulander, S. Borg, K.G. Kjellin Abnormal Microheterogeneity of Transferrin in Serum and Cerebrospinal Fluid in Alcoholism, *Acta Med. Scand.,* 204 (1978) 49-56.

[2] D. Sorio, E.F. De Palo, A. Bertaso, F. Bortolotti, F. Tagliaro Fluorescent Adduct formation with terbium: a novel strategy for transferrin glycoform identification in human body fluids and carbohydrate-deficient transferrin HPLC method validation, *Anal. Bioanal. Chem.,* 409 (2017) 1369-1378.

OC-20 - QUANTITATION AND PURITY ASSESSMENT OF EXTRACELLULAR VESICLES IN ISOLATES FROM *PECTOBACTERIUM CAROTOVORUM SP.* CULTURING MEDIA USING CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

Extracellular vesicles (EVs) are a group of liposome-like micro- and nanoparticles capable to transport proteins, lipids and nucleic acids. Depending on the cargo they carry, bacterial EVs can be used for communication between cells, transfer of genes or to supress immunological response of the host [1].

Isolation as well as characterization of EVs is a complex procedure that requires a number of sophisticated devices, consumes time and funds, and is a limitation of studies on EVs [1].

The presented work was aimed into the development of capillary electrophoresis (CE) method for the assessment of purity and content of EVs in isolates obtained from bacterial growth media using ultracentrifugation and filtration. The isolates were characterized with total protein content measurements, dynamic light scattering (DLS) technique, transmission electron microscope (TEM) and MALDI-TOF/TOF-MS analysis. The samples were further submitted to CE analysis which enabled to separate EVs from the impurities. The identity of the main peak was confirmed with fractionation of the sample and off-line, downstream DLS and TEM analyses.

The conducted research indicates the great potential of the developed method as a routine tool for quality control of EVs in scientific and commercial laboratories.

This work was supported by the National Science Centre of Poland (grant number 2016/21/D/ST4/03727).

REFERENCES

[1] A. J. Manning, M. J. Kuehn, Functional Advantages Conferred by Extracellular Prokaryotic Membrane Vesicles, J. Mol. Microbiol. Biotechnol. 23 (2013) 131–141.

YC-1 - CAPILLARY ELECTROPHORESIS BASED GLYCAN ANALYSIS OF TRYPTIC DIGESTED SERUM PROTEINS SEPARATED BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

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ABSTRACT

Lung adenocarcinoma is one the most common type of cancer in the world, yet it is difficult to distinguish from the early symptoms of chronic obstructive pulmonary disease (COPD), which is also high in mortality statistics. Patients with COPD also have an increased risk of developing primary lung cancer [1]. Early diagnosis of the disease is essential to increase the chances of survival for the patients. Filtering willingness can be significantly increased by developing non- or minimally invasive diagnostic procedures. These methods require the discovery of more specific molecular diagnostic biomarkers, which require the development of new analytical procedures.

In this study a new method is presented for analysis of the glycan structures of several high abundant serum proteins as potential biomarkers. Thereby, the molecular alterations caused by lung cancer or COPD can be detected. In the first step, serum proteins were digested with trypsin then the resulting peptides were separated into different fractions by hydrophilic interaction liquid chromatography (HILIC). The glycopeptides were identified, and further digested with PNGase F to release their N-glycans. The deliberated carbohydrates were labeled with APTS and analyzed by capillary electrophoresis (CE). The differences in their glycan profiles can help in distinction between lung cancer and COPD, which could greatly improve the accuracy of treatments of these diseases.

REFERENCES

[1] M. Szabo, L. Hajba, R. Kun, A. Guttman, E. Csánky, Proteomic and Glycomic Markers to Differentiate Lung Adenocarcinoma from COPD, *Current Medicinal Chemistry* (2018) 25: 1

YC-2 - ENVIRONMENTAL THREATS IN PREECLAMPSIA - OLD OBSERVATIONS AND NEW CONCLUSIONS

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ABSTRACT

Preeclampsia (PE) is a serious disorder typical for human pregnancy and it constitutes one of the reasons for elevated perinatal mortality [1,2]. PE is diagnosed in the presence of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (>300 mg/24 h) after the 20th week of pregnancy or, exceptionally, immediately after childbirth. It is estimated that it affects 2–8% of pregnancies worldwide [3,4]. It can be caused by various factors, but its etiology is not fully understood. Some studies suggest that oxidative stress, lack of immunological balance, inflammation factors, and endothelial dysfunction are involved in developing symptoms of PE [5,6]. Moreover, exposure to heavy and toxic metals can also play a significant role in the occurrence of PE [7]. It was found that an increase of 1 µg/dL Pb in blood was associated with a 1.6% increase in the likelihood of PE [8]. It is also suggested that higher copper/zinc ratios in plasma is associated with increased risk of PE [1], and hypozincemia in PE may result in the generation of oxidative stress by weakening antioxidant defence mechanisms [9].

Our research focuses not only on the comparison of preeclamptic and normotensive groups applying modern analytical techniques, but also on the attempt to explain problems and environmental risk factors for pregnancy complications, including PE.

REFERENCES

[1] M. Bakacak, M. Kılınç, S. Serin, Ö. Ercan, B. Köstü, F. Avcı, H. Kıran, G. Kıran, Changes in Copper, Zinc, and Malondialdehyde Levels and Superoxide Dismutase Activities in Pre-Eclamptic Pregnancies, *Med. Sci. Monit.* 21 (2015) 2414–2420.

[2] D.M. Folk, Hypertensive Disorders of Pregnancy: Overview and Current Recommendations, *J. Midwifery Women's Heal.* 63 (2018) 289–300.

[3] Duhig, B. Vandermolen, A. Shennan, Recent advances in the diagnosis and management of pre-eclampsia, *F1000Research.* 7 (2018) 242.

[4] American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in Pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy , *Obstet Gynecol*. 122 (2013) 1122-31.

[5] I.C. Weel, R.N. Baergen, M. Romão-Veiga, V.T. Borges, V.R. Ribeiro, S.S. Witkin, C. Bannwart-Castro, J.C. Peraçoli, L. De Oliveira, M.T. Peraçoli, Association between placental lesions, cytokines and angiogenic factors in pregnant women with preeclampsia, *PLoS One*. 11 (2016) 1–15.

[6] L.G. de Oliveira, A. Karumanchi, N. Sass, Preeclampsia: oxidative stress, inflammation and endothelial dysfunction, *Rev. Bras. Ginecol. Obstet*. 32 (2010) 609–16.

[7] J.P. Elongi Moyene, H. Scheers, B. Tandu-Umba, V. Haufroid, B. Buassa-Bu-Tsumbu, F. Verdonck, B. Spitz, B. Nemery, Preeclampsia and toxic metals: A case-control study in Kinshasa, DR Congo, *Environ. Heal. A Glob. Access Sci. Source*. 15 (2016) 1–12.

[8] A.E. Poropat, M.A.S. Laidlaw, B. Lanphear, A. Ball, H.W. Mielke, Blood lead and preeclampsia: A meta-analysis and review of implications, *Environ. Res*. 160 (2018) 12–19.

[9] S. Kumru, S. Aydin, M. Simsek, K. Sahin, M. Yaman, G. Ay, Comparison of Serum Copper, Zinc, Calcium, and Magnesium Levels in Preeclamptic and Healthy Pregnant Women, *Biol. Trace Elem. Res.* 94 (2003) 105–112.

YC-3 - MULTICAPILLARY GEL ELECTROPHORESIS ANALYSIS OF N-GLYCANS

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ABSTRACT

Nowadays, there are several glycoprotein based biotherapeutics on the pharmaceutical market, such as antibodies and fusion proteins. Due to the post-translational modifications oligosaccharides are attached to the Asn297 aminoacid of the above mentioned proteins. N-glycomic analysis of these products is essential since compositional changes in glycosylation of the Fc region can lead to discrepancies in serum half-life, immunogenicity, anti-inflammatory and effector functions. For instance, terminal sialylation influences anti-inflammatory mechanisms, while the presence of high mannose type oligosaccharides leads to faster clearance and concomitantly shorter serum half-life. Glycosylation should be considered as a critical quality attribute in the biopharmaceutical industry and be inspected during every step of the manufacturing process [1,2].

Deep structural determination of these new drug modalities requires an appropriate, accurate and fast method, which is able to succeed in high-throughput analysis. After endoglycosidase-based cleavage, the released N-glycans were labeled with a fluorescence dye (APTS) and were analyzed by LED induced fluorescence detector in a multicapillary gel electrophoresis device. The individual structures were identified with the help of their GU values [3].

REFERENCES

[1] L. Hajba, A. Szekrenyes, B. Borza, A. Guttman, On the glycosylation aspects of biosimilarity, *Drug Discov. Today*, 23 (2018) 616-625.
[2] M. Szigeti, J. Chapman, B. Borza, A. Guttman, Quantitative assessment of mAb Fc glycosylation of CQA importance by capillary electrophoresis, *Electrophoresis*, 39 (2018) 2340-2343.

YC-4 - ANALYTICAL STUDY OF PHOTODEGRADATION AND PHOTOTOXICITY OF TWO ANTIHISTAMINIC DRUGS, EMEDASTINE AND KETOTIFEN

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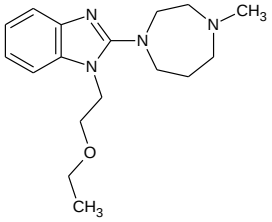
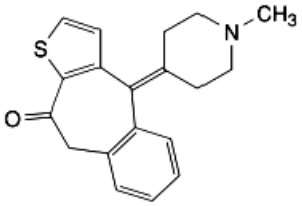
Emedastine inhibits H_1 receptors and interferes with mediator release from mast cells either by inhibiting calcium ion influx across basophil plasma membrane or by inhibiting intracellular calcium ion release within the cells. Ketotifen is also H_1 receptor antagonist that stabilizes mast cells, inhibits platelet-activating factor and acts as an eosinophil inhibitor [1].



Emedastine



Ketotifen

UV/VIS spectra of the both drugs showed maxima above 280 nm. Therefore, their photoreactivity was studied by irradiation with UV/VIS light [2]. In the next step, a standard reactive oxygen species (ROS) assay was developed as an alternative method for phototoxicity evaluation [3].

Experiments were performed using UV/VIS irradiation at doses of 459, 918, 1377, 1836, 2295, 2754, 19277 and 57831 kJ/m^2 kJ/m^2 in a Suntest CPS PLUS chamber (Atlas, Germany). The working solutions of emedastine and ketotifen were prepared in buffers of pH 3, 7 and 10. The stressed samples were quantitatively determined using two HPLC methods. Finally, the ROS test based on malondialdehyde (MDA) determination was used to indirect estimation of potent phototoxicity with quinine as a positive standard.

Photodegradation of emedastine and ketotifen was in the range 30-40% and 30-100% respectively, depending on the pH value. Under UV/VIS irradiation, emedastine and ketotifen were shown to potentiate peroxidation of linolenic acid and to generate MDA. Both drugs showed lower photoreactivity than quinine but significant enough for further examination.

REFERENCES

[1] K.L. Randall, C.A. Hawkins, Antihistamines and allergy, *Aust. Prescr.* 41 (2018) 41-45.
[2] I. Ahmed, S. Ahmed, Z. Anwar, M. Ali Sheraz, M.Sikorski. Photostability and Photostabilization of Drugs and Drug Products, *Int. J. Photoenergy*, Volume 2016, Article ID 8135608, 19 pages.
[3] K. Kim, H. Park, K. Lim, Phototoxicity: its mechanism and animal alternative test methods, *Toxicol. Res.* 31 (2015) 97-104.

YC-5 - DEVELOPING SEPARATION GELS AND METHODS FOR THE ANALYSIS OF BIOTHERAPEUTIC PROTEINS VIA CAPILLARY-SDS GEL ELECTROPHORESIS COUPLED ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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ABSTRACT

Capillary-SDS gel electrophoresis (CE-SDS) is one of the mostly used analytical purity checking methods in the biopharmaceutical industry using UV or fluorescent detection. Coupling of CE-SDS with electrospray ionization mass spectrometry (ESI-MS) would provide very important structural information for therapeutic protein examination [1]. With this aim in view, CE-ESI-MS is an exceedingly reliable, modern and high resolution system combination when considering the analysis of monoclonal antibodies (mAbs), fusion proteins and other biotherapeutic drug-related agents. Protein purification and solubilization on the other hand, requires the use of ionic detergents, yet these substances cause ionization suppression for both polar and apolar compounds in ESI-MS, based on the assumption that ESI brings out the formation of SDS clusters and cause space-charge effects in the ion trap [2]. This phenomena makes the electrospray process non compatible for MS analysis. As a denaturing agent, sodium dodecyl sulfate binds to the proteins with high affinity providing all molecules an equal net negative charge and a similar charge-to-mass ratio, but due to its severely MS interfering attribute SDS must be replaced with other detergents or eliminated prior to MS detection [3]. The goal of our research group was to develop a new gel buffer system for CGE separations and an on-line method for the removal of SDS from the background electrolyte (BGE) prior to MS analysis.

REFERENCES

[1] J. A. Loo, H. R. Udseth, and R. D. Smith - Peptide and protein analysis by electrospray ionization-mass spectrometry and capillary electrophoresis-mass spectrometry. *Analytical Biochemistry*, 179(2), (1989) 404-412.

[2] P. Hommerson, A.M. Khan, G.J. de Jong, G.W. Somsen, - Comparison of electrospray ionization and atmospheric pressure photoionization for coupling of micellar electrokinetic chromatography with ion trap mass spectrometry, *J. Chromatogr.* A 1204 (2008) 197-203.

[3] L. S. Hernández, C. Montealegre, S. Kiessig, B. Moritz, and C. Neusüß - In-capillary approach to eliminate SDS interferences in antibody analysis by capillary electrophoresis coupled to mass spectrometry, *Electrophoresis*, 38 (2017) 1044-1052.

YC-6 - GLYCOMIC ANALYSIS OF HUMAN SERUM FROM LUNG CANCER, COPD AND THEIR COMORBIDITY PATIENTS BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

Lung cancer (LC) and chronic obstructive pulmonary disease (COPD) are among the leading causes of mortality worldwide. Their high mortality rate is caused by misdiagnosis since it is difficult to distinguish LC, COPD, and their comorbidity based on symptoms only. In addition, the presence of COPD increases the risk of lung cancer development. Commonly applied diagnostic methods, including biopsy, are invasive and often serve late results in many cases. Therefore, it is important to develop a non-invasive molecular diagnostic method capable of predicting the presence of the actual ailments (lung cancer, COPD or their comorbidity) even in early stage. Recently, glycobiomarker research on serum sample utilization gained increasing importance. In this study pooled human serum samples were investigated by capillary electrophoresis-laser-induced fluorescence assay. Samples were from lung cancer (90), COPD (90) and comorbidity of COPD with lung cancer (90) patients. Sample pooling was applied in order to minimize information loss of species below the detection threshold and improve efficiency of the measurements. In this study 61 N-glycan structures were identified from healthy human serum. The N-glycosylation profiles of the pooled samples were quantitatively compared against pooled sample of 18 healthy individuals. Based on the reported comparative study, a dozen glycan structures were identified as potential glycobiomarker panel, revealing significant changes (>33% relative peak area change) between the pathological and control samples.

YC-7 - PHENOLIC COMPOSITION, ANTIOXIDANT ACTIVITY AND ACETYLCHOLINESTERASE INHIBITORY OF *MORUS ALBA* L. COMMERCIAL SAMPLES. A COMPARATIVE STUDY

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ABSTRACT

There is a growing interest in herbs and plant food supplements that represent a source of nutritional antioxidants, providing a potential strategy to diversity and enrich the daily diet. The present study aimed to evaluate phenolic composition and antioxidant activity of hydromethanolic and water extracts prepared from the commercial samples of mulberry white (*Morus alba* L.). Moreover, acetylcholinesterase (AChE) inhibitory of water extracts of mulberry white were determined. A simple and rapid high performance liquid chromatography (HPLC) method has been used for separation and determination of seven major phenolic acids (gallic, chlorogenic, rosmarinic, vanillic, *p*-coumaric, caffeic and ferulic) and three major flavonoids (quercetin, rutin, apigenin) in mulberry white commercial samples. Besides, total phenolic (TPC), total flavonoid (TFC), total phenolic acid (TPAC) and L(+)-ascorbic acid (ASA) contents were determined by spectrophotometric technique. The antioxidant activity was assessed by DPPH• scavenging activity and ferric reducing/antioxidant power (FRAP) assay, while AChE inhibitory activity was assayed by Ellman's method adopted to 96 wells microplate. The results showed that the water extracts were richer in phenolic compounds than hydromethanolic extracts of mulberry white, however rutin and chlorogenic acid were found to be predominant constituents in both extracts, while *p*-coumaric and vanillic acids were obtained in the lowest concentrations. The water extracts had also higher antioxidant activity than hydromethanolic extracts, but they did not show inhibitory activity against AChE. Besides correlation analysis showed high positive correlation between ferulic acid and AChE inhibitory activity in water extracts and correlation between the pairs: ascorbic acid and caffeic acid, ascorbic acid and total phenolic contents, and chlorogenic acid and total flavonoid contents in hydromethanolic extracts of mulberry white. Concluding, the mulberry white beverages could be an important dietary source of natural antioxidants with nutritional and pharmaceutical importance.

YC-8 - DRUG DELIVERY SYSTEMS FOR MEDICAL APPLICATIONS

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ABSTRACT

Drug delivery systems (DDS) are used in order to achieve a higher therapeutic effects of medicaments in a specific diseased site with minimal toxicological effect. Conventional methods of administration of a drug substance do not fully use the therapeutic effect of medicaments. This is due to the distribution of the drug in the body, which begins usually in the oral route. Controlling the kinetics of drug release provides an improvement in the effectiveness of the therapeutic substance and reduces the severity of side effects. In addition, DDS often also allow the active substance to be delivered accurately to the affected site [1]. DDS are designed to alter the pharmacokinetics and biodistribution of their associated drugs, or to function as drug reservoirs (for example as sustained release systems) [2].

Microorganisms have the ability to adhere on the surface of the medicals materials and next can create a biofilm on this surface. Bacteria's biofilm provides protection for the bacteria against antimicrobial agents, antibodies and defences of the human body. Over 65% of all human infections have been estimated to be biofilm-related. Bacterial cells that are an integral part of biofilm are up to 1000-fold more resistant to antimicrobial agents compared to planktonic form of bacteria [3, 4, 5].

In this study, two types of polymer coatings were prepared and applied as biopsy needles surface. High performance liquid chromatography occurred as a very good solution for monitoring of drug release from antimicrobial coatings and it was used in these studies.

REFERENCES

[1] R.C. Barbaresso, I. Rău, R.G. Zgârian, A. Meghea, M.V. Ghica, Niflumic acid-collagen delivery systems used as anti-inflammatory drugs and analgesics in dentistry, *Comptes. Rendus. Chim*., 17 (2014), 12-17.
[2] T.M. Allen, P.R. Cullis, Drug Delivery Systems: Entering the Mainstream, *Science*, 303 (2004), 1818-1822.
[3] M. Jamal, A. Wisal, A. Saadia, J. Fazal, I. Muhammad, A. N. Muhammad, H. Tahir, A. Muhammad, R. Muhammad, A. K. Muhammad, Bacterial biofilm and associated infections, *J Chinese Med Assoc*., 81 (2018), 7-11.
[4] D. Kowalczuk, G. Ginalska, J. Golus, Characterization of the developed antimicrobial urological catheters, *Int. J. Pharm.*, 402 (2010), 175–183
[5] E. Dayyoub, M. Frant, S. R. Pinnapireddy, K. Liefeith, U. Bakowsky, Antibacterial and anti-encrustation biodegradable polymer coating for urinary catheter, *Int. J. Pharm.*, 531 (2017), 205-214.

YC-9 - IONIC LIQUIDS AS AMPLIFIERS OF EXTRACTION EFFICIENCY OF SELECTED BIOGENIC AMINES FROM URINE SAMPLES BEFORE CAPILLARY ELECTROPHORESIS SEPARATION

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ABSTRACT

Ionic liquids seem to be a good alternative to traditional organic solvents because they are characterized by high chemical and physical stability, low volatility and wide specific application and reusability [1]. Biogenic amines are involved in the most common pathologies for human diseases. Determining the level of amines is important and helpful in the diagnosis of complex and varied types of cancer. In this research, to investigate the usefulness of the ionic liquid to improve the extraction efficiency of selected BAs from human urine samples using solid phase microextraction (SPME) combinated with micellar electrokinetic chromatography (MEKC). The developed SPME-MEKC technique allowed to determine the levels of BAs in the urine samples of patients. This finally allowed the signal intensity to be increased from 9 to 21 times for the tested BAs. The values of LODs were found to be 0.08 µg/mL for NA, 0.09 µg/mL for L-Tryp and 0.16 µg/mL for DA, A and L-Tyr. Whereas, the LOQ, defined as the lowest concentration which can be detected with the precision expressed by relative standard deviations (RSD%) below 15%, was 0.25 µg/mL for NA and L-Tryp and 0.5 µg/mL for DA, A and L-Tyr. The developed SPME with the addition of 1-ethyl-3-methylimidazolium tetrafluoroborate IL to the desorbing phase can be used for satisfactory simultaneous isolation of five biogenic amines: DA, A, NA, L-Tryp and L-Tyr, from real urine samples. It was confirmed that after the process of validation, the developed SPME-MEKC method meets all the FDA and ICH criteria for analytical methods [2].

REFERENCES

[1] N. Miękus, I. Olędzka, N. Kossakowska, A. Plenis, P. Kowalski, A. Prahl, T. Bączek, Ionic liquids as signal amplifiers for the simultaneous extraction of several neurotransmitters determined by micellar electrokinetic chromatography, *Talanta* 2018; vol. 186, s. 119-123.

[2] N. Kossakowska, I. Olędzka, A. Kowalik, N. Miękus, P. Kowalski, A. Plenis, E. Bień, A. Kaczorowska, M., A. Krawczyk, E. Adamkiewicz-Drożyńska, T. Bączek, Application of SPME supported by ionic liquids for the determination of biogenic amines by MEKC in clinical practice, *Journal of Pharmaceutical and Biomedical Analysis* 173 (2019) 24-30.

Abstracts of Poster Presentations

PS-1 - EPITACHOPHORESIS – NEW TOOL FOR LARGE VOLUME CONCENTRATION

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ABSTRACT

During the last years, concentration and purification of biomolecules such as nucleic acids (NAs), proteins, peptides, metabolites, and small organic ions from the complex matrix and large sample volumes are requested and widely studied. Isotachophoresis has emerged as an alternative to the solid phase extraction protocols for quantitative, easy-to-automate, and gentle NA extraction from various sample types [1]. To address the large volume sample capacity, we have developed a new device with the circular design of the separation channel based on the moving boundary electrophoresis principle. The device is suitable for focusing large sample volumes (up to 15 milliliters) and provides high recovery and concentrates the DNA in a microliter volume collection cup. In a non-sieving separation media, all DNA fragments can be focused in one zone. Whereas the migration velocity of the moving boundary and also widths of analyte zones in steady state is not constant, we introduce term epitachophoresis for this mode of migration [2].

The developed device is possible to operate in three different modes – constant power, constant voltage, and constant current. In current work, we studied the velocity of the moving boundary in all three operation modes experimentally and theoretically.

REFERENCES

[7] Datinská, V., Voráčová, I., Schlecht, U., Berka, J., Foret, F., J. Sep. Sci. 2018, 41, 236-247.

[8] Foret, F., Datinská, V., Voráčová,I., Novotný, J., Gheibi, P., Berka, J., Astier, Y., Anal. Chem. 2019, 91, 11, 7047-7053.

PS-2 - SIMPLIFY MODIFICATION MAPPING AT THE INTACT PROTEIN LEVEL WITH ON-LINE SEPARATION AND ANALYSIS BY CE-MS

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ABSTRACT

Performing on-line, high-resolution separation of proteoforms prior to mass spectrometry (MS) can be difficult. Often, one must either implement an immunocapture enrichment strategy and/or lengthy cation exchange, pre-fractionation steps prior to MS analysis. In this presentation, we demonstrate the use of capillary electrospray – mass spectrometry (CE-MS) methods for high-resolution separation of intact proteoforms.

We will use protein standards to demonstrate how CESI-MS separations of intact proteins differ from separations obtained by standard reverse phase LC-MS analysis.

We then move on to examples where this approach has been applied to analysis of biological and pharmaceutical samples where CE-MS has been used to separate and identify proteoforms where charge differences aid the separation by CE.

PS-3 - ISOLATION AND FRACTIONATION OF EXOPOLYSACCHARIDE PRODUCED BY CYANOBACTERIUM *NOSTOC* SP.

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ABSTRACT

Cyanobacteria (blue-green algae) are cosmopolitan photosynthetic prokaryotic microorganisms, which produce a large spectrum of metabolites located in cells, on their surface or released into their environment [1]. Exopolysaccharides (EPSs) are one of the most important excreted compounds, due to their special physico-chemical properties and biological activities. They can be produced in the form of a pure polysaccharide, proteoglycan or glycoprotein conjugates depending on the type of cyanobacteria [2, 3].

The isolation of EPSs from culture media, purification and subsequent fractionation play a main role in the sample processing. The obtained fractions are used for further physico-chemical characterization and structure elucidation of native EPSs [2].

In this study, EPS was isolated from a culture medium of the cyanobacterium *Nostoc* sp. (strain Hindák 2004/6). Conventional methods of isolation (ethanol precipitation) and fractionation (ion-exchange chromatography) were used. Obtain fractions were further characterised by chromatographic methods (GPC, GC-MS) and by NMR spectroscopy.

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REFERENCES

[1] T. Encarnação, A.C.C.C. Pais, M.G. Campos, H.D. Burrows, Cyanobacteria and microalgae: a renewable source of bioactive compounds and other chemicals, *Sci Prog,* 98 (2015) 145-168.

[2] C. Delattre, G. Pierre, C. Laroche, P. Michaud, Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides, *Biotechnol Adv,* 34 (2016) 1159-1179.

[3] S. Pereira, A. Zille, E. Micheletti, P. Moradas-Ferreira, R. De Philippis, P. Tamagnini, Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly, *FEMS Microbiol Rev,* 33 (2009) 917-941.

PS-4 - CHARACTERIZATION OF PROTEIN-PEPTIDE COMPOSITION OF ROYAL JELLY

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ABSTRACT

Royal jelly is a complex secretion produced by the hypopharyngeal glands of worker bees. It is exclusive nourishment for honeybee queen larvae, providing all nutrients essential for reproduction. Immunomodulating, anti-inflammatory, antioxidant, and antibacterial activities of royal jelly make it a valuable product for functional food and dietary supplements [1]. However, allergens contained in royal jelly may cause serious reactions, including life-threating anaphylaxis.

The goal of this study was the analysis of the protein-peptide composition of the royal jelly based on advanced mass spectrometry strategies. Samples of royal jelly were purified and concentrated using ProteoMiner (Bio-Rad) protein enrichment kit. All of the four obtained fractions were digested with trypsin and analyzed with nanoLC-MALDI-TOF/TOF MS (Bruker Daltonics) system. The processing of the acquired MS spectra and identification of protein/peptide compounds were performed using advanced bioinformatics tools.

The methodology proposed for this study allowed for the identification of new hypothetical proteins contained in royal jelly that may have important functions in the development of allergy in humans. Enhancing knowledge of the royal jelly composition may significantly improve understanding of both: mechanism of allergy in humans and influence on the development of bee queen larvae. Therefore, there is a need for the identification of new proteomic compounds and determination of their functions. Because of the promising results received in this study, further investigation of the royal jelly will be performed.

This work has received financial support from the Polish National Science Centre (grant number: 2016/23/D/NZ7/03949).

REFERENCES

[1] M.F. Ramadan, A. Al-Ghamdi, Bioactive compounds and health-promoting properties of royal jelly: A review, *J Funct Foods.*, 4 (2012) 39-52.

PS-5 - CAPILLARY ELECTROPHORESIS AND MALDI-TOF MS FOR RAPID AND RELIABLE IDENTIFICATION OF VIRUSES

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Virus detection and identification in complex biological samples is one of the major tasks in modern medicine. Identification of viruses usually relies on combination of methods based on cell culture, electron microscopy and antigen or nucleic acid detection. However, many of the currently used methods are expensive and time-consuming and they may lack specificity and sensitivity.

In recent years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been described as a fast and reliable technique for identification of various microorganisms including viruses. Nevertheless, sample preparation is still a crucial step in the analysis of various microorganisms in biological samples. Capillary electrophoretic (CE) methods can be advantageously used for both the concentration and separation of microorganisms. Moreover, several on-line combinations of concentration techniques, such as transient isotachophoresis (tITP) and stacking-sweeping in micellar electrokinetic chromatography (MEKC), were used to improve efficiency of CE analyses.

The objective of this study is to demonstrate the ability of method combining CE in a capillary etched with supercritical water with MALDI-TOF MS for fast and reliable identification of viruses. The viruses were first dynamically adhered onto the roughened part of the separation capillary prior their on-line concentration and separation using tITP/ MEKC. The individual fractions were then collected from the capillary and off-line analyzed by MALDI-TOF MS.

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PS-6 - BIOSENSOR FOR DETERMINATION OF CATHEPSIN S BASED ON SURFACE PLASMON RESONANCE

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ABSTRACT

Cathepsin S (CTSS) is a cysteine protease that is strongly expressed in malignant tissues. The maximum catalytic activity is at pH 6.5-7.5. CTSS has a strong proteolytic activity that causes degradation of the extracellular matrix of the connective tissue [1 -3]. SPRi is a sensitive, label-free technique that can provide data on adsorption and / or desorption phenomena in real time that occur on the metal / dielectric surface.

Two biosensors sensitive to cathepsin S were constructed, constituting the basis of new analytical methods for its determination. One of the biosensors uses the antibody as a receptor, while the other uses an inhibitor. AFM microscopy confirmed the formation of subsequent layers of biosensors. The analytical parameters of the cathepsin S methods were determined, which are based on these biosensors. They are characterized by good selectivity, precision and accuracy. The limit of detection was obtained at 0.04 ng / mL for the biosensor with antibody and 0.14 ng / mL for the biosensor with the inhibitor. The limit of quantification was 0.28 ng / mL for the biosensor with antibody and 0.84 ng / mL for the biosensor with the inhibitor. Both methods are also consistent, as evidenced by the Pearson coefficient (r = 0.997).

REFERENCES

[1] Cathepsin S Controls Angiogenesis and Tumor Growth via Matrix-derived Angiogenic Factors. B. Wang, J. Sun, S. Kitamoto, et al. J Biol Chem. 2006; 281(9): 6020 – 6029.

[2] S. Gupta, R. K. Singh, S. Dastidar, et al. Cysteine cathepsin S as an immunomodulatory target: present and future trends. Expert Opin Ther Targets. 2008; 12(3): 291 – 299.

[3] H. Kirschke, B. Wiederanders. Cathepsin S and related lysosomal endopeptidases. Methods Enzymol. 1994; 244: 500 – 511.

PS-7 - SYNTHESIS OF NEW FLUORESCENT LABELS TO IMPROVE GLYCAN ANALYSIS

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ABSTRACT

Sensitive analysis of glycans is significantly hampered by the lack of chromogenic or fluorescent groups in the glycan structures, as well as, their poor ionization properties. To overcome some of these disadvantages, the common analytical strategies in glycomics include among others derivatization of glycans.

The presented study proposed a concept of a new type of reactive, fluorescent and ionizable derivatization reagent in order to achieve the highly sensitive and selective detection of glycans. Therefore, we have designed a label carrying three tertiary amino groups and a carbonyl-reactive functionality on the pyrene core as the ideal skeleton.

The applied synthetic procedures started with simple pyrene derivatives (1-aminopyrene, 1-hydroxypyrene, and corresponding trisulfonates) and included two general parts: 1/ formation of aryl tris(chlorosulfonate) with subsequent nucleophilic substitution with diamine and 2/ multistep modification of aromatic amino or hydroxy group to the more reactive one (aliphatic primary amino or hydrazinyl groups).

This work was supported by the Grant Agency of the Czech Republic (18-00062S) and the institutional research plan (RVO: 68081715).

PS-8 - OPTIMIZATION OF PRESSURIZED HOT WATER EXTRACTION FOR ISOLATION OF ALLERGEN PROTEINS FROM ALMONDS

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ABSTRACT

An increasing demand for rapid, inexpensive and efficient allergen extraction method has emerged in recent years. Pressurized hot water extraction (PHWE) has become popular for plant proteins isolation. In the presented contribution, optimization of PHWE parameters for efficient almond protein allergens isolation was carried out, followed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Non-defatted/defatted fine almond powder was mixed with inert glass material in ratio 1:10, packed into 11ml extraction vessel and extracted for 15 minutes at pressure of 15 MPa and with temperature range 40 °C–120 °C to determine the optimal extraction temperature. Further, four extraction times (2, 5, 10 and 15 min) at optimized temperature were tested. Finally, the effect of extraction cycle numbers from 1 to 5 was studied at optimized conditions. It has been found that allergen extraction is more efficient for defatted compared with non-defatted samples. A negative effect on allergen isolation was observed with increasing temperature, 40 °C was optimum for both sample types. Extraction of allergens was quite fast when most of the allergens were extracted within 2 min; no significant increase/decrease in allergens content was detected while the extraction time was prolonged. Additional extraction cycles improved the extraction results minimally, two cycles with duration 2 min were sufficient to maximize allergen recovery. We suggest these optimal PHWE conditions: temperature 40 °C, extraction time 2 min, one cycle of extraction at 15 MPa for both types of samples.

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PS-9 - THE APPLICATION OF SPRI BIOSENSORS FOR DETERMINATION OF FIBRONECTIN IN NATURAL SAMPLES

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ABSTRACT

Fibronectin (FN) is one of the biologically active species which may be of importance as a potential biomarker. It is a glycoprotein that is essential component of the extracellular matrix (ECM). FN is present in plasma and in various types of tissue and plays important role in cell adhesion, growth, migration, differentiation, wound healing and embryonic development [1]

The aim of the study was to applied Surface Plasmon Resonance Imaging biosensors for the fibronectin detection in natural samples. The biosensor was based on the interaction of fibronectin with specific antibody [2]. Determination of fibronectin concentration was carried out in saliva samples from patients with oral diseases, plasma from patients with endometriosis, ovarian cancer, colon cancer and children with malnutrition. The control group contained saliva and plasma samples of potentially healthy people. A multiple increase in the concentration was observed in patients with endometriosis and ovarian cancer before the removal of an endometrial cyst / ovarian tumor. However, after removing the endometrial cyst / tumor of the ovary, a decrease in the concentration of fibronectin was observed with the passage of time after surgical intervention. Patients with colon cancer also had increased levels of fibronectin compared to the control group. The concentration of fibronectin in children with malnutrition was lower compared to healthy people.

Our results identify that determination of fibronectin concentration by using SPRI biosensors may be a promising method for safely and noninvasively diagnostics of diseases related with disorder in turnover of fibronectin.

REFERENCES

[1] P. Singh, C. Carraher, J.E. Schwarzbauer, Assembly of fibronectin extracellular matrix. *Annu. Rev. Cell Dev. Biol*., 26 (2010) 397-419.

[2] A. Sankiewicz, L.Romanowicz, M. Pyc, A. Hermanowicz, E.Gorodkiewicz, SPR imaging biosensor for the quantitation of fibronectin concentration in blood samples. *J. Pharm. Biomed. Anal.*, 150 (2018) 1-8.

PS-10 - COMPARISON OF OLIGOSACCHARIDE LABELING EMPLOYING VARIOUS DERIVATIZATION CHEMISTRIES

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ABSTRACT

Traditional oligosaccharide/glycan analysis involves multistep sample preparation, where labeling represents a key step before analysis using either liquid chromatography (LC) or capillary electrophoresis (CE). Labeling has several purposes such as enhancing sensitivity of different analytical detection (UV, fluorescence), since most oligosaccharides do not contain chromophoric or fluorophoric moieties. Nowadays the majority of CE/LIF-based analysis of oligosaccharides and glycans employed a labeling step using 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS).

In this work, we compare oligosaccharide labeling by two negatively charged fluorescent labels, APTS and Cascade Blue hydrazide. Effectiveness of the labeling chemistries were investigated by maltopentaose and 4-hydroxybenzaldehyde followed by LC/UV and LC-MS analysis. Finally, the more effective hydrazone formation technique was applied for oligosaccharide and *N*-linked glycan analysis by CE/LIF.

PS-11 - DETERMINATION OF ORGANIC ACIDS IN HONEYBEE VENOM USING MASS SPECTROMETRY-BASED METHODOLOGY

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ABSTRACT

Honeybee (*Apis mellifera*) venom is a natural product that poses a danger to human health, but also have therapeutic properties. Peptides and proteins constitute the majority of its dry mass [1]. However, there are also multiple low-molecular-weight compounds present in honeybee venom, which have various biological functions [2]. The aim of the study was to quantify low-molecular-weight organic acids present in honeybee venom.

In the project, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used. Chromatographic separation was accomplished by the application of a Synergi Hydro–RP column. Multiple reaction monitoring mode and negative ion mode was used. Honeybee venom samples were dissolved in water, sonicated and centrifuged. Then, due to different concentration levels of the studied organic acids in venom, two sample preparation methods were employed: solid phase extraction or dilution with solvent A. The LC-MS/MS methodology was validated to prove its reliability.

This is the first study that provided specific data on the content of organic acids in honeybee venom. The developed method enabled quantitative analysis of eight organic acids in venom samples: fumaric acid, glutaric acid, 2-hydroxybutyric acid, kynurenic acid, malic acid, malonic acid, citric acid and succinic acid. Among them, the highest abundance of citric acid was found. Moreover, the variability in the organic acid content in venom samples was observed. Factors influencing the changes in venom composition need to be studied and the composition needs to be controlled, i.a. to ensure the safety of using venom as a product for medical formulations.

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REFERENCES

[1] J. Matysiak, J. Hajduk, F. Mayer, R. Hebeler, Z.J. Kokot, Hyphenated LC-MALDI-ToF/ToF and LC-ESI-QToF approach in proteomic characterization of honeybee venom, *J. Pharm. Biomed. Anal.*, 121 (2016) 69-76.

[2] A. Klupczynska, M. Pawlak, Z.J. Kokot, J. Matysiak, Application of metabolomic tools for studying low molecular-weight fraction of animal venoms and poisons, Toxins (Basel), 10 (2018) E306.

PS-12 - AMMONIUM SALTS HIJACK N-GLYCAN SEPARATION

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ABSTRACT

At enzymatic N-glycan release with the endoglyosidase PNGase F, the suggested buffer components are controversial. Kuster *at al.* among others showed that ammonium acetate and ammonium bicarbonate buffers caused shifts in peak area ratios, generating false results.[1] Despite of these earlier reports, some recent methods still suggest ammonium salts based buffers for PNGase F release [2-5]. To clarify this contradiction, the effect of different ammonium salt containing buffers was examined on signal strength and peak area distribution.

Ammonium bicarbonate, ammonium acetate, sodium hydrogen carbonate, ammonium chloride and ammonium carbonate salts were used in 20 mM and 50 mM concentrations at 7.5 pH for PNGase F digestion. Sodium phosphate buffer and water were used as blank. Human IgG1 samples were digested overnight at 37°C. Using these buffers the samples were labeled with ATPS fluorophore dye [6] and analyzed by capillary electrophoresis.

All type of ammonium salts based buffers, except of ammonium chloride, resulted shifted peak area ratios compared to the control. The FA2G2S2 and FA2BG2S2 structures had better relative fluorescence intensity than that of the control group. For the FA2 glycan the peak area decreased. All ammonium based buffers were in 50 mM concentration; however ammonium chloride buffer has already caused shifts in peak area ratios at 20 mM concentration.

REFERENCES

1. Küster, B. and D.J. Harvey, *Ammonium containing buffers should be avoided during enzymatic release of glycans from glycoproteins when followed by reducing terminal derivatization.* Glycobiology, 1997. 7(2): p. vii-ix.
2. An, H.J., A.H. Franz, and C.B. Lebrilla, *Improved capillary electrophoretic separation and mass spectrometric detection of oligosaccharides.* J Chromatogr A, 2003. 1004(1-2): p. 121-9.
3. Triguero, A., et al., *Chemical and enzymatic N-glycan release comparison for N-glycan profiling of monoclonal antibodies expressed in plants.* Anal Biochem, 2010. 400(2): p. 173-83.
4. Kolarich, D., et al., *Comprehensive glyco-proteomic analysis of human alpha1-antitrypsin and its charge isoforms.* Proteomics, 2006. 6(11): p. 3369-80.
5. Abdul Rahman, S., et al., *Filter-aided N-glycan separation (FANGS): a convenient sample preparation method for mass spectrometric N-glycan profiling.* J Proteome Res, 2014. 13(3): p. 1167-76.
6. Váradi, C., C. Lew, and A. Guttman, *Rapid magnetic bead based sample preparation for automated and high throughput N-glycan analysis of therapeutic antibodies.* Anal Chem, 2014. 86(12): p. 5682-7.

PS-13 - SPE-MEKC METHOD AS A TOOL FOR SIMULTANEOUS DETERMINATION OF METANEPHRINES IN URINE SAMPLES

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ABSTRACT

Due to the growing incidence of cancer, many laboratories search effective methods of diagnosis and treatment controling. In many neuroendocrine tumors, there is an increase in the secretion of catecholamines by tumor cells. In this cases, in the body fluids could be observed increases the concentration of catecholamines and their metabolites [1,2].

In this study metanephrine and normetanephrine, metabolites of epinephrine and norepinephrine, in human urine samples using solid-phase extraction assisted micellar electrokinetic chromatography (MEKC) was applied [3]. In the first step of this experiment optimization of sample preparation was investigated. For isolation of metanephrine and normetanefrine from urine samples solid phase extraction (SPE) with hydrophilic-lipophilic (HLB) columns and with methanol as eluent was selected as the best approach. This method was more efficient than the SPME technique and proved to be more effective in cleaning the sample matrix of ballast substances and in the isolation of analytes from the sample matrix. Then, the analytes were separated by MEKC method. A separation buffer consisted of 50 mM SDS, 5 mM borax, 150 mM boric acid and 15% methanol. This method was linear from 0.05 to 1.0 µg/mL for both analytes with accuracy of 90.2-118.0% and 82.0-100.8%, respectively. Finally, method was used to analyze urine samples from pediatric patients with neuroendocrine tumors such as *neuroblastoma*, Wilms tumor, *ganglioneuroblastoma* and *rhabdomyosarcome*.

REFERENCES

[1] T. Hervet, E. Grouzmann, S. Grabherr, P. Mangin, Determination of urinary catecholamines and metanephrines in cardiac deaths, *Int. J. Legal Med.* 130 (2016) 995-1001.

[2] A.G. Ellis, P.T. Zegalinski, K.E. Coleman, M.J. Whiting, Dilute, derivatise and shoot: Measurement of urinary free metanephrines and catecholamines as ethyl derivatives by LC-MS/MS, *Clin. Mass Spectr.* , 4-5 (2017) 34-41.

[3] N. Miękus, I. Olędzka, N. Kossakowska, A. Plenis, P. Kowalski, A. Prahl, T. Bączek, Ionic liquids as signal amplifiers for the simultaneous extraction of several neurotransmitters determined by micellar electrokinetic chromatography, *Talanta* 186 (2018) 119-123.

PS-14 - COULD IgG N-GLYCOME OF MOTHER BE A POTENTIAL BIOMARKER FOR PREDISPOSITION TO OBESITY AND DIABETES IN OFFSPRING?

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ABSTRACT

IgG molecules influence the development of the newborn's immune system and it may modify the functional activities of their genes as an epigenetic factor. Maternal IgG have potential roles in the early life of infants, it passes though the placenta to the fetus by the neonatal Fc receptor (FcRn) [1]. Changes in the conserved N-glycosylation of IgG molecules at Asn297 could modify the activity and lifetime of the antibodies secreted against various antigens [2]. Earlier studies have been reporting on relationships between the N-glycosylation profile of IgG and the body mass index [3]. In the past decades obesity has become an epidemic disease, and it is a potential risk factor via chronic inflammation for obese pregnant women could develop gestational diabetes mellitus (GDM) and their infants often born with macrosomia [4], infant hypoglycemia and insulin resistance.

Sprague-Dawley rats were weaned from their mothers at 3 weeks of age, fed with high fat high sugar (HFHSD) or standard diet (SD). The 9 weeks old SD or HFSD female rats were mated with mature SD male rats. The N-glycosylation profiles of obese and normal weight pregnant rats' placenta, serum and their newborn's serum will be investigated.

We hypothesize that maternal IgG from obese mothers could pass the genetic predisposition for obesity to the infants through placental transport. The analytical glycomics investigations in obese pregnant rat model could prove this concept were carried out by capillary electrophoresis with laser induced fluorescence detection and will be presented.

REFERENCES

1. Simister, N.E. and A.R. Rees, *Isolation and characterization of an Fc receptor from neonatal rat small intestine.* Eur J Immunol, 1985. **15**(7): p. 733-8.

2. Kobata, A., *The N-linked sugar chains of human immunoglobulin G: their unique pattern, and their functional roles.* Biochim Biophys Acta, 2008. **1780**(3): p. 472-8.

3. Nikolac Perkovic, M., et al., *The association between galactosylation of immunoglobulin G and body mass index.* Prog Neuropsychopharmacol Biol Psychiatry, 2014. **48**: p. 20-5.

4. Xiong, X., et al., *Gestational diabetes mellitus: prevalence, risk factors, maternal and infant outcomes.* Int J Gynaecol Obstet, 2001. **75**(3): p. 221-8.

PS-15 - BIOANALYSIS OF SILICONE AND POLYACRYLATE TRANSDERMAL PATCHES – ADHESIVENESS AND DRUG RELEASE

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ABSTRACT

Polyacrylates are commonly used in pressure sensitive adhesive (PSA) formulations [1]. Recently, there has been growing interest in silicone-based drug delivery devices because of thoroughly investigated biocompatibility of these elastomers. However, the potential use of the material is limited by possibility of drug-polymer interactions, what can result in poor performance of the patch, including drug release and adhesiveness [2].

The main aim of this study was to investigate the possibility of incorporating three model drugs (cytisine Cyt, indomethacin Ind, and testosterone Tst) to silicone elastomer and to compare release rates from this matrix with observed for the polyacrylate films (Duro-Tak 87-2852, Henkel), both intended for transdermal drug delivery. Additionally, adhesive properties of the patches were evaluated.

Cyt, Ind and Tst were successfully incorporated in both type ofadhesive patches, which were prepared by blending in planetary mixer (Thinky, Japan) and casted into films 100 μm thick. Comparative analysis of the release of Cyt, Ind and Tst from silicone versus polyacrylate films was conducted using *in vitro* dissolution model (USP apparatus 5, paddle over disk at 37°C, medium: water, phosphate buffer pH 7.4 or 1% dodecyl sulphate sodium solution, respectively).

In the *in vitro* model for each active substance over 8% and 12% of the total dose was released after 24 h, from silicone and polyacrylate matrices, respectively. Adhesive properties of the polyacrylate patches measured in a peel test increased considerably in the presence of Ind, whilst the drug added to silicone formulations had minimum effect. No effect of Cyt or Tst addition on adhesiveness of the polyacrytate patches was noted.

REFERENCES

[1] Strasinger C., Raney S. G., Tran D. C., Ghosh P., Newman B., Bashaw E. D., Ghosh T., Shukla C. G. Navigating sticky areas in transdermal product development Journal of Controlled Release 233 (2016) 1–9

[2] Colas, A., Curtis, J., Hoffman, A. S., Schoen, F. J., Lemons, J. E. (2013). B - Silicones A2 - Ratner, Buddy D. I (pp. 82-91): Academic Press.

PS-16 - PRE-TREATMENT AND ELUTION OF AMINO ACIDS FROM DRIED BLOOD SPOTS FOR DIRECT DETERMINATION BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

Amino acids are basic structural units of proteins in human body and concentrations of free amino acids in body fluids are important indicators of primary and secondary metabolisms. Combination of CE with contactless conductivity detection (C^4D) is a well-established analytical scheme [1] and its applications to analyses of amino acids in body fluids were described in several publications [2]. Analysis of amino acids requires an extensive sample pre-treatment, which usually involves protein precipitation and subsequent centrifugation of the precipitated proteins. For the amino acid analysis in newborn screening, capillary blood in form of dry blood spot (DBS) is currently the biological samples of choice [3]. Nevertheless, no comprehensive study, which would address the issue of pre-treatment and elution of amino acid from DBSs was presented.

In this contribution, the process of sampling the DBSs and their subsequent elution with deionized water, methanol, acetonitrile, and optionally with mixtures thereof is shown. Various effects of the co-eluted matrix components on CE-C^4D were observed for the selected elution solutions and are comprehensively described. The CE-C^4D method was optimized for baseline separation of biogenic amino acids and indicated excellent analytical parameters and high stability of the separation system. CE-C^4D was used for the direct analysis of the DBS eluates from healthy donors with amino acid concentrations at physiological levels. Finally, the method was applied to the determination of selected amino acids from DBS samples, which are related to common inborn metabolic disorders, such as maple syrup urine disease and phenylketonuria.

REFERENCES

[1] P. Kubáň, P.C. Hauser, 20th anniversary of axial capacitively coupled contactless conductivity detection in capillary electrophoresis, *TrAC – Trends Anal. Chem.* 102 (2018) 311-321.

[2] K. Málková, E. Samcová, K. Štulík. Rapid monitoring of arrays of amino acids in clinical samples using capillary electrophoresis with contactless conductivity detection, *J. Sep. Sci.* 33 (2010) 2394-2401.

[3] K. R. Chalcraft, P. Britz-McKibbin. Newborn Screening of Inborn Errors of Metabolism by Capillary Electrophoresis−Electrospray Ionization-Mass Spectrometry, Anal. Chem. 81 (2009) 307-314.

PS-17 - A FIELD AMPLIFIED SAMPLE INJECTION (FASI) COUPLED WITH HYDROPHOBIC INTERACTION ELECTROKINETIC CHROMATOGRAPHY (HIEKC) METHOD FOR THE SIGNAL ENHANCEMENT OF SELECTED HYDROPHOBIC ANTIBIOTICS

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ABSTRACT

Electromigration techniques provide a high separation efficiency and thus are suitable for the analysis of complex hydrophobic mixtures of structurally similar compounds, which pose a number of difficulties when using other, most popular analytical methods. In our study, a field amplified sample injection and hydrophobic interaction electrokinetic chromatography (FASI-HIEKC) method has been developed for the separation of five macrolide antibiotics: spiramycin, ivermectin tylosin, josamycin, rapamycin, and one ansamycin drug – ryfamycin. HIEKC technique utilizes surfactants below their critical micelle concentration (CMC) in the presence of organic solvent to induce hydrophobic interactions between the surfactant monomers and the lipophilic analytes. By manipulating both injection sample and electrolyte composition, their pH value, and molarity, the systematic approach to maximise analyte differential electrophoretic mobility has been employed. The effect of the sample diluent and injection mode on the capillary pre-concentration techniques such as field amplified sample injection were investigated. Also, the influence of the injection of a water plug on the quantity, symmetry and high of signal analytes was demonstrated. The all analytes were completely resolved in less than 10 min in a 75 µm I.D. x 50 cm length fused-silica uncoated capillary. The analysis was performed in a 20 m*M* phosphate buffer (pH 7.1) with 60% (v/v) acetonitrile and an applied voltage of 25 kV was selected to effect the separation. The established method was validated and confirmed to be applicable to the determination of the active ingredients in a commercial pharmaceutical preparations.

REFERENCES

[1]. Kowalski, P., Olędzka, I., Plenis, A., Miękus, N., Pieckowski, M., Bączek, T., Combination of field amplified sample injection and hydrophobic interaction electrokinetic chromatography (FASI-HIEKC) as a signal amplification method for the determination of selected macrocyclic antibiotics. *Anal. Chim. Acta,* 1046 (2019)192-198.

PS-18 - DETERMINATION OF KETAMINE BY ACETONITRLE BASED SAMPLE STACKING IN COATED CAPILLARIES

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ABSTRACT

A new electrophoretic sample stacking for a sensitive determination of ketamine and its metabolites in clinical samples is introduced [1-3]. Blood plasma, or microdialysates of living tissues are laboratory pre-treated by addition of acetonitrile to clinical sample before its injection to the separation capillary. The addition of acetonitrile not only removes proteins, but also decreases the electric conductivity of highly salinized clinical samples and enables its injection into separation capillary in large volume. Then the separation voltage is switched on with the simultaneous forcing the rest of acetonitrile out of the capillary by electroosmotic flow that moves in opposite direction to electrophoretic migration of analytes. For this purpose, specially coated fused silica capillaries treated by polycationic polymers are prepared and newly tested. It is possible to improve LOD ten or hundred times by application of the described acetonitrile-based stacking and directly determine the submicromolar concentration of ketamine and its metabolites in combination with non-selective contactless conductivity detection. The described procedure was used to monitor the anaesthetic ketamine and its metabolites for study of the pharmacokinetics of the drug in laboratory rats. Ketamine is a common drug used in human and veterinary medicine that acts as an antagonist to NMDA, or opioid receptors. In contrast to other anaesthetics, it stimulates the cardiovascular and respiration centre and has substantial analgesic effects. Broader use in clinical practice is prevented by undesirable effects in the form of mental disturbances, such as terrifying dreams, disorientation and sensorial and perceptive illusions.

REFERENCES

[1] P. Tůma, P. Heneberg, Š. Vaculín, D. Koval, Electrophoretic large volume sample stacking for sensitive determination of the anti-microbial agent pentamidine in rat plasma for pharmacological studies, *Electrophoresis* 39 (2018) 2605-2611.

[2] P. Tůma, M. Bursová, B. Sommerová, R. Horsley, R. Čabala, T. Hložek, Novel electrophoretic acetonitrile-based stacking for sensitive monitoring of the antiepileptic drug perampanel in human serum, *J. Pharm. Biomed. Anal.*, 160 (2018) 368-373.

[3] P. Tůma, B. Sommerová, Š. Vaculín, Rapid electrophoretic monitoring of the anaesthetic ketamine and its metabolite norketamine in rat blood using a contactless conductivity detector to study the pharmacokinetics, *J. Sep. Sci.*, 42 (2019) 2062-2068.

PS-19 - AN ON-LINE PRECONCENTRATION STRATEGY FOR THE ELECTROPHORETIC DETERMINATION OF SELECTED PRESERVATIVES IN PHARMACEUTICALS

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ABSTRACT

A chemical compound, in order to become a preservative, must meet certain requirements established from the point of view of the patient's safety and maintaining the proper quality of the drug. The acceptable daily intake (ADI) for preservatives is determined by the Joint FAO / WHO Expert Committee, therefore the development of analytical methods of preservatives especially for parabens in pharmaceuticals has practically demanded for consumer health. In this study, a large volume sample stacking (LVSS) with polarity switching and cyclodextrin electrokinetic chromatography (CDEKC) method has been developed for the simultaneous separation and determination of 8 preservatives: methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BP), isobutylparaben (IBP), sorbic acid (SA), benzoic acid (BA), p-hydroxybenzoic acid (PHBA) in pharmaceuticals. The effects of some typical parameters such as sample volume, applied voltage, composition and pH of the running buffer and organic modifier concentration were examined and optimized. Moreover, the impact of type and concentration of cyclodextrin as electrolyte modifiers was also investigated. The detection limits of analytes for the elaborated LVSS-CDEKC method were found to be in 0.8 – 5 ng/mL range, which were around 500 times lower than normal CDEKC without preconcentration technique. All analytes were completely resolved in less than 10 minutes in an uncoated fused-silica capillary of 75 µm internal diameter (I.D) x 50 cm length. The electrophoretic separations were performed in a 25 mM tetraborate and 2 mM α-cyclodextrin system (pH=9.3) with an applied voltage of 25 kV. The established method was validated and confirmed to be applicable for the determination of the preservatives in a quality control of selected pharmaceuticals.

PS-20 - METHOD DEVELOPMENT FOR QUANTITATIVE ANALYSIS OF MODIFIED DEOXYNUCLEOSIDES AND NUCLEOSIDES IN BIOLOGICAL MATRICES

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ABSTRACT

Modified nucleosides and deoxynucleosides are products of RNA and DNA turnover. They are not metabolized and cannot be utilized for synthesis of RNA and DNA molecules. They are excreted in unchanged form. Consequently, a correlation between their elevated level in urine and pathophysiological disorders development can be expected. Increased level of modified nucleosides and deoxynucleosides was observed in such diseases as: hepatocellular carcinoma, breast cancer or urogenital cancer [1].

The aim of the study is the targeted metabolomics analysis of 11 modified nucleosides and deoxynucleosides in urine and plasma samples collected from bladder cancer patients with the use of LC-QqQ/MS technique. Since proper sample treatment influences obtained results significantly, the first task of the research covered the development of sample preparation procedure. This included optimization of separation conditions and solid-phase extraction procedure (SPE). Different chromatographic conditions were compared, including: type of stationary phase, flow rate, column temperature and gradient programme. According to SPE, differences in the sugar moiety between nucleosides and deoxynucleosides cause differences in their extraction ability and consequently difficulties with sorbent selection that allows for the extraction of nucleosides and deoxynucleosides at once. Previously, nucleosides and deoxynucleosides were more often analyzed separately. The goal was to develop method for simultaneous extraction of nucleosides and deoxynucleosides from urine and plasma. Different sorbents were evaluated by their selectivity, recovery and ability to extract modified nucleosides and deoxynucleosides. Method based on selected sorbent was further optimized.

REFERENCES

[9] M. Patejko, W. Struck-Lewicka, D. Siluk, M. Waszczuk-Jankowska, M. J. Markuszewski, Urinary nucleosides and deoxynucleosides, *Adv. Clin. Chem*, 83 (2018) 1-51

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PS-21 - A SIMPLE AND EFFICIENT PROCEDURE FOR CAPILLARY BLOOD SPIKING WITH BASIC AND ACIDIC DRUGS FOR SUBSEQUENT DRIED BLOOD SPOT ANALYSIS

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ABSTRACT

A low-cost, simple, and attractive technique for blood collection, called dry blood spot (DBS) sampling, offers a considerably less invasive alternative to intravenous collection of blood for bioanalysis. [1] Since the introduction of the DBS technique, several challenges have been faced especially with respect to quantitative analysis. Proper application of an internal standard (IS) into DBS has been recognized as one of the major challenges. This is particularly due to the minute volumes of the blood samples, analyte-blood interactions, inhomogeneous distribution of analytes in the DBS and DBS inhomogeneity, quick process of clotting, etc., which may result in low repeatability and accuracy of the subsequent analysis [2].

In the actual contribution, a simple process was developed and optimized for effective IS addition and DBS elution. A group of acidic and/or basic drugs, with different chemical characteristics, was added to the collected capillary blood in different ways and the mixture was transferred onto the DBS collection card (903®Whatman™). Various elution procedures were also examined and are discussed. Direct injection of the eluted DBS sample into CE-UV ensured a rapid and reliable determination of the drugs without the need for any additional pretreatment steps (extraction, preconcentration). The contents of analytes in the dissolved eluates were determined and recoveries >70% were achieved for the optimized elution systems with satisfactory repeatability (RSD <5%).

REFERENCES

[1] P.A. Demirev, Dried Blood Spots: Analysis and Applications, *Anal. Chem.* 85 (2013) 779−789

[2] P. Abu-Rabie, P. Denniff, N. Spooner, J. Brynjolffssen, P. Galluzzo, G.Sanders, Method of Applying Internal Standard to Dried Matrix Spot Samples for Use in Quantitative Bioanalysis, *Anal. Chem*. 83 (2011) 8779–8786

PS-22 - DEVELOPMENT OF EXTRACTION SORBENT FABRICATED BY 3D-PRINTING USING PETG-CARBON NANOTUBES COMPOSITE

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Microextraction is widely used for simplification of sample matrix and preconcentration of analyte. The most commonly used type of microextraction is SPME, however its bioanalytical application is limited, mainly due to economical reason.

The main goal of this study was to investigate the possibility to apply PETG-carbon nanotubes (PETG-CNT) composite as sorbent for extraction. PETG-CNT can be easily processed by 3D printing to achieve desired shape of a sorbent. CNT possesses sorption ability, however it is crucial to expose them on the surface of the sorbent, e.g. by sacrificial rinsing of PETG with dichloromethane. The extraction method is divided into one-hour sorption in an aqueous solvent and then desorption is carried out in a solution of acetonitrile in water (8:2, v/v). The extraction efficiency is then tested using an LC-MS equipped with a single quadrupole on the C18 column. The preliminary results of imipramine microextraction showed 37% efficiency in water samples. Such method is very time efficient as printing of 12 samples and assign takes less than 30 minutes.

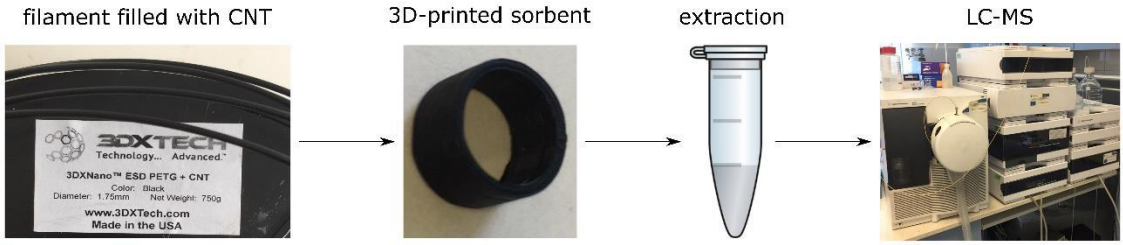

Fig. 1. General scheme for implementation of PETG-CNT composite into drug extraction.

We demonstrate fabrication, processing and extraction procedure aimed to determine imipramine and several other drugs using PETG-CNT sorbent [Fig.1.]. Water-based samples were extracted using reversible sorption to carbon nanotubes executed by changed lipophilicity of solvent and further processed by LC-MS analysis. In results we discuss parameters that influence extraction recovery and performance of our newly developed extraction protocol. The main advantage of the proposed method is low costs comparing with classic SPME approaches as well as ability to design sorbent that meets current needs, eg. mixing by magnetic stirrer.

PS-23 - A NEW METHOD FOR EPIRUBICIN ANALYSIS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTOR IN HUMAN PLASMA AND URINE AND ITS APPLICATION TO REAL SAMPLES

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ABSTRACT

Epirubicin as a doxorubicin epimer with lower risk of heart failure is widely used in the treatment of oncological patients. The development of a rapid, sensitive and simple method determination of epirubicin in biological fluids may provide information about drug pharmacokinetics and optimize patient therapy [1,2]. In this assay, liquid chromatography method with fluorescence detector (LC-FL) was used to determine of epirubicin in human urine and plasma. Preparation of samples included an extraction procedure and additional protein precipitation using 40% $ZnSO_4$ for urine samples. Solid phase extraction with Supel Select HLB cartridge and mixture of dichloromethane:2-propanol:methanol (2:1:1, *v/v/v*) to elute the adsorbed analyte, was used. Chromatographic analysis was performed on a Synergi Hydro – RP 80A (150 × 4.6 mm, 4 µm) column with mobile phase consisting of 40 mM phosphate buffer (pH 4.1) and acetonitrile (69:31, *v/v*). The excitation and emission wavelengths of the FL detector were 497 nm and 557 nm, respectively. The method was validated according to the criteria of the International Conference on Harmonization and Food Drug Administration. Linearity of this method was obtained in the range of 1-10000 ng/mL for urine and 1-1500 ng/mL for human plasma with a limit of detection of 0.25 ng and limit of quantification of 1 ng/ml for both matrices. Next, the method was used to analysis urine and plasma samples taken from a patient after 6-hour infusion of epirubicin at a dose of 150 mg/m^2. The results prove, that this method can be successfully used for drug monitoring therapy of patients with cancer and also in clinical studies.

REFERENCES

[3] A. Plenis, A. Frolow, N. Rekowska, I. Olędzka, P. Kowalski, E. Bień, A.M. Krawczyk, E. Adamkiewicz-Drożyńska, T. Bączek, Determination of Bendamustine in Human Plasma and Urine by LC-FL Methods: Application in a Drug Monitoring, *Chromatographia*, 79 (2016) 861-873.

[4] O. Maliszewska, A. Plenis, I. Olędzka, P. Kowalski, N. Miękus, E. Bień, M.A. Krawczyk, E. Adamkiewicz-Drożyńska, T. Bączek, Optimization of LC method for the quantification of doxorubicin in plasma and urine samples in view of pharmacokinetic, biomedical and drug monitoring therapy studies, *J. Pharm. Biomed. Anal.*, 158 (2018) 376-385.

PS-24 - DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN HUMAN BREAST MILK USING 3D-PRINTED SORBENT FOR EXTRACTION PRECEDING GC-MS ANALYSIS

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Human breast milk (HBM) is a natural biofluid containing nutrients and other bioactive components, which are necessary for newborn development. World Health Organization (WHO) recommends exclusive breastfeeding up to 6 months of age, with continued breastfeeding along with appropriate complementary foods up to two years of age or beyond [1]. However, atmospheric pollution and diet constituents can lead to the accumulation of polycyclic aromatic hydrocarbons (PAHs) in human milk, which in consequence can influence a baby's health. The main source of these compounds is tobacco smoke, smog and fried food [2]. In this regards it is important to monitor the concentration of PAHs in human milk and improve women's knowledge about the impact of environment and lifestyle on the quality of milk and the infant well-being.

In this work, we demonstrate the analytical approach for the determination of 16 PAHs previously reported to occur in human milk. The procedure of sample preparation is based on 3D-printed LAY-FOMM60®-based sorbent for SPME extraction (Fig. 1.). The sorption and desorption time, type of extraction solvent, temperature and shaking rate were optimized for the effective isolation of PAHs from the HBM sample. GC-MS was used for the separation, identification, and quantification of PAHs.

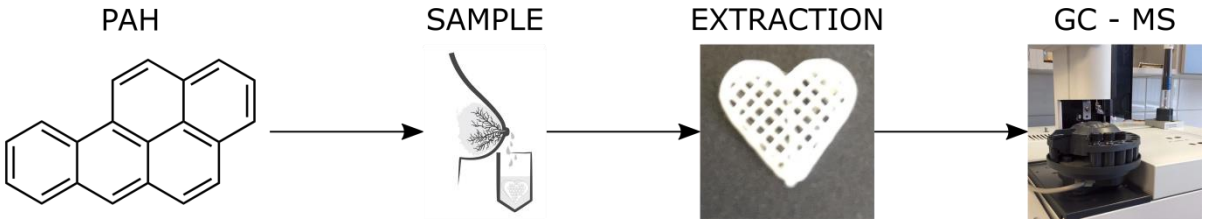

Fig. 1. General scheme for the determination of polycyclic aromatic hydrocarbons (PAHs).

The developed extraction method ensures a low cost, fast and robust analysis of PAHs in human milk. The use of this procedure allows reducing the volume of organic solvent in comparison to LLE methods. The applicability of the extraction method was demonstrated by the determination of PAHs in HBM samples collected by women from Pomeranian Voivodeship.

REFERENCES

[1] https://www.who.int/topics/breastfeeding/en/

[2] J. Pulkrabova, M. Stupak, A. Svarcova, P. Rossner, A. Rossnerova, A. Ambroz, R. Sram, J. Hajslova, Relationship between atmospheric pollution in the residential area and concentrations of polycyclic aromatic hydrocarbons (PAHs) in human breast milk, *Science of the Total Environment*, 562 (2016) 640-647.

PS-25 - ITP ANALYSIS OF AMINE DERIVATIVES OF ADAMANTANE

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ABSTRACT

The amine derivatives of adamantane (Amantadine, Rimantadine, Memantin) are widely used as antiviral drugs against various strains of flu [1] and/or for treatment of Parkinson disease. SQ109 based on etylenediamine-skeleton of adamantane is active against both drug susceptible and multi-drug-resistant tuberculosis bacteria while it is assumed that adamantane skeleton is responsible for biological activity as consequence of its high lipophilicity and hydrophobicity.

The development of determination of above mentioned compounds is paid high attention [1]. The analytical methods of molecular spectroscopy (absorption, luminescence, NIR), flow-injection analysis and potentiometry with ISE were tested [1-2] while hyphenated chromatographic methods, mostly HPLC-MS, were employed for analysis of biological samples [1]. In addition, capillary electromigration techniques (electrophoresis, isotachophoresis - ITP) were utilized for analysis of these compounds in mixture [2-4] and their sufficient resolution in CZE was achieved by addition of cyclodextrines when inclusion complexes of high stability are formed [4,5]. The application of cyclodextrins as complexing agents in capillary ITP is less often than in CZE, therefore the new analytical method for determination of Amantadine and Rimantadine in mixture was developed.

REFERENCES

[1] M.J. Navas, A.M. Jiménez, Analytical methods to determine Anti-Influenza Drugs, *Crit. Rev. Anal. Chem.* 41 (**2011**) 81-97.

[2] A.L. Revilla, J. Hamáček, P. Lubal, J. Havel, Determination of rimantadine in pharmaceutical preparations by capillary zone electrophoresis with indirect detection or after derivatization, *Chromatografia* 47 (**1998**) 433-439.

[3] R. Jannasch, Capillary isotachophoresis - a new Method in drug analysis. 3. Analytical capillary isotachophoresis for the determination of Amantadine and Rimantadine, *Pharmazie* 41 (**1986)**, 478-482.

[4] N. Reichová, J. Pazourek, P. Polášková, J. Havel, Electrophoretic behavior of adamantane derivatives possessing antiviral activity and their determination by capillary zone electrophoresis with indirect detection**,** *Electrophoresis* 23 (**2002)**, 259-262.

[5] P. R. Vashi, I. Cukrowski, J. Havel, Stability constants of the inclusion complexes of beta-cyclodextrin with various adamantane derivatives. A UV-Vis study. *South Afr. J. Chem.* 54 (**2001)** 1-18.

PS-26 - ADVANCES IN STEROID ANALYSIS ENABLED BY 3D-PRINTED SORBENTS

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ABSTRACT

Modern bioanalysis, which involves the quantitative and qualitative determination of small-molecule endogenous and exogenous substances in biological samples, is a powerful and useful tool that can generate valuable information related to many areas connected with human health and quality of life. Recent research, showing the immense potential of 3D printing, compelled our group to explore how this technology could be applied to techniques used in analytical chemistry. In particular, 3D printing offers three promising advantages: availability, low cost of materials and equipment, and the ability to fabricate objects of nearly any shape to suit the needs of a given application. In our first report we demonstrated that a commercial 3D material (LAY-FOMM) can function as a chemically active object that enables the reversible sorption of endogenous steroids.

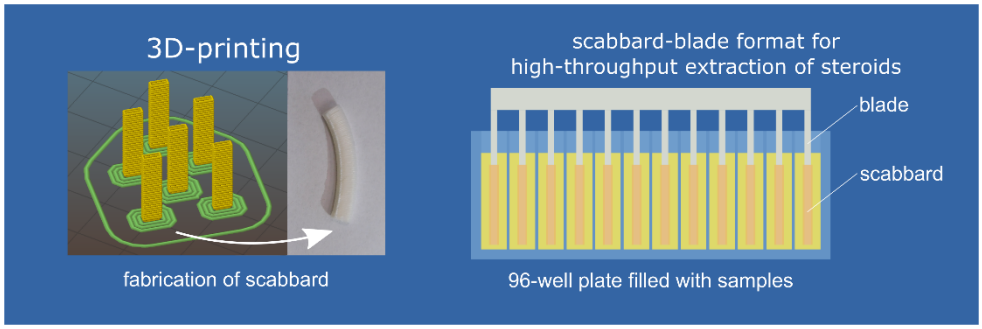

Fig.1. General information on scabbard-blade sorbent format for steroid extraction.

Next we used a 3D printer to fabricate sorbents with a scabbard-like shape for use with a 96-blade system, which, along with the use of a 96-well plate, allows multiple extractions to be performed simultaneously (Fig.1.). In order to assess the relative benefits of this 3D printed approach, we compared the performance of the proposed LAY-FOMM-based sorbent to that of the widely used C18 sorbent. Although the LAY-FOMM sorbent showed lower extraction recovery rates than the C18 sorbent, all of the other validation parameters suggest that it is suitable for use in high-throughput analysis of steroids in human plasma [1].

REFERENCES

[1] M. Belka, L. Konieczna, M. Okońska, M. Pyszka, S. Ulenberg, T. Bączek, Application of 3D-printed scabbard-like sorbent for sample preparation in bioanalysis expanded to 96-wellplate high-throughput format, Anal. Chim. Acta, 1081 (2019) 1-5.

PS-27 - ASSESSMENT OF AMINO ACID LEVELS IN NON-INVASIVE AND INVASIVE SAMPLES OBTAINED FROM BARIATRIC PATIENTS USING LC-MS/MS METHOD

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ABSTRACT

Background:

The analysis of free amino acids (AAs) in biological samples is important for diagnosing the health of individuals, because their concentrations are known to vary with various diseases [1-4]. In this study, reliable and high-throughput analytical method for AAs analysis in different biological matrices was proposed and applied to assess their concentration in non-invasive collected breath condensate samples (EBC) obtained from patients before bariatric treatment.

Materials and methods:

A robust and sensitive LC-ESI-MS/MS method for identification and quantification of free amino acids in exhaled breath condensate, plasma, saliva and urine samples, has been developed. Due to the presence of highly polar groups (-NH_2, -OH, -COOH) and their non-volatile nature, amino acids need to be derivatized with propyl chloroformate before LC-MS/MS analysis.

Results:

In our developed method, the separation of 36 of physiological amino acids was achieved in 18 min. It was observed that arginine, glutamine, serine, proline, hydroxyproline, glycine, threonine, alanine, proline, methionine, aspartic acid, histidine, lysine, norvaline, glutamic acid, tryptophan, leucine, phenylalanine, isoleucine, asparagine, cystine, cysteine and tyrosine levels are higher in plasma and saliva with reference to breath condensate samples. Only valine concentration are comparable in both saliva and EBC samples.

Conclusion:

The proposed LC-MS/MS method is rapid, sensitive and can easily be performed in aqueous media. Further it does not require any pre-purification, cleanup and/or lyophilization steps before analysis. The method developed has wide applications for the routine analysis of amino acids in non-invasive breath samples, without the need for blood collection.

REFERENCES

[1] Chace D.H., Hillman S.L., Lorey F., Cunningham G.C., *Clin. Chem.* 44, 2405–2412 (1998).

[2] Wang T.J., Larson M.G., Vasan R.S. et al., *Nat. Med.* 17, 448-454 (2011).

[3] Yosida H., Kondo K., Yamamoto H. et al., *J. Chromatogr. B* 998-999, 88-95 (2015).

[4] Konieczna L., Pyszka M., Okońska M., Niedźwiecki M., Bączek T., *J. Chromatogr. A* 1542, 72-81 (2018).

PS-28 - AMINO ACIDS AS NON-INVASIVE BIOMARKERS IN ACUTE RESPIRATORY DISTRESS SYNDROME

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ABSTRACT

Background: Acute respiratory distress syndrome (ARDS) is a live threatening condition which require intensive care including mechanical ventilation. There are numerous diseases that can lead to ARDS. Some causes are connected with infection (e.g. sepsis, pneumonia) while some are non-infection problems (e.g. trauma, acute pancreatitis) [1]. Exhaled breath condensate (EBC) is a non-invasive biological sample that can be obtained from mechanically ventilated patients as well as from spontaneously breathing individuals. There are known some biomarkers which may answer some clinical questions about ARDS patients [2]. The aim of our study was to investigate if amino acids might expend our knowledge about ARDS and help in clinical decision-making process towards ARDS patients.

Materials and methods: EBC was obtained from patients undergoing mechanical ventilation due to ARDS as well as from healthy individuals. Sampling was performed with Turbo DECCS equipment in -10^{o}C. Amino acids profiling was performed with LC/MS/MS after derivatization with propyl chloroformate.

Results: parameters connected with respiration (PaO2, pH) as well as function of particular organs (creatinine, SOFA, CRP) are correlated with levels of acidic amino acids (Asp, Glu) – higher concentration in EBC of these compounds are correlated with better general condition. In addition, levels of sulphuric amino acids in EBC correlates with better lung function among patients.

Conclusion: This pilot study suggest that amino acids can be important as biomarkers of ARDS and perhaps can help in monitoring of these patients. Further studies should answer if these compounds are truly significant in ICU management.

REFERENCES

[1] B. A. McNicholas, G. M. Rooney, J.G. Laffey,. Lessons to learn from epidemiologic studies in ARDS, *Current Opinion in Critical Care*, 24 (2018) 41-48.

[2] S. M. Metwaly, A. Cote, S. J. Donnelly, M. M. Banoei, A. L. Mourad, B. W. Winston, Evolution of ARDS biomarkers: Will metabolomics be the answer? *American Journal of Physiology. Lung Cellular and Molecular Physiology* 315 (2018), L526-L534.

PS-29 - DECREASED LEVEL OF VITAMIN D IN OBESITY PATIENTS MEASURED BY THE LC-MS/MS METHOD

Anna Krawczyńska[1], Lucyna Konieczna[1], Maria Skrzypkowska[2], Janusz Siebert[3], Magdalena Reiwer-Gostomska[3], Piotr Gutknecht[3], Łukasz Kaska[4], Justyna Bigda[4], Maria Proczko-Stepaniak[4], Tomasz Bączek[1]

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ABSTRACT

Background:

Obesity is associated with micro- and macronutrients or vitamins level [1]. One of the most important is vitamin D, its deficiency may cause osteoporosis, osteomalacia, autoimmune diseases (type 1 diabetes, Crohn's), schizophrenia, depression or cancer (breast, colon etc.) [1,2]. The principal aim of the study was to develop and validate of reliable quantification method for vitamin D levels at low sub-nanogram concentrations using small volume of plasma (250 µL). Plasma samples intended for research were collected from 60 patients qualified for bariatric surgery at the Department of General, Endocrinological and Transplant Surgery of GUMed.

Materials and methods:

The optimized LC-MS/MS method in positive mode preceded by dispersive liquid-liquid microextraction (DLLME) technique was applied for the determination of six vitamins: cholecalciferol (D_3), calcifediol ($25OHD_3$), calcitriol $1,25(OH)_2D_3$, ergocalciferol (D_2), ercalcidiol $25OHD_2$, alfacalcidol ($1\alpha OHD_3$), calcitriol $1\alpha,25(OH)_2D_3$ in human plasma samples within 15 minutes. Mobile phase was consisted of water (phase A) and acetonitrile (phase B), both with 0.1% formic acid, flow rate 0,3 mL/min. Separation was performed using Pursuit 3 (150 x 3,0 mm) column thermostated at 40°C temperature. The parent and daughter m/z ions were monitored by MS/MS in SRM mode.

Results: Obese subjects had significantly lower vitamin D concentrations than did age-matched control subjects. BMI was inversely correlated with plasma vitamin D concentrations in obese subjects.

Conclusion:

Obesity-associated vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D from cutaneous and dietary sources because of its deposition in body fat compartments.

REFERENCES

[1] M. Wacker and M. F. Holiack, Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation, *Nutrients* 5 (2013) 111–148.

[2] C. W. Compher, K. O. Badellino, and J. I. Boullata, Vitamin D and the bariatric surgical patient: A review, *Obes. Surg.* 18 (2008) 220–224.

PS-30 - THE EFFECT OF OMEGA-LOOP GASTRIC BYPASS SURGERY ON SERUM AMINO ACIDS CONCENTRATION

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ABSTRACT

Obesity is associated with changes in amino acid (AA) profiles [1,2], some of which are linked to gut microbiota dysbiosis [1], insulin resistance, cardiovascular disease risk or dyslipidaemia [2]. Omega-loop gastric bypass (OLGB) is a type of bariatric surgery used in obesity treatment and it leads to remission of type 2 diabetes and improvement of blood cholesterol, triglycerides and C-reactive protein levels [3]. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyse AAs in serum of patients with morbid obesity, before and 6-9 months after the surgery, to assess OLGB impact on circulating AAs. The control group were lean, healthy subjects. Glucose metabolism was evaluated, and we found correlation between branched-chain amino acids (BCAAs) and homeostatic model assessment (HOMA) index of insulin resistance ($p = 0.007$). Differences in AAs profiles were assessed using one way-analysis of variance (ANOVA) followed by principal component analysis (PCA). The pre-OLGB patients were differentiated form other groups due to high levels of BCAAs (leucine and isoleucine), L-2-aminobutyric acid and glutamic acid and lowered tryptophan, ornithine, taurine, aspartic acid and proline levels (PC2, 8,3%; PC3 6.2%). Contrastingly, post-OLGB patients were similar to controls. This suggests that OLGB restores correct AAs concentrations, which may in turn contribute to further positive effects such as the normalization of glucose metabolism.

REFERENCES

[10] R. Liu, J. Hong, X. Xiaoqiang et al., Gut Microbiome and Serum Metabolome Alterations in Obesity and After Weigh-Loss Intervention, *Nat Med* 23 (2017) 859-868.

[11] W. Hu, L. Sun, Y. Gong et al., Relationship between Branched-Chain Amino Acids, Metabolic Syndrome, and Cardiovascular Risk Profile in a Chinese Population: A Cross-Sectional Study, *Int J Endocrinol* 2016 (2019) 8173905.

[12] A. Mika, P. Stepnowski, L. Kaska et al., A Comprehensive Study of Serum Odd- and Branched-chain fatty acids in patients with excess weight, *Obesity* 24 (2016) 8;1669-1676.

PS-31 - OPTIMIZATION OF ANALYTICAL CONDITIONS FOR THE ANALYSIS OF SELECTED IMMUNOSUPPRESSANTS IN SERUM SAMPLES

Anna Roszkowska[1], Alina Plenis[1], Ilona Olędzka[1], Piotr Kowalski[1], Natalia Miękus[1,2], Natalia Treder[1], Tomasz Bączek[1]

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ABSTRACT

Immunosuppressive drugs (immunosuppressants) are mainly used in transplantations, however, due to their anti-inflammatory and anti-proliferative properties, the application of those drugs in coronary stents has gained increasing attention over the last years. Immunosuppressants have narrow therapeutic window and strong plasma protein binding. Moreover, the concentration of drugs released from coronary stents into the bloodstream is very low, therefore it is necessary to monitor the level of those drugs in individual patients.

The aim of this work was to develop sensitive and efficient analytical method for the extraction and determination of immunosuppressants in biofluids. In order to select the most efficient extraction approach, three techniques: SPE (solid phase extraction), SPME (solid phase microextraction) [1] and DLLME (dispersive liquid-liquid microextraction) [2] were used for the extraction of selected immunosuppressants (tacrolimus (TAC), novolimus (NOV), sirolimus (SIR) and everolimus (EVE)) from PBS solution and serum samples. The analysis were carried out by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with the use of Kinetex® C18 column (50 x 2.1 mm, 1.7 µm) in a positive ionization mode. The total analysis time was 6.5 min. The mobile phase A was composed of water with 20 mM ammonium acetate and 0.5% formic acid, and mobile phase B was composed of methanol with addition of 20 mM ammonium acetate and 0.5% formic acid. The most efficient extraction method for analysis of immunosuppressants in serum samples turned out to be DLLME with the use of 1 mL mixture of ethanol (dispersing solvent) and chloroform (extraction solvent) in a ratio of 8:2 (v/v). Alternatively, SPME with C18 extraction phase and a mixture of methanol, acetonitrile, and water (40:40:20, v/v/v) with 0.1% formic acid used as desorption mixture could be applied for the extraction of drugs from biofluids.

REFERENCES

[1] A. Roszkowska, M. Tascon, B. Bojko, K. Goryński, P.R. Dos Santos, M. Cypel, J. Pawliszyn, Equilibrium ex vivo calibration of homogenized tissue for in vivo SPME quantitation of doxorubicin in lung tissue, *Talanta* 183 (2018) 304–310

[2] L. Konieczna, A. Roszkowska, M. Niedźwiecki, T. Bączek, Hydrophilic interaction chromatography combined with dispersive liquid-liquid microextraction as a preconcentration tool for the simultaneous determination of the panel of underivatized neurotransmitters in human urine samples, *JCA* 1431 (2016) 111-121.

PS-32 - A RAPID AND SIMPLE METHOD FOR ESTIMATION OF CHEMICALLY-INDUCED MITOCHONDRIAL TOXICITY IN *CHLAMYDOMONAS REINHARDTII* CELLS

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ABSTRACT

The development of science and technology is associated with the intensive use of various chemical compounds, including herbicides, detergents or pharmaceuticals. Large amounts of chemicals reach natural ecosystems *via* municipal or industrial sewage. Assessment of toxic potential of the chemicals towards plants and animals is one of the most important tasks of eco-monitoring and eco-protection.

Mitochondrial membrane potential (MMP), monitored *in vivo* in mammalian cells, is one of the indicators of chemicals cytotoxicity. To measure MMP, the fluorescent dye JC-1 is commonly used. However, using JC-1 in plant or algal cells can be, hindered by the presence of the cell wall – a potential barrier for dye molecules, and chlorophyll, which exhibits autofluorescence.

The aim of our study was to develop the complete protocol for MMP measurement in the cells of the model green alga *Chlamydomonas reinhardtii,* as a tool for estimation of chemically-induced mitochondrial toxicity. To confirm the usefulness of the developed protocol, we used diclofenac (DF), a non-steroidal anti-inflammatory drug that is a common contaminant detected in water reservoirs worldwide and that has been found to be toxic for plants and algae. The results indicate that our protocol enables observations of MMP interruptions, caused by DF in *Chlamydomonas reinhardtii* cells, and that MMP was reduced in dose dependent manner. The statistical analyses confirmed that the results are reproducible, and that the method is sensitive and suitable for toxicological studies.

This work was supported by the National Science Centre, Poland [grant UMO-2016/23/B/NZ9/00963].

PS-33 - THE APPLICATION OF LC-FL METHODS FOR THE QUANTIFICATION OF SELECTED ANTHRACYCLINE ANTIBIOTICS IN HUMAN PLASMA AND URINE SAMPLES

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ABSTRACT

Anthracycline antibiotics, such as doxorubicin and idarubicin, are widely used drugs in a therapy of various types of cancer, including leukemia, Hodgkin's disease, and lung cancer, among others. However, clinical utility of these cytostatic drugs is hampered by serious side effects mainly related to the cardiotoxicity. Therefore, it is important to monitor the level of these agents in biological fluids in order to optimize the efficacy of therapy while minimizing drug's adverse effects [1,2].

The aim of the study was to develop simple, accurate, and precise liquid chromatography with fluorimetric detection (LC-FL) for quantification of doxorubicin and idarubicin in human plasma and urine samples. In addition, various sample preparation procedures, namely protein precipitation, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) were tested for effective extraction of drugs from biological matrices, and finally SPE with HLB cartridges was selected.

The chromatographic conditions for the analysis of anthracycline drugs by LC-FL were optimized. The most effective separation of analytes was achieved using a Discovery HS-C18 column and a mixture of acetonitrile and 0.1% formic acid in water as mobile phases. The developed LC-FD methods were validated according to the FDA and ICH requirements. The linearity was confirmed over the concentration range of 1-1000 and 1-25000 ng/mL for doxorubicin in plasma and urine samples, respectively. For idarubicin, the linearity range was 1-750 and 1-200 ng/ml for plasma and urine samples, respectively. The absolute extraction recoveries were above 81.86% for both drugs. Finally, the optimized SPE-LC-FL protocols were successfully applied for monitoring of doxorubicin and idarubicin levels in biological samples of pediatric cancer patients.

REFERENCES

[1] A. Plenis, A. Frolow, N. Rekowska, I. Olędzka, P. Kowalski, E. Bień, M. Krawczyk, E. Adamkiewicz-Drożyńska, T. Bączek, *Chromatographia*, 79 (2016) 861-873.

[2] O. Maliszewska, A. Plenis, I. Olędzka, P. Kowalski, N. Miękus, E. Bień, M. Krawczyk, E. Adamkiewicz-Drożyńska, T. Baczek, *J. Pharm. Biomed. Anal.*, 158 (2018) 376-385.

PS-34 - POSTOPERATIVE CHANGES IN SERUM FREE FATTY ACIDS CONCENTRATION AFTER LAPAROSCOPIC SLEEVE GASTRECTOMY AND OMEGA LOOP GASTRIC BYPASS

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ABSTRACT

Introduction. Serum free fatty acids (FFA) are chronically elevated in obese people as a result of dysfunctional lipid metabolism and increased lipolysis in adipose tissue, that leads to an inflammatory state, lipotoxicity, hypertriglyceridemia and insulin resistance [1]. Laparoscopic sleeve gastrectomy (LSG) and omega loop gastric bypass (OLGB) are the bariatric procedures with comparable short-term weight loss and metabolic effects. Recent studies showed that in patients undergoing LSG the FFA levels decreased 3 months after surgery and normalize approximately one year after surgery [2,3]. About the OLGB such data is not available. Therefore, the aim of this study was to compare serum FFA levels in patients before and 3 months after OLGB and LSG.

Methods. Serum non-esterified fatty acids (NEFA) in 15 obese patients undergoing LSG and 22 patients undergoing OLGB were determined using a HR Series NEFA HR (2) assay kit prior to bariatric surgery and 3 months after.

Results and conclusion Three months after bariatric operations a significant reduction (P <0,001) in FFA levels were found both with LSG and OLGB compared with pre-surgery concentrations. A more significant decrease was observed in the case of the OLGB (53.4%) compared with LSG (36.6%). A greater decrease in the FFA concentration may be associated with a reduction in the time of passage of food and fat absorption in case of OLGB, which may indicate that OLGB leads to better improvement of lipid metabolism compared to the LSG but the mechanisms require further study.

REFERENCES

[1] A. Mika, L. Kaska, M. Proczko-Stepaniak, A. Chomiczewska, J. Swierczynski, R.T. Smolenski, T. Sledzinski, Evidence That the Length of Bile Loop Determines Serum Bile Acid Concentration and Glycemic Control After Bariatric Surgery, *Obes. Surg.*, 28 (2018) 3405–3414.

[2] J.M. Ramón, S. Salvans, X. Crous, S. Puig, A. Goday, D. Benaiges, L. Trillo, M. Pera, L. Grande, Effect of Roux-en-Y Gastric Bypass vs Sleeve Gastrectomy on Glucose and Gut Hormones: A Prospective Randomised Trial, J. *Gastrointest. Surg.*, 16 (2012) 1116–1122.

[3] C. Lin, V. Våge, S.A. Mjøs, O.M. Kvalheim, Changes in Serum Fatty Acid Levels During the First Year After Bariatric Surgery, *Obes. Surg.*, 26 (2016) 1735–1742.

PS-35 - DIRECT IMMERSION-SINGLE DROP MICROEXTRACTION COUPLED IN-LINE WITH CAPILLARY ELECTROPHORESIS FOR THE ANALYSIS OF IBUPROFEN, NAPROXEN AND KETOPROFEN

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ABSTRACT

Direct immersion (DI)-single drop microextraction (SDME) is one of the liquid phase sample pretreatment techniques in-line coupled with capillary electrophoresis (CE). It was applied for the sensitive analysis of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, naproxen, and ketoprofen.

The acidic analytes in an acidified sample donor solution were extracted into a basic acceptor drop phase (sodium tetraborate titrated with 1 M NaOH to pH 9.8) covered with a thin organic layer (n-octanol) attached to the tip of a separation capillary. Using a coating solution (5 vol% ODTS and 0.1 vol% acetic acid in ethanol) as described in the previous report [1,2], the inlet tip surface of the capillary was hydrophobically coated to ensure the stability of the drop throughout the entire extraction. The in-line SDME was carried out at 25°C with a commercial PA 800 Plus CE instrument equipped with an absorbance detector at 214 nm.

Obtained results demonstrated that 3-phase DI-SDME was simple and efficient for NSAID enrichment and matrix removal. Due to the small dimensions of the acceptor and the organic phases (which was optimized taking into consideration of the drop stability, enrichment factors, and reproducibility), the enrichment factors of 40-100 were obtained from a 5-min extraction.

SDME in-line coupled with CE using a simple UV/VIS detector could serve as a new alternative green analytical method, which requires only small amounts of sample and solutions and consumes less time (compare to standard off-line extraction protocols). Indeed, SDME-CE can be considered a promising approach for the analysis of NSAIDs in bio-fluid samples or drug-contaminated drinking water supplies.

REFERENCES

[1] Choi, K., Kim, S. J., Jin, Y. G., Jang, Y. O., Kim, J. S., Chung, D. S., Anal. Chem. 2009, 81, 225-230.

[2] Choi, K., Kim, J., Chung, D. S., Bioanalysis 2011, 3, 799-815.

PS-36 - ANALYTE FOCUSING BY MICELLE COLLAPSE FOR LIQUID EXTRACTION SURFACE ANALYSIS COUPLED WITH CAPILLARY ELECTROPHORESIS OF NEURAL ANALYTES ON A SOLID SURFACE

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ABSTRACT

Liquid extraction surface analysis (LESA) has an advantage of directly sampling analytes on a surface [1], thus avoiding unnecessary dilution by homogenization of the bulk sample commonly practiced in solid sample analysis [2]. By combining LESA with capillary electrophoresis (CE), the additional advantage of separating analytes before detection can be accomplished [3]. For neutral molecules, micellar electrokinetic chromatography (MEKC) needs to be used [4]. Since the detection sensitivity of CE in general suffers from the small capillary dimension, analyte focusing by micelle collapse (AFMC) was employed [5] for enhanced extraction in LESA and sample preconcentration for MEKC. In addition, using a commercial CE instrument, the LESA process was performed much faster and more reliably compared to our first demonstration of LESA-CE using a homemade CE setup [3]. Three neutral water-insoluble pesticides sprayed on an apple skin were directly extracted, preconcentrated, and analyzed by the automated LESA-AFMC-MEKC with high sensitivity in ten minutes. The relative standard deviations of the migration times and peak heights were 0.8–2.1% and 1.2–3.0%, respectively when ametryn was used as an internal standard. The limits of detection obtained with UV absorbance at 200 nm were 1.8–6.4 ppb.

REFERENCES

[1] Comi, T. J., Makurath, M. A., Philip, M. C., Rubakhin, S. S., Sweedler, J. V., MALDI MS Guided Liquid Microjunction Extraction for Capillary Electrophoresis−Electrospray Ionization MS Analysis of Single Pancreatic Islet Cells, *Anal. Chem.*, 89 (2017) 7765-7772.

[2] Zhang, S., Yang, X., Yin, X., Wang, C., Wang, Z., Dispersive liquid–liquid microextraction combined with sweeping micellar electrokinetic chromatography for the determination of some neonicotinoid insecticides in cucumber samples, *Food Chem*., 133 (2012) 544-550.

[3] Sung, I. H., Lee, Y. W., Chung, D. S., Liquid extraction surface analysis in-line coupled with capillary electrophoresis for direct analysis of a solid surface sample, *Anal. Chim. Acta*, 838 (2014) 45-50.

[4] Quirino, J. P., Terabe S., *Science*., Exceeding 5000-Fold Concentration of Dilute Analytes in Micellar Electrokinetic Chromatography, 282 (1998) 465-468.

[5] Quirino, J. P., Neutral analyte focusing by micelle collapse in micellar electrokinetic chromatography, *J. Chromatogr. A*, 1214 (2008) 171–177

PS-37 - MICROFLUIDIC CHIP FOR ANALYSIS WITH PHOTON-UPCONVERSION NANOPARTICLES

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ABSTRACT

Microfluidic devices, combined with various bioanalytical and optical sensors, present an example of a high-throughput sampling tool in many chemical, biological, and biomedicine applications. In droplet-based devices trains of water microdroplets (1 pL to 1 μL) serve as vessels for delivering drugs and nutrients as well as for encapsulating of biologically active particles and cells. Stabilized microdroplets can be incubated off-chip and reintroduced into the other microfluidic environment for further processing and analysis [1].

Upconverting nanoparticles (UCNPs) represent an ideal luminescent label since they possess negligible autofluorescence and high photostability compared to the organic fluorophores or quantum dots. Moreover, the excitation wavelength (near-infrared) can be easily separated from the emission, which can be tuned by the doping lanthanide ions. Recently, UCNPs were introduced for digital immunoassays [2, 3] providing higher sensitivity than conventional approaches thanks to the excellent properties of UCNPs. The main benefits in the combination of droplet microfluidics with photon-upconversion nanoparticle sensing are high-throughput screening in biochemical applications, ease of automation, and only minute consumption of reagents.

Here we report novel instrumentation including open-source epiluminescence detector for recording photon-upconversion luminescence from water microdroplets generated in a polydimethylsiloxane (PDMS) microfluidic chip. As a model biochemical experiment, the activity of β-glucosidase was measured. The introduced approach opens new possibilities for automation of digital bioaffinity assays and single cell experiments [4].

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REFERENCES

[1] L. R. Shang, Y. Cheng, Y. Zhao, Emerging Droplet Microfluidics, *J. Chem. Rev.* 117 (2017) 7964-8040.

[2] H. H. Gorris, O. S. Wolfbeis, Photon-Upconverting Nanoparticles for Optical Encoding and Multiplexing of Cells, Biomolecules, and Microspheres, *Angew. Chem.-Int. Edit.* 52 (2013) 3584-3600.

[3] A. Hlaváček, Z. Farka, M. Hübner, V. Horňáková, D. Němeček, R. Niessner, P. Skládal, D. Knopp, H.H. Gorris, Competitive Upconversion-Linked Immunosorbent Assay for the Sensitive Detection of Diclofenac, *Anal. Chem.* 88 (2016) 6011-6017.

[4] Z. Farka, M.J. Mickert, A. Hlaváček, P. Skládal, H.H. Gorris, Single Molecule Upconversion-Linked Immunosorbent Assay with Extended Dynamic Range for the Sensitive Detection of Diagnostic Biomarkers, *Anal. Chem.* 89 (2017) 11825-11830.

PS-38 - DEVELOPMENT OF TRIMETHYLSILYL ACETATE BASED COATINGS FOR BIOAPPLICATIONS USING PLASMA OF RF CAPACITIVELY COUPLED DISCHARGE

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ABSTRACT

Plasma-polymerized organosilicon coatings have been playing an important role in many research studies due to their wide range of applications. These materials are perspective for industrial applications as protective coatings of metals and plastic substrates, anti-reflection coatings for solar cells, water-repellent surfaces, barrier films etc. [1-5].

Surfaces based on organosilicon precursors prepared by PECVD also exhibit specific properties that make them interesting for bioapplications as well. Surfaces modified by depositing organosilicon coating optimizing cell attachment, cell proliferation and protein adsorption have a great potential in BioMEMS microfabrication, treatment of cell-culture dishes and biosensors or creation of implants with biocompatible surface [6-9].

In the present study, low pressure RF capacitively coupled discharge in mixture of trimethylsilyl acetate (TMSA) monomer with oxygen, argon or nitrous oxide was used to develop thin solid films suitable for medical applications. This study is focused primarily on research of properties of resulting coatings (chemical composition, surface wettability and surface structure) in dependence on discharge parameters. Resistance of plasma polymers to liquid environment is the critical parameter that determines usability of these materials for bioapplications, Since reactions of organosilicon-based coatings to liquid medium may result in changes of chemistry and undesirable delamination effect, stability of deposited TMSA plasma polymers in phosphate buffered saline (PBS) was verified as well. The aim of present work is to optimize PECVD process to create organosilicon coatings doped by C=O functionalities with sufficient stability in PBS medium.

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REFERENCES

[1] L. Zajíčková, V. Buršíková, Z. Kučerová, D. Franta, P. Dvořák, R.Šmíd, V. Peřina, A. Macková, Deposition of protective coatings in rf organosilicon discharges, *Plasma Sources Science and Technology*, 16 (2007) S123–S132.

[2] Y.-M. Ko, H.-C. Choe, S.-C. Jung, B.-H. Kim, Plasma deposition of a silicone-like layer for the corrosion protection of magnesium, *Progress in Organic Coatings*, 76 (2013) 1827–1832.

[3] V. Buršíková, P. Sládek, P. Sťahel, L. Zajíčková, Improvement of the efficiency of the silicon solar cells by silicon incorporated diamond-like carbon antireflective coatings, *J. Non-Cryst. Solids*, 299–302 (2002) 1147–1151.

[4] Y. S. Choi, J. S. Lee, S. B. Jin, J. G. Han, Super-hydrophobic coatings with nano-size roughness prepared with simple PECVD method, *J. Phys. D: Appl. Phys.*, 46 (2013).

[5] M. Shahpanah, S. Mehrabian, M. Abbasi-Firouzjah, B. Shokri, Improving the oxygen barrier properties of PET polymer by radio frequency plasma-polymerized SiO_xN_y thin film, *Surf. Coat. Technol.*, 358 (2019) 91–97.

[6] A. Elagli, K. Belhacene, C. Vivien, P. Dhulster, R. Froide-vaux, P. Supiot, Facile immobilization of enzyme by entrap-ment using a plasma-deposited organosilicon thin film, *J. Mol. Catal. B: Enzym*, 110 (2014) 77–86.

[7] S. Sarapirom, J. S. Lee, S. B. Jin, D. H. Song, L. D. Yu, J. G. Han, C. Chaiwong, Wettability effect of PECVD-SiO_x films on poly(lactic acid) induced by oxygen plasma on protein adsorption and cell attachment, *J. Phys.: Conf. Ser.*, 423 (2013).

[8] J. Wei, T. Igarashi, N. Okumori, T. Igarashi, T. Maetani, B. Liu, M. Yoshinari, Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts, *Biomed. Mater.*, 4 (2009) 045002.

[9] G. R. Prasad, S. Daniels, D. Cameron, B. McNamara, E. Tully, R. O'Kennedy, PECVD of biocompatible coatings on 316L stainless steel, *Surf. Coat. Technol.*, 200 (2005) 1031–1035.

PS-39 - ETHANOL SENSOR BASED ON Eu(III) TERNARY COMPLEX OF DO3A LIGAND

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ABSTRACT

1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (H_3DO3A) as a heptadentate ligand forms a stable complex with Ln(III) aqua ions [1]. The ternary [Ln(DO3A)L] complex exhibits a high luminescence due to the antenna effect, which leads to the sensitization of Ln(III) luminescence by a fluorophore (*e.g.* L = picolinic/isoquinolic acid) via efficient energy transfer from the ligand to the Ln(III) ion. The utilization of ternary Eu(III) and Tb(III) complexes as selective dual luminescence/electrochemical sensors for the determination of bicarbonate using a substitution reaction was reported [1-2]. Recently, this idea was utilized for indirect selective determination of urea [3]:

$$\text{Urea} + 2\ H_2O \rightarrow 2\ NH_4^+ + CO_3^{2-}$$

catalyzed by the urease enzyme, whose activity can be also estimated.

Analogously, this approach was applied for the selective luminescence determination of ethanol using oxidation of NAD^+ catalyzed by the alcohol dehydrogenase enzyme (ADH, see Figure 1):

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH^+ + H^+$$

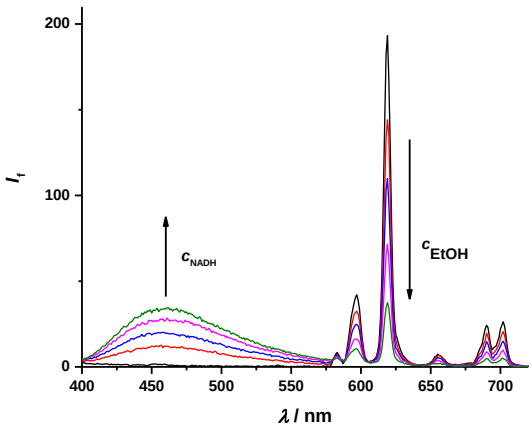

Figure Luminescence spectra of the Eu(III) ternary complex in the presence of ethanol and NAD^+ cofactor.

REFERENCES

[1] J. Vaněk, P. Lubal, P. Hermann, P. Anzenbacher P., Jr., Luminescent Sensor for Carbonate Ion Based on Lanthanide(III) Complexes of 1,4,7,10-Tetraazacyclododecane-1,4,7-Triacetic Acid (DO3A), *J. Fluorescence* 23 (2013) 57-69.

[2] J. Vaněk, F. Smrčka, P. Lubal, I. Třísková, L. Trnková, Dual carbonate sensor based on Eu(III) complex of DO3A ligand, *Monatsh. Chem.* 147(2016) 925-934.

[3] F. Smrčka, P. Lubal, M. Šídlo, The urea biosensor based on luminescence of Eu(III) ternary complex of DO3A ligand, Monatsh. Chem. 148(2017) 1945-1952.

PS-40 - FREE FLOW ISOTACHOPHORESIS DNA PURIFICATION IN CHANNEL FROM DISPOSABLE NON-WOVEN FABRIC BED

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ABSTRACT

Application of the discontinuous buffer systems for analysis of compounds in electrophoresis has been used in analytical methods for many decades. Currently, there has been a renewed interest in isotachophoresis due to its remarkable potential in extraction and focusing of nucleic acids. Our laboratory has been using free-flow electrophoresis in nonwoven beds for a number of electrophoretic modes of separation. In this work we propose free flow isotachophoresis in a strip of nonwoven fabric (approx. 4 x 170 x 1 mm) for purification and concentration of DNA. For analysis we used a lab-made platform published earlier [1]. The concentrating effect is presented using purified dsDNA extracted from salmon. Concentrating effect, determined with DNA gel electrophoresis, reached up to 50 times depending on the volume of a collected fraction. The part of the strip containing the DNA was marked by three dyes with mobilities selected to surround the mobility of dsDNA. The method was further tested for purification of dsDNA from yeast (*Saccharomyces cerevisiae*) cellular debris originating from sonication of yeast cells. The concentrating effect of the free flow isotachophoresis was reduced by broadening of dsDNA rich fraction caused by co-migrating simple organic acids coming from the sample matrix. Purification of dsDNA from proteins from sonicated yeast cells was observed with the proposed method.

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REFERENCES

[1] F. Duša, K. Šlais, New solution IEF device for micropreparative separation of peptides and proteins., Electrophoresis. 34 (2013) 1519–25. doi:10.1002/elps.201200485.

PS-41 - COMBINED PHOTOMETRIC DETECTOR UTILIZING LIGHT EMITTING DIODES, 50 nL SILICA CAPILLARY CELL, AND CCD SPECTROMETER

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ABSTRACT

Many LC methods utilize absorbance and fluorescence detectors connected in series as a versatile detection strategy for quantitation of target fluorophores and non-fluorescent molecules within single run. However, the use of two bulky detectors is not a useful strategy for capillary LC (column I. D. typically less than 300 μm), where detector should be equipped with a miniaturised detection cell and positioned as close as possible to the column outlet to keep an extra column band broadening within acceptable limits. An effort to integrate absorbance and fluorescence monitoring into a single detection cell and detector device is thus a logical step. Here we present a compact photometric detector design which uses light emitting diodes (LEDs) as the light source, customized 50 nL L-shaped silica capillary detection cell [1], and CCD spectrometer as the light detector. 265 nm LED is used as the light source for measurement of absorption while 365 nm and 470 nm LEDs are used for fluorophore excitation. Presented results demonstrate 1 mm effective optical path of the detection cell and extremely low level of stray light such that upper limit of dynamic range is more than 2.5 AU. Response of the detector is linear over three orders of magnitude of concentration. Detection capabilities are demonstrated by LODs of uracil, anthracene and fluorescein of 2×10^{-6} M, 1×10^{-8} M, and 1×10^{-9} respectively.

REFERENCES

[1] J. Šesták, J. Planeta, V. Kahle, Nanolitre-scale cell based on L-shaped silica capillary and optical fibre for absorption photometric detection in capillary liquid chromatography, *Anal. Chim. Acta,*, 1073 (2019) 99-108.

PS-42 - TEMPERATURE EFFECTS IN CAPILLARY SODIUM DODECYL SULPHATE GEL ELECTROPHORESIS

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Recent developments in new generation therapeutic proteins has become widespread. Complementing traditional low molecular weight drug therapies with therapeutic proteins such as monoclonal antibodies, fusion proteins, etc., for optimized treatment can be established against haematological and solid tumors as well as autoimmune and inflammatory diseases.[1] Checking the purity of therapeutic proteins and detecting any structural ambiguities is very important for the biopharmaceutical industry. Capillary gel SDS electrophoresis (SDS-CGE) is a high efficiency bioanalytical tool with rapid analysis time and low volume sample requirement thus an excellent tool for efficient characterization of therapeutic proteins.

According to previous studies, the choice of proper separation temperature in CE plays an important role in altering separation efficiency and selectivity. Migration times and peak asymmetry of the proteins were studied as a function of capillary temperature ranging 15-50ºC in SDS-CGE. While the migration time of the SDS-protein complexes decreased by increasing the temperature, the peak symmetry and efficiency increased with elevated temperature. Arrhenius plots were also generated to investigate the temperature dependence and activation energy requirement of protein separations in CGE-SDS.

REFERENCES

[1] Zhao, S.S. and D.D. Chen, Applications of capillary electrophoresis in characterizing recombinant protein therapeutics. Electrophoresis, 2014. 35(1): p. 96-108.

PS-43 - RETENTION AND SELECTIVITY OF SULFOBETAINE-STATIONARY PHASE IN METHANOL- AND ISOPROPANOL-RICH MOBILE PHASES

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ABSTRACT

The sulfobetaine-capillary monolithic columns (0.1mm x 150mm) were prepared by acidic hydrolysis of tetramethoxysilane in the presence of polyethylene glycol and urea and modified to zwitterionic-stationary phase by [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium hydroxide [1]. These columns were used to evaluate an effect of the mobile-phase composition on retention and selectivity of sulfobetaine-stationary phase employing acidic, basic and neutral compounds as test analytes.

The effects of the content of organic solvents (acetonitrile, isopropanol and methanol) or their mixtures in mobile phase on separation of selected analytes were evaluated under isocratic elution conditions. An addition up to 10 % (v/v) of methanol or isopropanol to the acetonitrile-rich mobile phase caused only minor changes in retention and selectivity of sulfobetaine-stationary phase. On the other hand, methanol- as well as isopropanol-rich mobile phases significantly influenced the separation of test analytes.

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REFERENCES

[1] D. Moravcová, J. Planeta, V. Kahle, M. Roth, Zwitterionic silica-based monolithic capillary columns for isocratic and gradient hydrophilic interaction liquid chromatography, *J. Chromatogr. A*, 1270 (2012) 178–185.

PS-44 - BURIED MICROFLUIDIC CHANNEL FOR SEGMENTED FLOW ANALYSIS

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ABSTRACT

Analytical systems based on microfluidics, represent a fast-developing area of research aimed at developing powerful, portable, and high throughput systems for a wide variety of applications. [1]. Here, we present a buried microfluidic channel with an integrated optical system and a thermal gradient to perform melting curve analysis [2,3]. The fabrication process of the microfluidic buried channel within a silicon wafer was based on a single level lithography and a combination of deep reactive ion etching, creating a trench that is conformably coated with optically transparent parylene. Afterward a femtosecond laser photoablation of the trench bottom is used to create the opening for isotropic Si etching by XeF_2. The working principle of device was demonstrated on the determination of melting temperature of dsDNA-SYBR Green complex in a segmented flow and the calculation of heat transfer at different flow rates. During signal processing we apply deconvolution and evaluation through several calibration procedures. Such microfluidic system has a great potential in diagnostics, DNA, protein and drug research.

REFERENCES

[1] D. Czaplewski, M. Zalalutdinov, B. Schmidt, Fabrication of flexible polymer tubes for micro and nanofluidic applications, J. Vac. Sci. Technol B., 20 (2002) 2459–2465

[2] I. Gablech, J. Somer, Z. Fohlerová et al. Fabrication of buried microfluidic channels with observation windows using femtosecond laser photoablation and parylene-C coating, Microfluidics and Nanofluidics 22 (2018) 105

[3] B.-W. S, P. Neuzil, C. Fang, et al in The 6th International Conference on Microtechnologies in Medicine and Biology, ed. O. Guenat, Lucerne, SWITZERLAND, 2011

PS-45 - SIMULTANEOUS DETECTION OF PEACH AND APRICOT DNA BY MULTIPLEX REAL-TIME PCR-HRM WITH INTERCALATING DYE

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ABSTRACT

Multiplex real-time PCR-HRM with specific primers and intercalating dye was suggested as a new method for authentication of products containing buffalo meat by Sakaridis et al [1]. It has since been used for authentication of other meat products and medicinal plant products, but not for authentication of fruit-based foodstuffs. In this work we tested this method with DNA isolated from peach and apricot fruits and with two primer pairs, whose specifity was verified by real-time PCR-HRM [2, 3]. The multiplex real-time PCR-HRM was carried out with equal amounts of DNA from both fruit species, and its sensitivity was tested with decreasing amounts of each DNA (1 ng, 100 pg, 10 pg, 1 pg). We detected peach DNA in assays with 1 ng to 10 pg of DNA, and apricot DNA was detected in assays with 1 ng to 100 pg of DNA. We were able to detect two disctinct amplicones in all assays in which both peach and apricot DNA was detected.

REFERENCES

[1] SAKARIDIS, Ioannis, Ioannis GANOPOULOS, Anagnostis ARGIRIOU a Athanasios TSAFTARIS. A fast and accurate method for controlling the correct labeling of products containing buffalo meat using High Resolution Melting (HRM) analysis. *Meat Science*. 2013, **94**(1), 84-88.

[2] SHANG, Ying, Pengyu ZHU, Kunlun HUANG, Weihong LIU, Wenying TIAN, Yunbo LUO a Wentao XU. A peach (Prunus persica)-specific gene, Lhcb2, used as an endogenous reference gene for qualitative and real-time quantitative PCR to detect fruit products. *LWT - Food Science and Technology*. 2014, **55**(1), 218-223..

[3] HAASE, Ilka, Philipp BRÜNING, Reinhard MATISSEK, Markus FISCHER, Wenying TIAN, Yunbo LUO a Wentao XU. Real-Time PCR Assays for the Quantitation of rDNA from Apricot and Other Plant Species in Marzipan. *Journal of Agricultural and Food Chemistry*. 2013, **61**(14), 3414-3418.

PS-46 - RECENT DEVELOPMENTS OF THE GUCAL APPLICATION

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ABSTRACT

Capillary electrophoresis (CE) is playing an increasingly important role in the recently emerging field of biologics. While the necessary CE instrumentation is well developed, the number of the available bioinformatics tools are limited. The GUcal app automatically calculates the glucose unit (GU) values for all sample components of interest in an electropherogram with a concomitant database search based for structural assignment [1]. The GU value calculation utilizes the well-accepted conventional concept, based on the comparison of the migration times of unknown analyte peaks with a mixture of homologous oligosaccharides (usually dubbed as "ladder") to provide migration time normalization. Direct comparison of GU values of commercially available standards (e.g., internal, bracketing or both) against GUs of unknown sample components of interest is one of the essential tasks of database mediated glycan structure assignment. However, one of the most important aspects of such structure identification is the size, reliability, and relevance of the database used for the search. The original GUcal app database had 32 entries representing all N-glycans released from the conserved ASN_{297} site of human IgG. We have recently expanded this database by integrating all publicly available CE data in the Glycostore collection (https://www.glycostore.org/) [2,3]. Thus, GUcal can now automatically search the closest matching glycan structures in the capillary electrophoresis based glucose unit subset of this large glycobiology database. The GUcal app is freely available online - www.gucal.hu - and readily facilitates capillary electrophoresis based glycan analysis.

REFERENCES

[1] Jarvas, G., M. Szigeti, and A. Guttman, GUcal: An integrated application for capillary electrophoresis based glycan analysis. Electrophoresis, 2015. 36(24): p. 3094-6.

[2] Abrahams, J.L., M.P. Campbell, and N.H. Packer, Building a PGC-LC-MS N-glycan retention library and elution mapping resource. Glycoconj J, 2018. 35(1): p. 15-29.

[3] Campbell, M.P., et al., GlycoBase and autoGU: tools for HPLC-based glycan analysis. Bioinformatics, 2008. 24(9): p. 1214-6.

PS-47 - THE STUDY OF NATURAL HONEYBEE PRODUCTS USING TARGETED METABOLOMICS METHODS

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ABSTRACT

Honey bee (*Apis mellifera)* is a species whose genome has been fully mapped. Thus, natural bee products (such as honeybee venom, pollen or royal jelly) could be the best source of novel pharmacologically active compounds to investigate due to the fact that it is possible to study these product across different 'omics' such as genomic, proteomic and metabolomic. Metabolomics studies of bee products could potentially contribute to better understanding composition and their biological activity which still remains not fully described. Therefore the performed studies aimed at analyzing selected groups of low molecular mass compounds in natural origin bee products using targeted metabolomics methods.

The analyses were carried out using 1260 Infinity (Agilent) high performance liquid chromatograph coupled with 4000 QTRAP (Sciex) triple quadrupole mass spectrometer. The metabolites quantitation was accomplished through the aTRAQ Kit for Amino Acid Analysis (Sciex) and AbsoluteIDQ p180 kit (Biocrates). The applied methods allowed to analyze over 180 metabolites from different chemical compounds classes such as: amino acids and biogenic amines, glycerophospholipids, sphingolipids and monosaccharides.

The presented research provides new information about the composition of natural bee products as well as the concentration of selected low molecular metabolites. It could contribute to the in-depth characterization of studied natural products, which are responsible for many biological and toxicological effects and also have nutritional properties. Thus, they are increasingly used in natural medicine and apitherapy.

The project received support from the National Science Centre, Poland (grant number:

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PS-48 - INFLUENCE OF 14-METHYLPENTADECANOIC ACID ON THE EXPRESSION OF LIPID METABOLISM-RELATED GENES IN LIVER HEPATOCELLULAR CARCINOMA CELLS (HEPG2)

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ABSTRACT

Fatty acids (FAs) play an important role in human metabolism, however still little is known about certain FA classes, which are present in human blood at relatively low concentrations. Some of them enhance inflammation and oxidative stress [1]. They might also modify expression of genes connected with lipid metabolism [2]. The example of such a group are branched chain fatty acids (BCFAs). BCFAs may take part in pathogenesis of certain diseases, and on the other hand, they can exert anticancer properties [3] [4] [5]. Changes in BCFAs levels are known to be present in obesity – a condition associated with chronic inflammation, oxidative stress and altered lipid metabolism. Furthermore, there is an inverse correlation between BCFAs and triacylglycerol levels [6].

The aim of our study was to assess the influence of one of BCFAs – 14-methylpentadecanoic acid (14-MPA) – on the expression of genes related to lipid metabolism: *FASN*, *SREBP1* and *SCD1* in HepG2 cells.

Cells were incubated with 14-MPA for 48 hours and then collected to isolate RNA. After performing reverse transcription, levels of mentioned genes expression were determined using real-time PCR. We observed lowered expression of *FASN*, *SREBP1* and *SCD1* in cells treated with 14-MPA in comparison to control cells.

Moreover, we confirmed the ability of HepG2 cells to intake 14-MPA from the medium by determination of lipid composition of collected cells and media using GC/MS. The amount of 14-MPA in medium after incubation was lower than prior to, while much higher in cells, which confirms the intake of investigated FA.

REFERENCES

[1] J.Y. Lee, K.H. Sohn, S.H. Rhee et al., Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4, *J Biol Chem*, 276 (2001) 16683–16689.

[2] T.A. Burns, A.K.G. Kadegowda, S.K .Duckett et al., Palmitoleic (16:1 cis-9) and cis-vaccenic (18:1 cis-11) acid alter lipogenesis in bovine adipocyte cultures, *Lipids*, 47 (2012) 1143.

[3] Y. Yang, L. Shangpei, X. Chen, H. Chen, M. Huang, J. Zheng, Induction of apoptotic cell death and in vivo growth inhibition of human cancer cells by a saturated branched-chain fatty acid, 13-methyltetradecanoic acid, *Cancer Res.*, 60 (2000) 505–509.

[4] S. Wongtangtintharn, H. Oku, H. Iwasaki, T. Toda, Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells, *J. Nutr. Sci. Vitaminol*., 50 (2004) 137–143.

[5] A. Poulos, Lipid metabolism in Zellweger's syndrome, *Prog Lipid Res*, 28 (1989) 35-51.

[6] A. Mika, P. Stepnowski, Ł. Kaska, M. Proczko, P. Wiśniewski, M. Śledzinski, T. Śledzinski, A comprehensive study of serum odd- and branched-chain fatty acids in patients with excess weight, *Obesity*, 24 (2016) 1669-1676.

PS-49 - COMPARISON OF THE CONCENTRATIONS OF METABOLIC PROFILES OF PTERINE COMPOUNDS IN URINE SAMPLES FROM PATIENTS WITH BLADDER, RENAL AND PROSTATE CANCER

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ABSTRACT

Pteridines constitute a large and structurally varied group of natural compounds involved in the biosynthetic pathways of cofactors and vitamins. What is crucial for the diagnostic function is the fact that normal somatic cells metabolize pterins differently from cancer cells. The monitoring pteridines level may have prospective value for controlling the cause of the malignant process [1].

The first goal of the presented research was a comparative analysis of metabolic profiles of selected pterin compounds between the control group of healthy people and patients with bladder, kidney or prostate cancer. The second aim of the study was a comparative analysis of pterin compounds between the studied cancer diseases. Methodology of research involved the preparation of urine samples by oxidation using I2/KI and filtered through a 0,22 μm. The chromatography analysis with using a column LiChrospher 60 RP-select B and fluorescent detector allowed for the separation and quantitative analysis of six pterins.

The obtained results indicate the possibility of differentiating healthy people and patients with prostate, kidney and bladder cancer, based on the concentration of selected pterin compounds. Statistical analysis also showed statistically significant differences between the selected pterin compounds and the investigated cancers of the urinary tract system.

REFERENCES

[1] P. Kośliński, R. Bujak, E. Daghir, M.J. Markuszewski, Metabolic profiling of pteridines for determination of potential biomarkers in cancer diseases, *Electrophoresis* 32(211) 2044-54,

PS-50 - UNDERSTANDING DISTRIBUTION OF METABOLOMICS DATA: IDENTIFYINGSTRUCTURE AND ANALYSIS OF BIMODAL METABOLOMICS DATA

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ABSTRACT

Identifying the distribution of metabolomics data is a key element for every data analysis. The distribution is important to consider if our goals are to understand the metabolomics data and to ensure that we can generate unbiased coefficients and mean values that generate reasonable predictions.

Metabolomics data generated from GC-MS or LC-MS experiments are usually non-normally distributed. Identifying metabolites with binomial distribution (recognized by the presence of two modes, each with a characteristic peak) is an important task in metabolomics data analysis as this distribution may occur either naturally from group separation, or may indicate problems with identification. Regardless of its origin a useful tool for the analysis is binomial regression which has the flexibility to fit various distributions.

The purpose of this study is to illustrate how to simply identify the structure of metabolomics data exemplified on 9 randomly selected metabolites using basic plots and how to model bimodally distributed data using a binomial regression (Bayesian Regression Models using 'Stan' – *brms* package). We also perform "ranking and selection" to identify metabolites with the greatest contribution for group separation.

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PS-51 - BAYESIAN WORKFLOW IN STATISTICAL ANALYSIS OF ISOCRATIC CHROMATOGRAPHIC DATA

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ABSTRACT

Optimization of separation conditions is an important step in the development of any analytical method in RP HPLC. The use of mathematical models to predict retention times of analytes can significantly improve the process of seeking conditions leading to the desired separation.

The aim of this work is to present a scheme of construction and analysis of the Bayesian hierarchical model of predictions of retention times of analytes [1,2]. The presented model describes publicly available chromatographic data of 1026 compounds measured under isocratic conditions in various acetonitrile contents [3]. The Stan program coupled with R were used as the platform for data analysis. These are free and generally available tools, which enable full Bayesian inference with Markov Chain Monte Carlo sampling. This methodology allows, among others, to incorporate prior knowledge about the likely values of model parameters as well as to share information across analytes. In this work we used a nonlinear Neue's model to describe the relationship between retention factor and acetonitrile content in the mobile phase. The model was parametrized in terms of retention factor in 100% water, retention factor in 100% acetonitrile and curvature coefficient. Lipophilicity was considered as the only predictor. The final model turned out to be well calibrated with the data and gives insight into behavior of analytes in the chromatographic column.

REFERENCES

[1] Kubik, Ł., Kaliszan, R., & Wiczling, P. (2018). Analysis of Isocratic-Chromatographic-Retention Data using Bayesian Multilevel Modeling. Analytical Chemistry, 90(22), 13670–13679.

[2] Gabry, J., Simpson, D., Vehtari, A., Betancourt, M., & Gelman, A. (2019). Visualization in Bayesian workflow. *Journal of the Royal Statistical Society: Series A (Statistics in Society)*, *182*(2), 389–402.

[3] http://www.retentionprediction.org/hplc/database/.

PS-52 - INFLUENCE OF SENSOR STRUCTURE ON SELECTED ANALYTICAL PARAMETERS IN TECHNIQUE OF SURFACE PLASMON RESONANCE – REVIEW

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ABSTRACT

The presentation is a theoretical summary of different sensor structures applied in Surface Plasmon Resonance technique. SPR is used in the quantitative and qualitative analysis of biomarkers and other biological active substances [1-4]. SPR imaging version called SPRI is also promising analytical tool for the determination of biologically active substances, including cancer markers. Both techniques can compete to other analytical techniques present on the market, e.g. enzyme immunoassay ELISA test, in terms of selectivity, analysis errors, as well as the time and cost of a single determination. Key point of SPRI analysis is a specific and highly selective sensor for a given biomarker, which has to be created. Markers are captured by a specific antibody or inhibitor or aptamer from serum or other body fluid, which results in an analytical signal generated by Surface Plasmon Resonance (SPRI) technique. The presentation includes characteristics of the sensors being developed, e.g. its structure, which relates to a specific type and order of different metal layers and basic substrates used. Additionally, process of materials selection will be presented. In the literature there have been found different researched sensors with several various structures, such as glass coated with different noble metal layers: mostly gold or silver containing an adhesive spacer of chromium or titanium. Furthermore, other inorganic compounds such as titanium nitride [5], indium tin oxide [6, 7] or silica [8] have been applied, in order to improve sensor performance, e.g. plasmonic effect influencing analytical signal strength or even affecting other analytical parameters such as sensitivity of biomarker determination.

REFERENCES

[1] E. Gorodkiewicz, H. Ostrowska, A. Sankiewicz, SPR imaging biosensor for the 20S proteasome: sensor development and application to measurement of proteasomes in human blood plasma, Microchimica Acta 175 (2011) 177-184

[2] A. Sankiewicz, P. Laudanski, L. Romanowicz, A. Hermanowicz, W. Roszkowska-Jakimiec, W. Debek, E. Gorodkiewicz, Development of surface plasmon resonance imaging biosensors for detection of ubiquitin carboxyl-terminal hydrolase L1, Analytical Biochemistry, 469 (2015) 4-11

[3] E. Gorodkiewicz, A. Sankiewicz, P. Laudanski, Surface plasmon resonance imaging biosensors for aromatase based on a potent inhibitor and a specific antibody: Sensor development and application for biological material, Central European Journal of Chemistry 12 (2014) 557-567

[4] E. Gorodkiewicz, Surface plasmon resonance imaging sensor for cathepsin determination based on immobilized Cystatin, Protein Peptide Letters 16 (2009) 1379-1385

[5] A. H. El‑Saeed, K. A. E. Hameed, M. Y. Azab, S. S. A. Obayya, Highly sensitive SPR PCF biosensors based on Ag/TiN and Ag/ZrN configurations, Optical and Quantum Electronics (2019) 51:56

[6] S. M. Gan, P. S. Menon, N. R. Mohamad, N. A. Jamil, B. Y. Majlis, FDTD simulation of Kretschmann based Cr-Ag-ITO SPR for refractive index sensor, Materials Today: Proceedings 7 (2019) 668–674

[7] K. Shah, N. K. Sharma, V. Sajal, SPR based fiber optic sensor with bi layers of indium tin oxide and platinum: A theoretical evaluation, Optik 135 (2017) 50–56

[8] R. D'Agata, G. Spoto, Surface plasmon resonance imaging for nucleic acid detection, Anal. Bioanal. Chem. 405 (2013) 573–584

PS-53 - APPLICATION OF QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS APPROACH WITH THE USE OF AB INITIO AND SEMI-EMPIRICAL MODELING METHODS IN ANALYSIS OF SELECTED ANTIMICROBIAL SULFONAMIDES

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ABSTRACT

A group consisting of 23 sulfonamides exhibiting antimicrobial activity was analyzed with the application of quantitative structure–activity relationships (QSAR) method. The purpose of this study was to show the common and differentiating characteristics of the analyzed chemical structures alike physicochemically as well as pharmacologically based on the quantum-chemical calculations both *in vacuo* and in the aquatic environment together with their internal biological activity. The semi-empirical (RM1 method) and *ab initio* (RHF optimization) level of in silico molecular modeling was performed for calculations of statistically significant molecular descriptors and quantum-chemical indices to compare obtained results. The relationship between the structure and biological activity and physicochemical parameters data was able to class and describe analyzed molecules and the applied chemometric approaches (principal component analysis, factor analysis, and multiple regression analysis) revealed the influential features of the tested structures responsible for the antimicrobial activity [1].

REFERENCES

[1] P. Kawczak, L. Bober, T. Bączek, The comparison of semiempirical and ab initio molecular modeling methods in activity and property evaluation of selected antimicrobial sulfonamides, *Med. Chem. Res.*, 28 (2019) 778–787.

PS-54 - METABOLOMICS-BASED ELUCIDATION OF CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) constitutes a gradual loss in kidney function over months or years. The disease represents a major public health issue and its prevalence is estimated to equal 5-15% of the general population. Metabolomics can help elucidate pathology of various disorders and reveal new hypotheses about possible mechanisms of the disease.

In this study, the non-targeted metabolomics approach was applied to evaluate potential metabolic differences between patients with CKD (n=30) and healthy (n=30) group. Patients were suffering stage 3 (n=14) and 4 (n=16) CKD. Liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) with positive and negative ion electrospray ionisation was utilized to acquire serum metabolic profiles. To improve metabolite coverage, serum samples were also analysed with triple quadrupole gas chromatography mass spectrometry (GC-MS) equipped with electron ionisation (EI) source.

Obtained data were subjected to univariate and multivariate statistical analyses. p-value, selectivity ratio (SR) and variable importance in projection (VIP) led to selection of metabolites significantly differentiating CKD patients and healthy controls. Moreover, ANOVA was applied to compare two stages of the disease and healthy controls. In both comparisons, the predominant chemical group was acylcarnitines which take part in fatty acids oxidation and were previously associated with other renal dysfunctions. Correlation of metabolites with glomerular filtration rate (GFR) was also checked with the use of Spearman's rank correlation coefficient. Among the most highly correlated compounds were: myo-inositol, sorbitol and decatrienoylcarnitine.

To sum up, we found that serum metabolic fingerprints of CKD patients differ from healthy group. Hence, untargeted metabolomics seems to be useful approach to propose and develop both diagnostic and prognostic metabolic indicators of CKD.

PS-55 - DOE-BASED SIMPLIFICATION OF SAMPLE PREPARATION PROCEDURE IN UNTARGETED GC-MS METABOLOMICS

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ABSTRACT

Many studies proved the potential of metabolomics approach for explanation of development mechanisms of various diseases. While untargeted metabolomics might provide putative list of metabolites differentiating studied groups (healthy volunteers/cancer patients), targeted approach enables to measure these differences quantitatively. The objective of the study was to simplify a method for preparation of urine samples for untargeted metabolomics analysis by means of GC-MS. Since sample preparation step for GC-MS is usually very complicated and time-consuming, implementation of experimental design (Design of Experiments, DoE) was reasonable.

DoE consists of making specific, controlled modifications in a studied system in order to create mathematical model allowing to predict how monitored responses are affected by applied modifications [1]. In other words, the use of DoE approach allows to screen the most important factors, predict relationships between them and generate the most optimal settings in order to get the most favourable response while saving the time spent on the optimization and reducing the cost of analysis. Its main advantage is the ability to provide optimal parameters' settings by performing a minimal number of experiments.

Urine sample preparation procedure was assessed using Fractional Factorial Design (FFD) based on the evaluation of time- and temperature-dependent parameters, such as: incubation time, evaporation temperature, time of vortex-mixing, etc. FFD was implemented as a screening procedure in order to evaluate the significance of the tested variables and propose simplified and less time-consuming procedure. The influence of studied factors on extraction of selected metabolites of different physico-chemical properties was also examined.

REFERENCES

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

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PS-56 - METABOLOMIC STUDIES IN SEARCH FOR ETHYL ALCOHOL ABUSE BIOMARKERS IN BLOOD AND EVALUATION OF THE USEFULNESS OF THE OBTAINED RESULTS IN FORENSIC MEDICINE

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ABSTRACT

Metabolomics is a branch of science which focuses on the analysis of low molecular weight compounds in biological fluids [1][2]. Homeostasis aberration results in qualitative and quantitative changes of metabolites which identification may become useful in medico-legal diagnostics and in determining the cause of death [3].

Based on literature data, it has been observed that the concentration of such molecules as gamma-glutamyltransferase (gamma-GT), ethyl glucuronide (EtG), ethyl sulphate, phosphatidyl ethanol (PEth) and fatty acid ethyl esters (FAEE) changes with the time elapsed since the last alcohol consumption [4] [5].

The main objective/aim of the presented study is to search for and determine the concentration levels of human plasma metabolites indicating the excessive consumption of ethanol. The research will be carried out on biological material from the Department of Forensic Medicine of the Medical University of Gdańsk. The target and control samples will be determined using high-performance liquid chromatography combined with mass spectrometry with ionization by electrospraying with a time of flight analyzer (HPLC-ESI-TOF/MS, Agilent Technologies). Afterwards, potential biomarker compounds will be quantitatively analyzed.

It is expected that these studies will allow defining limits of metabolites characteristic for particular models of ethanol consumption, and the results will be used to determine the cause of ambiguous death.

Developing and validating an optimal test method and setting limit values for ethanol consumption metabolites could be useful and provide a valuable source of information in explaining the cause of death [6] when post-mortem ethanol concentrations were not determinable.

REFERENCES

[1] "Metabolomika – współczesny trend badawczy w medycynie i farmacji," pp. 14–17, 2018.

[2] A. Słowikowska, B. Toczyłowska, R. Cichoń, and P. Hendzel, "Metabolomika — chemiczny „odcisk palca" i istotny element medycyny spersonalizowanej," Folia Cardiol., vol. 11, no. 4, pp. 353–358, 2016.

[3] R. J. Dinis-Oliveira, "Metabolism and metabolomics of opiates: A long way of forensic implications to unravel," J. Forensic Leg. Med., vol. 61, no. May 2018, pp. 128–140, 2019.

[4] T. M. Maenhout, M. L. De Buyzere, and J. R. Delanghe, "Non-oxidative ethanol metabolites as a measure of alcohol intake," Clin. Chim. Acta, vol. 415, no. November 2018, pp. 322–329, 2013.

[5] R. Z. Litten, A. M. Bradley, and H. B. Moss, "Alcohol biomarkers in applied settings: Recent advances and future research opportunities," Alcohol. Clin. Exp. Res., vol. 34, no. 6, pp. 955–967, 2010.

[6] G. G. Harrigan, G. Maguire, and L. Boros, "Metabolomics in alcohol research and drug development.," Alcohol Res. Health, vol. 31, no. 1, pp. 26–35, 2008.

PS-57 - COMPERATIVE STUDY ON DISINTEGRATION TIME OF ORODISPERSIBLE FILMS CONTAINING INCORPORATED PARTICLES

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ABSTRACT

Orodispersible polymer films (ODFs) allow to administer active substance in a solid dosage form easily because of the rapid disintegration in saliva. Although the most popular approach is to prepare ODF with active substance dissolved, a drug may be presented in the film in form of large particles (pellets, granules) [1]. There is no clear regulatory requirements for disintegration time in Ph.Eur. The time limit is considered usually less than 180 s as required by the Ph.Eur for orodispersible tablets or 30 s required by the FDA. When *in vivo* experiments are performed with the ODFs, high standard deviations of the disintegration times are obtained [2].

The aim of this study was to establish the influence of incorporated particles on disintegration time of ODFs and their surface properties - roughness. Two methods were compared: in a glass dish and using the Ph.Eur. tester with a special holder. The influence of placebo pellets concentration (20 - 45%) and their size: CL100 (156 μm) and CL200 (276 μm) was evaluated. The influence of crospovidone on addition was determined. Orodispersible films were produced using: hypromellose and a glycerol as a plasticizer. Solvent casting method (CAMAG, Muttenz) was used with dosing gap of 300 - 800 μm.

The effect of the incorporated pellets on the disintegration time depended on the concentration and the size of the pellets as well as on the size of the dosing gap. ODFs with larger particles in high concentration (45%) disintegrated faster. The addition of crospovidone at low concentrations (0.15 - 0.45% w/w) did not significantly affect disintegration time.

REFERENCES

[1] C. Woertz, P. Kleinebudde, Development of orodispersible polymer films containing poorly water soluble active pharmaceutical ingredients with focus on different drug loadings and storage stability, *Int. J. Pharm*., 493 (2015) 134-145.

[2] I. Speer, D. Steiner, Y. Thabet, J. Breitkreutz, A. Kwade, Comperative study on disintegration methods for oral film preparations, *Eur. J. Pharm. Biopharm.*, 132 (2018) 50-61.

PS-58 - DRUG RELEASE PROFILES AS A MARKER OF CHANGES IN LIPID MICROPARTICLES CAUSED BY SPRAY DRYING

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Solid Lipid Microparticles (SLM) are multi-compartment drug carriers which allow for the prolonged release of active substance (API) with the aim to increase its effectiveness [1,2]. SLM prepared with hot emulsification method could be applied as liquid dispersion or after spray drying in the form of dry powder for reconstitution.

The goal of the study was to examine the impact of spray drying process on the distribution of API in the microspheres. Any changes in localization of API in the formulation (in the lipid core, in interphase or in the dispersing liquid) will affect the release profiles of API and such tests were performed. As model APIs cyclosporine and spironolactone were used.

The *in vitro* drug release was investigated in the membrane-free system after mixing SLM with the acceptor fluid and incubating under agitation at 37 °C [2]. Concentration of APIs was determined by HPLC.

During the experiments it has been shown that the spray drying process of SLM dispersion may lead to faster release of the active substance. It was observed that API redistribution depended on the lipid type and API. When the lipid matrix was Compritol, SLM released about 30% more cyclosporine within 6 hrs than before drying. This phenomenon was not observed when the lipid matrix was composed of stearic acid. In SLM with spironolactone, some differences in the release profiles were observed independently of the lipid type.

The obtained results indicate that the release studies are very important tool in the assessment of interactions of API with lipid in microspheres and they allow to detect changes that have occurred during processing, e.g. drying.

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REFERENCES

[1] M. Sznitowska, E. Wolska, H. Baranska; et al. The effect of a lipid composition and a surfactant on the characteristics of the solid lipid microspheres and nanospheres (SLM and SLN), *Eur. J. Pharm. Biopharm.*, 110 (2017) 24-30.

[2] E. Wolska, M. Sznitowska, J. Chorążewicz; et al. Ocular irritation and cyclosporine A distribution in the eye tissues after administration of Solid Lipid Microparticles in the rabbit model, *Eur. J. Pharm. Sci.*, 121 (2018) 95-105.

PS-59 - IN VITRO DISSOLUTION TESTS FOR ENTERIC-COATED MINITABLETS ADMINISTERED IN HYDROGELS OR IN CAPSULES

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ABSTRACT

Minitablets (MTs) are multiparticulate solid oral dosage forms dedicated for paediatric patients and people with dysphagia [1]. MTs (diameter 1-3 mm) are administered as a variable number of subunits, which facilitates the selection of a dosage appropriate to the age of the patient. MTs can also be considered as alternative for pellets, administered in capsules or as sprinkles [2].

Enteric-coated MTs with diclofenac (diameter 2 mm - dose 1.2 mg or 3 mm - dose 3 mg) were successfully obtained in fluid bed coating system using aqueous dispersion of Eudragit L 30D55.

The aim of the study was to examine simple hydrogels and hard gelatine capsules as standard universal carriers for MTs and to optimize dissolution test. In vitro dissolution tests were performed in two apparatus (1- basket, and 2- paddle) with a rotation of 50 rpm at 37 ± 0.5 °C. The tests were carried out with 900 ml of 0.1 mol/L HCl for 2 h and then with 900 ml of pH 6.8 phosphate buffer for 1 h. Additionally, release rate of diclofenac from MTs placed in a hard gelatine capsules, 0.5% carbomer gel (Carbopol 974P NF, Lubrizol) or 2% carmellose sodium gel (Carboxymethylcellulose sadium salt, Sigma) were examined. Single dose containing 24 mg of diclofenac sodium (8 MTs 3mm and 20 MTs 2 mm) in about 4.5 g of a gel or one capsule were tested.

The study showed that diclofenac is released faster from MTs when the paddle apparatus was used. No effect of the capsule shell on the release rate of the diclofenac has been demonstrated. Although it was possible to obtained fast release of diclofenac from MTs mixed with carbomer gel, with some delay in relation to the capsule, the use of carmellose sodium gel caused a significant delay in the release rate. The results showed difficulties in choosing a universal gel vehicle which can facilitate drug administration without affecting biopharmaceutical properties of the primary formulation.

REFERENCES

[1] A. Kluk, M. Sznitowska, et al, Can preschool-aged children swallow several minitablets at a time? Results from a clinical pilot study, *Int. J. Pharm.*, 485 (2015) 1-6.

[2] F.L. Lopez, C. Tuleu, et al, Effect of formulation variables on oral grittiness and preferences of multiparticulate formulations in adult volunteers, *Eur. J. Pharm. Sci.*, 92 (2016) 156-162.

PS-60 - FLUORESCENCE MICROSCOPY IN TRANSDERMAL DISTRIBUTION OF HESPERIDIN

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ABSTRACT

A natural flavanone-type flavonoid, hesperidin is wide spread in nature. It presents a range of pharmacological effects: anti-infammatory, cardiovascular protection, antioxidant activity have been reported [1]. Recent studies focused on hesperidin multiple cutaneous functions. The most important benefits of hesperidin application are wound healing, UV protection, antimicrobial, inhibition of melanogenesis, antimelanoma cancer [2], though transdermal distribution of hesperidin has never been estimated.

In our study hesperidin (10µg/ml) was applied on human dorsal skin for 24h (*ex vivo*). Afterwards skin slices were analyzed under fluorescence microscope. As hesperidin gives fluorescence in different range than skin macromolecules, the distribution of hesperidin in individual layers of the skin was possible [1, 3]. It was indicated, that stratum corneum is not a barrier for hesperidin, witch passes through and accumulates in dermis.

REFERENCES

1. A. Garg, S. Garg, L.J. Zaneveld, A.K. Singla, Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. *Phytother Res* 15 (2001) 655–669.
2. M.Q. Man, B. Yang, and P.M. Elias, Benefits of Hesperidin for Cutaneous Functions. *Evidence-Based Complementary and Alternative Medicine*, vol. 2019 (2019), Article ID 2676307.
3. R. Ochocka, A. Hering, J. Stefanowicz– Hajduk, K. Cal, H. Barańska. The effect of mangiferin on skin: Penetration, permeation and inhibition of ECM enzymes. *PLoS ONE* (2017) 12(7).

PS-61 - SIMULTANOUS SEQUENTIAL ANALYSIS OF ^{210}Po, ^{210}Pb and U (^{234}U, ^{238}U) ISOTOPES IN CALCIUM AND MAGNESIUM SUPPLEMENTS

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ABSTRACT

Polonium ^{210}Po ($T_{1/2}$=138.376 days) and radiolead ^{210}Pb ($T_{1/2}$=22.2 years) appear at the end of the decay-chain of uranium ^{238}U and are radio-ecologically interesting natural elements to investigate due to their high radiotoxic characteristics. Uranium is widely spread in nature, occurs in over 160 minerals, locally at high concentrations. Isotopes ^{234}U and ^{238}U occur naturally in uranium decay chain; both of them are alpha emitters of low radioactivity and radiotoxicity.

The aim of this pioneer study was to investigate the most popular calcium and magnesium supplements as a potential additional source of polonium ^{210}Po, radiolead ^{210}Pb and uranium (^{234}U, ^{238}U) in human diet. The analysed Ca and Mg pharmaceutics contained their organic or inorganic compounds; some from natural sources as shells, fish extracts, or sedimentary rocks. The objectives of this research were to investigate the naturally occurring ^{210}Po, ^{210}Pb, ^{234}U and ^{238}U activity concentrations in calcium and magnesium supplements, find the correlations between their concentration in medicament and the element chemical form, and calculate the effective radiation dose connected to analysed supplement consumption.

To achieve the goal of the study, the simultaneous sequential separation radiochemical method was used. Polonium ^{210}Po was deposited directly from the sample and deposited on silver disc. After this process, the sample was left for 6 months. After this time, from the ^{210}Pb present in the sample, as a result of its decay, an equilibrium amount of ^{210}Po was formed. The measurement of ^{210}Po increment in the alpha spectrometer allowed for direct ^{210}Pb activity calculation. After the second ^{210}Po deposition and measurement targets preparation for ^{210}Pb activity determination, the sample was prepared for uranium separation. Uranium fraction was separated and purified using anion exchange resins. Three different resins were used – the first allowed to separate uranium from thorium and plutonium, the second and the third were used to purify the uranium fraction.

On the basis polonium ^{210}Po, radiolead ^{210}Pb and uranium (^{234}U, ^{238}U) content calculated in analysed calcium and magnesium supplements, the effective radiation doses were estimated. Obtained data showed there is no serious radiological risk connected to uranium ingestion with calcium and/or magnesium supplements.

PS-62 - SLAG OBTAINED IN THE CIRCULATING FLUIDIZED BED COMBUSTION TECHNOLOGY AS AN ADSORBENT FOR METAL IONS RECOVERY

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ABSTRACT

Every year, industry produces millions of tons of heavy metals throughout the world. This activity contributes to the growing pollution of the aquatic environment, and thus its degradation [1]. Hence, recently there has been growing interest in the problem and the search for methods of sewage treatment from heavy metals. Among many industrial techniques, adsorption processes may be alternative using cheap wastes, such as fly ash and slag obtained as a result of combustion of municipal and industrial sewage sludge [2 - 4]. Currently, more and more thermal conversion installations are being built due to many benefits, including heat recovery, significant reduction of waste mass and avoiding storage.

The slag used in the research is waste generated in the modern circulating fluidized bed combustion (CFBC) technology, which nowadays is regarded as the most effective method of combustion of municipal sewage sludge in the European Union. In the first stage physical and chemical properties of the material were described, including bulk density, grain size distribution, SEM-EDS analysis, thermogravimetry, zeta potential, BET adsorption and desorption, pore volume (BJH), FT-IR and SEM images analysis. Next, adsorptive properties of slag in relation to selected metal ions were examined in batch experiments. Consequently, the results showed high adsorption efficiency and capacity. To conclude, it should be noted that the obtained results give reasonable grounds to continue work in this area. Thus, the waste material may be a cheaper alternative to more expensive industrial adsorbents in wastewater treatment processes.

REFERENCES

[1] S.V. Yadla, V. Sridevi, M.V.V. Chandana Lakshmi, Adsorption performance of fly ash for the removal of lead, *Int. J. Eng. Res. & Technol.*, 1 (2012) 1-7.

[2] T. Kalak, J. Dudczak, R. Cierpiszewski, Adsorption behaviour of copper ions on elderberry, gooseberry and paprika waste from aqueous solutions, *Proceedings of 12th International Interdisciplinary Meeting on Bioanalysis (CECE)*, Brno, Czech Republic, (2015) 123-127.

[3] T. Kalak, R. Cierpiszewski, Adsorptive removal of copper and cadmium ions using fly ash resulting from CFBC technology, *Proceedings of 15th International Interdisciplinary Meeting on Bioanalysis (CECE)*, Brno, Czech Republic, (2018) 177-181.

[4] T. Kalak, A. Kłopotek, R. Cierpiszewski, Effective adsorption of lead ions using fly ash obtained in the novel circulating fluidized bed combustion technology, *Microchem. J.*, 145 (2019) 1011–1025.

PS-63 - WHAT SECRETS HIDES CAT'S FUR (FELIS CATUS)?

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Cats are mammals that have accompanied humans since the dawn of history.[1]

Their coat, next to a set of many different colours and patterns, may be the answer whether the accumulation of radioactive elements depends on determinants such as race, age, coat length, weight, origin, place of residence, sex and food consumed. The aim of the research was to determine the level of bioaccumulation of the ^{210}Po isotope in the fur of 11 different feline breeds (European, Ragdoll, Persian, Scottish Fold, Siberian, Russian Blue, Neva Masquerade, Cornish Rex, Norwegian Forest, British, Main Coon). Concentration of the ^{210}Po in the cat's hair ranged from 0.208 ± 0.021 to 15.458 ± 2.733 Bq / kg (with an average value of 4.15 ± 0.45 Bq / kg).

Conducted research has shown that the bio - accumulation of ^{210}Po within the cat's hair depends on its length, breed and the food consumed. The largest accumulation of this radionuclide was found in the coat of a long haired Maine coon cats and cats which were fed with wet food. On the other hand, the smallest amounts was found in the hair of the short - haired Russian blue breed. From the statistical point of view, factors such as gender and place of residence of the cats were not relevant in the terms of administrative (territorial) divisions of Poland. However, factors such as age, weight and location of the cat may have a significant influence on the final results.

[1] John Pickrell: Oldest Known Pet Cat? 9,500-Year-Old Burial Found on Cyprus (ang.). National Geographic, 2004-04-08.

PS-64 - MELLIFEROUS PLANTS AS A SOURCE OF ^{210}PO IN HONEY FROM CENTRAL AND SOUTHERN POLAND

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ABSTRACT

It is estimated that about 20,000 bee species live on Earth, but only a few managed to domesticate and obtain honey and wax in economically significant quantities. Reared by beekeepers Apis cerana, Meliponini and Apis mellifera. Bee honey is created by combining floral nectar or honeydew with enzymes and formic acid produced by bees in their digestive. Over the centuries, the role of honeybees in human life has changed significantly, they were mainly kept for valuable honey, today with increase of agricultural production they are used to pollinate monoculture crops. This is a direct result of numerous impurities in honey such as residues of veterinary medicines, agrochemicals or the proximity of industrialized areas. The aim of the study was to determine the concentration of ^{210}Po in honey samples obtained for analysis from selected voivodships of Poland and to examine whether the type of melliferous plants has a significant impact on the bioaccumulation of this radionuclide in the analyzed research material.

PS-65 - ACIDIC AND ENZYMATIC HAIR DEGRADATION FOR MERCURY SPECIATION ANALYSIS

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Hair is known to store the toxic compounds absorbed by living creature. Mostly inorganic (iHg) and methyl mercury (MeHg) are bound. One of the most efficient and specific for MeHg extractions is application of 2M HCl [1]. The enzymatic extraction needs milder conditions, and might be used for extraction of organic toxins [2]. We present the results of acidic and enzymatic extraction of mercury species from hair reference material IAEA-086 obtained by HPLC-ICP-MS as a reference method.

The acidic extraction was carried out with 2M HCl at room temperature for selected periods of time. We have not noticed significant difference between the results of total extraction carried out within 1h up to 4h. The four hours extraction of MeHg resulted in 83-86 % yield and 8-12% of iHg. The enzymatic digestion was carried out with proteinase K in phosphate buffer. The yields reached 15% after one and three hours. The overnight extraction gave lower yields 10% and 6% for iHg and MeHg respectively.

Having proved selective and almost quantitative MeHg extraction from hair, we aimed to couple the extraction procedure with less expensive spectrometric detectors than ICP-MS. Therefore, the atomic absorption spectrometry (AAS) was employed. Two approaches have been compared: 1) determination of MeHg in liquid extracts by single purpose mercury analyser (AMA-254) and 2) volatile species generation (VSG)-AAS based on MeHg conversion to methyl mercury hydride by $NaBH_4$. Comparison of analytical figures of merit for Hg speciation analysis by all methods, i.e. HPLC-ICP-MS, VSG-AAS and AMA-254 will be discussed.

[1] M. Čejchanová, V. Spěváčková, K. Kratzer, K. Wranová, V. Spěváček & B. Beneš, Determination of Mercury and Methylmercury in Hair of the Czech Children's Population, *Biol Trace Elem Res* 121 (2008) 97–105.

[2] K. Saisho, E. Tanaka and Y. Nakahara. Hair Analysis for Pharmaceutical Drugs. I. Effective Extraction and Determination of Phenobarbital, Phenytoin and Their Major Metabolites in Rat and Human Hair, *Biol. Pharm. Bull.* 24 (2001) 59—64.

PS-66 - THE IMPACT OF ATMOSPHERIC PRECIPITATION ON THE CONTENT OF ^{210}Po IN SELECTED HERBS SPECIES

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Among naturally occurring radioisotopes, a highly toxic alpha-radiation emitter, namely ^{210}Po and its precursor, the beta radiation emitter ^{210}Pb, are particularly important. Since 70-80% of the world's population uses unconventional medicine and treats it as a primary health care, it is worth determining whether the consumption of herbal preparations is safe from radiological point of view. The subject of the research was to determine an influence of atmospheric precipitation on the content of the radioactive isotope polonium ^{210}Po in selected species of commonly used herbs collected from various areas of Poland and from the cultivation of potted herbs (basil, sage, peppermint, lemon balm) and soil. In the case of one kind of herbs, the washing process was conducted, and the potential impact of atmospheric precipitation on the content of analyzed isotope was estimated.

The results of ^{210}Po activity determination in the analyzed herb samples showed that the highest concentration of ^{210}Po activity was observed for lemon balm leaves collected in Ryki (Lubelskie Voivodeship) (14.4 mBq/kg w.w.), and the highest ^{210}Po activity among the herbs was recorded for roots of a medical sage (0.52 mBq/kg w.w.). In the case of nettle, where the washing process was applied, the concentration of polonium activity ^{210}Po in leaves decreased by 8%. The analysis of the results showed that there are statistically significant differences in the content of ^{210}Po between plants collected from different regions of Poland and potted plants cultivated without the influence of external factors.

PS-67 - IS COW MILK GOOD INDICATOR OF ENVIRONMENT POLLUTION?

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ABSTRACT

Food safety and quality are particular interest to both public health institutions and the consumers themselves. The presence of chemical contaminations, including radionuclides, is one the criteria for assessing the safety of products intended for consumptions. The aim of the work was to determine the ^{210}Po concentration in milk samples originating from the Warmian-Masurian, Podlaskie, Lesser Poland and Silesian provinces of northern and southern Poland, as well as to estimate the harmfulness of the tested product to human health based on the designated annual effective dose. From the radiochemical point of view, so far, there is a lack of competent and full studies on whether cow's milk can be a good bioindicator of the natural environment. The average ^{210}Po concentration in cow milk samples were 40,53±0,59 [mBq/dm^3] for Lesser Poland, 38,82±0,77 [mBq/dm^3] for Silesian, 19,19±1,38 [mBq/dm^3] for Warmian-Masurian and 24,18±1,51 [mBq/dm^3] for Podlaskie Voivodeships. The values of ^{210}Po concentration and annual effective dose in voivodeships of southern Poland are twice higer than vlaues of these parameters in voivodeships of northern Poland (39,68±0,68 [mBq/dm^3] and 0,167±0,003 [µSv/rok]; 21,68±1,44 [mBq/dm^3] and 0,091±0,006 [µSv/rok] respectively). The obtained results research show that higher values analyzed radioniclide for southern areas of Poland can be connected with farms location and the proximity of communication routes. Pollution caused by dry and wet atmospheric perticipation are very important too. The differences between values of analyzed radionuclide of ^{210}Po in cow milk samples from different regions of Poland show that cow milk is good biondicator of environment pollution.

PS-68 - CAPILLARY ELECTROPHORESIS AS AN ALTERNATIVE TECHNIQUE FOR DETERMINATION OF NON-CHROMOPHORIC POLYPHOSPHONATES

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ABSTRACT

Liquid chromatography is widely used analytical technique to separate a broad range of analytes due to their selectivity and specificity. Usually UV, is a common method for detecting compounds of interest using either a variable wavelength detector or a diode array detector. But, what are the alternatives if UV detection just doesn't offer enough for specificity, sensitivity and application perspective and when studied analytes simply lacks a chromophore? Sometimes pre-column or post-column derivatization of the sample, among many other alternative approaches, may be employed. However, such application may not always be an attractive option, as it can be slow and time-consuming. In this case, capillary electrophoresis (CE) is increasingly recognized as an important separation technique because of its speed, efficiency, reproducibility, ultra small sample volume, low consumption of solvent, and easy removal of contaminants. Application of CE system with UV indirect detection mode can prove to be a more effective approach then derivatization in the analysis of non-chromophoric analytes by HPLC.

The organophosphonates are a group of both synthetic and biogenic organophosphorus compounds, characterized by the presence of a single, covalent, carbon to phosphorus (C-P) bond. Because of high energy of dissociation, this bond is very stable and it is extremely resistant to chemical hydrolysis, thermal decomposition, and photolysis. Therefore, they serve as components of washing powders, additives to surfactants, antiscaling and anticorrosive agents, regulators of cement hardening and so on. As a consequence of wide and increasing industrial applications, thousand tons of organophosphonates are introduced every year into the environment. The lack of information on the fate of phosphonates in the environment is linked to analytical problems, namely to the lack of methods of their determination at trace concentrations in environment. Thus, sensitive, selective and reliable analytical methods are required to monitor amounts of phosphonates in various type of samples. Thus, the aim of this project was to develop an effective method for determination the group of organophosphorus compounds – very polar and non-chromophoric polyphosphonates from aqueous samples using capillary electrophoresis with UV-VIS direct and indirect detection modes. A mixture of studied compounds (i.e. ATMP, GBMP, HEMPA,) were separated using selected background electrolyte. During the studies, the attempts have been made to optimized conditions CE separation step (the type, concentration and pH of BGE, addition of surfactant and an organic solvent).

PS-69 - HYDRODISTILLATION PARAMETERS AFFECT PHTHALIDE CONTENT IN APIUM GRAVEOLENS ESSENTIAL OIL

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Celery (*Apium graveolens*) is a popular vegetable, spice and medicinal aromatic plant. All parts of celery contain phthalide-rich essential oil which determines the characteristic aroma and biological properties of the plant. Phthalide constituents, represented by 3-n-butylphthalide, sedanolide and sedanenolide, possess anti-inflammatory, anti-carcinogenic and neuroprotective properties [1], thus warranting the development of natural drugs based on *A. graveolens* oil. However, phthalide content of celery oil is highly variable and depends on size reduction and hydrodistillation time [2,3]. Given this, the aim of the study was to evaluate the effects of selected parameters of essential oil production, including the type of apparatus used [pharmacopoeial Clevenger (CL) or Likens-Nickerson (LN) type], process time, size reduction, salt effect and enzyme pretreatment, on essential oil content and its composition. The experiments were conducted using celery seeds and phthalide content of the samples was determined by HPLC. LC-DAD-ESI-MS analyses revealed the presence of 3-n-butylphthalide, sedanenolide and sedanolide in the analysed oils. The increase of distillation time from 3 to 6 h resulted in oil yields 8% and 30% higher for Clevenger and LN apparatus, respectively. At the same time, the amount of oil obtained using LN apparatus was 33% and 63% higher (for 3 and 6 h process, respectively), as compared to CL method. The increase in oil yield was accompanied by elevated phthalide content in the volatile fraction. The concentration of 3-n-butylphthalide raised by 10 and 8% (for CL and LN, respectively), reaching up to 27% of volatile oil composition. 3-n-Butylphthalide content was positively affected by size reduction/seed grinding (27% increase) and salt effect (further increase, up to 57%).

[1] A. Kokotkiewicz, M. Łuczkiewicz, Celery (*Apium graveolens* var. *dulce* (Mill.) Pers.) Oils. In: Preedy, V.R. (Ed.), *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press (2016) 325-338.

[2] M.P. Jain, J.K. Sama, S.M. Jain, V.K. Sharma, B. Singh, An improved method for the recovery of essential oil from Celery seeds, *Indian J. Chem. Technol.*, 10 (2003) 370-372.

[3] H.B. Sowbhagya, S.R. Sampathu, N. Krishnamurthy, Evaluation of size reduction on the yield and quality of celery seed oil, *J. Food Eng.* 80 (2007) 1255-1260.

PS-70 - TLC OF ACTIVE COMPOUNDS FROM SELECTED *POPULUS* LEAVES

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ABSTRACT

Leaves from some poplars are herbal remedy traditionally used in the treatment of rheumatism and prostate inflammation [1,2]. Flavonoids and phenolic acids besides salicin and their derivatives are the main groups of biologically active compounds [1]. The aim of our work was to optimize the HPLC-MS and 2D-TLC separations of poly- and simple phenols present in *Populus alba, P. nigra, P. candicans* and *P. tremula* leaves and evaluate their anti-inflammatory activity by the use of TLC bioautographic tests - DPPH, ryboflavin-NBT test and inhibithion of xanthine oxidase.

The developed HPLC-MS separation was carried out on a Kinetex C18 (100 × 2.1mm, 2.6 um) column under gradient elution A- water/ trifluoroacetic acid (100/0.1, v/v), B- acetonitrile/ water/ trifluoroacetic acid (50/50/0.1, v/v/v): from 5 to 40% B in A+B (tG = 40 min) and 40 to 100% B in A+B (tG = 40 to 55 min) , T = 20°C, v = 0.2 ml/min.

The 2D-TLC separation was carried out on the TLC Silica gel 60 plates (10 cm × 10 cm) with the mobile phase composed of formic acid/ water/ methylethyl ketone/ ethyl acetate (1:1:3:5; v/v/v/v) in the first direction and the mixture of chloroform/ methanol/ water/ formic acid 70:30:2:2 (v/v/v/v) in the second.

The presence of compounds with antioxidant activity in the TLC autobiography using DPPH and riboflavin-NBT test and anti-inflammatory activity in the xanthine oxidase inhibition test in the leaves of *Populus nigra, P. alba, P. candicans* was shown. Among the active substances, rutin, isoquercitrin, cynaroside and chlorogenic acid in the material from black poplar, rutin and chlorogenic acid in white poplar, isoquercitrin and chlorogenic acid in *Populus candicans.*

REFERENCES

[1] B.-E.van Wyk, M. Wink, *Medicinal Plants of the World*, MedPharm, (2004) Wrocław.

[2] D. Benedec, I. Oniga, B. Muresan, A.C. Mot, G. Damian, A. Nistor, R. Silaghi-Dumitrescu, D. Hanganu, M. Duma, L. Vlase, Contrast between water- and ethanol-based antioxidant assays: aspen (*Populus tremula*) and black poplar (*Populus nigra*) extracts as a case study, *J. Food Qual.* 37 (2014) 259-267.

PS-71 - THE DIFFERENTIAL PROTEOMICS OF *SALMONELLA ENTERICA* SSP. *DIARIZONAE* EXPOSED TO HUMAN SERUM

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ABSTRACT

The complement is the crucial element of innate immune system. Pathogens can gain resistance against bactericidal action of the complement utilizing several molecular mechanisms. One of them is decorating bacterial outer cell surface with lipopolysaccharides (LPSs), which can act as a molecular mimicry due to presence of multiple sialic acid residues [1,2]. The bacteria from *Salmonella* genus, which can contain sialic acid in the O-antigen, are one of the most common causes of salmonellosis, which is regarded as a worldwide public health problem. Overwhelming *Salmonella* infections can lead to sepsis, in development of which complement activation plays an important role [3].

Previously the link between *Salmonella* serogroup O48 strains LPS length and their resistance to bactericidal activity of human serum was established [4]. Moreover, multiple passages in human serum lead to substantial changes in bacterial outer membrane proteome [5], however methodology utilized in previous study (2D gel electrophoresis followed by in gel digestion and mass spectrometry spot analysis) lacked the sensitivity to get the detailed picture of induced changes.

In present study we examined changes in proteome of PCM 2511 *Salmonella enterica* ssp. *diarizonae* strain passaged in human serum [4]. The outer membrane proteome was isolated and prepared according to protocols established earlier [5]. In order to quantify changes in the proteome we utilized Tandem Mass Tags (TMT) – a powerful tool in differential proteomic analysis [6].

REFERENCES

[1] E. Severi, D.W. Hood, G.H. Thomas. Sialic acid utilization by bacterial pathogens. *Microbiology* 153 (2007) 2817–2822 (DOI 10.1099/mic.0.2007/009480-0)

[2] A.P. Moran, M.M. Prendergast, B.J. Appelmelk. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. *FEMS Immunology and Medical Microbiology* 16 (1996) 105-l 15

[3] M.M. Markiewski, R.A. DeAngelis, J.D. Lambris. Complexity of complement activation in sepsis. J. *Cell. Mol. Med.* 12 (2008) 2245–2254.

[4] A. Pawlak, J. Rybka, B. Dudek, E. Krzyżewska, W. Rybka, A. Kędziora, E. Klausa, G. Bugla-Płoskońska, Salmonella O48 Serum Resistance is Connected with the Elongation of the Lipopolysaccharide O-Antigen Containing Sialic Acid. Int J Mol Sci. 2017 Sep 21;18(10). (doi: 10.3390/ijms18102022)

[5] B. Dudek, E. Krzyżewska, K. Kapczyńska, J. Rybka, A. Pawlak, K. Korzekwa, E. Klausa, G. Bugla-Płoskońska, Proteomic Analysis of Outer Membrane Proteins from Salmonella Enteritidis Strains with Different Sensitivity to Human Serum. PLoS One. 2016 Oct 3;11(10):e0164069. (doi: 10.1371/journal.pone.0164069)

[6] A.Thompson, J. Schäfer, K. Kuhn, S. Kienle, J. Schwarz, G. Schmidt, T. Neumann, C. Hamon. Tandem Mass Tags: A Novel Quantification Strategy for Comparative Analysis of Complex Protein Mixtures by MS/MS. Anal. Chem.20037581895-1904 (https://doi.org/10.1021/ac0262560).

PS-72 - PLASMA FREE AMINO ACID PROFILING IN LEUKEMIA PATIENTS BY UHPLC-ESI-MS/MS METHOD USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR SAMPLE PREPARATION

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ABSTRACT

Redistribution or translocation of plasma free amino acids (PFAAs) to support visceral or tumour protein synthesis is an essential feature in cancer patients [1].

The aim of presented study was to develop a sensitive UHPLC-ESI-MS/MS method with an easy sample preparation step based on dispersive liquid-liquid microextraction (DLLME) as a preconcentration tool for the extraction of 26 free amino acid from plasma samples.

DLLME is a modern and attractive sample pretreatment technique, that exhibits many advantages including simplicity, rapidly, efficiency and low costs. DLLME is also in line with green chemistry principles and uses small amounts of organic solvents.

This application of developed UHPLC-ESI-MS/MS method provides a new strategy in the contemporaneous bioanalysis of highly polar compounds in complex biological matrices, such as plasma. The obtained results of the elaborated method with DLLME have great potential as a diagnostic tool in bioanalysis of the panel of free amino acid in order to diagnose disease and to monitor therapy.

REFERENCES

[1] L. Hong-Shiee, L. Jenq-Chang, L. Po-Huang , W. Shan-Tair, C. Wei-Jao, Plasma free amino acid profile in cancer patients, *Semin. Cancer Biol.*, 15 (2005) 267-276.

PS-73 - ISOLATION AND IDENTIFICATION OF GENUS *FRAGARIA* DNA IN COSMETIC PRODUCTS

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ABSTRACT

Cosmetic products with ingredients of plant origin promises beneficial effects on the customer health. It is possible to detect presence of plant DNA in the cosmetic product in very small quantities using techniques of molecular biotechnology, and, thus to detect the presence of a potential allergen that may negativelly affects the health of sensitive individuals. One of these allergens is cinnamyl alcohol [1] presented for example in fruits that belong to genus *Fragaria*. In this study gene-specific primers encoding cinnamyl alcohol dehydrogenase were used for detection of presence of *Fragaria x ananassa* DNA in leave-on cosmetic product [2]. Purified DNA was isolated and the specific sequence was amplified using real-time PCR [3]. The presence of amplicons of specific size and melt temperature was confirmed using agarose electrophoresis and HRM analysis.

REFERENCES

[1] C.Villa, R. Gambaro, E. Mariani, et al., High-performance liquid chromatographic method for the simultaneous determination of 24 fragrance allergens to study scented products. *Journal of Pharmaceutical and Biomedical Analysis*, 44 (2007) 755-762

[2] D.J. Sargent, A. Rys, S. Nier, et al. The development and mapping of functional markers in Fragaria and their transferability and potential for mapping in other genera. *Theoretical and Applied Genetics*, 114 (2006) 373-384.

[3] D.J. Sargent, J. Clarke, D. W. Simpson, et al. An enhanced microsatellite map of diploid Fragaria. *Theoretical and Applied Genetics*, 112 (2006) 1349-1359.

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PS-74 - COMPARISON BETWEEN PLASMA AND URINE METABOLIC PROFILES FROM PROSTATE CANCER PATIENTS

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Prostate cancer (CaP) is the second most common cause of cancer death in men. Such high mortality is related mainly to histological variability of CaP and a non-specificity of the available diagnostic methods. Currently used diagnostic marker of CaP – prostate specific antigen (PSA) brings many false negative results particularly in the range of 4-10 ng/ml (25-40%). Additionally, the knowledge of CAP pathogenesis is still not totally explained.

In the present study, comparison of plasma and urine metabolic fingerprints from prostate cancer patients (n=43) and healthy volunteers (n=40) was carried out. The analyses were performed using two complementary analytical platforms, GC-EI-QqQ/MS in a scan mode and LC-ESI-TOF/MS. The obtained data sets underwent data pretreatment methods including alignment, filtration, normalization and scaling. Next, the uni-and multivariate statistical methods were applied like t-test, U Mann-Whitney test as well as Principal Component Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA). The obtained statistically significant metabolites were selected according to adjusted *p value* (FDR *p value* < 0.05). As a result, statistically significant metabolites among studied groups of samples were proposed to be considered as potential markers in CaP recognition. After identification, the statistically significant urinary metabolites revealed to be mainly linked to tricarboxylic acid (TCA) cycle, carbohydrates conversions, nucleosides degradation and polyamines metabolic pathway. In regard with statistically significant metabolites obtained from plasma samples, they were mainly involved in fatty acids and amino acids metabolic pathways. The obtained results will be subsequently verified using quantitative targeted approach.

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PS-75 - THE USE OF METABOLOMICS IN IDENTIFYING METABOLIC ALTERATIONS IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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Polycystic ovary syndrome is endocrine and multifactorial disorder, which is diagnosed in about 10% of women of reproductive age, therefore is one of the main reasons of female infertility. The pathogenesis of polycystic ovary syndrome is still unknown. PCOS is connected with ovulatory dysfunction and polycystic ovaries appearance and also with higher level of androgens concentration.

The use of the metabolomic approach in a field of PCOS aims to identify specific metabolic pathways potentially involved in the pathophysiology of this disorder. Determination and comparison of the metabolic profiles of serum samples obtained from women with PCOS and healthy controls was the main aim of this study. Untargeted metabolomic analysis was performed with the use two complementary analytical techniques: liquid chromatography and gas chromatography coupled with mass spectrometry. The identification metabolites specific for biochemical pathways disturbed in PCOS was the next step. The differences of metabolic profiles in both groups of women was developed with the use of univariate and multivariate statistical analysis.

A few metabolites connected with metabolic disorders of amino acids, carbohydrates, steroid hormones, lipids, purines and citric acid cycle were identified during untargeted metabolomics analysis. Understanding the pathomechanism of this syndrome and the identification of new potential biomarkers of PCOS will contribute to its early recognition and effective treatment.

PS-76 - DESIGN, SYNTHESIS, RADIOLIGAND BINDING STUDIES AND CHIRAL SEPARATION OF POTENTIAL PYROGLUTAMIDE-BASED P2RX7 RECEPTOR ANTAGONISTS

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P2X7 receptor (P2X7R) is a homotrimeric high concentration sensitive ATP-gated ion channel expressed by a wide variety of cells. Data from gene knock-out studies or experimental results from its activation allowed to consider this receptor as an appealing therapeutic target for treating a number of diseases including cancers, neurological disorders, autoimmune diseases and chronic pain [1]. As the activation of the P2X7R is considered as a key regulatory element of the inflammasome complex and linked to the enhanced secretion of the pro-inflammatory cytokine interleukin1, much research has focused on the discovery of potential antagonists of this receptor [2]. In our lab, we have designed, synthesized and studied, using HEK293 cells stably expressing the human P2X7R, the binding affinity of analogues of new P2RX7 potential antagonists issued from pyroglutamic acid derivatives. Using a ligand-based approach, a pharmacophore model was selected as fitting at best the molecules of the reference dataset. Exhibiting two hydrogen bond acceptors and two lipophilic features, this pharmacophore query was used to filter our proprietary chemical library. Then, investigations were focused on the modulation of the positions 1 and 5 of the lactam ring, the exploration of the conformational space available in position 3 of the lactam ring and the replacing of the pyroglutamide scaffold. These pyroglutamide derivatives were assessed in a competitive radioligand binding study. Among these molecules, eight of them exhibited interesting equilibrium inhibition constants (Ki between 44.00 nM and 3.11 µM) in radioligand binding assays using [$^{(3)}$H]A804598 as the reference ligand. In our test, five compounds even show a better affinity than the GSK1370319A S enantiomer, a compound patented by the GSK company for its antagonistic properties against the P2X7R. A docking study with some of the synthesized pyroglutamides revealed that these compounds were allosteric modulators of P2RX7.

In conclusion, better understanding of potential P2RX7 antagonist interactions with P2RX7 has been provided, and pyroglutamides have been shown as potential drug-candidates for inflammation-related diseases.

REFERENCES

[1]E De Marchi, E Orioli, D Dal Ben, E Adinolfi, P2X7 Receptor as a Therapeutic Target, Adv Protein Chem Struct Biol. (2016) 104:39-79.

[2]J-H Park, Y-C Kim, P2X7 receptor antagonists: a patent review (2010-2015), Expert Opin Ther Pat (2017) 27 (3): 257-267.

PS-77 - ULTRA SENSITIVE QUANTIFICATION OF MONOCLONAL ANTIBODIES AND ADCS IN MOUSE PLASMA USING TRAP ELUTE MICROLC MS/MS METHOD

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ABSTRACT

LC-MS has been routinely adopted for biologics quantitation serving as the orthogonal technology to the traditional ligand binding assays (LBAs). As the amount of biological sample that can be collected from a small animal is limited, studies requiring ultra-low-level detection demonstrate the importance of MicroLC-MS methodologies. Herein we introduce a hybrid LBA/MicroLC-MS/MS workflow for ultra-sensitive quantification of Trastuzumab Emtansineand SILuLiteSigmaMAbuniversal antibody (SILuLite) in mouse plasma. This method can be simply transferred to quantitation assays for any other human mAbsin an animal matrix with minimum modification.

REFERENCES

1. Zhang F., Li Y., etc, Quantification of Trastuzumab in Rat Plasma using an Improved Immunoaffinity-LC-MS/MS Method, SCIEX Technical Note

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