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editors



ABSTRACT BOOK

20th International Interdisciplinary Meeting on Bioanalysis
CECE 2026

Hotel Tatra**** Bratislava, Slovakia

21 – 23 October 2026



COMENIUS
UNIVERSITY
BRATISLAVA

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**20th International Interdisciplinary Meeting on Bioanalysis
CECE 2026**

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Abstract Book

2026
Comenius University Bratislava



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Preface

Dear colleagues,

It is a great pleasure to welcome you at the 20th International Interdisciplinary Meeting on Bioanalysis CECE 2026 in Bratislava, Slovakia.

Continuing the long and successful tradition of CECE conferences, this meeting serves as a unique platform bringing together scientists from diverse areas of bioanalytical sciences who may not frequently meet within the same scientific forum. The conference fosters interdisciplinary exchange of knowledge, stimulates new collaborations, and promotes innovation across a broad range of research fields.

The scientific program reflects the dynamic development of modern bioanalysis. The program features plenary and keynote lectures delivered by internationally recognized experts, oral presentations, and interactive poster sessions designed to encourage scientific discussion and networking. Particular attention is devoted to supporting early-career researchers through the CECE Young Scientists section, which provides PhD students and young scientists with an opportunity to present their work, develop communication skills, and potentially establish future scientific collaborations.

We are especially delighted that CECE is being held in Slovakia for the first time. Bratislava, situated at the crossroads of Central Europe, offers a unique blend of rich history, cultural heritage, and an environment for modern scientific development. We hope that both the scientific program and the social events will provide participants with valuable professional experiences as well as memorable opportunities to discover the atmosphere, traditions, and hospitality of Slovakia's capital city.

This abstract book presents the contributions of researchers from various scientific areas. We sincerely thank all authors, speakers, participants, sponsors, and members of the organizing and scientific committees for their contributions and support, which have made CECE 2026 possible.

We hope that the conference will inspire fruitful discussions, foster new collaborations, and contribute to further progress in the field of bioanalysis.

Welcome to CECE 2026, and we wish you a successful and enjoyable conference in Bratislava!

Katarína Maráková and Jana Lavická
chair and co-chair of the conference

Bratislava 21st October 2026

The Medal of Jaroslav Janák

Since 2014, the **Jaroslav Janák Award for the Development of Analytical Chemistry** has been awarded by the Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic. Named after the inventor of the gas chromatograph (patented in 1952), the founder of the institute (1956), and its long-term director, the medal is awarded to scientists who have significantly contributed to the development of separation sciences.

In **2026**, the **Medal of Jaroslav Janák** goes to **Dr. Marián Koval'**. Marián graduated from Comenius University in 1975 and earned his CSc. (PhD-equivalent) degree in 1982 with a dissertation entitled "*Isotachophoresis in Non-Aqueous Media*".

From 1981 to 1983, he worked at the Research Institute of Viticulture and Enology in Modra, focusing on the application of isotachophoresis (ITP) to wine analysis. In 1982, he joined the Institute of Radioecology and the Utilization of Nuclear Technology, later renamed Villa Labeco, in Spišská Nová Ves, as a senior research scientist. He played a major role in translating ITP technology into practical analytical instrumentation and in developing the first ZKI 01 ITP analyzer, which received the highest award at the international Incheba trade fair in 1983. In 1985–1986, he led an international team developing a new generation of ITP analyzers, resulting in the ZKI 02, which entered production in 1990. He subsequently played a leading role in the development and commercialization of successive generations of ITP analyzers, including fully automated systems. More than 500 of these instruments were manufactured, and are now used in dozens of countries worldwide. He is the author of several patents, inventor's certificates, industrial designs, and dozens of scientific papers in international peer-reviewed journals. He has presented at numerous international conferences and, through courses he organized or co-organized, has helped train hundreds of specialists in isotachophoresis. His career combines scientific expertise, technological innovation, and successful instrument development, with a lasting contribution to the advancement and worldwide application of isotachophoresis in analytical chemistry. I wish to congratulate Marián on his achievements and thank him for all his work for the analytical chemistry science and community.



Medal of Jaroslav Janák



Dr. Marián Koval'

Franta Foret

DAY 1 | 21ST OCT, 2026 | Wednesday

10:00-14:00		Registration
14:00-14:30		Conference opening Award of the Medal of Jaroslav Janak
Session 1 <i>Chair: Katarína Maráková</i>		
14:30-15:10	WP1	Cell-Type-Specific Response to Alpha-Synuclein Overexpression Reveal Divergent Post-Translational Mechanism Relevant to Parkinson's Disease <u>Dominika Fričová</u> <i>Comenius University Faculty of Medicine, Bratislava, Slovakia</i>
15:10-15:50	WP2	Beyond the Expert: Translating Capillary Electrophoresis innovation into robust, patient-centric pharmaceutical Quality Control <u>Cari Sängner - van de Griend</u> <i>Kantisto BV, Netherlands</i>
15:50-16:10		Coffee Break
Session 2 <i>Chair: František Foret</i>		
16:10-16:40	WK1	Characterization of Advanced Drug Delivery System Using Multimodal Liquid Chromatography <u>Kevin Schug</u> <i>University of Texas at Arlington, TX, USA</i>
16:40-17:10	WK2	Matrix Effects in LC-MS: Why the Evaluation Strategy Matters? <u>Lucie Nováková</u> <i>Charles University, Faculty Of Pharmacy in Hradec Králové</i>
17:10-17:30	WO1	LC-MS method development from the perspective of TDM-focused laboratory <u>Martin Kertys</u> <i>Comenius University Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia</i>
17:30-17:50	WO2	Singlet Oxygen as a Reactive Footprinting Probe in Structural Mass Spectrometry <u>Michael Volný</u> <i>University of Chemistry and Technology, Department of Analytical Chemistry, Prague, Czechia</i>

17:50-18:00	Sponsor talk	Hermes LabSystems – Sponsor talk <u>Ján Hrouzek</u> <i>Hermes LabSystems s.r.o, Bratislava, Slovakia</i>
18:00-20:00	Welcome reception Poster session	

DAY 2 | 22ND OCT, 2026| Thursday

8:00-8:30	Registration
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Session 3

Chair: Cari Sängér - van de Griend

08:30-09:00	ThK1	Capillary Gel Electrophoresis: Past, Present and Future <u>András Guttman</u> <i>University of Debrecen, Faculty of Medicine, Horváth Csaba Memorial Laboratory of Bioseparation Sciences, Center for Translational Medicine, Hungary</i>
09:00-09:30	ThK2	Enabling Low-Input Proteomics Using Orthogonal Nanoflow Liquid Phase Separation Techniques Coupled to Mass Spectrometry <u>Michal Greguš</u> <i>Northeastern University, Barnett Institute of Chemical and Biological Analysis, Department of Chemistry and Chemical Biology, Boston, MA, USA</i>
09:30-09:50	ThO1	Smart Samplers for LC–MS: Advancing Protein Bioanalysis <u>Trine Grønhaug Halvorsen</u> <i>University of Oslo, Department of Pharmacy, Oslo, Norway</i>
09:50-10:10	ThO2	Phytochemical and Biological Potential of Selected <i>Astragalus</i> Species in the Context of Modern Cosmetic and Pharmaceutical Applications <u>Alina Plenis</u> <i>Medical University of Gdansk, Department of Analytical Chemistry, Gdańsk, Poland</i>
10:10-10:30	ThO3	Automated Long-Term Monitoring for Permeation and Toxicology Studies <u>Hana Sklenářová</u> <i>Charles University, Faculty of Pharmacy in Hradec Králové, Czech Republic</i>
10:30-10:50	Coffee Break	

Session 4

Chair: Jana Havlíková

10:50-11:20	ThK3	Towards Precision Medicine through Minimally Invasive Bioanalysis <u>Tomasz Bączek</u> <i>Medical University of Gdańsk, Department of Pharmaceutical Chemistry, Gdańsk, Poland</i>
11:20-11:40	ThO4	Deep-UV Laser Ablation as a Versatile Tool for Ambient Mass Spectrometry <u>Filip Gregar</u> <i>Palacký University, Faculty of Science, Department of Analytical Chemistry, Olomouc, Czech Republic</i>
11:40-12:00	ThO5	Hi-Fidelity N-Glycan Profiling by Capillary Electrophoresis <u>Gabor Jarvas</u> <i>University of Pannonia, Veszprém, Hungary</i>
12:00-12:20	ThO6	Enhanced Capillary Electrophoretic Analysis of Tryptophan Pathway Metabolites through Optimized Sample Preparation and Fluorescence Detection <u>Ilona Olędzka</u> <i>Medical University of Gdańsk, Department of Pharmaceutical Chemistry, Gdańsk, Poland</i>
12:20-12:30	Sponsor talk	Supercritical Fluids in Bioanalysis: From Online Extraction to Chiral SFC-MS/MS on a Single Platform <u>Richard Hodoši</u> <i>Shimadzu Slovakia, Bratislava, Slovakia</i>
12:30-13:30	Lunch	

Session 5

Chair: Kevin Schug

13:30-13:45	YS1	Size-Exclusion Chromatography as a Tool for Simultaneous Isolation and Protein Corona Modulation of Extracellular Vesicles <u>Ondrej Moravek</u> <i>University of Pardubice, Pardubice, Czechia</i>
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13:45-14:00	YS2	LA-ICP-MS: An Effective Tool for Studying Metal Distribution in Cells <u>Laura Tesárová</u> <i>Comenius University Bratislava, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Bratislava, Slovakia</i>
14:00-14:15	YS3	Comorbidity-Associated N-Glycome Profile Shifts: A Preliminary Study Evaluating COPD and Diabetes in Lung Cancer Patients <u>Beatrix Kiss</u> <i>University of Pannonia, Research Institute of Biomolecular and Chemical Engineering, Veszprém, Hungary</i>
14:15-14:30	YS4	The Potential of Saliva as a Sample Matrix for Therapeutic Drug Monitoring <u>Nela Žideková</u> <i>Comenius University Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia</i>
14:30-14:45	YS5	Comparative N-Glycan Profiling of Urinary and Recombinant Human Chorionic Gonadotropin by CZE-LEDIF and HILIC-HRMS <u>Chi-Phong LY</u> <i>University of Paris-Saclay, Institut Galien Paris-Saclay, France</i>
14:45-15:00	YS6	Development of CE-LIF Approaches for Sugar and Oligosaccharide Profiling in Food and Biological Samples <u>Joanna Bulesowska</u> <i>Medical University of Gdańsk, Department of Pharmaceutical Chemistry, Gdańsk, Poland</i>
15:00-15:20	Coffee break	
Session 6 Chair: Vladimír Havlíček		
15:20-15:35	YS7	Charting the Brain Endocannabinoid Landscape by UHPLC-ESI-MS/MS <u>Giulia Bottai</u> <i>University of Modena and Reggio Emilia, Department of Life Sciences, Modena, Italy</i>
15:35-15:50	YS8	Flexible Multi-Criteria Gradient Scoring for Targeted LC-MS/MS Method Optimization <u>Michal Kašpar</u> <i>University of Pardubice, Czech Republic</i>

15:50-16:05	YS9	Rapid Voltammetric Analysis of Pharmaceutical Samples <u>Marek Vaško</u> <i>Comenius University Bratislava, Faculty of Sciences, Department of Analytical Chemistry, Bratislava, Slovakia</i>
16:05-16:20	YS10	Urinary PSA N-Glycan Profiling with Capillary Electrophoresis for Prostate Cancer Detection <u>Ákos Szegedi</u> <i>University of Debrecen, Faculty of Medicine, Horváth Csaba Memorial Laboratory of Bioseparation Sciences, Research Center for Translational Medicine, Debrecen, Hungary</i>
16:20-16:35	YS11	Sensor-Integrated Body-on-a-Chip Platform Technology for Modern, Animal-Free Toxicology <u>Mateo Vasconez Martinez</u> <i>Technical University of Vienna, Cell Chip Group, Vienna, Austria</i>
19:00-22:00	Dinner DOCK7 <i>(Pribinova 32, 821 09 Bratislava)</i>	

DAY 3 | 23RD OCT, 2026 | Friday

Session 7

Chair: Michal Greguš

09:00-09:30	FK1	High-Resolution Mass Spectrometry for Identifying Novel Bioactive Pollutants in Water <u>Andrea Vojs-Staňová</u> <i>Comenius University Bratislava, Faculty of Natural Sciences, Department of Analytical Chemistry, Bratislava, Slovakia</i>
09:30-10:00	FK2	When Antibiotics Are Not Enough: Resistance and Persistence in Obligate Intracellular Pathogen <i>Coxiella burnetii</i> <u>Ľudovít Škultéty</u> <i>Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia</i>
10:00-10:20	FO1	Exploring the Analytical Landscape of Bioactive Endogenous Compounds: The <i>p</i>-Cresol Paradigm <u>Federica Pellati</u> <i>University of Modena and Reggio Emilia, Department of Life Sciences, Modena, Italy</i>
10:20-10:40	FO2	Library-Driven Automated Selection of Fragment Ions and Collision Energies for Quantitative Mass Spectrometry <u>Gergő Bodnár</u> <i>Thermo Fisher Scientific; Bratislava, Slovakia</i>

		Application of Gas-Phase Electrophoresis (nES GEMMA Instrumentation) in Exhaled Breath Condensate (EBC) Characterization
10:40-11:00	FO3	<u>Victor Weiss</u> <i>Technical University of Vienna, Institute of Chemical Technologies and Analytics, Vienna, Austria</i>
11:00-11:20	Coffee break	
Session 8 <i>Chair: Jana Lavická</i>		
11:20-11:50	FK3	Epitachophoresis – from Concept to Commercialization <u>František Foret</u> <i>Czech Academy of Sciences, Institute of Analytical Chemistry, Bioanalytical Instrumentation, Brno, Czech Republic</i>
11:50-12:30	FP1	Noninvasive Diagnosis of Invasive Infectious Diseases <u>Vladimír Havlíček</u> <i>Czech Academy of Sciences, Institute of Microbiology, Prague, Czechia</i>
12:30-12:50	Conference closing Prize announcement	
13:00-14:00	Lunch	

Plenary lectures

CELL-TYPE-SPECIFIC RESPONSES TO ALPHA-SYNUCLEIN OVEREXPRESSION REVEAL DIVERGENT POST-TRANSLATIONAL MECHANISMS RELEVANT TO PARKINSON'S DISEASE

Miraj Ud Din Momand¹, Petra Majerova¹, Diana Mjartinova², Natalia Maruskinova²,
Karolina Albertusova¹, Michael Dobrota², Lubica Fialova¹, Sara Stefankova⁴,
Petar Podlesniy⁵, Muhammad Khalid Muhammadi¹, Miroslav Balaz^{6,7},
Dominika Fricova^{1,2,3*}

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³ Department of Neurology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia

⁴ International College of Cardiology, Košice, Slovakia

⁵ Institute of Biomedical Research of Barcelona (IIBB-CSIC), Barcelona, Spain

⁶ Laboratory of Cellular and Molecular Metabolism, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

⁷ Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

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Alpha-synuclein (α -syn) overexpression is widely used to model early pathogenic mechanisms in Parkinson's disease (PD); however, the role of cellular context in determining α -syn toxicity remains insufficiently understood. We generated stable human neuronal models expressing GFP-tagged wild-type (WT) or A53T mutant α -syn at low and high expression levels in SH-SY5Y neuroblastoma cells and ReNcell VM ventral midbrain neural progenitor cells. We evaluated cellular viability, oxidative stress, mitochondrial morphology and bioenergetics, apoptosis-associated markers, intracellular α -syn distribution, and post-translational modifications. A53T α -syn induced a consistent toxic phenotype in both cellular models, characterized by increased oxidative stress, mitochondrial dysfunction, impaired respiratory capacity, and activation of early apoptotic markers, particularly at high expression levels. In contrast, WT α -syn produced divergent, cell-type-specific effects. In SH-SY5Y cells, WT α -syn increased metabolic activity and enhanced basal and maximal mitochondrial respiration, suggesting an adaptive metabolic response. Conversely, ReNcell VM cells displayed elevated oxidative stress and reduced mitochondrial metabolic activity, basal respiration, maximal respiration, and spare respiratory capacity following WT α -syn overexpression. A53T α -syn exhibited a punctate, aggregate-like intracellular distribution and increased serine-129 phosphorylation, whereas WT α -syn remained predominantly diffuse. Notably, ReNcell VM cells showed a more prominent and diverse post-translational modification profile of α -syn, even in the absence of overt WT α -syn aggregation. These findings demonstrate that α -syn-induced neuronal dysfunction is shaped not only by mutation status and expression level, but also by the intrinsic properties of the cellular environment. This dual-cell platform may facilitate investigation of early, cell-type-specific mechanisms of α -syn pathology and support the development of targeted therapeutic approaches for PD.

Acknowledgement

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Momand, M. U. D. et al. Alpha-Synuclein Overexpression Triggers Divergent Cellular Responses and Post-Translational Modifications in SH-SY5Y and ReNcell VM Models. *Cell. Mol. Life Sci.* 2026, 83 (1), 123.

WP2

Beyond the Expert: Translating Capillary Electrophoresis innovation into robust, patient-centric pharmaceutical Quality Control

Cari E. Sanger – van de Griend^{1,2,3*}, Ewoud van Tricht^{1,3} and Jakob Haglof²

¹*Kantisto B.V., the Netherlands*

²*Uppsala Universitet, Sweden*

³*BioQC, United Kingdom*

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The application of capillary electrophoresis technologies in the (bio)pharmaceutical industry continues to expand. However, successful implementation in a manufacturing environment, where methods must be operated beyond a small group of technology experts, requires a strong commitment to best practices and Analytical Quality by Design (AQbD). At the same time, simplicity and practicality should be maintained through the KISS (Keep It Simple and Straightforward) principle. This lecture will highlight recent developments, discuss key considerations in method and technology development for (bio)pharmaceutical quality control, and emphasize the ultimate goal of ensuring patient safety and product quality.

Noninvasive diagnosis of invasive infectious diseases

Vladimír Havlíček*

Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

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Invasive infection diagnostics poses a bioanalytical problem: informative molecules originate in poorly accessible tissues, whereas clinically useful measurements should preferably be made in serially obtainable biofluids. We combine chromatographic mass spectrometry, high-resolution imaging, elemental analysis and immunoassays to translate pathogen chemistry into fluid-phase biomarkers.

In a prospective study of 148 high-risk patients with haematological malignancies (37 proven/probable invasive aspergillosis, 111 controls), urinary triacetylfusarinine C normalized to creatinine (uTafC/Cr) was quantified by targeted liquid chromatography-mass spectrometry (LC-MS), while serum galactomannan and pentraxin-3 were measured by enzyme immunoassays. uTafC/Cr and galactomannan each detected 51.4% of cases without false positives in available controls; their combination increased sensitivity to 67.6%, and addition of pentraxin-3 increased case detection to 83.8% with lower specificity.

We then examined why biomarker timing matters. High-performance LC coupled to Fourier-transform ion cyclotron resonance MS resolved gliotoxin, bis(methylthio)gliotoxin and higher sulfur congeners across zinc-controlled *Aspergillus fumigatus* cultures, while RT-qPCR/RNA sequencing characterized the effect of AfuPmV-1 and inductively coupled plasma MS quantified zinc in endotracheal aspirates. The data support gliotoxin as a late, zinc-sensitive, ecology-dependent marker.

The same analytical logic extends across pathogens. In experimental pulmonary mucormycosis, high-resolution MALDI mass spectrometry imaging localized rhizoferrin and homorhizoferrin to infected lung foci; targeted, matrix-matched LC-MS transferred these tissue signals into serial urinary measurements from day 2, with rhizoferrin also detected in human bronchoalveolar lavage. In experimental *Escherichia coli* meningoencephalitis, MALDI imaging mapped aerobactin and salmochelin-related metabolites together with host metal-sequestration proteins, while corresponding bacterial siderophores and host antimicrobial markers were recovered from cerebrospinal fluid.

Together, these studies illustrate a workflow from spatial discovery and high-resolution structural assignment to targeted biofluid quantitation and orthogonal host-response markers for monitoring invasive infection.

Acknowledgement

I thank all co-authors and clinical collaborators who contributed to the studies summarised here.

Keynote lectures

CHARACTERIZATION OF ADVANCED DRUG DELIVERY SYSTEMS USING MULTIMODAL LIQUID CHROMATOGRAPHY

Kevin A. Schug^{1*}, Brady Drennan¹, Phat Tien Dinh¹ and Rebecca Taylor¹

¹*Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington TX, USA*

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Contemporary drug delivery systems for pharmaceutical therapeutics are significantly more sophisticated than traditional small molecule drugs. They require a multitude of tests to establish critical quality attributes, and this need is time- and resource-intensive. To address this need, we are developing a multimodal liquid chromatography system that aims to be versatile in terms of reducing the number of analyses needed to characterize different drug delivery systems. The analytical system comprises a trapping column, which can be used to segregate different drug components. Components that pass through the trap are directed to one set of analytical column(s) and detector(s). Trapped components can then be back eluted to another set of column(s) and detector(s). The choices of trap column, analytical columns, and detectors are variable depending on the goals of the analysis. To characterize the degradation of an extended-release polymeric drug delivery system, a restricted access media trap column has been used to pass high molecular species for subsequent size exclusion analysis and then send trapped species for reversed-phase separation (1). The hyaluronic acid polymer conjugated to a protein ophthalmic drug was monitored under alkaline and enzyme degradation conditions over time to characterize the release of different drug system components. The multimodal liquid chromatography system has most recently been extended to characterization of lipid nanoparticles (LNPs) and RNA-containing LNPs. For this application, a spongy monolith modified with polyethyleneimine was developed to pass and partially separate LNPs (2). The primary goal of this work is to develop a platform for direct characterization of encapsulation efficiency. Unencapsulated components will be trapped on the modified spongy monolith, whereas LNPs pass through and are captured on a reversed-phase separation system to break the LNPs and determine payload and lipid components. This talk will provide an overview of multimodal liquid chromatography and its development for determining different critical quality attributes in different advanced drug delivery systems.

Acknowledgement

The authors would like to thank Genentech for support in the development and testing of multimodal liquid chromatography, which includes providing the polymeric drug delivery system for investigation.

References

- (1) Drennan, B. et al. Characterization of hydrogel drug delivery system stability using column-switching multimodal liquid chromatography – mass spectrometry. *Anal. Chem.* 2025, 97, 21827-21834.
- (2) Drennan, B.W. et al. Ion Exclusion Chromatography of RNA–Lipid Nanoparticles using Polyethyleneimine-Modified Spongy Monoliths. *Anal. Chim. Acta* 2025, 1383, 344852.

MATRIX EFFECTS IN LC-MS: WHY DOES THE EVALUATION STRATEGY MATTER?

Lucie Nováková^{1*}, Hana Kočová Vlčková¹, Kateřina Plachká¹, Frantisek Švec¹

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Matrix effects (ME) remain a major challenge in quantitative LC-MS analysis of complex samples, and their evaluation is an essential part of method development and validation. Two quantitative strategies are most widely used: the post-extraction addition approach, recommended by the EMA/ICH guideline and considered the reference method, and the comparison of standard and matrix calibration curve slopes. However, whether these approaches provide comparable results had not been systematically assessed.

Both strategies were compared for 26 compounds in serum after protein precipitation using UHPLC-ESI-MS/MS in positive and negative ionization modes. Because the slope-based approach inherently depends on the regression model, unweighted, 1/X- and 1/X²-weighted linear regression, as well as logarithmic transformation, were evaluated. The choice of calibration model markedly affected ME estimation, particularly for compounds with ME above 20%. While none of the models overestimated ME compared with post-extraction addition, the slope-based approach frequently underestimated it, in some cases by hundreds of percent. This discrepancy arises because calibration curve slopes reflect only concentration-dependent rotational ME, whereas concentration-independent translational ME shifts the calibration curve intercept.

Therefore, a new equation was proposed to calculate translational ME from the difference between standard and matrix calibration curve intercepts, related to the response at the LLOQ. The total ME, obtained as the sum of slope- and intercept-derived contributions, closely matched the reference values. The applicability and robustness of the proposed approach were further demonstrated using an independent set of compounds in plasma, urine, and apple juice and two UHPLC-MS/MS platforms from different manufacturers. Differences from the reference values were predominantly within $\pm 20\%$, irrespective of matrix type, instrumental platform, or weighting scheme. The proposed equation is currently not applicable to logarithmic calibration models.

These findings demonstrate that neglecting translational ME can lead to substantial underestimation of matrix effects and potentially misleading conclusions during method validation.

Acknowledgement

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- (1) Kočová Vlčková, H. et al. Comprehensive Investigation of Matrix Effect Evaluation Approaches for Reliable Quantification in Bioanalysis Using UHPLC-MS/MS. *Anal Chim Acta* 2026, 1413, 345642.

CAPILLARY GEL ELECTROPHORESIS: PAST, PRESENT AND FUTURE

Andras Guttman^{1,2*}

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Capillary gel electrophoresis (CGE) remains a powerful platform for the high-resolution separation of nucleic acids, SDS–protein complexes and complex carbohydrates, offering rapid analysis, automation, high efficiency, and excellent reproducibility. These strengths continue to support its use in genomics, proteomics, glycomics, biopharmaceutical quality control, and clinical diagnostics. Recent advances in capillary coatings, polymer matrices, and detection technologies are expanding CGE beyond traditional DNA sequencing and monoclonal antibody analysis. Emerging applications include characterizing multispecific antibodies, fusion proteins, viral capsid proteins, large DNA vectors, mRNA lipid nanoparticles, and vaccine plasmid topology. In parallel, coupling capillary electrophoresis with mass spectrometry is creating new opportunities in proteomics, glycomics, lipidomics, and metabolomics. Despite competition from next-generation sequencing and liquid chromatography, CGE retains distinct advantages in cost-effectiveness, sizing precision, analytical robustness, and its green chemistry feature. Future developments are expected to center on advanced separation concepts including capillary gradient gel electrophoresis, multicapillary platforms, lab-on-a-chip technologies, and point-of-care applications. This presentation highlights past endeavors, the current state, emerging applications, and future trajectory of CGE, demonstrating its continued relevance as a versatile technology in modern analytical and bioseparation science.

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- (1) Guttman, A. et al. Capillary Gel Electrophoresis 2022, Elsevier, Amsterdam, The Netherlands, ISBN: 978-0-444-52234-4.

ENABLING LOW-INPUT PROTEOMICS USING ORTHOGONAL NANOFLOW LIQUID PHASE SEPARATION TECHNIQUES COUPLED TO MASS SPECTROMETRY

Michal Gregus^{1*}, Thao Tran¹, Yunfan Gao¹, Noah Gould¹, Anne-Lise Marie¹ and Alexander R. Ivanov¹

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Despite major improvements in sampling techniques and mass spectrometry (MS) instrumentation, omics profiling (e.g., proteomics and glycomics) of amount-limited clinically and biomedically relevant samples, such as single cells, organelles, rare cell isolates, microneedle tissue biopsies, and liquid microbiopsies, remains a major analytical challenge. Standard proteomic and glycomic methods, developed for substantially larger sample volumes and higher protein and glycan amounts, do not transfer directly to low-input samples at the single-cell, pg-, ng-, and low-μg-scale.

High-sensitivity omics workflows pose technical challenges at each single step, from sample collection and preparation to data acquisition and analysis. This presentation will illustrate breakthrough MS-based analytical workflows for robust and sensitive analysis of extremely low-input biological samples, with an emphasis on ultra-low flow (ULF) liquid-phase separation techniques, such as liquid chromatography (LC) and capillary electrophoresis (CE), coupled to high-resolution MS.

Our ULF LC-based platform utilizing in-house prepared ultra-narrow-bore capillary columns, including monolithic and porous layer open tubular (PLOT) columns (5–50 μm internal diameters) operating at flow rates ≤50 nL/min, will be described. In bottom-up proteomic (BUP) analysis, monolithic columns enabled identification of $2,348 \pm 42$ protein groups and $10,062 \pm 285$ peptide groups for 1 ng of a HeLa digest, using a 1 h gradient at 12 nL/min (Orbitrap Fusion Lumos)¹. Pilot top-down proteomic (TDP) analysis of six HeLa cells using PLOT columns yielded, on average, 29 ± 3 proteins and 38 ± 2 proteoforms per run, and 44 proteins and 68 proteoforms in total².

CE-based separations hyphenated with MS will also be presented, covering the analysis of peptides, released glycans, and intact proteins. By processing intact cells directly inside the separation capillary, online with CE-MS/MS, we eliminated sample handling and transfer steps. We applied this workflow for TDP and glycomic analysis of low numbers of mammalian cancer cells (<10) and single cells^{3,4}.

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TOWARDS PRECISION MEDICINE THROUGH MINIMALLY INVASIVE BIOANALYSIS

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Recent advances in bioanalysis increasingly emphasize analytical strategies capable of obtaining biologically meaningful information while minimizing disturbance of investigated systems. This lecture presents two complementary research pathways connected by a common objective: translating minimally invasive sampling into molecular phenotyping and biomedical applications.

The first pathway focuses on biocompatible solid-phase microextraction (SPME) as a microsampling tool for *in vivo* and *ex vivo* investigations. Applications include direct monitoring of phytocannabinoids in living *Cannabis* plants and region-specific profiling of endocannabinoids in selected brain structures. These studies demonstrated that microsampling can provide physiologically relevant molecular information while reducing sample preparation, solvent consumption, and disruption of biological systems.

The second pathway addresses the development of non-invasive bioanalytical platforms based on exhaled breath condensate (EBC). Highly sensitive LC-MS/MS methodologies for amino acid profiling, supported by national, international, and European patents, enabled targeted metabolic phenotyping in human studies. The development of a dedicated EBC collection system with active temperature control and standardized sampling conditions established a technological foundation for reproducible clinical investigations. These platforms were subsequently applied in paediatric leukaemia, obstructive sleep apnoea and bariatric surgery-related metabolic studies.

Although focused on chemically distinct classes of analytes, both research directions share a common philosophy: obtaining clinically relevant molecular information using minimally invasive or non-invasive sampling strategies. Together, they illustrate a transition from monitoring selected bioactive compounds in living systems toward targeted molecular phenotyping across multiple biological matrices, providing new opportunities for translational bioanalysis, precision diagnostics, and personalized medicine.

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HIGH-RESOLUTION MASS SPECTROMETRY FOR IDENTIFYING NOVEL BIOACTIVE POLLUTANTS IN WATER

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Environmental waters contain complex mixtures of anthropogenic chemicals, including pharmaceuticals, industrial compounds, pesticides, metabolites, and numerous substances of unknown identity. Their composition is continuously modified by natural processes as well as by water and wastewater treatment. Biological treatment, ozonation, photochemical and other advanced treatment processes can efficiently remove parent contaminants, but may simultaneously generate degradation and transformation products with different persistence, mobility, toxicity, and biological activity. Consequently, disappearance of the parent compound does not necessarily correspond to elimination of environmental risk. Newly formed products may retain biological activity or, in some cases, exhibit greater toxicological relevance than their precursors. High-resolution mass spectrometry (HRMS), combined with targeted analysis, suspect screening, and non-target analysis, provides a powerful platform for detecting and structurally characterizing known contaminants, metabolites, and previously unrecognized transformation products. A major challenge remains the prioritization of environmentally and biologically relevant compounds among thousands of detected features. Effect-directed analysis can address this limitation by integrating chemical analysis with bioassays and chromatographic fractionation. Selective affinity-based approaches, such as pull-down assays, can further enrich compounds interacting with specific biological targets, including receptors and transport proteins. The integration of passive sampling, effect-based testing, fractionation, affinity enrichment, and LC–HRMS therefore provides a comprehensive strategy for linking chemical occurrence with biological effects. Such workflows extend water-quality assessment beyond conventional target monitoring and parent-compound removal, supporting the identification of emerging bioactive pollutants and a more complete evaluation of the chemical and biological safety of water-treatment processes.

Acknowledgement

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When antibiotics are not enough: Resistance and persistence in obligate intracellular pathogen *Coxiella burnetii*

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Antimicrobial resistance represents an increasing One Health challenge, linking human and veterinary medicine with environmental reservoirs of infection. However, treatment failure cannot always be explained by classical, genetically encoded antibiotic resistance. This is particularly relevant for obligate intracellular pathogens, whose specialized intracellular lifestyle, slow growth and ability to enter metabolically restricted states can markedly reduce antimicrobial efficacy.

Coxiella burnetii, the causative agent of Q fever, provides an illustrative example. Doxycycline remains the cornerstone of treatment, yet eradication of the organism may be difficult, particularly during persistent infection requiring prolonged therapy. Our studies indicate that exposure of *C. burnetii* to doxycycline induces a coordinated physiological response that enables bacterial survival without necessarily requiring conventional resistance mechanisms. Under doxycycline stress, bacterial replication is strongly inhibited but viable organisms remain capable of resuming growth after removal of the antibiotic. Ultrastructural analysis further demonstrates a shift from the metabolically active large-cell variant toward the smaller, environmentally resistant small-cell variant.

Proteomic analysis revealed extensive remodeling of the bacterial proteome during doxycycline exposure. Particularly affected were proteins associated with protein synthesis, stress responses, maintenance of cell-envelope integrity, protein translocation, pH homeostasis and detoxification of oxidative stress. Increased abundance of these proteins suggests that *C. burnetii* responds to antibiotic pressure by reducing metabolic activity while simultaneously reinforcing cellular protection and damage-control mechanisms. Among these responses, the envelope-associated proteins DegP and TolB emerge as particularly interesting candidates for therapeutic targeting.

These observations highlight an important distinction between antimicrobial resistance and persistence. Successful treatment of intracellular bacterial infections may therefore require approaches that target not only essential bacterial processes but also the mechanisms that permit survival during antibiotic exposure. Combination therapies, pathogen-specific targets, photodynamic antimicrobial strategies and vaccination all offer complementary possibilities. Understanding bacterial persistence may ultimately be as important as discovering new antibiotics for improving the control of Q fever and other intracellular infections.

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EPITACHOPHORESIS – FROM CONCEPT TO COMMERCIALIZATION

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Most bioanalytical workflows begin with a critical step: efficient sample pre-separation. Among the available approaches, non-affinity electrophoretic methods offer a powerful strategy that exploits differences in electromigration to selectively separate and enrich target analytes from complex biological matrices. Building on this principle, we recently introduced epitachophoresis (ETP) as a promising electrophoretic technique capable of concentrating and separating nucleic acids directly from milliliter-scale samples. The ETP arrangement enables exceptionally high, virtually unlimited concentration factors.

Our work systematically evaluates key experimental parameters, including the size, shape, and composition of zone-stabilizing media, as well as device architectures optimized for handling large sample volumes. Traditional slab gel electrophoresis commonly employs polyacrylamide or agarose gels as sieving and stabilizing matrices. While effective, these materials inherently restrict the size of nucleic acids that can be concentrated. To overcome these limitations, we explored large-pore-stabilization materials and leveraged advanced 3D printing technologies to fabricate rigid stabilizing manifolds that suppress liquid flow during epitachophoresis. A variety of 3D-printed stabilizing manifold geometries were evaluated to support analyte focusing in both anionic and cationic modes. Remarkably, depending on device geometry, the system can concentrate ionic components from sample volumes ranging from 1 to 50 mL into a collection volume of 150 μ L or less. The concentration process is rapid, typically requiring only a few minutes up to one hour, depending on the stabilization medium and applied electrical power.

Using this platform, we successfully demonstrated single-run isolation of DNA and RNA from biologically relevant samples, including challenging materials such as formalin-fixed paraffin-embedded (FFPE) tissues. Although the system was initially designed for nucleic acid extraction and focusing, our results highlight the broader analytical potential of ETP. In particular, this presentation will explore its application to the separation and enrichment of additional biomolecules, including peptides and proteins, opening new possibilities for high-performance bioanalytical sample preparation. In this communication, we present the development of the novel instrumentation, from the device concept to a commercial system for processing crude samples using discontinuous electrophoresis in a circular configuration.

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Orals and Young Scientist Talks

LC-MS METHOD DEVELOPMENT FROM THE PERSPECTIVE OF A TDM-FOCUSED LABORATORY

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Liquid chromatography-mass spectrometry (LC-MS) has become a cornerstone analytical platform for the quantification of drugs, metabolites, and biomarkers in complex biological matrices. Successful LC-MS method development requires a systematic strategy that balances analytical sensitivity, selectivity, robustness, and operational practicality. In therapeutic drug monitoring (TDM) and clinical laboratories, additional challenges include high sample throughput, short turnaround times, workflow automation, and sustained instrument performance. Well-designed LC-MS methods not only fulfil regulatory and quality requirements but also generate reliable data that support clinical decision-making, biomedical research, and the advancement of personalised medicine. Continuous method optimisation remains essential to ensure long-term performance and adaptability to evolving analytical needs.

N-Lactoyl amino acids (Lac-AAs) have recently emerged as biologically relevant metabolites implicated in metabolic regulation, exercise physiology, mitochondrial dysfunction, diabetes, and other pathological states. Nevertheless, progress in this field has been hindered by the limited availability of sensitive and validated targeted analytical methods for their quantification in biological samples.

Here, we describe the development and full validation of a simple, rapid, and robust LC-MS/MS method for the simultaneous quantification of five Lac-AAs (*N*-lactoyl-tyrosine, *N*-lactoyl-valine, *N*-lactoyl-leucine, *N*-lactoyl-phenylalanine, and *N*-lactoyl-tryptophan) in human plasma. The method incorporates several innovative analytical features, including baseline chromatographic resolution of the isomeric pair *N*-lactoyl-leucine and *N*-lactoyl-isoleucine, preparation of a surrogate plasma matrix using solid-phase extraction, and straightforward enzymatic synthesis of deuterium-labelled internal standards. Together, these advances provide a practical and reliable analytical tool for investigating the physiological and pathological roles of Lac-AAs in clinical and translational research.

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Singlet oxygen as a reactive footprinting probe in structural mass spectrometry

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Protein footprinting is a powerful tool for structural characterization of proteins and their complexes that examines conformational changes by determining which parts of a protein are solvent-accessible or involved in interactions, by introducing stable, site-specific covalent modifications and then identifying their positions. By comparing the footprint of a protein in different states, changes in the protein topography can be detected and interpreted, often in the context of other structural information. The protein footprint is created by modifying either backbone or side chains of the protein by a suitable chemical probe^{1,2}. Here we demonstrate new method that allows probing protein structure by exposing protein molecule to singlet oxygen, a known reactive oxygen species, which results in fast oxidative modifications³. In our experiments, the singlet oxygen was generated *in-situ* under controlled conditions using photodynamic effect. To perform the experiments, we have built a mixed-flow apparatus, which operated as follows: Two lines (solution of the analysed molecule and a photosensitizer solution) were pumped using syringe pumps and mixed in a T-junction, flow of mixed solutions continued into an optical flow-cell where it was irradiated by a 650nm laser. Singlet oxygen was formed and let to immediately react with the peptide or protein. The reaction was quenched and the flow of oxidized products was collected for subsequent mass spectrometry analysis. The method was evaluated using several model peptides/proteins (e.g., angiotensin, myoglobin...). Amino acid residues susceptible to oxidation by singlet oxygen were identified, and the reaction mechanism and conditions were investigated. The effects of pH and composition of the reaction buffer were also examined. During method development, unusual or previously unreported oxidative modifications were observed. The results demonstrate that singlet oxygen labelling yields information that correlates with that obtained by established covalent labelling methods and can report on structural features and their changes in proteins.

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SMART SAMPLERS FOR LC–MS: ADVANCING PROTEIN BIOANALYSIS

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Dried blood spot (DBS) sampling has gained a large interest in bioanalysis. In this approach a small volume (< 50 µL) of biological sample is collected on a piece of filter paper, before the sample is dried and sent to the lab for analysis. The simplicity makes the technique suitable in in patient-centric settings where the patient or caretakers can collect samples at home.

DBS can be used for sampling of a variety of analytes. It is the standard sampling method in screening of inborn errors in newborns as well as an accepted sampling method in doping analysis. The focus in this presentation will be on determination of proteins from dried biological samples using liquid chromatography tandem mass spectrometry (LC-MS/MS). This field is still in its infancy and extensive and time-consuming sample preparation is often required especially when determining low abundance protein markers. The essential sample preparation steps typically include protein proteolysis for bottom-up analysis and affinity extraction to selectively enrich the target protein or its proteotypic peptide.

In our research we focus on integrating these tedious sample preparation procedures in the sampling step to save time and labour once the samples reach the analytical laboratory. We transform the sampling unit to a smart sampler, capable of performing sample preparation at the point of sample application and drying. The samplers are prepared by binding proteolytic enzymes for tryptic digestion or antibodies for affinity extraction to the sampling material^{1,2} [1, 2]. As a result, only a simplified preparation process is needed in the laboratory prior to analysis. In this paper, an overview of recent progress in smart sampling will be given by showcasing both proteolytic - and affinity samplers.

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PHYTOCHEMICAL AND BIOLOGICAL POTENTIAL OF SELECTED *ASTRAGALUS* SPECIES IN THE CONTEXT OF MODERN COSMETIC AND PHARMACEUTICAL APPLICATIONS

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The genus *Astragalus* represents one of the largest groups of medicinal plants and is recognized as a rich source of biologically active secondary metabolites, including flavonoids, phenolic acids, saponins, and polysaccharides. The growing interest in naturally derived bioactive compounds has increased the scientific focus on *Astragalus* species as potential ingredients for cosmetic, dermatological, nutraceutical, and pharmaceutical applications. The aim of the presented studies was to evaluate the phytochemical composition and selected biological properties of several *Astragalus* species, including *A. membranaceus*, *A. cicer*, *A. glycyphyllos*, *A. strictispinus*, and *A. macrocephalus* subsp. *finitimus*.

Different morphological parts of the plants (leaves, flowers, fruits, and roots) and various extraction solvents were analyzed in order to determine their chemical profiles and biological activities [1-3]. High-performance liquid chromatography (HPLC) analyses revealed the presence of numerous phenolic compounds, among which quercetin, gallic acid, naringenin, and apigenin were predominant. The investigated extracts demonstrated diverse antioxidant potential evaluated using DPPH, ABTS, FRAP, and CUPRAC assays, with several samples exhibiting strong radical scavenging activity correlated with their phenolic content. Furthermore, selected extracts showed antibacterial activity, particularly against *Staphylococcus aureus*, while moderate inhibitory effects toward enzymes associated with skin aging and neurodegenerative processes, including tyrosinase, acetylcholinesterase, and butyrylcholinesterase, were also observed.

The obtained results indicate that the studied *Astragalus* species constitute promising sources of multifunctional phytochemicals with potential applications in modern cosmetic and pharmaceutical formulations. Their antioxidant, antimicrobial, and enzyme-inhibitory activities suggest possible use in skin-protective, anti-aging, and therapeutic products [4]. Thus, *Astragalus* species can be considered as interesting natural resources for innovative bioactive preparations.

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AUTOMATED LONG-TERM MONITORING FOR PERMEATION AND TOXICOLOGY STUDIES

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A novel flow-programming setup based on the SIA concept was proposed for on-line monitoring of cell membrane permeation [1]. 3D printed module was developed and used for the automation of the monitoring permeation kinetics of the marker rhodamine 123 by sampling the apical and basolateral compartments of a 3D printed permeation module every 10 min. Then, the differences in profiles obtained in combination with the P-glycoprotein transporter inhibitor (verapamil) on the efficiency of a marker transport across a cell monolayer was investigated to express the interaction of exogenic compounds with cell membrane transporters in real-time. Employing the 3D-printed module, permeation testing was shortened from 8 h in the conventional module to 2 h. We also show that monitoring Rho123 levels in both compartments enables to calculate the amount of Rho123 accumulated inside cells without the need of cell lysis. Later, a toxicity assays based on the release of *Metridia* luciferase upon interaction with toxic substances and thus changes in cell membrane permeability were carried out [2]. Model substances for acute toxicity from the group of cholic acids – chenodeoxycholic and deoxycholic acids – were tested. The obtained kinetic profiles were evaluated based on the delay between the addition of the toxic substance and the increase of the chemiluminescence signal in the acceptor compartment.

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Deep-UV Laser Ablation as a Versatile Tool for Ambient Mass Spectrometry

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Ambient mass spectrometry has emerged as a powerful approach for the rapid analysis of complex samples with minimal or no sample preparation. Among laser-based ambient techniques, laser ablation electrospray ionization (LAESI) enables direct sampling of surfaces under atmospheric conditions and provides access to molecular information while preserving the spatial distribution of analytes (1).

With the introduction of the remote deep-ultraviolet laser-based approach, rDUVLAESCI (2), the capability of laser-based techniques has been extended. This approach combines deep-UV laser ablation with secondary ionization of the generated aerosol in a hybrid electrospray ionization–atmospheric pressure chemical ionization (ESI–APCI) environment. Using a 193 nm laser enables absolute material desorption directly from the substrate, bringing the technique closer to quantitative ambient analysis. The combination of complementary ionization mechanisms broadens analyte coverage and enables the detection of compounds with substantially different physicochemical properties, while retaining molecular information through predominantly soft ionization processes.

The capabilities of rDUVLAESCI were evaluated using a range of complex samples and analytical applications, including latent fingerprints and biological tissue sections. Particular attention was given to mass spectrometry imaging, where the technique was used to visualize the spatial distribution of endogenous and exogenous compounds in complex biological matrices. The approach was further applied to the detection and imaging of psychoactive substances and their residues in biological samples, demonstrating its potential for trace-level analysis.

Overall, rDUVLAESCI represents a versatile extension of ambient mass spectrometry, combining direct surface sampling, broad molecular coverage, and spatially resolved analysis. The presented applications highlight its potential for rapid chemical profiling and molecular imaging of complex real-world samples.

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HI-FIDELITY N-GLYCAN PROFILING BY CAPILLARY ELECTROPHORESIS

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Capillary electrophoresis has become one of the most important analytical platforms for high-resolution glycan profiling; however, reliable structural assignment requires robust normalization of migration times and accurate calculation of glucose unit values. Conventional GU determination utilizes an oligosaccharide ladder for capillary calibration, while a subsequent development introduced the so called three-internal-standard approaches to improve reproducibility and reduce the need for full ladder-based calibration.

However, a major limitation of the conventional fluorescent glycan analysis methods is the requirement for removal of excess labelling reagent prior to separation. Such purification steps may result in structure-dependent analyte losses and therefore alter the measured glycan profile. To address this issue, an electroosmotic-flow-assisted capillary zone electrophoresis strategy and the so called CATRIS background electrolyte system were developed, enabling online electrokinetic sample clean-up and purification-free analysis of APTS-labelled carbohydrates. Comparison of purified and non-purified samples indicated that sample clean-up may cause non-uniform glycan losses, supporting the need for workflows that minimize off-line sample manipulation.

Building on these developments, a new GU calculation approach will be introduced in this presentation that uses two internal standards together with numerical determination of a virtual electroosmotic-flow reference. The method is independent of capillary length and provides substantially improved precision. This concept forms the analytical basis of a next-generation data evaluation platform designed to integrate GU assignment, automated peak integration, and standardized data processing.

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ENHANCED CAPILLARY ELECTROPHORETIC ANALYSIS OF TRYPTOPHAN PATHWAY METABOLITES THROUGH OPTIMIZED SAMPLE PREPARATION AND FLUORESCENCE DETECTION

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The tryptophan metabolic pathway generates biologically important compounds involved in neurotransmission, immune regulation, and the pathogenesis of numerous neurological and metabolic disorders. Reliable determination of these metabolites remains challenging due to their low physiological concentrations and the complexity of biological matrices.

This study aimed to develop and evaluate sensitive capillary electrophoretic methods for the determination of selected tryptophan pathway metabolites, including serotonin (5-HT), 5-hydroxytryptophan (5-HTP), and 5-hydroxyindoleacetic acid (5-HIAA). Particular emphasis was placed on improving analytical sensitivity through optimization of sample preparation procedures and laser-induced fluorescence (LIF) detection.

Micellar electrokinetic chromatography (MEKC) conditions were optimized, enabling baseline separation of all analytes within 10 min based on separation buffer composed of 10 mM borax, 50 mM SDS, and 10% (v/v) methanol. For CE-LIF analysis (488/520 nm), fluorescent derivatization with 1 mM 5-DTAF was optimized with respect to pH, buffer composition, and reaction temperature. The performance of the developed CE-LIF procedure was compared with a UV-based approach employing extraction and preconcentration strategies. The highest signal enhancement factor values were obtained using SPE with Strata X-C columns and were from 6.9-fold for 5-HIAA to 16.5 for 5-HTP.

The optimized CE-LIF method demonstrated enhanced analytical performance, providing 10-fold lower limits of detection than UV detection and achieving LOD values of 3 ng/mL for 5-HTP and 5-HIAA and 15 ng/mL for 5-HT. These sensitivity levels cover concentration ranges relevant for the CE-LIF determination of endogenous tryptophan pathway metabolites in biological samples. Good linearity ($R^2 > 0.993$) and precision ($RSD < 14.2\%$) were obtained, while recoveries in model biological matrices ranged from 60.0% to 120.0%.

The results demonstrate that optimized sample preparation combined with CE-LIF detection substantially improves the analytical performance of capillary electrophoretic profiling of tryptophan pathway metabolites and provides a promising platform for future bioanalytical and clinical studies.

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Size-Exclusion Chromatography as a Tool for Simultaneous Isolation and Protein Corona Modulation of Extracellular Vesicles

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Extracellular vesicles (EVs), including exosomes, are nanoscale mediators of intercellular communication that carry proteins, lipids and nucleic acids reflecting the physiological and pathological state of their cells of origin (1). Their high diagnostic potential is therefore closely linked to the quality of their isolation from complex biological matrices. Although a range of standard isolation techniques is available, they often suffer from limited yield and insufficient purity, partly due to the co-isolation of non-vesicular components and the presence of an interfering surface-associated protein corona (2,3). To address these limitations, this study explores low-pressure size-exclusion chromatography (SEC) not only as a separation technique, but also as a tool for stripping the protein corona through tailored mobile phase composition. Building on findings by Tóth et al. (2025) demonstrating the influence of ionic strength on corona, we investigated whether similar principle can be applied during SEC to simultaneously isolate EVs and reduce the adsorbed protein layer (3). EVs were fractionated by high-ionic-strength phosphate buffer. The presence of EVs in eluted fractions were evaluated by dynamic light scattering and by WB analysis with immunochemical detection of CD63 and CD81. We demonstrated that this approach enhances specific epitope accessibility and improves the reliability of downstream affinity-based analyses, positioning SEC as a dual-function method that integrates EV isolation with protein corona removal or reduction.

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LA-ICP-MS: An Effective Tool for Studying Metal Distribution in Cells

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Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) has emerged as a powerful analytical tool for high-resolution elemental bio-imaging and single-cell quantification. The widespread clinical use of nickel-based orthopedic alloys carries an inherent risk of implant wear and corrosion, leading to the release of metallic ions that migrate into surrounding tissues and induce cytotoxicity [1, 2, 3]. This study investigates the intracellular distribution and accumulation of nickel within human osteoblasts, while evaluating the modulatory role of magnesium as a protective agent. To overcome matrix effects and ensure accurate quantification, ablation parameters were optimized, and calibration strategies utilizing Certified Reference Materials deposited on PVDF membranes as well as gelatin gel standards were validated [4, 5]. Quantitative multi-elemental imaging of ⁶⁰Ni and ³¹P—evaluated using specialized data reduction and visualization software such as Ilaps and Iolite [6, 7]—revealed that nickel successfully penetrates the cellular membrane and exhibits preferential accumulation within the cell nucleus [2]. Furthermore, exposing cells to varying concentrations of magnesium demonstrated its competitive, protective effect against nickel-induced toxicity [8]. Quantitative analyses demonstrated that varying concentrations of magnesium influence the amount of nickel capable of entering the cell and penetrating its cytoplasm [8]. These findings highlight LA-ICP-MS as an effective platform for studying metal-cell interactions and provide quantitative insights into how essential elements can mitigate heavy metal toxicity in biological systems [1, 8].

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Comorbidity-Associated *N*-Glycome Profile Shifts: A Preliminary Study Evaluating COPD and Diabetes in Lung Cancer Patients

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Aberrant glycosylation frequently accompanies malignant, metabolic, and chronic inflammatory diseases however, it remains unclear how coexisting conditions influence disease-associated alterations in circulating *N*-glycan profiles [1,2]. To address this question, this preliminary study examined the influence of chronic obstructive pulmonary disease (COPD) and diabetes mellitus (DM) on serum *N*-glycan profiles in 40 patients with lung cancer (LC), divided into four groups (LC, LC+DM, LC+COPD, and LC+COPD+DM; n = 10 each). Released and fluorescently labelled *N*-glycans were profiled by capillary electrophoresis with laser-induced fluorescence detection. Nineteen peaks were selected for quantitative analysis. Peak-wise factorial models assessed DM, COPD, and their association while accounting for age and gender. When the peaks were analyzed individually, Peak 12, tentatively assigned as A3G(4)3S(6)2 by GU matching (median GU 7.23), was the only peak that remained significant after multiple-testing correction ($\beta = 0.27$, FDR $q = 0.01$; HC3 FDR $q = 0.02$; partial $\eta^2 = 0.34$). Descriptively, its mean relative peak area (%) was approximately 21% higher in DM-positive patients, while the adjusted model confirmed the DM association on the logit scale. Likewise, analysis of the complete *N*-glycan profile revealed a significant association with DM (PERMANOVA $R^2 = 0.06$, $p = 0.02$), whereas no significant COPD or DM $\times$ COPD association was detected. F(6)A2B showed an exploratory DM-associated signal but did not remain significant after conventional multiple-testing correction. Our findings suggest that diabetes may be associated with a modest but detectable shift in the serum *N*-glycome of patients with lung cancer, with A3G(4)3S(6)2 representing the strongest peak-specific finding in this cohort.

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The potential of saliva as a sample matrix for therapeutic drug monitoring

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Therapeutic drug monitoring (TDM) is an important tool in personalised medicine. It involves measuring drug levels in biological matrices and, together with appropriate clinical interpretation, can support individualised dosing and optimise pharmacotherapy. TDM mostly relies on blood-based sampling; however, this approach has several limitations, including the need for trained healthcare personnel, patient attendance at a healthcare facility, invasiveness, and patient discomfort. Therefore, alternative biological matrices, such as saliva or urine, are increasingly being explored for TDM.

Saliva and oral fluid collection is non-invasive, easy, and well tolerated, and patients or their caregivers can collect samples at home. This may be particularly beneficial for specific patient populations, including infants, children, and elderly people. As only unbound drug enters the saliva, salivary concentrations may provide a more representative indicator of systemic exposure. However, saliva's suitability for TDM depends on the drug's physicochemical and pharmacokinetic properties, and a reliable relationship between salivary and plasma concentrations is essential for meaningful clinical interpretation. Currently, salivary TDM is used mainly for several antiepileptic medications, such as carbamazepine, lamotrigine, levetiracetam, and pregabalin.

In the present work, we aim to evaluate the potential of using oral fluid in clinical practice to improve treatment monitoring and simplify sample collection, which can be performed by patients at home. We developed an LC-MS/MS method for the quantification of albendazole and its two main metabolites, albendazole sulfoxide and albendazole sulfone, in oral fluid. For the first time, we provide a correlation between plasma and oral fluid levels in patients with alveolar echinococcosis treated with albendazole.

Thus, we can conclude that saliva and oral fluid may be suitable for routine TDM and may help improve clinical management.

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COMPARATIVE N-GLYCAN PROFILING OF URINARY AND RECOMBINANT HUMAN CHORIONIC GONADOTROPIN BY CZE-LEDIF AND HILIC-HRMS

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Human chorionic gonadotropin (hCG) is an essential glycoprotein hormone produced by trophoblastic cells that supports early pregnancy by maintaining the corpus luteum and enabling maternal-fetal communication.¹ hCG is heavily glycosylated, containing four N-glycosylation sites (two on each subunits) and four O-glycosylation sites on the beta subunit. Carbohydrate moieties account for up to 25-35% of its molecular weight. The glycosylation of hCG varies throughout pregnancy, and has been shown to change under pathological conditions². In this context, detailed hCG glycosylation analysis is crucial for diagnostic applications. Hereby, we present an N-glycan analysis workflow of urinary and recombinant hCG standards (u-hCG/r-hCG). Complementary techniques, including capillary electrophoresis (CE) coupled with light-emitting diode induced fluorescence (LEDIF) and hydrophilic interaction liquid chromatography coupled with high resolution mass spectrometry (HILIC-HRMS) were implemented to strengthen characterization.

For CE analysis, N-glycan analysis of u-hCG and r-hCG standards were obtained by Capillary Zone Electrophoresis (CZE). To our knowledge, this is the first application of CZE to released N-glycans of hCG, revealing vastly different profiles. To enhance detection sensitivity and resolution, we relied on dual electrokinetic preconcentration, combining large volume sample stacking (LVSEP) and transient isotachopheresis (t-ITP).³ The effects of concentration, injected volume, pH and ionic strength of electrolyte solutions involved in the stacking process were evaluated to find the best compromise between high detection sensitivity and good resolution. The optimized LVSEP-tITP approach yielded a three-fold resolution gain compared to initial conditions, with enrichment factor up to 400, and good repeatability (RSD on migration time <3%, n=3), allowing analysis of glycoprotein samples down to the µg/mL range with high glycan coverage. In parallel, HILIC-HRMS workflow provided complementary compositional and structural insights on N-glycans present in both hCG standards. The mass spectra demonstrated significant difference in fucosylation (r-hCG: 4% and u-hCG: 43% of the total N-glycan abundance) and sialylation (r-hCG: 64%/u-hCG:87%).

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Development of CE-LIF approaches for sugar and oligosaccharide profiling in food and biological samples

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Carbohydrate profiling by capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) was developed as a single analytical platform whose complexity increased progressively from food analysis to clinical glycomics. The central concept was the use of 8-aminopyrene-1,3,6-trisulfonic acid (APTS) labeling to follow the transition from high-concentration free sugars, through low-abundance oligosaccharides, to enzymatically released protein glycans. Honey was selected as the initial, relatively simple model matrix. Glucose, mannose, and maltose were efficiently derivatized at 50 °C using APTS and 2-picoline borane, a less toxic alternative to sodium cyanoborohydride. Fructose showed poor and irreproducible labeling because of its lower ketose reactivity. The method was linear over 0.5–10 mM ($R^2 > 0.994$), with an LOD of 0.16 mM and recoveries of 93.47–119.75%. However, excess APTS caused substantial background, while SPE and SPME reduced reagent-related signals at the cost of losses of highly hydrophilic analytes. The workflow was then transferred to commercial milks, introducing a more complex matrix and lower-abundance oligosaccharides. Acetonitrile deproteinization produced stronger analyte signals than ethanol. Cow's milk was dominated by lactose, whereas lactose-free milk showed predominant glucose and galactose signals. Rice beverages contained glucose, maltose, maltotriose, and higher maltooligosaccharides, while oat beverages were characterized by an intense maltose signal together with glucose, maltotrioses, maltotetraoses, and maltopentoses. In both matrices, abundant sugars frequently masked minor oligosaccharides, demonstrating the need for more selective sample preparation. The final stage extended the platform to IgG-derived N-glycans released with PNGase F and labeled with APTS/2-picoline borane. At the current stage, relatively high amounts of model IgG are required to achieve sufficient sensitivity. Further optimization of glycan release, purification, labeling, and signal enhancement is therefore essential before transfer to human serum and differentiation of glycan profiles among patient groups, including infectious and inflammatory conditions.

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CHARTING THE BRAIN ENDOCANNABINOID LANDSCAPE BY UHPLC–ESI–MS/MS

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Endocannabinoids (ECBs) are bioactive lipid mediators and key components of a complex signaling network involved in the modulation of central nervous system function, including neuronal communication and synaptic transmission. Primarily mediated through CB1 and CB2 receptors, endocannabinoid signaling modulates neurotransmitter release and contributes to physiological and pathological processes including pain, neuroinflammation, and neurodegeneration¹. Reliable quantification of ECBs in neural tissues is therefore essential to elucidate their biological relevance and investigate their potential involvement in central nervous system (CNS) disorders. Despite the availability of analytical methods for biological fluids, their accurate determination in brain tissue remains challenging due to their endogenous occurrence and lipophilic nature within a highly complex lipid-rich matrix^{2,3}.

The present study focused on the development and validation of a targeted UHPLC–ESI–MS/MS method for the simultaneous quantification of 11 endocannabinoids and related lipid mediators in brain tissues, with application to distinct cerebral regions. Sample preparation and chromatographic conditions were optimized by comparing different liquid–liquid extraction systems and stationary phases. Method validation included evaluation of linearity, accuracy, precision, and recovery at multiple concentration levels.

Chromatographic separation was achieved on a reversed-phase C₁₈ column using a Shimadzu LC-40 X3 UHPLC system coupled to a SCIEX 6500+ QTRAP mass spectrometer operating in ESI-MS/MS mode. The optimized protocol provided reproducible extraction and good quantitative performance. Quantification was successfully performed in murine brain samples and across distinct cerebral regions. Ethyl acetate–hexane showed comparable extraction performance to toluene–MTBE, supporting its potential as a safer alternative. Overall, the validated method provides a robust analytical tool for regional profiling of brain endocannabinoids and related lipid mediators.

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FLEXIBLE MULTI-CRITERIA GRADIENT SCORING FOR TARGETED LC–MS/MS METHOD OPTIMIZATION

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Development of targeted LC–MS/MS methods for large analyte sets requires simultaneous consideration of chromatographic performance and mass-spectrometric acquisition constraints. To support objective method selection, we developed a flexible Gradient Score (GS) framework that integrates several chromatographic and MS/MS-related descriptors into a single normalized response.

The approach was evaluated using more than 60 phenolic standards analyzed by reversed-phase LC coupled with triple-quadrupole tandem mass spectrometry. In contrast to the original GS formulation (1), which relied on a fixed set of parameters, the present version allows the user to select the most relevant subcriteria and assign their relative importance according to the optimization objective. The available descriptors include gradient time, overlap of scheduled MRM windows, resolution of predefined critical analyte pairs, peak-width performance, and analyte distribution across the chromatographic window. Individual descriptors are transformed by min–max normalization and combined into a weighted score, enabling direct comparison and ranking of experimental conditions.

The framework was applied to stationary-phase screening and subsequent optimization of gradient profiles, including both linear and segmented gradients (2). This workflow enabled the identification of conditions that provided a balanced compromise between separation quality, analysis time, and acquisition feasibility. The configurable GS concept therefore represents a practical and transferable tool for rational development of targeted LC–MS/MS methods and is readily adaptable to different analytical priorities and compound classes.

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RAPID VOLTAMMETRIC ANALYSIS OF PHARMACEUTICAL SAMPLES

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Boron-doped diamond (BDD) is a promising electrode material for the fabrication of electrochemical sensors, mainly due to its exceptional electrochemical properties and wide range of applications in analytical chemistry.¹ In this work, a screen-printed BDD sensor (SP-BDD) was investigated. The sensor consisted of a BDD working and auxiliary electrode, together with a quasi-reference silver/silver chloride (Ag/AgCl) electrode without an internal electrolyte, prepared by electrochemical oxidation of Ag.² The SP-BDD sensor was utilized in combination with a 3-D printed flow-through electrochemical cell. This configuration enabled the evaluation of the sensor performance, stability, and reliability during voltammetric analyses.

Electrochemically active pharmaceutical substances were chosen as model analytes for method optimization. The optimization involved investigating the influence of supporting electrolyte composition, pH, and scan rate on the voltammetric response of the target analyte. Potential interferents affecting the analysis of pharmaceutical substances, particularly in multi-component samples, were also investigated. In addition, the long-term stability of the BDD sensor was evaluated, and key analytical performance parameters (repeatability, limit of detection (LOD), limit of quantification (LOQ), and linear dynamic range (LDR) were assessed.

The obtained results indicate that the SP-BDD sensor, in combination with the flow-through electrochemical cell, is suitable for rapid and reliable voltammetric analysis of pharmaceutical samples. Furthermore, the proposed miniaturized and partially automated analytical platform shows strong potential for the development of fully automated pharmaceutical analysis in accordance with green analytical chemistry principles.

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URINARY PSA N-GLYCAN PROFILING WITH CAPILLARY ELECTROPHORESIS FOR PROSTATE CANCER DETECTION

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Prostate cancer is one of the most common cancers among men in Europe. Its diagnosis is based on serum PSA measurement, which can lead to unnecessary biopsies due to its limited specificity. Changes in the PSA N-glycan pattern during neoplastic transformation may provide diagnostic information independent of PSA concentration. We aimed to compare urinary PSA N-glycan profiles in histologically confirmed prostate cancer and benign prostatic hyperplasia (BPH). To date, 32 patients have been included (23 prostate cancer, 9 BPH). Urine samples were collected immediately after robot-assisted radical prostatectomy in prostate cancer patients and also before surgery in BPH patients. PSA N-glycan profiles were analyzed by capillary electrophoresis. On the electropherograms, we identified 20 peaks that were highly reproducible based on their migration times. Group differences in individual and paired peak proportions were assessed using the Mann–Whitney U test, and the most reliable candidates were evaluated by ROC analysis. The relative proportion of the ~6.48-minute peak was significantly lower in prostate cancer than in BPH ($p=0.0073$; AUC 0.812, sensitivity 91.3%, specificity 66.7%). The ratio of the two strongest peaks (~7.67 and ~8.92 minutes) was also significantly lower in prostate cancer ($p=0.0029$; AUC 0.845, sensitivity 60.9%, specificity 100%). Based on our results, the PSA N-glycan profile detectable in urine identified promising biomarker candidates for distinguishing between prostate cancer and BPH. Validation of the ~6.48-minute peak and the ~7.67/~8.92-minute peak ratio in a larger cohort is warranted. The results suggest that the PSA N-glycosylation profile may, in the future, serve as a noninvasive diagnostic tool to aid prostate cancer detection and support biopsy decision-making, thereby potentially reducing unnecessary biopsies.

SENSOR-INTEGRATED BODY-ON-A-CHIP PLATFORM TECHNOLOGY FOR MODERN, ANIMAL-FREE TOXICOLOGY

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Organophosphates (OP) are pervasive components in pesticides and chemical weapons such as Sarin gas (Perry et al. 2020). It has been shown that prolonged exposure to OPs can result in severe neurological disorders and has proven to be fatal (Dorandeu et al. 2023; Perry et al. 2020). Therefore, regular environmental monitoring to detect OP concentrations is essential in agricultural applications and the military sector to ensure the safety of personnel and civilian populations (Bhattu, 2021; Dorandeu et al. 2023). State-of-the-art analytical detection methods such as mass spectroscopy and liquid chromatography require time-consuming pre-treatments and costly reagents and are not suited for in-field usage (Bhattu et al. 2021).

There is an urgent need for enhanced methods to assess environmental organophosphate neurotoxicity without endangering human or animal lives (Dorandeu et al. 2023). This study aims to develop a multi-organ microfluidic platform for in-field deployment capable of acute toxicity evaluation, a Body-On-A-Chip model able to mimic the pathway of OPs through the skin, into the body and finally arriving at the brain.

BodyTox 2.0 present a sensor-integrated body-on-a-chip platform technology for symptom-relevant toxicological analyses in the environmental and CBRN fields. The project showcases the current state of microfluidic human models with integrated real-time sensors for investigating neurotoxic and environmentally relevant pollutants.

Looking ahead, the platform addresses future applications in risk and symptom modelling for security research, disaster relief, and defence applications. Ongoing follow-up projects are expanding the system with additional organ and sensor modules, as well as AI-supported sensor analytics for the most complex *in vitro* toxicological questions.

Acknowledgement

The technological basis of the detector was developed in the cooperative FFG FORTE project “BodyTox 2.0” and “Living Sensor 1.0” in cooperation with the BMLV/Austrian Armed Forces and financed by the BMF.

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EXPLORING THE ANALYTICAL LANDSCAPE OF BIOACTIVE ENDOGENOUS COMPOUNDS: THE *P*-CRESOL PARADIGM

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p-Cresol is both an environmental contaminant and an endogenous metabolite primarily produced from L-tyrosine by gut microbiota, particularly by *Clostridium* species. Following intestinal absorption, it undergoes extensive hepatic first-pass metabolism, generating mainly *p*-cresyl sulfate (*p*CS) and, to a lesser extent, *p*-cresyl glucuronide (*p*CG). These metabolites circulate predominantly bound to plasma proteins and are eliminated through the kidneys. Increasing evidence suggests the involvement of *p*-cresol and its metabolites in central nervous system (CNS) dysfunction and in neurodevelopmental, neuropsychiatric and neurodegenerative disorders, including autism spectrum disorder (ASD), post-traumatic stress disorder (PTSD), Alzheimer's disease (AD) and Parkinson's disease (PD). Nevertheless, the occurrence and regional distribution of *p*-cresol in the brain remain largely unexplored.

To overcome this issue, a highly sensitive high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) method was developed and validated for the first time for targeted quantification of *p*-cresol in brain tissues, together with an efficient extraction procedure. This analytical approach was applied to: characterize the distribution of *p*-cresol across different brain regions in adult male and female C57BL/6J mice and compare cortical levels of *p*-cresol among three mouse strains, namely C57BL/6J, CD-1 IGS, and BTBR T+Itpr3tf/J, which is a widely used preclinical model for studying idiopathic ASD; to investigate the effect of acute and chronic administration of *p*-cresol in C57BL/6J and BTBR T+Itpr3tf/J; to assess the level of *p*-cresol and its metabolites in an *in vivo* model of AD based on transgenic 5xFAD mice.

These findings demonstrate a heterogeneous basal distribution of *p*-cresol across brain regions and highlight significant strain-dependent variations in its brain levels. These results provide also the first comprehensive characterization of *p*-cresol in the brain, establishing solid proof for exploring mechanistic links between gut-derived metabolites and brain dysfunction and paving the way for novel insights into its contribution to neurodevelopmental and neurodegenerative diseases.

Library-Driven Automated Selection of Fragment Ions and Collision Energies for Quantitative Mass Spectrometry

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Introduction:

Optimization of fragment ions and collision energies is essential for developing targeted quantitative mass spectrometry methods, but conventional optimization requires experimental measurements for individual compounds and can be resource intensive. We developed an automated approach using experimentally acquired spectra in the mzCloud spectral library to identify candidate fragment ions and corresponding collision energies for quantitative analysis. The workflow ranks MS2 fragment ions by abundance and evaluates their collision-energy-dependent behavior, providing up to five high-intensity fragments with preferred fragmentation conditions. Reusing curated spectral-library data can reduce experimental effort during quantitative method development.

Methods:

An automated algorithm retrieves and evaluates high-resolution accurate-mass (HRAM) MS2 data available in mzCloud. Spectral experiments undergo quality-control steps including evaluation of the number and distribution of collision-energy measurements and fragmentation behavior of individual product ions. Fragment-ion breakdown curves are analyzed to characterize ion responses and identify suitable fragmentation conditions. For datasets meeting the quality criteria, normalized collision energy (NCE) values can be converted to collision energy (CE) to facilitate transfer across mass-spectrometer platforms. Batch analysis is supported using InChIKey identifiers.

Preliminary Data:

The workflow was evaluated using mzCloud data covering approximately 32,000 compounds from reference and automatically processed libraries. The starting dataset contained 3,462,578 MS2 spectra from 200,482 ion-tree experiments. Following quality filtering, 168,486 experiments fulfilled the criteria for further evaluation. Experiments were retained only when fragmentation data contained adequate collision-energy measurements with sufficient coverage of the CID or HCD energy range. Particular emphasis was placed on product-ion breakdown curves, which provide the basis for selecting fragment ions and preferred collision energies. The current implementation supports conversion of NCE-derived conditions to CE for multiple instrument configurations, including Tribrid™ and Hybrid™ mass spectrometers, facilitating translation of library-derived fragmentation information to triple-quadrupole quantitative workflows.

Novel Aspect:

A spectral-library-driven workflow transforms existing high-resolution MS/MS data into automatically selected product ions and collision-energy conditions for quantitative method development, reducing compound-specific experimental optimization.

APPLICATION OF GAS-PHASE ELECTROPHORESIS (nES GEMMA INSTRUMENTATION) IN EXHALED BREATH CONDENSATE (EBC) CHARACTERIZATION

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Retrieving a representative, unbiased sample directly and non-invasively from the lung of a patient followed by its analysis is an attractive strategy to gain information on the health status of an individual. In this respect we focussed on collection of exhaled breath condensate (EBC) in combination with gas-phase electrophoresis on a nano electrospray gas-phase electrophoretic mobility molecular analyzer (nES GEMMA). This latter instrument enables characterization of (bio-)nanomaterials in their native state in respect to their size, size distribution and particle number concentration [1] in accordance with recommendations of the EC for nanomaterial characterization.

In the first part of our study we worked with a pool of n=15 volunteers, demonstrating the applicability of nES GEMMA for EBC characterization focussing on possibilities and limitations of the instrumentation [2]. Based on our findings, we then continued in a clinical setting characterizing EBC of n=5 lung cancer patient and n=5 control samples, respectively. Despite the low number of donors in the second part of our study we could show differences in nES GEMMA spectra from healthy and patient donors. Although further work needs to be directed towards in-depth characterization of EBC and detected EBC components, we are convinced that EBC analysis via nES GEMMA will be a valuable tool in lung cancer diagnosis in the future.

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Integrated Mass Spectrometric Evaluation of Tributyltin Salicylate and Tributyltin Propionate as Novel RXR Ligands with Anti-Cancer Potential in Breast Cancer Cells.

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Organotin(IV) compounds have attracted considerable attention due to their ability to induce apoptosis through the intrinsic mitochondrial pathway, making them promising candidates for anticancer therapy. In the present study, the in vitro effects of two triorganotin(IV) derivatives, tributyltin propionate and tributyltin salicylate, were evaluated using the human triple-negative breast cancer cell line MDA-MB-231. Macejova et al. (2026) have shown that these compounds act as synthetic ligands of nuclear retinoid X receptors (RXRs), which regulate genes involved in cell proliferation, differentiation, and apoptosis. To investigate their molecular effects, changes in the cellular protein expression profile were analyzed using two-dimensional gel electrophoresis followed by MALDI-TOF mass spectrometry, with particular emphasis on heat shock proteins (HSPs). HSPs are molecular chaperones frequently overexpressed in cancer cells, where they maintain protein homeostasis, promote tumor progression, suppress apoptosis, and contribute to chemo resistance. Treatment with both triorganotin(IV) derivatives resulted in significant downregulation of several HSP family members, indicating disruption of cellular stress-response mechanisms. These findings suggest that HSPs may represent important molecular targets of tributyltin compounds and contribute to their anticancer activity. Overall, the results provide insight into the biological effects of triorganotin(IV) derivatives and support their further investigation as potential anticancer agents targeting stress-response pathways in breast cancer.

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DETECTION OF TiO₂ NANOPARTICLES GENOTOXICITY AT DIFFERENT pH SIMULATING GASTROINTESTINAL TRACT

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Titanium dioxide (TiO₂) is one of the most extensively produced and utilized nanomaterials due to its wide range of technological, medical, and economic applications. However, the increasing presence of TiO₂ nanoparticles (NPs) in the environment has raised concerns regarding their potential adverse effects on human health (1). Because TiO₂ NPs are commonly found in food products, food packaging materials, and drinking water (2), they can readily enter the human gastrointestinal tract, where they are exposed to environments characterized by varying pH conditions. Such pH fluctuations may significantly influence the stability, dispersion, and biological activity of nanoparticles, thereby affecting their toxicity and interactions with biomolecules.

The aim of this study was to investigate the effect of TiO₂ nanoparticles incubated under pH conditions simulating those found in the gastrointestinal tract on the structure of DNA. DNA damage was evaluated using a DNA biosensor and a biosensing approach with electrochemical detection, while the obtained results were further validated by UV-Vis spectrophotometry. Cyclic voltammetry measurements revealed damage to DNA immobilized on a glassy carbon electrode (DNA/GCE biosensor) of up to 10%. The most sensitive and effective method for assessing the genotoxic effects of TiO₂ NPs was the biosensing approach based on monitoring changes in the current response of DNA residues using differential pulse voltammetry, which detected alterations of up to 47.6%. The strongest influence of TiO₂ nanoparticles on DNA was observed at pH 8.0, particularly in relation to guanine residues (3). These findings were confirmed by UV-Vis spectrophotometric analyses, which demonstrated hyperchromic and bathochromic shifts in the absorption spectra.

Overall, the results indicate that pH conditions similar to those encountered in the gastrointestinal tract can significantly modulate the genotoxic potential of TiO₂ nanoparticles and their interactions with DNA, highlighting the importance of considering physiological environmental factors when evaluating the biological safety of nanomaterials.

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COMBINATION OF RAMAN SPECTROSCOPY AND ACOUSTOFLUIDICS: TOWARDS CHEMICAL IDENTIFICATION OF FINE MICROPLASTICS

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Microplastics (MPs) are plastic particles ranging in size from approximately 1 μm to 5 mm. Due to the widespread use and persistence of plastic materials, MPs have become ubiquitous environmental contaminants.¹ Although their effects on human health are still not fully understood, it is already evident that they are related to the material composition of microplastics as well as their size and shape. Therefore, reliable methods for the comprehensive characterization of microplastics are becoming increasingly important.

Raman spectroscopy is a powerful vibrational analytical technique that provides molecular fingerprints of materials while requiring minimal sample preparation and enabling non-destructive analysis. An additional advantage of Raman spectroscopy is its ability to detect relatively small particles, down to the submicrometer range. This is particularly relevant for microplastics, as particle size may influence their potential biological effects and their deposition within the respiratory system. However, its inherently weak Raman scattering limits its practical application and consequently results in low sensitivity. Surface-enhanced Raman spectroscopy (SERS) can overcome this limitation by utilizing metallic nanostructures, resulting in a significant enhancement of the Raman signal. This makes SERS a promising method for detecting microplastic particles, especially when they are present in low concentrations.

Acoustofluidics is an emerging technique that uses acoustic waves for contactless manipulation with particles in liquids. This can be useful for confining the particles for feasible detection by spectroscopic methods. In this work, we aim to investigate the capacity of SERS to detect 1 μm polystyrene microspheres and PET fragment-like particles. Furthermore, we explore a potential acoustofluidic approach for improving their detection by concentrating the particles within a sample droplet.

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CAPILLARY GEL ELECTROPHORESIS OF DNA FRAGMENTS USING WATER-SOLUBLE HYDROPHILIC POLYMERS

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The growing use of nucleic acid-based therapeutics requires rapid, high-resolution analytical methods for quality assessment and quality control. In this study we developed a dual-network hydrogel-based physical copolymer for capillary gel electrophoresis by combining hydroxyethyl cellulose (HEC), which provided efficient molecular sieving, with interpenetrating polyvinylpyrrolidone (PVP), which reduced electroosmotic flow. Boric acid in the background electrolyte stabilized the HEC matrix through spiroborane bridge formation, while the addition of glycerol enhanced separation resolution. The resulting tetrahydroxyborate-stabilized HEC–PVP copolymer readily separated double-stranded DNA restriction fragments ranging up to 10 kilobase pairs and even resolved some fragments of identical length. Its analytical utility was further demonstrated by monitoring plasmid DNA integrity. The developed matrix provides high-resolution separation of nucleic acids and offers a promising approach for the analytical characterization of gene therapy products in biopharmaceutical applications.

THE USE OF BIOPHYSICAL TECHNIQUES IN THE CHARACTERIZATION OF BIOPHARMACEUTICALS

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Newly developing and routinely produced biopharmaceuticals like antibodies, protein vaccines and other biomacromolecules have to be characterized and for this we can use biophysical techniques. These range from elaborate structure determining techniques as X-ray crystallography, cryo electron microscopy, nuclear magnetic resonance spectroscopy (NMR), atomic force microscopy and small angle X-ray scattering to the methods elucidating oligomeric state and secondary structure of proteins as dynamic light scattering, mass photometry, circular dichroism (CD) spectroscopy and Fourier transform infrared spectroscopy (FTIR). The interactions of biomacromolecules are measured using the surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), biolayer interferometry (BLI), flow induced dispersion analysis (FIDA), microscale thermophoresis and stopped flow methods. The stability of macromolecules is measured using differential scanning calorimetry (DSC) or differential scanning fluorimetry (DSF). All of these methods are available at the Institute of Neuroimmunology or other institutes participating in the project Advanced Protein Biotechnology Consortium (APBC), which allowed for a recent upgrade of the infrastructure. The use of the biophysical methods will be showcased on the examples of Alzheimer's disease related tau proteins and antibodies.

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CHARACTERIZATION OF EXTRA PEAKS IN PHYTOCHELATIN PROFILING IN PLANT SAMPLES

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Phytochelatins (PCs) are a specific group of plant thiol peptides that play a key role in the detoxification of heavy metals. They have a linear structure consisting of repeated γ -glutamylcysteyl units terminated with a glycine residue. Currently, the most widely used and sensitive method for routine analysis of PCs utilizes fluorescent labeling with monobromobimane, which reacts with the sulfanyl groups to give stable fluorescent products that enable detection at picomolar levels.

The samples containing PCs were analyzed by reverse-phase (C18) chromatography hyphenated with fluorescence ($\lambda_{\text{exc}}/\lambda_{\text{em}}$ 390/480 nm) or mass spectrometry ($[M+H]^+$, $[M+2H]^{2+}$) detection.

During the optimization of the labeling protocol and analytical method, some unexpected peaks were observed in chromatograms. These can be divided into three groups according to their origin. MS/MS experiments confirmed the presence of partially labeled PCs, over-labeled PCs, and isophytochelatins in the sample. The formation of partially labeled and over-labeled PCs was confirmed employing PC standards. Phytochelatin derivatives (isophytochelatins) were identified as $(\gamma\text{GluCys})_n$, $(\gamma\text{GluCys})_n\text{Ser}$, $(\gamma\text{GluCys})_n\text{Gln}$, $\text{Cys}(\gamma\text{GluCys})_n\text{Gly}$, and $(\gamma\text{GluCys})_n\text{Asn}$.

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MAPPING THE GLYCOME IN ALZHEIMER'S DISEASE: A GLYCOMICS APPROACH TO BIOMARKER DISCOVERY

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and remains a major challenge for early and reliable diagnosis. Current diagnostic approaches increasingly rely on established protein biomarkers, including amyloid beta (A β), tau and phosphorylated tau. However, additional molecular information may improve our understanding of disease-associated changes and contribute to the identification of complementary biomarkers.

Glycosylation is one of the most abundant post-translational modifications and plays important roles in protein folding, stability, molecular recognition, cell signalling and immune regulation. Alterations in glycosylation have been associated with several pathological processes relevant to Alzheimer's disease, including neuroinflammation, altered protein processing and protein aggregation.

The aim of this work is to explore glycomics-based strategies for mapping glycosylation changes associated with Alzheimer's disease and to evaluate their potential for biomarker discovery. Particular attention is focused on lectin-based glycoprofiling, including lectin assays and lectin microarray platforms, which enable the analysis of multiple glycan features such as sialylation, fucosylation, galactosylation, branching and mannose-containing structures. These approaches can be applied to biological samples as well as to selected AD-associated proteins and glycoproteins.

The integration of lectin-based glycoprofiling and multivariate data analysis may help identify glycan patterns associated with Alzheimer's disease. These disease-associated glycosylation changes could contribute to the identification of novel biomarker candidates and support the future development of improved bioanalytical approaches for Alzheimer's disease.

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Impact of Carrier Ampholytes on Vesicle Stability During Capillary Isoelectric Focusing

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Capillary isoelectric focusing (cIEF) is a powerful separation technique for analyzing the isoelectric point of vesicles.¹ However, it often deals with the instability of vesicular samples during analysis. Taylor dispersion analysis (TDA)² was used to assess vesicle integrity under cIEF conditions with two different broad-range carrier ampholytes (CAs), Pharmalyte 3–10 and AESlyte SH 3–10. The study evaluated synthetic POPC:DOPE:Chol liposomes, biological liver-derived vesicles, and mRNA-vaccine lipid nanoparticles (LNPs). TDA sensitively reported hydrodynamic radius changes reflecting membrane stability. Across 0.1–2 % (w/v) CA concentrations, POPC:DOPE:Chol vesicles remained stable until the highest concentration (vesicle signal loss at 2 %), whereas liver-derived vesicles were disrupted even at 0.1 %, demonstrating high ampholyte sensitivity. This was confirmed by the lack of a liver-derived vesicle signal in cIEF. Vaccine LNPs retained detectable populations throughout all tested conditions, and their successful cIEF separation was achieved in both CA systems, yielding reproducible apparent pI values (6.16–6.36). While Pharmalyte 3–10 maintained high pH-gradient linearity, AESlyte SH 3–10 exhibited minor distortion in the acidic range. Ultimately, TDA provides a quantitative framework for optimizing cIEF conditions and pre-screening CA–lipid compatibility prior to nanoparticle analysis.

Acknowledgement

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Postpartum IgA and IgG N-Glycosylation in the Perspective of Maternal Obesity and Gestational Diabetes

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Gestational diabetes mellitus (GDM) and maternal obesity are associated with metabolic and immunological changes, including chronic low-grade inflammation, some of which may persist into the postpartum period. Immunoglobulin N-glycosylation is a sensitive molecular feature that may reflect metabolic and immunological conditions. However, different immunoglobulin classes have distinct biological functions and N-glycosylation profiles and may therefore exhibit different patterns of glycosylation changes in response to metabolic and inflammatory conditions. In this study, we examined postpartum IgA and IgG N-glycosylation patterns in relation to maternal obesity and GDM. Maternal serum samples were collected 12 weeks postpartum from women classified into four groups according to obesity and GDM status: healthy normal-weight, healthy obese, GDM, and GDM+obese (n=15/group). IgA and IgG were isolated from pooled maternal serum samples and analyzed by capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) after enzymatic N-glycan release and fluorescent labeling. The individual N-glycan structures and derived glycosylation traits were evaluated across the four maternal groups.

The two immunoglobulin classes showed distinct but partially overlapping N-glycan patterns across the groups. In case of IgG, analyses of sialylation to neutral structure (SF/NF) and core-fucosylation to the total N-glycosylation (cF/TNG) revealed that obesity may be more strongly associated with postpartum IgG N-glycosylation differences than GDM. IgA also exhibited group-related changes in individual glycans, while more detailed glycosylation traits revealed pronounced differences in antennarity, galactosylation, and sialylation. Sialylation- and galactosylation-related traits showed a non-monotonic pattern, with generally higher levels in obesity and lower levels in groups with preceding GDM.

These findings suggest that postpartum IgA and IgG N-glycosylation may reflect maternal metabolic status in different ways, highlighting the potential value of examining multiple immunoglobulin classes as molecular markers of postpartum immunometabolic alterations

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CHARACTERIZATION OF HUMIC ACIDS AND THEIR FRACTIONS BY RP-HPLC METHOD USING STEPWISE AND LINEAR GRADIENT ELUTION

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Humic substances (HSs) are ubiquitous natural materials occurring in huge amounts in soils, sediments and waters as a product of the chemical and biological transformation of animal and plant residues. HSs are also complex mixture of amorphous, yellow to black colored, hydrophilic, polyelectrolyte-like, polydisperse macromolecules and probably not their two molecules are identical. With respect to their supramolecular structure, there is still a lot of controversy about their nature. Reversed-phase high-performance liquid chromatography (RP-HPLC) as prevailing chromatographic method has achieved in the field of analysis and characterization of HSs only very limited application. In most cases authors used conventional organic solvents (methanol, acetonitrile) typical for RP-HPLC separation of small molecules together with weakly pH buffered aqueous part of mobile phase. However, these mobile phases did result in moderate satisfaction with HS samples [1,2].

In this work, RP-HPLC using combination of step-wises and linear gradient of dimethylformamide (DMF) in buffered aqueous mobile phase and a wide-pore (30 nm) octadecylsilica column was evaluated for purpose of characterization of RP-HPLC fractionated humic acids [3,4].

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AUTOMATED LAB-IN-SYRINGE DISPERSIVE SOLID PHASE MICROEXTRACTION USING DISSOLVABLE SORBENT FOR DETERMINATION OF ALKYLPHENOLS

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An overview of a fully automated method for preconcentration of selected alkylphenols, namely 4-nonylphenol, 4-heptylphenol, and 4-tert octylphenol, in water samples will be presented. The method was based on solvent-assisted dispersive solid phase microextraction (dSPE), which was automated via the flow-batch technique Lab-In-Syringe (LIS)¹ and coupled online to HPLC with diode array detection. Iron (III) thenoyltrifluoroacetate complex², was used as a novel dissolvable sorbent. A 40 µL of pre-dissolved sorbent was dispersed in the sample solution inside the void of an automatic syringe pump upon which it precipitated and yielded a solid nanostructured sorbent. The sorbent was efficiently retained on a melamine foam packing at the syringe inlet where the analytes were adsorbed. Afterthat, the analytes and sorbent were dissolved in a methanolic ascorbic acid solution for elution, and the eluent was directly injected to online coupled HPLC for analysis. Parameters influencing the method including extraction pH, volume of sorbent solution, stirring rate, sample volume, extraction time, eluent volume, and transfer volume to HPLC were optimized. The automated method demonstrated excellent analytical performance, with linear working ranges from 1 to 1000 µg/L, sub-ppb detection limits, and recoveries between 98.3% and 110%. By operating sample pretreatment and chromatographic separation in parallel, the system achieved a sample throughput of 4.5/h, high repeatability (RSD < 5%), and preconcentration factors of 16.4 to 21.2. With an AGREE greenness score of 0.59, this novel LIS-dSPE approach offers a robust, reproducible, and environmental friendly for alkylphenol monitoring.

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SERS AND SALDI MS IMAGING: TOWARD ORTHOGONAL IDENTIFICATION OF BIOMOLECULES

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Surface-enhanced Raman spectroscopy (SERS) investigates the molecular structure of biomolecules adsorbed on silver nanostructures through the enhancement of Raman scattering.¹ As a non-destructive technique, SERS is ideally suited for integration with surface-assisted laser desorption/ionization mass spectrometry (SALDI MS).² Because SERS and SALDI MS are based on fundamentally different physicochemical principles, they generate highly orthogonal datasets, providing complementary information. This work presents the development of a sample preparation protocol for multimodal imaging using SERS and SALDI MS.

A sputtering method was employed to rapidly produce a discontinuous silver nanoisland layer over a large sample area e.g. 1×1 cm. Key parameters relevant to multimodal imaging were evaluated, including the homogeneity of the nanoisland layer by scanning electron microscopy and its chemical stability by SERS measurements of model biomolecules. Indeed, silver oxidation induced by atmospheric gases was identified as a critical factor influencing substrate performance. To prevent oxidation, an argon-atmosphere chamber was constructed to protect silver-treated samples during SERS measurements. Furthermore, the chamber was equipped with a humidification unit facilitating gentle interaction between analytes and the silver nanoislands. The composition of the humidification solvent mixture was also systematically optimized. The performance of both SERS and SALDI MS for imaging the spatial distribution of proteins, e.g. myoglobin, in matrices of varying complexity was investigated. SERS measurements were performed using an InVia Raman spectrometer (Renishaw), while SALDI MS analyses were conducted with a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a subatmospheric MALDI source (MassTech Inc.).

The proposed method enables detailed sample characterization through complementary multimodal analysis, demonstrating the compatibility and potential of combined SERS and SALDI MS.

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ASSESSMENT OF GREEN SOLVENTS AS ALTERNATIVES TO ACETONITRILE FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY OF INTACT PROTEINS

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Growing awareness of environmental issues has accelerated the adoption of green analytical chemistry, including the development of novel high-performance liquid chromatography (HPLC) methods aimed at replacing toxic organic solvents and fluorinated mobile-phase additives, such as acetonitrile (ACN), methanol (MeOH), and trifluoroacetic acid (TFA), with more sustainable alternatives including ethanol, isopropanol, and ethyl lactate. However, the wider implementation of green solvents in HPLC is limited by their higher viscosity, elevated UV cut-off wavelengths, and different elution characteristics compared with conventional solvents. These properties may lead to increased backpressure, reduced separation efficiency, longer analysis times, and lower detection sensitivity. Furthermore, the retention of proteins is highly sensitive to mobile-phase composition due to the “on-off” elution mechanism, whereby even slight changes in eluent strength may cause a sudden desorption of the analyte from the stationary phase (1).

A standard mixture of seven intact proteins with various isoelectric points and molecular masses was separated by gradient elution on a Dionex UltiMate 3000 Series UHPLC System using Restek Viva C4 (2.1 × 100 mm, 300 Å, 5 µm) and Waters XBridge Protein BEH C4 (2.1 × 100 mm, 300 Å, 3.5 µm) columns. The reference method employed a water–ACN gradient (20–90%) containing 0.2% (v/v) formic acid and 0.2% (v/v) difluoroacetic acid in mobile phase, with a run time of 16 min (2). Alternative greener methods employed water–ACN (20–90%), water–ethanol (5–60%), and water–isopropanol (5–60%) gradients containing 0.2% (v/v) formic acid, with run time ranging from 16 to 30 min. The column temperature was maintained at 40 °C, and the mobile phase flow rates of 0.3 or 0.4 mL/min were used to optimize system pressure. Although the use of green solvents resulted in lower separation efficiency, satisfactory chromatographic performance was still achieved. The application of green solvents in protein analysis is therefore promising; however, further research is needed to fully exploit their potential and overcome the remaining challenges.

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DEVELOPMENT AND VALIDATION OF HPLC-UV METHOD FOR CEFIDEROCOL ANALYSIS

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Cefiderocol is a siderophore cephalosporin antibiotic with significant activity against multidrug-resistant Gram-negative pathogens. Its unique chemical structure enables active transport across the outer membrane, allowing more efficient penetration into bacterial cells. (1) This study aimed to develop and validate a reliable HPLC-UV analytical method for the determination of cefiderocol in bulk solution with evaluation of key validation parameters including specificity, linearity, precision, accuracy, and sensitivity. Chromatographic analyses were performed using a reverse-phase high-performance liquid chromatography (RP-HPLC) system coupled with a UV detector. The separation was carried out on a Sunfire C18 column. The mobile phase consisted of 0.1 % formic acid in LC–MS grade water (A) and acetonitrile (B). Gradient elution was applied with initial mobile phase conditions of 99:1. The flow rate was set to 1.0 mL/min, and the column temperature was maintained at 40 °C. Sample injection was performed with an injection volume of 10 µL. Detection was achieved using UV spectrophotometry at a wavelength of 260 nm. A reverse-phase HPLC-UV method for cefiderocol quantification was developed and validated using a C18 column with UV detection at 260 nm. Chromatographic separation was achieved within 18 min, with a cefiderocol retention time of 4.91 min. Linearity was confirmed over the concentration range of 4.15–66.5 µg/mL ($r^2 = 0.9997$). Intra- and inter-day precision expressed as RSD were 0.5% and 3.1%, respectively. The experimentally determined LOD and LOQ values were 0.052 µg/mL and 0.208 µg/mL, respectively. The developed method was successfully applied to the analysis of bulk solution samples. The developed HPLC-UV method enables reliable and reproducible quantification of cefiderocol with satisfactory sensitivity, precision, accuracy, and specificity and is suitable for routine analytical application in the determination of cefiderocol and other structurally similar β -lactam antibiotics in bulk solutions, pharmaceutical dosage forms, and plasma samples.

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Analytical profiling of biliary bile acids using solid-phase microextraction for assessment of liver graft viability

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Background: Normothermic ex vivo liver perfusion (NEVLP) provides an opportunity to assess graft function before transplantation by monitoring metabolic activity. Bile represents a particularly informative matrix; however, its complex composition and high analyte concentrations pose challenges for conventional sample preparation. This study aimed to develop SPME-based analytical strategies for comprehensive and rapid profiling of biliary bile acids (BAs) during the peritransplantation period.

Methods: A high-throughput thin-film microextraction coupled with liquid chromatography–mass spectrometry (TFME-LC-MS) method was optimized and validated for the quantification of 14 BAs in bile. In parallel, a direct SPME–microfluidic open interface–mass spectrometry (SPME-MOI-MS) approach was evaluated for rapid analysis of BA isobar groups without chromatographic separation. Both approaches were applied to bile samples collected from porcine heart-beating donors (HBD, n=5) and donation-after-circulatory-death (DCD, n=5) grafts subjected to 90 min of warm ischemia and subsequent NEVLP and transplantation.

Results: The validated TFME-LC-MS method demonstrated linearity across 0.1–1000 ng/mL ($R^2 > 0.99$), with LLOQs of 1–10 ng/mL and acceptable intra- and inter-day accuracy and precision. Matrix effects did not exceed 12%, while analyte stability ranged from 86–109%. Longitudinal profiling revealed distinct BA dynamics associated with ischemia and the subsequent stages of perfusion and reperfusion. Notably, DCD grafts showed altered levels of TCA, GCA, GCDCA, TCDCA, TLCA and TDCA at selected time points. The direct SPME-MOI-MS approach generated results consistent with LC-MS, with correlations exceeding $R=0.87$ for most monitored isobar groups.

Conclusions: SPME-based workflows enable sensitive, reproducible and complementary assessment of biliary BA profiles during liver transplantation. The combination of quantitative TFME-LC-MS with rapid SPME-MOI-MS analysis may provide a practical analytical approach for dynamic, near-real-time evaluation of graft function and ischemic injury during NEVLP.

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POSTER PRESENTATION: MALDI-TOF Mass Spectrometry Imaging of *Zophobas morio* Larvae Fed with Polystyrene

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Plastic pollution and persistent microplastics necessitate new biological degradation strategies. Superworms (*Zophobas morio*) may be an effective invertebrate model for polystyrene (PS) breakdown. While plastic consumption is well documented, spatial molecular adjustments across larval organs remain largely unexplored. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging (MALDI-TOF MSI) was implemented to map tissue-specific biomolecular and peptide changes in cryosections of larvae fed expanded polystyrene (EPS 100) for 28 days relative to standard-diet controls. Larvae were sacrificed in precooled ethanol on dry ice and embedded in a non-interfering gelatin–carboxymethylcellulose (CMC) matrix, which avoided the polymer background typical of commercial freezing compounds. Longitudinal 10 µm whole-body cryosections were thaw-mounted onto indium tin oxide (ITO) slides, followed by ethanol washing and automated aerosol deposition of α -cyano-4-hydroxycinnamic acid (HCCA). A parallel set of sections received a dedicated on-tissue treatment tailored for in situ peptide mapping. Measurements were performed in positive linear mode (m/z 1,000–20,000 Da) at 50 µm lateral resolution. Principal component analysis (PCA) revealed distinct clustering between control and PS-fed cohorts, demonstrating that dietary plastic introduces pronounced biochemical divergence confined mostly to the digestive tract rather than somatic tissues. Non-parametric Mann–Whitney testing confirmed statistically significant ($p < 0.05$) signal increases in the PS group for key ions, prominently m/z 2424.5, 2848.4, 3611.6, and a dominant peak at m/z 3723.3 Da localized within the gut lumen and epithelium. Peptide-targeted cryosections resolved discrete spatial remodeling of low-molecular-weight fragments along the intestinal barrier. These results confirm MALDI-TOF MSI as a reliable, label-free tool for in situ evaluation of larval gut adaptation during synthetic polymer degradation.

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Label-Free Impedimetric Immunosensor for Exosome Detection

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Exosomes, nanoscale extracellular vesicles (EVs) with diameters ranging from approximately 30-150 nm, are secreted by almost all cell types. They are present in all body fluids and play a crucial role in intercellular communication (1). They are also involved in numerous physiological pathways, including tissue remodelling, homeostasis regulation, and neuronal development, as well as in pathological conditions including thrombosis, inflammation, cancer progression etc. Their main biological function is the transfer of bioactive cargo derived from parental cells, including proteins, lipids and nucleic acids (miRNAs, mRNAs, and others), which can affect the behavior of recipient cells (2). This ability makes exosomes attractive candidates for the detection, monitoring, and prognosis of various diseases, including cardiovascular, neurodegenerative disorders, or cancer. Exosomes represent promising biomarkers for non-invasive diagnostic applications. Common methods for their detection and characterization are ELISA, Western blotting, flow cytometry etc (3,4).

Electrochemical impedance spectroscopy (EIS) is a highly sensitive technique for monitoring changes at the electrode-solution interface. It is based on the application of a low-amplitude alternating potential to an electrochemical system and analysis of resulting response. It allows label-free and non-destructive detection of biomolecular interactions occurring on or near the electrode surface (5).

In this work, we present a label-free immunosensor for exosome detection based on electrochemical impedance spectroscopy (EIS), employing boron-doped diamond electrodes modified with anti-CD81 antibodies.

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Microelution solid phase extraction using green solvents for top-down CE-MS protein analysis

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In recent years, there has been a growing demand for making the analytical processes sustainable and environmentally friendly. As a result, it is essential to evaluate the environmental impact of both established procedures and newly developed methods. Identifying and addressing processes that contribute to environmental harm is crucial for minimizing their ecological impact. In proteomics, sample preparation is a critical step involving various extraction methods for the isolation of target proteins from biological fluids. Traditionally, these methods use large volumes of organic solvents, such as acetonitrile, which are known to be dangerous and challenging to dispense.

According to green analytical chemistry principles, the environmental impact of the methods can be reduced either by miniaturizing the processes or by replacing organic solvents with less toxic alternatives. In our work we investigated the use of environmentally friendly solvents (ethanol, isopropyl alcohol, acetone, and organic carbonates) for microelution solid-phase extraction (μ SPE) to isolate low molecular weight proteins spiked into pooled human serum, plasma, urine, and saliva. Additionally, the impact of acidic modifiers in elution solvents, various eluate treatment strategies, and various μ SPE devices were evaluated. Following extraction, the eluate could be directly, after a simple dilution, injected into capillary electrophoresis-mass spectrometry, eliminating the need for nitrogen evaporation and preventing unnecessary analyte loss.

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ANALYSIS OF INFLIXIMAB USING MULTISEGMENT INJECTION CE-DAD WITH SAMPLE PRECONCENTRATION

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Monoclonal antibodies (mAbs) are currently widely used in clinical practice. Due to their complex protein structure, mAbs are susceptible to degradation under suboptimal handling and storage conditions throughout the entire chain from manufacturing to administration. Besides this, strong evidence supports the use of therapeutic drug monitoring (TDM) for mAbs, thereby highlighting the need for robust and reliable quantitative analytical methods.

Based on the reviewed literature, mAbs are predominantly quantified using bottom-up LC-MS/MS approaches. In contrast, clinical practice in Slovakia relies mainly on ELISA-based assays for TDM, despite the potential advantages of capillary electrophoresis (CE).

The method used in this study builds on prior optimisation of the BGE: 2 M acetic acid + 5 % ACN, with the primary aim of increasing analytical throughput. For multisegment injection, the optimised parameters included spacer injection length and maximum number of sample segments. In addition, two online preconcentration strategies: transient isotachopheresis (tITP) and field-amplified sample stacking (FASS) were investigated and compared using infliximab at concentration 0.05 mg/mL.

Both tITP and FASS led to a rapid improvement in peak resolution. Optimal conditions for both preconcentration approaches consisted of a 50 s sample and a 200 s spacer injection at 50 mbar, using 3 sample segments. For tITP, the optimal ammonium acetate concentration enabling effective preconcentration was 75 mM.

In summary, MSI-CE-DAD combined with sample preconcentration was applied, to the best of our knowledge, for the first time to the analysis of a monoclonal antibody. The proposed approach increases sample throughput and contributes to a “greener” analytical workflow. Further method development and optimisation of MSI-CE will include implementation of MS detection. Future applications of a fully validated method may include quality control or TDM of mAbs.

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EXPOSE! - A PLATFORM FOR GAS, AEROSOL, AND PARTICULATE TOXICOLOGICAL EXPOSURE FOR AIR-INTERFACING TISSUES

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Air-interfacing tissues such as airway and mucosal epithelia are primarily exposed to environmental toxicants via gases, aerosols, and particulate matter. In real-world settings, these exposures occur as complex, low-concentration mixtures such as urban smog and fine dust, with adverse effects often emerging only after prolonged exposure. Toxicological studies rely on animal models or long-term clinical observations, highlighting the need for improved phase-specific exposure strategies *in vitro*. Air–liquid interface (ALI) cultures provide a physiologically relevant basis for such studies by exposing the apical epithelial surface directly to air while maintaining nutrient supply from the basal compartment. Compared with continuously submerged cultures, ALI conditions promote epithelial differentiation and the development of more representative barrier and mucosal phenotypes[1]. However, controlled exposure of these models remains challenging, particularly for aerosols containing either liquid droplets or solid particles [2]. Deposition is governed by aerosol generation, particle or droplet size, transport, and impaction, requiring exposure systems capable of delivering reproducible doses without disrupting the epithelial interface.

To address these limitations, a modular, pressure-driven exposure platform is proposed to enable controlled application of liquid aerosols and solid particulate matter to air-interfacing tissues. Complementary *in situ* exposure to complex environmental gaseous pollutants is achieved via thermodesorption-based enrichment. The current system demonstrates sensor integration for monitoring temperature, relative humidity, gas concentration, pressure, and flow, enabling characterization of enrichment and transport. Initial operation of a self-regulating thermodesorption unit confirms the feasibility of controlled adsorption–desorption cycles for trace-gas handling. Bronchial, nasal, and buccal epithelial barrier models are currently being established under ALI conditions. Integration of these tissue models with the aerosol delivery and thermodesorption-based gas enrichment modules will provide a versatile exposure platform for investigating complex, multiphase environmental exposures *in vitro*.

Acknowledgement

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OPTIMIZATION OF PRECONCENTRATION TECHNIQUES FOR THE ANALYSIS OF MONOCLONAL ANTIBODIES BY CAPILLARY ELECTROPHORESIS

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Capillary electrophoresis (CE) enables rapid, high-resolution separations with low sample and solvent consumption. However, CE has limited detection sensitivity due to the injection of very small sample volumes. Preconcentration techniques can increase the amount of analyte in the capillary and improve its detectability.^{1,2} These include dynamic pH junction (DPJ) and transient isotachopheresis (tITP), applicable to proteins, peptides, and samples with high salt content.^{3,4} This study aimed to optimize and compare these techniques for the analysis of the monoclonal antibodies (mAbs) infliximab (IFX) and rituximab (RTX) and assess their effects on analyte amount in the capillary and detector response. Separations were performed by CE using 2 M acetic acid as the background electrolyte (BGE) and UV detection at 280 nm. Optimization included selecting suitable concentrations of ammonium bicarbonate (AmBi) for DPJ and ammonium acetate (NH₄Ac) for tITP. Sample injection time and the effects of organic solvent addition were also investigated. The selected conditions were subsequently applied to reduced forms of IFX and RTX. For DPJ, 10 mM AmBi and a 300 s injection time were suitable, whereas 250 mM NH₄Ac and a 300 s injection time were selected for tITP. Addition of acetonitrile (ACN) to BGE provided more favorable results than methanol (MeOH) with both techniques. For DPJ, 1% ACN was selected for intact IFX, whereas 2.5% ACN provided a higher detector response and more favorable separation for the reduced form. For tITP, 2.5% ACN was selected; however, applying the optimized conditions to reduced forms of IFX and RTX did not yield complete separation of the mAb subunits. Adjusting the preconcentration conditions increased detector response in the analysis of IFX and RTX, while applying these techniques to reduced forms revealed differences in the effectiveness of DPJ and tITP while further optimization will be performed.

Acknowledgement

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PORTABLE HPLC SYSTEM FOR MOBILE LABORATORY - ANALYSIS OF HAZARDOUS COMPOUNDS

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High-performance liquid chromatography (HPLC) is a widely used separation technique for analysing non-volatile substances, including thermolabile and high-molecular-weight substances soluble in water or other common solvents, e.g., drugs, toxins, environmental pollutants, and many others. Laboratory HPLC systems are used in forensic, medical, pharmacological or environmental analysis; however, these bulky instruments are limited to laboratory use. Advances in the miniaturisation of components have already enabled the development of portable systems applying the advantages of HPLC in field laboratory environments, enabling to perform the analysis at the point of need. We present a miniaturised portable HPLC instrument system consisting of a simplified capillary LC scheme capable of gradient elution and using commercially available capillary microcolumns. The system is equipped with a newly developed compact optical detector with a unique capillary detection cell and CCD spectrometer capable of simultaneous multi-wavelength UV-Vis absorbance and fluorescence monitoring [1]. We applied this system for the analysis of selected CWA surrogates and degradation products, toxins, drugs, polycyclic aromatic substances and other toxic substances. The presented results demonstrate the capability of a miniaturised capillary liquid chromatograph to perform fast and repeatable separation of toxic compounds in standard mixture and/or in complex matrices. The research follows with the application of the portable HPLC for other hazardous agents of concern.

Acknowledgement

This work was supported by the Security Research Programme of the Ministry of the Interior of the Czech Republic (project No. VB02000015) and by the Czech Academy of Sciences (Institutional research plan RVO:68081715).

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Micro-pipette tips based on nanofibers for the extraction of mycotoxins

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Sample preparation is still the most demanding step of the entire analysis. Current research is focused on developing new extraction methods for sample pretreatment before analysis. It is desirable to simplify and miniaturize this procedure as much as possible. Solid phase extraction (SPE) is a popular method because it offers a wide range of modifications, mainly through the development of new sorbents and materials. Nanomaterials are starting to gain considerable interest in this area, and therefore research is devoted to their use in a number of applications such as industry, biomedicine or environmental analysis (1). An indisputable advantage of nanomaterials as extraction sorbents is their surface to volume ratio (2). In our study, we focused on nanofiber discs for direct extraction, that miniaturize the extraction volumes and eliminate other source of errors. The target group of analytes of interest were mycotoxins ochratoxin A and zearalenone. Mycotoxins are responsible for causing physiological, biochemical and pathological changes in almost all living organisms. Some of them are listed as carcinogens (ochratoxin A). Our results present micro-pipette tips extraction (SPE-PT) on several different nanomaterials in the form of discs, that showed various extraction yields. The highest extraction efficiency ($\geq 80\%$) for both toxins provided (PCL (polycaprolactone), PCL + ZrO₂ (40%), PA6 (polyamide 6)) while the lowest results were observed for (PPS (polyphenylene sulfide), PBT + MB (polybutylene terephthalate), PCL + MB). Both mycotoxins were quantified using liquid chromatography with fluorescence detection.

Acknowledgement

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SPME-Based Chemical Biopsy for Early Complication Assessment in Kidney Transplantation

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The shortage of donor organs remains a major challenge in transplantation, increasing the need to expand the donor pool, including through the use of extended criteria donors. However, kidneys from higher-risk donors are more susceptible to ischemia-reperfusion injury and early post-transplant complications, potentially affecting graft function. Early assessment of graft status and identification of patients at increased risk may therefore support timely clinical decision-making and improve post-transplant care.

This study employed solid-phase microextraction (SPME)-based chemical biopsy for direct assessment of kidney graft status and early identification of post-transplant complications. The SPME approach enabled minimally invasive graft sampling, while thin-film microextraction provided rapid plasma preparation for complementary assessment of systemic changes. Chromatographic separation coupled with high-resolution mass spectrometry was used to characterize kidney tissue and recipient plasma. The feasibility of rapid targeted lipid determination using SPME coupled with a microfluidic open interface to mass spectrometry (SPME-MOI-MS) was also preliminarily explored. Multivariate and machine-learning approaches were applied to identify compounds associated with early graft dysfunction and evaluate their relevance for risk assessment and postoperative monitoring.

The analyses identified candidate compounds associated with early graft dysfunction, including amino acids and their derivatives, nucleotides, organic acids, peptides, and lipids. Compounds detected in donor kidney tissue contributed to pre-transplant graft risk assessment, while changes in recipient plasma provided complementary information during the early postoperative period. Preliminary SPME-MOI-MS experiments demonstrated the feasibility of rapid determination of selected lipids, supporting the development of rapid targeted analysis.

SPME-based chemical biopsy enables minimally invasive, direct assessment of kidney graft status and, combined with complementary plasma analysis, provides information relevant to early complication risk assessment and graft monitoring. Targeted analysis and rapid SPME-MOI-MS may further support the development of clinically applicable methods for kidney transplantation.

Acknowledgement

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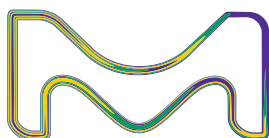
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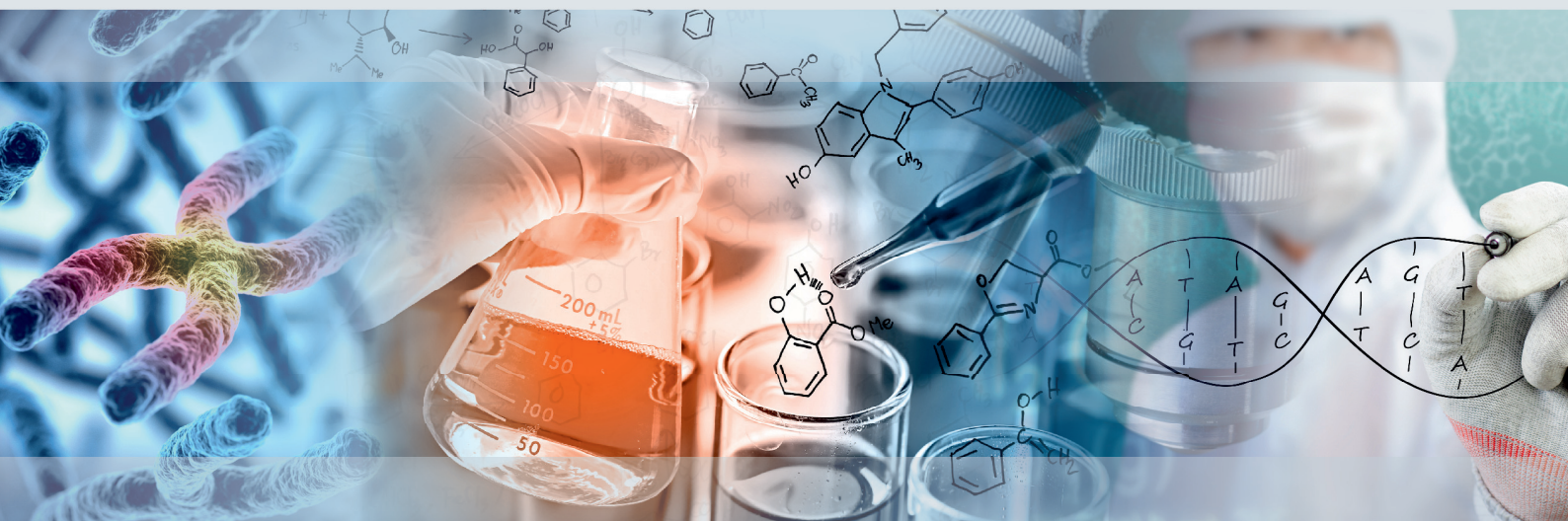
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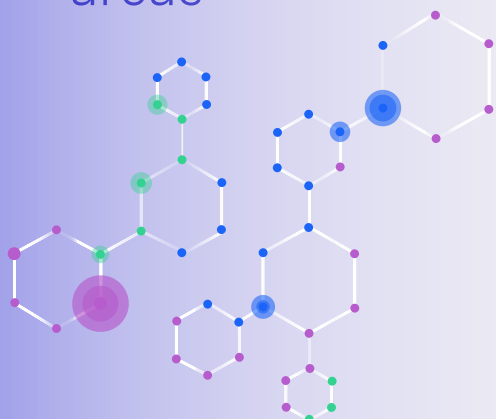
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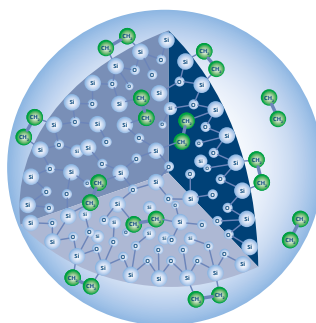


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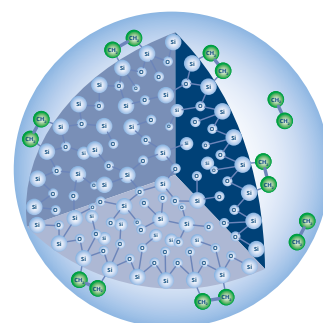
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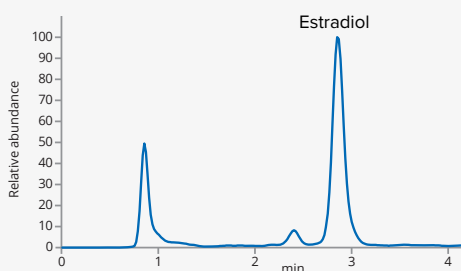
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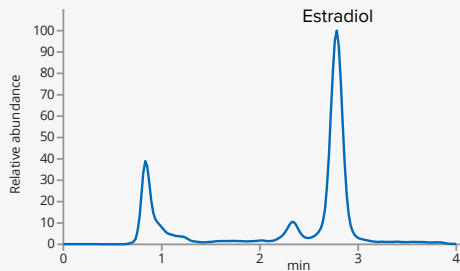
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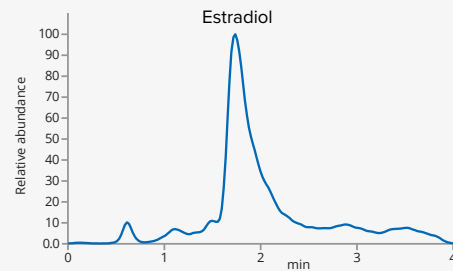
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