7th Scientific Conference of Polish Metabolomics Society

Bialystok, 4-6th November 2020

BOOK OF ABSTRACTS

7th Scientific Conference of Polish Metabolomics Society

Metabolomics Circle

Book of abstracts

Białystok 2020

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Dear Colleagues,

We are pleased to welcome you to the 7th Scientific Conference of the Polish Metabolomics Society – Metabolomics Circle 2020. Due to the travel restrictions and social distancing requirements caused by the COVID-19 pandemic, the Metabolomics Circle is now held as a virtual event from 4th to 6th November 2020.

The meeting will be broadcast live, with both pre-recorded and real-time sessions. The questions will be asked through the chat and delivered to the speaker by the chairmen and moderators.

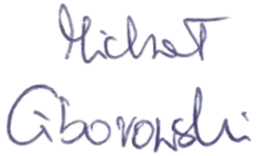
Despite the current epidemic situation, we are proud to present an outstanding scientific program, with two keynote speakers and four invited speakers, reporting the main analytical advances in the field of metabolomics together with the most impacted and relevant examples of its application. The program also includes 21 oral presentations, 18 short oral presentations and 32 poster communications, a selection of which will be discussed in four sessions of oral presentations, two sessions of short oral presentations and two-day poster session. All these presentations will be complemented with four discussion panels. The invited experts will express their opinions on crucial and debatable matters in the field. To spread these excellent researches, all the papers presented at this conference may be submitted to the "Advances in Medical Sciences", the official journal of the Medical University of Bialystok (IF_{2019}=2.570).

Before two days of talks and presentations, a set of extensive workshops, addressed especially to early career researchers will be available. Moreover, short oral presentations and e-posters will be evaluated, and the best talks and posters will be awarded.

Vendors will have a chance to advertise their newest products and solutions via short videos available at the conference webpage. We would also like to express our gratitude to all the companies for their participation and financial support of the conference.

Finally, we would like to thank all of the participants for their contribution as well as for attending the meeting, especially taking into account the current epidemiological situation. We are aware that many of you have had difficulties to find the time to attend this event. We did everything in our power to ensure that your participation will be scientifically successful and that you will enjoy attendance at the Metabolomics Circle 2020!

On behalf of the Organizing Committee,
the Scientific Committee of the Metabolomics Circle 2020
and the Polish Metabolomics Society,



Assoc. Prof. Michał Ciborowski
President of the Local Organizing Committee

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MSc. Katarzyna Miniewska

MSc. Ewa Paszkowska

Dr. Karolina Pietrowska

MSc. Tomasz Pieńkowski

MSc. Julia Siemińska

4th November 2020

WORKSHOPS

	Moderators: Patrycja Mojsak, Marta Kordalewska
09.00-09.30	**Tomas Kovalczuk**, Leco Poland
	High Performance GC- and GCxGC-TOFMS metabolomics-based approach for the discovery of potential cancer biomarkers in plasma
09.30-09.45	**Tomas Kovalczuk**, Leco Poland
	Aroma Profiling of Coffee with GC, GC×GC, and TOF MS
09.45-10.00	Q&A
	Moderators: Joanna Godzien, Michal Ciborowski
10.00-10.45	**Anas Kamleh**, Thermo Fisher Scientic
	Setting a new standard for analytical rigor and confident identifications in metabolomics
10.45-11.00	Q&A
	Moderators: Ewa Paszkowska, Adrian Godlewski
11.00-12.25	**Manuel Kratzke**, Biocrates
	Standardized metabolomics kits for quantitative and reproducible results. Kit workflow in the laboratory and the MetIDQ™ software
12.25-12.40	Q&A
	Moderators: Joanna Godzien, Michal Ciborowski
12.40-15.25	**Lukasz Nowicki**, Perlan Technologies
	Targeted and non-targeted data mining techniques for exploring high resolution spectro-chromatographic data; Alignment, normalization and filtering the data for efficient compound identification in metabolomics research
15.25-15.40	Q&A
15.45-16.45	**Isabel Riba**, Waters Corporation
	Ion mobility MS/MS data processing in complex matrixes
16.45-17.00	Q&A

5th November 2020

09.30–10.00	Conference Opening

SESSION I – *Analytical advances*

Chairmen: Michal Ciborowski, Michal Markuszewski

PLENARY LECTURE

10.00-10.35	**Coral Barbas,** Universidad CEU-San Pablo, Madrid, Spain
	In source fragmentation a tool for improving metabolite identification in untargeted Metabolomics

	ORAL PRESENTATIONS
10.35-11.00	**Jerzy Adamski,** Helmholtz Zentrum München, Neuherberg, Germany Metabolomics for biomedical research
11.00-11.25	**Magdalene Reinkensmeier,** Bruker Daltonik GmbH Highly reproducible 4D-Metabolomics using PASEF on a timsTOF Pro
11.25-11.50	**Hafiz Suleria,** The University of Melbourne, Australia LC-ESI-QTOF-MS/MS characterization of phenolic compounds from Australian grown avocados and their antioxidant potential
11.50-12.15	**Emily Armitage,** Shimadzu Corporation, Manchester, UK MS/MS untargeted metabolic profiling approach to study ethanol toxicity in mouse cerebral Tissue
12.15-12.40	**Craig Wheelock**, Karolinska Institute, Stockholm, Sweden An automated high-precision workflow for untargeted LC-MS-based urinary metabolomic epidemiology
	DISCUSSION PANEL I
12.50-13.40	**Metabolomics in biomedicine** Experts: Barbara Bojko, Boguslaw Buszewski, Michal Markuszewski, Piotr Mlynarz Moderators: Ewa Slominska, Wieslaw Wiczkowski
13.40-14.30	*Coffee break/Poster session*
14.30-15.45	**Short presentations session I** Chairmen: Marta Kordalewska, Patrycja Mojsak
	DISCUSSION PANEL II
15.45-16.35	**Metabolomics versus metadata and other omics** Experts: Stanislaw Deja, Beata Malachowska, Kamil Myszczynski, Ivana Stanimirova-Daszykowska Moderators: Michal Daszykowski, Andrzej Eljaszewicz
	SESSION II - ***Application of metabolomics*** **Chairmen: Wojciech Miltyk, Ryszard Smolenski**
	ORAL PRESENTATIONS
16.35-17.00	**Wojciech Barg,** Wroclaw Medical University, Poland Employing metabolomics in medicine - a clinician's point of view
17.00-17.20	**Natalia Drabinska,** Polish Academy of Sciences, Olsztyn, Poland Towards the identification of antibioticresistant bacteria causing urinary tract infections using volatile organic compounds analysis-a pilot study
17.20-17.45	**Shaun Bilsborough,** Agilent Technologies Determination of metabolomic changes involved in C2C12 rat myoblast differentiation into myotubes using discovery and targeted workflows
17.45-18.05	**Marek Michalowski**, Pro-Environment, Poland HCS and *In Vivo* imaging applications as a necessary part of targeted therapy development

18.05-18.30	**Stanislaw Deja,** UT Southwestern Medical Center UnACCeptable? - the interplay between fat oxidation and gluconeogenesis
	6th November 2020
	SESSION III – *Lipidomics* **Chairmen: Joanna Godzien, Rafal Szewczyk**
	PLENARY LECTURE
09.00-09.40	**Maria Federova,** University of Leipzig, Germany Analytical solutions for deep lipidome profiling
	ORAL PRESENTATIONS
09.40-10.05	**Tomas Cajka,** Institute of Physiology CAS, Czech Republic A workflow for combined analysis of the lipidome, metabolome, and exposome
10.05-10.25	**Wojciech Luczaj**, Medical University of Bialystok, Poland Lipidomic characterization of keratinocytes from skin of psoriatic patients exposed to cannabidiol followed by UVB irradiation
10.25-10.45	**Mariusz Aleksander Bromke,** Wroclaw Medical University, Poland Profiling of free fatty acids in lymph: a technical advance
10.45-11.05	**Malgorzata Szultka-Mlynska,** Nicolaus Copernicus University, Poland "Omics" applications of EC-MS
	DISCUSSION PANEL III
11.15-12.05	**Metabolomics in practical terms** Experts: Emilia Fornal, Tomasz Ligor, Lukasz Nowicki, Wojciech Wojtowicz Moderators: Joanna Godzien, Mariusz Aleksander Bromke
12.05-13.20	**Short presentations session II** Chairmen: Natalia Drabinska, Bartlomiej Kalaska
13.20-14.30	*Coffee break/Poster session*
	DISCUSSION PANEL IV
14.30-15.20	**Metabolomics in the non-academic sector** Experts: Tomasz Bienkowski, Damian Smuga, Rafal Szewczyk, Jerzy Wisniewski Moderators: Andrzej Malkowski, Piotr Mlynarz
	SESSION IV - *Analytical advances II* **Chairpersons: Maciej Stopa, Andrzej Eljaszewicz**
	ORAL PRESENTATIONS
15.20-15.45	**Liang Li,** University of Alberta, Edmonton, Alberta, Canada Recent advances in Chemical Isotope Labeling (CIL) LC-MS for high-coverage quantitative metabolome analysis

15.45-16.10	**Tomas Kovalczuk,** LECO EATC Berlin, Germany Advantages of GC- and GCxGC- TOF MS in metabolomic research
16.10-16.35	**Piotr Mlynarz,** Wrocław University of Science and Technology, Wroclaw, Poland Cancer cells metabolomics studies. The effect of hypoxia, normoxia and cultivation time
16.35-17.00	**Anthony Zerlin,** Agilent Technologies How to efficiently remove lipids and proteins from your metabolomic samples for better LCMS results
17.00-17.25	**Joanna Bogusiewicz,** Nicolaus Copernicus University in Torun, Poland Spatially resolved profiling of human brain *in vivo*. Chemical biopsy as a new tool in neuroscience research
17.25-18.05	Conference Closing

SHORT PRESENTATIONS	
5th November 2020 **(14.30-15.45)**	
14.30-14.38	**Anna Michalska-Falkowska,** Medical University of Bialystok, Poland The significance of systematic biobanking for reliable metabolomics research
14.38-14.46	**Radik Mametov,** Nicolaus Copernicus University, Torun, Poland Polypyrrole based coating materials for SPME fibers to be used in analysis of VOCs as potential colorectal cancer biomarkers
14.46-14.54	**Karolina Zuchowska,** Nicolaus Copernicus University, Torun, Poland Quantitative determination of piperacillin and imipenem in plasma and bronchoalveolar lavage with SPME-LC-MS/MS
14.54-15.02	**Natalia Platosz,** Polish Academy of Sciences in Olsztyn, Poland Methylated cyanidin glucuronide is the main anthocyanin compound found in sheep's blood plasma and cerebrospinal fluid after intraruminal administration of chokeberry preparation
15.02-15.10	**Marika Franczak,** Medical University of Gdansk, Poland Nanochromatography with mass detection: a new way to measure level of angiotensins in human plasma
15.10-15.18	**Adrian Arendowski,** Rzeszow University of Technology, Poland Gold nanoparticle-based laser desorption/ionization mass spectrometry in serum and urine metabolomics analyses of renal cell carcinoma
15.18-15.26	**Agata Sumara,** Medical University of Lublin, Poland Application of high resolution mass spectrometry and chemometric method as a tool to detect sesame seeds oil
15.26-15.34	**Kacper Jablonowski,** Medical University of Bialystok, Poland Comparison of nutraceutical effect of Cystoseira abies marina and Rosemarinus Officinalis on oxidative stress in T1DM animal model: the changes in the levels of phospholipids and ether-phospholipid
15.34-15.42	**Szymon Plewa,** Poznan University of Medical Sciences, Poland Towards spatial distribution of lipids – matrix-assisted laser desorption/ionization mass spectrometry imaging of ovarian tumour tissue – the pilot study
6th November 2020 **(12.10-13.25)**	
12.10-12.18	**Sinemyiz Atalay,** Medical University of Bialystok, Poland Proteomic profile of skin keratinocytes from rats exposed to UVA/B radiation and treated with cannabidiol
12.18-12.26	**Alicja Braczko,** Medical University of Gdansk, Poland The development of LC/MS method for cystine determination in peripheral blood leukocytes and new challenges in laboratory diagnosis and monitoring of cystynosis in Poland
12.26-12.34	**Agnieszka Gegotek,** Medical University of Bialystok, Poland Proteomic approach to metabolic changes in psoriasis vulgaris

12.34-12.42	**Maria Jacewicz**, Waters Corporation, Warsaw, Poland
	A one second screening of cyanobacteria utilizing laser REIMS technology
12.42-12.50	**Ewelina Maslak,** Nicolaus Copernicus University, Torun, Poland
	Culturomics approach for diabetic foot infection diagnosis
12.50-12.58	**Karolina Mielko,** Wroclaw University of Science and Technology, Poland
	Comparison of microorganisms disintegration methods for metabolomics analysis by using 1H NMR
12.58-13.06	**Paulina Mierzejewska,** Medical University of Gdansk, Poland
	An unusual nicotinamide derivative – 4-pyridone-3-carboxamide ribonucleoside (4PYR) as a novel endothelial toxin and oncometabolite
13.06-13.14	**Magdalena Muszynska,** Biotechnology Line Leader Pro-Environment Polska Sp. z o.o.
	Bispecific antibodies - a new look at the molecular interactions analysis
13.14-13.22	**Julia Mironenka,** University of Lodz, Poland
	The influence of 2,4-D on the lipid peroxidation of Trichoderma harzianum

POSTER SESSION

5th November 2020 (13.40-14.30)

6th November 2020 (13.25-14.30)

- **Monika Beszterda**, Poznan University of Life Sciences, Poland
  Important remarks concerning LC/ESI-MS analysis of biochanin A and prunetin
- **Magdalena Buszewska-Forajta**, Medical University of Gdansk, Poland
  The potential role of lipids in prostate cancer based on FFPE tissue samples with the use of MALDI-TOF
- **Weronika Brzozowska**, University of Szczecin, Poland
  Biosynthesis of three-dimensional diatomaceous biosilica doped with titanium ions
- **Anna Czajkowska**, Medical University of Bialystok, Poland
  Relative Dependency Analysis of metabolomics data obtained for aqueous humour samples collected from diabetic and non-diabetic patients during cataract surgery
- **Joanna Dawidowska**, Medical University of Gdansk, Poland
  The role of intervention in improving hemodialyzed patients adherence to hypotensive therapy
- **Mariusz Fleszar**, Wroclaw Medical University, Poland
  Development of Mass Spectroscopy assay for Nε-(1-Carboxymethyl)-L-Lysine, Nε-(1-Carboxyethyl)-L-lysine in human plasma
- **Magdalena Gregorczyk**, Medical University of Gdansk, Poland
  Amino acid and nucleotide profile in plasma, kidney, and liver of mice with Huntington's disease
- **Ahsan Hameed**, Medical University of Bialystok, Poland
  Differences in the metabolic response to a high-carbohydrate meal between lean and overweight men – a metabolomics study
- **Piotr Jakubow**, Medical University of Bialystok, Poland
  Neurometabolite changes in patients with complex regional pain syndrome using proton magnetic resonance spectroscopy
- **Daria Janiszewska**, Nicolaus Copernicus University, Torun, Poland
  Application of hyphenated techniques for the determination and identification of antibiotics and microorganisms from clinical samples
- **Agnieszka Klupczynska**, Poznan University of Medical Sciences, Poland
  A study of free amino acid composition of different honeybee products
- **Anna Kozub**, Medical University of Lublin, Poland
  LC/HRMS-based metabolic fingerprinting and multivariate analysis in authentication of meat
- **Tomasz Kulesza**, Mossakowski Medical Research Centre Polish Academy of Sciences, Poland
  The influence of the diabetic environment on phosphate homeostasis in podocytes
- **Marcin Lipinski**, Medical University of Gdansk, Poland
  Simultaneous determination of lopinavir, saquinavir, and ritonavir in human plasma using liquid chromatography-ion trap mass spectrometry
- **Szymon Macioszek**, Medical University of Gdansk, Poland
  Evaluation of gastrointestinal stromal tumour sample preparation procedure for LC-MS untargeted metabolomic analysis
- **Katarzyna Miniewska**, Medical University of Bialystok, Poland
  Postprandial changes in plasma metabolome of non-diabetic men are related to genetic susceptibility to type 2 diabetes
- **Hanna Mojska**, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland
  AAMA and GAMA levels in urine as markers of acrylamide exposure from the diet and tobacco smoke
- **Aneta Ostrozka-Cieslik**, Medical University of Silesia in Katowice, Poland
  Effect of thyrotropin on kidney damage of stored before transplantation by hypothermic perfusion
- **Alicja Pakiet**, University of Gdansk, Poland
  The effect of one anastomosis gastric bypass on serum fatty acid profile in patients with morbid obesity

- **Karolina Pietrowska**, Medical University of Bialystok, Poland
  Metabolomics reveals differences in aqueous humor composition between patients with and without pseudoexfoliation syndrome
- **Grzegorz Przybyla**, University of Opole, Poland
  Paracetamol - a drug with new and multidirectional mechanisms of action
- **Natalia Pudelko-Malik**, Wroclaw University of Science and Technology, Poland
  Application of advanced metabolomics methods for the determination of fatty acids in seeds oils
- **Badr Qasem**, Wroclaw University of Science and Technology, Poland
  The 1H NMR studies for monitoring of unique changes in metabolic response of fibrosarcoma cell line on oxygen availability and cultivation time
- **Lidia Radko**, National Veterinary Research Institute in Pulawy, Poland
  Interactions of veterinary drugs: enrofloxacin and fipronil *in vitro* study
- **Anna Rajska**, Medical University of Gdansk, Poland
  Searching for changes in metabolome of women with polycystic ovary syndrome with the use untargeted metabolomics
- **Anna Roszkowska**, Medical University of Gdansk, Poland
  Optimization of analytical approach based on SPME-LC-MS/MS for the analysis of selected endocannabinoids in brain regions
- **Paulina Samczuk**, Medical University of Bialystok, Poland
  Biochemistry of death – application of metabolomics in forensic medicine
- **Jolanta Strzelecka**, Medical University of Warsaw, Poland
  QEEG as a possible biomarker for ASD diagnosis
- **Marta Tomczyk**, Medical University of Gdansk, Poland
  Use of stable isotopomers and liquid chromatography with mass spectrometry (LC/MS) for cardiac substrate preference analysis in mice
- **Renata Wawrzyniak**, Medical University of Gdansk, Poland
  Untargeted metabolomics of early vascular aging in hypertensive patients
- **Izabela Wojtczak**, Nicolaus Copernicus University, Poland
  Metabolic insertion of biosilica obtained from diatoms with cerium ions
- **Magdalena Zabielska-Kaczorowska**, Medical University of Gdansk, Poland
  Label-free quantitative SWATH-MS proteomic analysis of adult myocardial slices *in vitro* after biomimetic electromechanical stimulation

KEYNOTE SPEAKERS

Coral Barbas

Universidad CEU-San Pablo, Madrid, Spain

Maria Fedorova

Universität Leipzig, Germany

INVITED SPEAKERS

Tomaš Čajka

Institute of Physiology CAS, Czech Republic

Liang Li

University of Alberta, Edmonton, Alberta, Canada

Hafiz Suleria

The University of Melbourne, Australia

Craig Wheelock

Karolinska Institute, Stockholm, Sweden

Prof. Coral Barbas is Full Professor of Analytical Chemistry at the Chemistry and Biochemistry Department of the Faculty of Pharmacy at Universidad CEU San Pablo in Madrid and Director of the "Centre for Metabolomics and Bioanalysis" (CEMBIO) at this Faculty. She is also Director of CEU International School of Doctorate, Visiting Professor at Imperial College London and at Medical University of Bialystok. She received the medal of Bialystok Medical University, the Medal of the Belgian Society of Pharmaceutical Sciences and she was named to the 2016 Power List, the 50 Most Influential Women in Analytical Chemistry in the World by The Analytical Scientist. She is currently president of the new board of the Royal Spanish Chemistry Society in the territorial section of Madrid.

The subject of scientific interests and pioneering discoveries of Professor Barbas is research on the application of highly advanced technologies of analytical chemistry in metabolomic analysis. Pioneering research in this field concerns the improvement of separation methods and large-scale data management strategies for the identification and quantification of metabolites. Her group is a world leader in implementing the multiplatform approach (GC-MS, LC-MS and CE-MS) in the metabolomic analysis of cardiovascular diseases, diabetes, obesity, cancer, bacterial and other infections.

Dr. Maria Fedorova is a group leader at the Institute of Bioanalytical Chemistry at the Faculty of Chemistry and Mineralogy, at the University of Leipzig. Her research is focused on the development and optimization of chromatography and mass spectrometry methods for the analysis of lipids and their modified forms.

Dr. Fedorova's group works on the implementation of high throughput LC-MS methods in the discovery lipidomics, targeting in-depth identification and quantification of human lipidome in variety of tissues. By combining lipidomics data with investigation of related proteins and protein post-translational modifications *via* systems medicine approach, Dr. Fedorova aims to provide a deeper understanding of the pathophysiology of obesity, insulin resistance, type 2 diabetes and cardiovascular diseases. Another aspect of Dr. Fedorova's research is the development and application of mass spectrometry-based methods for analysis of protein and lipid modifications, including lipid-protein adducts. She is interested in understanding the role of redox lipid and protein signalling in the onset and progression of human disorders associated with chronic inflammation, including cardiovascular and metabolic diseases.

Dr. Fedorova is a Vice-Chair of the COST Action EpiLipidNET, Chair of the International HNE Club, member of LIPID MAPS Advisory Board and member of the steering committee of Lipidomics Standards Initiative as an Interest Group within International Lipidomics Society. She is also a member of Society for Free Radical Research Europe, Society of Redox Biology and Medicine, and Metabolomics Society.

Assoc. Prof. Tomáš Čajka is the head of the Laboratory of Translational Metabolism (research lab) and the Laboratory of Metabolomics (core lab) at the Institute of Physiology of the Czech Academy of Sciences, Prague. Previously he worked at the University of Chemistry and Technology, Prague. Additionally, he worked at the West Coast Metabolomics Center at the University of California, Davis, USA. His research activities include metabolomics, lipidomics, exposomics, fluxomics, cheminformatics, and chemometrics. He is interested in creating innovative approaches using cutting-edge LC–MS technologies to combine untargeted and targeted metabolomics methods, extending the coverage of spectral libraries and identification of unknowns by using *in-silico* fragmentation software, and introducing these tools in biomedical research resulting in the development of clinical medicine. The laboratory is certified and operations are compliant with Good Laboratory Practice (GLP) guidelines.

Dr. Liang Li obtained his B.Sc. degree in Chemistry from Zhejiang (Hangzhou) University, China, in 1983, and his Ph.D. degree in Chemistry from the University of Michigan, Ann Arbor, USA, in 1989, under the direction of Professor David M. Lubman. After graduation, he started to work at the Department of Chemistry at the University of Alberta, Edmonton, Alberta in July 1989. He has also worked as an Adjunct Professor of Biochemistry Department, Faculty of Medicine, since January 2008. He is a co-PI of The Metabolomics Innovation Centre (TMIC) mainly supported by Genome Canada. He was a co-PI of the Human Metabolome Database (HMDB) Project; his laboratory created the HMDB MS/MS spectral library of the endogenous human metabolites that has been used world-wide by the metabolomics community for unknown metabolite identification based on spectral matches. He is a Co-Director of TMIC since July 2019. He is a founder of Nova Medical Testing, Inc., a university spin-off company focusing on developing mass spectrometry based analytical solutions for medical and health diagnostic applications, including targeted diagnostic-panel analysis and global biomarker analysis. Dr. Li is an elected fellow of the Royal Society of Canada (Academy of Science) (2019). He received the Young Explorers Prize from the Canadian Institute for Advanced Research (CIAR), which was given to Canada's top twenty researchers aged forty or under in science and engineering (2002) as chosen by a panel of international judges. He was the recipient of the Rutherford Memorial Medal in Chemistry from the Royal Society of Canada (2003). He was one of the seven researchers selected as Brightest Minds 2016 by Canadian Institutes of Health Research. He is a fellow of the Chemical Institute of Canada since 2001. Dr. Li has published more than 280 research papers mainly in the area of analytical mass spectrometry. He has delivered many invited lectures including Hong Kong Polytechic University Distinguished Lecture in 2011, Distinguished Lectureship on Cerebrating the 50th Anniversary of Hong Kong Baptist University Faculty of Science in 2011, and Thermo Fisher Scientific Distinguished Scientist Lecture at the University of Montreal in 2013.

Dr. Hafiz Suleria works in the School of Agriculture and Food as a McKenzie Fellow. He has completed his Postdoctoral Research from the Department of Food, Nutrition, Dietetics and Health, Kansas State University, USA.

Previously, he was awarded Alfred Deakin Postdoctoral Research Fellowship at the Deakin University, Australia. He did his PhD from the University of Queensland in a collaboration with Translational Research Institute, UQ School of Medicine and Commonwealth and Scientific and Industrial Research Organization, Australia. Before joining the UQ, he worked as a Lecturer in the Department of Food Sciences, Government College University Faisalabad, Pakistan. He also worked as a Research Associate in "PAK-US Joint Project" at the National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan and the Department of Food Science, University of Massachusetts, USA funded by the Higher Education Commission, Pakistan and the Department of State, USA. His research concentrates on food science and nutrition particularly in screening of phytochemicals/bioactive molecules from different plants, marine and animal sources. His research interest includes isolation, purification and characterization of bioactive compounds using various cutting-edge techniques followed by their *in vitro* bioactivity, *in vivo*, cell culture and animal studies. Hafiz Suleria has published more than 100 peer-reviewed scientific papers in different high reputed journals. He is also in collaboration with more than five universities where he is working as a co-supervisor/special member for PhD and Postgraduate students and also involved in joint publications, projects and grants.

Assoc. Prof. Craig Wheelock works in the Department of Medical Biochemistry and Biophysics at the Karolinska Institute in Sweden, where he founded the Integrative Molecular Phenotyping Laboratory (*www.metabolomics.se*). He is also a visiting professor at the Gunma Institute for Advanced Research (GIAR) in Japan, where he leads the Karolinska International Open Laboratory in metabolomics.

Research in the Wheelock laboratory is focused on the use of mass spectrometry-based approaches to perform molecular phenotyping at the population level. The interests of the group are centered on understanding the pathophysiology of inflammatory respiratory diseases, particularly asthma. An emphasis of the group is the targeted analysis of lipid mediators (e.g., eicosanoids, sphingolipids) and investigating their role in the etiology of inflammatory disease. As part of our molecular phenotyping efforts, we develop the analytical, statistical and informatics methods necessary to concatenate and interrogate 'omics-based data structures.

He is a member of the European Respiratory Society (ERS) Science Event Working Group and a consultant on the NIEHS funded Children's Health Exposure Analysis Resource (CHEAR) project at Mount Sinai, New York. When not balancing his time between Sweden and Japan, he enjoys teaching his kids to kayak and play nicely with others.

DISCUSSION PANELS

Metabolomics in biomedicine

The aim of this panel is to present applications of metabolomics in biomedicine and available research strategies. The discussion will focus on main challenges associated with the planning of metabolomics studies. Panelists will share their opinion on different approaches in collection of metabolome samples (quenching, sampling *in vivo* and *in vitro* models), the use of unique and normalized methods in the context of the balance between the metabolome coverage and unknowns, as well as challenges and perspectives facing Metabolome-wide association studies. Among invited experts, there are representants of various approaches and methodologies, what is a starting point for an interesting discussion. The debate will be conducted by Assoc. Prof. Barbara Bojko, Prof. Boguslaw Buszewski, Prof. Michal Markuszewski and Prof. Piotr Mlynarz. The talk will be moderated by Prof. Ewa Slominska and Assoc. Prof. Wieslaw Wiczkowski.

Assoc. Prof. Barbara Bojko, professor of Nicolaus Copernicus University in Torun. Her studies are mainly focused on the implementation of new analytical solutions based on microextractions' techniques into clinical diagnostics and pharmaceutical research. She is particularly interested in low- and non-invasive tissue analysis in oncology, organ transplantation and translational medicine. She has published over 100 papers in peer-reviewed journals obtaining over 2600 citations.

Prof. Boguslaw Buszewski, the head of Department of Environmental Chemistry and Bioanalysis and the head of Sepatative Bioanalytical Methods Centre BioSep, Nicolaus Copernicus University in Torun. Corresponding member of the Polish Academy of Sciences. His numerous scientific interests include pharmaceutical, medical, and biochemical analysis, proteomics, and metabolomics with the use of state-of-the-art instrumental techniques in the laboratory environment and in the field. He is one of the most frequently cited Polish chemists (over 18 000 citations) (h-index=56).

Prof. Michal Markuszewski, the head of Department of Biopharmacy and Pharmacokinetics, Medical University of Gdansk. The areas of his research are bioanalytics, particularly metabolomics, pharmaceutical analytics, and pharmacokinetics. He is a co-author of over 130 original papers in international scientific journals.

Prof. Piotr Mlynarz, Department of Biochemistry, Molecular Biology and Biotechnology. He is in charge of the team specialized in the use of NMR spectroscopy supported with chemometrics and statistical methods in metabolomics research. He applies metabolomics in environmental diagnostics, biotechnology, and microbiology, as well as authentication and analytics of agricultural produce and food products. He has published over 90 scientific articles, receiving almost 1500 citations.

Metabolomics, other omics, and metadata

The purpose of this panel is to present issues related to the integration of data from different omics platforms. These problems will be discussed taking into account biological, analytical, and software aspects. Experts will assess reliability of fusion of omics data considering available tools and solutions aiding data integration process. For this purpose, aspects associated with the correct interpretation of observed metabolome changes, challenges concerning omics data integration, and problems related to software will be discussed. Among invited experts, there are adherents of both biological and statistical approach, what guarantees presenting multidimensional nature of data fusion problem. Following scientists will take part in the debate: Dr. Stanislaw Deja, Dr. Beata Malachowska, Dr. Kamil Myszczynski, and Assoc. Prof. Ivana Stanimirova-Daszykowska, the talk will be moderated by Prof. Michal Daszykowski and Assoc. Prof. Andrzej Eljaszewicz.

Dr. Beata Malachowska, a medical doctor by training, currently works as an omics data analyst in the Department of Radiation Oncology at Albert Einstein College of Medicine in New York. She started her academic work in the Department of Biostatistics and Translational Medicine at the Medical University of Łódź. She is co-author of over 40 publications and laureate of numerous scientific awards and grants: Student Nobel in medical sciences, Gold OTIS, scholarship of the Polpharma Scientific Foundation, L'Oréal-UNESCO For Women in Science Award, and START grant of Foundation for Polish Science.

Dr. Kamil Myszczynski, bioinformatics specialist at the Institute of Animal Reproduction and Food Research of Polish Academy of Science in Olsztyn and University of Warmia and Mazury in Olsztyn. He works on the analysis of data acquired from high-throughput methods such as the genome, transcriptome, and DNA micromatrix sequencing. He is also interested in data integration in the cross-talk between genome, transcriptome, proteome, and metabolome.

Assoc. Prof. Ivana Stanimirova-Daszykowska, Professor of the University of Silesia in Katowice. She conducts research on developing stable chemometrics methods which will enable data analysis with missing values and incorporation of experimental error information into the data analysis process. She works on methods allowing the integration of information obtained from data derived from different measurement sources and analytical platforms (data fusion). She implements developed methods in, among others, metabolomics, metabonomics, and analysis of food and pharmaceutical products.

Dr. Stanislaw Deja, UT Southwestern Medical Center in Dallas. His research is focused on metabolic pathways (including hepatic metabolism) control and regulation. He specializes in the application of isotopic-labelled metabolites in tracing metabolic fluxes *in vivo* and *ex vivo*. He is particularly interested in the use of a combination of NMR and MS techniques to precisely model the activity of metabolic networks and their disorders in terms of obesity.

Metabolomics in practice

The aim of this panel is to present critical assessment and evaluation of the most frequently used techniques (LC-MS, GC-MS, NMR) in terms of their potential and limitations for targeted as well as non-targeted analyses. These problems will be discussed, considering laboratory and data analysis aspects. Panelists will express their opinion on available sample preparation methods, with the particular focus on the automatization of this process, data acquisition optimization in both targeted and non-targeted analyses, and crucial points in the data treatment and metabolites identification. Invited experts are representatives of targeted as well as non-targeted approach, and users of the various analytical platforms, what is not only a starting point of profound discussion but also a possibility to familiarize the audience with practical aspects of each approach. Assoc. Prof. Emilia Fornal, Assoc. Prof. Tomasz Ligor, Dr. Lukasz Nowicki and Dr. Wojciech Wojtowicz will take part in this debate which will be moderated by Dr. Joanna Godzien and Dr. Mariusz Bromke.

Assoc. Prof. Emilia Fornal, analytical chemist, an expert on liquid chromatography and mass spectrometry, professor of the Medical University of Lublin. She works in the Chair and Department of Pathophysiology at the Medical University of Lublin. Her scientific research is focused on the application of liquid chromatography and mass spectrometry in medicine, pharmacy, and food science. She has extensive experience in the identification of new psychoactive substances, metabolomics and proteomics analyses.

Assoc. Prof. Tomasz Ligor, an analytical chemist by training. He works in the Department of Environmental Chemistry and Bioanalysis at Nicolaus Copernicus University in Torun. The main area of his scientific interests is gas chromatography, mass spectrometry, volatile organic compounds, volatolomics as well as methods for biological samples preparation and search for volatile biomarkers of neoplastic diseases.

Dr. Wojciech Wojtowicz, biotechnologist by training, Wroclaw University of Science and Technology graduate, currently member of the team of Chemometrics and Analytical Technology at University of Copenhagen. For the last eight years, he has been conducting studies on the application of metabolomics with the use of magnetic resonance spectroscopy in the analysis of neoplastic diseases. Presently, he works on automatization and improvements of preparation processes and data analysis in metabolomics.

Dr. Lukasz Nowicki, PhD in biological sciences with specialization in biochemistry. For over 9 years he has been working as an application engineer in Perlan Technologies Poland. He is involved in the development and optimization of targeted and non-targeted LC-MS and GC-MS methods. He also conducts training on the use of Agilent's LC-MS and GC-MS analytical instruments and related software.

Metabolomics in non-academic sector

This panel is aimed to present applications of metabolomics in non-academic areas and the differences in its implementation in comparison to the academic environment. The discussion will identify key challenges associated with the implementation of metabolomics studies in pharmaceutical, medical, and biotechnological sectors. The panelists will comment on the differences between routine methods and those developed for publication purposes, problems with commercialization and R&D labour market expectations towards researchers. Among the invited experts, there will be representatives of both commercial and academic sector what guarantees a substantive discussion and a chance to learn challenges and opportunities offered by cooperation with the non-academic community. The debate will be attended by Dr. Tomasz Bienkowski, Dr. Damian Smuga, Dr. Rafal Szewczyk and Dr. Jerzy Wisniewski, and the conversation will be moderated by Dr. Andrzej Malkowski and Prof. Piotr Mlynarz.

Dr. Tomasz Bienkowski, PhD in chemical sciences with a specialization in mass spectrometry. For over 20 years he has been promoting and developing this technique. Currently, he is chairman of the board and co-owner of Masdiag Laboratory which uses modern analytical techniques, mainly LC-MS/MS, in routine medical diagnostics. He is co-author of numerous scientific articles and patent applications.

Dr. Damian Smuga, by passion and professionally associated with preclinical development of innovative therapeutic substances. He is the leader of ADMET Team at Celon Pharma S.A. He conducts and coordinates the work of the research group concerned with the analysis of new chemical compounds, including their characteristics during preclinical drug development. His scientific interests are focused on QSAR and QSPR analysis, development and advancement of analytical methods as well as targeted and non-targeted metabolomics.

Dr. Rafal Szewczyk, PhD in biological sciences with specialization in microbiology-biotechnology. He is the scientific director of LabExperts. His interests are focused on proteomics and metabolomics of biological processes, searching, identification and profiling of different biomarkers, modern microbiological diagnostics and biodegradation of xenobiotics. He has over 20 years of work experience with various separation techniques, mass spectrometry as well as in a microbiological laboratory.

Dr. Jerzy Wisniewski, PhD in medical sciences. From the beginning of his scientific career connected with Wroclaw Medical University. He works on LC-MS technique application in various studies in the field of metabolomics and proteomics. For several years he has been cooperating with the business community, performing internships and projects in companies located in Wroclaw Technology Park.

KEYNOTE LECTURES

In Source Fragmentation A Tool For Improving Metabolite Identification In Untargeted Metabolomics

M. Mamani-Huanca, A. Gradillas, A. Gil de la Fuente, Á. López-Gonzálvez, C. Barbas

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In-source fragmentation (ISF) is a phenomenon that takes place at the intermediate pressure region between the ion source at atmospheric pressure and the vacuum chamber of a mass spectrometer. Although electrospray ionization (ESI) is regarded as one of the softer ionization technologies with lower production of in-source fragment ions, fragmentation still occurs, sometimes in an unintentional way. It happens both in liquid chromatography (LC) and capillary electrophoresis (CE) and therefore this is a topic of interest for researchers in untargeted metabolomics.

Although it is a known phenomenon, many questions are still open: optimal conditions for in-source fragmentation; fragmentation patterns produced under those conditions; their reproducibility and capability for systematization and rationalization; potential independency of the matrix, and – probably the most important – potential use of the provided information for the identification of unknowns in untargeted metabolomics.

In this presentation we respond to those questions and apply our knowledge to study the identification of modified amino acids[1]. The alteration of modified amino acid (MAA) profiles in biological samples is related to important cellular, physiological, and pathological processes. The diagnostic ions (DIs) presented and our in-house fragment library allowed us to establish a workflow for targeted extraction of MAAs. We present key examples showing successful findings such as the identification of N2-methyl-l-lysine, which provides insight into the lysine methylome. The experimental results presented prove that the use of ISF data, when combined with a thorough study of the fragmentation mechanisms, constitutes an informative source of accurate molecular identity. Our study was performed using CE-MS, however the background knowledge is applicable both to CE and LC.

References:

[1] Mamani-Huanca M. et al. Unveiling the Fragmentation Mechanisms of Modified Amino Acids as the Key for Their Targeted Identification. *Anal Chem.* 2020 Apr 7;92(7):4848-4857.

Analytical solutions for deep lipidome profiling

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Human metabolic disorders including obesity, diabetes, cardiovascular diseases, cancer and neurodegeneration are multifactorial and characterized by complex alterations in biomolecular and signalling networks challenging their early diagnosis, prognosis and selection of optimal pharmacological intervention strategies. Identification of reliable markers can accelerate clinical developments by (*i*) enhancing the monitoring of disease progression and changes in clinical status, (*ii*) measuring the impact of the treatment or therapeutic interventions on the course of the disease, and (*iii*) providing information on drug-target interactions. In addition, the systematic multidimensional (multi-Omics) vision of human diseases can guide the development of personalized, predictive, preventive and participatory medicine (P4 Medicine). Lipids represent a particularly promising markers as a majority of metabolic diseases are characterized by disbalance in lipid metabolism. Modern MS-based lipidomics provides the possibility to identify and quantify hundreds of individual lipids present in cells, tissues and biological fluids aiming to decipher the molecular mechanisms of lipids actions and biological roles. However, full understanding of lipid metabolism, signalling, and membrane properties in a particular tissue or cell type requires deep lipidomics profiling based on the combination of several analytical and computational strategies. Here I describe an example of analytical workflow for in-depth lipidome characterization with the focus on selecting optimal extraction, fractionation, LC-MS/MS and computational strategies.

ORAL PRESENTATIONS

Metabolomics for biomedical research

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Metabolomics addresses a comprehensive view on metabolites depicting biological and non-enzymatic processes. The metabolomics requests integrative application of epidemiology, analytics and bioinformatics and provides information on specific metabolic signatures in health and disease. Metabolomics was instrumental in describing processes of healthy aging, endocrine homeostasis in transgender individuals and diabetes type 2. Unexpected kinetics in lipid metabolism and cross-talks between urea cycle and TCA were discovered.

Highly reproducible 4D-Metabolomics using PASEF on a timsTOF Pro

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The highly confident annotation of metabolites requires the combination of many properties of the ion. In this study, we compared the results from multiple systems using PASEF acquisition. PASEF utilizes trapped ion mobility spectrometry (TIMS) separation, which separates metabolites based on their shape. In utilizing the fast PASEF MS/MS mode, up to x10 more compounds can be fragmented producing more MS/MS spectra which are "mobility cleaned" using TIMS separation, allowing for the unambiguous determination of a metabolite. Moreover, PASEF gives CCS values for each ion, allowing an additional parameter to be used for identification of structure. The determined CCS values were highly reproducible for intra- and inter-lab measurements (< 1%) proving that CCS values in libraries can be reliably used. This was further confirmed by comparing the measured CCS value to those already in the literature, showing similar trends and a deviation of <1%. PASEF acquisition mode was used to separate co-eluting compounds and utilize the additional separation dimension to give clean MS/MS spectra. On average 15 MS/MS spectra were acquired per PASEF scan. This results in a high coverage of precursor peaks in each MS spectrum, leading to about 80% of metabolomics features with assigned MS/MS spectra. A further central topic of the presentation will be the inter-lab comparison of the determined CCS values. For all identified metabolites, the CCS values determined by TIMS deviated by < 1% on average from published DT-IMS reference CCS values (e.g. for Theobromine, Riboflavine, 1-Methyladenosin, Caffeine), showing the ability to compare CCS values determined by TIMS with DT-IMS values from earlier studies. The CCS value deviation filter was combined with filters for retention time deviation, precursor mass error, precursor isotopic pattern and MS/MS spectrum in an Annotation quality (AQ) scoring symbol of the MetaboScape software, that has been used for data processing. This reliability provides high confidence in annotations of target molecules and shows the benefit of PASEF spectra for metabolomics experiments.

LC-ESI-QTOF-MS/MS screening and characterization of phenolic compounds from Australian grown avocados and their antioxidant potential

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Avocado is a common tropical fruit known for its nutritional and health benefits. Number of polyphenol-rich avocado by-products are produced during processing in the food and cosmetics industry, especially peels and seeds. From an economic point of view, the use of these by-products would be beneficial. The comprehensive understanding of phenolic profile in avocado by-products is limited. In this experiment, unripe Hass, ripe Hass, Wurtz, and Reed were selected for research. liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS) was used to separate and identify the polyphenols in edible and non-edible parts of four kinds of avocados, and their antioxidant capacity was evaluated by various antioxidant test methods, including total phenolic, flavonoid, and tannins content, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, and total antioxidant capacity assay. The results of the component analysis showed that the extract mainly contained four classes of phenolic compounds: flavonoids, lignans, phenolic acids, stilbenes. In the distribution of phenolic compounds in the samples, the differences among varieties existed. Some polyphenols were only found in one avocado variety. The different antioxidant activities of the extracts also indicated the difference of phenolic profiles among different samples. Compared with the seed and pulp extracts, the peel extracts have higher total phenolic content and antioxidant activity. Then, the characteristics of phenolic compounds in the extract of avocado were characterized by high-performance liquid chromatography with photodiode array detector (HPLC-PAD). In general, it is suggested that avocado seeds and peels are superior sources of polyphenols, possess antioxidant effects, which are favorable to health, and can be used as functional food ingredients or antioxidant additives.

Keywords: Functional foods, polyphenols, avocado and antioxidant potential

MS/MS untargeted metabolic profiling approach to study ethanol toxicity in mouse cerebral tissue

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Alcoholic liver disease remains a leading cause of chronic liver disease, with morbidity and mortality continually increasing. In recent years, biomarkers discovered through metabolomics have shed light on the biochemistry of this disease, arousing interest in ethanol-induced toxicity in other tissues in the body. In this study, the metabolic effects of acute (short-term) and chronic (long-term) exposure to ethanol have been investigated in mouse cerebral tissue using LC-QTOF-MS/MS with fast scanning data independent acquisition (DIA).

The long-term (8 week) and short term (11 day) experiments consisted of C57BL/6 mice (8-10 weeks old) separated into groups for control and *ad libitum* feeding with a *Lieber-DeCarli* ethanol diet containing daily 5% alcohol 99%. In the short term experiment, a single dose of 25% alcohol was administrated by oral gavage at day 5 of the intervention and at the last day of experiment 6 hours before sacrifice. The experiment was conducted in agreement with current national and European legislation (N. 2015/1992, ΠΔ 56/2013, European guideline 2010/63) and cerebral tissues were collected post-mortem. Untargeted metabolic profiling analysis was performed on cerebral tissue extracts using LC-QTOF-MS/MS. MS and 44 DIA-MS/MS scans were performed at 50 spectra per second within a mass range of m/z 50-1000, resulting in a total cycle time of less than 1 second. Collision energy spread was applied to acquire precursor and product ion data in the same scan to support metabolite ID. Authentic standards were also analysed. Univariate statistical analysis revealed that acute exposure decreased arachidonic acid, docosahexaenoic acid, glycerophosphocholine, CDP-choline, adenine, adenosine, cytidine, creatine, creatinine, valine and taurine and some phospholipids. Chronic exposure decreased acetyl-carnitine, adenine and adenosine with phospholipids and ceramides, while many amino acids were increased along with hypoxanthine, xanthine, oxoproline, choline, creatinine, and docosahexaenoic acid.

Using this LC-QTOF-MS/MS method with fast scanning DIA, data was generated for biologically relevant precursors in a single untargeted experiment, rendering DIA advantageous over other MS/MS methods for metabolite ID. Significant differences in metabolite responses were identified, showing metabolomics can be a useful tool for understanding the toxic effect of alcohol consumption.

An automated high-precision workflow for untargeted LC-MS-based urinary metabolomic epidemiology

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Urine is a non-invasive biofluid that is rich in polar metabolites and well suited for metabolomic epidemiology. However, the physiological concentration of urine can differ >10-fold due to variable individual hydration levels, which can pose a major challenge in large-scale untargeted LC-MS metabolomics. Although there are many urine normalization methods (e.g., specific gravity; SG), most rely upon manual sample preparation, which is not practical for large cohorts. Here, we present an automated LC-MS workflow for untargeted urinary metabolomics in a 96-well plate format and provide metrics for data quality assessment. We developed an automated method to measure SG using a refractive index detector (RID), with accuracy within 85-115% and <3.4% precision. Bland-Altman statistics comparing the RID method with a hand-held refractometer showed a mean deviation of -0.0001 SG units (limits of agreement: -0.0014 to 0.0011) for 842 urine samples. We then implemented an automated liquid handler, which provided an estimated 7-fold reduction in operator time, translating to 1.5 instead of 10.5 days of sample preparation for an 842-sample cohort. We also developed two complementary HILIC chromatography methods for positive and negative ionization on a high-resolution QToF operating in DIA mode. Five exogenous technical internal standards (tIS) were incorporated in each polarity to monitor data quality. Following signal drift correction by cubic spline regression, we obtained CV_{QC} <5% and CV_{sample} <16% for 4/5 tIS in a cohort of 842 urine samples and 248 pooled QCs in each polarity. Data processing was performed using MS-DIAL and the CorrDec spectral deconvolution algorithm based upon an in-house library of >450 analytical standards and *in silico* spectra from MSFinder. We annotated >600 metabolites including endogenous compounds, xenobiotics and drugs. This platform is suitable for performing urinary untargeted metabolomic epidemiology, which is an important advance for applications in population-based molecular phenotyping.

Employing metabolomics in medicine - a clinician's point of view

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In medical practice, diagnosis and treatment are based on a diagnostic pyramid. This comprises main patient's complains, medical anamnesis including past medical history, physical examination, lab tests & imaging, and targeted diagnostic procedures. This allows for a gradual and more precise diagnosis, from the initial diagnosis, through the working one, to the final diagnosis and treatment. The importance of each individual symptom (biomarker) for making the final diagnosis is based on the assessment of its sensitivity (the probability that the symptom is present in the disease) and specificity (the probability that the symptom can occur in another disease). Pathognomonic (characteristic only for a particular disease) symptoms are rare, thus sensitivity and specificity of a variety of biomarkers must be analyzed together. Hypothetical diagnoses are rejected one by one until the most probable one is found. For a computer aided diagnosing, most often "the receiver operating characteristic (ROC)" attempt is employed. The ROC curve is created by plotting the true positive rate (TPR) against the false positive rate (FPR) at various threshold settings for each investigated biomarker. Guidelines and recommendations in practical medicine are based on data from meta-analyzes of randomized trials. Unfortunately, repeatability, validation, and standardization of metabolomic methods in medicine are still far insufficient, thus meta-analyzes employing metabolomics are almost entirely absent. Consequently, the application of metabolomics in medical practice is very limited so far.

Contrary to everyday medical practice, metabolomics is already important in experimental medicine. Metabolic studies in medicine require close cooperation of physicians, biochemists, and biostatisticians. Tasks for medics include designing the metabolomic experiment i.e. defining the research hypothesis, the study purpose, end points, inclusion/exclusion criteria, and sampling the biological material. Assessing the metabolome at the cellular level gives great opportunities to study the pathomechanisms of the disease, its phenotyping, and stratification of the disease progression. It has also been shown that metabolome determination can be predictive for diagnosis even over a dozen years before clinical symptoms appear.

Conclusions:

(1) Without repeatability, standardization, and validation -omic methodologies cannot be put into medical practice.
(2) The -omic approach seems to be an ideal tool for new treatment options based on therapies addressed to cell metabolism.
(3) The -omic approach allows effective phenotyping and stratifying of diseases. Thus, it also seems to be an ideal tool for tailoring the treatment for an individual patient.
(4) If simplified and less expensive, the -omic methodologies could be ideal for large-scale population screening for diseases early detection and prevention.
(5) The widespread use of -omics in medicine is only a matter of time.

Towards the identification of antibiotic-resistant bacteria causing urinary tract infections using volatile organic compounds analysis – a pilot study

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The incidence of infectious diseases caused by antibiotic-resistant bacteria is increasing worldwide and is described as the biggest threat of the 21st century. Inappropriate identification of bacteria and the empiric prescribing of antibiotic is common practice in many countries, leading to the antibiotic-resistance crisis. Therefore, there is a strong need to develop new diagnostic tools, allowing fast and precise identification of antibiotic-resistant bacteria in medical practice. The main aim of this study was to evaluate the applicability of volatile organic compounds (VOCs) analysis in the identification of antibiotic susceptibility in common bacteria responsible for urinary tract infections (UTIs). First, the incubation conditions were optimized to obtain the maximum number of VOC detected which next allowed the assessment of differences in VOCs profiles between sensitive and resistant strains of E. coli causing UTIs. VOCs in the headspace above bacterial cultures has been analysed using solid-phase microextraction together with gas chromatography-mass spectrometry (GC-MS). The analysis of VOCs in the headspace above the bacterial cultures allowed the distinguishing of resistant and sensitive bacteria based on the abundance of six VOCs with 85.7% overall accuracy. Bacterial isolates were rapidly analysed using GC-MS in a much shorter time compared to traditional susceptibility tests. The results of this preliminary study are promising, and with development could lead to a practical, faster diagnostic method for use in routine microbiology. Further work with large sample sizes will potentially enable the development of diagnostic algorithms applicable to routine other methods of volatile detection.

Determination of Metabolomic Changes Involved in C2C12 Rat Myoblast Differentiation into Myotubes Using Discovery and Targeted Workflows

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Myoblasts are round, nucleate cells that are derived from muscle-associated satellite cells and are the proliferative precursor to muscle fibers. For this investigation, we used a mouse cell line model as an example for exploring metabolomic discovery and targeted data mining workflows using Q-TOF mass spectrometry. C2C12 is a murine myoblast cell line that is easily propagated in high-nutrient culture. Upon serum starvation, C2C12 cells fuse and form multinucleate myotubes that have many characteristics of myofibers *in vivo*. These cells are no longer proliferative and switch metabolism due to their need for ATP generation and less metabolic intermediate production. This system provides an interesting experimental contrast between proliferative myoblasts and quiescent-like myotubes. A metabolomics differential analysis experiment was performed using a 6546 Q-TOF MS coupled to a 1290 Infinity II LC system. Due to the polar nature of metabolites being analyzed, they are not well-retained by reversed-phase chromatography, so HILIC chromatography was used. After the data was collected, Agilent metabolomics software tools were used to perform differential analysis and to identify the metabolites. To be considered identified the compounds must match the mass, isotope pattern and retention time or match the MS/MS spectrum of an authentic standard in the METLIN library. Structural correlation was used to elucidate metabolites that did not have MS/MS spectra available. Using both PCA and hierarchical clustering, clear differences were determined in the glycolysis and TCA metabolic pathways of the myoblasts and myotubes. A comparison was made between a discovery workflow and a hypothesis-driven targeted approach in determining changes in the metabolomes of the two models.

HCS and *In Vivo* imaging applications as a necessary part of targeted therapy development

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Modern medicine more and more often uses technologies that enable defining therapies aimed directly at cells related to the disease process or dysfunction of a particular patient. A comprehensive assessment of the effectiveness of the therapy, as well as potential side effects, is extremely important. Therefore, targeted therapies are increasingly being verified prior to application at the level of cell cultures or model organisms.

The development of cellular imaging techniques and *In Vivo* imaging of laboratory animals allows for the analysis of the effect of therapy on the morphology, biochemistry and physiology of individual cells, as well as the observation of the effect of therapy on the target cells implanted into the model organism. The use of fluorescence and luminescence marking techniques guarantees high sensitivity and resolution as well as the possibility of standardizing the imaging process and data analysis.

The lecture will present examples of applications in the field of High Content Screening cellular imaging and *In Vivo* imaging of laboratory animals, used in the process of developing targeted therapies, as well as hardware solutions enabling this type of research.

UnACCeptable? - the interplay between fat oxidation and gluconeogenesis

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Carbohydrates and lipids are primary energy sources in mammals; thus, their oxidation and synthesis are carefully controlled. The balance between these pathways is perturbed in non-alcoholic fatty liver disease (NAFLD) and diabetes. Excess dietary carbohydrates are converted to fatty acids and contribute to liver triglyceride through the pathway of de novo lipogenesis (DNL). This pathway also suppresses hepatic fatty acids oxidation to ketones or carbon dioxide. Therefore, activation of hepatic beta-oxidation with simultaneous inhibition of DNL is a promising therapeutic strategy for treatment of NAFLD and lowering liver fat. However, the fate of energy produced in this process tends to be overlooked. We hypothesized that accelerated fatty acid oxidation may increase the amount of reducing equivalents and ATP produced and unintentionally alter other metabolic processes in liver. Using a medium scale stoichiometric network of liver metabolism, we tested the effects of elevated fat oxidation on hepatic pathways. The model suggested that excess energy can result in activation of gluconeogenesis (GNG). We tested our predictions experimentally using various high fat diets and recently developed acetyl-CoA carboxylase (ACC) 1 and 2 liver specific double KO mice (LDKO). Loss of ACC1/2 depleted malonyl-CoA resulting in lower DNL and simultaneously increased fat oxidation by activation of carnitine palmitoyltransferase I (CPT-1) but resulted in elevated blood glucose. Glucose labeling by 2H2O and [U-13C3]lactate was higher in LDKO hepatocytes, indicating elevated gluconeogenesis. This effect was dependent on the availability of long chain fatty acids suggesting that loss of malonyl-CoA and activation of CPT-1 promote GNG. Finally, LDKO mice had elevated hepatic fluxes *in vivo* including glucose production. Using targeted LC-MS/MS analysis of liver tissues, LDKO mice were found to have massive accumulation of liver citrate and other TCA cycle intermediates, and significantly elevated hepatic energy charge. Thus, activation of fat oxidation in liver lowers lipid accumulation, but has downstream metabolic effects on energetically dependent pathways, such as GNG, that may need to be recognized in the context of disease such as diabetes.

A workflow for combined analysis of the lipidome, metabolome, and exposome

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Liquid chromatography–mass spectrometry (LC–MS) is the preferred technique in metabolomics, lipidomics, and exposomics. However, the true breadth of a metabolome, lipidome, and exposome cannot be captured by a single extraction method or instrumental platform. Hence, the main task is to cover complex lipids, polar metabolites, and exposome compounds using as few platforms as possible while maintaining the requisite precision and accuracy for the metabolite classes detected by the chosen platforms.

We here introduce an LC–MS workflow **LIMeX** for the simultaneous extraction of complex **LI**pids, polar **Me**tabolites, and **eX**posome compounds in human plasma. The sub-groups of compounds are isolated using an 'all-in-one' extraction with a methanol/methyl tert-butyl ether mixture and water. Analysis of complex lipids is conducted using reversed-phase LC (RPLC) in positive and negative electrospray (ESI) mode while polar metabolites and exposome compounds are separated using hydrophilic interaction chromatography (HILIC) in ESI(+) and RPLC in ESI(–). Simultaneous acquisition of MS1 and MS/MS spectra in data-dependent mode is used for each platform[1,2]. The acquired raw data files are processed using user-friendly MS-DIAL 4 software covering 117 lipid subclasses (lipidomics workflow)[3] or over 1 million MS/MS spectra for 31,000+ compounds (metabolomics workflow).

For NIST SRM 1950 human plasma, calculated concentrations for selected polar metabolites were in agreement with the NIST certificate. As regards complex lipids, we compared our results with LIPID MAPS[4] and NIST Inter-lab comparison study[5]. A good agreement between calculated concentrations and consensus location from these studies was achieved for most of the reported lipids demonstrating that even a single point calibration can provide accurate data. Overall, 500+ complex lipids, 100+ polar metabolites, and tens of exposome compounds (mainly food components and pharmaceutical drugs) in plasma for human cohort studies can be reported.

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Lipidomic characterization of keratinocytes from skin of psoriatic patients exposed to cannabidiol followed by UVB irradiation

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Psoriasis is an inflammatory skin disorder with relatively limited treatment options that are not fully effective. One of these treatments is UVB phototherapy, which increases the oxidative modifications of phospholipids in the cell membrane of the skin. Cannabidiol (CBD), a natural phytocannabinoid with antioxidant and anti-inflammatory properties, has been suggested as a potential therapeutic agent in psoriasis. Thus, we carried out a lipidomic analysis on the keratinocytes of healthy individuals and patients with psoriasis irradiated with UVB and treated with CBD. Our lipidomic approach included phospholipid and ceramide profiling. Our results showed that in psoriatic keratinocytes, most classes of phospholipids, including phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) and ether-linked phosphoethanolamine (PEo), were down-regulated, while SM (sphingomyelin) was up-regulated. Observed increase in SM content was consistent with decreased level of CER[NDS], the main ceramide fraction in keratinocytes of psoriatic patients. These changes were accompanied by an increase in the negative zeta potential, which indicates translocation of PS to the outer layer of the membrane. Treatment of psoriatic keratinocytes with CBD led to down-regulation of PC, PS, as well as up-regulation of some PEo and SM, and zeta potential. However, UVB irradiation of psoriatic keratinocytes resulted in up-regulation of PC, PC plasmalogens, PEo and decreased in the negative zeta potential, while exposure of these cells to CBD led to decrease in the level of SM. Consequently, our results suggest that CBD can modulate phospholipid metabolism in keratinocyte *in vitro* in two different ways. This phytocannabinoid induces pro-apoptotic changes observed in psoriatic keratinocytes. On the other hand, CBD regulates transepidermal water loss and antioxidant defense of UVB-irradiated keratinocytes from psoriatic patients, which may have therapeutic value. Nevertheless, further research is needed to confirm and accurately explain observed changes.

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Profiling of free fatty acids in lymph: a technical advance

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Background: Lymph is a unique biofluid, but for a long time it has been regarded as compositionally overlapping with blood plasma. In the recent decade this notion has been challenged, partially thanks to '-omic' techniques. Lymph's rate of production as well as constitution depends greatly on factors such as diet, physical activity or ones pathophysiological condition. On the level of constituting molecules (proteins and metabolites) lymph composition carries fingerprints of extracellular matrix processing, tissue growth and remodeling, cellular metabolic/catabolic activities as well as other tissue-specific 'omic' signatures. In our research project presented here, we have established a method of fatty acid measurements with use of LC-MS, which we've applied to study the content of free fatty acids in human lymph.

Methods: The lymph was collected from patients who underwent surgical procedures of lymph nodes removal (n=62). Data on history of hypertension (n=28) in the studied population as well as the BP and treatment drugs were collected. In our straightforward procedure, aliquots of lymph (800 µl) were mixed with an internal standard (arachidonic acid D11, 15 ng) and the mixtures were extracted twice with 800 µl of methyl-tert-butyl-ether (MtBE). Isolated metabolites were not derivatized. The separation was carried out in an AQUITY UPLC system with use of CSH C18 Column. The connected Time-of-Flight mass analyzer (Waters' Xevo GS Q-ToF) recorded mass spectra in negative polarity mode. Multivariate analysis, modelling and classifying decision trees were applied in analysis of the measurement results.

Results: Levels of 41 analytes were recorded in lymph. The most abundant fatty acids in human lymph were octadecenoic acid (FA18:1), octadecadienoic acid (FA18:2), hexadecanoic acid (FA16:0), hexadecenoic acid (FA16:1), octadecanoic acid (FA18:0), tetradecanoic.acid (FA14:0), and arachidonic acid (FA20:4). Very high inter-individual variance of fatty acid content in lymph was observed. The fatty acid profiles were not characteristic enough to differentiate strata in our studied population. The extraction method can be applied to other types of body fluids while the LC-MS method could be used in analysis of free or lipid-bound fatty acids.

"Omics" applications of EC-MS

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Redox reactions are integral parts of many cellular processes. They are therefore extensively studied *in vitro* and *in vivo*. Electrochemistry (EC) represents a purely instrumental approach to characterize direct and indirect effects of redox reactions on bioorganic molecules, such as peptides, proteins, and endogenous and exogenous small molecules as well as nucleic acids. In addition to direct infusion electrospray ionization (ESI)–mass spectrometry (MS), hyphenated techniques such as liquid chromatography–mass spectrometry (LC–MS) are often applied for comprehensive characterization of reaction mixtures generated by EC experiments. EC–LC–MS represents a fast, automatable, and "green" approach that can be used in a variety of "omics" disciplines. Biotransformation reactions involving xenobiotics are usually intended to make them more polar and, thus, enable a more rapid excretion. Biotranformation can lead to loss or gain of activity. Furthermore, oxidoreductases such as the members of the cytochrome P450 superfamily generate structural complexity during natural product biosynthesis, and they play a central role in the biotransformation of drugs and toxins ("phase I metabolisms"). Biotransformation reactions involving xenobiotics are usually intended to make them more polar and, thus, enable a more rapid excretion. Biotranformation can lead to loss or gain of activity. EC–ESI–MS represents a versatile tool for studying redox processes involving different kinds of bioorganic molecules, such as peptides, proteins, endogenous, and exogenous small molecules as well as nucleic acids. EC–ESI–MS is a purely instrumental approach. EC enables the fast, automated, and cost-effective generation of redox reaction products, and is a "green chemistry" technique. EC–ESI–MS represents a very useful complement to *in vitro* and *in vivo* techniques in metabolomics, proteomics, genomics, and related areas of application. Despite the applicability of EC in general and EC–MS in particular to "omics" this approach is relatively new and therefore unknown to analytical chemists. Nevertheless, the fast growing number of EC–MS publications illustrates the power of this technology and will help to create a broader acceptance and use in life science applications.

Recent advances in Chemical Isotope Labeling (CIL) LC-MS for high-coverage quantitative metabolome analysis

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A key step in metabolomics is to perform relative quantification of metabolomic changes among different samples. High-coverage metabolome profiling will benefit research in systems biology and disease biomarker discovery, but is currently a challenging task. To increase coverage, traditionally, multiple analytical tools and methods are used to generate a combined metabolome data set. However, this approach requires heavy investment on equipment and high analytical skills and expertise. In this presentation, I will discuss a simple and robust platform for in-depth metabolome analysis based on chemical isotope labeling (CIL) liquid chromatography (LC) mass spectrometry (MS). I will discuss the research strategies behind the development of the instrumental platform and standardized workflows for sample preparation, sample analysis, data processing and results analysis. I will then use several examples of applications to showcase the CIL LC-MS platform for disease biomarker discovery research as well as biological studies.

Advantages of GC- and GCxGC- TOF MS in metabolomic research

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Although the basics of Time of flight mass spectrometers (TOF MS) were established in 30s of 20th century, due to the lack of fast electronic the renewal of this technology was postponed to early 90s of last century. At this time the enormously fast, but extremely sensitive, first commercially available GC-TOF MS systems were introduced to the market. The nature of TOF MS technology, such as fast and sensitive acquisition of unskewed MS spectra, was followed by implementation advanced mathematical algorithms of data mining – automated peak find, deconvolution and scripting.

The potential of marketed fast GC-TOF MS instruments equipped with ion source not requiring its cleaning was later on extended by their combination with comprehensive gas chromatography (GCxGC).

The increased nowadays demands for analytical instrumentation can be summarized as: "One run covering all analyst's requirements, such as target and non-target screening, qualitative and quantitative capabilities along with easy and fast hardware-software handling and reasonable data file sizes". Such requirements were kept in mind within the latest instrument development. Come to see the achievements in high-speed GC- and GCxGC-TOF MS analyses, maintaining not only extremely high sensitivity, but also excellent mass accuracy for your metabolomics research.

Cancer cells metabolomics studies, effect of hypoxia, normoxia and cultivation time

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Metabolomics studies of cell cultures enable to perform an advance analysis of low molecular weight compounds, thus allows to identify of perturbed biochemical pathways by different chemical and / or physical external stimuli. In our lab we performed *in vitro* experiments on HT1080 Fibrosarcoma cell line in Eagle's minimal essential medium applying different cultivation conditions by modifying oxygen concentration, namely: 1% O2, 6% O2 and 21% O2, in various time points 12h, 24h and 36h. Additionally we have used the reverse hypoxia and reverse normoxia conditions. For all experiment the following steps were performed: (a) optimizing the cell density for NMR experiments; (b) sample preparation including medium and cell pellet (each time point in triplicate); (c) elaboration of metabolic profiles based NMR spectroscopy; (d) metabolites identification and bioinformatics data analysis. We have determined extracellular and intracellular metabolomes within cell metabolism changes by deregulation of oxygen bioavailability.

How to efficiently remove lipids and proteins from your metabolomic samples for better LCMS results

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A good plasma metabolomics sample preparation strategy is vital to generating high quality and reproducible results. The strategy must address quenching metabolism and effectively extracting metabolites for LC/MS analysis. Unfortunately, the preparative steps for biological samples including plasma have not been standardized, validated, nor automated. The preparation of large numbers of samples for basic and translational research studies can require impractical amounts of manual labor, and may suffer from processing inconsistencies. Additionally, metabolite concentrations in plasma are inherently the product of both metabolic and catabolic processes, which are catalyzed by cell- and blood-based enzymes. The accurate measurement of relative metabolite concentrations fundamentally requires that the residual activity of any such enzyme be minimized or eliminated upon sample collection1. Collectively, these hurdles can limit the fidelity of downstream quantitative analyses, and preclude day to-day and lab-to-lab comparisons.

In an effort to address these hurdles, we have developed an automated sample preparation protocol to analyze metabolites from plasma that precipitates plasma proteins to quench enzymatic activity, depletes lipids, and extracts metabolites ahead of the LC/MS analysis. Protein precipitation is accomplished by modifying the sample solution to impede noncovalent intramolecular bonds required to maintain hierarchical protein ultrastructure. Adding organic solvents or other reagents that disrupt intramolecular electrostatic interactions are two common protein precipitation strategies. With respect to catabolic and metabolic processes that may persist and alter metabolite concentrations after sample collection, protein ultrastructure must be disrupted so that precipitation is accompanied by enzymatic inhibition. Finally, for the purposes of metabolite analyses, the protein precipitation strategy must also maintain metabolite solubility, and be compatible with metabolite extraction workflows.

We tested two classic protein precipitation strategies for their ability to yield consistent quantitation of a broad array of plasma metabolites under conditions simulating basic or translational research settings in which the metabolite extraction step may be delayed. This Technical Overview provides results for both trichloroacetic acid (TCA) based and methanol:ethanol based (M:E) precipitation strategies, and their ability to withstand delays in subsequent extraction steps2. We developed our automation protocol using the Agilent Bravo for metabolite extraction from plasma to:

(1) Improve longitudinal reproducibility
(2) Simplify the process,
(3) Decrease variability

Finally, we evaluated this workflow with LC/MS by assessing its reproducibility within and between days using a pooled human plasma standard. The LC/MS work was performed using the Agilent MRM-based LC/MS/MS platform for central carbon metabolites.

Spatially resolved profiling of human brain *in vivo*. Chemical biopsy as a new tool in neuroscience research

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Biochemical analysis of brain tissue was always challenging because of the limited access to this biological material. Consequently, low invasive tool which allows obtaining analytes for neuroscientific or diagnostic purpose is highly desired. The goal of this preliminary study was to assess the applicability of solid phase microextraction (SPME) for *in vivo* characterization of white and grey matter in human brain tissue immediately before biopsy. The group of 8 patients were included into the study. The experiment was approved by Bioethical Committee in Bydgoszcz (KB 142/2017). The samplings were performed with the use of SPME device consisted from four fibers enabling extraction of metabolites from two matters simultaneously. In order to avoid additional brain damage, SPME fibers were inserted in the planned trajectory the biopsy needle. After collecting all the samples metabolomic and lipidomic analysis were performed with use of LC-MS platform. The results showed that wide range of compounds were extracted and the amount of small molecules is similar in both structures. The metabolomic analysis revealed that in addition to amino acids, ureas and metabolites involved in drug metabolism, lipids are predominant in the brain independently to the sampled matter. Based on this observation, more in depth lipidomic analysis were carried out. Analysis of the lipidome revealed relatively weak separation of brain structures but indicated higher amount of lipids in the white matter which is known to be more active metabolically compared to the grey matter. However, extended studies should be continued on a larger cohort to draw solid conclusions about the biochemistry of the live human brain. This proof-of-concept experiment, was the first attempt to *in vivo* characterization of metabolite profile of white and grey matter of human brain. The results confirmed that SPME coupled with LC-HRMS could be successfully used for the spatial resolution analysis of brain composition in patients during brain tumor biopsy.

Acknowledgements:
The work was supported by the Kościuszko - edycja II grant entitled "Metabolomic and lipidomic profiling of white and gray matter of cerebral cortex using novel technology based on chemical biopsy coupled to mass spectrometry" from Polish Ministry of Defence.

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SHORT ORAL PRESENTATIONS

The significance of systematic biobanking for reliable metabolomics research

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Metabolomics, as the research branch dedicated to identification of small-molecule and unstable metabolites, strongly depends on quality of primary samples used for analysis. The quantity of each metabolite may be altered by multiple factors, like oxygen, changes of temperature, UV light. Thus, measurements of individual metabolites may be highly dependent on procedures of sample handling. To accurately answer research questions through metabolomics, reliable biorepositories are necessary to provide the high-quality specimens. Therefore, biobanks representing highly organized collection of samples along with comprehensive set of data, should be recognized as crucial source of specimens for metabolomics analyses. Biobanks, where collection are conducted by professionally trained specialists in accordance with Standard Operating Procedures, are dedicated to managing biospecimens for research purposes. Precise documentation of every collection step is an essential feature of quality control and assurance. Researchers involved in the analysis of metabolome need to pay attention to the source of samples, including the steps of collection and storage, since the preanalytical conditions play important role in acquisition of reliable results and affect the experimental bias. Frequently, samples used in metabolomics research are collected according to different procedures, so the formation of Best Practice Guidelines exclusively for metabolomics biobanking constitutes the vital issue to obtain more homogenous repository, irrespective of which biobank samples have been processed at. Accordingly, validated quality control measures are essential part of biobanking process to ensure the quality of samples dedicated to metabolomics applications. Analyses of metabolites in clinical samples are used to create the predictive value of metabolomics profiles for individual disease. Moreover, multiple studies are focused on linking the metabolomics data with a variety of clinically relevant phenotypes and data acquired from other omics applications to proceed with implementation of precision medicine. Through supporting the academic and research community with data from omics analysis, we are able to boost the biomarkers discovery and better understanding of diseases development.

Polypyrrole based coating materials for SPME fibers to be used in analysis of VOCs as potential colorectal cancer biomarkers

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Solid-phase microextraction is one of most commonly used extraction techniques. Nowadays SPME method of sample preparation procedures are used in large range of analytical tasks including environmental applications, food analysis, clinical, pharmaceutical and biotechnological applications. However, a new trends and directions in SPME has still been developing. One of them is development of new coating materials. Nowadays the most common coating materials for commercial SPME fibers include PDMS, DVB, CAR, CAR/PDMS, DVB/CAR/PDMS, PA. After development and introduction of solid-phase microextraction techniques in 1990 a few groups of lab-made materials (metal-organic frameworks, conductive polymers, molecular imprinted polymers, ionic liquids) were and successfully introduced. In this study polypyrrole coatings were directly electrodeposited onto metal wires by cyclic voltammetry with application of different types of counter ions and solutions. Obtained polymers were characterized by different techniques such as scanning electron microscopy (SEM), FTIR, low temperature nitrogen adsorption, thermal gravimetric analysis. The designed fibers were utilized for extraction of different types of VOCs commonly found in different types of biological matrices. Extraction, separation and identification parameters were optimized. LOD, LOQ, enrichment factor of SPME fibers with polypyrrole coatings were determined for each analyte. The designed fibers will be eventually used as sampling tools for extraction of VOCs, markers of colorectal cancer.

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Quantitative determination of piperacillin and imipenem in plasma and bronchoalveolar lavage with SPME-LC-MS/MS

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Severe bacterial infections caused by multi-drug resistant (MDR) strains, such as Ventilation-associated pneumonia (VAP) are the leading cause of morbidity and mortality among the ICU patients. VAP occurs in 9-27% mechanically ventilated patients of ICU and its mortality rate is about 30-70%. In the case of infections caused by pathogens with reduced sensitivity to antibiotics, or patients suffering from Ventilation-Associated Pneumoniae exhibiting altered drug pharmacokinetics, a higher drug dose may be required to yield clinical effect. An effective antimicrobial therapy shall be personalized and based on continuous monitoring of antibiotic concentration, e.g. in plasma or alveolar fluid. The currently used methods for therapeutic drug monitoring rely on liquid chromatography coupled with either UV or MS detection, which require time-consuming sample preparation and relative long run time significantly reducing their applicability in hospital practice. Due to this reason, effective methods for rapid determination of the dynamically changing concentration profiles of antibiotics in the blood are sought. Here we present a new method for quantitative determination of piperacillin and imipenem in human plasma and alveolar fluid enabling estimation of degree of antibiotic penetration into the site of infection. For this purpose the conditions of Thin-Film solid phase microextraction were optimized to preconcentrate both β-lactams directly from human specimens. Also the conditions of LC-MS/MS analysis were optimized to ensure highly selective and sensitive determination of analytes in complex matrix. All plasma and BAL samples were received from mechanically ventilated patients hospitalized at Department of Anesthesiology and Intensive Care Unit (10th Military Research Hospital and Polyclinic in Bydgoszcz, Poland) within the project receiving financial support from National Science Centre (grant no. 2017/26/D/NZ6/00136). The determined concentrations of antibiotics in plasma before and after infusion were within the range of 72.32-339.78 mg/L for piperacillin and of 4.79-27.07 mg/L for imipenem, respectively. The evaluated degree of drug penetration to the alveolar fluid was in the range of 1.88-7.95% piperacillin and of 1.49-11.15% for imipenem.

Methylated cyanidin glucuronide is the main anthocyanin compound found in sheep's blood plasma and cerebrospinal fluid after intraruminal administration of chokeberry preparation

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The study of anthocyanin compounds stems from their strong antioxidant activity and wide applicability in the prevention. So far, no studies have been carried out to determine the penetration of various forms of anthocyanins and their metabolites through the blood-cerebrospinal fluid barrier (BCSF-B), as well as the transformation of these bioactive compounds during the processes of passing through this barrier. Therefore, to be able to verify that anthocyanins can have a potential beneficial effect on the brain, it is first necessary to determine if these compounds are absorbed and in what form they are present in the body fluids after ingestion. Ewes (n = 16) were intraruminal administered with anthocyanin preparations of chokeberry at a dose of 10 mg cyanidin/kg of body weight in an experiment that lasted for 10 hours. Before and at specific time intervals after administration of the preparations, blood from the jugular vein, urine, and cerebrospinal fluid (CSF) from third ventricle of the brain were collected. The obtained samples pre-treatment were carried out by solid-phase extraction. The concentration of native anthocyanins and their metabolites were analyzed using micro-HPLC-MS/MS. Before chokeberry administration, body fluids did not contain anthocyanins compounds. After this berry intake, 21 anthocyanins were discovered with micro-HPLC-MS/MS in body fluids. Native and conjugated derivatives (methylated, sulfonated, glucuronidated) were identified in the plasma, urine and CSF. Interestingly, methylated cyanidin glucuronide was the main anthocyanin found in sheep blood plasma (31%) and CSF (20%). The study showed that anthocyanins penetrate across BCSF-B and their changes in profile and concentration in CSF result from the fluctuation of these pigments concentration in blood plasma and various abilities of cyanidin derivatives present in blood plasma to permeate across. Results enable tracking anthocyanins biological fate after these pigments intake (from absorption through forms of presence in blood plasma and urine to penetration into CSF) which may help to determine the biological properties of these compounds including the potentially neuroprotective activities.

Nanochromatography with mass detection: a new way to measure level of angiotensins in human plasma

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Background and aim: Angiotensins are small peptides that are part of the renin-angiotensin-aldosterone system (RAAS) involved in e.g. regulation blood pressure and managing water-electrolyte balance. Disorders of RAAS, e.g. angiotensin II hyperactivity, may lead to ischemic heart disease, hypertension, or renal failure. The main aim of this research was to develop a reliable method of different angiotensin peptide profiling in human blood samples, using nanochromatography with mass detection (nLC-MS/MS).

Methods and results: Four representatives of angiotensin family: I, II, 1-7, IV, and AngII [Asn1, Val5] internal standard were used to prepare standard curves in buffer A (1% acetic acid in H2O) and matrix (pooled plasma). This standards' set was used also for further validation, i.e. assessing repeatability, matrix effect, and analyte recovery. Quantitative assessment of angiotensins was performed in blood samples collected from eight healthy volunteers. Plasma was mixed with protease inhibitors cocktail and internal standard, following by acetonitrile protein precipitation. The supernatant was lyophilized and reconstituted in buffer A before analysis. Following separation on a C18 column in the RP regime, detection and quantification of angiotensins' signals were based on three product ions per parent molecular ion in SRM (Selected Reaction Monitoring) mode for each analyte. The analysis was performed on a Dionex 3000 UltiMate liquid nano chromatography (Thermo Scientific) connected to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific). Calibration curves spread from 5 to 1000 pg/mL (8 points) for each angiotensin. Correlation coefficients for most compounds (in plasma and phase A) were >0.9. Exception was Ang(1-7) with R2 = 0.52. AngI levels in patient's plasma ranged from 26.4 to 34.5 pg/mL, AngII 9.4-574.0 pg/mL, and AngIV 28.4-539.0 pg/mL (n=3).

Conclusion: These preliminary studies are a solid base for further method development and validation. Our results require confirmation and replication, nevertheless, they show a possibility of clinically useful diagnostic methods, which could be helpful for investigating RAAS-associated pathologies.

Gold nanoparticle-based laser desorption/ionization mass spectrometry in serum and urine metabolomics analyses of renal cell carcinoma

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Renal cell carcinoma (RCC) is heterogeneous group of cancers divided into several subtypes: clear cell (80% of cases), papillary (10%), chromophobe (5%), medullary and collecting duct (below 1%) and other unclassified subtypes (~5%) according to WHO classification. RCC is a very aggressive and often fatal disease. As many as 30% of patients have metastases to other organs at the moment of diagnosis, so this type of tumor is still a very significant challenge. This is due to its relatively late detection, a problem in which specific biomarkers for kidney cancer could be of great importance. Proteomic approach predominates in current strategies of cancer biomarker search and although several RCC protein biomarkers have been proposed, but they suffer from low sensitivity and specificity. Cancer is a disease that alters cell metabolism, so it seems that the appropriate approach will be the metabolic profiling of kidney tissue and bioliquids such as serum and urine. One of the methods used to detect biomarkers is laser desorption/ionization mass spectrometry (LDI MS). It is an analytical technique based on ionization of a substance present in the sample after being irradiated with the laser pulses. Ions that form are then separated in accordance with their mass to charge ratio and then detected. The most commonly used LDI method is matrix-assisted laser desorption/ionization (MALDI). However, MALDI spectra contain a high chemical background below m/z 1000 due to the use of organic matrices. For small molecules such as metabolites, surface-assisted laser desorption/ionization (SALDI) and specifically – nanostructure-based techniques, for example gold nanoparticle-enhanced target (AuNPET), are generally better suited. During the presentation experimental results in the subject of kidney cancer metabolic biomarker research using gold nanoparticles-based laser mass spectrometry will be shown.

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Application of high resolution mass spectrometry and chemometric method as a tool to detect sesame seeds oil

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Sesame (*S. indicum* L.) is an annual plant and comes from the Pedaliaceae family. At the beginning sesame was cultivated only in Asia, but currently it is growing in many countries around the world, nonetheless Asia and Africa are the leaders of sesame production. Because of sesame nutrition properties, its seeds were recommended as a snack for children. Due to the high content of fat in sesame seeds – about 37-63%, this plant is a good source to obtain sesame seeds oil. The most popular technique to produce sesame seeds oil is cold-pressing method, which provides high quality of product with bioactive compounds like fatty acids, polyphenols, tocopherols, sterols, carotenoids and lignans. Composition of this products provides that oil is not only used in food, but also in pharmaceutical and cosmetic industry. However, popularity of this product and its availability induce researchers to pay attention to the side effect after consuming products which contain sesame. Over the course of recent years, a significant increase in allergy caused by sesame seeds has been observed. That is why it is important to care about consumer health and aim to develop analytical approaches, enabling to detect the content of sesame. In this study, metabolic analysis was performed by high-performance liquid chromatography coupled to Q-TOF mass spectrometry supported by the chemometric data processing in order to detect ions characteristic for cold-pressed sesame seeds oil. This analysis enables to distinguish sesame seed oil from cold-pressed oils from other plants.

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Comparison of nutraceutical effect of *Cystoseira abies marina* and *Rosemarinus Officinalis* on oxidative stress in T1DM animal model: the changes in the levels of phospholipids and ether-phospholipids

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Streptozotocin-induced type 1 diabetic rats (STZ-T1DM) are commonly used as an animal model of diabetes and oxidative stress. This work is a continuation of our previous studies in which the impact of rosemary (R) and cystoseira (C) extracts on urine metabolites of STZ-T1DM rats have been studied[1,2]. We have already shown that under administrated doses, both extracts have antioxidant properties with no signs of toxicity. To expand the information about metabolites from primary metabolism and to obtain a more complex view of biochemical changes under treatment applied, plasma samples were analyzed. Six experimental groups were studied, i.e. 3 control groups receiving placebo (CV), R extract (CR), and C extract (CC) and 3 respective diabetic groups (DV, DR, DC). Deproteinized plasma samples were fingerprinted using RP-HPLC-QTOF-MS. After data processing and filtration, a comparative analysis was performed. Metabolites significant in comparison CV vs DV were tracked in the comparisons DV vs DR and DV vs DC to look for the effect of the treatment on diabetes/oxidative stress evoked changes. In total, the signal of 33 features was found increased, and 32 decreased in diabetes in comparison to controls. Majority of these signals were assigned as lipids with glycerophospholipids (GPL) as the major group. Importantly, different groups of GPL behaved differently: the signals of LysoPCs were found decreased in T1DM, while signals of LPEs were increased. Applied treatment was partially but significantly reversing these changes, however with different extent for R and C treatment. In all cases the therapeutic effect was higher for C than R. These differences were even more noticeable for ether LPCs: C was significantly reversing observed decrease, while for R this effect was minor. These findings are essential, especially that ether-lipids serve as endogenous antioxidants: C efficiently reduced amount of ROS, therefore diminish of ether-lipids was smaller. Obtained results clearly illustrate antioxidative properties of tested treatments and portrait the potential of nutraceutical intervention in highly oxidizing conditions.

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Towards spatial distribution of lipids – matrix-assisted laser desorption/ionization mass spectrometry imaging of ovarian tumour tissue – the pilot study

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Analysis of the biological fluids, provides information on the compounds circulating in the body, making the information about their real origin uncertain. Thus, technique which enables visualization of the biomolecules may be useful for a proper interpretation of the processes ongoing within the tissue - at the site of disease. In the recent years, some lipid compounds have been proposed as potential cancer biomarkers[1]. MALDI instruments, originally designed for proteomics, can be successfully applied in studying of lipids. Moreover, this technology may also be helpful for simultaneous detection and visualization of a wide range of compounds for studying area-specific alterations within analyzed tissue section. The presented research aimed at investigation of lipid compounds using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) approach for an ovarian tumour tissue obtained during serial sectioning. The 2,5-Dihydroxybenzoic acid (DHB) matrix was applied to the tissue sample - ovarian fibrothecoma using an automated system "ImagePrep" matrix applying station (Bruker). The analysis was carried out using UltrafleXtreme MALDI-TOF/TOF (Bruker) mass spectrometer, under control of flexControl and flexImaging software (Bruker). The proposed methodology allowed for the visualization of a remarkable diversity of the potential lipids compounds within the analyzed tissue section of ovarian fibrothecoma. The study allowed us to prove the utility of MALDI-MSI approach for the analysis of a spatial distribution of lipids and visualizing their distribution on the tissue section. However the complexity of the lipidomic analysis and lipid diversity entail the necessity of further in-depth studies. Nevertheless, the presented technique of studying of intact tissue, which allows visualization of a biomolecules distribution within the analyzed tissue section, that may lead to a better understanding of a molecular background of a human diseases.

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Proteomic profile of skin keratinocytes from rats exposed to UVA/B radiation and treated with cannabidiol

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The non-psychoactive phytocannabinoid - cannabidiol (CBD), due to its anti-inflammatory and antioxidant properties, has great therapeutic potential that may help to avoid the harmful metabolic effects of UV radiation. Therefore, the aim of this study was to analyze the effect of CBD on the proteomic profile of skin keratinocytes in nude rats exposed to UVA / UVB radiation. The results obtained from the SDS-PAGE/nanoHPLC/QexactiveOrbiTrap analysis indicated that UVA/B irradiation as well as topical application of CBD significantly modifies the expression of proteins involved in inflammation, redox balance and apoptosis in rat skin keratinocytes. Moreover, UVA/B radiation radically increases the expression of the cytoprotective transcription factor - Nrf2 and its transcription efficiency, as evidenced by the increased level of superoxide dismutase (Cu, Zn-SOD). Moreover, both types of radiation significantly alter the expression level of proteins that are involved in the regulation of cytokine-mediated signaling/inflammatory responses such as nuclear receptor coactivator 3 and paralemmine 3, while CBD partially prevents these changes. In addition CBD prevents changes in the UVA/B-induced apoptotic pathways (mainly related to Bcl-2 and transforming growth factor-beta). Topical application of CBD has also been found to help normalize the expression of keratinocyte proteins, which are important in cellular metabolism, by modeling their biosynthesis and degradation. Based on the obtained results, it can be concluded that CBD prevents changes in the proteostasis of keratinocytes exposed to UV radiation. Therefore, CBD can be suggested as a protective compound in counteracting UV-induced metabolic changes in epidermal keratinocytes.

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The development of LC/MS method for cystine determination in peripheral blood leukocytes and new challenges in laboratory diagnosis and monitoring of cystynosis in Poland

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Background: Cystinosis is a lysosomal storage disorder caused by mutations in CTNS gene that encodes carrier protein for cystine. This leads to irreversible damage to organs, especially the kidneys. For now, determination of cystine concentration in white blood cells (WBC) is a key biochemical test for the diagnosis and therapeutic monitoring of cystinosis.

Aims: This study aimed to develop and validate LC/MS method without derivatization for cystine determination and to compare the cystine levels in mixed-leukocytes, polymorphonuclear neutrophil granulocytes (PMN) and peripheral blood mononuclear cells (PBMC) in patient with cystinosis.

Methods and results: A method for the collection, transport and blood processing has been developed. WBC, PBMC and PMN were gathered from venous blood collected to sodium citrate tubes using discontinuous gradient of histopaques. Cell pellet after purification and centrifugation was resuspended in N-ethylmaleimide to avoid interference from cysteine. Then internal (homoarginine and 13C2 L-cystine) and external (L-cystine) standards were added. Samples were deproteinized and dried with speed-vac evaporator. The residue was dissolved in distilled water and analyzed by LC/MS. Mass detection was accomplished in positive ion mode by MRM of the precursor→product ion transitions m/z. Coefficients of determination for all calibration curves were > 0.99. In patient with treated, well-monitored cystinosis a concentration of endogenous cystine was 1.08±0.32 nmol/mg prot in WBC fraction, 1.22±0.07 in PMN and 0.32±0.03 in PBMC (mean±SD; n=3). By comparison, the concentration of cystine in healthy control WBC is below 0.25 nmol/mg prot.

Conclusion: The determination of the cystine concentration in peripheral blood total leukocytes by LC/MS is a good test for the diagnosis and treatment monitoring of cystinosis that was for the first time implemented for Polish patients in our laboratory. However, its results are highly dependent on the variability of the proportions between individual WBC fractions. It is particularly important to search for new plasma markers for the diagnosis and monitoring of cystinosis that bypass the multi-stage procedure of material processing.

Proteomic approach to metabolic changes in psoriasis vulgaris

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Psoriasis vulgaris is a chronic, immune-mediated inflammatory skin disease manifested by accelerated exfoliation of the epidermis. Occurred changes on the skin not only reduce the comfort of the patients' life, but also contribute to the development of depressive states. Moreover, the accompanying continuous inflammation slowly debilitating the whole organism. The lack of complete knowledge of the main factors predisposing an individual to the appearance of psoriatic lesions, has recently led to the search for modifications in biochemical pathways participating in the development of this disease. Therefore, there is still looking for mechanism leading to the development of psoriasis, as well as its potential biomarkers. Very promising are the data comparing the proteomic profiles of cells from psoriatic patients, including not only skin cells, such as keratinocytes and fibroblasts, but also peripheral blood lymphocytes and the surrounding them plasma. Proteomic analysis identified proteins whose levels are altered as the disease progresses, and thus suggested metabolic pathways involved in the development of psoriasis. These include proteins involved in the inflammatory response, redox homeostasis, signal transduction, transcription/translation regulation, proteolytic processes, and lipid metabolism. Moreover, some of the changes seen in skin cells appear to be caused by changes in the blood and vice versa. Proteomic analysis also allowed to determine the structure of selected proteins, determining their functionality and activity. As a consequence, it will explain the role of certain proteins in excessive exfoliation of the epidermis. The conducted analyzes also allowed to identify protein modifications by lipid peroxidation products (such as 4-HNE, 4-ONE, MDA) and the impact of these changes on the functioning of skin cells. As a consequence, it can be concluded that multidirectional proteomic analysis allowed for the compilation of a full picture of changes occurring in the course of psoriasis development, which may be useful not only in the assessment of the severity of the disease, but may also contribute to the development of more effective therapies.

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One second screening of cyanobacteria utilizing laser REIMS technology

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Laser desorption- rapid evaporative ionization mass spectrometry (LA-REIMS) is a recent addition to the field of ambient analysis techniques which allows chemical information to be obtained directly from an object with minimal sample preparation[1]. Here, we report the first instance of the application of this technique to a collection of 26 cyanobacterial strains maintained in culture. With cyanobacterial biomass collected onto a glass-fiber filter(Whatman GF/C), each analysis consisted of >five repeats of one second laser pulse, with the aerosol generated passing into the REIMS1 source attached to a Q-ToF mass spectrometer (Xevo G2-XS, Waters, Wimslow). The resultant chemical fingerprint of the different species and strains were processed and analysed using multivariate analysis. Both unsupervised and supervised methods were employed with clear differences observed for each sample within the group. Upon building a statistically significant dataset within custom written software, it was possible to build a model and classify the cyanobacteria based on their spectral fingerprint in real-time. Using this approach, cyanobacteria of unknown species and origin can be projected into the compiled model to obtain an answer on the one second time scale. Questions regarding biological variation, bacterial toxicity and minimum cell numbers are still under investigation, but this advancement in immediate screening shows huge promise and further work is underway.

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Culturomics approach for diabetic foot infection diagnosis

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Diabetic foot syndrome is one of the most serious health complications of diabetes. It is estimated that half of the patients with diabetic foot ulcer develop an infection defined by a common term diabetic foot infection (DFI) which raises serious health consequences. Diagnosis and selection of effective therapy of DFI is now a major challenge for both clinicians and scientists around the world due to the wide spectrum of potential pathogens involved in the development of DFI and often polymicrobial nature of infection. The solution to this problem may be to use a culturomic approach - optimization of culture conditions using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) to overcome culture-based method limitations. The main goal of the study was to evaluate the utility of the 10 common microbiological culture media for reflection of the microbiological background of the 16 DFI samples using the culturomics approach. As a result, received 204 bacterial isolates representing 18 different species – 8 G(-) and 10 G(+). Most patients were dominated by polymicrobial infections (81%). The analysis revealed great differences between tested culture media in terms of the number of isolated G(+) and G(-) bacterial species. The largest number of G(+) species was isolated on blood agar (BLA) while the use of the chromagar orientation (CHRA) enabled detection of all identified G(-) species. The best DFI background coverage obtained using vancomycin resistant enterococi agar (VRE)-59%, glucose bromocresol purple agar (BCP) -56%, and CHRA -55%. The use of a set of two media allowed to increase the recovery of the microbiological background of the samples by an average of 17%, and in the case of 3 media by another 9%. The most useful media for DFI investigation was CHRA and VRE in case of which simultaneous use gave 82% coverage which increased up to 88% when universal tryptic soy agar was included. Performed studies proved that the culturomics approach can improve the accuracy of the DFI microbiological background reflection if suitable media are selected and could be the fast and reliable diagnostic tool for taken the appropriate treatment of the infection.

Comparison of microorganisms disintegration methods for metabolomics analysis by using 1H NMR

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Scientists are looking for a tool that will allow them to effectively, quickly, accurately and low-cost determine the type of bacteria. Finding such a method of identifying microorganisms will significantly accelerate the implementation of treatment and allow for better effectiveness. One part of metabolomic experiment is disintegration methods with have a big influence on the results[1,2]. In this experiment six bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Corynebacterium glutamicum*, *Bacillus cereus*, *Enterococcus feacalis*) were analyzed. All samples were prepared in five repetitions. For this analysis three different methods of cell disintegration were compared (sonication, sand mill and Tissue Lyser) and the methanol-water extraction method (1:1) was chosen. Results showed that the metabolites concentration is changing depends on used disintegration method. The sand mill proved to be the best method for two strains (*E. coli* and *E. faecalis*). Sonication proved to be the best method for three strains (*K. penumoniae*, *P. aeruginosa* and *C. glutamicum*). Tissue Lyser was the best for *B. cereus*. Differences are more likely due to different cell wall structures. Results were provide also that each bacteria strain have they own metabolomic profile. PCA and OPLS models showed good grouping of different strains. Proper disintegration method and conditions for selected material may be crucial for the experiment results. It may be very significant for samples containing different species of microorganism. These results provide that NMR (currently MS is widely used) can also be a method for identifying and distinguishing bacterial strains.

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An unusual nicotinamide derivative – 4-pyridone-3-carboxamide ribonucleoside (4PYR) as a novel endothelial toxin and oncometabolite

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Our recent studies identified a novel path of nicotinamide metabolism that involve 4-pyridone-3-carboxamide-1-ß-D-ribonucleoside (4PYR) and demonstrated its cytotoxic effect in endothelium. This study tested effects of 4PYR and its metabolites on the metabolism changes and metastasis in experimental models of breast cancer. Mice were divided into three groups: 4T1(injected with mammary 4T1 cancer cells), 4T1+4PYR(4PYR-treated 4T1 mice) and control. Animals were maintained for 2 or 21 days. Lung metastasis and endothelium function were analyzed together with the assessment of blood nucleotides (including 4PYR and its ATP-like and NAD-like derivatives), plasma amino acids, nicotinamide metabolites and vascular ecto-enzymes of nucleotide catabolism. Furthermore, effect of 4PYR on PARP-1 and SIRT1 activities and NAD concentration was evaluated in cultured 4T1, MDA-MB 231 and MCF-7 cells. The increase in blood 4PYR in 4T1 was observed already after 2 days. 4PYR and its metabolites were noticed after 21 days in 4T1 only. Higher blood 4PYR concentration was linked with enhanced lung metastases in 4T1+4PYR vs. 4T1. Decreased L-arginine and higher asymmetric-dimethyl-L-arginine plasma concentrations as well as enhanced vascular ecto-adenosine deaminase activity were observed in 4T1+4PYR vs. 4T1 and control, indicating endothelial dysfunction in 4PYR-treated group. Vascular relaxation caused by flow-dependent endothelial activation in 4PYR-treated mice was over 20% lower as compared to control. In addition, increased permeability of lung endothelial cells was observed after 4PYR treatment in comparison to control. Decreased nicotinamide, but enhanced nicotinamide metabolites concentration were noticed in 4T1 vs. control. Reduced N-methylnicotinamide and further increase in Met2PY were observed in 4T1+4PYR vs. 4T1 and control. In cultured 4T1, MDA-MB 231 and MCF-7 cell experiment 4PYR treatment reduced PARP-1 and SIRT1 activities. 4PYR formation is accelerated in cancer and increased concentration of this molecule induces profound metabolic disturbances that may affect cancer progression and especially metastasis, most likely through impairment of endothelial homeostasis. We suggest that 4PYR and its derivatives could be considered as new class of oncometabolites.

Bispecific antibodies – a new look at the molecular interactions analysis

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The measurement of kinetic rates and avidity binding in the simultaneous engagement of two antigens is key to optimizing for bispecific antibody target specificity early in the development process. The quantitative analysis provides insight on how to adjust the individual affinities of the bispecific antibody arms, so that the most favorable cooperative action is achieved, specifically maximal on-target and minimal off-target antibody binding.

To achieve this, a new biosensor was used, that uses nanolevers made of DNA to create functional structures on the surface of the chip. The biosensor mimics the presentation of two different target antigens on the surface of a cancer cell and enables the detection of dual-color fluorescence for simultaneous kinetic studies of single and dual-binding of bispecific antibodies.

The influence of 2,4-D on the lipid peroxidation of *Trichoderma harzianum*

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Trichoderma is one of the most studied genera of fungi because of its practical and ecological significance. It is widely used to protect plants against pathogens and increase their resistance against abiotic stress. This fungus is also known to reduce the negative impact on plants caused by chemical stress. In recent years, herbicides have been increasingly used as a selection tool for plant crops. The herbicide 2,4-D (2,4-dichlorophenoxy acetate) has been used widely since XIX century to control dicotyledonous weeds. It has long been regarded as less toxic to non-target organisms, including soil microorganisms. The aim of the study was to investigate the influence of the popularly used 2,4-D on the defense reaction and lipid peroxidation of *T. harzianum*. To identify if 2,4-D caused lipid peroxidation in the examined strain, the amounts of the peroxidation product and, the level of oxygenated fatty acids, was determined. In each phase of growth, the amount of thiobarbituric acid-reactive substances (TBARS) was higher in the herbicide treated sample. Moreover, statistically significant differences were observed in the content of oxylipins, the main changes concerned 9-oxoODE, 13-oxoODE and 13-HPODE in both of the tested time points. The obtained results show that 2,4-D increased lipid peroxidation in *T. harzianum* liquid culture.

E-POSTERS

Important remarks concerning LC/ESI-MS analysis of biochanin A and prunetin

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In recent years the nutritional and bioactive properties of foods are being intensively investigated with a view to control, in addition to food quality, their possible influence on human health. Isoflavones are a subclass of flavonoids and the most common phytoestrogens – phytochemicals with similar effects as estradiol hormones. Among them biochanin A, occurring in high concentrations in soy and red clover, has attracted particular attention of scientists due to its biological activity such as anticancer, antidiabetic, and anti-inflammatory activity. Prunetin, an isomer of biochanin A, is less common than biochanin A; however, it has also shown biological activity, such as modifies inflammation response, which are pivotal determinants of longevity and improves intestinal epithelial barrier function. Nowadays, LC/ESI-MS methods seem to be the most useful ones widely used for the flavonoid identification, among them biochanin A and prunetin. In order to reliably identify a given compound by LC/ESI-MS, not only retention times but also fragmentation pattern of the analyzed compound must match well those of the standard compound. Ipso facto, the main aim of the study was an attempt to facilitate the distinction between biochanin A and prunetin during analysis using the LC/ESI-MS method. LC/ESI-MS analysis of phytoestrogens usually requires the use of reversed-phase conditions and the most common is acidified CH3CN/H2O gradient. We have found that under these conditions prunetin and biochanin A have similar retention times. Biochanin A and prunetin can be analyzed in positive and negative ion modes, but in the last one they have almost identical fragmentation patterns. In our study, the general fragmentation patterns of biochanin A and prunetin were identical, irrespective of collision induced dissociation (CID) conditions. The product ions had different relative abundances, however, from the qualitative point of view, the most abundant product ions are identical (have identical m/z values). It is reasonable that product ions which can be used to differentiate between biochanin A and prunetin are for biochanin those at m/z 253,153, 152 and for prunetin those at m/z 257, 168, 167.

Biosynthesis of three-dimensional diatomaceous biosilica doped with titanium ions

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In the search for innovative solutions to modern technologies, and especially in the design and manufacture of new nanocomposite inorganic materials, microorganisms as "natural microtechnologists" may be rich inspiration. In this aspect, diatoms seems to be one of the most spectacular examples thanks to their outstanding ability to synthesize amorphous silica (silica exoskeletons) with hierarchical three-dimensional structure. The amazing variety of precision 3D silica structures with their own original openwork morphology is represented by siliceous frustules of more than 100 000 known species of diatoms. These unique capabilities of diatoms produce considerable interest because of the prospect of controlled biosynthesis of three dimensional ordered silica structures by growing microorganisms in artificial conditions. The main aim of this study was to evaluate the ability of the diatom species Pseudostaurosira trainorii to metabolically insert soluble titanium from culture medium into the structure of their amorphous silica cell walls, by cultivation of selected diatom species under laboratory conditions. The culture of diatom species was cultivated using Erlenmeyer flasks with Guillard's F/2 growth medium with soluble titanium concentrations from 2.5 mg/l to 90 mg/l. The idea of incorporating selected elements in silica exoskeletons of diatoms is based on known capabilities of silicon to isomorphous substitution of these elements in the structure of silicate minerals as well as the opportunities of diatom cells to metabolic inserts of the different metals in their silica frustules. The morphological and structural features, elemental composition, structure, photoluminescence properties and thermal stability of the obtained biosilica was examined using a set of techniques comprising scanning electron microscopy, transmission electron microscopy, photoluminescence spectroscopy, IR spectroscopy, thermogravimetry, etc.

The potential role of lipids in prostate cancer based on FFPE tissue samples with the use of MALDI-TOF

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Prostate cancer is one of the most common hormone-dependent cancer type in men. Recent studies indicate that androgen receptor regulates CaP development. Moreover it is assumed that, metabolism of lipids has a significant impact on the cancer cells growth. On the other hand exact cellular mechanisms of cancerogenesis remain unknown. Proper understanding of pathogenesis may support quick diagnosis and enhance the therapy, however, requires to determine wide spectrum of metabolites with different physico-chemical properties. In this terms, MALDI-TOF approach focused on direct determination of compounds with different properties seems to be interesting. The group of compounds that play a significant role in CaP development are lipids. For this reason monitoring of phospholipids with the use of alternative imaging spectrometric technique, namely MALDI-TOF may provide a new knowledge of CaP pathogenesis. However, due to the fact that MALDI-ToF analysis is dedicated to proteomics, there is a need to optimize sample preparation procedure. Another problem is lack of knowledge about the factors influencing lipid characterization with the use of MALDI. The main goal of the study was to select the more efficient and reliable protocol for lipidomic analysis with use of MALDI-TOF-MS/MS protocol. For this reason two matrixes for MALDI-TOF analysis and two extraction protocols were tested. Finally the optimized protocol was applied for simultaneous determination of phospholipids in 80 prostate tissue samples (40 samples of CaP and 40 controls). The obtained results were statistically analyzed with use of univariate and multivariate statistical methods. Within the study, alteration in lipid metabolism especially phospholipids was shown. These lipids were assigned as metabolites with the best discriminative power for two tested group.

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Relative Dependency Analysis of metabolomics data obtained for aqueous humour samples collected from diabetic and non-diabetic patients during cataract surgery

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Cataract, an ocular lens opacity associated with a decrease in visual acuity, is a major cause of vision impairment and blindness worldwide. Epidemiological studies demonstrated that patients with diabetes mellitus are up to five times more likely to develop cataract than non-diabetic individuals. Growing evidence suggests that a longer duration and poor metabolic control of diabetes are the main risk factors for cataract formation[1].

Aqueous humour samples collected during cataract surgeries from 16 patients with diabetes and 19 non-diabetic controls were analyzed with LC-QTOF-MS (in both ion modes) using hydrophilic interactions (HILIC) and reversed-phase (RP) chromatography[2].

A novel machine learning classifier, known as Relative Dependency Analysis[3] (RDA) was applied to identify discriminative patterns in the data. The algorithm induces a decision tree with splitting nodes that consider relative fraction changes between metabolites in each patient. RDA appeared insensitive to the commonly used data normalization and standardization procedures, and rules generated by this classifier based on relations within a small group of compounds can serve as biological switches.

The RDA analysis included all data sets: HILIC(+), HILIC(−), RP(+), and RP(−). Performed tests showed that RDA was capable of identifying patterns composed of the rules with 2 to 6 metabolic features with the accuracy of 91.4%, 100%, 97.1%, and 94.2%, respectively, each of the data sets. The rules used by RDA, included new metabolic features, previously identified as non-significant on univariate or multivariate analyses used commonly for metabolomics data. Upon their identification, these new signals might improve our knowledge of the relationship between diabetes and increased risk of cataract development. The bioinformatical approach used in this study could minimize the influence of interindividual metabolic differences on the statistical outcome. Application of RDA to metabolomics data could add considerably to the results of a typical statistical analysis, identifying additional relationships between metabolites that play a role in the pathogenesis of a given medical condition.

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The role of intervention in improving hemodialyzed patients adherence to hypotensive therapy

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Adherence to therapeutic recommendations is particularly important in the treatment of chronic diseases like chronic kidney disease (CKD) and arterial hypertension. Both of these conditions significantly increase the incidence of cardiovascular disease (CVD) and the mortality rate in comparison to the general population. Proper blood pressure control diminishes pathophysiological changes in the cardiovascular system in patients with CKD. In the era of highly effective hypotensive drugs, one of the most important elements of effective therapy is adherence to therapeutic recommendations. Good cooperation between the medical personnel and the patient, and thus a high level of adherence, to large extent depends on the patient's knowledge of the essence of his or her disease, the principles of non-pharmacological and pharmacological therapy management, as well as of the consequences of poor cooperation in this area. It seems that in patients with CKD, especially in haemodialysed patients, the simultaneous introduction of educational measures, simplification of the treatment regimen, self-monitoring of blood pressure and support from medical personnel and family can significantly improve patients' medical adherence. The aim of the study was identification of the commonly used hypotensive drugs and their metabolites in blood of haemodialysed patients in order to check the influence of planned intervention on the patients' compliance to prescribed drugs. The intervention was carried out by discussion with a patient on the importance of adherence to physicians recommendations. The simultaneous identification of selected 12 drugs (metoprolol, nitrendipin, nebivolol, telmisartan, carvedilol, ramipril, amlodipin, doxazosine, bisoprolol, candesartan, lercanidipin, enalapril) was performed with the use of high performance liquid chromatography coupled with time of flight mass spectrometry (HPLC-TOF-MS). The subsequent study aims to examine to what extent such an intervention could improve adherence to therapeutic recommendations for the use of hypotensive drugs in the group of haemodialysed patients.

Development of Mass Spectroscopy assay for Nε-(1-Carboxymethyl)-L-Lysine, Nε-(1-Carboxyethyl)-L-lysine in human plasma

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Advanced glycation end-products (AGEs) are complex compounds formed during nonenzymatic reactions of reducing sugars or lipid oxidation products with amino groups of proteins by so called Maillard reaction. AGEs are associated with various pathological states, i.e. atherosclerosis, diabetes neurodegeneration, inflammation, tumors, age-related pathologies and septic shock. Nε-(Carboxymethyl) lysine (CML), Nε-(carboxyethyl) lysine (CEL) are typical AGEs, which have been associated with sepsis and sepsis shock. AGEs are mediated as signaling compounds by receptor for advanced glycation end-products (RAGE) considered as a crucial component innate immune response in sepsis. Many analytical methods have been proposed for analysis of CML, CEL. They are based either on chromatographic techniques including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography coupled with fluorescence detector (HPLC-FLD) or analysis using enzyme immunoassays (ELISA). ELISA techniques are associated with limitations such as lack of specificity, risk of matrix interference. Methods which overcomes this limitations are those utilizing LC-MS technique. Due to high polarity of CML and CEL this compounds are difficult to retain on reversed phase columns. Wherefore many methods utilize chromatographic separation on HILIC columns, for reversed phase chromatography commonly nonafluoropentanoic acid (NFPA) has been used as an ion-pairing agent or derivatization with 9-fluorenylmethyl chloroformate has been employed. Here, we present LC-MS method of for the simultaneous analysis of CML and CEL in human plasma with propyl chloroformate as derivatization reagent.

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Amino acid and nucleotide profile in plasma, kidney, and liver of mice with Huntington's disease

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Purpose: Huntington disease is autosomal dominant neurodegenerative disorder caused by the mutant protein huntingtin where polyglumine tract is overexpressed. N-acetylglutamate regulates, among others, carbamoylphosphate synthetase, in the urea cycle, so we examined systemic amino acid transformations in the liver and kidney. In addition, since amino acids can be precursors to, among others, NAD, we examined nucleotide transformations and also plasma amino acid levels following administration of nicotinamide riboside.

Material and methods: Eight-month-old R6/1 with developed Huntington's disease model mice and C57BL/J control mice were used for the study. Nucleotides were determined by HPLC. Amino acids were determined by LC-MS TSQ Vantage. Mice were injected 250 mg/kg body mass nicotinamide riboside for four weeks and then amino acid concentrations in plasma were studied.

Results: Tryptophan, methionine and asparagine concentrations were unchanged in Huntington's disease model mice plasma. Nicotinamide riboside administration significantly decreased the amino acid concentrations in plasma, only tryptophan remained unchanged while methionine and aspartate increased. Concentrations of arginine and tryptophan in liver, did not change in Huntington's mice. Concentration of glycine, valine and leucine in the kidney differed from the way the other amino acids were changed. ATP levels were lowered in the liver and kidney medulla of mice with Huntington's disease, did not change in kidney cortex . The concentration of NAD and NADP did not change in the liver and kidney cortex, while it was decreased in the kidney medulla of mice with huntington disease.

Conclusions: The lack of changes in the concentration of arginine formed in the urea cycle may indicate the body's defense mechanism against the excessive amount of urea formed. Nicotinamide riboside brought the amino acid profile closer to that of healthy mice. Administering it as a supplement could improve the situation of changes in the amino acid profile of people with Huntington's disease.

Differences in the metabolic response to a high-carbohydrate meal between lean and overweight men – a metabolomics study

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Background: The relationship of HC meal intake with metabolic syndrome is still not fully explained. Metabolomics has the potential to indicate metabolic pathways altered by HC meal, which may improve our knowledge regarding the mechanisms by which HC meal may contribute to metabolic syndrome development.

Objectives: The short-term and immediate postprandial metabolic response to HC meal intake was evaluated and compared between lean and overweight/obese (OW/OB) healthy men.

Methods: Two groups of non-diabetic male participants (i.e. OW/OB and lean, age: 23-38 years, BMI: from 23.8 to 30.8), each consisting of 12 subjects, underwent a randomized, controlled, and cross-over one-time HC-meal-challenge trial. On the two different visits (with 2-3 weeks of break between them) the participants received standardized HC meal (89% of energy from carbohydrate, 11% protein, and 0% fat) or normal-carbohydrate (NC) meal (45% carbohydrate, 30% protein, 25% fat). Fasting (0 min) and postprandial blood samples were collected at predefined time points (i.e. 30, 60, 120, 180 min) and untargeted plasma metabolomics was performed using LC-QTOF-MS. For each metabolic feature, based on its intensity change in time, the area under the curve (AUC) was calculated. Obtained AUC values were forwarded for multivariate statistical analysis.

Results: The OW/OB individuals exhibited more pronounced changes in the metabolic response to HC meal intake compared to NC meal consumption. After the HC meal significantly different AUC for metabolites from several classes was observed. Among others, an increase of lysophosphatidylcholines (from +35 to +185%, variable importance in the projection (VIP) range 1.1-1.9), lysophosphatidylethanolamines (from +133 to +255%, VIP 1.4-2.2), long-chain fatty acids (from +54 to +133%, VIP 1.2-1.7), or leukotrienes (from +84 to 216%, VIP 1.4-1.9) and decrease of sphinganine (from -60 to -40%, VIP 1.3-1.6) in OW/OB compared to lean individuals was observed.

Conclusion: Obvious postprandial metabolic differences were noted between lean and OW/OB individuals receiving HC meal. Metabolic pathways differentially affected by the HC meal belong to phospholipids and glucose metabolism, sphingomyelin signalling, as well as purines/pyrimidines and aldosterone catabolism

Neurometabolite changes in patients with complex regional pain syndrome using proton magnetic resonance spectroscopy

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Complex regional pain syndrome (CRPS) develops after an injury, heart attack, stroke, sprain or fracture that affects part of the body in adults and children. The condition is thought to be a malfunctioning of the peripheral and central antinociceptive system. Early correct diagnosis is thought to be important in preventing progression to avoid long-lasting disability. There is no single laboratory test to diagnose CRSP. *In vivo*, tissue damage is associated with elevated concentrations of cytokines and NGF, which stimulate the release of pro-inflammatory neuropeptides, substance P, and calcitonin gene-related peptide by peripheral neurons, inducing retrograde depolarisation of small-diameter fibres (C and Aδ). The result is peripheral sensitisation, with vasodilation and protein extravasation, which explain the symptoms and severity of the clinical manifestations. According to literature data on proton magnetic resonance spectroscopy (1H-MRS), depletion levels for N-acetylaspartate and other identifiable chemicals in brain regions can be a marker CRPS syndrome. Reduced level of N-acetylaspartate in dorsolateral prefrontal cortex and increased level of myo-inositol in orbitofrontal cortex of the patient with intractable severe pain is find in CRPS. In our study we performed MRI scans with a magnetic field strength of 3.0T using standard MRI sequences and 1H-MRS in the Independent Department, Laboratory of Molecular Imaging of the Medical University of Bialystok. 1H-MRS was performed using the single voxel spectroscopy method, where the assessment of metabolic disturbances is possible in a small tissue volume. The voxels was located symmetrically in both hemispheres of the brain in patients suffered from CRPS within the following locations: insula, thalamus, basal ganglia and postcentral gyrus. The following metabolites was assessed: N-acetylaspartate (NAA) - a marker of neuron density, choline (Cho) - an indicator of myelin breakdown products, and myo-inositol (mI) - a marker of microglia activation. To broaden the knowledge about changes in MR imaging studies in the course of CRPS we would like to assess marker microglia aviation in patients experience a remission of symptoms if specific opioid CNS therapy start early after diagnosis.

Application of hyphenated techniques for the determination and identification of antibiotics and microorganisms from clinical samples

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Microorganisms occupy all known ecosystems – from natural environments, those changed by humans, to living organisms. The presence of a microbiome is necessary for the proper functioning and development of the body. Understanding the physiology and genetics of pathogenic bacterial species is important for disease prevention and faster microbiological diagnosis. Biochemical, serological or chemotaxonomic methods and also, in recent years, spectroscopic, spectrometric, and genomic tools are routinely used to identify microorganisms in the context of clinical microbiology, One of the most frequently used methods for the identification of microorganisms is the MALDI TOF-MS technique. In this study our culture media were used non-selective (BHI and MHA) and selective (BCP and VRE). The obtained results allowed to conclude that bacteria of the genus Staphylococcus and Enterococcus had the largest share in postoperative wound infections. Additionally, universal media allowed for the elimination of the greatest number of bacteria types and positively influenced the quality of the obtained spectra in the MALDI TOF-MS analysis. The use of all microbiological media allowed to the isolation of 16 species belonging to 10 types of bacteria. Not only the influence of the medium, but also the culture conditions on the isolation of bacterial cells and their correct identification were analyzed. The results obtained during the examination of the conditions of sample preparation in the MALDI TOF-MS marking of microorganisms allowed us to conclude that a 4-hour incubation of bacterial cells allows for their identification. The results show, however, that the most optimal incubation time is 6h in the liquid TSB medium. The second part of the research was to develop an analytical procedure for the determination and identification of antibiotics and their potential metabolites. Additionally, for the isolation and enrichment of selected compounds in case of microextraction by packed sorbent, sorbent C18 and acetonitrile: methanol:water (5:3:2; v/v/v) were used as elution medium. It has been proven that the use of mass spectrometry for the determination and identification of potential metabolites of selected antibiotics is possible.

A study of free amino acid composition of different honeybee products

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Due to their nutritional, prophylactic, and therapeutic properties, honeybee (Apis mellifera) products (HBP) have been used since ancient times. They are complex mixtures that contain a huge variety of bioactive compounds, such as proteins, peptides, carbohydrates, minerals, biogenic amines, and other metabolites. However, the composition of HBP and their biological activity remains not fully investigated[1]. The study aimed to identify and quantify free amino acids occurring in three HBP: venom, pollen, and royal jelly. A validated method employing a liquid chromatograph coupled to a triple quadrupole mass spectrometer was used. For sample preparation, an amine-reactive isotope-coded tag (aTRAQ reagent) was used. The applied method enabled quantitative analysis of both proteinogenic and non-proteinogenic amino acids in the HBP solutions. In the first step, a preliminary study of a selection of a solvent for the preparation of HBP solutions was conducted. Moreover, an assessment of the variability of free amino acid profiles of HBP was also performed. The study allowed us to obtain a detailed amino acid profile of each HBP covering more than 30 compounds. In honeybee venom and pollen, proline was the most abundant amino acid. In royal jelly, lysine and proline were the dominant constituents of the determined metabolite profile. Among pollen samples, differences in amino acid composition occurred depending on the botanical origin of the samples. Among venom samples, we measured variations in amino acid levels when comparing either different years or different months of venom sample collection. The presented research provided new information about the metabolome of the selected HBP. Our study revealed a high abundance of proline in HBP. Proline-rich peptides represent an important group of insect antimicrobial peptides, and proline residue was indicated to be responsible for the antimicrobial activity of peptides derived from HBP[2]. Metabolomics studies of HBP should be continued to obtain their most comprehensive metabolite profiles.

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LC/HRMS-based metabolic fingerprinting and multivariate analysis in authentication of meat

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Food authentication is a rapidly growing field due to increasing public awareness concerning food quality and safety. In recent years, food frauds and adulterations have increased significantly. This practice is motivated by fast economical gains and has an enormous impact on public health[1].The mislabeling of meat species can expose consumer to risks associated with meat allergies, and can lead to economic losses[2]. The accidental or intentional blending of meat from different species is becoming a worldwide problem[3].The development of advanced analytical methods is crucial to enable efficient testing for food authenticity. High-resolution mass spectrometry based metabolic fingerprinting combined with chemometrics represents a valuable tool for detection of food adulterations.

In this work, we study by means of UHPLC high resolution mass spectrometrythe metabolic profiles of meat species, which are the most prone to adulteration. Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was employed for the analysis of meat extracts and metabolic fingerprints were obtained. The results of mass profiling and multivariate analyses (Principal Component Analysis - PCA, Partial Least Square - Discriminant Analysis PLS-DA) of the acquired high-resolution mass spectrometry (HRMS) data allowed the determination of metabolic features differentiating the examined meats indicating that LC/MS-based metabolic fingerprinting can be used for the detection of meat adulterations.

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The influence of the diabetic environment on phosphate homeostasis in podocytes

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Inorganic phosphate (Pi) and pyrophosphate (PPi) are two key substances in a calcification process, i.e. a deposition of calcium phosphate salts in tissues in a form of hydroxyapatite. Pi is one of the building blocks of hydroxyapatite and also a mineralization promoter, while PPi is the strongest inhibitory factor in this process. Pathological calcification of soft tissues may occur in the final stage of chronic kidney disease caused by diabetic nephropathy. The hyperphosphatemia present at that time contributes to the dysfunction of kidneys, in particular a glomerular filtration barrier. An important element of this barrier are foot processes of podocytes – glomerular epithelial cells. It should be noted that podocytes are located in an urinary space of Bowman's capsule, which lacks osteopontin or albumin – known inhibitors of calcification. PPi can be a protective factor for podocytes by controlling the phosphate balance in the glomerular filtrate and preventing excessive formation of hydroxyapatite. The aim of the study is to investigate the influence of the diabetic environment on phosphate homeostasis in podocytes and how it affects the function of these cells. To establish this, the activity of the two main enzymes producing intracellular Pi and PPi was measured – tissue nonspecific alkaline phosphatase (TNAP) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1), respectively. Podocytes cultured in high glucose concentration (HG) showed an increase in TNAP activity as compared to control cells (0.201 ± 0.003 vs 0.173 ± 0.007 nmol/min/mg protein; $n = 7$; $p = 0.001$). However, no changes in the activity of the NPP1 enzyme were found. By using western blot analysis, a decrease in the amount of type III sodium-dependent phosphate transporters – PiT 1 and PiT 2 was observed in podocytes cultured in HG. Additionally, the exposure of the PiT 1 transporter in the podocyte plasma membrane was reduced in HG. Also a decrease in the amount of extracellular PPi was observed in HG conditions. The above data suggest that the high glucose conditions, observed in diabetes, cause changes in Pi/PPi balance and promote calcification processes in podocytes.

Simultaneous determination of lopinavir, saquinavir, and ritonavir in human plasma using liquid chromatography – ion trap mass spectrometry

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Saquinavir, ritonavir, and lopinavir are viral protease inhibitors that were developed for and are widely used in human immunodeficiency virus (HIV)-related disease. These compounds are currently tested in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. We aimed to develop liquid chromatography/mass spectrometry (LC/MS) procedure for the accurate determination of the blood concentration of these therapeutics. Samples of human plasma were protein precipitated with zinc sulfate in methanol/water solution. Extracts were analyzed with reversed-phase chromatography connected to electrospray ionization (ESI) ion source of ion trap mass detector operating in MS and MS/MS modes. Calibration curves demonstrated good linearity from 0.01 to 10 µg/mL and acceptable reproducibilities and recoveries. The procedure described proves that a very basic ion-trap LC/MS system could be used for selective, rapid, and precise determination of antiviral protease inhibitors.

Evaluation of gastrointestinal stromal tumour sample preparation procedure for LC-MS untargeted metabolomic analysis

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In analytical method validation process, robustness is the ability of an analytical procedure to achieve undeniable results despite introducing minor changes in experimental conditions. Quality assurance is essential in metabolomic analysis to ensure that the acquired data are of high quality. Tissue is a particularly challenging type of biological matrix in metabolomic analysis due to complex sampling procedure, normalization, homogenisation, and metabolite extraction. In the present study, we applied the concept of robustness testing for evaluation of the sample preparation method of a gastrointestinal stromal tumour for metabolomic analysis with the use of HPLC-TOF/MS and two complementary LC columns (RP and HILIC). The Plackett-Burman plan was used, according to which 15 measurements were taken for ten modifiable factors. The variables included in the plan were: the volume of methanol used for extraction, the volume of MTBE added to the sample, the time of shaking, the volume of water used for extraction, the time of centrifugation, the volume to be evaporated, the evaporation temperature and the volume of solvent added after sample concentration. The selected factors occurred at two levels, and additionally, central points were introduced, in which parameter values were as in the evaluated method. The sum of signal intensities measured in the samples and the values of two principal components in the PCA analysis were chosen as the responses. The study shows that the critical stage of tissue sample extraction procedure is the temperature of solvent evaporation in the centrifugal concentrator. Instability of evaporation temperature proved to impact the value of the second principal component in the PCA model built on LC-MS data collected in HILIC analysis, in negative ionisation mode. Methylguanosine, acetylcarnitine, or fumaric acid were the most sensitive to fluctuations in evaporation temperature. According to the research results, strict control of the critical factor should be performed. The condition of the centrifugal concentrator used during the extraction procedure should be verified before each project to ensure correct vacuum and temperature levels in the device.

Postprandial changes in plasma metabolome of non-diabetic men are related to genetic susceptibility to type 2 diabetes

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Single nucleotide polymorphism (SNP) in the prospero homeobox 1 (PROX1) gene is a strong genetic susceptibility factor for diabetes mellitus type 2 (T2DM) and impaired β cell function. However, the role of PROX1 gene in T2DM development still remains unclear. Therefore, this study aimed to use gas chromatography-mass spectrometry (GC-MS) based metabolomics to evaluate postprandial changes in plasma metabolites after the high-carbohydrate (HC) and normo-carbohydrate (NC) meal-challenge-tests in non-diabetic men with different PROX1 genotypes.

Thirteen contestants (5 with high risk (HR) and 8 with low risk (LR) genotype) participated in HC and NC meal-challenge-tests. Fasting (0' min.) and postprandial (30', 60', and 120' min. after meal intake) plasma samples were fingerprinted with GC-MS. After data pretreatment, identification and quality assurance protocol, 52 metabolites were forwarded for statistical analysis. The Wilcoxon signed-rank test was performed using the R software environment (version 4.0.0, https://www.R-project.org/). Multiple comparisons were corrected using the Benjamini-Hochberg procedure.

GC-MS based metabolomics revealed that LR genotype carriers did not demonstrate any alterations between fasting and postprandial metabolites level (neither after LC, nor HC meal). However, HR genotype carriers exhibited changes between fasting and postprandial level of 7 metabolites (mainly carboxylic acids and monosaccharides) but only after HC meal.

The results of our study led to the conclusion that HC meal challenge test provoked changes in plasma metabolites in HR genotype, but not LR genotype. More detailed studies and pathways analyses are required to determine the exact mechanism of this process and its role in T2DM development.

AAMA and GAMA levels in urine as markers of acrylamide exposure from the diet and tobacco smoke

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Acrylamide (AA) is a 'probably carcinogenic to humans' (Group 2A, IARC 1994) monomer that can form in heated starchy food. Cigarette smoking appears to be the second most important source of exposure to AA. The main metabolite of AA and responsible for its carcinogenic effect is glycidamide. Both of these compounds are conjugated with glutathione and further metabolised to form mercapturic acids: N-Acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA). Aim: Determination of AAMA and GAMA levels in urine by LC-MS/MS. Assessment whether AAMA and GAMA levels in urine are suitable marker to estimate the exposure to acrylamide from food and tobacco smoke.

Study group: Twelve healthy volunteers (6 women and 6 men), aged 27 to 62, who gave their written consent to participate in the study. Three participants smoke cigarettes (6 to 25 cigarettes/day). Study design: During the first 3 days, participants were on their usual diet, followed by a 1-day diet without AA-sourced foods ("white diet") and smokers did not smoke. This was followed by another 3 days of their typical diet. On the last day of the experiment, participants consumed mainly food, which is a source of dietary AA ("FF diet"). Each day food consumption data over 24 hours was collected and participants provided the morning urine sample.

Methods: AAMA and GAMA levels in urine was determined using validated LC-MS/MS method.

Results: The median AAMA and GAMA levels in urine after the day of "white diet" is significantly ($p < 0.05$) lower compared to AAMA and GAMA levels in urine after typical diet days and "FF diet" day. Estimated based on AAMA and GAMA levels in urine (metabolomic approach), exposure to AA from the typical diet was on average 0.42 µg/kg b.w./day and was similar to the exposure estimated for the entire Polish population (0.47 µg/kg b.w./day) using the probabilistic approach. We found significantly ($p < 0.05$) higher levels of acrylamide metabolites in the urine of smokers compared to non-smokers, regardless of the type of diet.

Conclusion: Our results confirm the status of AAMA and GAMA as markers of exposure to acrylamide present in the diet and tobacco smoke.

Effect of thyrotropin on kidney damage of stored before transplantation by hypothermic perfusion

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Thyrotropin (TSH, Thyroid-stimulating hormone) is a hormone that is secreted in the anterior of pituitary gland. It stimulates the thyroid gland to produce thyroxine (T4) and triiodothyronine (T3). It has many unique properties, including the stimulation of proliferation and hypertrophy of thyrocytes. TSH influences on the proper functioning of the kidneys. It affects the renal blood flow, glomerular filtration, electrolyte pumps, secretory and absorptive capacity of tubuli, and kidney structure. The aim of the study was to analyse the protective effect of thyrotropin, as a component of Biolasol® solution (Biochefa, Sosnowiec, Poland), on the prevention of ischemia-reperfusion injury of nephrons. The solution was modified by the addition of ascorbic acid (0.088g/l) and TSH at a dose of 1 µg/L. A model of isolated kidneys of the Great White Poland pigs was used for the study. Grafts were rinsed with Biolasol solution (control), Biolasol + Vit. C (B1) and Biolasol + Vit. C + TSH (B2). Then the kidneys were stored for 48h under hypothermic conditions (4°C) and flushed again. The parameters of renal function in the collected perfundates were analyzed: LDH, protein, creatinine, urea and electrolytes. TSH affects the effectiveness of kidney perfusion and preservation. It was found that the activity / concentration of markers of renal function was lowest in Biolasol + vit .C + TSH perfundates. Thyrotropin as a component of the Biolasol preservation solution has a positive effect on the protection of the kidneys during ischemia-reperfusion.

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The effect of one anastomosis gastric bypass on serum fatty acid profile in patients with morbid obesity

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Treatment of obesity by bariatric surgery can result in metabolic benefits. One anastomosis gastric bypass (OAGB) is a type of minimally invasive bariatric procedure that is highly effective for weight loss and improves glucose homeostasis in patients. Our recent study[1] utilized chemometric methods to show that OAGB also leads to the normalization of serum amino acid concentrations in patients with morbid obesity, to patterns similar to those found in healthy control subjects. Alteration of fatty acids (FAs) composition and metabolism is a well-established feature of obesity, however the impact of OAGB on the FA profiles is not well understood. Therefore, the aim of our study was to examine if serum FA profiles normalize as a long-term effect of OAGB procedure. The study included 46 patients with morbid obesity and 29 healthy control subjects. Serum was collected from patients with morbid obesity before and 6-9 months after OAGB. Serum FA profiles were analysed by GC-MS. The comparison of FA profiles by standard statistical analysis revealed significant differences in some FAs between patients with morbid obesity and non-obese controls as well as significant FAs changes in post-OAGB patients. The principal component analysis (PCA) analysis showed that FA profiles differ significantly between controls and subjects with morbid obesity. However, the OAGB did not lead to normalization of FA profiles in subjects post treatment to the patterns found in healthy controls. These results suggest that patients that underwent OAGB surgery should be supplemented with some FAs (e.g. polyunsaturated, branched-chain or odd-chain FAs) in order to reconstitute the appropriate serum FA profiles.

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Metabolomics reveals differences in aqueous humor composition between patients with and without pseudoexfoliation syndrome

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Pseudoexfoliation (PEX) is a stress-induced elastosis accompanied by excessive production of microfibrils, and their deposition in the anterior segment of the eye, mainly lens capsule; what can be observed during a routine ophthalmic examination. It is considered a major risk factor for accelerated cataract formation, cataract surgery complications, and development of glaucoma, which untreated or inadequately treated may lead to blindness. The pathogenesis of PEX has not been fully elucidated yet. To evaluate the actual biochemical status of the eye and reveal metabolic pathways dysregulated by an ocular disease, aqueous humor (AH) can be analyzed. AH is a transparent fluid that fills the anterior and posterior chambers of the eye. It maintains the intraocular pressure and provides nutrition for the avascular ocular tissues. Determination of metabolites in AH characteristic to PEX may provide valuable information about the molecular mechanisms behind this ocular disorder. Consequently, this study aimed to compare the metabolic composition of aqueous humor between the patients with and without PEX undergoing cataract surgery. AH samples obtained from 34 patients (15 with and 19 without PEX) were analyzed by LC-QTOF-MS using reversed-phase and HILIC chromatography. As a result, eleven statistically significant metabolites were identified. In patients with PEX, a decrease of amino acids and their derivatives (−19 to −31%, VIP 1.38–2.38), as well as two acylcarnitines hydroxybutyrylcarnitine (-29%, VIP=1.24) and decatrienoylcarnitine (-46%, VIP=1.89), were observed. Contrary, indoleactaldehyde was increased (+96%, VIP=2.64) in AH of patients with PEX. Among other significant metabolites, two known antioxidants ascorbic acid (-33%, VIP=2.11) and hydroxyanthranilic acid (-33%, VIP=2.25), as well as possessing anti-inflammatory activity S-adenosylmethionine (-29%, VIP=1.93), can be mentioned. Obtained results indicate that PEX can be associated with oxidative stress and inflammation, as well as disturbances in the processes of cellular respiration and energy production in the mitochondria. Implementation of non-targeted metabolomics provided a better insight into the still not fully-understood pathogenesis of PEX.

Paracetamol – a drug with new and multidirectional mechanisms of action

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Paracetamol (acetaminophen) is the most commonly used over-the-counter (OTC) drug in the world. Despite its popularity and use for many years, the safety of its application and its mechanism of action are still unclear. For a long time, it was thought to act on similar cyclooxygenases to non-steroidal anti-inflammatory drugs. However, paracetamol has no significant anti-inflammatory activity. Therefore, in recent years, extensive research has been carried out on this drug. Currently, it is believed that paracetamol is a multidirectional drug and at least several metabolic pathways are involved in its analgesic and antipyretic action. It mainly includes the involvement on the endocannabinoid system and serotonergic pathways, influence on transient receptor potential (TRP) channels and to a protein variant of COX-1. Additionally, inhibits T-type Cav3.2 calcium channels and voltage-gated Kv7 potassium channels. It also exerts an impact on L-arginine in the nitric oxide (NO) synthesis pathway. However, not all of these effects have been clearly confirmed. Therefore, our aim of the study was to gather information on the latest research on the effects of paracetamol and its metabolites. Work based on own article and others: https://doi.org/10.1111/1440-1681.13392

Application of advanced metabolomics methods for the determination of fatty acids in seeds oils

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The composition of fatty acids in plant oils is the main factor determining their chemical, physical and biological properties. Therefore, the structural and quantitative analysis of lipid compounds is crucial in selecting potential application of oil . Oils extracted from seeds have a different fatty acid profile, even between dissimilar species of the same type of plant. The lipid composition of oils is dominated by unsaturated fatty acids. Compounds belonging to the group of conjugated fatty acids (CFA) are of particular interest. Their biosynthesis is possible only by specific plant species, therefore the oils obtained from them have a unique profile of fatty acids. An important group of CFA are conjugated isomers of α-linolenic acid (CLnA,) which occur only in seeds of plant. Recent years have brought more reports about the health-promoting properties of CLnA acids. They constitute an important research subject, especially in therapeutic applications, in particular: anti-cancer, anti-atherosclerotic and anti-inflammatory . The most frequently used analytical methods for their determination of their quantitative and qualitative analysis are based on mass spectrometry, often in combination with gas chromatography (GC) or liquid chromatography (LC). In this approach, separation of CLnA isomers is difficult, due to their weak stability. For that reason, it is necessary to looking for new methods of fatty acids analysis . In this study an alternative methodology for the determination of fatty acids in vegetable oil using nuclear magnetic resonance (NMR) spectroscopy is presented. In this approach, sample derivatization is not required and time of analysis is significantly shorter. Omitting the esterification step protects the CLnA acids from isomerization reactions and allows to check the real oil composition. Accurate and insightful analysis of 1D: 1H and 13C NMR spectra and 2D: 1H, 1H COSY and 1H, 13C HSQC spectra allows for unambiguous identification of the type of isomer found in the tested material, even if they have a similar structural.

The 1H NMR studies for monitoring of unique changes in metabolic response of fibrosarcoma cell line on oxygen availability and cultivation time

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The growth ratio and development of cell culture strongly depend on the availability of oxygen and accessible nutrients, which can change continuously during cultivation time. The viability parameters depend strongly on the phenotype of investigated cells. Usually *in vitro* studies used atmospheric oxygen concentration (21%). While during hyperoxia conditions, the oxygen level is much higher, than human tissue between 1% to 6%. In the blood that leaves the lung, it contains oxygen roughly over 10% but in most tissues, the oxygen concentration should be around be 6%. In contrast in most solid tumors, which experience hypoxia as a pathologic condition the oxygen partial pressure drops below 1%[1]. In our study, we focused on the metabolites profile changes on cell pellet and cell culture medium during normoxic, hypoxic, and hyperoxic conditions. At the same time the reverse approach conditions were applied to the cultivated cells, namely: from 1% O2 hypoxia 12h incubation to 6% O2 normoxia "rev.normoxia"; from 6% O2 normoxia 12h incubation to 1% O2 hypoxia "rev.hypoxia". as a consequence of angiogenesis and metastasis. We confirm that there is a pre-state phenotype (hypnormoxic State) depend on cultivation time and oxygen availability. We have determined extracellular and intracellular metabolomes within cell metabolism changes by deregulation of oxygen bioavailability.

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Interactions of veterinary drugs: enrofloxacin and fipronil *in vitro* study

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Particular public attention is focused on the presence of veterinary drugs in the food of animal origin and in the environment. The presence of these chemotherapeutic agents in varying concentrations can pose a serious problem in human exposure to these drugs. There are more and more opinions in the literature that interactions between veterinary drugs are a new, unrecognized problem in veterinary toxicology. The problem is exacerbated by the lack of data on human exposure to a mixture of different veterinary drugs present in food and the ecosystem. Enrofloxacin is the most commonly used antimicrobial veterinary drug in Poland. Fipronil belongs to the phenylpyrazole group, is registered as a veterinary medicine, and is most commonly used to control ectoparasites in animals. The concentrations of both veterinary drugs are detected in food, environment, and plants. The aim of the study was verify the occurrence of cytotoxic interactions between two veterinary drugs: enrofloxacin and fipronil in humans. The cellular model was cultured human hepatoma, HepG2. Cytotoxic evaluation was performed after 72h incubation, using four methods (MTT, NR, TPC, LDH). The existing nature of drug-drug interaction was assessed using the combination index (CI). It has been shown that the tested drugs are characterized by low toxicity, and the toxic effects increased with the increase of the concentrations of tested drugs in their mixtures. The EC20 concentrations were: for the mixture (1:1) from 3.3 to > 5 µg/ml; for a mixture (1:2) from 1.6 to 4.3 µg/ml; for a mixture (2:1) from 2.4 to > 5µg/ml. The nature of the interaction between enrofloxacin and fipronil was concentration-dependent and led to a strong synergistic effect of both drugs. Obtained results suggest that mixtures of veterinary drugs show higher cytotoxicity than the effects of individual drugs.

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Searching for changes in metabolome of women with polycystic ovary syndrome with the use untargeted metabolomics

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PCOS is multifactorial disorder, which is diagnosed in about 10% of women of reproductive age. It is connected with ovulatory dysfunction and polycystic ovaries appearance and also with higher level of androgens concentration. Despite such a high incidence, the pathogenesis of polycystic ovary syndrome still remains unexplained. The use of the metabolomic approach in a field of PCOS aims to identify specific metabolic pathways potentially involved in the pathophysiology of this frequent 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 conducted with the use two complementary analytical techniques: liquid chromatography and gas chromatography coupled with mass spectrometry. Next step was identification metabolites specific for biochemical pathways disturbed in PCOS. Univariate and multivariate statistical analysis showed the difference of metabolic profiles in both groups of women. During the untargeted metabolomic analysis of serum samples, a few metabolites connected with metabolic disorders of amino acids, carbohydrates, steroid hormones, lipids, purines and citric acid cycle were identified. Understanding the pathomechanism of this syndrome and the identification of new potential biomarkers of PCOS will contribute to its early recognition and effective treatment.

Optimization of analytical approach based on SPME-LC-MS/MS for the analysis of selected endocannabinoids in brain regions

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Introduction: The endogenous cannabinoid system is involved in several physiological processes, including energy metabolism and memory processes. This system consists of different types of receptors (e.g., CB1 and CB2), ligands and enzymes involved in the metabolism of endocannabinoids (ECBs). The purpose of this study was to optimize the SPME-LC-MS/MS methodology for analysis of four selected endocannabinoids, namely anandamide (AEA), 2-arachidonoyl glycerol (2-AG), N-arachidonoyl dopamine (NADA), 2-arachidonyl glyceryl ether (2-AGe) in selected brain regions. In addition, the level of receptors of endocannabinoid system, namely CB1 and CB2 in brain samples was estimated, and correlated with the level of ECBs.

Methods: After optimization of selected conditions of sample preparation based on solid-phase microextraction (SPME), SPME biocompatible probes with C18 coating (extraction phase) were applied for the sampling of 3 brain regions (cerebellum, cerebral cortex and striatum) of female and male rats. C18 probes were introduced into selected parts of brain for 30 min static extraction, and then obtained extracts were subjected to LC-MS/MS analysis. In addition, immunohistochemical staining of brain tissue sections with the use of antibodies against CB1 and CB2 receptors was performed.

Results: SPME conditions for the analysis of endocannabinoids in brain samples were optimized, and 30 min extraction time and 60 min desorption time with the use of a mixture of MetOH/IPA (50:50, v/v) were selected for the analysis of endocannabinoids in brain regions. 2-AG and AEA were extracted from all analyzed brain regions, however, the level of 2-AG was significantly higher in comparison to the level of AEA. NADA and 2-AGe were detected only in selected brain samples. The CB1 receptors predominated in analyzed tissue sections.

Conclusions: High-throughput method based on SPME-LC-MS/MS was developed for the analysis of trace level of selected ECBs in intact (non-homogenized) brain samples. Optimized analytical conditions along with the correlation with immunohistochemical results are the basis for further analysis of the level of endocannabinoids in brain samples, and also in selected brain structures of living organisms (*in vivo* SPME).

Biochemistry of death – application of metabolomics in forensic medicine

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Somebody said once that "in this world nothing can be said to be certain, except death and taxes". Truly, death affects all people worldwide, which makes research about death so universal and widely applicable. However, besides the fact, that death accompanies humanity from ages, our knowledge about death it is still very limited. The main aim of the research is the application of metabolomics to evaluate and understand the modulations of small metabolites that take place after death. The experiment was performed on animal (domestic pig) blood samples with time, anticoagulant presence, temperature, and humidity as factors taken into account. The moment of the death and then dozen time points (from a few hours till a few days after it) were selected to examine chosen time intervals. **Metabolic fingerprinting was performed by use of liquid chromatography with mass spectrometry detection** (QTOF, model 6550 Agilent Technologies, Santa Clara, CA, USA). Visible differences in hemolysis and hematocrit levels linked to the presence of anticoagulant were observed in examined groups during material collection and preparation. Performed instrumental and statistical analysis allowed to indicate over thousand metabolites modulated in different time intervals, of which over 200 was statistically significant. Detected metabolites were linked with many biochemical pathways, including sphingolipid metabolism, aminoacyl-tRNA biosynthesis, phenylalanine, tyrosine and tryptophan metabolism, porphyrin metabolism, histidine metabolism, sucrose metabolism, glycerophospholipid metabolism, valine, leucine and isoleucine degradation, biosynthesis of unsaturated fatty acids, and others. Some of these processes are probably effects of cells degradation, others can be linked to decay and caused by bacteria metabolism and activity. These findings suggest that death causes wide range scale changes in the cell as well as whole organism metabolism. Some of them will be linked with host biochemistry, others will be the effect of external factors as micro-organisms activity. More studies are needed to explain the processes behind this phenomenon.

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QEEG as a possible biomarker for ASD diagnosis

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Objective: The aim of our study is to analyze QEEG as a potential biomarker for all frequencies in all areas of the brain, and compare it with a group of healthy subjects. An important factor is the quantitative evaluation of alpha, beta, theta, and delta waves amplitudes in the QEEG study in children with ASD, depending on the age of the patient.

Methods: The study analyzed the EEG records of patients diagnosed in the EEG Laboratory of the University Clinical Hospital in Warsaw, Poland. The examination was carried out with the use of the Elmiko device according to the international 10–20 protocol. Power spectra for each lead were obtained with the Fast Fourier Transformation algorithm. All patients enrolled in the study were assigned to either "autism" (36 subjects) or "headache" groups (68 subjects). Prior to the EEG, all patients underwent imaging studies (CT or MRI of the head) in order to exclude organic causes of their medical complaints.

Conclusion: Our analysis confirmed that there are differences in the spread of brain waves in people with ASD compared to healthy individuals. In fact, alpha and beta waves may be the leading factors to be assessed in EEG testing in children with suspected ASD. This is necessary and reasonable from the point of view of pediatric neurology, pediatrics, psychology, psychiatry and medical related sciences and more, in order to develop standards that determine the appropriate characteristics of brain bioelectric function in children with ASD.

Keywords: biomarker, autism, algorithm.

Use of stable isotopomers and liquid chromatography with mass spectrometry (LC/MS) for cardiac substrate preference analysis in mice

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Heart is an organ characterized by prominent metabolic flexibility for utilizing all carbon substrates, including carbohydrates, fatty acids, ketones and amino acids. It is well known that cardiac substrate preference could be coordinated by factors such as the availability of individual substrates, hormonal activity but also might be deregulated in pathological conditions. However, so far there is no simple *in vivo* method to investigate changes in substrates use in cardiac metabolism in mice. Thus, the aim of the study was to develop method for the analysis of cardiac substrate preference using stable 13C glucose, 13C valine , 13C leucine isotopomers and mass spectrometry. Our assay allowed for investigation of the glucose flux to pyruvate as well as the entrance of the acetyl-CoA (formed from glucose as well as branch chain amino acids (BCAA) such as valine and leucine) to Krebs cycle by analysis of 13C alanine and 13C glutamate enrichment in mice heart. Briefly, analysis was based on subcutaneous injection of glucose or BCAA isotopomers. After 60 min of injection mice were anesthetized and hearts were freeze-clamping. At the same time, blood samples were collected from a tail vein before and after 1 hour of injection. Afterwards, mice hearts were extracted with perchloric acid while the blood extraction was performed with acetone. The method was validated in mice after glucose/insulin, iodoacetate, ranolazine or trimetazidine injection that demonstrated expected shifts in cardiac metabolic substrate use. Our method allowed fast and simple estimation of cardiac glucose and BCAA use in mice and has potential for analysis of changes in pathology and after pharmacological treatments.

Untargeted metabolomics of early vascular aging in hypertensive patients

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Arterial stiffening is a hallmark of early vascular aging (EVA) syndrome and an independent predictor of cardiovascular morbidity and mortality. In this case-control study we sought to identify plasma metabolites associated with EVA syndrome in the setting of hypertension. An untargeted metabolomic approach was used to identify plasma metabolites in an age-, BMI-, and sex-matched groups of EVA (n = 79) and non-EVA (n = 73) individuals with hypertension. After raw data processing and filtration, 497 putative compounds were characterized, out of which 4 were identified as lysophosphaditylcholines (LPCs) [LPC (18:2), LPC (16:0), LPC (18:0), and LPC (18:1)]. A main finding of this study shows that identified LPCs were independently associated with EVA status. Although LPCs have been shown previously to be positively associated with inflammation and atherosclerosis, we observed that hypertensive individuals characterized by 4 down-regulated LPCs had 3.8 times higher risk of EVA compared to those with higher LPC levels (OR = 3.8, 95% CI 1.7–8.5, P < 0.001). Our results provide new insights into a metabolomic phenotype of vascular aging and warrants further investigation of negative association of LPCs with EVA status. This study suggests that LPCs are potential candidates to be considered for further evaluation and validation as predictors of EVA in patients with hypertension.

Metabolic insertion of biosilica obtained from diatoms with cerium ions

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Diatoms are single-celled algae of microscopic size (from 5 μm to 500 μm). They can live singly or form colonies where each cell is a separate entity. The greatest peculiarity and most important feature of diatoms is the structure and composition of the cell wall. Diatom frustules are perforated with periodical pore systems, thus creating unique openwork and three-dimensional structures. The possibilities of 3D structure silica biosynthesis can be extended by chemical modification of diatoms in the process of growth. The synthesis carried out by these microtechnologists and the possibility of adding other elements to their structure, makes it possible to obtain a modern material. Therefore, it seems ideal to combine the unique structure of biosilica with the unique properties of elements from the rare earth group. Since the 1950s, when the first applications for REE were developed, there has been a constant increase in demand for this group of raw materials. This is due to the discovery of new applications for them. Cerium is used in modern technologies so-called " high-tech". It is used in the production of automotive catalysts, various electronic devices such as phones, tablets and LEDs, as well as in the reduction of nitrogen oxide consumption in catalytic processes. Cultivation of selected species of diatoms in laboratory conditions opens the perspective of controlled biosynthesis of 3D orderly silica structures doped with rare earth metals. The aim of the research work is the synthesis of openwork biosilica with a three-dimensional structure doped with cerium. The realization of this task consists in the cultivation of diatoms of the species *Pseudostaurosira trainorii* under laboratory conditions and *in vivo* admixture of cerium ions when manipulating the culture conditions. Characteristics of morphological and structural features, physicochemical properties, as well as the content and distribution of the admixed element were analyzed after a series of instrumental methods.

Label-free quantitative SWATH-MS proteomic analysis of adult myocardial slices *in vitro* after biomimetic electromechanical stimulation

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Conventional *in vitro* cell cultures are widely available, but results obtained with this model are not directly applicable to the *in vivo* environment. A possible solution to overcome this problem could be use of tissue slices with a preserved architecture, multi-cellularity and many physiological functions of the intact heart. This approach creates a bridge between the the cellular and *in vivo* studies. Additional factor that may impact relevance is electrical activity. Aim of this work was to characterize changes in proteomic patterns in cardiac slices that occur during its long term culture and electrical stimuli.

Methods: Rat myocardial slices were stretched to sarcomere lengths (SL) within the physiological range of 1.8–2.4 μm. Electromechanically stimulated slices were compared with slices cultured without electromechanical stimulation (unloaded and nonstimulated - TW) on a liquid–air interface and with fresh myocardial slices (0 h - C). Qualititative (relative) proteomic pattern analyses were conducted in four groups: control (C) and three investigated groups - TW, 1.8 and 2.2 using a label-free SWATH-MS technique on high resolution microLC-MS/MS TripleTOF 5600+ system (SCIEX). The acquired MS/MS spectra from IDA LC-MS/MS analyses of the rat heart samples were searched with use of UniProt Rattus norvegicus database (version from 15.05.2018) using Paragon algorithm incorporated in ProteinPilot 4.5 (SCIEX) software.

Results: In order to visualize changes in the level of protein expression between group C and group 22, a SWATH MS analysis was performed. The protein library consisted of 393 proteins, 310 of which were successfully quantified. Based on statistical analysis using the student's t-test and assuming a significance cut-off point with $p < 0.05$, we selected a list of 77 proteins whose concentration change between groups C and 22. These include for example: NADH-ubiquinone oxidoreductase, Ckmt2 protein, MICOS complex subunit, creatine kinase M-type.

Conclusions: This study demonstrated proteomic pattern shift between heart slices that were stretched, slices cultured without electromechanical stimulation and with fresh myocardial slices. Most prominent changes were observed in proteins related to mitochondria respiration and energy metabolism.

WORKSHOPS

LECO - Tomas Kovalczuk

High Performance GC- and GCxGC-TOFMS metabolomics-based approach for the discovery of potential cancer biomarkers in plasma.

Aroma Profiling of Coffee with GC, GC×GC, and TOF MS.

The assurance of quality and validity of generated data is nowadays strongly requested for any scientific output from a metabolomic research. Such data can be only obtained with both (i) high resolution GC and (ii) high performance MS instrumentation.

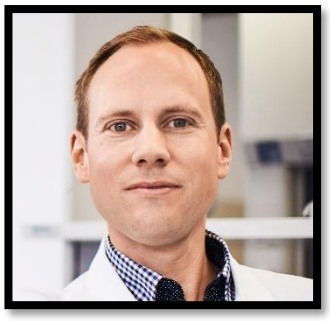
The workshop will be focused on the application of comprehensive two dimensional gas chromatography hyphenated to Time of Flight mass spectrometry in non-targeted analysis in metabolomic studies. Attendees will be introduced to the principles and advantages of employed techniques (GCxGC, TOF MS) along with used approaches (peak find & spectral deconvolution algorithms, GCxGC data alignment) that will be demonstrated on two case studies focused on (i) annotation of candidate cancer biomarkers in human plasma and (ii) coffee profiling.

Thermo Fisher Scientific – Anas Kamleh

Setting a new standard for analytical rigor and confident identifications in metabolomics.

During the workshops the trainees will learn about semi-targeted metabolomics workflow using a new compact HRAM Orbitrap mass spectrometer designed for exceptional usability and equipped with analytical capabilities for small molecule analysis. Trainees will learn how to discover MS instrumentation designed for exceptional usability resulting in increased productivity, recognize which analytical conditions generate high quality insightful metabolomics results, and explore innovations for small molecule analysis that lead to confident and trustworthy data. The workshop is addressed to the metabolomics researchers implementing large scale analyses and MS practitioners employing small molecule untargeted approaches for comprehensive analysis, but also to the scientists new to the field of metabolomics applying biomarker discovery, global profiling, general phenotyping.

Biocrates – Manuel Kratzke



Standardized metabolomics kits for quantitative and reproducible results. Kit workflow in the laboratory and the MetIDQ™ software.

During the workshops the trainees will learn how to uncover the phenotype of an individual from just a few microliters of sample material. The main subject of the workshop is the AbsoluteIDQ® p180 kit, a simple and high-throughput tool for basic, clinical, and epidemiological research. Attendees will learn about the suitability of the kit which can be used on all stages of biomedical research – from cell culture and rodent models to large-scale epidemiological studies. Part of the training is the familiarization with the MetIDQ™ software is part of the kit. It guides through the registration of the plate layout, sample analysis and quantification right up to the assessment of data quality, data normalization and exports for statistical evaluations.

Perlan Technologies – Łukasz Nowicki

Targeted and non-targeted data mining techniques for exploring high resolution spectro-chromatographic data. Alignment, normalization and filtering the data for efficient compound identification in metabolomics research.

During the workshops the trainees will learn about untargeted and targeted techniques of spectro-chromatographic data mining using Agilent MassHunter Qualitative software. For untargeted data mining the trainees will learn about the Molecular Feature Extraction algorithm for the LC-QTOF high resolution data, and the deconvolution algorithm for the GC-MS data. The workshop participants will learn about the use of compound databases and libraries in targeted data mining workflows. With the Agilent Mass Profinder software the participants will learn about spectro-chromatographic data alignment and filtering. The main goal of the workshops is to learn about the ways to simplify screening workflows with HRAM Q-TOF MS or GC-MS with the use of the latest software tools from Agilent.

Waters – Isabel Riba

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Ion mobility MS/MS data processing in complex matrixes.

During the workshops, the trainees will learn how to use Vion system, automatically calibrated devices and one software platform to acquire and process high-resolution MS data that can be used in targeted or non-targeted metabolomics, proteomics and lipidomics. During the workshop, easy to use workflows will be presented based on a true experiment of samples extracted from strawberries and raspberries. An overview on Vion IMS spectrometer will be given, including IMS Q-TOF analyzer schematics, discussing Ion Mobility Spectrometry and the Collision Cross Section parameter, presenting MSe experiment and explaining Lock Mass Ion Source principles. Software UNIFI and its main functionalities will be introduced. Trainees will learn how to create analysis methods in UNIFI software, including natural products analysis, covering such aspects of features annotation as structure elucidation or possible fragmentation.

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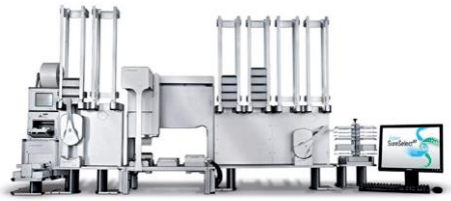
Najlepsze rozwiązania

Firma PERLAN Technologies Polska Sp. z o.o. rozpoczęła działalność w listopadzie 1999 roku pod nazwą Agilent Technologies Poland Sp. z o.o. w ramach realizacji strategicznego planu podziału firmy Hewlett Packard.

Od maja 2002 roku firma działa pod nazwą PERLAN Technologies Polska Sp. z o.o. i jest autoryzowanym dystrybutorem działu Life Science and Chemical Analysis firmy Agilent Technologies Inc.

Dostarczamy aparaty, akcesoria oraz kompletne rozwiązania umożliwiające odbiorcom identyfikację i prowadzenie badań tysięcy substancji, określając ich skład, budowę, właściwości fizyczne, chemiczne i biologiczne, analizękomórkową, metaboliczną oraz analizę DNA i RNA.

Oferujemy między innymi:



Rozwiązania umożliwiające automatyzację dowolnego procesu laboratoryjnego

Oferujemy rozwiązania do automatyzacji pracy laboratoryjnej począwszy od prostych instrumentów a skończywszy na kompletnych systemach wyręczających pracę ludzi. W wyniku połączenia innowacyjnej inżynierii i wysokich standardów jakości Agilent Technologies projektuje i dostosowuje urządzenia rewolucjonizujące badania biotechnologiczne, farmaceutyczne i genomiczne.

Zapytaj o ofertę — dowiedz się więcej >

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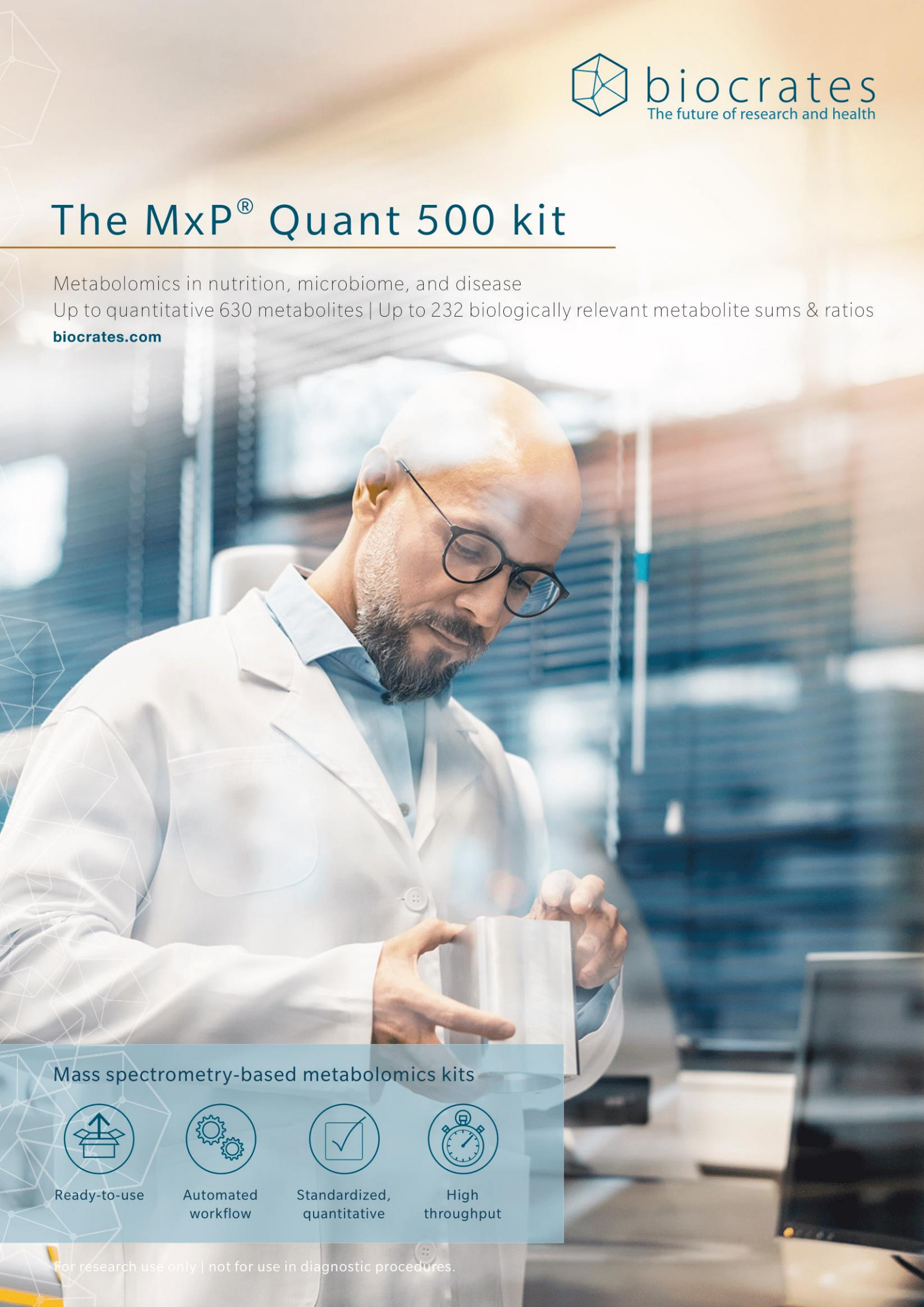

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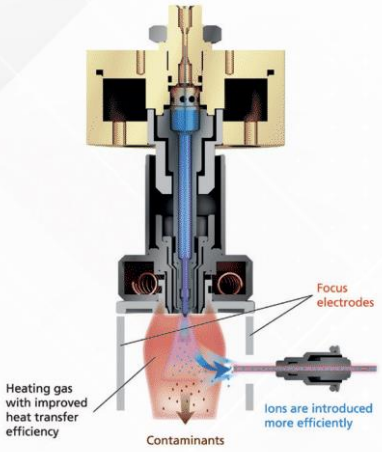
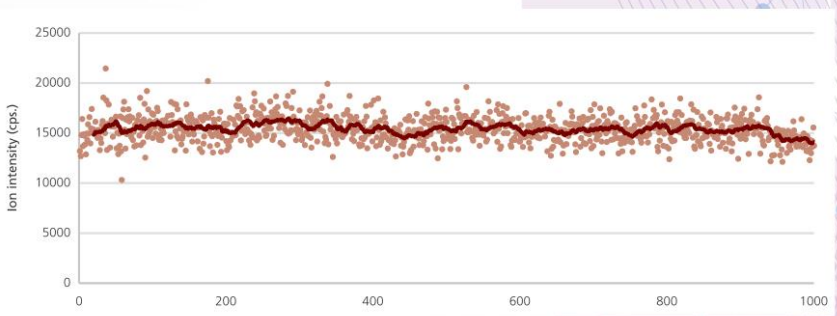
Zaawansowane oprogramowanie do analizy wieloskładnikowej LC-MS/MS i GC-MS/MS



Najnowsze źródło ESI z elektrodami ogniskującymi.



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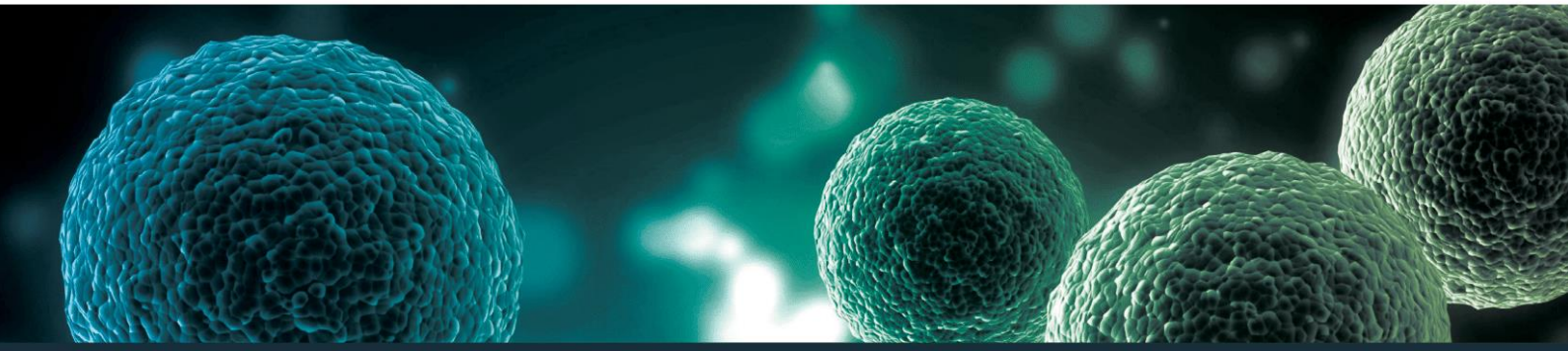

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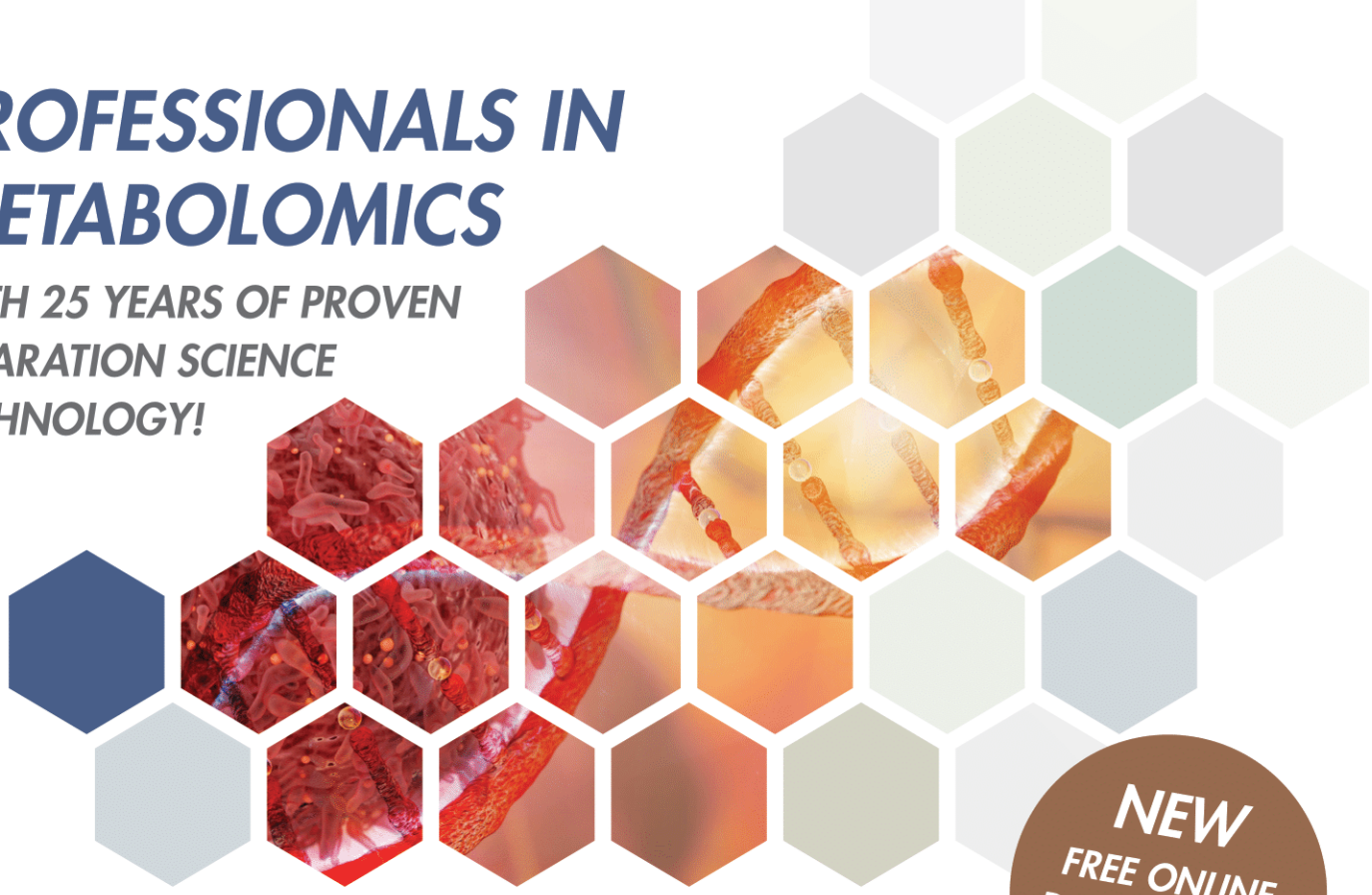

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Naszymi klientami są laboratoria przemysłowe, naukowe, medyczne, inspektoraty nadzoru, zakłady komunalnych (badanie wody i ścieków). Oferujemy szeroką gamę produktów dla laboratoriów fizykochemicznych i mikrobiologicznych, w tym odczynników, materiałów eksploatacyjnych, sprzętu i mebli laboratoryjnych renomowanych producentów, m.in.:

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Nasza oferta obejmuje:

- Projekty laboratoriów: przygotowanie projektu, fachowe doradztwo, meble laboratoryjne: stoły laboratoryjne i dygestoria, szafy bezpieczeństwa do przechowywania odczynników chemicznych i butli z gazem
- Urządzenia do dozowania i miareczkowania: pipety, biurety, titratory, dozowniki butelkowe
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- Chromatografia: chromatografy, generatory gazów, kolumny, strzykawki, filtry strzykawkowe, naczynka
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Zapewniamy doświadczony serwis, obsługę gwarancyjną i pogwarancyjną sprzętu laboratoryjnego a także specjalistyczne szkolenia naszych klientów.

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Firma Merck KGaA jest najstarszym na świecie koncernem biologiczno-chemicznym & farmaceutycznym, z ponad 300-letnią tradycją, założonym w 1668 roku w Niemczech w miejscowości Darmstadt. Firma zatrudnia ~50 000 pracowników, którzy reprezentują 129 różnych narodowości i pracują w oddziałach umiejscowionych w 66 krajach na całym świecie.

W 2010 roku Merck połączył się z firmą Millipore, a w 2015 z Sigma Aldrich. W rezultacie, czego powstał nowy dział "Life Science", który oferuje ~300 000 produktów pozwalających zwiększyć efektywność pracy laboratorium oraz zoptymalizować procesy produkcji.

Merck Sp. z o.o. i Sigma-Aldrich Sp. z o.o. reprezentują dział Life Science międzynarodowej grupy Merck KGaA w Polsce. Dostarczają na rynek polski wysokiej jakości produkty, kompleksowe rozwiązania, usługi serwisowe i zaplecze aplikacyjne dla każdego laboratorium badawczego, kontroli jakości oraz narzędzia do optymalizacji procesów produkcyjnych.

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