14th Balaton Symposium

on High-Performance Separation Methods

September 7-9, 2025, Hotel Azúr****, Siófok, Hungary

Book of Abstracts

Hungarian Society
for Separation Sciences

14th Balaton Symposium
on High-Performance Separation Methods

Lectures

September 7-9, 2025
Hotel Azúr**, Siófok, Hungary**

L-01 | Molecules that change shape during GC analysis: unusual behaviour explained by use of GC×GC

Philip J. Marriott

Australian Centre for Research on Separation Science, School of Chemistry, Monash University, Clayton, Australia

Presenter email: philip.marriott@monash.edu

Comprehensive two-dimensional gas chromatography (GC×GC) offers additional separation and peak capacity compared to one-dimensional GC (1DGC). The 2D contour plots in GC×GC provide intriguing insight into unusual chemical processes, often not evident in 1DGC.

An on-column rearrangement of a molecule's structure, where each structure can be separated by the column, could be reversible ($A \rightleftharpoons B$) or irreversible ($A \rightarrow B$); e.g., represented by isomerisations, rearrangements or decompositions. The on-column process constitutes progressive conversion of one species into the other (controlled by thermodynamics and kinetics), and in GC×GC, this generates a 'streak' pattern in the 2D contour plot. In 1DGC, for a complex mixture, these streaks may be completely 'transparent'; in GC×GC they are clear and unambiguously apparent. With judicious choice of phases and conditions, different streak patterns will be delineated, and this depends also on whether a reversible or irreversible process occurs.

We first observed this many years ago in 1DGC for sterically hindered aromatics; migration of a $Cr(CO)_3$ group in unsymmetrical aromatics; cis/trans isomers in $Cr(tfa)_3$; and the 'textbook' case of oximes (RCH=NOH). By using GC×GC we have examined *E*/*Z* oxime isomerisation including use of chiral columns in ^{1}D and ^{2}D, rearrangements in various essential oil samples (e.g. for germacrene compounds) [1] and recently, irreversible pyrethrin→isopyrethrin for changes both during injection and on-column [2]. Most recently we studied band shapes for Cope rearrangements in Myrrh oil [3].

This presentation will introduce on-column 1DGC processes above. Then GC×GC results for oxime and *Chrysanthemum cinerariifolium* extracts, and the 'band shapes' seen in Myrrh essential oil (e.g. germacrene ⇌ elemene; furanodiene ⇌ curzerene). For *C. cinerariifolium*, of the six natural pyrethrins, two (pyrethrin I and pyrethrin II) undergo irreversible thermal isomerisation to the 'iso-compound', due to a shift in conjugated bonds. Effects of inlet temperature, oven temperature, oven temperature program (isothermal vs. gradient) and flow rate will be examined with a focus on qualitative interpretation of results.

References:

[1] Marriott P.J., et al. Shape-shifting molecule behaviour during comprehensive two-dimensional gas chromatography analysis – A review. *Journal of Chromatography Open*. 5. 100125, (2024).

[2] Byrnes N.A., et al. Exploring thermal isomerisation in gas chromatography analyses using natural pyrethrins: comparison of comprehensive two-dimensional and one-dimensional gas chromatography. *Journal of Chromatography A*. 1708, 464369, (2023).

[3] Lim R. et al. The observation of band shapes for shape-shifting molecule transformations in comprehensive two-dimensional gas chromatography. *Journal of Chromatography* A, Accepted for Publication.

L-02 | Two dimensions, one goal - finding the best chromatographic response function for two-dimensional liquid chromatography

Gerben B. van Henten, Jelle Alderden, Tijmen S. Bos, **Bob W.J. Pirok**

Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands

Two-dimensional liquid chromatography (2D-LC) is a powerful analytical technique that enables the separation of highly complex mixtures by combining two orthogonal separation mechanisms. However, its practical implementation remains hindered by the complexity of method development, which involves multiple interacting parameters across both dimensions. Bayesian optimization (BO) has emerged as a promising strategy for automated method development in 2D-LC, particularly because it does not rely on explicit retention models or peak tracking. A critical yet often overlooked factor in BO workflows is the choice of chromatographic response function (CRF), which quantifies separation quality and drives the optimization process.

In this study, a comprehensive evaluation of various CRFs, both single-objective and multi-objective, was conducted within a BO framework using purely in silico chromatograms simulated via retention modeling. Each CRF was assessed based on its optimization landscape, its sensitivity to chromatographic features such as resolution, its ability to guide BO toward meaningful chromatographic improvements and robustness.

Thoughtful CRF design, balancing chromatographic insight with computational tractability, is key to unlocking the full potential of BO for practical, high-performance 2D-LC method optimization. In this presentation, the results of this study will be presented. The importance of CRF selection in automated method development workflows for 2D-LC will be explained.

L-03 | Single-particle electrophoresis with in-plane nanofluidic devices

Stephen C. Jacobson

Department of Chemistry, Indiana University, Bloomington, United States
Presenter email: jacobson@iu.edu

We are developing integrated micro- and nanofluidic devices to probe individual nanoscale particles, e.g., virus capsids, liposomes, extracellular vesicles, and inorganic particles, with improved spatial and temporal resolution. To develop these measurement systems, we design in-plane architectures into which a range of functions can be integrated, e.g., reactions, filters, separations, sensors, and sorters. We use resistive-pulse sensing as a label-free, nondestructive technique to characterize the physical properties of individual particles, including their size, shape, electrophoretic mobility, and zeta potential. To improve measurement precision, multiple nanopores are fabricated in series, and to improve temporal response, reactions are coupled directly with the nanopore detection region. Moreover, these in-plane devices are easily coupled with fluorescence lifetime measurements to extract additional information from particles.

L-04 | Microfluidics-based electro-membrane extraction using a micro-pillar stabilized liquid membrane

Jörg P. Kutter[1], Anna Thu Hoai Nguyen[1], Nickolaj J. Petersen[1], Stig Pedersen-Bjergaard[1,2]

[1] *Dept. of Pharmacy, University of Copenhagen, Copenhagen, Denmark*
[2] *Dept. of Pharmacy, University of Oslo, Blindern, Oslo, Norway*

Sample preparation is an essential part of many analytical procedures, for clean-up or enrichment. In case of very small sample volumes or complex matrices, sample preparation is required to even allow a subsequent separation and detection to provide meaningful data.

Electromembrane extraction (EME) is a microextraction technique, where target compounds are extracted from a sample solution, across an organic phase, and into an aqueous acceptor solution [1]. Mass transfer and extraction are facilitated by an electrical field sustained across the organic phase. Downscaling and implementing this technique into a microfluidic device not only reduces sample and reagent volumes, but also enhances extraction efficiency and speed. EME also allows so-called "soft extractions", which are characterized by low recovery, yet high enrichment, and can thus be used where solutions need to be probed while essentially not disturbing any chemical equilibria (e.g., in microphysiological systems ("organ-on-chip")).

Traditionally, the organic liquid membrane is a supported liquid membrane (SLM), where a small volume of organic solvent is immobilized in a porous polymeric membrane stacked between the donor and acceptor compartments. However, when transitioning into the microchip format, this geometry is not optimal for quick exchange or renewal of the organic phase.

Here, we developed a microfluidic device incorporating a free liquid membrane (FLM) instead of the traditional SLM, i.e., direct interfaces between aqueous and organic phases without the need for an actual, physical membrane. To achieve this, the microfluidic channels need to be properly designed to provide stable interfaces under stagnant and flowing conditions, as well as during the application of a voltage while extracting. This is possible in a miniaturized format by exploiting geometry, surface tension, viscosity and chip materials with adequate contact angles. Specific designs including micro-pillar rows between the phases, and performance data will be discussed in the presentation.

Reference:

[1] Hansen, FA et al., J. Sep. Sci., 45, 2021, 246-257.

L-05 | Comparative evaluation of reversed-phase and hydrophilic interaction liquid chromatography columns for untargeted profiling of bioactive compounds in *Hypericum perforatum*

Martina Catani[1], Davide Barboni[1], Desiree Bozza[1],
Natasha Damiana Spadafora[2], Nicoletta Bianchi[3], Chiara De Luca[1],
Simona Felletti[2], Alberto Cavazzini[1,4]

[1] *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy*
[2] *Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy*
[3] *Department of Translational Medicine, University of Ferrara, Ferrara, Italy*
[4] *Council for Agricultural Research and Economics, CREA, Rome, Italy*

The achievement of a comprehensive profiling of plant metabolites has long represented a challenge, not only due to their wide-ranging abundances but also as a result of their considerable chemical diversity. Recent advances in highly sensitive liquid chromatographic (LC) techniques, particularly when coupled with high resolution mass spectrometry (HRMS), have established metabolomics as a key approach for the analysis of thousands of non-volatile metabolites in crude natural extracts. Nevertheless, the different polarities of primary and secondary metabolites often limit the efficacy of conventional reversed-phase liquid chromatography (RPLC) in providing exhaustive compound coverage. To address this limitation, orthogonal separation techniques such as hydrophilic interaction liquid chromatography (HILIC) should be employed as a complement to RPLC.

In this work, four columns with identical geometrical specifications but with different stationary phase chemistry (one reversed-phase C18 and three different HILIC adsorbents) were employed for the untargeted analysis of bioactive compounds contained in *Hypericum perforatum*. The columns were evaluated not only in terms of chromatographic performance but also based on their ability to resolve challenging isobaric compound pairs of isobaric compounds. Finally, the integration of RPLC and HILIC data enabled a more comprehensive characterisation of the metabolites associated with the plant.

L-06 | Possibilities of nanoparticle columns for ultra-high-performance LC

Hanrong Wen, Sebastiaan Eeltink, **Ken Broeckhoven**

Vrije Universiteit Brussels, Brussel, Belgium

The continuous demand for higher performance, high-throughput separations in the field of proteomics and metabolomics to resolve several hundred samples a day, requires to further push the limits of separation performance in liquid chromatography. The Knox-Saleem limit gives the upper limit of separation performance for a fully optimized system (column length, flow rate and particle size) and shows that improved column packing quality (lower h_{min}), higher operating pressure (ΔP_{max}) and more permeability column structures (reduced flow resistance ϕ) are the most straightforward options for further advancement. However, as the current limitations of 3D printing resolution currently do not allow us to obtain perfectly ordered and open structures with sufficiently small feature size, the use of packed bed columns will remain the mainstay in LC for the foreseeable future. To achieve superior performance to contemporary LC systems, lower particles sizes than those currently available, i.e. in the sub-micron range, will be required, especially if extremely high operating pressures (2000 bar and above) are envisioned. Based on the kinetic plot theory, for example, a 10-fold reduction in analysis time for N=100,000 can be achieved when using 500 nm nanoparticles operated at 3000 bar. An additional advantage of the use of nanoparticles is their tendency towards self-assembly into highly-ordered crystalline-like structures. However, a trade-off exists for these highly ordered, but dense packings (low porosity) due to their high flow resistance. The required very high operating pressures for use in short columns (<5-10 cm) also dictates the use of capillary column formats to alleviate the effects of viscous heating.

To achieve such massive gains in separation speed and thus sample throughput, several hurdles need to be overcome. First of all, packing procedures need to be optimized, tuning slurry (colloid) stability, and packing conditions to assemble 'perfectly' packed nano-columns. In addition, the effect of column and particle diameter, their respective ratio, and colloid concentration was investigated. These optimizations allowed to obtain robust nanoparticle columns through convective assembly of stable colloids with free choice of particle sizes and capillary diameters. SEM imaging showed no significant change of column bed structure after grafting. Surprisingly, the flow resistance of the nanoparticle column was found to be similar to that of conventional packed columns ($\phi \approx$ 600-800). A 4.5 cm C_{18} 750 µm nanoparticle column was tested on Vanquish-timsTOF (ΔP_{max} = 150 MPa) with a BSA digest separation, showing good run-to-run repeatability (RSD of residence times < 0.2%). The potential of these columns was further highlighted with a HeLa digest separation, showing its potential for low-input and fast gradient peptides separations.

L-07 | Luck and strange!

Imre Sallay

Osaka Soda, Osaka Nishi-ku, Japan

The traditional division between clean, easy to purify synthetic peptide processes and recombinant peptide purification (always messy, treacherous and complicated) just disappeared due to great advances in synthetic technology and the science behind.

Nasty surprises are in store for the challengers jumping in the fray for manufacturing weight loss peptides in super large scale.

GLP-1 molecules can be even worse than insulin and insulin analogs when it comes to self-aggregation/fibrillation of the peptide.

The fibrillation of the GLP-1 peptides during the purification step will be discussed. Fibrillation being a self-accelerating process gets so bad that the aggregated peptide molecules block the flow of mobile phase, driving up the back pressure of the HPLC column prohibitively high.

To make the peptide purification process profitable, the fouled-up columns need to be cleaned to gift the expensive stationary phase new life.

The Cleaning In Place of the silica opens a new Pandora's box. CIP (a good CIP) can remove the aggregated peptide layer from the silica, but the cleaning is done in so aggressive solutions that they start to damage the silica!

The CIP is the Necessary Evil in the peptide purification process of the GLP-1 peptides.

The pros and cons of the CIP are discussed in details.

L-08 | Adsorption studies in supercritical fluid chromatography operated with alternative solvents as a tool for greener chiral separations

Simona Felletti[1], Chiara Nosengo[2], Chiara De Luca[2], Martina Catani[2], Alberto Cavazzini[2,3]

[1] *Dept. of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy*
[2] *Dept. of Chemistry, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy*
[3] *Council for Agricultural Research and Economics, Rome, Italy*
Presenter email: simona.felletti@unife.it

Chemistry is moving towards more sustainable techniques that adhere to the 12 principles of green chemistry. This shift necessitates the use of non-toxic, eco-friendly solvents and methods or techniques that minimize the waste produced.

In this field, supercritical fluid chromatography (SFC) has (re)emerged as an optimal alternative to liquid chromatography for the green transition in both analytical and preparative scale chromatography [1]. Indeed, the employment of CO2 as mobile phase is particularly advantageous in terms of both sustainability and chromatographic efficiency, in contrast to LC. This makes SFC particularly attractive for separating chiral compounds, especially in the pharmaceutical industry. Nevertheless, despite these advantages, the complete understanding of the influence of the mobile phase properties on the adsorption behavior of analytes is still not fully accessed. This complicates the accurate modeling and prediction of chromatographic behavior under SFC conditions, limiting the ability to optimize and scale up SFC processes effectively [2].

Recently, dimethyl carbonate (DMC) has been explored as a non-toxic, green co-solvent in SFC [3]. However, fundamental studies on its use under SFC conditions have never been documented so far. In this work, the effect of the co-solvent type (DMC vs methanol) and concentration (0-10%) on adsorption properties of Whelk-O1 chiral selector has been investigated for trans-stilbene oxide (TSO) racemic mixture.

Results of this study will be helpful to fully assess the potential of SFC in various applications.

References:

[1] G. Guiochon, A. Tarafder, Journal of Chromatography A, 1218 (2011) 1037–1114
[2] M. Enmark, P. Forssén, J. Samuelsson, T. Fornstedt, Journal of Chromatography A, 1312 (2013) 124–133
[3] D. Folprechtová, et al., Analytica Chimica Acta 1332, (2024) 343380

L-09 | The dynamic balances in HPLC: An unconventional application of temperature to optimize ionizable solute separation

Krisztián Horváth[1], Barnabás Soós[1,2], Róbert Kormány[2]

[1] *Analytical Chemistry Research Group, University of Pannonia, Veszprém, Hungary*
[2] *Drug Substance Analytical Development Division, Egis Pharmaceuticals PLC, Budapest, Hungary*

Column temperature (CT) is a critical parameter in reversed phase high performance liquid chromatography (RP HPLC), traditionally leveraged to adjust retention times and reduce eluent viscosity. While its effects on analyte diffusion and mass transfer are well established, its potential role in modulating selectivity - especially via indirect pH shifts - remains underexplored. This study investigates the interplay between CT and selectivity for ionizable compounds, with particular emphasis on analytes whose pKa values lie near the working pH of the mobile phase.

We show that, beyond its classical uses, CT can serve as a practical tool for fine tuning chromatographic selectivity by exploiting the temperature dependence of solute pKa. We present a mathematical model that links temperature, pH, and retention, and validate it in a case study of structural isomers (*ortho* , *meta* , *and para* substituted analogues) of the same compound, where controlled CT adjustments reversed their retention order.

This strategy is particularly relevant to pharmaceutical impurity profiling, where early elution of impurities—prior to the peak of the active pharmaceutical ingredient (API)—can enhance detectability through improved resolution and signal to noise. Overall, the findings highlight an often overlooked opportunity to deploy CT not only for performance optimization but as a selectivity altering parameter in method development.

L-10 | Employing multidimensional design space modeling across frequent chromatographic challenges—published use cases

Arnold Zöldhegyi[1], Barnabás Soós[2], Krisztián Horváth[3], Imre Molnár[1], Róbert Kormány[2]

[1] *Molnár-Institute for Applied Chromatography, Berlin, Germany*
[2] *Egis Pharmaceuticals Plc., Budapest, Hungary*
[3] *University of Pannonia, Center for Natural Sciences, Faculty of Engineering, Veszprém, Hungary*

In high-performance liquid chromatographic (HPLC) applications, the development of efficient, robust, and well-transferable methods is a fundamental requirement. Analytical procedures must deliver consistent performance not only during initial implementation but also throughout the later stages of their lifecycle—including unforeseen future applications.

Despite this need, robustness issues are frequently reported across the industry, often traceable to poor initial method design, such as incorrectly selected working conditions or insufficient control of critical method attributes. In this context, modeling tools built on systematic knowledge—aligned with Analytical Quality by Design (AQbD) principles—have proven particularly valuable, both for proactively eliminating potential issues and for reactively troubleshooting existing failures.

Among expert software solutions, the DryLab 4 modeling suite stands out by uniquely and effectively combining chromatographic theory with the minimal number of experiments necessary to understand a given separation problem. From a practical standpoint, the modeling process is logical and streamlined: the experimentally calibrated Design Space (DS) generated by the software encompasses all separation options within the studied system. The resulting digitized knowledge base offers extensive opportunities for in-model investigations, such as evaluating parameter settings, assessing method transferability, and robustness testing. Moreover, these unique modeled fingerprints are inherently suited for systematic characterization and comparison of complete separation systems, including the assessment of some unaccounted variables commonly encountered in industrial practice.

Through several peer-reviewed examples, we aim to showcase a sound, systematic modeling strategy for addressing specific challenges associated with dynamic nature of separations. These include: (1) identifying suitable replacement columns and workable operating conditions; (2) comparing various column chemistries and competitive stationary phases; (3) evaluating batch-to-batch inconsistencies; (4) mitigating HPLC system-to-system differences; and (5) managing transferability challenges arising from differences between volatile and non-volatile buffer systems within identical pH-settings.

These case studies clearly demonstrate that, alongside appropriate instrument and stationary phase selection, a detailed, multidimensional understanding of the interactions among method parameters—and their consistent fine-tuning—is essential for achieving and maintaining consistent analytical method performance.

L-11 | Excess adsorption isotherms of methanol and acetonitrile from aqueous solutions on silica-based stationary phases

Evgenii Mashkov, Tamás Kristóf, Krisztián Horváth

University of Pannonia, Center for Natural Sciences, Faculty of Engineering, Veszprém, Hungary

The interfacial behavior of solvent mixtures plays a critical role in chromatographic retention mechanisms. This study investigates the excess adsorption isotherms of methanol–water and acetonitrile–water binary systems on bare silica stationary phases. The experimental component employed the minor disturbance method (MDM) implemented on a high-performance liquid chromatography (HPLC) system equipped with a refractive index detector. A series of injections was conducted under varying solvent compositions to quantify the excess adsorption of each component. The resulting isotherms revealed pronounced solvent-specific interactions: water exhibited strong preferential adsorption under organic-rich conditions, while methanol and acetonitrile showed increased adsorption primarily in water-rich compositions, demonstrating distinct competitive behaviors for available surface sites.

To complement these observations, molecular dynamics simulations for the acetonitrile–water system were performed using the RASPA software package. Two amorphous silica models characterized by distinct surface hydrophilicity were applied to capture the influence of surface chemistry on solvent structuring and orientation. Simulations yielded molecular density profiles and orientational distributions that provided insight into the microscopic organization of acetonitrile–water mixtures at the silica interface. Notably, the simulation results qualitatively supported the experimental trends, confirming the preferential enrichment of water molecules on hydroxylated surfaces under acetonitrile-rich conditions and enhanced retention of acetonitrile on less hydrophilic, dehydroxylated surfaces. This convergence underscores the utility of integrating chromatographic measurements with molecular modeling to achieve a more comprehensive understanding of solvent–surface interactions.

Although some quantitative differences were observed between experimental and simulation data due to methodological and structural simplifications, both approaches consistently captured the key physicochemical phenomena governing retention.

L-12 | From HILIC to PALC on a single column: Uncovering the hidden complexities of retention mechanisms

Ivett Bacskay[1], Mikołaj Dembek[2], Alessandro Buratti[3], Martina Catani[3], Szymon Bocian[2], Attila Felinger[1,4]

[1] *Department of Analytical and Environmental Chemistry and Szentágothai Research Center, University of Pécs, Pécs, Hungary*
[2] *Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland*
[3] *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy*
[4] *Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary*
Presenter email: bacskay@gamma.ttk.pte.hu

Chemically bonded Diol-P-R stationary phases containing both hydrophobic and hydrophilic ligands can be used in per-aqueous liquid chromatography (PALC) and may also be suitable for hydrophilic interaction liquid chromatography (HILIC). When the stationary phase possesses multiple types of functional groups, different interactions contribute to retention during separation. According to chromatographic theory, the retention mechanisms of PALC and HILIC should be fundamentally different.

Retention mechanisms can be reliably investigated using nonlinear chromatographic techniques, besides analysing retention data of highly diluted samples at the analytical scale. The processes occurring at the interface between the mobile and stationary phases can be described and understood through the determination of nonlinear equilibrium isotherms, for which we applied the inverse method.

Accurate determination of the column's hold volume is key to correctly interpret the adsorption isotherms. Overestimating the total hold-up volume can result in significant errors when calculating overloaded peaks and adsorption isotherms. In contrast, underestimating the total porosity has little to no effect on the predicted band profiles, although it may suggest a more heterogeneous model.

In our work, we determined the void volume of polar-functionalized stationary phase columns using several chromatographic techniques, and investigated how the value of total porosity affects the shape of adsorption isotherms determined by the inverse method. We found that both reversed phase and hydrophilic interaction chromatographic processes can occur on these stationary phases, depending on the composition of the mobile phase.

L-13 | Innovative chromatographic strategies for the characterization of mRNA-based drug products

Davy Guillarme[1,2], Athanasios Tsalmpouris[1,2], Marc Francois-Heude[3], Jean-Francois Cotte[3], Camille Malburet[3], Jonathan Maurer[1,2]

[1] *School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland*
[2] *Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Geneva, Switzerland*
[3] *mRNA Center of Excellence, Sanofi, Marcy l'Etoile, France*
Presenter email: davy.guillarme@unige.ch

Messenger RNA (mRNA) has emerged as a crucial platform for vaccines and novel therapeutics, offering new options for the prevention of infectious diseases and the treatment of cancer. mRNA offers numerous advantages, such as high yields from cell-free in vitro transcription, cost effectiveness, and excellent safety profile. However, these molecules are particularly difficult to analyze, due to their large size (1,000-5,000 nucleotides), highly anionic phosphate backbone, strong hydrophilicity, and propensity for complex secondary structures. Comprehensive characterization of these macromolecules and their lipid nanoparticle (LNP) formulations requires advanced chromatographic approaches capable of resolving intact transcripts from their impurities, and check encapsulation efficiency with high sensitivity and reproducibility.

Here, we report the development and optimization of four different chromatographic strategies for mRNA-bas5ed drug products. First, ion-pair reversed-phase liquid chromatography (IP-RPLC) was adapted for large RNA integrity assessment. Thirteen alkylamine ion-pair reagents (weak to strong) were systematically evaluated across pH 6–8, temperatures of 45–85 °C. In the end, by mixing several moderately hydrophobic IP reagents, marked improvements in selectivity and peak shape for RNA species up to 6 kb were observed. Second, size-exclusion chromatography (SEC) was evaluated using various ultra-wide-pore (1,000 Å) columns from different providers. Despite limited resolving power intrinsic to pore-based separation of such large polymers, we showed the applicability of SEC for high- and low-molecular-weight species contained in two different mRNA products of different sizes. Next, an anion-exchange chromatography (AEX) method was established to quantify LNP encapsulation efficiency (EE). A glycine/NaCl mobile-phase system (pH 10.1–11) incorporating Triton X-100 allow reducing mRNA carryover, improved recovery, and discriminated free, surface-associated, and fully encapsulated mRNA. The results were critically compared with the reference method, Ribogreen for 20 candidate vaccines (1–2.5 kb). Finally, hydrophilic interaction chromatography (HILIC) was used for direct online LNP disruption, intact mRNA integrity analysis and measurement of EE. Using BEH amide columns, we proved that on-line and off-line disruption yielded equivalent EE for commercial LNP products (Comirnaty, Spikevax), with EE of 95.7 % (HILIC), 94 % (RiboGreen), and 96.7 % (AEX).

Together, these chromatographic methods offer an interesting toolkit for the characterization of mRNA products, facilitating regulatory compliance and accelerating pipeline development.

This work was jointly funded by the University of Geneva and Sanofi. Marc François-Heude, Jean-François Cotte, and Camille Malburet are/were employees of Sanofi and may hold shares and/or stock options in the company. Davy Guillarme, Athanasios Tsalmpouris, and Jonathan Maurer declare no competing interests.

L-14 | Automated method development in two-dimensional liquid chromatography for the characterization of polymers

Tijmen S. Bos[1,2], Bob W.J. Pirok[1,2]

[1] *Analytical Chemistry Group, Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, The Netherlands*

[2] *Centre for Analytical Sciences Amsterdam (CASA), Amsterdam, The Netherlands*

Although 2D-LC offers powerful capabilities for resolving highly complex mixtures, its broader application has been hindered by the labor-intensive and time-consuming nature of method development. To overcome this limitation, several research groups focus on integrating chemometric techniques and machine learning algorithms to streamline and accelerate this process. The aim is to facilitate wider access to the benefits of 2D-LC across diverse scientific and industrial applications.

In this light, our group has designed an automation framework "AutoLC" [1], to enable closed-loop, iterative method optimization for samples with unknown or complex compositions. This system operates by processing experimental data to inform the selection of new method parameters, repeating this cycle until a specified performance criterion is met. Key algorithmic components include peak tracking, multi-start regression, retention modeling, and Bayesian optimization, which collectively allow for automated and interpretable decision-making in method development.

While this approach and others have demonstrated strong performance in small molecule analysis, extending it to the characterization of synthetic and biological polymers introduces significant challenges. Unlike conventional analytes, polymeric systems are often represented by continuous or multimodal distributions rather than discrete peaks. As such, the development of novel distribution-sensitive detection algorithms, tailored chromatographic descriptors, and appropriate objective functions is required. These efforts are supported by the integration of high-resolution separation techniques with advanced computational tools capable of extracting meaningful chemical information from complex datasets.

This presentation will discuss these challenges and recent advancements aimed at bridging the gap between the theoretical potential and practical application of 2D-LC in polymer science, with implications for enhancing analytical workflows in both research and industrial settings.

Reference:

[1] T.S. Bos, J. Boelrijk, S.R.A. Molenaar, B. van 't Veer, L.E. Niezen, D. van Herwerden, S. Samanipour, D.R. Stoll, P. Forré, B. Ensing, G.W. Somsen, B.W.J. Pirok, Chemometric Strategies for Fully Automated Interpretive Method Development in Liquid Chromatography, Anal Chem, 94 (2022), pp. 16060-16068, 10.1021/acs.analchem.2c03160

L-15 | All you need is: 2LabsToGo-Eco! Fast, sustainable access to critical analytical-toxicological data

Gertrud Morlock, Wolfgang Schwack, Maria Romero, Kevin Jakob, Johannes Schmidt, Thomas Nimmerfroh

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

How to avoid exposure to mutagenic, genotoxic, and cytotoxic compounds in daily life? Fast knowledge on such critical analytical data is gained by planar separations hyphenated with planar bioassays [1]: no kiss on lipstick lips, reduce to eat red meat, avoid skin creams, wound-healing creams, perfumes, *etc*. [2, 3].

To start with the groundbreaking technology providing insights into sample safety and quality, the 2LabsToGo-Eco [4] is an affordable option. It consolidated the analytical laboratory and biological/toxicological laboratory in a sustainable all-in-one system. Designed and developed not only for reliable screening but also quantitative analyses, it exploited open-source technologies, 3D printing, miniaturization, portability, flexibility, adaptability, effect-directed detection, accessibility, affordablity, environmental friendliness, dematerialization, and sustainability. For rapid assignment and identification of prioritized hazardous compounds a 12D hyphenation [5] was demonstrated.

The prioritization strategy is disruptive because it shifts the focus from simply separating and identifying all chemical components – which often results in overwhelming data about unknown compounds with unclear biological relevance – to actively detecting the biological/toxicological effects of compounds within complex samples. This approach directly highlights which substances are biologically active or hazardous, answering critical questions about sample safety and benefits that conventional analytical methods frequently miss.

Acknowledgements: Lecture is dedicated to Csaba Horváth, Professor of Chemical Engineering at Yale University.

References:

[1] Morlock, G.E. Chemical safety screening of products – better proactive, J. Chromatogr. A 1752 (2025) 465946
[2] Morlock, G.E., Zoller, L. Fast unmasking toxicity of safe personal care products, J. Chromatogr. A 1752 (2025) 465886
[3] Morlock, G.E., Heil, J. Fast unmasking hazards of safe perfumes, J. Chromatogr. A 1754 (2025) 465959
[4] Romero, M.C.O., Jakob, K., Schmidt, J., Nimmerfroh, T., Schwack, W., Morlock, G.E. Consolidating two laboratories into the most sustainable lab of the future: 2LabsToGo-Eco, Anal. Chim. Acta 1367 (2025) 344103
[5] Schreiner, T., Ronzheimer, A., Friz, M., Morlock, G.E. Multiplex planar bioassay with reduced diffusion on normal phase, identifying androgens, verified antiandrogens and synergists in botanicals via 12D hyphenation, Food Chem. 395 (2022) 133610

L-16 | Novel streamlined approaches for olive oil authentication using SFC-MS and 2D-LC-MS

Irene Caño Carrillo, Bienvenida Gilbert-López and **Juan F. García-Reyes**

University of Jaén, Jaén, Spain

The authentication of virgin olive oil (VOO) remains a significant challenge in the context of food quality control and fraud detection. The unsaponifiable fraction is commonly analyzed to assess both the quality and authenticity of VOO, with particular emphasis on its sterol and triterpenic alcohol content. Conventional methods for this analysis often require significant time and involve complex procedures such as saponification, fractionation, and derivatization. In this presentation, two novel streamlined approaches based on advanced chromatographic techniques coupled to mass spectrometry (MS), including two-dimensional liquid chromatography (2D-LC) and supercritical fluid chromatography (SFC), are presented. These methodologies have enabled the direct analysis of key minor components in olive oil with minimal sample pretreatment, thereby reducing solvent consumption and overall preparation time. The application of 2D-LC-MS has been reported for the detailed profiling of triterpenic alcohols, free sterols, and steryl esters in a single run without the need for saponification and derivatization, offering a more comprehensive characterization of olive oil samples. Meanwhile, SFC-MS has proven to be a rapid, environmentally sustainable, and efficient strategy for the simultaneous assessment of a broader range of unsaponifiable compounds, including sterols, triterpenic alcohols, and hydrocarbons such as squalene and stigmasta-3,5-diene, the latter being a crucial marker for VOO authentication. Therefore, employing diverse and advanced analytical strategies enhances the accuracy and effectiveness of olive oil authentication by providing detailed chemical information, streamlining sample preparation, and reducing environmental impact.

L-17 | Ion exchanger-based chiral stationary phases applied for enantioselective separations

István Ilisz, Gábor Németi, Antal Péter, Róbert Berkecz

Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary

Within the pharmaceutical sector, chirality has become a pivotal element in drug development and commercialization. Thus, there is a notable need for dependable analytical methods to measure enantiomeric purity, as well as to aid in pharmacological research, pharmacodynamic assessments, and clinical investigations. High-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) stands out as one of the most effective techniques for separating chiral compounds, applicable at both analytical and preparative stages. Over recent years, numerous chiral compounds have been studied and proposed as potential CSPs. Notably, CSPs based on ion exchangers have shown particular efficacy in enantioseparating ionizable analytes. Quinine- and quinidine-based ion-exchange CSPs have garnered significant attention due to their distinctive structural characteristics, which incorporate both basic and acidic functional groups alongside chiral centers with defined stereochemistry. These traits allow for highly efficient and selective enantiomer interactions, rendering these CSPs essential in HPLC-based enantioselective analyses. This lecture aims to showcase enantiomeric separations achieved using ion-exchange CSPs with quinine and quinidine-based selectors. The study investigates how various factors, such as the composition of the mobile phase solvent, types of basic additives, counter-ion concentrations, and the chiral selector's structure, impact enantioselectivity. Additionally, a thermodynamic evaluation using a simplified van't Hoff methodology is discussed.

Acknowledgements: This work was supported by the National Research, Development and Innovation Office-NKFIA through project K137607. Project no TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

L-18 | Advancing metabolomics with novel analyzer technology, intelligent data acquisition and data processing

Luka Milivojević

Thermo Fisher Scientific, Breda, The Netherlands
Presenter email: luka.milivojevic@thermofisher.com

Since its introduction, back in 2005, Orbitrap™ mass analyzer has enabled science to solve some of the most challenging analytical problems due to high quality data it provides. In 2023 we have introduced a novel Astral™ analyzer to the world of mass spectrometry. Both analyzers are part of Thermo Scientific™ Orbitrap Astral series of mass spectrometers which makes these instruments dual HRAM (High Resolution Accurate Mass) analyzer devices. As they work in parallel each contributes with its own specific advantages. While large molecule workflows are almost always challenging due to molecule size and complexity, also small molecule analysis can be incredibly challenging once we start untargeted or even targeted analysis of samples that contain numerous compounds in difficult matrixes. A good example of that is metabolomics. Orbitrap Astral series mass spectrometers, due to their high performance, accuracy, precision and speed are perfect for complex metabolomic workflows and are together with intelligent data acquisition and intelligent data processing advancing these workflows to a new level. We will show some examples of how we can increase sample throughput to meet large cohort demands while providing high quality quantitative and qualitative results with more depth in unknown analysis.

L-19 | The greening of liquid chromatographic methods

Oktawia Kalisz[1], Marek Tobiszewski[2], **Szymon Bocian**[1]

[1] *Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland*

[2] *Department of Analytical Chemistry, Faculty of Chemistry and EcoTech Center, Gdańsk University of Technology, Gdańsk, Poland*

Liquid chromatography is a technique that is gaining increasing interest and application. At the same time as the increase in the number of analyses performed, the amount of organic waste produced increases. Therefore, new methods and materials are being sought to achieve sustainable chromatography or so-called green chromatography.

Due to the harmful effects of organic solvents on humans and the environment, "green" technologies are being developed. Separation techniques, including chromatographic techniques, rely on the use of large amounts of organic solvents. As a result, there is growing interest and demand for research into the separation of mixtures under environmentally friendly conditions, through the reduction of solvent consumption and the use of safer solvents.

There are many ways to make liquid chromatography "green". These include: decreasing the dimensions of the HPLC column to use smaller flows that reduce the amount of organic waste generated; operating at elevated temperatures; replacing organic solvents with other, more "green" solvents such as water, ethanol, dimethyl carbonate, ethyl lactate, or supercritical CO_2. Among the above-mentioned, separations performed using pure water as the only eluent in HPLC are the most environmentally friendly; however, it is the most difficult to perform. There is no doubt that the future of sustainable chromatographic methods will be alternative solvents, with dimethyl carbonate, ethanol, and ethyl lactate at the forefront. The green aspects of chromatography are the main issue of this presentation.

L-20 | Use of green solvents for the isolation of melittin from bee venom

Chiara Nosengo[1], Amin Tabesh, Chiara De Luca[1], Amir Faraji[1], Hassan Rezadoost[2], Alberto Cavazzini[1,3], Simona Felletti[4], Martina Catani[1]

[1] *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy*
[2] *Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran*
[3] *Council for Agricultural Research and Economics (CREA), Rome, Italy*
[4] *Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy*
Presenter email: chiara.nosengo@unife.it

Honeybee venom is a complex mixture of enzymes, peptides, lipids, and small molecules and it is widely used in traditional medicine. Among all the compounds, melittin, a 26 amino acids peptide, representing about 50 % of the dry venom exhibits several bioactive functions such as anticancer, anti-inflammatory, and antimicrobial properties. However, to exploit the full potential of this compound it is necessary to find suitable purification methods able to remove the principal allergens of bee venom: Phospholipase A2 and apamin [1]. Nevertheless, in literature the isolation of this compound was only obtained at low yield, or with the employment of complicated procedure making the entire purification process solvent and time consuming [2]. For this purpose, the most promising technique is preparative reversed phase chromatography with the use of acetonitrile (ACN) as organic modifier thanks to its well-known chromatographic properties. However, this solvent is classified as toxic, in addition, significant amount is present into the waste eluting from the chromatographic run. In this context, following the principles of Green (analytical) Chemistry, it is crucial to develop new sustainable and more effective chromatographic methods able to recover the highest product possible, and at the same time, reduce the solvents consumption and enhance the operator safety. One of the options is the replacement of ACN with greener alternatives such as ethanol (EtOH) and isopropanol (IPA), or more recently dimethyl carbonate (DMC). As a matter of fact, DMC has been shown to be suitable for the purification of therapeutic peptide under RP conditions with similar performance to ACN. Moreover, the elution strength of DMC was demonstrated to be roughly 2.5 times higher than ACN, with a noticeable reduction in the total amount of organic modifier used [3], [4].

In this study, the feasibility of different organic modifiers for the isolation of melittin from bee venom has been evaluated in terms of final yield, total purity and organic solvent consumption, moreover, the overall greenness was then accessed through the application of different green metrics.

References:

[1] F. Sobral, A. Sampaio, S. Falcão, M.J.R.P. Queiroz, R.C. Calhelha, M. Vilas-Boas, I.C.F.R. Ferreira, Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal, Food Chem. Toxicol. 94 (2016) 172–177. https://doi.org/10.1016/j.fct.2016.06.008.

[2] A.C.L. Teoh, K.H. Ryu, E.G. Lee, One-step purification of melittin derived from Apis mellifera bee venom, J. Microbiol. Biotechnol. 27 (2017) 84–91. https://doi.org/10.4014/jmb.1608.08042.

[3] D. Bozza, C. De Luca, S. Felletti, M. Spedicato, F. Presini, P.P. Giovannini, M. Carraro, M. Macis, A. Cavazzini, M. Catani, A. Ricci, W. Cabri, Dimethyl carbonate as a green alternative to acetonitrile in reversed-phase liquid chromatography. Part II: Purification of a therapeutic peptide, J. Chromatogr. A. 1713 (2024) 464530. https://doi.org/10.1016/j.chroma.2023.464530.

[4] Felletti, S., Spedicato, M., Bozza, D., de Luca, C., Presini, F., Giovannini, P. P., Carraro, M., Macis, M., Cavazzini, A., Catani, M., Ricci, A., & Cabri, W. (2023). Dimethyl carbonate as a green alternative to acetonitrile in reversed-phase liquid chromatography. Part I: Separation of small molecules. *Journal of Chromatography A, 1712*. https://doi.org/10.1016/j.chroma.2023.464477

L-21 | Dissecting the single cell proteome: From sample preparation to data interpretation

Alienke van Pijkeren[1], Kristoffer Basse[1], Stefan van Teijlingen[1], Surina Chuckaree[1], Jing Zheng[1], Mohhamed H. Kaniyar[2], Omar G.R. Bringas[2], John LaCava[2,3], **Péter L. Horvatovich**[1], Guinevere S.M. Lageveen-Kammeijer[1]

[1] *Analytical Biochemistry, Groningen Institute of Pharmacy, University of Groningen, Groningen, The Netherlands*

[2] *European Research Institute for the Biology of Ageing, University Medical Centre Groningen, Groningen, The Netherlands*

[3] *Laboratory of Cellular & Structural Biology, The Rockefeller University, New York, United States*

Single cell proteomics (SCP) is emerging as a transformative approach to dissect cellular heterogeneity by enabling proteome-level insights at single-cell resolution. Despite rapid advances, SCP remains analytically and computationally challenging due to low signal intensities, sample scarcity, and the lack of optimized data processing strategies. Many available software tools were originally developed for bulk proteomics and are often suboptimal for SCP-specific data characteristics. Robust analytical and bioinformatic workflows tailored to SCP are therefore essential to ensure data quality, reproducibility, and biological interpretability.

This work presents the development of a reliable SCP workflow combining single-cell isolation with the CellenONE system, optimized low-volume sample preparation, and label-free LC-MS/MS analysis using the timsTOF SCP platform. Given the low signal intensity in SCP datasets, a systematic evaluation of data processing strategies was undertaken to assess their effect on identification depth, quantification precision, and downstream statistical interpretation.

Data acquired from A549 and HeLa cells were analyzed using Spectronaut and DIA-NN software under three conditions: analyzing cells separately (Individual), together (Matching), or alongside high-input carrier samples (Boosting). Additionally, a benchmarking dataset composed of mixed HeLa, yeast, and *E. coli* digests at single-cell-equivalent concentrations was processed through over 80,000 workflows combining different methods for peptide precursor and protein quantification, normalization, imputation, and differential expression analysis (DEA).

Results demonstrated that the choice of data analysis strategy significantly influences identification rates and data completeness. The Matching approach improved data consistency and reduced cell-to-cell variability, whereas Boosting enhanced detection of low-abundance proteins but reduced per-cell quantification accuracy. Among downstream methods, sum-based protein quantification paired with single-value imputation improved DEA performance, particularly when processed using DIA-NN. The finalized SCP pipeline was applied to investigate proteomic alterations in ORF1p knock-down N2102Ep cells.

This work contributes to the establishment of standardized, reproducible pipelines for single-cell proteomics. Current efforts focus on expanding analytical depth, investigating post-translational modifications at the single-cell level such as single cell glycomics, and integrating SCP with spatially resolved techniques. Future developments aim to combine deep single-cell proteomics with mass spectrometry imaging and laser microdissection (LMD) to achieve spatially resolved, high-content proteomic profiling in complex tissue environments.

L-22 | Direct injection ESI-MS analysis of proteins with high matrix content: Utilizing Taylor–Aris dispersion

Attila Gáspár, Ruben Szabó, Cynthia Nagy

Dept. of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary
Presenter email: gaspar@science.unideb.hu

We recently demonstrated the effectiveness of Taylor–Aris (TA) dispersion in reducing interferences typically encountered during electrospray ionization-mass spectrometric (ESI-MS) analysis of therapeutic proteins without pre-separation or purification [1]. The approach, easily implemented using commercial CE-MS systems, enables direct injection analysis through the use of submicroliter sample volumes introduced into a 65 cm long, 50 µm i.d. capillary under 1 bar pressure. This method, termed Taylor–Aris dispersion-assisted mass spectrometry (TADA-MS), is particularly well-suited for analyzing proteins with low diffusion coefficients in complex matrices containing small organic and inorganic compounds. In addition to highlighting the key features of TADA-MS, the presentation will emphasize its advantages in native protein analysis: (i) significantly enhanced sensitivity due to reduced matrix effects, (ii) the use of more "native-like" analytical conditions, (iii) compatibility with direct injection of non-MS-friendly matrices, and (iv) a considerable simplification and economization of the workflow is ensured [2].

To address the common issue of protein adsorption to capillary walls - leading to peak distortion and poor reproducibility - we also introduce a simple strategy that minimizes wall interactions. When the analysis is carried out rapidly, the leading portion of the parabolic flow profile avoids contact with the wall, allowing a significant fraction of the sample to remain in the center of the capillary [3].

Acknowledgements: The authors acknowledge the financial support provided to this project by the National Research, Development and Innovation Office, Hungary (K142134).

References:

[1]. Szabo R., Nagy C., Gaspar A.: Direct Injection ESI-MS Analysis of Proteins with High Matrix Content: Utilizing Taylor–Aris Dispersion. Angew. Chem. Int. Ed. (2024), e202318225.

[2]. Szabo R., Gyemant Gy, Nagy C., Andrasi M., Gaspar A.: Taylor–Aris Dispersion-Assisted Mass Spectrometry for the Analysis of Native Proteins, Anal. Chem. 2024, 96, 28, 11309-11317

[3]. Szabo R., Andrasi M., Gaspar A.: Fast Taylor Dispersion Analysis for Minimizing Protein Adsorption Effects, Anal. Chem. 2025, doi: doi.org/10.1021/acs.analchem.5c02250

L-23 | Artificial intelligence in HPLC-MS: From retention time prediction to *de novo* structure determination

Ágnes Révész, Dániel Szabó, **László Drahos**

HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

This presentation explores the applications of artificial intelligence (AI) and machine learning in high-performance liquid chromatography-mass spectrometry (HPLC-MS). We discuss fundamental AI concepts, including neural networks and their theoretical capability to learn complex relationships based on universal approximation theorems.

Key applications demonstrated include: retention time prediction with high accuracy, automated chromatographic peak detection using deep learning models, AI-assisted HPLC method development, and advanced MS/MS spectrum interpretation for molecular identification. We present four main MS/MS spectra identification strategies, ranging from database matching to generative AI models for de novo structure determination.

Recent breakthroughs include self-supervised learning on massive spectral datasets, creating foundation models for mass spectrometry that can detect molecular features without explicit training—similar to advances in natural language processing.

Finally, a case study on elucidating a *Cyclocybe cylindracea* derivative highlights how AI tools such as MetFrag and SIRIUS can reduce structural-determination time by orders of magnitude compared with manual workflows.

L-24 | Phytochemicals in food and plant samples - how to solve analytical challenges

Irena Vovk[1], Vesna Glavnik[1], Maja Bensa[2], Breda Simonovska[1], Etil Guzelmeric[3], Nisa B. Sen[3], Péter G. Ott, Ágnes M. Móricz[4], Simona Strgulc Krajšek[5]

[1] *Laboratory for Food Chemistry, National Institute of Chemistry, Ljubljana, Slovenia*
[2] *Research Institute of Faculty of Health Sciences, Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia*
[3] *Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Kayisdagi Cad., Istanbul, Türkiye*
[4] *Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary*
[5] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
Presenter email: irena.vovk@ki.si

Phytochemicals are a large group of compounds, naturally occurring in plants. Phytochemicals have diverse chemical structures and a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory, enzyme-inhibitory effects, enhancement of immune response or cell-to-cell communication, lowering blood pressure and cholesterol level. Many phytochemicals consumed on a daily basis with our diet are still unknown. Despite their growing use in food supplements and functional foods, many of these compounds remain unidentified or poorly characterized due to analytical challenges. Chromatographic techniques play an important role in analysis and discovery of phytochemicals in different matrices. This presentation will address the development and optimization of chromatographic and hyphenated techniques—such as high-performance thin-layer chromatography (HPTLC-densitometry, HPTLC-image analysis, HPTLC-MS/(MS), HPTLC-EDA) and high-performance liquid chromatography ((U)HPLC-UV/Vis, (U)HPLC-MS/(MS))—for both targeted and non-targeted analyses of phytochemicals in complex plant and food matrices. Analyses of phytochemicals in diverse samples such as food supplements, food waste, bee pollen, flower pollen, invasive alien plant species (tree of heaven, Russian vine, Chinese knotweed, Japanese knotweed, giant knotweed, Bohemian knotweed), etc. will be discussed. Examples will show challenges related to the lack of commercial standards and standard reference materials, lack of chromophores, isomeric structures and stability of the analytes. Furthermore, challenges in non-targeted (HP)TLC–EDA analyses of antimicrobial compounds, antioxidants and enzyme inhibitors in crude extracts prepared from different parts of invasive alien plant species, flower pollen and bee pollen were mostly related to the influence of the stationary phase, detection reagents and detection modes. Profiling based on HPTLC–EDA–image analysis showed a big potential in studies of phytochemicals. The integration of complementary chromatographic techniques with EDA has proven indispensable for the analysis of phytochemicals in food and plant samples.

Acknowledgements: This study was supported by the Slovenian Research and Innovation Agency (ARIS; research core funding No. P1-0005; projects: J4-50138, J4-4561, N1-0235, V3-24049; bilateral project BI-TR/20-23-004; "Young Researchers" program - Maja Bensa), the Scientific and Technological Research Council of Türkiye (TÜBİTAK; project No: 119N569) and the National Research, Development and Innovation Office of Hungary (NKFIH; project SNN139496).

L-25 | Non-target screening and bioassay-guided isolation of bioactive components monitoring by high-performance thin-layer chromatography based methods

Ágnes M. Móricz[1], Márton Baglyas[1,2], Anna Cselőtey[1,2], József Balla[3], József Bakonyi[1], Ildikó Schwarczinger[1], Zoltán Bozsó[1]

[1] *Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary*
[2] *Semmelweis University, Doctoral School, Budapest, Hungary*
[3] *Department of Inorganic and Analytical Chemistry, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary*
Presenter email: moricz.agnes@atk.hu

High-performance thin-layer chromatography (HPTLC) enables rapid screening of botanical extracts, obtaining chemical fingerprints, running of non-target effect-directed analyses (EDA), and characterization of the compounds in the bioactive zones by HPTLC–HRMS/MS. Starting from a complex matrix, the most efficient way to isolate the compounds responsible for an antimicrobial effect is bio-assay guided fractionation and purification. It means that the isolation procedure, which includes several separation steps, is interlaced by biomonitoring steps that ensure skipping inactive fractions of the sample and leading to the pure active agent(s). A biomonitoring tool is preferred to be a reliable, fast, and high-throughput bio-screening method. HPTLC–EDA fulfills these requirements and parallel HPTLC–derivatizations and (HP)TLC–UV/Vis/FLD–MS analyses ensure the follow-up of the compounds of interest.

In our studies as monitoring tools HPTLC–antioxidant and HPTLC–antibacterial (*Bacillus subtilis*) assays were used and the bioactive isolates were obtained by a series of flash chromatography and/or semi-preparative HPLC separations. Structure elucidation were performed by nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC)–MS, and flow injection analysis (FIA)–HRMS/MS. The antioxidant or antimicrobial activity of the isolated compounds was assessed by microplate assays. With this procedure, nine antioxidant caffeate esters of fatty alcohols were identified in black locust (*Robinia pseudoacacia*) stem bark, and among them one has been reported in *R. pseudoacacia* stem bark, five are described for the first time in this species, and three are new natural compounds. Furthermore, five new labdane diterpenes were found in a goldenrod flower.

Acknowledgement: This work was partially supported by the National Research, Development and Innovation Office of Hungary (SNN139496).

L-26 | Possibilities and challenges of the annotation and quantification of lipid A molecules from gram-negative bacteria

Ágnes Dörnyei[1], Viktor Sándor[2], Anikó Kilár[2]

[1] *Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Hungary*
[2] *Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary*
Presenter email: dornyei@gamma.ttk.pte.hu

Lipid A is the endotoxic center of lipopolysaccharide (LPS), which is found in the outer membrane of Gram-negative bacteria. It plays a crucial role in bacterial physiology and immune recognition. Accurate annotation (structural identification) and quantification of lipid A are essential for understanding bacterial diversity, antibiotic resistance, and host-pathogen interactions. However, these tasks are complicated by the structural diversity and chemical lability of these specific saccharolipid compounds, as well as their affinity for a wide variety of cations.

Recent advancements in mass spectrometry-based lipid A analysis focus on MALDI-TOF screening and structural elucidation through tandem mass spectrometry combined with separation techniques. We are dedicated to developing strategies in this latter area. Natural mixtures of lipid A often contain closely related isomeric species that may co-elute during LC-MS/MS or CE-MS/MS analysis. This co-elution can result in chimeric MS/MS spectra, which complicates accurate annotation. In this lecture, we will demonstrate how positive electrospray ionization tandem mass spectrometry (ESI-MS/MS) has become a powerful tool for the identification and quantification of lipid A phosphorylation regioisomers.

L-27 | Characterization of pea and milk proteins to support research on enhancing nutritional value

Mercy Mukite, Judit Tormási, Mária Berki, Rita Tömösközi-Farkas, Enikő Horváth-Szanics, **László Abrankó**

Hungarian University of Agriculture and Life Sciences (MATE), Institute of Food Science and Technology, Budapest, Hungary
Presenter email: abranko.laszlo.peter@uni-mate.hu

Food proteins are essential nutrients in human nutrition. Beyond the quantity consumed, increasing attention is being paid to protein quality, which is primarily determined by amino acid composition and digestibility—key factors influencing biological value. The nutritional quality of a protein is also influenced by, for example, the solubility of the protein, which can indirectly affect digestibility.

Understanding and optimizing these protein characteristics is an emerging focus in food and nutrition science. In this work, we present two examples that demonstrate the application of separation-based analytical techniques in supporting developments in this field. The first example highlights the use of SDS-PAGE and HPLC-UV methods to assess the changes in solubility and digestibility of pea proteins. The second example involves the characterization of milk protein profiles from A2 genetic variant cattle carrying the modified β-casein, using HPLC-UV, and the detection of the digestion-derived peptide β-casomorphin-7 (BCM-7) via UHPLC-ESI-MS/MS. These analytical tools provide valuable insights into protein functionality and support targeted strategies for enhancing the nutritional quality of food products.

Acknowledgements: This work was financially supported the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences (MATE) and the MATE - Proof of Concept and the 2020-1.1.2-PIACI-KFI-2021-00305 projects.

L-28 | A comparison of the fatty acid composition and nutritional quality indices of meat products and their plant-based alternatives

Tamás Marosvölgyi[1], Viktor Koczka[2,3], Tímea Dergez[1], Éva Szabó[3]

[1] *University of Pécs, Faculty of Medicine, Institute of Bioanalysis, Pécs, Hungary*
[2] *University of Pécs, Faculty of Health Sciences, Doctoral School of Health Sciences Pécs, Hungary*
[3] *University of Pécs, Faculty of Medicine, Department of Biochemistry and Medical Chemistry Pécs, Hungary*
Presenter email address: marosvolgyi.tamas@pte.hu

Objectives and Study: Plant-based diets are becoming increasingly popular with the growing interest in healthy lifestyles and more sustainable eating habits. A whole-food, plant-based diet has positive health effects. At the same time, the food industry has developed a variety of alternative products that differ significantly from a whole-food plant-based diet in their composition and degree of processing. The aim of this study was to compare the fatty acid composition and fatty acid-based nutritional quality indices of ground beef-based foods from different manufacturers and their fully plant-based alternatives.

Methods: We investigated six plant-based and four beef hamburger patties and one plant-based and one beef minced meat product, each with three different expiry dates. Total lipid contents were determined by gravimetry. Fatty acid composition was determined by GC-FID (C6:0-C26:0). Essential fatty acid index, n-3/n-6 polyunsaturated fatty acid ratio, unsaturation index, atherogenicity index, thrombogenicity index, hypocholesterolemic/hypercholesterolemic ratio were also calculated from the fatty acid values. Statistical analysis was performed using SPSS 29.0, with Kruskall-Wallis and Mann-Whitney tests. Significance values were adjusted by Bonferroni correction for multiple testing. A p-value of less than 0.05 was considered statistically significant.

Results: The fat content of the plant-based (10.25% [8.60%; 14.87%] median [Q1; Q3]) and animal-based products (19.67% [16.16%; 26.68%]) was significantly different ($p<0.01$). Significantly different fatty acid composition profiles were observed between investigated for both animal and plant-based product groups and within the plant based product group. There were significant differences between plant-based and animal-based products for each calculated lipid nutritional index within each food group.

Conclusion: Lipid content and fatty acid composition of plant-based meat alternatives are significantly different from the animal-based food, leading to differences in nutritional values. Further analytical and clinical studies are needed to provide a more accurate and comprehensive picture of the long-term health effects of the foods included in this study.

L-29 | Revival of slalom chromatography: A promising alternative to gel electrophoresis for large DNA/RNA analysis and purification

Fabrice Gritti, Matthew Lauber, Kevin Wyndham

Waters Corporation, Milford, United States

Slalom chromatography (SC) is a separation technique originally discovered in 1988 [1,2], recently revitalized using bio-inert (metal column hardware modified with a hydrophilic vapor deposited surface and diol-bonded particle surface), ultra-high-pressure liquid chromatography (UHPLC) columns and systems for analyzing large DNA/RNA fragments up to 30 kbp [3]. The first commercial SC column (Waters GTxResolveTM 250 Å Slalom Column, MaxPeakTM Premier Technology, 2.5 µm, 4.6 × 300 mm 250 Å) became available in 2025. The SC separation mechanism is an out-of-equilibrium process governed by the weak entropic elasticity of double-stranded DNA/RNA and the shear forces generated in the interparticle spaces of UHPLC columns. Unlike gel permeation chromatography (GPC), SC elutes smaller DNA fragments first and larger ones last.

In this presentation, we first revisit the recently established SC separation mechanism, grounded in the fundamental physical properties of DNA/RNA biopolymers (force-extension relationships) and laminar flow dynamics (shear forces) in packed beds, to identify the most relevant applications in cell and gene therapy, including DNA restriction mapping, plasmid topology analysis, and mRNA vaccine dsRNA impurity characterization. In the second part, we explore the practical advantages of SC in terms of ease of use, speed, resolution, and analytical accuracy/precision for DNA/RNA analysis and purification. Its overall performance is compared to the current gold standard: agarose gel electrophoresis (AGE).

SC was benchmarked against AGE for the separation of several DNA samples, including a 1 kbp DNA ladder (1.0–10.0 kbp), λ-DNA BstEII digest (1.3–8.5 kbp), λ-DNA HindIII digest (2.0–23.1 kbp), and λ-DNA Monocut Mix (1.5–48.5 kbp). SC is shown to be a simpler (standard UHPLC method), significantly faster (20×), higher peak capacity (2×), more sensitive (~1-5 ng detection limit), and more accurate/precise (±0.5% retention time) alternative to AGE for determining the length and size of DNA/RNA fragments > 3 kbp (Figure 1). This work introduces SC as a powerful bioanalytical and preparative technique for rapid, high-resolution characterization of nucleic acids in plasmid and mRNA manufacturing workflows.

References:

[1] J. Hirabayashi, K.-I. Kasai, Slalom chromatography: a new size-dependent separation method for DNA, Nucleic Acid Res. Symp. Ser., 20, 1988, 67-68.

[2] B. Boyes, D. Walker, P. McGeer, Separation of large DNA restriction fragments on a size-exclusion column by a nonideal mechanism, Anal. Biochem., 70, 1988, 127-134.

[3] F. Gritti, Retention mechanism in slalom chromatography: Perspectives on the characterization of large DNA and RNA biopolymers in cell and gene therapy, J. Chromatogr. A, 1743, 2025, 465691.

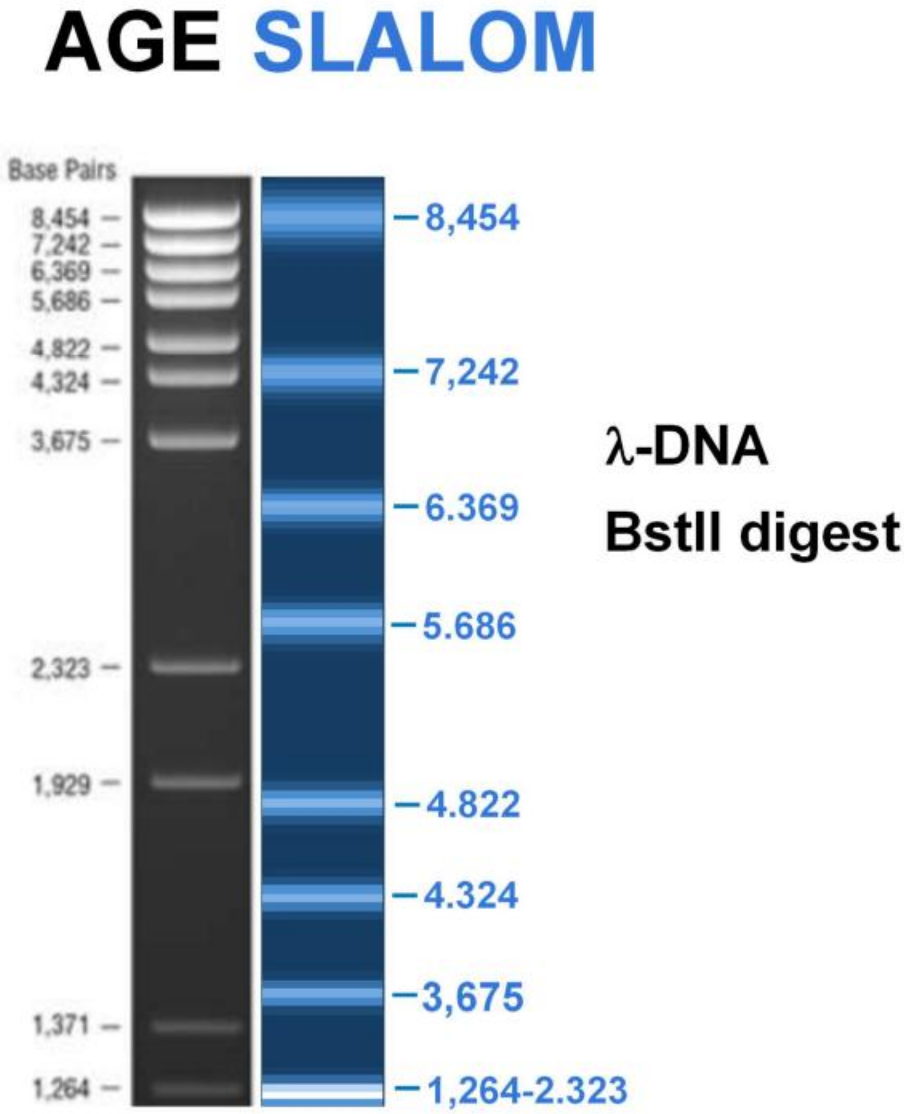

Figure 1: Comparison between Slalom Chromatograph (3 min run) and Agarose Gel Electrophoresis (60 min run) in DNA restriction mapping applications.

L-30 | Technoeconomic and sustainability evaluation of 3D printed monolithic adsorbers for integrated clarification and capture of therapeutic antibodies

Simone Dimartino[1], Mariachiara Conti[2], Yuki Abe[3], Andrew Sinclair[3], James Pullen[4]

[1] *Institute for Bioengineering, The School of Engineering, The University of Edinburgh, Edinburgh, United Kingdom*

[2] *Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, Tennessee, United States*

[3] *Biopharm Services Ltd, Chesham, United Kingdom*

[4] *Fujifilm Biotechnologies, Billingham, United Kingdom*

Monoclonal antibodies (mAb) are the dominant class of approved biopharmaceuticals, owing their success to their specificity to bind a virtually infinite variety of antigens. Large scale mAb manufacturing has been enabled by the development of a robust Protein A chromatography platform process. During the past 20 years, traditional protein A chromatography resins have seen only incremental improvements (such as enhanced binding capacity and alkaline stability of protein A) and are now run at maximum capacity to cope with the increase in mAb titres from upstream processing. Alternative convective media like membranes, nanofibres and monoliths have the potential to increase throughput thanks to unaffected performances at high flow rates, but generally suffer from low adsorptive performances and suboptimal fluid distribution.

With 3D printing technologies, the prospect of conceiving radical changes in chromatography adsorbents, such as new geometries and bespoke channels, has finally become a reality. The current 3D printing resolution of around 300 μm enables the fabrication of bespoke structures with flow through channels able to process fluids containing cells and/or cell debris, without risking particles' entrapment as it would occur in the interstitial voids of traditional packed beds or of monoliths. This potential removes the need for costly clarification procedures, usually performed in multiple rounds of filtration and/or centrifugation steps, before sending the harvest to the following chromatographic capture step. Thus, 3D printed monolithic adsorbers (PMA) could find application as integrated clarification and capture columns, combining into a single unit operation the functions currently performed by two distinct unit operations.

This work investigates the economic and sustainability advantages that the implementation of PMA could have in the biomanufacture of monoclonal antibodies (mAbs). A technoeconomic model that includes the PMA unit in the bioprocessing train is developed. After model calibration using experimental data of a prototype Protein A functionalized PMA, the economics and environmental burden arising from the combination of clarification and capture in a single unit operation are evaluated. The results obtained are compared against established mAb biomanufacturing processes, ultimately demonstrating that PMA have the potential to simplify bioprocess operations while at the same time producing Cost of Goods (CoG) savings, as well as a decrease in the process mass intensity (PMI) and carbon emissions.

L-31 | Carrier ampholyte-based isoelectric focusing in capillaries and gels with ion transport into electrode vessels where current density is different from that in the separation space is not a steady-state process, it is transient bidirectional isotachophoresis. Part 2

Gyula Vigh[1] and Bohuslav Gas[2]

[1] *Texas A&M University, College Station, United States*
[2] *Charles University, Prague, Czech Republic*

In 1961, Svensson described the separation of ampholytic compounds according to their isoelectric points (p*I* values) in temporally and spatially stable natural pH gradients that he created from carrier ampholytes (CAs), *in situ*, in a sucrose density gradient that eliminated thermal convection [1]. He called the process isoelectric focusing (IEF) and developed its theoretical model that stated that the extreme p*I* carrier ampholytes (that accumulated in distinct bands in front of the electrodes, at high concentration) created a diffusive flux into the separation space that balanced the electrophoretic flux bringing them back from the separation space to the electrodes, eventually establishing a steady-state process.

When polyacrylamide gel slabs and rods were later introduced for the reduction of thermal convection [2], the resulting pH gradients became temporally and spatially unstable [3], just as they did in capillary IEF [4]. Over time, the accumulating experimental evidence, together with the insight gleaned from the emerging dynamic simulation programs [5,6] led to the currently generally accepted two-stage model of the modern IEF experiment [7] stipulating that in the first, rapid focusing phase a well-defined pH gradient is formed that, in the second phase, is altered by slow isotachophoretic processes which gradually remove the extreme p*I* CAs, changing the span and shape of the pH gradient.

In my lecture at the 2023 Balaton Symposium I demonstrated, based on computer simulations completed with the newly released Simul 6 IEF/ITP program ([8], freeware at https://www.simul6.app), that in open IEF systems where all ions can enter the electrode vessels a single electrophoretic mechanism, transient, bidirectional isotachophoresis (TBD-ITP) operates from the first to the last moment of current flow. TBD-ITP is a process (i) whose outcome is determined by the ionic mobilities, pK_a values and loaded amounts of all ionic and ionizable - components; (ii) is constrained by both the total amount of charge transported through the separation space and the migration distance available for the leading ions, and (iii) can never reach steady-state with respect to the spatial coordinates of the separation channel.

In this lecture, using the key historic development, I will show that the separation science community - including me – should have long recognized, based on first principles, without computer simulations, that the modern IEF process *(i)* can never be a steady-state process, *(ii)* does not have an innate, inalienable two-step nature and *(iii)* does not have a preordained, decipherable natural end point, only an arbitrary, user-selected termination point, because it is a TBD-ITP ampholyte separation process.

References:

[1] Svensson, H. *Acta Chem Scand*. 1961;15:325.
[2] Leaback D, Rutter AC. *Biochem Biophys Res Commun*. 1968;32:447.
[3] Miles L, Simmons JE, Chrambach A. *Anal Biochem*. 1972;49:109.
[4] Hjerten S, Zhu MD. *J Chromatogr*. 1985;346:265.
[5] Palusinski OA, Allgyer TT, Mosher RA, BierM, Saville DA. *Biophys Chem*. 1981;13:193.
[6] Schafer-Nielsen C, Svendsen PJ. *Anal Biochem*. 1981;114:244.
[7] Thormann W, Mosher RA. *Electrophoresis*. 2021;42:814.
[8] Vigh, G, Gas, B. *Electrophoresis* 2023;44:667.

L-32 | Do we know what we want?

Peter Schoenmakers, Gerben van Henten, Bob Pirok

van 't Hoff Institute for Molecular Science (HIMS), University of Amsterdam, and Centre for Analytical Sciences Amsterdam Amsterdam, The Netherlands

The current excitement around artificial-intelligence (AI) and machine-learning (ML) methods brings renewed interest in computer-aided, or even fully automated method development for chromatography[1]. There are exciting new possibilities, including Bayesian optimization and reinforcement learning[2,3]. However, an often-cited bottleneck is our inability to define clear goals for the process. Developers of method-development strategies say they don't know what chromatographers want.

In this lecture the questions that will be addressed are "Do we (as chromatographers) know hat we want?" or "Are we (as chromatographers) able to bring across what we want"? We will consider when and where we can define clear goals, such as wanting to achieve sufficient separation in the shortest possible time and whether we can translate such goals in simple mathematical terms. A number of different optimization criteria, or chromatographic response functions (CRFs), will be compared. We will use examples to demonstrate how the goals we define affect the outcome of method optimization procedures.

As a final objective, we want to bridge the gap between the language of chromatographers and that of software developers.

References:

[1] T. S. Bos, J. Boelrijk, S. R. A. Molenaar, B. Van 'T Veer, L. E. Niezen, D. Van Herwerden, S. Samanipour, D. R. Stoll, P. Forré, B. Ensing, G. W. Somsen and B. W. J. Pirok, *Anal. Chem.*, 2022, **94**, 16060–16068.

[2] J. Boelrijk, S. R. A. Molenaar, T. S. Bos, T. A. Dahlseid, B. Ensing, D. R. Stoll, P. Forré and B. W. J. Pirok, *J. Chromatogr. A*, 2024, **1726**, 464941.

[3] A. Kensert, G. Desmet and D. Cabooter, *J. Chromatogr. A*, 2024, **1713**, 464570.

14th Balaton Symposium
on High-Performance Separation Methods

Posters

September 7-9, 2025
Hotel Azúr****, Siófok, Hungary

P-01 | Mathematical modeling of Taylor-Aris dispersion-assisted mass spectrometry for the study of operating conditions

Ruben Szabó[1], Attila Felinger[2,3,4], Krisztián Horváth[5], Attila Gáspár[1,6]

[1] *Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary*
[2] *Department of Analytical and Environmental Chemistry and Szentágothai Research Center, University of Pécs, Hungary*
[3] *HUN-REN–PTE Molecular Interactions in Separation Science Research Group, Hungary*
[4] *Institute of Bioanalysis, Medical School, University of Pécs, Hungary*
[5] *Research Group of Analytical Chemistry, University of Pannonia, Hungary*
[6] *HUN-REN Balaton Limnological Research Institute, Hungary*

An essential technique for protein sample analysis is electrospray ionization mass spectrometry (ESI-MS). As most matrix components are incompatible with this technique, sample purification or hyphenation of the MS to a separation technique is usually necessary. Taylor-Aris dispersion was recently demonstrated to enable the direct injection analysis of large molecules present in ESI-MS incompatible matrices, such as monoclonal antibodies present in their pharmaceutical formulations or native protein complexes in phosphate-buffered saline solution. This approach enables simple, fast, and cost-effective protein analyses [1, 2].

Establishing the ideal operating conditions for these analyses can be challenging as numerous parameters can affect the outcome of these measurements such as mobilizing pressures, capillary geometry, sample volume, analyte and matrix concentrations and characteristics. Therefore, the aim of this work was to develop a mathematical model that is capable of describing TADA-MS measurements under different flow and operating conditions.

Formulas describing Taylor Dispersion Analyses are known in the literature and we found that by adjusting these to account for the finite injected sample volume, the taylograms expected under different flow conditions can be described [3]. The signal-suppressing effects during ionization caused by the matrix components were described according to the work done by the Consortium for Top-Down Proteomics [4]. The final model enabled the in-silico investigation of the effects of operating conditions for Taylor-Aris Dispersion Assisted Mass Spectrometry, and a simple formula for the suggested injection volume was presented [5].

References:

[1] R. Szabo, C. Nagy, A. Gaspar, *Angew. Chem. Int. Ed.*, **2024**, 63, e202318225.
[2] R. Szabo, G. Gyemant, C. Nagy et al. *Anal. Chem.*, **2024**, 96, 11309-11317.
[3] H. Cottet, J. P. Biron, M. H. Martin, *Analyst.*, **2013**, 139, 3552–3562.
[4] D. P. Donnelly, C. M. Rawlins, C. J. DeHart et al. *Nat. Methods.*, **2019**, 16, 587–594.
[5] R. Szabo, A. Felinger, K. Horváth, A. Gaspar, *Talanta.*, **2026**, 296, 128435.

P-02 | Static gradient: The new CPC method for generic APIs: optimizing capex and opex for purification 10 tons of API per year

Katalin Radvánszky[1], **Gábor Nagy**[2], Attila Decsi[1]

[1] *LiLiChro, Gyál, Hungary*
[2] *TAPI, Debrecen, Hungary*
Presenter email: gabor.nagy@tapi.com

The biggest cost in industrial liquid-liquid chromatography is solvents and their handling. Therefore, for industrial-scale separations, it is important to keep solvent handling and use as simple as possible.

TAPI and LiLiChro have started joint development work on a $3B market for the purification of a generic formulation.

Solvent mixtures are usually equilibrated in the lab. As we usually do not use the same amount of stationary phase as mobile phase, there is always a (usually stationary phase) reserve. This is not a problem for small quantities, but in an industrial environment it is a loss.

In an industrial application the primary objective is to reduce costs, so this loss should be minimized. We have developed a system where solvent balancing is not necessary. During the development work it became clear that this method not only achieved the original objective, but also more than that, namely, to increase the yield.

This method is generally applicable to the purification of other active substances and both extraction and costs (capex opex) can be optimized, and this method development without equilibration also opens up more scope for the CPC technique, as we have not yet found any developments without equilibration.

In order to optimize costs, the need for a full recalibration has been examined in more detail. We could not find any articles on this in the CPC literature - so we started our own experiments.

Our experiments led to the following improvements:

- the stationary phase calibration can be omitted
- the equilibration of the mobile phase can be replaced by the addition of a more soluble component of the stationary phase
- increased removal of contaminants eluting before and after the product
- recovery per run increased from 50% to 70%.

In our presentation we would like to demonstrate this method. It is not a panacea, of course, but hopefully it will be useful to others. We call it static gradient.

P-03 | Pressure-regulated liquid chromatography

Diána Lukács, Olga Ulbert, Kriszián Horváth

Center for Natural Sciences, Analytical Chemistry Research Group, University of Pannonia, Veszprém, Hungary
Presenter email: lukacs.diana@mk.uni-pannon.hu

With the continuous development of the infrastructure of liquid chromatography separations (e.g. columns, ultra high pressure devices), the possibilities for method development are expanding significantly. However, along with the former, new questions are arising about the effects and possible controllability of the physico-chemical processes taking place in the background of the separations.

In addition to the use of UPLC and UHPLC systems, the effect of temperature and pressure, especially on the retention behaviour of large components (e.g. proteins), is receiving particular attention. Column thermostatting, i.e. controlling the temperature profile of the columns, is a common practice in liquid chromatography, while the control of pressure changes is not widely used in practice.

In recent years, there have been several publications [1-3] on the application of column pressure regulation as a new method development direction. However, to effectively exploit the former option, in-depth knowledge of the processes taking place in the column is necessary.

The aim of our work is to investigate and model the effects of controlled column pressure on retention and peak shape, and thus on separation efficiency. In our research we apply different theoretical pressure gradient programs (linear and nonlinear, positive and negative) to calculate peak profiles of components with different molecular sizes and predict the chromatograms expected from instrumental measurements. In our model calculations, we use a combination of the generally accepted linear solvent strength and equilibrium dispersive models in liquid chromatography, thus obtaining a detailed picture of the retention/elution processes in columns under controlled pressure.

References:

[1] Fekete, S., Fogwill, M., & Lauber, M. A. (2022). Pressure-enhanced liquid chromatography, a proof of concept: Tuning selectivity with pressure changes and gradients. *Analytical Chemistry*, *94*(22), 7877-7884.

[2] Fekete, S., & Lauber, M. A. (2023). Theoretical study on solute migration and band broadening occurring in pressure-enhanced liquid chromatography. *Journal of Chromatography A*, *1692*, 463872.

[3] Lardeux, H., Guillarme, D., Imiołek, M., Fekete, S., & Lauber, M. (2023). Pressure–Enhanced Liquid Chromatography as a Suitable Approach to Improve Selectivity for Large Molecule Separations. *LCGC*, 41(s4) (2023), 28-34.

P-04 | Investigation of HPLC enantioseparations of pyrimidine and diaminopyrimidine derivatives on polysaccharide–type chiral stationary phases utilizing green solvents

Gábor Németi[1], Tam Le Minh[2], Zsolt Szakonyi[2], Antal Péter[1], István Ilisz[1]

[1] *Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary*
[2] *Institute of Pharmaceutical Chemistry, University of Szeged, Szeged, Hungary*
Presenter email: nemeti.gabor@szte.hu

The investigation of chiral pharmacons, the synthesis of pure enantiomers, and the separation of racemic mixtures are of paramount importance. Nowadays, one of the best options for separation is the use of chiral stationary phases, which are available for both analytical and preparative scale applications.

The multimodal polysaccharide-type chiral selectors have become the most commonly used group of chiral stationary phases. In addition to normal-phase separations, reversed-phase and polar-organic, as well as polar-ionic chromatographic modes employing "greener" solvents offer numerous advantages, such as often enabling faster and sufficiently efficient separations compared to normal-phase conditions.

Pyrimidine possesses significant biological importance, and many pharmaceutical effects of its derivatives are known. For example, halogenated pyrimidine derivatives may exhibit antiviral, antifungal, and anticancer properties. Similarly, compounds containing the α-methylene-γ-lactone group represent important pharmaceutical agents. By combining these two compound classes, novel potential drug candidates can be obtained, whose enantiomeric separation constitutes a crucial step in drug development processes.

In this work, immobilized polysaccharide-type chiral stationary phases were employed for the separation of enantiomer pairs of pyrimidine derivatives. The effect of mobile phase composition was investigated primarily in polar-organic mode using various alcohols. The immobilized-type selector also allowed the examination of the effect of solvents with lower environmental impact, e.g., ethyl acetate and dimethyl carbonate. Structure–retention relationships between the chiral selector and selectands, as well as their influence on chromatographic parameters, were analyzed to investigate the mechanistic aspects of chiral recognition. The effect of temperature was also studied, and van't Hoff plots were constructed to evaluate the thermodynamic characteristics.

Acknowledgments: This work was supported by the National Research, Development and Innovation Office-NKFIA through project K137607. Project no. TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme. This work was also supported by the ÚNKP-23-3-SZTE-191 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

P-05 | Ultra high-throughput LC-MS/MS workflow for Red blood cell partitioning assay

Dalma Fuderer, Zoltán Urbán, Anna Lídia Mészáros

Charles River Laboratories Hungary Kft., Veszprém, Hungary
Presenter email: dalma.fuderer@crl.com

Red blood cell (RBC) partitioning plays a critical role in understanding the pharmacokinetic properties and intracellular distribution of drugs. Certain compounds, such as chloroquine, preferentially accumulate within RBCs, potentially affecting both efficacy and toxicity. In contrast, drugs like metoprolol exhibit a more uniform distribution between plasma and cellular components.

In this study, the RBC partitioning of metoprolol and chloroquine was assessed using a simplified high-throughput LC-MS/MS method. Whole dog/rat/minipig blood was incubated with the analytes and centrifuged to separate plasma from RBCs. Both fractions underwent protein precipitation with acetonitrile containing carbutamide as the internal standard. Quantitation was based on the analyte-to-IS (TA/IS) peak area ratios, which were compared between the plasma and RBC fractions. The plasma-to-RBC ratio was calculated from these relative responses.

Our results corresponded with previously published data, both for metoprolol and chloroquine. To further enhance throughput, we evaluated the method's performance on two platforms: a conventional LC-MS/MS system and a high-throughput dual-arm LC-MS system (ADDA). The ADDA platform allows for staggered injections, effectively reducing sample run times as low as 15 seconds per sample with a 30 seconds method. We were able to reduce measurement time per compound (12 samples each) to approximately 6 minutes, enabling the screening of up to 200 compound per day. This high-throughput performance was evaluated in comparison with a conventional single-arm LC-MS/MS platform, highlighting its analytical advantages in the RBC partitioning workflow. This rapid assay format is particularly suitable for screening purposes and high-throughput settings.

P-06 | Impact of mobile phase property variations on retention and mass-transfer kinetics in UHPLC

Olga Ulbert[1*], Diána Lukács[1], Zsolt Ulbert[2], Krisztián Horváth[1]

[1] *Center for Natural Sciences, Analytical Chemistry Research Group, University of Pannonia, Veszprém Hungary*

[2] *Research Center for Biochemical, Environmental and Chemical Engineering, Department of Process Engineering, University of Pannonia, Veszprém Hungary*

Ultra-high-performance liquid chromatography (UHPLC) is widely recognized for its superior separation efficiency and rapid analysis times, achieved through the use of high pressures and small particle sizes. However, the extreme operating conditions inherent to UHPLC significantly influence the physical and chemical properties of the mobile phase as it traverses the column. As the mobile phase enters the column under high inlet pressures (often exceeding 1000 bar), a substantial pressure drop occurs along its path. Concurrently, frictional heating results in an increase in the local mobile phase temperature. These pressure and temperature gradients induce complex changes in mobile phase density and viscosity, which in turn affect analyte diffusion, mass transfer, and overall chromatographic performance.

In this work, UHPLC retention and adsorption-desorption kinetics of different analytes are studied as a function of mobile phase properties (temperature, density, viscosity, surface tension, pH, hydrophobicity, compressibility and molar volume). Physicochemical property estimation is carried out by the Aspen Plus V14 software widely applied by process engineers.

Our analysis reveals that elevated temperatures reduce viscosity and increase diffusivity, potentially improving mass transfer kinetics but also altering retention behavior. Simultaneously, density and compressibility changes can impact solute partitioning between the mobile and stationary phases. Variations in surface tension and molar volume further modify solute-solvent interactions, potentially leading to shifts in selectivity and resolution.

These findings underscore the necessity of considering in-column mobile phase property variations when optimizing UHPLC methods, especially for temperature- and pressure-sensitive analytes. By characterizing these property changes, we provide a more comprehensive understanding of analyte behavior during UHPLC separations and propose strategies for improved method robustness and reproducibility. Future work will focus on quantitative modeling of these gradients and their integration into predictive retention and selectivity models.

P-07 | Invisible? Let's Colour! Alternative methods for determination of Cis-trans isomers by HPLC

János Hegedűs, Viktória Doszpoth, **Éva Kalmár**

Chromatography Team, Process Chemistry Laboratory, Gedeon Richter Plc. Budapest, Hungary

Nowadays, for the unambiguous characterization of many pharmaceutical active ingredients, it is essential to identify and confirm the active isomeric form. Therefore, during analytical testing, the purity must be demonstrated with respect to isomeric impurities as well. This requirement may arise as early as the intermediate stages of synthesis, particularly when the goal is the selective production of a specific isomer.

Furthermore, during analytical testing, the purity must be demonstrated with respect to isomeric impurities as well. This requirement may arise as early as the intermediate stages of synthesis, particularly when the goal is the selective production of a specific isomer.

However, early-stage intermediates often pose significant challenges for analytical chemists. Even if the separation of an isomeric pair is not inherently difficult, these building blocks may lack chromophores, rendering them undetectable by conventional UV detectors. In addition to developing a separation method with adequate resolution and selectivity, the issue of detection must also be addressed—fortunately, several strategies are available for this purpose.

If alternative detection techniques are not feasible, derivatization becomes the method of choice to render the compound detectable—essentially, "Let's colour!" the molecule. Our model compound exhibits both aforementioned analytical challenges: it exists as a cis-trans isomeric forms and lacks a chromophore, making it invisible to UV detection. We present a method development study for the determination of cis-trans ratio using four different derivatizing agents, followed by the optimization of the most suitable method and its implementation into an injector program.

P-08 | Utilizing the Linear Solvent Strength (LSS) model on porous graphitic carbon stationary phases

Barnabás Soós[1,2], Róbert Kormány[1], Krsiztián Horváth[2]

[1] *Drug Substance Analytical Development Division, Egis Pharmaceuticals PLC, Budapest, Hungary*

[2] *Department of Analytical Chemistry, University of Pannonia, Veszprém, Hungary*

Porous graphitic carbon (PGC) stationary phases offer unique advantages in high-performance liquid chromatography (HPLC), including exceptional thermal and pH stability, as well as distinctive selectivity profiles. These characteristics make PGC a promising alternative for separating mixtures containing both polar and non-polar compounds, such as allopurinol and its European Pharmacopoeia (Ph. Eur.) impurities. However, the atypical retention behavior of PGC phases raises questions about the applicability of conventional retention models, such as the linear solvent strength (LSS) model.

This study investigates the retention mechanism of allopurinol and its five Ph. Eur. impurities (ImpA–E) on a PGC stationary phase. A comprehensive experimental design was implemented, involving variation of column temperature (30–90 °C), mobile phase pH (2.0–3.0), and gradient time (5–20 min). Retention data were modeled using the LSS approach, and retention parameters were fitted with a custom Python script. The results demonstrated that LSS modeling yields high accuracy on PGC phases, with predicted retention times deviating less than ±3% from measured values.

Further method development was carried out using DryLab® 4 software, leveraging a three-dimensional design space (gradient time, temperature, pH) to optimize separation. An optimal working point was identified at pH 2.0, 60 °C, and a 6-minute linear gradient. Experimental validation confirmed the excellent agreement between predicted and observed chromatograms for all six components.

This work provides strong evidence that LSS-based retention modeling is applicable to PGC chromatography despite its non-conventional retention mechanisms. The developed method is fast, robust, and capable of resolving allopurinol and its impurities in a single run. The approach offers a generalizable framework for the systematic development of HPLC methods using PGC phases and expands the scope of modeling tools beyond traditional reversed-phase systems.

P-09 | The Chromatography Compass ver. 2.0 is here!

Daniele Di Siena, Imre Sallay

Daiso Fine Chem GmbH, Düsseldorf, Germany

The demand for peptide APIs is growing on an unpredicted rate. Peptide purification processes are extremely challenging due to increased scale and the treacherous nature of the new peptides (fibrillation).

Designing and running purification processes needs special knowledge and expertise and the chance for success is very low when analytical HPLC work practices are applied.

The keyword for a successful purification process is PRODUCTIVITY.

Productivity itself is difficult to define other way then "The sum of Purity, Yield and Load" in the process. The right choice of silica grade is crucial.

Clear understanding on the SEPARATION MODE in action in different stationary phases is a must.

The brilliant CHROMATOGRAPHY COMPASS concept is back with significant upgrade: stationary phases representing in an even more pronounced way the underlying differences between the separation modes.

Ample examples show the differences and help to understand the science behind separation of peptides.

P-10 | Spectroscopic and chromatographic investigation of an unknown impurity of the packiging of an intermediate

Miklós Dékány, János Kóti, Zsófia Dubrovay, Zoltán Béni

Spectroscopic Research Department, Gedeon Richter Plc., Budapest, Hungary
Presenter e-mail: m.dekany@gedeonrichter.com

A pharmaceutical impurity of an intermediate can have significant impact on the final quality of the drug product. Due to the potential safety risk of the undesired pharmacological or toxicological effect might associated to these impurities, besides the active pharmaceutical ingredient (API) the quality of the synthetic intermediates used in the manufacturing process of the API must be adequately controlled as well.

Recently, bluish discoloration of a few plastic bags used for packaging of an otherwise white synthetic intermediate was encountered. Due to the risk of failure of a large-scale API production, a detailed analytical investigation was initiated. As part of this study, our department was requested to investigate the problem by IR, MS and NMR spectroscopic techniques with the aim of gaining some structural information on the blue unknown.

This spectroscopic study will be presented here focusing mainly on the difficulties one can encounter when dealing with the structural identification of colored unknowns in unknown quantity. Based on our spectroscopic results, a mixture of some trimeric compounds, most probably derived from an antioxidant leaching from the polyethylene bag, was suggested as plausible root cause of the observed discoloration.

P-11 | Investigating the relationship between fatigue and anxiety in young elite basketball players using subjective and objective methods

András Szilárd Gáspár, Nelli Farkas, **Anita Bufa**

Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary

Athletes are frequently exposed to conditions that can lead to increased psychological stress. Beyond the impact of physical load, stress may negatively influence performance, general well-being, and contribute to a higher risk of injury.

In our study, we examined the relationship between anxiety, cognitive fatigue, and physical fatigue during the autumn competitive season among young elite basketball players in the U16–U18 age categories, female (n = 23) and male (n = 27). Subjective methods included the State-Trait Anxiety Inventory (STAI-Y) and the Visual Analog Scale (VAS) and as objective measures, we analyzed urinary steroid profiles and creatinine levels at six time points: before and after the first, middle, and final games of the season.

Our results indicated no significant differences in playing time between boys and girls ($p = 0.282$). Pre-game measures revealed no significant differences between female and male athletes in anxiety ($p = 0.117$), cognitive fatigue ($p = 0.561$), or physical fatigue ($p = 0.831$). However, post-game scores showed significantly higher fatigue levels in girls (cognitive fatigue: $p = 0.015$; physical fatigue: $p < 0.001$). We observed that fatigue was correlated with anxiety (cognitive: rho = 0.394, $p < 0.01$; physical: rho = 0.224, $p = 0.018$) as well as with objective urinary biomarkers. Cognitive fatigue was associated with creatinine (rho = 0.212, $p < 0.01$), 16-OH-DHEA (rho = -0.143, $p = 0.03$), 11-O-PT (rho = -0.215, $p < 0.01$), THA (rho = 0.135, $p = 0.04$), THF (rho = 0.145, $p = 0.03$), and β-CL (rho = 0.146, $p = 0.02$). Physical fatigue was correlated with creatinine (rho = 0.259, $p < 0.01$), 16-OH-DHEA (rho = -0.135, $p = 0.04$), and 11-O-PT (rho = -0.249, $p < 0.01$). Anxiety scores from the STAI-Y were significantly associated only with aCL (rho = 0.245, $p = 0.01$).

Our findings suggest that subjectively reported anxiety, cognitive fatigue, and physical fatigue are meaningfully associated with objectively measured urinary steroid and creatinine levels, providing valuable insight into the psychophysiological state of young elite athletes during competitive periods.

P-12 | Investigation of glycosaminoglycan alterations in lung cancer

Domonkos Pál[1,2], Gábor Tóth[3], Simon Sugár[1], Kata Dorina Fügedi[3], Dániel Szabó[3,4], Ilona Kovalszky[5], Dávid Papp[4,6], Gitta Schlosser[6], Tamás Tornóczky[7], Tünde Harkó[8], Judit Moldvay[8,9], László Drahos[3], Lilla Turiák[1]

[1] *MTA-HUN-REN TTK Lendület (Momentum) Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
[2] *Semmelweis University Doctoral School, Budapest, Hungary*
[3] *MS Proteomics Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
[4] *Hevesy György PhD School of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary*
[5] *1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary*
[6] *MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, ELTE Eötvös Loránd University, Budapest, Hungary*
[7] *Department of Pathology, Faculty of Medicine and Clinical Center, University of Pécs, Pécs, Hungary*
[8] *National Korányi Institute of Pulmonology, Budapest, Hungary*
[9] *Pulmonology Clinic, Albert Szent-Györgyi Medical School, University of Szeged, Deszk, Hungary*

Lung cancer is the second most commonly diagnosed cancer worldwide and remains the leading cause of cancer-related death. Carbohydrates, including glycosaminoglycans (GAGs), are increasingly recognized as valuable diagnostic biomarkers in cancer and other diseases. During tumor progression, GAGs often undergo significant changes in both their abundance and structural features.

In this study, we carried out a comprehensive glycomics analysis focusing on chondroitin sulfate (CS) and heparan sulfate (HS) disaccharides in small cell lung cancer (SCLC) and various subtypes of non-small cell lung cancer (NSCLC). We analyzed both tumor tissues and the adjacent non-tumorous regions. Our further objective was to investigate the CS disaccharide characteristics of lung adenocarcinoma samples harboring genetic alterations in the EGFR, KRAS, and ALK genes, as well as in wild-type (WT) tissue. We performed enzymatic digestion on formalin-fixed paraffin-embedded (FFPE) lung tissue sections to break down the GAG polysaccharide chains into the disaccharide building units. The resulting CS and HS disaccharides were extracted from the tissue surface, purified, and then separated using self-packed capillary columns via hydrophilic interaction liquid chromatography and weak anion exchange (HILIC-WAX) in combination with ammonium formate salt gradient HPLC-MS methods. We quantified total CS and HS levels, along with various sulfation patterns, by integrating the chromatographic peaks of the individual disaccharides. The statistical analyses and data visualization were conducted using R and Python to compare total and relative disaccharide abundances, as well as derived metrics like the 6-*O*-/4-*O*-sulfation ratio for CS and *N*-/*O*-sulfation ratio for HS.

We observed significant differences in both the quantity and sulfation patterns of CS and HS disaccharides between tumor and tumor-adjacent tissue. CS levels were approximately twice as high in tumor regions, whereas total HS levels remained largely unchanged. However, the average degree of sulfation increased notably across all tumor phenotypes. In adenocarcinoma specifically, the 6-*O*-/4-*O*-sulfation ratio of CS was elevated compared to other tumor types, and *O*-sulfated HS disaccharides were more abundant in tumor samples overall. By examining the different genetic alterations in the case of lung adenocarcinoma, only a few significant differences emerged between the sample groups. Notably, the 4-*O*-sulfated disaccharide D0a4 was found in significantly lower abundance in the EGFR mutation group compared to the ALK and WT groups. The distribution of non-sulfated, mono-sulfated, and di-sulfated disaccharides followed similar trends across all genetic subtypes. Additionally, principal component analysis (PCA) showed no clear clustering among the different groups based on disaccharide profiles.

These findings suggest that the most pronounced changes in CS disaccharide profiles occur between tumor and adjacent normal tissues, rather than between different lung cancer subtypes or genetic alterations in adenocarcinoma.

Acknowledgment: Funding from the National Research, Development and Innovation Office (OTKA FK131603) is acknowledged.

P-13 | Determination of polycyclic aromatic hydrocarbons in breast milk samples from hungarian mothers using HPLC–FLD

Tímea Dergez[1], Anikó Kőnig-Péter[1], Bernard Collins Anditi[2], Dénes Szerencsés[2], István Szabó[3], Mátyás Wahr[1], Viktória Poór[2]

[1] *Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary*
[2] *Doctoral School of Chemistry, Faculty of Sciences, University of Pécs, Pécs, Hungary*
[3] *Department of Environmental Toxicology, Hungarian University of Agricultural and Life Sciences, Gödöllő, Hungary*
Presenter email: timea.dergez@aok.pte.hu

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants known for their carcinogenic potential. Infants may be exposed to PAHs through breast milk following maternal exposure via inhalation, ingestion, or skin contact. In this study, breast milk samples from 50 volunteering Hungarian mothers were analyzed to determine their PAH levels using high-performance liquid chromatography with fluorescence detection (HPLC–FLD). The carcinogenic risk to infants was assessed using an Incremental Life Risk Calculation (IRCL) model. Participating mothers signed an informed consent form and completed a questionnaire to provide information about their education, residence, dietary habits, Body Mass Index (BMI), smoking, and other relevant factors that may influence PAH levels in breast milk. Based on the questionnaire responses, we explored potential associations between maternal characteristics and the concentration or profile of PAHs detected.

PAHs were detected in 48 of the 50 samples, with phenanthrene being the most frequently identified compound (92%). The concentration of total PAHs ranged from nondetectable levels to 78 ng/mL. Fluorene, phenanthrene, and pyrene were the predominant compounds, while dibenzo(a,h)anthracene was not detected in any sample. Although most samples indicated low risk for infants, two exceeded the acceptable threshold. Questionnaire data suggested that lifestyle factors such as maternal diet, living environment, and education level may influence PAH contamination in breast milk.

Keywords: *polycyclic aromatic hydrocarbon; HPLC–FLD; breast milk; carcinogenic risk; IRCL*

P-14 | Proteomic and phosphoproteomic analysis of prostate adenocarcinoma and benign prostate hyperplasia tissue samples

Krisztián Márk Karvaly[1,2], Fanni Bugyi[1], Tibor Szarvas[3,4], Tamás Ditrói[5], Péter Nagy[5,6,7], Lilla Turiák[1]

[1] *MTA-HUN-REN TTK Lendület (Momentum) Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*

[2] *ELTE Eötvös Loránd University, Budapest, Hungary; Faculty of Science, Institute of Chemistry, Budapest, Hungary*

[3] *Department of Urology, Semmelweis University, Budapest, Hungary*

[4] *Department of Urology, University of Duisburg-Essen, Essen, Germany*

[5] *Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary*

[6] *Department of Anatomy and Histology, HUN-REN–UVMB Laboratory of Redox Biology Research Group, University of Veterinary Medicine, Budapest, Hungary*

[7] *Chemistry Coordinating Institute, University of Debrecen, Debrecen, Hungary*

Presence or development of diseases can cause changes in protein content or post translational modifications (PTMs). One of the most investigated PTM is phosphorylation. During protein phosphorylation, a phosphate group is attached to the side chain of a protein. Prostate cancer (PCA) is one of the most prevalent cancers among males, and the risk of developing PCA increases with age. Due to limitation in current screening and diagnostic techniques, new approaches are needed. Proteomic analysis of prostate cancer tissue samples can reveal potential novel diagnostic markers.

In our research, we performed on-surface proteolytic digestion on 77 formalin-fixed paraffin-embedded tissue samples derived from patients with benign prostate hyperplasia (BPH, n = 15) or PCA (n = 62). The resulting peptides were purified using solid phase extraction (SPE). From each sample, a proteomic fraction containing mainly non-phosphorylated peptides and a phosphoproteomic fraction enriched in phosphopeptides were prepared using TiO_2 stationary phase. Both fractions were purified with SPE prior to HPLC-MS/MS measurements. Phosphoproteomic fractions were analyzed using data-dependent acquisition, whereas data-independent acquisition was employed for proteomic fractions.

We investigated proteins, and phosphorylation sites that were significantly altered between BPH and PCA samples in general, and PCA samples with different risk stratifications, like Gleason, CAPRA, D'Amico stratification. In the phosphoproteomic analysis 42 phosphorylation sites showed significant differences between the BPH and PCA samples. 10-30 phosphorylation sites were significantly different among BPH samples and PCA samples with different risk stratification. Such as, 23 phosphorylation sites were significantly altered between BPH and the low-risk Gleason group. In the proteomic analysis 903 proteins were significantly different between BPH and PCA samples, furthermore 378-1008 proteins were significantly different among BPH samples and PCA samples with different risk stratification. For example, 845 proteins showed significant differences between BPH and the high-risk CAPRA group.

We also performed cancer specific survival analysis on the identified proteins. By fitting univariate Cox-proportional hazards models, 112 proteins were found to be significantly associated with survival. Multivariate Cox-proportional hazards models revealed 89 proteins as potentially significant independent predictors of survival after adjusting for the CAPRA score, whereas 83 proteins were revealed when the model was adjusted for the D'Amico score.

Acknowledgments: Phosphoproteomics samples were run at the ISTA LSF Mass Spectrometry Facility, part of the Scientific Service Units of ISTA. This project was supported by the Lendület (Momentum) Program of the Hungarian Academy of Sciences (HAS, MTA). The project was implemented with the support from the National Research, Development and Innovation Fund of the Ministry of Culture and Innovation under the National Laboratories Program (National Tumor Biology Laboratory (2022-2.1.1-NL-2022-00010)) and the Hungarian Thematic Excellence Program (under project TKP2021-EGA-44) Grant Agreements with the National Research, Development and Innovation Office. This project has received funding from the HUN-REN Hungarian Research Network (grant 1500207).

P-15 | Cyclodextrin complexation of linear and cyclic diarylheptanoids

Ákos Bebesi, Tymon Yu, András Dohárszky, **Ida Fejős**

Department of Pharmacognosy, Semmelweis University, Budapest, Hungary
Presenter email: fejos.ida@semmelweis.hu

The physicochemical characterization and pharmacokinetic testing of potential herbal active ingredients is essential for effective and safe (phyto)therapy and optional drug development. Common alder (*Alnus glutinosa* L.) and european hornbeam (*Carpinus betulus* L.) contain diarylheptanoids, a group of plant secondary metabolites, that exhibits notable anticancer, anti-inflammatory, and antimicrobial effects, but are hindered by poor solubility and low stability. Cyclodextrins (CDs) are an excellent tool to overcome these problems: the polar nature of their outer surface ensures good water solubility, while their weakly hydrophobic inner cavity can accommodate apolar guest molecules through inclusion complex formation.

The aim of our work is to optimize the pharmacokinetic properties and bioavailability of several promising linear and cyclic diarylheptanoids by CD complexation.

The complex stability constants were determined by the environmentally friendly, low sample consuming affinity capillary electrophoresis, applying almost 30 native, neutral, positively, and negatively charged CD derivatives. Both the neutral and charged derivatives were able to form complexes with all 4 linear diarylheptanoids, oregonin, hirsutenon, rubranoside A and B, contrary to the cyclic plant metabolites. The cavity size, the substituent type and the degree of substitution also influenced the complex formation. The negatively charged subetadex and sugammadex were able to form the most stable complexes, exceeding 1000 M^{-1}. The result of the stability studies using selected CDs was in good agreement with the affinity capillary electrophoretic screening.

Based on our results, the "ideal excipients" were selected for the CD-based preparations of the promising plant compounds, oregonin and hirsutenon.

Keywords: *affinity capillary electrophoresis, inclusion complexation, bioavailability enhancement, oregonin, hirsutenon*

P-16 | Targeting lipid A modification pathways in *Shigella sonnei* with dephostatin

Laura Nagy

Institute of Bioanalysis, University of Pécs, Pécs, Hungary

75% of all surface of Gram-negative bacteria is covered with endotoxin molecules. These endotoxins, or lipopolysaccharides in other words, serve structural roles, forming a protective layer around the bacterium, but they also determine the ability to form biofilms, resistance to bacteriophages, and interactions with various antibiotics and the host's antimicrobial chemokines. While certain structural features are characteristic of a given strain, the composition of lipopolysaccharides can be influenced by environmental factors. The regulation of biosynthesis in response to environmental changes is particularly important in strains that live intracellularly, such Shigella sonnei, which must remain viable under very different conditions (both extracellular and intracellular).

The fine-tuning of lipopolysaccharide biosynthesis is possible via so-called two-component regulatory systems. Several of these two-component systems are known in bacteria, and some specifically regulate lipopolysaccharide biosynthesis: PhoP/Q and PmrA/B systems are the best known examples of that. These regulatory systems may influence the acylation pattern of lipopolysaccharides and place phosphoethanolamine or aminoarabinose moieties onto the molecule. These modifications impact the bacterium's resistance mechanisms, especially resistance to polymyxins.

Certain molecules are known in the literature to affect the PmrA/B and PhoP/Q systems. This poster presents the effect of dephostatin, which is likely to inhibit the PmrA/B system. It was investigated whether the bacterium is able to incorporate phosphoethanolamine and aminoarabinose when exposed to polymyxins (such as colistin, polymyxin B) in the presence of dephostatin.

P-17 | Determination of bioactive components from different growing condition microgreens *(Sinapis alba, Trigonella foenum graecum, Raphanus sativus longipinnatus)* by GCMS

Szabolcs B. Tóth, Lea Róde

Eszterházy Károly Catholic University, Institute of Biology, Department of Botany and Plant Physiology Eger, Hungary

Presenter email: b.toth.szabolcs@uni-eszterhazy.hu

Microgreens are the young seedlings of vegetables and herbs, harvested just after their first leaves (cotyledons) have developed, often with the addition of a few true leaves. They are not sprouts, which are harvested much earlier, and they are not fully grown vegetables or herbs. Microgreens are known for their concentrated flavors and nutritional value, making them a popular addition to various dishes.

Plant stress (biotic and abiotic stress) refers to any unfavorable condition or substance that negatively impacts a plant's metabolism, growth, or development. This can stem from various factors like inadequate water, extreme temperatures, insufficient or excessive nutrients, pest or disease infestations, or mechanical damage. Different plants thrive under varying growing conditions, including temperature, light, water, humidity, and soil type. Optimal conditions vary greatly by species, with some preferring full sun and well-drained soil while others flourish in shade with consistently moist soil. Bioactive compounds in plants are naturally occurring chemicals that can have positive effects on human health beyond basic nutrition. They are often secondary metabolites, meaning they are not directly involved in the plant's primary growth but have various beneficial properties. Examples include flavonoids, polyphenols, alkaloids, and terpenoids, which can act as antioxidants, anti-inflammatories, and immunomodulators.

In microgreens grown under different growing conditions (*Sinapis alba*, *Trigonella foenum graecum*, *Raphanus sativus longipinnatus*), the quality and quantity of bioactive components are also affected by the growing conditions. During the treatments we selected, we investigated the effect of lower temperatures compared to normal conditions, and the effect of iron as a vital trace element on the quantity and quality of bioactive components. derivatized (BSTFA/TMCS) samples were used for the GCMS study.

P-18 | Investigation of the bioactive compounds and antioxidant capacity of microgreens under different treatment conditions

Lea Róde, Szabolcs B. Tóth

Eszterházy Károly Catholic University, Institute of Biology, Department of Botany and Plant Physiology, Eger, Hungary
Presenter email: rodelea123@gmail.com

Microgreens are extremely rich in bioactive compounds, including minerals, vitamins and antioxidants, the consumption of which has beneficial physiological effects. The aim of our research was to investigate the antioxidant capacity of microgreens under different growing conditions and to identify the compounds responsible for the high antioxidant effect.

In our experiments, we used radish *(Raphanus sativus china rose)* and broccoli *(Brassica rapa var sylvestris)* microgreens. Five treatments were used during the study: continuous cold light (5000K), continuous warm light (2700K), "smart flowerpot" technology (automated watering and lighting), cold treatment in a climate chamber (constant 18°C, 16h light-8h darkness), and as a control group, the samples were placed in front of a glass window, simulating the light conditions of a home kitchen. The antioxidant capacity was determined by DPPH and FRAP methods, which were detected spectrophotometrically. The compounds of the microgreens were identified by GC-MS after derivatization (BSTFA/TMCS).

Evaluating the results, we found that the different treatments significantly affected the antioxidant capacity of both microgreen species. Broccoli *(Brassica rapa var sylvestris)* and radish *(Raphanus sativus china rose)* responded differently to the individual environmental factors.

Our results show that choosing optimal growing conditions can significantly influence the nutritional value of microgreens, especially their antioxidant capacity, which is also crucial for nutrient intake.

P-19 | What can make the difference of a urine bioanalytical method?

Éva Cseh, Mónika Sebestyén, Klára Terleczky-Horváth, Zoltán Juhász

Developmental Drug Metabolism & Pharmacokinetics, Gedeon Richter Plc., Budapest, Hungary

During Phase I clinical trials, it is often necessary to determine the concentration of the drug candidate molecule not only in human plasma but also in urine to characterize the renal excretion of the compound.

For the development of a bioanalytical method, we should take into consideration the characteristics of the sample matrix. In addition to the chromatography, the adsorption of an analyte to different materials can also be a critical factor in the evaluation of solubility and stability. An analyte can be adsorbed to the protein molecules in the matrix or to the inner wall of containers used for sample collection and processing in the clinical site or used for storage or preparation for analysis. Normally, human urine doesn't contain proteins. Therefore, an analyte is not able to bind to protein molecules in urine, but it can be adsorbed to the surface of the containers. So, the concentration of the analyte can be decreased in urine compared to plasma. This behaviour can cause a significant concentration loss even in the first steps of sample collection, when a volunteer's urine samples is poured from a collection container into a canister for pooling. This problem occurs when the water solubility of a molecule is insufficient. Usually detergents (e.g. Tween20) are used as anti-adsorb agents to to overcome it.

In our poster presentation we would like to show the main differences between human plasma and urine as matrices, and a feasible and unusual solution for preventing the decrease of the concentration of a drug molecule in human urine caused by surface adsorption.

With this solution we developed and validated an HPLC-MS/MS (High Performance Liquid Chromatography – Tandem Mass Spectrometry) method for the quantitative determination of the analyte in human urine.

P-20 | Gradient, reversed-phase HPLC method for simultaneous determination of chemical and enantiomeric impurities of dexibuprofen in a single run

Ali Mhammad[1,2], Gergely Dombi[1,2], Máté Dobó[1,2], Balázs Simon[1,2], Zoltán-István Szabó[3,4], Gergő Tóth[1,2*]

[1] *Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary*
[2] *Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary*
[3] *Department of Pharmaceutical Industry and Management, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Targu Mures, Romania*
[4] *Sz-Imfidum Ltd., Targu Mures, Romania*
* *Corresponding author: toth.gergo@semmelweis.hu*

In this work, a gradient reversed-phase HPLC method is presented for the simultaneous determination of four potential chemical impurities in dexibuprofen, as well as its chiral isomer, R-ibuprofen. Among nine polysaccharide-based chiral columns tested, enantioselectivity was observed only with the column containing cellulose tris(4methylbenzoate) under polar organic mode conditions. To enhance enantioresolution, a reversed-phase mode was employed by adding water to the mobile phase. Examination of the water content's impact on retention time revealed a typical reversed-phase retention behavior in alcohol–water mixtures, whereas acetonitrile–water mixtures displayed HILIC-like retention characteristics. A full factorial design optimization strategy was applied to identify the ideal conditions for determining organic and enantiomeric impurities in dexibuprofen. Under the optimized conditions—Lux Cellulose-3 stationary phase using a methanol:water:acetic acid 70:30:0.1 (v/v/v) mobile phase, at a flow rate of 0.5 mL/min and temperature of 35 °C—all target compounds were effectively separated with clear baseline resolution. The initial analysis required 60 minutes, primarily due to the prolonged retention of highly lipophilic ester impurities. To reduce the analysis time, a gradient elution strategy was developed, successfully cutting the runtime by half. Furthermore, the stability and robustness of the gradient system were confirmed by consistent performance over one hundred consecutive injections. Following regulatory validation guidelines, the developed HPLC method was effectively applied for the quality assessment of commercially available pharmaceutical formulations.

Keywords: *chiral switch; enantioseparation; chiral separation; polysaccharide; HPLC; Lux Cellulose-3*

P-21 | Machine learning-assisted retention time predictions on polysaccharide-based columns

Attila Imre[1,2], **Gergely Dombi**[2,3], Máté Dobó[2,3], Elek Ferencz[4], Balázs Balogh[2,5], Anna Vincze[2,3], Zoltán-István Szabó[6,7], György Tibor Balogh[2,3,8], Anita Rácz[9], Gergő Tóth G[2,3]

[1] *Center for Health Technology Assessment, Semmelweis University, Budapest, Hungary*
[2] *Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary*
[3] *Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary*
[4] *Emergency County Hospital Miercurea Ciuc, Service of Translational Medicine and Clinical Research, Romania*
[5] *Department of Organic Chemistry, Semmelweis University, Budapest, Hungary*
[6] *Department of Pharmaceutical Industry and Management, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Targu Mures, Romania*
[7] *Sz-imfidum Ltd.*
[8] *Department of Chemical and Environmental Process Engineering, Budapest University of Technology and Economics, Budapest, Hungary*
[9] *Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
Presenting author: dombi.gergely@semmelweis.hu

Enantioseparation continues to be a critical yet complex task in analytical chemistry, often requiring extensive experimentation to identify effective chromatographic conditions. High Performance Liquid Chromatography (HPLC) using polysaccharide-based chiral stationary phases (CSPs) is one of the most widely adopted technique for this purpose. However, traditional method development typically relies on a trial-and-error approach, which is time-consuming, costly, and inefficient. To address these limitations, we introduce a machine learning-based framework designed to accurately predict chromatographic outcomes and streamline the method development process for chiral separations.

Our study integrates multiple computational approaches, including Partial Least Squares (PLS) regression, neural networks (NN), and graph neural networks (GNN), in both individual and consensus modeling schemes. These models were trained on an extensive dataset of over 500 unique compounds, resulting in more than 1,300 experimentally determined retention times. The data spans four commonly used polar organic mobile phase conditions: Acidic Methanol, Basic Methanol, Acidic Acetonitrile, and Basic Acetonitrile.

In condition-specific prediction tasks, the consensus PLS-NN model delivered the best results, achieving R^2 values greater than 0.70 and RMSECV values below 0.40 across most solvent systems. For generalized prediction across all conditions, the GNN model demonstrated superior performance, with cross-validation results yielding an R^2 of 0.84 and RMSECV of 0.38. These findings were further supported by strong external validation results (R^2 = 0.84, RMSEP = 0.23), confirming the model's ability to generalize across chemical space and experimental conditions.

To make this approach accessible to the broader scientific community, we developed a freely available web-based tool at https://chiralscreen.com. This platform allows users to predict retention times, and the users can use these to predict enantioseparation and determine elution order, offering valuable guidance on selecting optimal starting conditions for method development.

In summary, our machine learning-driven platform represents a significant advancement in chiral HPLC method development, reducing reliance on empirical screening and enabling faster, more informed decision-making for enantioseparations.

Funding: This work was funded by the National Research, Development, and Innovation Office, Hungary (grant: NKFIH FK 146930).

Acknowledgements: This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Supported by the EKÖP University Research Scholarship Programme of the Ministry for Culture and Innovation from the Source of the National Research, Development and Innovation Fund.

P-22 | Bioassay-guided fractionation, isolation, and characterization of antibacterial compounds from the ethyl acetate extract of giant goldenrod (*Solidago gigantea* Ait.) flowers

Márton Baglyas[1,2,*], Zoltán Bozsó[1], Péter G. Ott[1], Ágnes M. Móricz[1]

[1] *Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary*
[2] *Doctoral School, Semmelweis University, Budapest, Hungary*
* *Corresponding author email: baglyas.marton@atk.hun-ren.hu*

Plants are considered an inexhaustible source of secondary metabolites possessing diverse structures and valuable biological activities. Giant goldenrod (*Solidago gigantea* Ait.), a plant native to North America and currently a highly invasive species in most parts of Europe, represents a largely untapped source of bioactive secondary metabolites. Although it is recognized as a medicinal plant and employed in phytotherapy due to its favorable pharmacological effects (diuretic, antiphlogistic, antioxidant, antispasmodic), its natural products remained poorly studied. As invasive species continue to pose serious ecological and environmental threats, exploring their potential benefits – such as utilizing *S. gigantea* for pesticide discovery – could provide a dual advantage: mitigating their spread while uncovering new agrochemical agents.

This work aimed to detect, isolate, characterize, and identify the antibacterial constituents of the ethyl acetate extract of the flowers of *S. gigantea*. Non-targeted, effect-directed screening for antibacterial components were carried out by thin-layer chromatography coupled with direct bioautography (TLC–DB) using a *Bacillus subtilis* bioassay. Fractionation was performed by preparative flash column chromatography but isolation required separations by semi-preparative, reversed-phase high-performance liquid chromatography (RP-HPLC). The structures of the isolates are to be elucidated by one- and two-dimensional nuclear magnetic resonance spectroscopy (NMR), high-resolution tandem mass spectrometry (HRMS/MS) and polarimetry. Minimal inhibitory concentration (MIC) values will be obtained from *in vitro* microdilution assays.

Five antibacterial compounds (1–5) were isolated from the ethyl acetate extract of *S. gigantea* flowers by a bioassay-guided procedure. Their antibacterial activity against Gram-positive *B. subtilis* bacteria has been proved so far using TLC–DB. The separation of two isomeric pairs (compounds 2–3 and 4–5, with molecular formulae $C_{25}H_{38}O_5$ and $C_{20}H_{28}O_6$, respectively) proved challenging due to their highly similar retention characteristics. Compound 1 was tentatively assigned as a monoglyceride previously identified from the leaves of *S. gigantea* [1]. Based on their chromatographic behavior, color upon derivatization with *p*-anisaldehyde sulfuric acid reagent, and HRMS/MS data, compounds 2–5 are presumed to be diterpenes, with compounds 2–3 supposedly to be angelate esters. Besides, several further bioactive compounds were detected in the flowers, which have previously been identified in the roots or leaves of the plant.

Acknowledgements: This study was supported by the National Research, Development and Innovation Office of Hungary (K128921 and SNN139496).

Reference:

[1] Baglyas, M.; Ott, P.G.; Bozsó, Z.; Schwarczinger, I.; Bakonyi, J.; Dlauchy, D.; Darcsi, A.; Varga, S.; Móricz, Á.M. Bioassay-Guided Isolation of *cis*-Clerodane Diterpenoids and Monoglycerides from the Leaves of *Solidago gigantea* and Their Antimicrobial Activities. *Plants* **2025**, *14*, 2152. https://doi.org/10.3390/plants14142152

P-23 | Greener solvents in reversed-phase liquid chromatography from evaluation in method development to practical application

Kalisz Oktawia, **Bocian Szymon**

Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland
Corresponding author email: oktawia.kalisz@doktorant.umk.pl

In recent years, modern society's awareness of global threats such as climate change and environmental issues has been growing steadily. Governments are promoting specific programs to enable sustainable development, such as the European Green Deal, which aims to achieve climate neutrality by 2050 and boost the economy through green technologies. For these reasons, green analytical chemistry (GAC) is increasingly recognized as a new and important area of green chemistry, where replacing harmful solvents with greener ones has become one of the main drivers of sustainability in laboratories and industry. However, GAC focuses on the environmental aspect, overlooking the financial impact or efficiency, which is contrary to the fundamentals of sustainable chemistry. Hence, white analytical chemistry (WAC), in addition to GAC principles, emphasizes the holistic adjustment of analytical procedures, also taking into account performance reliability and economic aspects [1].

Since the most common chromatographic system includes reversed-phase conditions, most wastewater comprises acetonitrile and methanol (MeOH), which are toxic, flammable, and hazardous to the environment and humans. For these reasons, replacing these solvents with greener alternatives such as ethanol or dimethyl carbonate (DMC) is becoming an urgent priority [1].

This work investigates the impact of mobile phase composition on the carbon footprint of ultrahigh-performance liquid chromatography (UHPLC) separations. We aim to demonstrate that environmentally friendly organic modifiers like ethanol (EtOH) and dimethyl carbonate (DMC) can achieve comparable chromatographic performance. This investigation contributes to developing green analytical chemistry by identifying effective and sustainable alternatives for UHPLC separations [2]. This study also introduces a novel analytical procedure for the determination of theobromine and caffeine in teas, utilizing, for the first time, dimethyl carbonate as both an eluent in solid-phase extraction (SPE) and a mobile phase constituent in UHPLC. This approach is compared to a reference method employing methanol in both extraction and HPLC analysis. Both developed methodologies are evaluated using tools such as AGREE, AGREEprep, ChlorTox, and the RGB model to compare their greenness and whiteness [3].

The study showed that it is possible to achieve equally satisfactory separations using alternative solvents and they highlight the potential of dimethyl carbonate to replace conventional organic solvents, such as methanol, without compromising the quality and effectiveness of analytical procedures in liquid chromatography.

References:

[1] O. Kalisz, M. Tobiszewski, A. Nowaczyk, S. Bocian, TrAC - Trends in Analytical Chemistry, 2024, 181, 1-16, 118007
[2] O. Kalisz, G. Hulicka, M. Tobiszewski, S. Bocian, Green Chemistry, 2025, 27, 3020
[3] O. Kalisz, M. Catani, S. Bocian, ACS Omega, 2025, 10, 12.

P-24 | Microfluidic method for determining total nitrogen in age-specific nutritional formulas

Fruzsina Balogh-Hartmann[1], Csilla Páger[1], Sára Nagy[2], Tamás Marosvölgyi[1], **Lilla Makszin**[1]

[1] *Institute of Bioanalysis, Medical School, University of Pécs, Hungary*
[2] *Department of Languages for Biomedical Purposes and Communication, University of Pécs, Medical School, Hungary*

This study presents a microfluidic approach to assess the total nitrogen content and electrophoretic profile of Foods for Special Medical Purposes (FSMPs), with a focus on analytical quality assurance. Unlike pharmaceuticals, FSMPs follow a simplified notification process prior to market entry, requiring only minimal safety and quality compliance. Therefore, independent analytical verification of their composition is critical.

We aimed to examine the impact of commonly used additives, including milk- and plant-based proteins, stabilizers, and vitamins, on nitrogen content, with particular attention to the contribution of components containing free amino groups (CFAGs), across FSMPs of various consistencies (liquid, powder, and yoghurt-like). Products formulated for both infants and adults were analyzed using an optimized microchip gel electrophoresis method (HSP 250), which provided repeatable and reproducible results.

The presence and distribution of CFAG fractions were significantly influenced by the additives used by different manufacturers. Statistical analysis revealed brand-specific differences across five FSMP categories. In several cases, the measured CFAG-derived nitrogen content exceeded the declared protein content on the label, a finding of relevance for individuals with kidney disease. A significant difference in nitrogen content was observed between infant and adult FSMPs in liquid form, whereas no such difference was found in powdered products. These findings highlight the importance of rigorous analytical control, particularly for nutritional products intended for therapeutic use.

Acknowledgements: This work was supported by the "Pécsi Tudományegyetem Kutatási Alap Pályázat" (grant number 020_2024_PTE_RK/22) and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

P-25 | Determination of Withaferin A in Ashwagandha-based dietary supplements using HPLC-MS/MS: insights into product variability and quality concerns

Csanád Vörös, **Orsolya Csernák**

Department of Pharmacognosy, Semmelweis University, Budapest, Hungary
Presenter email: csernak.orsolya@semmelweis.hu

Ashwagandha (*Withania somnifera*), also known as winter cherry, is a key herb in Ayurvedic medicine and has gained increasing popularity in the Western world as a dietary supplement. It is known for its adaptogenic properties and has been reported to support sleep, enhance physical performance, and alleviate stress-related symptoms. These effects make it particularly appealing to athletes, individuals with high-stress lifestyles, and those suffering from sleep disorders. The plant's therapeutic potential is primarily attributed to its bioactive constituents, especially the steroidal lactone compounds known as withanolides.

In this study, dietary supplements containing ashwagandha were investigated using a HPLC-ESI-MS/MS method. From the wide range of commercially available products, seven capsules from four different manufacturers and three powdered root products from three additional suppliers were analyzed to obtain a comprehensive overview of the labeled active compound content. As a reference material, we selected a powdered root sample obtained from Galke GmbH (Gittelde, Germany), a manufacturer certified according to BIO, Kosher, ISO 9001, ISO 14001, AEO, and GMP standards, providing a reliable benchmark for comparison.

Withaferin A—one of the most abundant withanolides in the plant—was chosen as the analytical standard for quantification. The developed HPLC-ESI-MS/MS method was validated according to the EMA guidelines for analytical method validation, assessing selectivity, accuracy, precision, recovery, carry-over, matrix effects, and stability. Due to its structural similarity to other withanolides, withaferin A enabled the detection and analysis of structurally related compounds with similar fragmentation patterns in MRM mode. Furthermore, our measurements were confirmed using high-resolution UHPLCESI-Orbitrap-MS/MS analysis, providing additional structural verification and supporting the robustness of our findings.

Overall, the chemical profiles of the powdered root products were all consistent with the reference sample. In contrast, the content and profile of bioactive compounds in the capsule formulations varied significantly, largely depending on the extraction methods used. Moreover, substantial differences were observed even between different batches from the same manufacturer. Our findings clearly demonstrate that the current lack of stringent regulation in the dietary supplement market can lead to considerable variability in product quality, posing a potential risk to consumers seeking consistent therapeutic efficacy. This underlines the pressing need for standardized quality control frameworks to ensure both the safety and reliability of dietary supplements, not only for products marketed for stress management and overall well-being.

P-26 | Optimization of ionic liquid-based capillary electrophoresis for carbohydrate analysis

Ibolya Madarászné Horváth, Viktor Sándor, Lilla Makszin, Csilla Páger

Institute of Bioanalysis, Medical School, University of Pécs, Hungary

The carbohydrate content of foods significantly influences their health effects, particularly in terms of glycaemic control and caloric intake. However, the analysis of carbohydrates presents several challenges. These arise primarily from the structural diversity and complexity of carbohydrates – especially polysaccharides – which complicates chromatographic separation. Furthermore, carbohydrates typically exhibit weak UV absorbance and poor ionization efficiency in mass spectrometry, making their detection difficult. A promising solution to these limitations is the use of ionic liquids.

Ionic liquids are considered green solvents due to their low volatility and recyclability. Their unique physicochemical properties make them valuable in various analytical applications, including carbohydrate analysis – particularly in separation techniques such as capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). In capillary electrophoresis, intermolecular interactions between ionic liquids and carbohydrates – such as hydrophobic, electrostatic interactions or hydrogen bonding – can enhance separation efficiency without the need for additional derivatization steps.

Our research focuses on developing a CE method employing an ionic liquid for the efficient separation of carbohydrates. Indirect UV detection was used, eliminating the need for carbohydrate derivatization prior to analysis. The present study investigates the effects of electrolyte composition, pH, and applied voltage on separation performance. The optimized method enables both qualitative and quantitative analysis of carbohydrates – including sucrose, lactose, maltose, glucose, fructose, galactose, arabinose, ribose, and mannose – within 9 minutes.

Acknowledgements: This work was supported by the "Pécsi Tudományegyetem Kutatási Alap Pályázat" (grant number 020_2024_PTE_RK/22) and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

P-27 | Identification of six antibacterial compounds isolated from tree of heaven (*Ailanthus altissima* (Mill.) Swingle) stem and trunk bark

Anna Cselőtey[1,2], Márton Baglyas[1,2], Nóra Király[1], Péter G. Ott[1], Vesna Glavnik[3], Irena Vovk[3], Ágnes M. Móricz[1]

[1] *Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary*
[2] *Semmelweis University, Doctoral School, Budapest, Hungary*
[3] *Laboratory for Food Chemistry, National Institute of Chemistry, Ljubljana, Slovenia*
Presenter email: cselotey.anna@atk.hun-ren.hu

Tree of heaven (*Ailanthus altissima* (Mill.) Swingle) is a deciduous tree native to Southeast Asia but has become invasive in many temperate regions of the world, such as Hungary and Slovenia, where it poses a major threat to the local biodiversity. The dried bark of the tree has been widely used in traditional Asian medicine to treat various illnesses, including asthma, epilepsy, and gastrointestinal and ophthalmic diseases.

In this work, we aimed to screen, characterize, and identify the antibacterial agents present in the stem and trunk bark of *A. altissima*.

The bioactive compounds were detected by thin-layer chromatography (TLC)–*Bacillus subtilis* bioassay and were characterized by chemical reagents (*p*-anisaldehyde and Dragendorff's reagent) and TLC–UV/Vis/FLD–heated electrospray ionization (HESI)-tandem high-resolution mass spectrometry (HRMS/MS). The bioassay-guided flash chromatographic fractionation and purification of antimicrobial compounds were followed by structure elucidation of the isolates using flow injection analysis (FIA)–HRMS/MS and NMR spectroscopy. The anti-*Bacillus* activity of the isolates was confirmed by microdilution assays determining their minimum inhibitory concentration (MIC).

In total, six bioactive compounds were isolated and identified from the bark extracts of *A. altissima*: (9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoic acid (**A1**) and (10*E*,12*Z*)-9-hydroxy-10,12-octadecadienoic acid (**A2**) from the stem bark; hexadecanedioic acid (**A3**), 16-hydroxyhexadecanoic acid (**A4**), and 16-feruloyloxypalmitic acid (**A5**) from the outer trunk bark; while canthin-6-one (**A6**) from the inner trunk bark. All isolates, except **A5**, showed antibacterial activity against *B. subtilis* in microplate assays, with **A6** exhibiting the strongest effect. To the best of our knowledge, this is the first study identifying **A1**-**A5** in *A. altissima*, also the first study reporting the antibacterial effects of **A3** and **A4**.

Acknowledgements: This study was supported by the National Research, Development and Innovation Office of Hungary (NKFIH; project SNN139496) and the Slovenian Research and Innovation Agency (ARIS; research core funding No. P1-0005, project N1-0235).

P-28 | Online SPE-HPLC-MS/MS determination of urinary DINCH plasticizer metabolites for exposure assessment in adult population

Róbert Góra[1], Renáta Górová[1], Helena Jurdáková[1], Ľubica Murínová[2]

[1] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Analytical Chemistry, Bratislava, Slovakia,*

[2] *Slovak Medical University, Faculty of Public Health, Department of Environmental Medicine, Bratislava, Slovakia*

Presenter email: robert.gora@uniba.sk

Plasticizer DINCH (1,2-cyclohexane dicarboxylic acid diisononyl ester; Hexamoll® DINCH) was introduced in 2002 and gradually replace phthalates used as plasticizers in production of plastic products, especially those coming into close contact with humans, mainly food packaging, toys and medicinal devices due to its more favorable toxicological profile [1]. However, in the case of high levels of exposure, hormonal changes, effects on kidney and liver function and on lipid metabolism were described. As biomarkers of exposure to DINCH, mainly metabolites hydroxy- (OH-MINCH) and carboxy- (cx-MINCH) monoesters are used [2,3]. Within the European Program for Human Biomonitoring (HBM4EU), the plasticizer DINCH was included to the substances of priority interest and the laboratory tests for harmonization of analytical procedure for the determination of DINCH metabolites in urine were done [4]. Exposure to DINCH in this study was assessed on the basis of concentration of oxidized metabolites in urine. Urine samples were analysed by combination of on-line solid phase extraction and HPLC-MS/MS method after deconjugation of metabolites from their glucuronide conjugates. The method was approved within the HBM4EU proficiency test scheme with Z-score $|Z| \leq 0.9$. Intraday and interday precisions were better than 4 % and 8 %, respectively, with limits of quantitation of 0.1 µg/L for cx-MINCH and 0.14 µg/L for OH-MINCH. Concentrations were calculated from matrix linear calibration curves in the range of 0.12 to 60 µg/L. Presented study provides very first results on exposure of Slovak adult population to DINCH plasticizer. Urine samples were collected from 300 adults aged 20-60 years from three regions of Slovakia. The detection frequency of at least one metabolite was 100 %. For adults we found median values of DINCH metabolites lower than in children, but similar to published values for older adolescents [5]. No interregional differences were found within Slovakia. The recommended safe limit for the sum of OH-MINCH and cx-MINCH metabolites of 4.5 mg/L was not exceeded. The maximum concentration determined in urine reached 48 µg/L. It can be concluded, that each participant was in contact with products containing DINCH. However, the level of exposure of the Slovak adult population to the plasticizer DINCH was found to be low and based on currently available scientific knowledge, no adverse impact on health is expected and no actions are currently necessary. However, due to the rapidly increasing production and use of this substance, it can be assumed that the exposure of the population to DINCH will increase in the near future. In accordance with the precautionary principle, regular biomonitoring is therefore necessary.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-20-0462.

References

[1] B.L. Wadey, J. Vinyl Addit. Technol. 9 (2003) 172.
[2] H.M. Koch, A. Schütze, C. Pälmke, et al., Arch. Toxicol. 87 (2013) 799.
[3] H. Jurdáková, R. Górová, Zdravotnícke listy 7 (2019) 6.
[4] M.E López,, T. Göen, H. Mol, et al., Int. J. Hyg. Environ Health.234 (2021) 234.
[5] Salamanca-Fernández E., Espín-Moreno L., Olivas-Martínez A., et al. (2024) Toxics 12, 642.

P-29 | *In vitro* permeability study of new KYNA analogues

Zahraa Ali[1], Bálint Lörinczi[2,3], István Ilisz[1], István Szatmári[2,3], Imola Wilhelm[4], László Vécsei[5,6], Róbert Berkecz[1,7]

[1] *Institute of Pharmaceutical Analysis, Faculty of Pharmacy, University of Szeged, Szeged, Hungary*
[2] *Institute of Pharmaceutical Chemistry and Research Group for Stereochemistry, Hungarian Academy of Sciences, University of Szeged, Szeged, Hungary*
[3] *Institute of Pharmaceutical Chemistry, Interdisciplinary Excellence Center, University of Szeged, Szeged, Hungary*
[4] *Institute of Biophysics, Biological Research Centre, Szeged, Hungary*
[5] *HUN-REN-SZTE Neuroscience Research Group, Hungarian Research Network, University of Szeged Danube Neuroscience Research Laboratory, Szeged, Hungary*
[6] *Department of Neurology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary*
[7] *Department of Forensic Medicine, Albert Szent-Györgyi Health Centre, University of Szeged, Szeged, Hungary*

Kynurenic acid (**KYNA**) is an endogenous neuroactive metabolite of the kynurenine pathway, which regulates several neurotransmitters such as dopamine, acetylcholine (Ach), glutamate, and γ-aminobutyric acid (GABA). Therefore, **KYNA** is involved in various neurological disorders such as Alzheimer's Dementia, Parkinson's disease, schizophrenia, Huntington's disease, and many others [1,2]. Even for COVID-19, **KYNA** represents an effective predictor of the acuteness and can inhibit the proliferation of cancer cells at high doses. [2, 3].

However, due to its limited permeability in the blood-brain barrier (BBB), **KYNA** cannot be administered as a drug externally. To overcome this limitation, newly synthesised **KYNA** analogues with better BBB permeability and enhanced pharmacokinetics were synthesised, and some of them have shown promising results in our previous research [1,4,5]. Nevertheless, determining **KYNA** analogues in biological samples is challenging due to their chemical diversity, functional groups, and varying concentrations in biological matrices [1, 4, 5]. Moreover, **KYNA** analogues' lipophilicity is a fundamental physicochemical characteristic dominating their pharmacokinetics and crossing the BBB [6]. No previous research reported LogP and LogD of **KYNA** analogues, and the analytical studies are limited.

This study aimed to develop a new analytical method and apply it to determine the concentration of **KYNA** analogues in an *in vitro* rat blood–brain barrier model.

An efficient targeted UHPLC-MS/MS method was developed and validated for the simultaneous determination of **KYNA** analogues in the brain homogenate. The mass spectrometric detection (MS/MS) was conducted using optimised electrospray ionisation (ESI) multiple reaction monitoring (MRM) mode. The flow injection method was used to optimise the ESI and MS/MS parameters and determine the quantifier and qualifier ions of the protonated **KYNA** analogues. The high-resolution tandem mass spectrometry MS/HRMS was used to study the fragmentation behaviour of **KYNA** analogues. LogP and LogD values were determined experimentally using the shake-flask method.

The total run time of the method was 10 minutes. The UHPLC-MS/MS method was validated entirely according to ICH guideline M10 bioanalytical method validation.

The developed UHPLC-MS/MS method was successfully applied to quantify **KYNA** analogues in an *in vitro* rat blood–brain barrier model.

Keywords: *Kynurenic Acid, UHPLC-MS/MS, Permeability, Kynurenic Acid Analogue*

Acknowledgments: Project no. TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme. This work was supported by National Research, Development, and Innovation Office-NKFIH through projects: National Laboratory of Translational Neuroscience (NLTN) Neurodevelopmental disorders" "Adult nervous system disorders" (RRF-2.3.1-21-2022-00011).

References:

[1] Molnár, K.; L˝orinczi, B.; Fazakas, C.; Szatmári, I.; Fülöp, F.; Kmetykó, N.; Berkecz, R.; Ilisz, I.; Krizbai, I.A.; Wilhelm, I.; et al. SZR-104, a Novel Kynurenic Acid Analogue with High Permeability through the Blood−Brain Barrier.Pharmaceutics 2021, 13, 61. https://doi.org/10.3390/pharmaceutics13010061

[2] Alves, L.d.F.; Moore, J.B.; Kell, D.B. The Biology and Biochemistry of Kynurenic Acid, a Potential Nutraceutical with Multiple Biological Effects. Int. J. Mol. Sci. 2024, 25, 9082. https://doi.org/10.3390/ijms25169082

[3] Roberts, I., Wright Muelas, M., Taylor, J.M. et al. Quantitative LC–MS study of compounds found predictive of COVID-19 severity and outcome. Metabolomics 19, 87 (2023). https://doi.org/10.1007/s11306-023-02048-0

[4] Kovács, V.; Remzső, G.; Körmöczi, T.; Berkecz, R.; Tóth-Szuki,V.; Pénzes, A.; Vécsei, L.; Domoki, F. The Kynurenic Acid Analog SZR72 Enhances Neuronal Activity after Asphyxia but Is Not Neuroprotective in a Translational Model of Neonatal Hypoxic Ischemic Encephalopathy. Int. J. Mol. Sci. 2021, 22, 4822. https://doi.org/10.3390/ijms22094822

[5] Domoki, F.; Tóth-Szűki, V.; Kovács, V.; Remzső, G.; Körmöczi, T.; Vécsei, L.; Berkecz, R. Differential Effects of Hypothermia and SZR72 on Cerebral Kynurenine and Kynurenic Acid in a Piglet Model of Hypoxic–Ischemic Encephalopathy. Int. J. Mol. Sci. 2023, 24, 14522. https://doi.org/10.3390/ijms241914522

[6] Arnott, J. A., & Planey, S. L. (2012). The influence of lipophilicity in drug discovery and design. Expert Opinion on Drug Discovery, 7(10), 863–875. https://doi.org/10.1517/17460441.2012.714363

P-30 | Methods for the analysis of per- and polyfluoroalkyl substances (PFAS) and the first comprehensive survey of PFAS contamination in Hungarian drinking water

Csilla Soros[1], **Máté Bakó**[1], István Szabó[2], Jeff Griffitts[2]

[1] *Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology, Department of Food Chemistry and Analytics, Budapest, Hungary*

[2] *Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Department of Environmental Toxicology, Gödöllő, Hungary*

Per- and polyfluorinated alkyl compounds (PFAS) pose significant environmental and health risks on a global scale. Our research focuses on analytical investigation methods and the determination of PFAS compound concentrations in Hungarian drinking water, surface water, and food products, with particular attention to the EU monitoring obligations that will come into effect from 2026.

PFAS compounds are synthetic substances that have been widely used worldwide since the 1940s, and due to their extraordinary stability, they are classified as persistent organic pollutants (POPs) in environmental registries. They are capable of bioaccumulation in living organisms through their binding to proteins, thus they also concentrate in human blood plasma, causing carcinogenic and immunosuppressive effects. The European Union continuously tightens the limit values for drinking water; currently, the maximum allowable concentration of all PFAS compounds is 0.1 μg/l in drinking water. This extremely low limit value presents a special analytical challenge.

International literature and regulations uniformly recommend high-sensitivity selective instrumental techniques such as (U)HPLC-MS/MS for multi-component PFAS analysis, but we find significant differences in sample preparation and in the applied stationary and mobile phases.

In poster format, we present to the audience our analytical method developed and validated last year, suitable for the simultaneous determination of 18 PFAS compounds. During method optimization, we paid special attention to the mobile phase composition, as this significantly influences detection sensitivity. We optimized the solid phase extraction (SPE)-based sample preparation procedure, which enabled 500-fold enrichment during the processing of 250 ml water samples. Thanks to this, we achieved a limit of detection (LOD) between 0.2-1.0 ng/l for the investigated components. We applied the validated method to examine 32 Hungarian drinking water samples. During our research, we are the first to publish domestic drinking water data, which contributes to European surveys.

Finally, we briefly present the winning application titled "PFAQuatic - Investigation of the environmental effects of certain PFAS compounds in surface waters, aquacultures, and related agricultural products" submitted to the HU-RIZONT International Excellence Research Cooperation Program (2024-1.2.3-HU-RIZONT) grant, which started in February 2025 at the Hungarian University of Agriculture and Life Sciences, in which we undertook the determination of 40 PFAS compounds (including ultra-short chain PFAS compounds) using isotope dilution technique in surface water and fish samples.

References:

[1] Kurwadkar, S., Dane, J., Kanel, S. R., Nadagouda, M. N., Cawdrey, R. W., Ambade, B., Struckhoff, G. C., & Wilkin, R. (2022). Per- and polyfluoroalkyl substances in water and wastewater: A critical review of their global occurrence and distribution. Science of The Total Environment, 809, 151003. https://doi.org/10.1016/j.scitotenv.2021.151003

[2] Panieri, E., Baralic, K., Djukic-Cosic, D., Buha Djordjevic, A., & Saso, L. (2022). PFAS Molecules: A Major Concern for the Human Health and the Environment. Toxics, 10(2), 44. https://doi.org/10.3390/toxics10020044

P-31 | Application of design of experiments for optimizing lipid separation in supercritical fluid chromatography

Yahia M. Kazmouz[1], Attila Felinger[1,2,3]

[1] *Department of Analytical and Environmental Chemistry and Szentágothai Research Center, Pécs, Hungary*
[2] *MTA-PTE Molecular Interactions in Separation Science Research Group, Pécs, Hungary*
[3] *Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary*

Lipids are a diverse group of hydrophobic biomolecules characterized by highly variable structures and physicochemical properties. They play essential roles as structural components of cell membranes and are involved in numerous biological functions. Consequently, alterations in lipid concentrations can reflect dysfunctions in the body and are often associated with various pathological conditions. Therefore, accurate lipid analysis is crucial [1].

Among the different lipid classes, we focused on optimizing a supercritical fluid chromatography (SFC) method for separating a mixture containing phosphatidylglycerols (PG), phosphatidylethanolamines (PE), and phosphatidylcholines (PC), which are representative glycerophospholipids. In addition, arachidonic acid and docosahexaenoic acid—main precursors of bioactive oxylipins—were included in the mixture. Due to the structural similarity of many lipid species and mediators, often differing by only a single double bond in their acyl chains, high-resolution separation methods are required. To achieve optimal separation, we employed response surface methodology (RSM) instead of the traditional one-factor-at-a-time approach. A central composite design (CCD), face-centered type, was used to investigate the effects of three parameters—gradient time, pressure, and methanol percentage—on the resolution and symmetry factor of the critical pair (arachidonic acid and docosahexaenoic acid).

The experimental design tested gradient times between 1.0 and 2.0 minutes, pressures from 125 to 225 bar, and methanol concentrations ranging from 2% to 5% (v/v), with each variable evaluated at three levels. The results indicated that pressure and gradient time had the most significant influence on the symmetry factor, while pressure primarily affected the resolution of the critical pair.

Reduced cubic models were required to adequately describe the relationship between these variables and the chromatographic responses, with coefficients of determination (R^2) not less than 0.9658 for all models. Moreover, significant interactions among the studied factors were observed, justifying the application of the design of experiments (DOE) strategy.

Based on the optimized models for resolution and symmetry, the selected SFC conditions for further experiments were 3.5% methanol (v/v), 125 bar pressure, and a gradient time of 1.5 minutes.

Reference:

[1] Chollet, C., Boutet-Mercey, S., Laboureur, L., Rincon, C., Méjean, M., Jouhet, J., ... & Touboul, D. (2019). Supercritical fluid chromatography coupled to mass spectrometry for lipidomics. Journal of Mass Spectrometry, 54(10), 791-801.