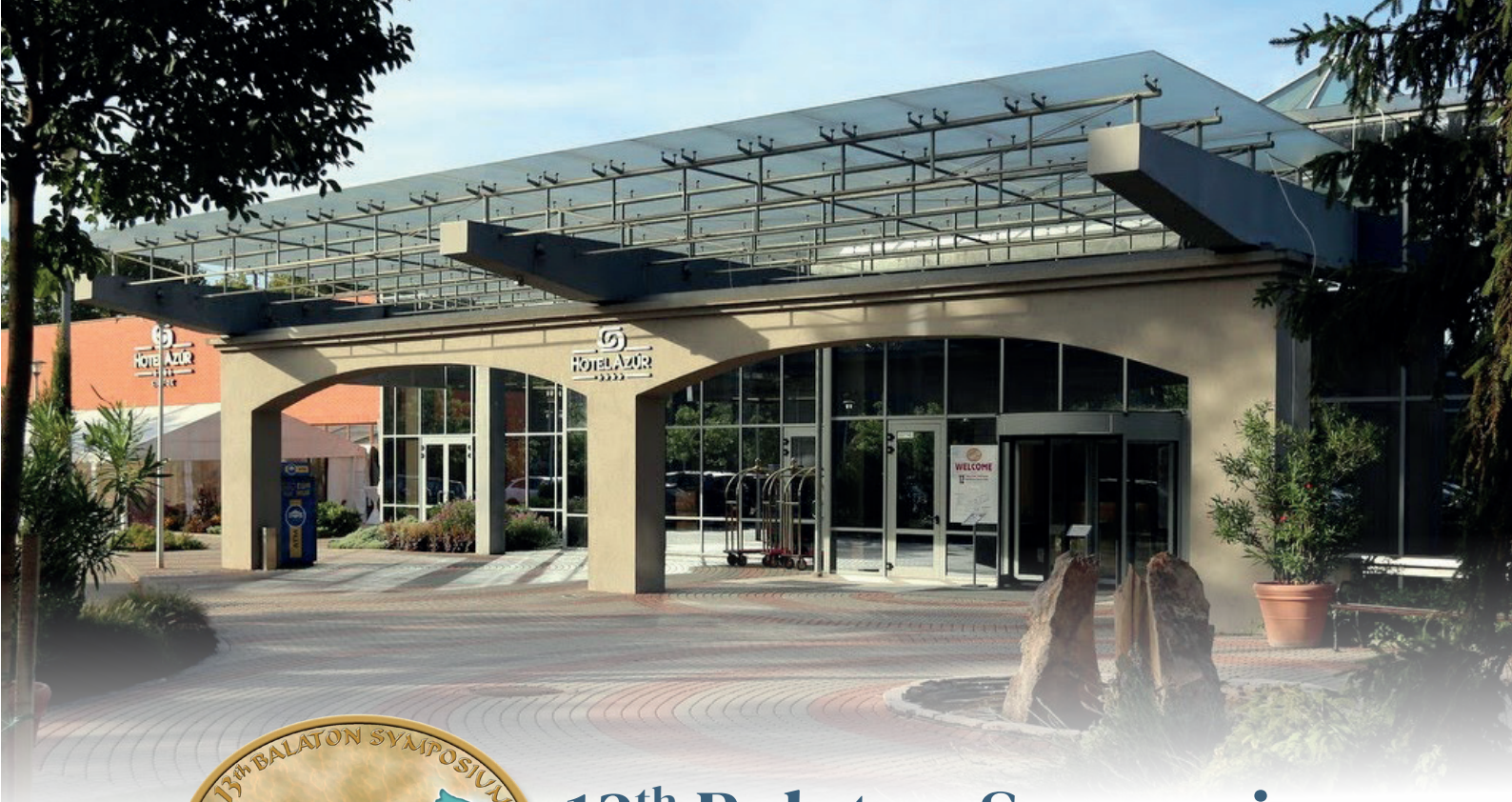

13th Balaton Symposium

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Book of Abstracts

Hungarian Society for Separation Sciences

Lectures

L-01 | Innovative chromatographic strategies to improve the characterization of oligonucleotides

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The emergence of new DNA or RNA-based therapies is opening up new perspectives for the treatment of genetic diseases. Among them, therapeutic oligonucleotides are enjoying growing success due to their high specificity for their target and their improved pharmacokinetic properties. Thus, although considered by regulatory agencies as small molecules, they also share characteristics with therapeutic proteins. Oligonucleotides are therefore a new class of pharmaceutical compounds requiring specific considerations.

In order to ensure the safety and efficacy of these new therapeutic molecules, their characterisation is essential and requires adapted and robust analytical methods. Reverse phase liquid chromatography with added ion pairing agents (IP-RPLC) is the reference method for the analysis of oligonucleotides, and HILIC is gaining in popularity.

In the present work, various strategies will be exposed to improve sensitivity, throughput, and selectivity when analyzing therapeutic oligonucleotides in IP-RPLC and HILIC modes. The goal of this presentation will be to highlight i) the importance of bioinert columns to limit adsorption of oligonucleotides, ii) the interest to use alternative column chemistries to improve selectivity, iii) the possibility to work with ultra-short columns of only a few mm to achieve high throughput separations, and iv) the use of pressure as an additional parameter to tune selectivity.

L-02 | Addressing materials and resolution challenges in the 3D printing of chromatography columns

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Additive manufacturing has recently transformed the chromatography landscape thanks to its capability to fabricate porous stationary phases with perfectly ordered structure. Industrial implementation of 3D printed stationary phases must now accelerate, with the goal to offer regular morphologies with improved separation performance which can easily integrate into the production line.

This presentation will delve into the recent strides taken to tackle the two primary challenges inherent in creating chromatography columns using 3D printing technology: i) the identification of materials compatible with both 3D printing processes and chromatographic operations, and ii) the rapid, high-resolution, and large-scale printing of such materials.

The initial segment of this work will outline formulations for the direct 3D printing of chromatography columns in a single step. These materials are rooted in methacrylate chemistry, rendering them compatible with Digital Light Processing (DLP) printing technology. Columns with anion and cation exchange modalities are obtained by incorporating functional monomers in the ink formulation, obviating the need for subsequent functionalization steps. A practical application will be showcased, detailing the capture and purification of c-phycocyanin, a protein of significant industrial relevance.

The second part of the talk will focus on multiscale control of 3D printed matrices, from mm to µm to nm, to rapidly achieve tuneable stationary phases for bioseparations. A different material formulation, employing epoxy chemistry to facilitate straightforward functionalization, will be introduced. The material's development, characterization, chemical derivatization, and subsequent evaluation for capturing and separating model proteins will be outlined. Impressively, these formulations offer an unprecedented level of control over morphology at sub-millimeter scales (achieving features as small as 50 µm for linear structures and 200 µm for complex geometries) and feature tunable porosity at sub-micrometer scales. Notably, these structures can be swiftly 3D printed in as little as one hour, enabling the creation of intricate large-scale models (up to 100 mL columns). The integration of anion and cation exchange ligands onto 3D-printed gyroid structures was accomplished, successfully demonstrating i) the separation of model proteins under dynamic conditions, and ii) the capture of proteins from a clarified cell harvest. These experiments exhibited dynamic binding capacities ranging from 5 to 16 mg mL^{-1} and yielded up to 86% purity in a single run.

These findings serve as a robust foundation for propelling the implementation and utilization of 3D-printed chromatography stationary phases to the forefront of practical applications.

L-03 | 4D hyphenated techniques reveal hazardous effects in food and cosmetics using the 2LabsToGo system

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The new open-source 2LabsToGo system offers the unique combination of a chemical and a biological laboratory. Exploring new technologies and new analytical strategies, a miniaturized, efficient, lean, sustainable, cost-saving, and freely available minilab (31 cm x 26 cm x 34 cm) for hyphenated high-performance planar liquid separation in combination with biological detection was developed [1]. Since it is open-source, the whole system can be adapted and optimized to one's own needs.

Complex samples can be analyzed in parallel comprehensively and non-targeted. They are automated applied to the adsorbent surface and separated via a minimized dosage system. LED detection at UV/Vis/FLD and tools for derivatization to widen the range of detectable compounds were integrated. The ability to apply cells on the adsorbent surface and to perform on-surface cell incubations including bioluminescence detection opened up new avenues. Thus, via a planar bioassay, the separated samples can be examined biologically to provide information about beneficial/harmful effects arising from complex samples. All relevant process steps are combined in this system, and its operation is very sustainable compared to the status quo.

The method performance and proper functionality of the 2LabsToGo system is demonstrated by various applications, such as screening of ergot alkaloids in rye and estrogen-like substances in wine and beer. The 2-Labs combination allows for a novel analytical strategy on compound prioritization [2,3] instead of separating everything. The combination of two disciplines on the same surface provides a 4-D hyphenation leading to important information and meaningful image-based profiles including data on the activity of compounds. It provides a better understanding and solves pressing challenges in the analytical field [4].

Acknowledgements

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L-04 | Developing an assay to determine impact of environmental conditions on the breeding of endangered species

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This project will look to develop an assay for the analysis of faecal matter from an endangered species to allow the monitoring of one or more hormones to determine the impact that social interactions between animals or other environmental factors have on the breeding process. Working with local conservation centres a method will be developed that will for the analysis of a series of critical stress hormones. This method will initially be developed using a combination of sample preparation and also chromatography couple to mass spectrometry. Once a method has been developed it will be transferred to a more simplified format that will be applicable to field studies.

Metabolomics is an emerging scientific discipline that can be used to identify biomarkers of interest for monitoring. Currently used in the pharmaceutical and medical fields, the use of metabolomics has seldom applied to animals – which would provide invaluable insights into the animals welfare. The ability to identify stress-related biomarkers, via non-invasive methods, would enable zoo keepers to better-manage the stress of their animals, increasing the likeliness of captive breeding success and beyond.

- Using metabolomics to identify stress-related biomarkers.
- Develop an assay to identify stress-related biomarkers from faecal samples.
- Share method to identify stress biomarkers from Black Rhino faecal samples using HPLC with Chester Zoo.

Using the assay/method developed during the project, Chester Zoo will be able to analyse faecal samples of Black Rhino's to identify stress-related biomarkers using HPLC. Accurately identifying stress in captive animals will enable zoo keepers to monitor stress levels and mitigate triggers. In time, monitoring levels of stress in captive animals will increase the success of captive breeding programmes and increase better welfare. The assay/method could be used to develop this approach in different species/target metabolites.

L-05 | Boosting chromatographic performance and productivity by thermal gradient-gas chromatography

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Following the quest for more performant, but at the same time faster separations, gas chromatography is suffering, like all chromatographic techniques from the "magic triangle" in chromatography, namely the paradigm that separation efficiency, separation speed and capacity cannot be all increased at the same time in one given system. While this fundamental limitation still cannot be overcome, an unconventional approach was introduced by Contreras more than a decade ago [1] which involved the use a (negative) thermal gradient to achieve excellent chromatographic separations on short capillary columns in very short separation times (typically less than 2 min).

This idea was later on improved and commercialized by Boeker et al. [2] who produced a cylindric device with a spiral grove in which the capillary column was placed. The cylinder was filled with a foam that created a defined resistance for air flowing through it. A forced flow of air was used to create a longitudinal temperature gradient along the GC column. This negative temperature gradient focuses the peaks of the compounds to be separated, while its change with time enables the elution of the analytes with excellent peak shape, narrow peak width and within very short retention times.

We have taken on and further improved this ground breaking idea of negative thermal gradient gas chromatography [3] and introduce – in contrast to the device of Boeker et al. – a modular concept for (negative) thermal gradient gas chromatography, (N)TG-GC, which can be used with any of the currently available standard gas chromatographs of major instrument manufacturers. With the implementation of this TG-GC device, commercial instruments are converted into high-speed, high performance separation devices whose performance is boosted. Examples will be presented from various fields of application where lengthy separations of 30-60 minutes in conventional mode can be shortened to 1-4 minutes separation time while essentially maintaining chromatographic resolution.

Using simulations of the chromatographic separation, we are able to predict which factors are critical in the optimization of the separation under TG-GC conditions. This allows to evaluate the applicability of different temperature profiles in TGGC and the proposition of different operational modes. These eventually lead to new and enhanced possibilities of using GC for high-throughput analysis, as well as for the monitoring of fast changing processes such as the monitoring of volatile organic compounds formed from the electrolyte of lithium ion batteries during a charge/discharge cycle.

Funding of this work by the Austrian Research Promotion Agency (FFG) under grant no. 879613 ("OPERION") is gratefully acknowledged.

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L-06 | Fundamental investigation of the extra column band broadening in nano-LC capillary tubing with different geometries

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With the push to ever smaller particle diameters, the introduction of core-shell particles, the ability to produce particles with narrower size distributions and the improvement of the packing procedures, packed bed UHPLC columns and capillaries produce such narrow peaks that separation performance is often determined by the extra-column band broadening (ECBB) effects. This problem is most stringent in the area of nano- and micro-LC, where column volumes are very small, and where, with the advent of e.g. microfabricated pillar array columns, very high-efficiencies are achievable, requiring very low dispersion fluidic connections. The aspects of ECBB have been studied in much detail in the past decades [1]. There is however still no fundamental basis to estimate or describe the effects of connecting capillaries with different geometries and dimensions, the dispersion due to capillary misalignment or the presence of void volumes in the fluidic path.

In this presentation, the results of a Computational Fluid Dynamics (CFD) study of these effects is presented. As representative cases, the extra dispersion caused in nano-LC capillary tubing with a different diameter, with a misalignment and with a dead zone in between are considered. The focus is on the typical flow rates ($0.25 \leq F \leq 2$ μL/min) and diameters ($d \leq 40$ μm) used in nano-LC. The CFD simulations allow to study the problem from a fundamental point of view, i.e., under otherwise perfect conditions (perfect alignment, zero dead-volume, perfect dispersionless detection). Flow rates, capillary diameters, diffusion coefficients and liquid viscosities have been varied over a range relevant for nano-LC (Reynolds-numbers $Re \leq 1$), with an extension made towards high-temperature nano-LC conditions ($Re \geq 10$ and more).

The extra dispersion caused in the different geometries has been quantified via a volumetric variance σ^2_{conn}, defined in such a way that the overall dispersion across the entire capillary system can be easily reconstructed from the known analytical solutions in the individual segments. When the two capillaries are longer than their diffusion entry length, covering most of the practical cases, σ^2_{conn} converges to a limiting value $\sigma^2_{conn,\infty}$ which varies approximately with the square of the flow rate. Under the investigated nano-LC conditions, the $\sigma^2_{conn,\infty}$-values are surprisingly small (e.g., on the order of 0.01 to 0.15 nL^2 in a 20 to 40μm connection) compared to the dispersion occurring in the remainder of the capillaries. Only when large dead zones are present, e.g. such as voids due to the use of improper ferrules or incorrect stem lengths, significant band broadening occurs and tailing peaks are obtained.

L-07 | Estimation and characterization of overloaded band profiles in ion-exchange separations

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Keywords: ion-exchange, overloaded peak profiles, isotherms

The retention behavior of compounds in a chromatographic system is governed by their isotherms. Beside the retention times, the type and shape of the isotherms determine the shapes of the overloaded peak profiles. In the case of convex, Langmuir-type isotherms, the overloaded peaks tail and the bands have sharp, straight fronts. For convex, BET-type isotherms, the peaks are fronting and the rears of the peaks are typically sharp and straight. In this work, the overloaded band profiles of ions are studied in ion-exchange chromatography by experimental and theoretical approaches. It is shown that even if all ions have Langmuir-type isotherms, both tailing and fronting band profiles can occur depending on the type of analytes and chromatographic conditions. Furthermore, under certain conditions, symmetric overloaded peak profiles may also appear. The implications of this phenomenon for analytical and preparative separation are discussed.

L-08 | New approach for the depletion of Δ^9-THC from CBD rich samples by simulated moving bed

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The growing popularity and medical use of Cannabis and hemp products, along with the change in the regulations, have contributed to increase the studies to find efficient and sustainable purification methods for cannabinoids from Cannabis extracts. Indeed, cannabinoids have many applications in fields, from medicine to food industry [1]. The two main target cannabinoids are Δ^9-tetrahydrocannabinol (Δ^9-THC), a psychoactive cannabinoid, and cannabidiol (CBD), a non-psychoactive cannabinoid. The presence of Δ^9-THC into commercial products must remain below critical regulatory concentrations because of its psychotropic effect. This points the attention on the need of fast, efficient, cost-effective and green methods for the depletion of Δ^9-THC from CBD rich samples. However, the purification of CBD is a challenging task due to the similar chemical structures and physical properties of cannabinoids.

Simulated Moving Bed (SMB) is known to be one of the most effective purification processes due to its low solvent consumption, internal solvent recycling and a better utilization of the stationary phase, if compared to traditional methods [2]. This process is based on multicolumn continuous countercurrent chromatography, where the feed is continuously loaded into the system and, thanks to the use of a series of valves, it is possible to mime the countercurrent movement of the stationary phase in the opposite direction of the mobile phase flow.

In this work, SMB has been applied for the depletion of Δ^9-THC from a CBD-rich extract. The separation performance, in terms of productivity, solvent consumption and environmental impact, of two SMB methods operated under reversed and normal phase elution modes has been evaluated and compared. Results indicate that the process using reversed phase mode can be successfully used as a green and sustainable alternative to commonly used methods for the purification of cannabinoids.

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L-09 | Separation of remdesivir diastereomers by centrifugal partition chromatography

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Remdesivir is a phosphoramidate nucleotide prodrug (ProTide) approved for the treatment of severe COVID-19 infection in 2020. It contains a chiral phosphorous center, with the current clinical compound being the S_P-diastereomer (GS-5734). However, the pharmacological relevance of the R_P-diastereomer is also considered significant. The chiral stationary phase (Lux Cellulose-2) utilizing the preparative HPLC method reported by Gilead Sciences is an expensive and hardly scalable step in the total synthesis of remdesivir. Thus, our goal was to develop a cost-efficient (achiral) centrifugal partition chromatographic approach for the efficient and scalable separation of remdesivir P-diastereomers.

First, more than 200 binary, ternary, and quaternary solvent systems were screened to find proper solubility, partition, and selectivity for the diastereomer pair. It was recognized that selectivity values as high as in the chiral environment could be achieved by carefully selecting the apolar organic solvents (e.g., ethyl acetate, heptane), cosolvents (e.g., methanol, methyltetrahydrofuran), solubilizing components (e.g., cyclodextrins, polyethyleneglycol), and pH. In addition, efficiency-enhancing operating modes of CPC, such as multiple dual mode (MDM) and trapping MDM, were developed to resolve the diastereomers. In this paper, performance, such as purity, yield, and productivity of laboratory and pilot-scale CPC methods, will be highlighted and compared in this challenging separation scenario.

L-10 | Influence of lignin-derived natural organic compounds on the separation of organic contaminants onto zeolites from water matrices

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Natural organic matter (NOM), a complex mixture of organic compounds, is commonly found in surface and groundwaters. Humic substances represent 40-80% of the NOM content and they are found in freshwater typically in a concentration range 1-25 mg/L [1]. Among this class of compounds, humic acids form a complex mixture of natural substances derived from the biodegradation of lignin; they are characterised by carboxyl and phenolic groups, and they are resistant to further degradation. p-Hydroxybenzaldehyde (p-HBA) and caffeic acid (CA) are monomers of humic acids that are commonly found in natural waters.

Toluene is a hydrocarbon belonging to the BTEX class (Benzene, Toluene, Ethylbenzene, Xylene) which is frequently detected in all environmental compartments due to its wide use as solvent, antiknock agent in gasoline, and for the production of benzene.

High-silica zeolites have already been proven to be efficient eco-friendly adsorbents for the removal of several organic contaminants from aqueous solutions [2]. To evaluate the possible use of these materials in water remediation applications, the adsorption of toluene in water matrices containing naturally found organic molecules, p-HBA and CA, was explored. Besides their natural occurrence, p-HBA and CA were chosen due to their molecular dimensions which are comparable with those of the contaminant selected and smaller than the pore size of zeolite.

In this work, zeolite ZSM-5, with MFI framework type topology and Si/Al ratio of 280, was chosen to investigate the adsorption of the selected organic compounds. Regarding the adsorption of the humic acids monomers, the systems ZSM-5 – p-HBA and ZSM-5 - CA were considered. Adsorption isotherms were determined at different pH values to establish the effect of this parameter onto the saturation capacity of the adsorbent materials.

Regarding the adsorption of the contaminant (TOL) from the aqueous matrix containing the natural humic acids monomers (p-HBA or CA), it resulted that the natural compounds do not show competitive behaviour: the adsorption of TOL was slightly affected by the presence of the ligninderived substances [3]. The higher selectivity of ZSM-5 for TOL with respect to p-HBA and CA was also confirmed from structural investigation of the selected zeolite loaded with single component solution of TOL and p-HBA or CA, and from a binary mixture containing both the contaminant and the humic acids monomer (i.e., TOL and p-HBA, or TOL and CA). Strong similarities between the X-ray powder patterns after adsorption of the mixture and after only TOL adsorption, indicate that the zeolite adsorb preferentially and selectively toluene, also in the presence of the natural compounds. These findings confirm the high selectivity of zeolites for the removal of organic contaminants from waters in presence of natural components.

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L-11 | To desalt or not to desalt: The significance of sample clean-up prior to CZE peptide mapping studies

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Bottom-up proteomics aims at the extensive characterization of protein samples with analytical platforms such as reversed-phase liquid chromatography (RPLC) or capillary electrophoresis (CE) coupled to tandem mass spectrometry (MS/MS). Sample clean-up prior to analysis is generally considered a necessity, however, the process can lead to peptide loss. Matrix removal is frequently done by solid-phase extraction (SPE) pipette tips containing C18 packing. In addition to removing the potentially interfering components from the digests, SPE is often used for off-line sample enrichment.

To investigate the impact of matrix removal, protein digests of varying complexity (albumin, human tear samples and yeast cell lysate) were analyzed with CE-MS before and after desalination with C18 SPE pipette tips. Moreover, samples containing added ammonium bicarbonate (AB) and acetonitrile (ACN) were also analyzed and their effect on on-line sample enrichment was evaluated. Results indicated that desalination, indeed, altered the peptide profile, samples typically lacked small, hydrophilic peptides that were not retained on the reversed-phase SPE packing. Non-desalinated digests offer a very attractive alternative to off-line enrichment, since the presence of salts can induce an on-line stacking phenomenon most probably via transient isotachophoresis. Stacking efficiency was further enhanced by the addition of ACN; sequence coverage values and number of protein hits increased.

ML-1 | Actual developments in HPLC modeling

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Modeling HPLC separations has a long tradition. It started 1986 as Lloyd Snyder started to model isocratic separations. Later modeling was extended for gradient methods in Reversed Phase Chromatography (RPC) and for Ion-Exchange Chromatography (IEC). Recent developments include HILIC, HIC, SFC and Robustness modeling. All techniques need experimental support to measure peak positioning, depending on pH, temperature, gradient time, salt- or additive concentration, etc. From the measured data a software (f.Ex. DryLab) can model a separation under a number of variable condition in seconds instead of hours.

The results are reliable, robust methods, which enable the fast development and market-entry of new and more efficient drug products. The lecture will present several case studies on modeling from actual developments.

L–12 | The role of radiochromatography in the pharmacokinetic and drug metabolism research

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Pharmacokinetic and metabolite profiling are indispensable part of drug discovery and development. The presentation overviews the modern tools of radiochromatography in preclinical and clinical pharmacokinetic and drug metabolism studies. The essential pharmacokinetic and drug metabolism studies of different species (mouse, rat, dog, rabbit, and human) provide information to the final drug registration process. The highly sensitive (pg/ml, fg/ml, at/ml) and selective hyphenated techniques (LC/Triple Quad-Jet Stream-ESI-MS and GC/MS-MS, etc.) required for the pharmacokinetic studies had replaced the conventional methods of detections such as GC and HPLC. During drug development radioactive isotope (beta and gamma single and/or double source)-labeled (^{3}H, ^{14}C, ^{99}Tc, ^{131}I) pharmacokinetic studies combined with new generation of triple-quad and high-resolution MS techniques (LC, CE, OPLC) are significant. Several related case studies will be introduced. The former Imaging Techniques (DAR, PIT) and the new generation of *in vitro – in vivo* Imaging Techniques (MALDI Imaging, nanoScan, PET/MRI in animal and human studies) will also be presented. A complex multi-step process will be illustrated from separation, purification, isolation to structure elucidation (GC-MS, LC-MS/MS, LC-NMR) of minor and major metabolites derived from animal and human biological matrices. The addition of these systems to the off-line and on-line separation and radioactivity detection possibilities of OPLC-DAR/PIT, OPLC-RD, HPTLC-DAR-MS and GC-RD, HPLC-RD and the combined multi-hyphenated techniques, OPLC-DAD-RD-MS/MS, OPLC-DAD-RD-NMR as well as LC-DAD-RD-MS/MS and LC-DAD-RD-NMR resulted in a new, flexible and rapid high-performance complex solution in the metabolism research.

L-13 | Role of serendipity in research and development: Vacuum jacketed column – mass spectrometry technology

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This presentation will illustrate the significant role that serendipity can play in research and development in the industry sector. It will cover a period of 8 years in the core research/fundamentals group at Waters Corporation where unanticipated events and observations have eventually led to the final development of a new liquid chromatography – mass spectrometry (LC-MS) technology. In particular, it is revealed how the concept of Vacuum Jacketed Columns (VJC) emerged and how this novel column technology was eventually found beneficial in LC-MS for maximum speed, resolution, and compound coverage in proteomics, metabolomics, and lipidomics. Proof-of-concept and user benefits of the newly designed VJC-MS prototype system will be illustrated and discussed in these different separation fields.

L-14 | Development of sample preparation and HPLC-MS methods for analyzing tissue-derived glycosaminoglycans

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Mass spectrometry analysis of tissue samples is widespread in biomarker research and holds great potential for mapping the biochemical processes underlying various diseases. Glycosaminoglycans (GAGs) are key molecules in structural development and signaling, therefore, they are appealing targets for a deeper understanding of molecular mechanisms. The most prevalent and structurally diverse classes of these sulfated polysaccharides are chondroitin sulfate (CS) and heparan sulfate (HS). Even after enzymatic digestion of the polysaccharide chain, they remain one of the most challenging compound groups in analytical chemistry due to their wide range of physicochemical parameters. Therefore, their comprehensive analysis in a single workflow requires extensive optimization, especially for the sampling of extremely small tissue regions (<10 µg total mass). Selected examples of the process of step-by-step workflow optimization will be shown in the presentation.

First, we optimized the on-tissue digestion of GAG chains, and we determined optimal sample handling conditions. Next, we developed solid-phase extraction (SPE) methods utilizing unique self-packed cotton-HILIC pipet tips and graphite spin tips. We found that the two stationary phases exhibited complementary behavior in terms of recoveries for the differentially sulfated disaccharides. Consequently, their sequential combination provides a high-recovery, unbiased purification method for CS and HS disaccharides. We also developed HPLC-MS methods for GAG disaccharide analysis. They are based on self-packed HILIC-WAX capillary columns operated with ammonium formate gradient for the elution of the differentially charged molecules.

The optimized workflow enabled us to characterize thin human tissue sections. We focused on cancerous malfunctions such as the differences between prostate cancer and benign prostate hyperplasia or deciphering intra- and intertumoral heterogeneity of different lung cancer cell types. In both above-mentioned cases, we found that the occupied sulfation positions of both CS and HS chains showed substantial differences among the compared sample groups.

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L-15 | A journey into zwitterionic teicoplanin-based superficially porous particles

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The wide applicability and versatility of macrocyclic antibiotics (like teicoplanin) have fostered their extensive use as chiral selectors in liquid chromatography. Many studies have been proposed about adsorption and interaction mechanisms between enantiomers and teicoplanin

selector from a molecular viewpoint [1,2]. However, it has been demonstrated that kinetic performance is highly influenced by experimental conditions. To shed light on this behaviour, all aspects and characteristics of this chiral selector need to be carefully taken into account and investigated from a fundamental perspective.

In this work, the effect of both the mobile phase composition (ACN/H_2O and MeOH/H_2O) and analyte chemistry (Methionine and Propionic Acid) on kinetic performance and thermodynamic properties of zwitterionic teicoplanin-based superficially porous particles (SPPs) has been evaluated.

More in detail, on the one hand, kinetic studies have been performed though the combination of both stop-flow and dynamic measurements. This permits to evaluate the impact of eddy dispersion, longitudinal diffusion, mass transfer resistance and adsorption-desorption kinetics on efficiency [3].

On the other hand, thermodynamic behavior has been investigated by calculating both absolute and excess adsorption isotherms. The study of adsorption isotherms allows to gain information on surface heterogeneity in terms of adsorption energy distribution, types of adsorption sites, preferential adsorption of one of the component of the binary mobile phase and to investigate if the mobile phase composition and the analyte type have an effect on both the binding constants and the enantioselectivity of the CSP.

Results revealed that eddy dispersion, adsorption-desorption kinetics and binding constants are strongly dependent on the analyte and on the organic modifier used in the mobile phase. Hence, the deep knowledge of CPS characteristics and the correct selection of experimental parameters can be helpful to obtain separations with very high efficiency or large enantioselectivity, depending on the need.

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L-16 | Thermal effects in (U)HPLC

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Introduction

Ever since the emergence of chromatography there has been a trend towards the usage of smaller particles. This trend is obviously driven by the fact that particle diameter is proportional to plate height and hence using smaller particles improves separation efficiency. Unfortunately, this trend also comes with the downside that higher inlet pressures are required to sustain a reasonable mobile phase flow rate. Besides an increase in equipment and energy cost, these higher pressures also lead to increased amounts of energy dissipated inside the column through viscous heating. As a consequence temperature rises and even worse, becomes non-uniform throughout the bed, which negatively affects separation efficiency. The present contribution summarizes the findings of a series of 4 studies [1-4] all on the subject of these thermal effects in (U)HPLC. Solutions to alleviate the effects of viscous heating found in literature are examined and new solutions are proposed.

Research design

Computational fluid dynamics (CFD) simulations were used to study thermal effects at the particle level, bed level and column level, as well as their influence on plate height.

Particle level - The influence of particle design on the thermal conductivity of the chromatographic bed was studied. Different stationary phase support materials, the presence and absence of a solid core and the usage of highly conducting cores were considered. Realistic values for particle conductivity were determined and used in the next study level.

Bed level - The effect of mobile phase conductivity and particle packing on the thermal conductivity of the chromatographic bed was investigated. Besides particle packed beds, monolithic structures were also considered. Realistic values for the bed conductivity were calculated and used in the following level of study.

Column level – Taking into account the influence of temperature and pressure on mobile phase viscosity and density, the interdependent mobile phase velocity field and temperature field were resolved and the influence of inlet pressure, column wall conductivity and thermal environment was investigated.

Plate height - The migration of an analyte peak through the bed was simulated to determine plate height values. The knowledge of the velocity field allowed to take into account velocity inhomogeneities. Simultaneously, the temperature field was used to include the effect of temperature on the analyte retention factor and diffusion coefficient.

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L-17 | Let's make chromatography more green

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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 while working with high-performance liquid chromatography apparatus is growing. Therefore, new methods and materials are being searched to achieve the so-called "green" chromatography.

Due to the harmful effects of organic solvents, affecting humans and the environment, "green" technologies are being developed. Separation techniques, including chromatographic techniques, are based on consuming large amounts of organic solvents. Thus, interest and demand for research towards separating mixtures under environmentally friendly conditions are increasing.

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; using mobile phase additives such as cyclodextrins or surfactants; stationary phases with shorter carbon chains; core-shell columns; operating at elevated temperatures; replacing organic solvents with other, more "green" solvents such as water, ethanol or supercritical CO_2. Among the above-mentioned, separations performed using pure water as the only eluent in HPLC are the most environmentally friendly. For such analyses to be carried out, it is necessary to apply appropriate separation conditions and unique columns, allowing for selective substance separation with such a highly polar solvent. The green aspects of chromatography are the main issue of this presentation.

L-18 | Replacement of acetonitrile with dimethyl carbonate as organic modifier in liquid chromatography

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Reversed-phase liquid chromatography (RPLC) is the most widely employed analytical method in (bio)pharmaceutical industry, where it is commonly used not only for analytical purposes, such as quality control of bulk drugs and formulation, but also for preparative scopes in industrial plants for the isolation of the target (bio)pharmaceutical with elevated purity. The most widely employed organic modifier in RPLC is acetonitrile due to its exceptional characteristics, such as high elution strength, UV transparency, high miscibility with water and low viscosity. However, acetonitrile is slightly toxic by acute exposure through oral intake, skin contact, and inhalation and it can be converted by the body into cyanide. [1]

For this reason, in the last year the greening of analytical methods has gained increasing interest in the pharmaceutical field, in order to reduce the environmental impact commonly associated with manufacturing process and to ensure health safety of operators. Indeed, especially in preparative conditions, the large amount of solvents commonly employed leads to the generation of high quantities of waste to be disposed. [2]

Beside alcohols, whose physico-chemical properties are well-known, also less common solvents have recently attracted the attention for this purpose. One of this is dimethyl carbonate (DMC), which is classified among the “recommended” solvents by the Innovative Medicines Initiative (IMI)-CHEM21, a European consortium promoting sustainable biological and chemical methodologies. [3] Indeed, DMC is nowadays produced though a clean process, it is biodegradable and it is not toxic. Although it is widely employed as solvent in novel applications related to supercapacitors, lithium batteries and other emerging devices for energy storage, DMC has been introduced as organic solvent for LC only in a recent paper where the authors demonstrated its applicability in LC hyphenated to inductively coupled plasma mass spectrometry platforms. [4] In this work, the possibility of using DMC as organic modifier in LC with UV-Vis detection will be discussed through a series of case studies including fundamental studies on retention in linear conditions up to applications in preparative conditions for the purification of pharmaceutically relevant peptides.

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L-19 | Tuning selectivity in reversed-phase chromatography applied for the separation of regioisomers of sugammadex related impurities

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Cyclodextrins has always been important excipients for food and pharma, however their therapeutic use discovered only lately. Among other blockbuster cyclodextrins, Sugammadex (SGM) found a particular market success, thanks to its unique ability to selectively encapsulate steroidal neuromuscular blocking agents, such as rocuronium or vecuronium. However, just like any pharmaceutical ingredients, during the chemical synthesis of SGM, the formation of process-related impurities, such as Mono-OH-SGM, Di-OH-SGM, Mono-halogen-SGM, etc., is almost inevitable. Thus, to guarantee the final product quality and to support the upstream synthetical processes, powerful, yet often time- and resource-intensive analytical methods (NMR, MS, IR) are frequently employed.

At the same time, reversed-phase high-pressure liquid chromatography (RP-HPLC) analysis in combination with simple optical detection can also provide with both efficient and economical solution for characterizing, identifying and quantifying these impurities. This being said, in our case, we aimed to maximize the separation process of relevant regioisomers of two different SGM-related impurities (Di-OH-SGM (Hexakis(6-deoxy-6-(2-carboxyethyl)thio)-gammacyclodextrin and OH-SH-SGM 6-Deoxy-6-thio-hexakis(6-deoxy-6-(2-carboxyethyl)thio)-gammacyclodextrin) on our HPLC-UV system, The presence of these regioisomers of the synthesized reference materials was already verified with orthogonal MS and NMR spectroscopic methods. Although the preliminary experiments on the selected C18 stationary phase already showed promising results, areas of suitable working conditions could not be discovered without contextualized knowledge. Hence, we applied a chromatography-based modeling software (DryLab) to systematically study and identify critical quality attributes (CQAs) of the separation system. The acquired model Design Space (DS) clearly showed that by fine-tuning relevant method parameters, optimal use of hardware to meet the required method goals (resolution, robustness and analysis time) could easily and flexibly be achieved.

The lecture will present this software-assisted development process, underlining the key benefits of a systematic, analytical Quality-by-Design (AQbD)-driven modeling approach in establishing fast and economic separation of regioisomers of SGM-related impurities.

L-20 | Challenges in chromatographic analyses of phytonutrients in food and plant samples

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Phytonutrients are a big group of compounds with different chemical structures and bioactivities (antioxidant activity, enhancement of immune response or cell-to-cell communication, lowering blood pressure and/or cholesterol level, etc.). Due to this diversity many phytonutrients consumed on a daily bases are still unknown. Several phytonutrients are nowadays marketed as active ingredients of food supplements (globally considered as food) or functional foods, although many of them have not yet been properly scientifically investigated. The use of phytonutrients in various products created a need for new source materials of these compounds. This created a great opportunity for valorization of invasive alien plant species, and plant waste materials from food industry, which is important for sustainable development. Methods based on chromatographic techniques are indispensable in this process of discovery of new plant materials as well as in development of new food products and control of food quality and safety.

This lecture will present challenges in development of methods based on high-performance thin-layer chromatography (HPTLC-densitometry, HPTLC-image analysis, HPTLC-MS/(MS) and high-performance liquid chromatography (HPLC-UV/Vis, (U)HPLC-MS/(MS)) for targeted and non-targeted analyses of phytonutrients present in invasive alien plant species, plant waste materials from food industry and food – including food supplements. Challenges related to stability of the analytes, lack of chromophores, isomeric structures and lack of commercial standards will be discussed.

Acknowledgements

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L-21 | HPTLC hyphenations as a key for preparative bioassay-guided isolation

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Screening, characterization, and isolation of bioactive compounds from natural sources can be achieved by bioassay-guided processes that comprise extraction, fractionation, and purification steps, all interlaced with biomonitoring [1]. High-performance thin-layer chromatography (HPTLC) combined with bioactivity assays ensures high-throughput, relatively cheap, and reliable effect-directed analysis (EDA). HPTLC-EDA is suitable to detect separated, individual compounds with a desired bioactivity (e.g. antibacterial, antifungal, enzyme inhibitory). To characterize the potent compounds, HPTLC-UV/Vis/FLD and HPTLC-mass spectrometry (MS) are very useful. Data about the compounds collected via HPTLC hyphenations is helpful for the development of (semi-)preparative liquid chromatographic techniques, such as normal and reversed-phase flash chromatography and reversed-phase semi-preparative HPLC.

We will present the transfer of the knowledge and methods (from HPTLC to preparative scale separation) with several examples of the bioassay-guided isolation process to discover plant-sourced bio-pesticides [e.g., 2,3]. We will show the richness of the invasive alien species in antimicrobial compounds that are potent candidates for agrochemical agents or lead compounds after appropriate modification and formulation.

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L-22| Application of high-performance separation techniques to the isolation of natural and semi-synthetic antitrypanosomal ecdysteroids

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Chagas disease, a life-threatening neglected tropical disease with the highest prevalence in the American continent, aggravates millions of patients' lives. The disease has an acute phase, lasting for several weeks and a chronic phase, which can be sustained for decades. In this phase, its pathogen, the protozoan *Trypanosoma cruzi* (*T. cuzi*) might cause cardiac, neurological, and gastrointestinal alterations. Due to limitations of the available therapy [1], new drug candidates are required. In this work, we present the investigation of fifty-two ecdysteroids' antitrypanosomal effects and semi-synthetic optimisation of the hit compounds. Significant antichagasic activities were exerted by some ecdysteroids: two dicinnamic esters and two compounds with an *E* or *Z tert*-butyl oxime ether moiety [2,3]. These two functional groups were identified as pharmacophores and incorporated into new 20-hydroxyecdysone (20E) derivatives. 20E was reacted with various cinnamic acid derivatives. Synthesis of the compounds yielded multiple esterified ecdysteroid, thereby multiple chromatographic steps were needed to obtain the pure compounds. RP-HPLC was applied on octadecylsilica or biphenyl stationary phases using aqueous methanol, acetonitrile and/or, in certain cases, THF as eluents. The optimized compounds possessed increased antitrypanosomal activity as compared to the first hits. The most potent derivatives were the C-2 monoester of ecdysteroid with *tert*-butyl oxime ether group at C-6 [4]. Further semi-synthesis and purification of new compounds are ongoing.

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L-23 | Boosting the purification performance of a challenging peptide separation by moving from reversed-phase to a mixed-mode stationary phase

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Peptides are a class of biomolecules with a great potential from the therapeutic point of view, because of their special biological properties. Industrially, the production stategies adopted, such as solid phase peptide synthesis or chemoenzymatic synthesis, produce both the target peptide and a series of impurities that must be removed. Preparative chromatography is the technique of choice for the large-scale purification of biomolecules, and it is generally performed in reversed-phase mode, using traditional hydrophobic adsorbents (e.g., C8 stationary phases). Adopting a single separation mode, however, is often unsufficient to reach the purity target imposed by regulatory agencies [1]. A solution is represented by mixed-mode columns, which are functionalised with two different ligands on the particle surface, exploiting two retention mechanisms in a single chromatographic step. Especially, when mixed-mode chromatography is used in attractive–repulsive mode, the repulsion performed by the dopant (meaning the ligand having smaller bonding density) on the analytes with the same charge sign push them to elute earlier, leading to narrower peaks [2].

This contribution illustrates a proof-of-concept study focused on the comparison of a hydrophobic adsorbent (C8) and a mixed-mode one (bearing mainly hydrophobic groups, C8, and 10% of a charged dopant) for the purification of a crude peptide mixture at preparative scale [3]. First of all, retention curves of the peptide with both columns have been investigated, to choose the range of organic solvent to work with along the gradient, during the purification on preparative scale. Thanks to more-favourable thermodynamics, it was found that, when collecting the whole peak excluding fractions of the peak tail, the mixed-mode column led to an increase in the recovery of roughly +15% with respect to the traditional hydrophobic column. The consequence is that the portion of the peak to be recycled (having an insufficient purity) is much smaller than with the C8, and this leads to higher productivity and lower solvent consumption. The chromatographic behavior of the peptide on both column at preparative scale has been modeled by studing its thermodynamics and adsorption isotherms; this can be exploited to predict peak shape and position even under different experimental conditions.

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L-24 | Greening downstream processing of biopharmaceuticals through multicolumn continuous preparative liquid chromatography and eco-friendly solvents

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Biopharmaceuticals are becoming increasingly important as a type of therapeutic treatment, due to their unique characteristics that cannot be replicated by conventional drugs. They are used for treating symptoms associated with various diseases, including cancer [1]. The synthesis of peptides involves the use of various methods, all of which produce not only the target active pharmaceutical ingredient (API) but also different types of impurities. Therefore, strict purity standards must be adhered to, which translates into the necessity of a downstream process. The current trends in analytical chemistry are focused to increase the sustainability of the purification processes. [1-2]. Pharmaceutical peptides are usually purified in reversed-phase preparative liquid chromatography (RP-HPLC) that involves the use of an apolar stationary phase and a polar mobile phase, usually consisting of a mixture of water and an organic modifier. Acetonitrile (ACN) has always been the preferred choice for its characteristics (good miscibility in water, excellent strength elution, etc.) but it is toxic to environmental and human health.

The environmental impact of downstream processing could be potentially reduced by means of different ways. The first one is the replacement of acetonitrile with alternative more eco-friendly solvents (e.g., alcohols) [2]. Another approach is to use multicolumn continuous chromatography platforms, which are based on the internal recycling of portions of the chromatogram, thus allowing for a reduction in solvent consumption with respect to corresponding single-column processes [2-3].

This work will show, through a series of case studies, how it is possible to reduce the environmental impact of purification approaches by using either alternative solvents to ACN, multicolumn continuous approaches, or a combination of both.

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L-25 | Novel application of LC-MS/MS and LC-DAD for the analysis of stimulants in dietary supplements

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Keywords: stimulant, doping, LC-MS/MS, LC-DAD, dietary supplement

The use of stimulants by both professional and amateur athletes is still in the center of interest in the world of sport. Although, these substances are banned by the World Anti-Doping Agency (WADA), they are very popular among athletes, which is also supported by WADA annual doping control statistics. Many top athletes try to find "legal" performance enhancing products with stimulating effect, especially dietary supplements (DSs) containing high amount of caffeine (CAF). DS's promising enhanced focus or better performance are widely commercialized in webshops, but they may be contaminated or intentionally adulterated with prohibited stimulants (PSs), thus instead of being safer, their use can provide high risk to a positive doping test. Consequently, our aim was to analyse these products.

The analyses of DSs were carried out by means of suitable chromatographic techniques. Our scopes were to quantify the legal active substance, CAF, by means of liquid chromatography coupled with diode array detectors (LC-DAD) and to detect eventual PSs (40 mostly occurring stimulants and narcotics) by means of LC coupled with tandem mass spectrometry (MS/MS). Our analytical methods were optimized considering the limit of detection, the selectivity, time and solvent consumption to obtain the most suitable ones for the target compound analysis. Furthermore, due to the high variety of the DS's forms and composition sample preparation phases were optimized for normal and swelling DSs. The development of highly sensitive and selective analytical techniques for PS analysis required the optimization of the MRM transitions and gas collision energies (CEs) of 40 target components and the internal standard, allowing their identification and detection at very low concentration (1-10 ng/g or mL) in commercially available DS. Among 45 analysed samples more than 30% of them contained almost one PS, mainly in the concentration range from ng/g to µg/g, while only in half of them was the CAF content in the range of ±10% respect to the labelled values.

The detection of PSs in DSs used by athletes is a crucial preventive step to avoid unintentional doping. Measurement of active compounds can help also in the evaluation of the cost/benefit of the usage of DSs. The developed methods can support the choice of safe and trustworthy DS used by elite athletes.

L-26 | Pipelines and systems for threshold avoiding quantification of LC-MS/MS data (PASTAQ)

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Introduction

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) remains one of the most popular tools for measuring organic compounds, with applications in proteomics and metabolomics. Lower intensity signals may contain relevant biological information and should not be discarded, independently of the identification status of quantified signal. In this work, we present a single-stage LC-MS(/MS) pre-processing pipeline, which extracts all compound relevant signals irrespective of identification annotation or abundances.

Approach

The Pipelines And Systems for Threshold Avoiding Quantification (PASTAQ) [1] offers a toolset for accurate quantification and pre-processing of DDA, DIA or MS1 only LC-MS(/MS) datasets. The pre-processing algorithms in PASTAQ operate on single-stage (MS1) data, with support for annotations and identifications linkage in the standard mzIdentML format such as obtained from MS-Fragger [2] or SearchGUI/PeptideShaker [3]. The code is freely available on GitHub: https://github.com/PASTAQ-MS/PASTAQ with MIT license.

PASTAQ is written in high-performance C++ code and offers Python bindings for the creation of tailored pipelines or integration with an existing ecosystem of mass spectrometry tools. This pipeline is simple to parametrize, requiring only to adjust the expected width of chromatographic peaks, type of mass spectrometry instrument used as well and its resolution at the reference m/z. PASTAQ performs isotopic peak detection, feature detection, retention time alignment and automatically generates a number of quality control figures that can be used to assess the quality of the data and preprocessing parametrization. PASTAQ does not apply thresholds for individual samples but extend dynamic range of compound signal by collecting signal that are frequently present in samples from at least one of the sample groups.

Results and discussion

The quantification performance of PASTAQ has been demonstrated on various proteomics benchmark datasets and on a publicly available human serum dataset. PASTAQ quantitative performance compared to the state-of-the-art MaxQuant software showed a reduction in the coefficient of variation (CV) of technical replicates and an increase in the number of quantified low intensity peptides. PASTAQ detected in human serum dataset gender related peptides and proteins. Example application for analyzing small molecule quantification is demonstrated for pharmacokinetic study of Ochratoxin A in mice.

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L-27 | Intact protein analysis by CZE-MS

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In most proteomics studies the bottom-up approach is employed, where the proteins are digested into peptides and then those are determined by tandem MS. Top-down mass spectrometry (TD-MS) can provide good insights into the proteoform variability and complexity since proteins are introduced into the MS system in their intact form without any chemical or enzymatic digestion or treatment. In TD-MS the molecular mass of the proteins can be determined and information on post-translational modification forms or the structure of the assemblies following proteolysis can be acquired.

The important element of the TD-MS instrumentation is the separation method with high resolution power for proteins. Even though several capillary electrophoresis modes can be employed for protein analysis, for TD-MS only the capillary zone electrophoresis (CZE) offers simple and efficient applicability with MS compatible electrolytes.

Analysis of intact proteins has been restricted by several difficulties, one of which being adsorption of proteins on the bare fused silica (BFS) capillary wall. To eliminate adsorption, one option is to employ BGE solutions with very low or high pH values and the other solution to use coated capillary. For the comparisons, a protein mixture comprising myoglobin, human insulin, human serum albumin, human hemoglobin and lysozyme was employed. Three different capillaries (BFS, dynamic successive multiple ionic-polymer layer (SMIL) and static linear polyacrylamide (LPA) coated) were compared based on their separation performances in their optimal operating conditions [1].

Measurements were carried out by a 7100 model CE instrument (Agilent) with UV and MS (maXis II UHR ESI-QTOF MS instrument, Bruker) detection. Different capillaries of 85 cm x 50 µm I.D. and 370 µm O.D. were used. UV detection was performed by spectrometric measurement (λ: 200 nm). Background electrolytes were 1 M formic acid (pH=1.8) or 50 mM acetate buffer (pH= 2.6 or 9.5), sheath liquid: 0.1% formic acid in 1:1 isopropyl alcohol.

In the lecture the major challenges of the intact protein analysis (maximazing the sensitivity with MS or UV detection and the separation efficiency) will be discussed. The proposed CZE-MS methods were applied in several diverse fields of the intact protein analysis. For instance human insulin is separated from its desamido-insulin isoforms, exhibiting the efficacy of CZE-MS to distinguish between components with mass differences of only 1 Da [2].

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L-28 | Assessment of nutritional value of food protein using digestion simulation

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Daily protein intake recommendations are typically assuming the consumption of food proteins of "average" composition. This practical approach cannot handle the fact that the nutritional value of different protein sources can greatly vary. The increasing consumption of nutritionally less valuable plant proteins would make it necessary to take into account the sometimes significantly different nutritional value of food protein sources. Nutritional value of food proteins can be described by various internationally accepted indicators, which are classically estimated using in vivo studies.

The aim of our work was to establish a rapid a low-cost platform based on digestion simulation and analytical methods harmonized for this, which can be applied for the in vitro estimation of the Digestible Indispensable Amino Acid Score (DIAAS), an acknowledged protein nutritional value indicator of food proteins.

The Infogest static protocol was used for digestion simulation and the amino acid composition was examined with the UHPLC-UV method after AQC derivatization following microwave-assisted digestion. Various commercially available meat products (sliced meat, salami, ham, sausage, hot dogs), as well as a milk protein concentrate (MPC), a plant-based meat imitation and an additional food (cooked pasta) were examined. In vitro DIAAS value calculated on the basis of the amino requirement pattern uniformly applicable to children over 3 years of age, adults and the elderly according to FAO guidelines. Our results were in good agreement with the limited available literature data for milk protein concentrate and some meat products. It was also shown that DIAAS of milk protein (> 140) and meat products (>120) were higher than that of cooked (34) pasta and vegan falafel made of chick pea (32). The developed analytical platform offers an efficient screening option for functional food development, since based on the provided DIAAS and limiting essential amino acid data the required compositional improvement of food proteins can be designed.

The funding of the OTKA K135294 grant is kindly acknowledged.

L-29 | Identification of a chimera mass spectrum of isobaric "lipid A" species

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Lipid A molecules belonging to the group of phosphoglycolipids are the organic components of the cell wall of Gram-negative bacteria. Lipid A, released from the outer membrane of the bacterial cell, can be characterized by a double effect when it enters the bloodstream of the host organism. Depending on its molecular structure, it can be either endotoxic (even deadly) or an immunostimulant (beneficial) for humans. Nowadays, tandem mass spectrometry is used for the qualitative and quantitative analysis of complex mixtures of lipid A molecules as well as the structural characterization of individual components that make up bacterial extracts. However, successful structural identification is significantly hindered by the simultaneous presence of structural isomers of the same mass (so-called isobaric molecules) in the sample. Practically, *ca.* 90% of the researchers examine these phosphoglycolipids in negative ionization mode. On the other hand, we found that the presence of phosphorylation isomers very often remains hidden during their negative-ion mass spectrometry analysis. At least the publications do not mention that isomers are also present. The complication is that the simultaneous fragmentation of two or even more ions of the same mass but different structures results in a so-called "chimera mass spectrum", and it is extremely difficult to recognize this phenomenon in the case of lipid A isomers differing only in the position of a single phosphate group. Identification of the minor isomer in negative ion mode is almost impossible, since the chimera mass spectrum closely resembles the fragmentation spectrum of the major isomer. Here, we propose two alternatives for the shotgun tandem mass spectrometry analysis of monophosphorylated lipid A in negative ion mode. One of them is the mass spectrometry analysis in positive ionization mode, by which we can easily recognize the presence of both isomeric species. The other is a non-aqueous capillary electrophoresis tandem mass spectrometry method, which can effectively separate isomeric lipid A species, thus avoiding the generation of a chimera mass spectrum.

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L-30 | Blood *N*-glycomic signatures of fibrosis in non-alcoholic fatty liver disease indicate low levels of global α2,3-sialylation

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Non-alcoholic fatty liver disease (NAFLD) is a disease of alarmingly increasing worldwide prevalence (25-30% of adult population), which can silently progress to non-alcoholic steatohepatitis (NASH) and associated fibrotic conditions. Increased liver fibrosis has been shown to be the most important feature associated with increased overall- and liver-related mortality and increased likelihood of developing liver-related complications, but current non-invasive diagnostics falls short on its early detection and staging. Glycosylation of blood proteins, that are mostly synthetized and glycosylated in the liver, may have the potential to address this unmet clinical need, as alterations to the blood protein *N*-glycome have been reported as hallmarks of liver pathologies. Encouraged by these earlier findings, here we explored total blood protein *N*-glycosylation of individuals with NAFLD/NASH and varying degree of fibrosis, with the aim to identify clinically translatable biomarkers allowing for early diagnosis.

We profiled the *N*-glycosylation of blood proteins on the released glycan level by a matrix-assisted laser-desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry-based method for a total of 75 NAFLD patients and 72 healthy controls in two independent cohorts. This platform enabled the unambiguous identification of more than 80 *N*-glycans including sialic acid linkage-isomers with high glycoform resolution.

The study found globally lowered α2,3-sialylation patterns that distinguished NAFLD from healthy controls in both cohorts. Upon further stratification of patients based on the degree of fibrosis, the found signature was found to be indicative for the histological manifestation of NASH-related fibrosis, and to certain extent reflective for its degree. The findings suggested a shift in glycan biosynthetic pathways, and analysis of two independent publicly available transcriptomics datasets underlined these assumptions, illustrated by reduced levels of the transcripts associated with the glycosyltransferase enzymes that tailor α2,3-sialylation.

In conclusion, our data sheds light on globally decreased α2,3-sialylation on circulatory proteins, offering the possibility for developing novel non-invasive diagnostic tests that facilitate early diagnosis of NASH-related fibrosis with potential to replace biopsies.

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Conflict of interest statement: T. Pongracz, M. Biewenga, N. de Haan, M. Wuhrer and M. Tushuizen are named inventors on a provisional patent application related to this work.

ML-2 | Introduction to the new Thermo Scientific Orbitrap Astral mass spectrometer

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Due to complexity, analysis of biological samples has always required high performance instrumentation that can provide high quality of data which then leads to meaningful and confident results. Parallel to that, diversity of such analyses dictates ever growing need for higher sample throughput required to gather enough information from different sources to identify specific molecules or processes that characterize respective conditions. In the last few years, we have also seen a shift to analyzing smaller sample quantities, driven by the interest in profiling the proteomes and metabolomes of individual cells. Consequently, mass spectrometry which among other techniques is used as key instrumentation for biological sample analysis is driven by a constant need for improvement and partially dictates the pace of research. While highest possible sensitivity is required to reach the required depth of analysis it is also critical to maintain throughput and chromatographic performance in order to generate sufficiently high-quality data from large data sets and draw meaningful conclusions. With the introduction of the Orbitrap Astral mass spectrometer we are moving these limits to a new level. Unique combination of the Thermo Scientific™ Orbitrap™ mass analyzer and Thermo Scientific™ Astral™ mass analyzer in synchronized acquisition provide mass accuracy, high resolution and high dynamic range of full scan spectra together with high speed and sensitive MS^2 spectra. In combination with Thermo Scientific™ Vanquish™ Neo UHPLC which provides precise low flow rates, stable low injection volumes and accurate gradient formation at high pressures, Orbitrap Astral delivers significantly deeper sample coverage while maintaining superior throughput. Both are necessary to get more complete, timely and meaningful results in current research while opening the doors to new possibilities.

L-31 | Understanding the fundamentals of chiral separations to design innovative applications

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The aim of this work is to discuss the fundamental aspects of chiral recognition mechanisms in liquid chromatography. We will compare different types of CSPs, such as macrocyclic antibiotics, brush-type, and various chiral analytes and chromatographic modes, and examine how particle geometry, chiral selector loading, and solvent effects influence the separation outcome and performance. We will also present some applications using synthetic brush-type CSPs with inverted chirality, demonstrating that enantioselectivity can be evaluated without requiring both enantiomers.

L-32 | Carrier ampholyte-based isoelectric focusing in capillaries and gels with ion transport into the electrode vessels is not a steady-state process, it is transient bidirectional isotachophoresis

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In 1961, Svensson described the isoelectric focusing (IEF) separation of ampholytic compounds according to their isoelectric points (p*I* values) in temporally and spatially stable pH gradients. He created the pH gradients from carrier ampholytes (CAs), *in situ*, in sucrose density gradients that mitigated the undesirable effects of thermal convection [1]. Seven years later, polyacrylamide gel slabs and rods were introduced to control thermal convection [2], though when they were used in Davies-type gel electrophoretic devices, the resulting pH gradients became temporally and spatially unstable [3]. The pH gradient proved unstable in capillary IEF as well [4]. Over time, the accumulating experimental evidence, together with the insight provided by the emerging dynamic simulation programs [5,6] led to the currently accepted two-stage model of the IEF experiment [7] which stipulates that the pH gradient is formed first, rapidly, in the focusing phase, but then, in the second phase, slow isotachophoretic processes gradually remove the extreme p*I* CAs, and alter the span and shape of the pH gradient.

In a triad of recent papers [8] we demonstrated, based on first principles and computer simulations completed with the newly released Simul 6 IEF/ITP program (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 that *(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 simulations obtained by Simul 6 IEF/ITP - we will show the salient features of the TBD-ITP ampholyte separation process.

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L-33 | LC method development - In computers we trust?

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Method development for liquid chromatography requires significant expertise. Algorithms and computer programs to aid the chromatographer started to emerge nearly 50 years ago and they have gradually gained more traction, thanks to many positive developments.

- Software tools have improved and new algorithms have become and are becoming available, including some that are derived from the exploding field of artificial intelligence.
- The computer power that is available in the laboratory at moderate to low costs has increased dramatically.
- LC hardware has improved and hyphenated systems with mass spectrometry (LC-MS) are increasingly available and affordable.

the other hand, LC methods have become more complex. LC-MS systems produce more data than conventional systems equipped with single-channel UV detectors. Comprehensive two-dimensional LC (LC×LC) methods require great amounts of time and effort if they are to be developed without contemporary software tools for method development

We have come to a point were instrument control and method development can be fully automated [1]. However, the question now arises whether such methods are as good – or better – as those diligently developed by skillful, experienced chromatographers. Can we trust the computers that produce methods for us?

Ultimately, the quality of the methods may depend on the knowledge and insight of the scientists that develop our software tools. It is essential that algorithms are fully transparent and that chromatographers understand their stengths, weaknesses and operational settings. Such aspects will be discussed in this presentaton.

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Posters

P-01 | Exploitation of the enantioselectivity space of coated amylose tris (3,5-dimethylphenyl-carbamate) in mixtures of 2-propanol and acetonitrile

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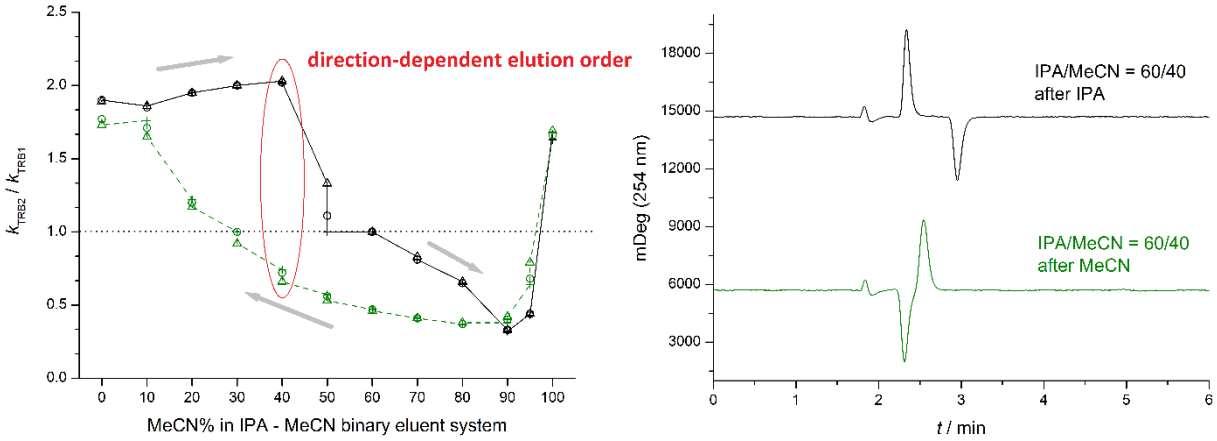
Chiral stationary phases (CSPs) with coated amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) selector have long been recognized for their excellent chiral recognition ability in liquid chromatography. The conformational versatility behind this feature is the source of their known hysteretic behavior, which has been previously observed in polar organic (PO) mode eluents containing 2-propanol (IPA). Hysteresis in mixtures of IPA and acetonitrile (MeCN), a typical PO mode eluent system, is promising for finding additional unique unexplored enantioselectivities.

Not only was the hysteresis detectable on ADMPC using mixtures of IPA and MeCN, but it was the typical behavior in a diverse set of test compounds. The difference in the retention time of the same analyte under conditions which only differed in the eluent history on the column can go up to 20-fold. The assumed hindered conformational changes of the selector were reflected in retention drift at certain eluent compositions. On the two sides of the transitions, distinct, useful states of the selector were detected. A series of IPA – MeCN compositions with defined pretreatment was selected and recommended as an extension of the preliminary, first choice method screening set that used only alcohols. The incorporation of a solvent possessing substantially different characteristics enhances the potential in practical applications, while keeping the technical simplicity. Stability and robustness of the additional states of the CSP were characterized. The examined columns of different brands shared the observed behavior. Kinetic stability of a column state is adequate for successful application.

The evaluated states of ADMPC provide multiple enantiorecognition potential by using mixtures of IPA and MeCN also considering the pretreatment of the column. Unprecedented double and triple elution order reversals along the composition range supported the versatility of the available states. Our findings further enhance the usefulness of ADMPC-containing CSPs. We provide instructions for the application of the widespread chiral selector in common eluent mixtures to avoid pitfalls regarding reproducibility and robustness.



P-02 | Characterization of the retention mechanism on polar embedded reversed phase columns

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Chemically bonded stationary phases which contain both hydrophobic and hydrophilic ligands can be used in reversed-phase liquid chromatography (RPLC) and as well as in hydrophilic interaction liquid chromatography (HILIC). Pure water is also suitable as a mobile phase. They also allow the separation of polar and non-polar analytes. Retention was investigated from different points of view on four homemade phosphodiester stationary phases with different non-polar ligands. These were alkyl chains of 10 and 18 carbon atoms, a benzyl group and a cholesterol molecule, which were named Diol-P-C10, Diol-P-C18, Diol-P-Benzyl and Diol-P-Chol.

The excess isotherms show that these stationary phases can also adsorb acetonitrile and water in significant amounts. This property ensures that retention occurs in both pure water and acetonitrile-rich mobile phases. The order of hydrophobicity of the stationary phases is as follows: Diol-P-Benzyl < Diol-P-C10 < Diol-P-C18 < Diol-P-Chol.

The retention of test molecules was also investigated. Adsorption isotherms for pure water and acetonitrile-rich mobile phase were determined by frontal analysis and inverse method. The saturation capacities could not be measured for the poorly soluble analytes. But it is clearly observed that there is a difference in the main chromatographic interaction for mobile phases with different polarities.

P-03 | Optimization of surface-sampling capillary electrophoresis – mass spectrometry for single cell metabolomics

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Capillary electrophoresis coupled to mass spectrometry (CE-MS) has been a well-established technique for metabolomic profiling of complex biological samples in liquid samples. Surface sampling CE-MS enables the acquisition of spatially defined chemical information from selected tissue regions[1], and holds great potential for investigating single cells sorted in well-defined arrays as well. The ability to chemically profile single cells is inevitable for a better understanding of biochemical mechanisms underlying various physiological conditions.

The surface sampling capillary electrophoresis mass spectrometry (SS-CE-MS) device has already been optimized and successfully applied to brain, spinal cord, kidney, and blood samples[1,2]. However, further developments focusing on sensitivity enhancement are necessary to implement SS-CE-MS in single cell metabolomics.

To achieve this, we performed a detailed optimization of the sheath liquid interface while monitoring the signal intensities of endogenous amino acid mixtures. We found that maintaining 0.18 - 0.22 µL/min flowrate was necessary for establishing a stable electrospray; larger flowrates caused unnecessary sample dilution and peak tailing. The composition of the sheath liquid was then thoroughly investigated by using several water-organic solvent (methanol, acetonitrile, isopropanol) mixtures doped with formic acid, ammonium formate, and ammonium acetate. We observed substantial differences in ionization efficiencies with regard to both the solvent composition and the type of the dopant. Our future aim is to utilize the optimized SS-CE-MS conditions to enable in-depth characterization of single cell metabolism.

Acknowledgments

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P-04 | An investigation of atropisomerism of Amenamevir and its intermediates by dynamic HPLC

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During drug discovery an ever growing interest lies in the stereoisomerism on the potential drug candidates. It has already been thoroughly proven that classical enantiomers with one or more chiral centers can have drastically different biological activity, pharmacodynamics and toxicity. Another largely overlooked type of stereoisomerism is atropisomerism, where stable stereoisomers, even enantiomers arise from the hindered rotation around a single bond. The interconversion of the different species don't require the breaking of any bonds, there is usually an equilibrium of the isomers, where the exchange rate may vary wildly, from minutes to years, and may depend on temperature, solvent and other factors.

Amenamevir (CAS 841301-32-4) is an antiviral drug against Herpes simplex (HSV-1, HSV-2) and Varicella zoster virus and used for the treatment of shingles. Amenamevir directly inhibit the viral helicase–primase enzyme complex, which is required for DNA unwinding and primer synthesis during replication. During the HPLC method development it was found that the API itself as well as its intermediates (Fig. 1, molecules 1-3 respectively) exhibited strange chromatographic features. From NMR and HPLC measurements it was concluded that it has arisen from the formation of atropisomers, where the rotation around the bond between the nitrogen and dimethylphenyl moiety is hindered. Temperature, pH and eluent dependence of the rate of interconversion was studied, and energy barriers were calculated.

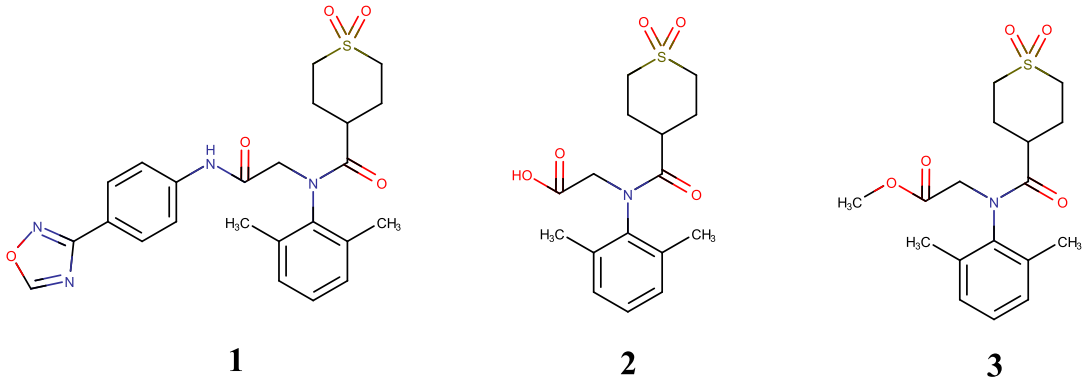

Figure 1
Amenamevir (1) and its intermadiates (2-3)

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P-05 | Challenges in effect-directed analyses of bioactives in invasive alien plant species

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Due to rapid spreading and large biomass, invasive alien plant species (e.g. Japanese knotweed, giant knotweed, Bohemian knotweed, Canadian goldenrod, giant goldenrod, tree of heaven, etc.) represent a major ecological (impact on biodiversity) and economic problem (destruction of infrastructure, etc.). In spite of many attempts, efficient eradication of invasive alien plant species has not been achieved. Rhizomes and flowers of Japanese knotweed are used for different food and health beneficial products sold in Asian markets. Resveratrol isolated from Japanese knotweed rhizomes is sold in food supplements even in Europe [1]. Different studies evaluate the applicability of the available biomass. Such studies reported antimicrobial clerodane diterpenes in giant goldenrod [2] and (-)-epicatechin as an important contributor to the antioxidant activity of Japanese knotweed rhizome bark [3]. Effect-directed analysis (EDA) in combination with thin-layer chromatography represents the quickest and the most cost-effective approach to discover the bioactive compounds in plant extracts. Several protocols for *in vitro* assays of researching bioactive compounds (e.g., antioxidants, antimicrobial compounds, acetylcholinesterase inhibitors, α-/β-glucosidase inhibitors, lipase inhibitors, and estrogenic endocrine disruptors) after the separation on the chromatographic plate are available in the literature. However, there is still a lot of room for optimization of these already established bioassay protocols, as well as for development of new bioassay methods that would enable studies of other activities. In this presentation challenges in nontargeted effect-directed analyses of bioactive secondary metabolites extracted from different parts of invasive alien plant species will be presented. The examples will include the influence of the stationary phase, detection reagents and detection modes (UV, Vis, FLD) on the effect-directed analyses of the extracts. The effect-directed analyses of antioxidants, antimicrobial compounds and enzyme inhibitors will be presented.

Acknowledgements

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P-06 | Antimicrobial potential of two diterpenes isolated from rough goldenrod (*Solidago rugosa* Mill.) against plant pathogens

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Plants are considered an inexhaustible source of secondary metabolites possessing diverse structures and valuable biological activity. Rough goldenrod (*Solidago rugosa* Mill.) is a plant being indigenous in America and naturalized as an alien in certain regions of Europe. Nevertheless, its bioactive natural products have been poorly studied.

This work aimed to detect, isolate, and identify the antimicrobial constituents of the ethanolic extract of *S. rugosa* roots and leaves. Their *in vitro* antimicrobial effects were also investigated against various Gram-positive (*Bacillus spizizenii*, *Clavibacter michiganensis* subsp. *michiganensis*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) and Gram-negative (*Xanthomonas arboricola* pv. *pruni*) bacterial as well as fungal (*Bipolaris sorokiniana*, *Fusarium avenaceum*) strains including phytopathogenic organisms.

Non-targeted, effect-directed screening for antimicrobial components were carried out by high-performance thin-layer chromatography coupled with direct bioautography (HPTLC–DB). Fractionation and isolation were performed by preparative flash column chromatography. The structures of the isolates were elucidated by one- and two-dimensional nuclear magnetic resonance spectroscopy (NMR), high-resolution tandem mass spectrometry (HRMS/MS) and polarimetry. Half-maximal inhibitory concentration (IC_{50}) values were obtained from *in vitro* microdilution assays.

Two compounds were detected and isolated from *S. rugosa* identified as (–)-hardwickiic acid (**1**) and (–)-abietic acid (**2**). This is the first report about the presence and abundance of **2** in *S. rugosa*. **1** and **2** exhibited strong inhibitory activity (IC_{50} = 1.0–5.1 µg/ml) against all studied Gram-positive bacteria. However, only weak antibacterial effect (IC_{50} = 201.2 and 166.6 µg/ml) was observed against the Gram-negative *X. arboricola* pv. *pruni* for **1** and **2**, respectively. **1** significantly inhibited the growth of *B. sorokiniana* with an IC_{50} of 3.8 µg/ml, and demonstrated moderate antifungal activity (IC_{50} = 73.5 µg/ml) against *F. avenaceum*. **2** appeared less potent against the examined fungal strains (IC_{50} = 165.5 and 120.6 µg/ml, respectively). After the antimicrobial activity enhancement of **1** and **2** by suitable synthetic modifications and formulations they can become more potent biopesticides.

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

P-07 | Development and validation of an LC-MS/MS method for the quantification of 2,3-epoxypropyl isopropyl ether in rat plasma

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2,3-epoxypropyl isopropyl ether is a frequently used material during epoxy resin manufacturing process. There is still little known about the attainable systemic concentration of such chemicals. Present work can provide valuable information to assist exposure assessment/biomonitoring in occupational safety studies.

To serve the bioanalytical need of a genotoxic study a sensitive and selective LC–MS/MS method had been developed and fully validated for the quantification of 2,3-epoxypropyl isopropyl ether in rat plasma.

The LC-MS/MS method worked with gradient elution [mobile phase A (20mM ammonium acetate in ultrapure water) B (acetonitrile)] on a YMC-Triart C18 column (3µm, 2.1x75 mm) with YMC-Triart C18 (3µm, 2.1x10 mm) guard column at 0.4 mL/min flow rate. The gradient program was as follows: initial conditions were 10% B; held at 10% B for 0.5 min, raised to 90% B over the next 3 minutes, held at 90% B for 0.5 min, decreased to 10% B over the next 0.5 min and held at 10% B for 0.5 min for re-equilibration of the system prior to the next injection. The column temperature was 30 °C and the injection volume was 10 µL. The autosampler temperature was 10 °C. 2-propanol was used as washing solvent to prevent carry-over. As internal standard neopentyl glycol diglycidyl ether was used since no stable isotope labeled analogue of the analyte was available. The Shimadzu LCMS-8060 triple quadrupole tandem mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode and the ammonium adduct form of the precursor ion was selected for fragmentation. MS acquisition was carried out in the ESI positive ionization mode.

The LLOQ for the analyte was 5 µg/mL over a 5–5000 µg/mL range. The plasma matrix did not cause any signal suppression. The mean recovery was 88.4 % and 85.4 % at high (4000 µg/mL) and low (15 µg/mL) level of concentration. The plasma samples are considered stable at -75°C ± 10°C for 38 days and can be subjected to 3 freeze-thaw cycles and for at least 4 hours on benchtop at room temperature.

Overall, a selective method was developed with a wide concentration range. In the future this method will be applied in a pilot study, where a single dose (1000 mg/kg) of 2,3-epoxypropyl isopropyl ether will be administered orally to rats and toxicokinetic plasma sampling will be performed at 6 time-points.

The experiment was founded by Doctoral School of Pharmaceutical Sciences (Semmelweis University), Research Centre for Natural Sciences and Toxi-Coop Limited Company.

P-08 | Characterization of oligosaccharides in breast milk collected from a mother with Sjögren's Syndrome by HPLC-MS/MS

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Human milk oligosaccharides (HMOs), the third largest solid fraction of human milk are of a great interest as they play a key role in many complex functions that promote infant health. In addition to their prebiotic action, they also have immunomodulatory functions and play an important role in brain development. The diverse functions of HMOs are provided by their diverse structures. Although HMOs are composed of only five different monosaccharide units (glucose, galactose, *N*-acetylglucosamine, fucose and *N*-acetylneuraminic acid), more than 200 different structures have been identified to date due to the variety of possible linkages. That makes their analytical characterization challenging.

The HMO pattern is highly influenced by the maternal Lewis phenotype, health, diet, and geographical distribution. The possible differences may be even more important in the case of maternal illness e.g. autoimmune diseases. In this study the comparison of the HMO pattern of healthy mothers and a mother with Sjögren's syndrome, an autoimmune disease affecting the exocrine glands was investigated. Oligosaccharides were separated by a porous graphitic carbon column and PGC-UHPLC-ESI-Orbitrap-MS/MS spectra of the samples were compared to corresponding databases. Quantification of the three most abundant isomeric pairs of HMOs (2'-fucosyllactose and 3-fucosyllactose, lacto-*N*-tetraose and lacto-*N*-neotetraose, 3'-sialyllactose and 6'-sialyllactose) in their alditol forms by a PGC-HPLC-ESI-MS/MS method was also carried out. The developed method was validated in terms of selectivity, accuracy, precision, recovery, carry-over, matrix effects and stability according to the EMA guideline on bioanalytical method validation.

We successfully identified the most abundant oligosaccharides by comparing the high resolution mass spectra with the corresponding database. However, our quantitative results suggest that the HMO content in the mother's milk with Sjögren's syndrome is much lower in colostrum, transitional and mature milk than in healthy ones, and the quantitative ratios do not follow the usual pattern.

P-09 | Cyclodextrin complexation study of Kratom Alkaloids

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Keywords: affinity capillary electrophoresis, cyclodextrins, inclusion complexation, bioavailability enhancement, indole alkaloid mitragynine

Kratom (*Mitragyna speciosa* Korth.) leaves have been used for centuries by Southeast Asian tribes for pain relief, wound healing, and nowadays, it is mainly consumed as a recreational drug due to its stimulant effect. Indole alkaloids, such as mitragynine and its derivatives are responsible for the therapeutic effects, however, these compounds are unstable and have poor water solubility, limiting their potential clinical use as “atypic opioid” analgesics with favourable side-effect profile. Cyclodextrins (CDs) are an excellent tool to improve bioavailability, as their inner cavity can accommodate guest molecules through inclusion complexation.

The aim of this work is to optimize the pharmacokinetic properties and bioavailability of mitragynine by CD inclusion complexation. The complex stability constants were determined by the environmentally friendly, low sample consuming affinity capillary electrophoresis, applying more than 30 native, neutral, positively, and negatively charged CD derivatives. The complex stabilities of mitragynine were compared with the complex formation ability of its 3 co-alkaloids, speciociliatine, speciogynine, and paynantheine. Both the neutral and negatively charged derivatives were able to form complexes with all 4 kratom alkaloids, and the cavity size, the substituent type and the degree of substitution also influenced the complex formation. The negatively charged sulfated- and sufoalkylated-beta-CD analogs were able to form the most stable complexes, exceeding 1000 M^{-1}. Based on our results, the "ideal excipient" was selected for a CD-based preparation, solubility, and stability studies.

P-10 | Matrix effects in cation exchange chromatography

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Keywords: matrix effect, analyte pH, ion chromatography

In ion chromatography studies, the matrix effect of other inorganic ions present in the sample is a well-known phenomenon. In our work, we studied the behavior of inorganic and organic ions in a system overloaded with ammonium ions. The measurements were carried out at different eluent concentrations to analyze not only the retention times but also other system performance parameters. In this system, which was significantly overloaded with ammonium ions, the behavior of the ions tested was also investigated by varying the temperature of the chromatographic column and the injected volume. Our results suggest that the observed effect is due to a combination of the pH change caused by the injected sample and the amount of ammonium ion introduced. We also demonstrate that, in a well-designed experiment, the addition of large amounts of ammonium hydroxide to the sample can improve the separation efficiency of organic and inorganic anions.

P-11 | Simplicity-driven method for the separation and quantification of basic molecules with HILIC HPLC-MS/MS

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The measurement of polar molecules with HPLC-MS is often surprisingly complicated. The bulk of chromatographic experiments is reversed-phase with standard C18 columns, which is unsuitable for polar molecules. Thus the standard setup should be altered accordingly. In many cases, it leads to certain pitfalls and ends up with unsatisfactory results. In this paper, we demonstrate an easy-tosetup method for the task, which offers a straightforward solution for the separation of polar basic compounds. The chromatographic efficiency is supported by separating fourteen components. Quantitative analysis is carried out on five representative candidates of them. We are going through the validation process to show that it applies to a wide range of polar analytes and is suitable for most practical applications.

P-12 | On the trail of photocatalytic degradation of fungicides

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As agricultural production grows, more and more diverse spray products are being marketed. These herbicides, pesticides and fungicides have poor biodegradation properties, leading to their accumulation in soil.

Penconazole ($C_{12}H_{15}Cl_2N_3$), one of the fungicides, is widely used to prevent fungal infection of fruits. The elucidation of the photochemical behaviour of this fungicide, which adheres to the surface of plants and is subsequently released into the water table, is a matter of interest from several points of view. On the one hand, direct degradation by sunlight significantly affects the biochemical cycles of nature, and on the other hand, it is necessary to complement the biological water purification methods e.g. with heterogeneous photocatalysis.

The photochemical stability of penconazole was investigated by visible light (Vis LED, λ_{max}=453 nm) and UV (UV LED, λ_{max}=371 nm) photolysis, supplemented with a commercially available TiO_2 catalyst. To monitor the processes occurring under the influence of illumination, the light absorption of the samples was recorded by spectrophotometry, and the pH and total organic carbon content of the solutions were measured. The concentration of the model compound and the resulting degradation products were determined by high pressure liquid chromatography-mass spectrometry (HPLC-MS) and ion chromatographic (IC).

It was found that penconazole does not decompose under visible and UV light without a catalyst under the experimental conditions used. In the presence of TiO_2, when the suspension is illuminated with UV light, the organic contaminant is mineralized. Ion chromatographic studies have shown that the hydrochloric acid formed from the chlorine content of the compound contributes significantly to the acidification of the liquid phase. Some degradation products of penconazole were identified by HPLC-MS method.

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P-13 | Microfluidic-based protein quantification in plant-based drinks derived from nuts

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The broad availability of plant-based milk alternatives (PBMAs) fulfills the demands arising from consumer-driven pressures, encompassing individuals with milk protein allergies and those consciously adopting animal-free diets seeking milk-like substitutes. The manufacturers of the plant-based drinks strive not only to replicate the desired taste and texture akin to cow's milk but also to achieve an optimal protein and nutrient composition that renders these products both tasty and nutritious.

PBMAs are made from various plant sources, including cereals, pseudo-cereals, legumes, nuts, seeds, or their combinations. This study focuses on the examination of nut-based plant drinks, specifically analyzing commercially accessible drinks comprised solely of the respective plant (one specific type), water, and salt. Coconut-based beverages from three different brands, as well as almond-based drinks from two different brands and one type of cashew-based beverage, were subjected to testing. Within each category, three products with varying expiration dates were selected to investigate potential variations in protein profiles.

Our findings revealed that despite all three types of milk drinks yielding five protein fractions during the separation process, the relative positions and proportions of these fractions were already distinct. In the case of the cashew-based plant drinks, the third protein fraction exhibited the largest time corrected area (identified as the globulin protein fraction), while for both brands of almond-based plant drinks, the fifth fraction displayed the largest area (identified as the prunin-2 protein fraction). However, in the case of the coconut-based plant drinks, we observed consistency only among two brands regarding the fraction with the largest area (in case of the fourth fraction), whereas one brand of coconut drinks demonstrated the third protein fraction (identified as albumin protein fraction) as more characteristic.

In our research, we developed a rapid and efficient microchip gel electrophoresis technique that proved suitable for quantifying the total protein content of these beverages. Furthermore, the observed variations in protein profiles present an opportunity for rapid quality determination.

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P-14 | Microfluidic analysis for determination of the protein content in legume-based plant drinks

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Owing to sensitivities towards specific components of cow's milk, such as lactose and milk protein, as well as the increasing avoidance of dairy products due to various dietary practices, there has been a remarkable upsurge in consumer demand for diverse plant-based milk alternatives (PBMAs). Simultaneously, manufacturers strive to develop plant-based alternatives that closely resemble the protein and nutrient composition of cow's milk.

Legume-based milk alternatives, such as soy and chickpeas, are the least similar in taste to the product they are intended to mimic, namely cow's milk. At the same time, soy is a rich source of protein, containing several types of protein, while chickpeas contain beneficial ingredients that contribute to additional health benefits.

In our study, we examined soy-based plant-based drinks from five different manufacturers and chickpea-based drinks from one manufacturer, each with three different expiry dates and no additives. To perform the measurements, we utilized microchip electrophoretic method coupled with a LIF (laser-induced fluorescence) detector, a rapid and automated analytical method that requires minimal sample volume.

In the analysis of the chickpea-based plant drink, we successfully isolated four distinct protein fractions, whereas in the case of soy-based drinks, a total of five fractions were separated. Among the soy-based drinks from the five different manufacturers, it can be observed that the electrophoretic profiles exhibit a high degree of similarity. This can be attributed to the fact that these drinks consist solely of soy, water, and salt without the addition of any other ingredients. In the case of chickpea-based plant drinks, the second fraction displayed the highest % of total time-corrected peak area, whereas in the case of soy-based drinks, the third fraction exhibited this characteristic.

The developed methodology represents a fast and efficient analytical approach for comparing the protein profiles of PBMAs and it demonstrates its applicability in accurately quantifying total protein content of these beverages.

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P-15 | Targeted UPLC-MS/MS method for the simultaneous determination of stilbenes derivatives in plant extract

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Stilbenes are non-flavonoid phenolic secondary metabolites found in various medicinal plants and food. Different biological activities have been linked to stilbenes as they proved to possess antioxidant, anti-inflammatory and antitumor effects. One of the famous stilbenes is the *trans* isomer of resveratrol which is produced in response to fungal infections. It is one of the extracted components of red wine. Several approaches have been developed to analyze resveratrol, while only a few data are available for determining other important stilbenes such as cis-miyabenol C, kobophenol A, carexinol A, and hopeaphenol in the literature.

In the present study, an efficient targeted ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) method was developed for the simultaneous determination of eight stilbene derivatives (resveratrol, cis-ε-viniferin, trans- ε-viniferin, cis-miyabenol C, kobophenol A, carexinol A, and hopeaphenol) in the plant extract. No analytical method has been reported to determine these stilbenes so far simultaneously.

All the investigated stilbenes showed higher ionization efficiency in negative electrospray mode than in positive mode. The MS/MS method was applied to study the fragmentation rules of stilbenes. For MS/MS detection, a multiple reaction monitoring (MRM) mode was optimized for quantifier and qualifier ions of all stilbenes. The developed reversed-phase UHPLC-MS/MS method was successfully applied to analyse stilbenes in plant extract using gradient elution with a total run time of 14 minutes.

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P-16 | HPLC Study of the Enantioselective separation of *β*-methyl-substituted amino acids applying ion exchanger-based chiral stationary phases

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High-performance liquid chromatography (HPLC), applying chiral stationary phases, is the most frequently utilized technique in resolving chiral analytes on an analytical scale. Several chiral stationary phases are available to achieve baseline resolution depending on the analyte's structure. In the case of ionic or ionizable compounds, the application of ion exchangers can be the best choice.

The enantioseparation of *β*-methyl-substituted amino acids was investigated utilizing *Cinchona* alkaloid-based ion-exchanger type chiral stationary phases. The effects of mobile phase composition on the enantioseparation were studied by applying mixtures of methanol and acetonitrile. The effect of quality and quantity of acid and base additives on the chromatographic properties were also investigated, as well as the effect of temperature. Relationships between the structure of the selector and selectand were evaluated based on the chromatographic parameters and elution orders of the stereoisomers. Ionic interactions were described by applying the simple stoichiometric displacement model.

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P-17 | Analysis of snake venoms with CZE-MS

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Venoms contain numerous biologically active compounds, primarily peptides and proteins, which have the potential to cause lethal effects [1]. When combined with capillary zone electrophoresis, the top-down mass spectrometric technique becomes highly effective in studying the structural and dynamic characteristics of intact proteins. This approach can be applied to complex protein molecules such as snake venom [2]. In our investigation, we examined three snake venoms sourced from distinct black mamba (Dendroaspis polylepis) specimens. Although two venom samples originated from snakes in the same geographic area, one of them was collected from a snake in a different location.

Analyses were conducted using a 7100 model CE instrument (Agilent) with UV and MS (maXis II UHR ESI-QTOF MS instrument, Bruker) detection. Fused silica capillaries of 85 cm x 50 µm I.D. and 370 µm O.D. was used. UV detection was carried out by on-capillary photometric measurement (detection wavelength: 200 nm). Background electrolytes were 1 M formic acid (pH=1.8), sheath liquid: 0.1% formic acid in 1:1 isopropyl alcohol. Our study involves the use of fused silica capillaries due to its simplicity and cheapness employing the background electrolytes with very low pH conditions to separate and characterize different components in snake venom samples originated from different geographic locations. We could achieve good precision, minimal adsorption and excellent separation efficiency. We observed similar separation patterns with CE-UV and CE-MS and the molecular masses of the proteins could be determined with MS detection.

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P-18 | The analysis of monoclonal antibodies with electrospray ionization mass spectrometry and subsequent spectral processing

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Mass spectrometry is one of the most important tools in the analysis of monoclonal antibodies (mAbs). Electrospray ionization is a prevalent technique that generates numerous charge states in the case of large molecules. The ionization of cetuximab was investigated and found to be promoted by the addition of acids, volatile organic solvents and the removal of the matrix components. The acquired spectra consist of many peaks corresponding to different charge states of the antibody. Different algorithms can assign charges to the peaks and create a zero-charge mass spectrum using these charges and m/z values, in a process called deconvolution. This deconvoluted spectrum is similar to each differently charged peak in the recorded spectrum but has higher signal-to-noise ratio and mass accuracy. Thisdepartm facilitates the identification of peaks and determination of masses corresponding to the peak maxima. The deconvolution parameters were studied in order to achieve ideal results and prevent the formation of artifacts. Three different deconvolution software were compared, which produced almost identical deconvoluted spectra. The accurate determination of peak maxima required smoothing of the spectra due to the noise present in the acquired spectra. Finally, a deconvoluted spectrum of trastuzumab was compared to the theoretical masses of common glycosylated forms.

P-19 | Ion-exchange chromatographic retention – ionic and molecular properties of analytes

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Ion chromatography techniques have long played a significant role in the qualitative and quantitative analysis of ionic and ionizable compounds. However, there are still less investigated areas related to the retention-elution processes of components.

In the classical description of ion-exchange chromatography, the basic parameters of the retention models are the mobile phase concentration, the nominal charge of the analyte and eluent ions, the theoretical ion-exchange capacity of the column bed, and the volume ratio of stationary and mobile phases. The former properties are easily quantifiable, and they are in the models as fixed parameters. The separation of the components is made possible by their different affinity for the resin, which is quantified by the selectivity constant. This empirical parameter describing the equilibrium distribution of analyte and eluent ions between the stationary and mobile phases, is typically quantified using iteration and/or adsorption isotherms from measured data.

The aim of this research is to develop a model that is suitable for predicting the expected retention of components under different conditions even without prior retention data. The column geometry, physical and chemical packing structure, eluent composition, and elution type are all included in the model.

Furthermore, an important part of the research is to explore the numerical relationship between the selectivity constant and the physical, chemical structure of the analyte. With the help of these structural descriptors, the selectivity constant can be modeled and the retention of the components predicted, supporting chromatographic method development under both standard and matrix-stressed conditions.

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P-20 | Chromatographic efficiency of pressure-induced gradient separations

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In chromatography, pressure can significantly influence the retention factors of the tested compounds. In liquid chromatography, this effect is primarily related to the change in the molecular volume of the solute during adsorption, which is remarkably large for large biomolecules such as peptides and proteins. As a result, the migration speed of the chromatographic bands varies spatially along the length of the column, which affects the amount of band broadening. During our work, based on theoretical considerations, we investigated the chromatographic efficiency under pressure-induced gradient conditions. By studying the retention factor and the migration rate, it can be established that components with the same retention time can show different migration patterns. The width of the initial band after injection is affected by the pressure gradient and results in significantly narrower initial bands for more pressure-sensitive compounds. In addition to the classical band broadening phenomena, the effect of the pressure gradient on the band broadening is remarkable. A positive velocity gradient results in extra bandwidth.

Our results clearly prove that the zones widen significantly at the end of the column if the molar adsorption volume change of the solute is large. If the pressure drop increases, this effect becomes even more significant. At the same time, the high exit speed of the lanes somewhat offsets the extra effect of the lane widening, but it cannot completely offset it. As a result, the separation efficiency of large biomolecules is significantly reduced due to the chromatographic pressure gradient. Under high-pressure liquid chromatography conditions, the degree of apparent loss of efficiency can reach up to 50% compared to the column's intrinsic efficiency.

P-21 | Impact of water use as additive on elution some polar compounds in supercritical fluid chromatography

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Supercritical fluid chromatography (SFC) is considered an environment-friendly approach due to utilizing carbon dioxide as the main eluent, in turn less consuming of organic solvents during the chromatographic process in comparable to high performance liquid chromatography (HPLC). Employing 100% CO_2 as mobile phase in SFC is limited due to the low polarity of CO_2, which is very close to that of liquid pentane or liquid hexane, hence it can only dissolve nonpolar compounds. Therefore, the majority of SFC experiments are carried out with alcohol modified - CO_2 such as methanol or isopropanol to improve the solubility of analytes in the mobile phase.

In our study we investigate the use of water as an additive in methanol-modified CO_2 for eluting some polar compounds from a hybrid silica column (Viridis BEH column 50 × 3 mm) in SFC.

According to the amount of added water to methanol we obtained different responses. Adding 1~2% of water to methanol leads to a positive role showing more symmetrical peaks and reducing the retention time for some compounds. Whilst introducing water amount higher than 3% to methanol was found to perturb the chromatographic results. Also, it was observed that the use of water as additive was not able to elute a strong basic solute such as propranolol HCl with an acceptable peak shape, thus it is required to use diethylamine (TEA) or diethylamine (DEA) as additive to elute propranolol from the hybrid silica column.

Improvement of the chromatographic peaks of the studied analytes by adding water to the methanol-modified CO_2 was assumed due to: i) adsorption of water on the stationary phase, consequently resembling the hydrophilic interaction liquid chromatography (HILIC) mechanism in liquid chromatography. ii) the availability of hydrogen bond acceptor / donor sites in the analyte structure.

P-22 | Scouting the application limits of different hold-up time markers in supercritical fluid chromatography

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The compressibility of carbon dioxide results in the variation of several chromatographic properties in supercritical fluid chromatography (SFC), since the mobile phase also undergoes significant expansion besides the pressure drop along the column. Density is the most critical property, but viscosity, thermal properties and solvation strength of the eluent are also affected, in addition to retention factors, column efficiencies, diffusivities and solubilities of the sample components. It is also understood that in SFC the set and true volumetric flow rates differ from each other, that can become an important issue when trying to translate retention times and hold-up times into retention volumes and hold-up volumes, respectively. Similarly, the method of hold-up time measurements is not as universal as in liquid chromatography.

Our work focuses on the application range of nitrous oxide as a hold-up time marker in supercritical fluid chromatography. This molecule has been suggested a decade ago to be used as unretained marker, something that the field of SFC was missing, since its beneficial properties make it an ideal candidate [1, 2]. Determination of the hold-up volume and actual volumetric flow rates have always been problematic in SFC due to the compressibility of carbon dioxide and one part of this is the difficulty of hold-up time measurements. Depending on the mobile phase, different methods have been used to measure the hold-up time with varying results. Nitrous oxide and other molecules have been compared in different conditions, mobile phases and stationary phases. In all cases, nitrous oxide gave the lowest elution times. However, detection was difficult in mobile phases containing 10% or more of organic modifier, because most solvents mask the signal of nitrous oxide. Interestingly, the choice of stationary phase also had a slight effect on detection.

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P-23 | The uncertainty of the van 't Hoff plots in chiral chromatography

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In chiral chromatography, the van 't Hoff plot (ln k vs. 1/T) is very popular for elucidating chiral separation mechanisms. However, the method has its own limits. One of the main problems is that the chromatographic system is not isobaric, there is a pressure drop along the column, thus the thermodynamic equilibrium is questionable. Another issue is that a chiral stationary phase is heterogeneous, it contains enantioselective and non-selective binding sites, so the retention factor (k) does not originate from the interactions of only one type of binding site [1, 2].

In our former work, where a chiral environment was modelled by connecting two reverse-phased columns, we showed that the flow-rate, the pressure, the column length and the type of the instrument also influenced the obtained result. Those measurements were mainly focused on reverse phase separations, so we continued our investigation in chiral environment. In our recent study, the retention of chiral solutes was studied using a chiral stationary phase by varying the flow-rate. Our results are consistent with previous works, the obtained results are greatly influenced by the applied flow-rate and pressure. The thermodynamic parameters did not change to the same extent in the case of the two enantiomers, so different conclusions can be drawn about the separation mechanism. The results obtained from the van 't Hoff plot can be useful in some cases, but the physical content of the numerical values of the parameters is debatable.

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P-24 | The impact of frits and column end structure on efficiency in liquid chromatography

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Sample band broadening in the immediate vicinity of the column ends can be characterized by column reversal experiments [1]. In the case of packed columns, measurements have shown that the columns are heterogeneous and some differences can be observed between the two ends of the column.

Column reversal has peak compression effect, the peaks obtained with reversed flow are always narrower and more symmetric than those without flow reversal, and therefore column reversal is suitable for determining the local plate height values of the columns and for detecting the difference between the two column ends.

We can conclude that shorter columns are more axially homogeneous than longer columns, so that column length is an influential factor in the column packing procedure.

Column reversal was also attempted with macromolecules to eliminate the effect of pores. Similar conclusions could be drawn, however, due to the complexity of the measurements, we will continue to perform the column reversal with small molecules in the following.

In addition to this, electron microscopy measurements were also carried out, where the purpose of the measurements was to determine whether any damage was visible on the frit or on the particles as a result of the column loading procedure. The inlet and outlet frit of five types of columns were examined. None of the cases showed visible damage to either the frit or the particles. However, the silica gel particles were visibly entrapped in the pores of the frit due to their size and the heterogeneous structure of the pressed frit. The particles embedded in the frit may also be responsible for the band broadening effect near the frit.

P-25 | HPLC-MS/MS method validation for determination of DINCH plasticizer metabolites in urine

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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. 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. As biomarkers of exposure to DINCH are used mainly hydroxy- (OH-MINCH) and carboxy- (cx-MINCH) cyclohexane-1,2-dicarboxylic acid monoesters. They are excreted in urine as glucuronide conjugates. Our aim was to implement and validate HPLC-MS/MS method for their determination in urine.

Analyses of urine samples were performed after hydrolysis of glucuronide conjugates of DINCH metabolites using hyphenated high-performance liquid chromatography with C18 stationary phase and isotope dilution tandem mass spectrometry. TSQ Quantum triple quadrupole mass spectrometer (ThermoFisher Scientific Inc.) was operated in negative mode. Monitored mass transition quantifiers for cx-MINCH, D8-cx-MINCH, OH-MINCH, and D8-OH-MINCH were 327.2→173.1, 335.2→173.1, 313.2→153.1, and 321.2→161.1, respectively.

Obtained parameters for presented method are summarized in Table 1. Intraday and interday precision was evaluated using six urine samples with known values of cx-MINCH (in the range of 1.09 - 14.6 $\mu g.l^{-1}$) and OH-MINCH (in the range of 1.09 - 23 $\mu g.l^{-1}$), measured in 5 replicates at three different days in a period of 1 month. Concentrations were calculated from matrix linear calibration curve in the range 0.12 to 60 $\mu g.l^{-1}$. Accuracy, expressed as the deviation from the reference values in control samples was evaluated as a Z-score in round tests and the requirement for quality approval was the interval of $|Z| \leq 2$.

Table 1. Parameter of presented method

Analyte	LOD ($\mu g.l^{-1}$)	LOQ ($\mu g.l^{-1}$)	Intra day CV (%)	Inter day CV (%)	*Z*-score	Calibration curve R^2
cx-MINCH	0.05	0.1	2.5 – 3.8	2.5 – 5.6	0.2 – 0.9	0.9980
OH-MINCH	0.03	0.14	2.4 – 3.4	5.1 – 8.2	0 – 0.8	0.9966

Elaborated method allows reliable determination of OH-MINCH and cx-MINCH metabolites of DINCH plasticizer with the precision better than 10% and accuracy expressed as Z-score in the range 0-0.9 vs. required value below 2. The achieved limits of quantification of 0.1 $\mu g.l^{-1}$ for cx-MINCH and 0.14 $\mu g.l^{-1}$ for OH-MINCH are in agreement with published data and satisfactory for the needs of biomonitoring studies.

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P-26 | Meconium pretreatment optimization for determination of DINCH plasticizer metabolites by HPLC-MS/MS

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Effort to minimize human exposure to harmful substances from the environment has driven their replacement with ones with more favorable toxicological profile. 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. However, as the DINCH production and usage have rapidly increased, the rate of exposure is increasing accordingly. It is important to focus biomonitoring especially on the population cohorts, for which exposure to chemicals has or may have more significant impact on development and health, such as during the prenatal development period.

Valuable biological matrix for examination of prenatal exposure to various harmful substances is meconium. Meconium is the first newborn stool with a semi-solid consistency. It is black-green in color and mostly odorless. It is composed of epithelial tissues, amniotic fluid, mucus, bile dyes and water. The secretion of meconium occurs only after birth, but it is formed in the fetus as early as the 12th week of pregnancy. The advantages of this matrix are noninvasive sampling and wide exposition window. On the other hand, meconium represents a very complex matrix, usually requiring time consuming and laborious pretreatment.

As biomarkers of exposure to DINCH are used mainly hydroxy- (OH-MINCH) and carboxy- (cx-MINCH) cyclohexane-1,2-dicarboxylic acid monoesters. The aim of our work was to study meconium sample pretreatment using liquid extraction with various organic solvents and solid phase extraction procedures prior to HPLC-MS/MS determination of these two metabolites. Triple quadrupole mass spectrometer with heated electrospray ionization source operated in negative mode was utilized for measurements. Pretreatment procedures were assessed for overall pretreatment effectiveness based on the recovery of cx-MINCH and OH-MINCH from the matrix and on the matrix effects in the ionization source of mass spectrometer.

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P-27 | Tandem mass spectrometry method for the diagnosis of inborn errors of creatine metabolism and transport

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Creatine deficiency syndromes (CDS) are a group of inborn errors of metabolism and include disorders of creatine synthesis and transport. A common feature of these syndromes is a lack of creatine in the brain, which causes neurological diseases. The CDS group includes two autosomal recessive disorders of creatine synthesis - arginine glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) enzyme deficiency, as well as an X-linked creatine transporter deficiency disorder (CTD). The determination of creatine and guanidinoacetic acid (GAA) as specific biomarkers of inborn errors of creatine metabolism and transport is significant from the point of view of establishing the correct diagnosis and treatment.

When determining the content of creatine in urine, the disadvantage is its unstability and increasing concentration over time due to bacterial contamination. If the creatine determination in urine sample is not carried out immediately after collection, it is necessary to freeze the sample immediately and reduce the number of thaws to a minimum.

This work is aimed at investigating the possibility of using a dry urine spot to determine the concentration of creatine, GAA and creatinine in urine sample using tandem mass spectrometry and testing the stability of these analytes in a liquid matrix and a dry urine spot at different storage temperatures.

The results show that GAA and creatinine are stable in the liquid matrix as well as in dry urine spot at room temperature and at 4°C. Creatine is stable at room temperature for only about 3 hours in liquid matrix, then its concentration increases and after 24 hours it is 3 to 4 times higher. In dry urine spot creatine is stable within 24 at room temperature, and more than three-fold increase in concentration was noted after a week, when stored at 4°C it was a one-and-a-half-fold increase after a week. All three analytes show a decrease in concentration when stored at -20°C in liquid matrix and dry urine spot as well due to precipitation, therefore sample sonication for redissolving these precipitates is recomended.

Using a certified reference material of lyophilized urine at three different concentration levels were evaluated intra-day precision (up to 2,9% for creatine, 3,8% for GAA and 2,1% for creatinine), inter-day precision (up to 3,4% for creatine, 4,4% for GAA and 3,0% for creatinine), and accuracy (up to 10.3%, 13,5 % and 6,1% for creatine, GAA and creatinine respectively) for a dry urine spot.

Detection limits for creatine, GAA and creatinine were at 0.005 mmol/l, 0.005 mmol/l and 0.12 mmol/l, and quantification limits at 0.018 mmol/l, 0.016 mmol/l and 0.40 mmol/l, respectively using a dry urine spot.

Dry spot urine samples of two patients with GAMT deficiency were also analyzed.

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P-28 | Determination of aflatoxin B_1 degradation with ozone-enriched medium by GC-MS technique

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Keywords: mycotoxins, aflatoxin B1, degradation, GC-MS.

Mycotoxins as secondary metabolites of fungi, the daily consumption is likely to be found in our foods. The total number of mycotoxins, estimated at about 300 to 500,000 pieces, of which only about 15-20 pieces of causing significant damage, poisonous toxins. Aflatoxins are a family of toxins produced by certain fungi that are found on agricultural crops (corn, peanuts, cottonseed, and tree nuts). The main fungi that produce aflatoxins are Aspergillus flavus and Aspergillus parasiticus, which are abundant in warm and humid regions of the world. Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage.

Aflatoxin B1 is commonly found on several foods and feeds in the temperate regions of Europe, Africa, Asia, America and Oceania. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional maximum tolerable daily intake for Aflatoxin B1 of 0.1-12 µg/kg.

The presence of mycotoxins in the end it would be possible to intervene more in the development of food crops, but unfortunately have the harvested crop are often infected with the inadequate and incomplete because of interference. The harvested crops, such as the mycotoxin content of grains used in food commodities mainly concentrated in the outer part of the shell. The outer shell is partly demolished or remove the toxin may be a good solution to reduce contaminated grain quantity toxins. The method developed is based on the oxidizing effect of ozone. The technique is simple and the ozone produced from grain contact between the outer shell enriched mycotoxins in part reacts with ozone, which produced a reaction to the toxin loses its toxic effects. The ozone production techniques were used, the corona-discharge technique.

During degradation experiments the quantity and quality of the Aflatoxin B1 were determined in ozone enriched medium with GC-MS technique, were analyzed the possible degradation products from degradation of toxin, which chemical structure and properties are significant because of the toxicity of resulting products.

P-29 | Structure and influence of antioxidant metal-chelating peptides

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Reactive oxygen species (or ROS) are essential highly reactive components for the proper functioning of the body, acting as signalling molecules to regulate biological and physiological process. However, the presence of endogenous or exogenous stresses (e.g., air pollutants, smoking, and UV-irradiations) can trigger dysregulation of ROS homeostasis, inducing the socalled oxidative stress reported to cause a wide variety of diseases. Over the last decades, research and development of antioxidant compounds has shown increasing interest, especially for their applications in nutrition and healthcare. While several biomolecules are well-known for their antioxidant properties (e.g., ascorbic acid, carotenoids), bio-sourced antioxidants have recently drawn considerable attention, especially bioactive peptides, mainly obtained by the hydrolysis process.

Our aim in this project was to synthesize peptides with appropriate structure, able of binding the iron(III) ion and acting as “indirect” antioxidant. Thus, we present herein a new series of bio-inspired antioxidant peptides derived from milk protein casein, the characterization of their complexes and of their antioxidant effect. We used various spectroscopic techniques (UV-vis, CD, Raman, NMR) and mass spectrometry to determine the composition, stability and structure of the iron(III)-complexes. We wondered whether these complexes were susceptible to quenching reactive oxygen species and how it took place, therefore we monitored the reactions under different conditions using the HPLC-MS technique.

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P-30 | Monitoring rat brain slice nimodipine level using a new targeted UHPLC-MS/MS method

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The calcium channel blockers can be classified into three main groups: phenylalkylamin, benzothiazepines, and dihydropyridines. Nimodipine (NMD) is the L-type voltage-gated second-generation 1,4-dihydropyridine calcium channel blocker. The NMD has a high lipophilic character (logP 3.41) which assists its penetration through the blood–brain barrier and is thus able to act on cerebral blood vessels resulting in sustained and conspicuous cerebral vasodilatation.

Over the years, several analytical methods were published to determine NMD levels in biological samples, mainly biofluids. Interestingly, only a few papers deal with NMD quantitation in the brain while the brain is the primary organ where the NMD exerts its effect. Therefore, our study aimed to develop a targeted UHPLC-MS/MS method for quantitatively determining NMD levels in rat brain tissue using a small sample quantity and a simple liquid–liquid extraction procedure. The UHPLC-MS/MS method was successfully validated and applied for the pharmacokinetic study of NMD in rat brain slices.

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P-31 | Planar multiplex assays differentiate effect responses providing true information

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Compounds in complex samples can quench the formed response signal of agonists using *in vitro* assays due to the co-presence of antagonists, cytotoxins and/or false-positives (physico-chemical quenching). The latter three mechanisms reduce the response signal measured via *in vitro* assays. This is not noticed, if not otherwise three separate *in vitro* assays are additionally performed for detection of antagonists, cytotoxins and/or false-positives, which is a very tedious effort. It was studied whether planar multiplex assays [1–3] could differentiate the responses and provide the information simultaneously.

Sample preparation was performed minimalistic to ensure sample integrity as much as possible. After automated application of the samples and planar separation, one agonist stripe and one end-product stripe were applied along each separated sample track. Then, the planar bioassay was applied on the chromatogram. Agonists were evident as bands. Antagonist reduced the agonist stripe. False-positives reduced the end-product stripe. Cytotoxins were evident via a respective substrate added to the culture after on-surface incubation. Synergists enhanced the agonist stripe. Thus, opposing effects were differentiated and not overlooked, which otherwise may mislead the interpretation and decision made for complex samples.

Multiplex bioassay profiling saved material and time since agonists, antagonists, false-positives, cytotoxins and synergists were detected simultaneously. It is fit for purpose with 20–30 min per sample depending on the assays (thereof 10% manual operation), Euro 1.20 consumables per sample, and Euro 2500 investment costs for the latest 2LabsToGo system with autosampler (previous prototypes [4,5]).

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P-32 | Proteomic and glycosaminoglycan characterization of ALK rearranged lung adenocarcinoma tissues

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Lung cancer is one of the most common types of cancer and is responsible for the most cancer-related deaths, making a detailed understanding of the molecular changes extremely important. In this study, we characterized the proteomic and glycosaminoglycan (GAG) composition of ALK rearranged lung tumor tissue regions based on 3 different characteristics: morphological classification, mucin and stroma content.

The study involved the examination of 22 regions (including 4 adjacent normal regions) on seven tissue sections using nanoUHPLC-MS/MS measurements after tryptic, chondroitinase-ABC, and heparin lyase digestion. Expression differences of proteins were investigated, and the biological functions of differentially expressed proteins were elucidated through protein interaction networks. Glycomic investigations were interpreted based on the total amount and sulfation patterns of chondroitin-sulfate (CS) and heparan-sulfate (HS) disaccharides produced during enzymatic digestion.

The differences between individual samples were examined using principal component analysis and hierarchical clustering. The results of these analyses revealed that adjacent normal regions were markedly different from tumor regions, while both the proteomic and GAG-omic profiles of tumor regions were strongly influenced by mucin content, with morphology having a secondary impact and stroma content having a little influence on these profiles. Due to the observed importance of the tumor mucin content, the results obtained by comparing regions with different mucin content will be presented in detail.

We found that differentially expressed proteins between medium (M2) and high mucin (M3) regions were involved in processes such as extracellular matrix organization, protein folding, and translation. A significant overexpression of five proteoglycan core proteins was observed in M3 regions compared to M2 regions, justifying the need to investigate the glycosaminoglycan chains. Regarding GAG analysis, an increase was observed in both the total amount and the average rate of sulfation in M3 tumors compared to M2 regions. M3 regions contained 3.5-fold more CS and 3.7-fold more HS than M2 regions, while the average rate of CS and HS sulfation increased by 2.0 and 1.4, respectively.

Overall, our pilot study highlights the role of tumor mucin content in both proteomic and glycosaminoglycan profiling and provides an excellent starting point for future studies of lung cancer.

P-33 | Phosphoproteomic analysis of lung- and prostate adenocarcinomas

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Analyzing the phosphoproteomic profiles specific to lung and prostate adenocarcinoma, two of the most frequently diagnosed cancers presents a significant opportunity to uncover kinase activities. These findings can reveal promising tissue-specific intervention points for different cancer types, leading to potential improvements in therapy response.

In the present pilot study mass spectrometry-based phosphoproteomic analysis was carried out on a total of 31 samples derived from formalin-fixed paraffin-embedded lung and prostate tissue sections. On-surface digestion was performed on small cancerous and tumor adjacent tissue regions. Differentially expressed proteins and phosphopeptides were evaluated using label-free quantitation and subsequent statistical analysis.

We have identified 13 disrupted kinases by kinase-substrate enrichment analysis. MAPK8, CDK5, and GSK3B exhibit opposite alterations between the two cancers analyzed. Furthermore, several members of the PAK family, involved in cytoskeletal signaling, showed heavily suppressed activities in both adenocarcinomas. Additionally, our study revealed significant changes in the expression of specific phosphoproteins, predominantly metabolite interconversion enzymes and cytoskeletal proteins, distinguishing lung and prostate cancer; these specific molecular signatures might be attractive targets for further in-depth investigations.

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P-34 | Analysis of chondroitin sulfate and heparan sulfate glycosaminoglycans in different lung cancer tissues

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Lung cancer, the second most commonly diagnosed cancer has the highest mortality rate among all tumor types. Carbohydrates are utilized as diagnostic biomarkers in various cancers. During tumor development, glycosaminoglycans (GAGs) undergo changes in their abundance and structure. Our objective was to conduct a comprehensive quantitative and qualitative glycomic study on samples of small cell lung cancer and different subtypes of non-small cell lung cancer, analyzing both tumor and tumor adjacent regions.

To examine disaccharides, we used tissue surface lyase digestion in our study. The CS and HS disaccharides extracted from the tissue surface were desalted, separated using self-packed capillary columns with hydrophilic interaction liquid chromatography and weak anion exchange (HILIC-WAX) mixed mode resin, and the detection was carried out with negative ionization mass spectrometry. We utilized recently developed HPLC-MS methods with separate ammonium formate salt gradients for the analysis of CS and HS disaccharides.

Several significant changes were observed in the content and sulfation patterns of GAGs between tumor and tumor adjacent regions. Although the alterations between the lung tumor phenotypes were not as remarkable, some differences could be identified. The abundance of CS was doubled in tumor samples, while the total content of HS did not show significant changes. Additionally, the average degree of sulfation significantly increased in all examined tumor phenotypes. Comparing adenocarcinoma samples to other lung tumor phenotypes, the 6-*O*-/4-*O*-sulfation ratio was increased. *O*-sulfated HS components were elevated in the tumor samples. These findings emphasize the importance of exploring the role of GAGs in lung cancer development, as several alterations were identified between tumor and tumor-adjacent tissue samples, as well as among different lung tumor phenotypes. Based on these results, future large-scale studies of HS and CS GAGs may reveal further significant correlations between structural and quantitative changes associated with cancer.

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P-35 | Transzferrin-anion komplexek fiziológiai hatása HeLa sejtekre

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A tumoros sejtek jelentősen több Fe^{3+} ion szükséglettel rendelkeznek a normál fiziológiájú sejtekhez képest [1]. A megemelkedett Fe^{3+} igény miatt több transzferrin receptort expresszálnak a sejtfelszínükön mint az egészséges sejtek, ezzel kielégítve vasigényüket. Ezt kihasználva kutatásunkat a szervezet fő vas szállító fehérjéjére a transzferrinre alapoztuk.

A transzferrinnek szüksége van anionos természetű molekulára, hogy két Fe^{3+} kötését biztosítani tudja, egyet-egyet mindkét lebenyén. Ezeket a kötéseket fiziológiás esetben a karbonát ion stabilizálja [2]. A karbonát iont helyettesíteni lehet karboxil-csoportot tartalmazó molekulákkal, melyek szignifikáns fiziológiai változásokat okozhatnak a sejtekben. Ezen effektusokra szerettünk volna kutatásunk során fényt deríteni különböző esszék alkalmazásával.

Kutatásunk célja transzferrin-anion komplexek tumoros sejtekbe történő juttatása, valamint fiziológiai hatásuk vizsgálata volt.

A munka a Richter Gedeon Nyrt. által létrehozott Richter Gedeon Talentum Alapítvány támogatásával „Richter Gedeon Kiválósági PhD Ösztöndíj” keretében készült.

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P-36 | Determination of endocannabinoids from human serum with LC-MS/MS method for the comparison of peripheral and spinal anesthesia

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Recent studies identifies five endogenous substances so called endocannabinoids (ECs) acting on CB1 and CB2 cannabinoid receptors. These ECs are anandamide (AEA), 1 and 2-arachidonoyl glycerol (1AG and2AG), arachidonoyl ethanolamine, (O-AEA), 2-arachidonyl glycerol ether (2AGe) and arachidonoyl dopamide (NADA). CB1 and CB2 receptors not only occur in the central nervous system, but they are widely distributed in various organs and tissues. Recent studies shows that endocannabinoids can also act on other receptors including transient receptor potential vanilloid type-1 (TRPV1) and orphan G-coupled receptors GPR55. This indicates that ECs can play different physiological roles both in central and in peripheral tissues. There are several structurally similar compounds like, oleic acid ethanol amide (OEA), palmitic acid ethanol amide (PEA), n-oleoyldopamine (OLDA) and stearic acid ethanol amide (SEA) that are not acting on CB receptors, but can affect EC actions.

Due to LC-MS/MS method allows the quantitative analysis of lipophilic and apolar metabolites with short analysis time and minimal sample preparation (no derivatization is needed), and can differentiate between similar compounds even from a complex matrix, it is widely used for the analysis of ECs. Since 2AG transforms easily in water solutions, such as human plasma into 1AG, the sample preparation should be made under low temperatures.

In our study a quantitative method has been developed using triple quadrupole mass spectrometer coupled to reverse phased liquid chromatography, for the simultaneous determination of AEA, 1AG, 2AG OEA, NADA, SEA, PEA and OLDA from human serum. The samples were taken from patients after surgical intervention involving peripheral or spinal anesthesia. The aim of the clinical study is the investigation of the correlation between the EC level expressed in the human body and the anesthetic method used during the intervention.

P-37 | Separation of bacterial lipooligosaccharides by non-aqueous CE-MS/MS

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Lipopolysaccharides (LPS) and lipoolygosaccharides (LOS) – also called endotoxins – are constituents of the outer leaflet of the outer membrane of Gram-negative bacteria. Native isolates of endotoxins comprise a high inherent heterogeneity, which contributes to the development of antibiotic resistance. This poster presents the application of a new non-aqueous CE-MS method to separate and online mass analyze the different constituents of underivatized, intact LOS isolates.

Non-aqueous CE (NACE) measurements were carried out with a 7100 CE system coupled to a 6530 Q-TOF MS (Agilent Technologies) equipped with an Agilent Jet Stream ESI interface. The non-aqueous background electrolyte (BGE) consisted of different mixtures of methanol, chloroform and acetonitrile, in the presence of triethylamine and acetic acid as ionic additives at varying concentrations.

Based on our previous results in the NACE separation of the hydrolyzed part of endotoxin (*i.e*. the lipid A region)[1] we developed a new electrolyte suitable for the separation and online fragmentation of intact LOS species. The migration of the amphipathic LOS ions was influenced both by the number of fatty acid chains (3 to 7) and by the number of phosphate (1 to 3) and sugar (2 or 3) residues. This resulted in the separation of several LOS species.

The experiments demonstrate for the first time the NACE separation and online fragmentation of small quantities of molecular LOS species in complex bacterial mixtures.

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P-38 | Monitoring tryptophan metabolites in mouse plasma

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Tryptophan (TRP) is an essential amino acid that is not only one of the building blocks of proteins but also serves as a precursor for the biosynthesis of many important bioactive compounds. A smaller proportion of TRP is converted into serotonin and melatonin, while a significant part of the TRP is metabolized via the kynurenine pathway including kynurenine, 3-hydroxykynurenine, 3-hydroxyantranilic acid, kynurenic acid (KYNA), xanthurenic acid, quinolinic acid and nicotinamide adenine dinucleotide. The SZR104 (*N*-(2-(dimethylamino)ethyl)-3-(morpholinomethyl)-4-hydroxyquinoline-2-carboxamide) as a synthesized novel aminoalkylated amide derivative of KYNA has a high permeability through an *in vitro* blood-brain barrier model. Since SZR104 is promising kynurenine analogues, the question arises as to what effect their administration into the body has on the TRP metabolism pathway.

The quantification of different metabolites in biofluids is challenging, especially when we would like to determine the small molecules with disparate physical properties (e.g. wide polarity range), low concentration endogenously, and other components in the matrix that can interfere with detection. Ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-MS/HRMS) is a specific and sensitive method for simultaneously determining targeted endogenous compounds.

In this study, SZR104 was intravenously administered to mice, and then blood samples were collected at different time intervals, thereby examining the change in the concentration of tryptophan metabolites in plasma. In our work, after rapid sample preparation procedure without any derivatization, the TRP and its 13 metabolites and SZR104 were successfully separated in mouse plasma within 9 minutes total run time. Standard addition with internal standard (KYNA-d5 and SZR72) calibration method was used for quantitative analysis.

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P-39 | Characterization of substances from slate bolete (*Leccinum duriusculum*) suitable for plant protection

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Major changes have taken in the agricultural sector in the European Union over the past decade. Many pesticides, among others insecticides, fungicides are continuously, systematically withdrawn from the market. One possible solution is the isolation of new natural compounds with novel action mechanisms that can be used in plant protection with the desired effect.

Among the basidiomycete mushrooms, large numbers of species produce different secondary metabolites with diverse bioactivity (e.g.: antioxidant, antibacterial, antifungal, etc.). The Slate Bolete, *Leccinum duriusculum* syn. *Boletus duriusculus* (Schulzer ex Kalchbr.) Singer is a common wild mushroom, which season lasts from May to November. As mycorrhizal fungus, it is not cultivated and can mostly be found in groups under poplar trees.

Our research focuses on the antibacterial and antifungal compounds of the fruiting bodies of the *L. duriusculum*. The pulverized mushroom sample was extracted by methanol, because this solvent gave the most efficient extraction of bioactive components. The raw extract also contained high amount of interfering components, so the extract was cleaned-up and fractionated by normal-phase flash chromatography.

The antibacterial and antifungal activity of the fractions were examined by TLC coupled with direct bioautography using different species of bacteria and microscopic fungi. The active fractions were further fractionated by RP-flash chromatography resulting in nearly clear isolates. The compounds with antimicrobial effects were characterised by TLC hyphenations including chemical derivatisation and TLC-MS. The collected data will help the identification of the isolates.

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P-40 | The effect of Closantel on lipopolysaccharide biosynthesis in *Shigella sonnei*

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Keywords: shigellosis, bacterial two-component system, lipopolysaccharide

Lipopolysaccharides are molecules that cover more than 70% of all Gram-negative bacteria. Lipopolysaccharide biosynthesis - like a variety of other pathways - is under the regulation of bacterial two-component systems. This project analyses the connection between the biosynthesis of lipopolysaccharides and a bacterial two-component system, suggesting new possible targets on Gram-negative bacteria.

Analyzing an interesting *Shigella sonnei* mutant, a hotspot was identified in lipopolysaccharide biosynthesis by the epimerase *gmhD*. Targeting the gene through the bacterial two-component system by Closantel resulted in a neutral effect.

As a conclusion, we can state that bacterial two-component systems can play a role in the regulation of LPS biosynthesis by controlling the expression of genes involved in the biosynthesis. While Closantel did not affect the expression of *gmhD*, we will continue to test other two-component system inhibitors.

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MP-1 | Trends and analytical solutions in nitrosamine analysis

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The determination of genotoxic nitrosamine content in pharmaceutical products has been an analytical and chromatographic challenge in the past 5 years. Considering the EMA and FDA recommended daily dose limits, very sensitive and selective instruments and methods are needed for the detection and quantitation of these impurities in such a small concentration range. While the measurement of "classic" (nitroso derivatives of common amines found in the solvents as impurities used for the synthesis) nitrosamines was challenging, their testing with the use of isotope labelled internal standards and LC-MS/MS and/or GC-MS/MS systems is becoming routine in our laboratory. In addition, active pharmaceutical ingredient related genotoxic nitrosamine impurities are receiving more and more attention and their measurement requires unique solutions in many cases. In case of measuring these molecules, ensuring adequate recovery is one of the biggest problems due to the matrix effect of the active pharmaceutical ingredient (API) and the excipients used for formulation. Both the API and the excipients are soluble in common extraction solvents used for LC and GC sample preparation in quantities which can cause ion suppression (or enhancement in rare cases) in the parts per trillion (ppt) concentration range. According to our experience the matrix effect caused by the API can be lowered with better chromatographic separation. The aim of this scientific poster is to present solutions for lowering the matrix effect during the method development phase of these synthesis related nitrosamine impurities in case better separation does not solve the problem.

MP-2 | Efficient and sensitive peptide mapping approach by µPAC columns with ultralow sample loading

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Peptide mapping is an important approach to analyze monoclonal antibodies for the identification of sequences, post-translational modifications and mutations. Traditional packed columns are usually used for peptide mapping at analytical flow rate with large sample loading. For improved separation and sensitivity, low flow chromatography has become the preferred LC method. Microfabricated pillar array columns (µPAC™) were introduced as an innovative technology for low flow. Here, peptide mapping is conducted using 50 cm µPAC Neo and 5.5cm High Throughput µPAC Neo columns. With only 20ng NISTmAb tryptic digest, 96.4% and 98.6% are achieved for heavy chain (HC) and light chain (LC) in 15 mins elution time using 50cm µPAC Neo column. The same sequence coverages are achieved in a 5 mins elution time using 5.5cm High Throughput µPAC Neo column[1].

Compared with packed bed (and monolithic) column technology microfabricated pillar array columns (µPAC™) are an innovative technology that enables high peak capacity separations at moderate LC pump pressures. Through the implementation of lithographic pattern transfer and deep reactive ion etching (DRIE) into silicon wafers, separation channels can be manufactured that contain micrometer sized silicon features that are perfectly positioned according to a pre-defined design. The introduction of perfectly-ordered separation beds eliminates any Eddy dispersion originating from heterogenous flow paths through the column and increases column permeability. It also provides high peak capacity separations at low flow rate with enhanced ionization sensitivity.

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MP-3 | Single-shot LC-MS workflow for comprehensive proteome identification on an orbitrap astral mass spectrometer

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Traditionally, deep proteomic profiling was achieved using MudPIT approach wherein the complex peptides were pre-fractionated either using ion-exchange chromatography or more recently high-pH reversed-phase fractionation followed by analysis of 24-48 fractions by LC-MS/MS. Early studies using ultra-long 3 hours gradients resulted in identification of 5,400 proteins. Further improvements in MS instrumentation resulted in proteome depth to 8,000-10,000 protein in ~2 hours runs. Here, we show that ultra-high proteome depths can be achieved using novel Astral mass analyzer using one hour gradient thereby significantly increasing sample throughput and maximizing instrument time for analysis of more samples in less time. Using µPAC Neo 50cm column, deep proteome coverage of eleven commonly used cell lines was achieved on a new Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer interfaced with Thermo Scientific™ Vanquish™ Neo UHPLC system.

Recent advances in mass spectrometry technologies have led to higher proteomic depths mostly using MudPIT-based MS analyses. Such approaches add considerable labor, requires high sample amounts and may not be feasible for processing tens or hundreds of samples. Recent reports on single-shot proteomic analyses showed identification of up to ~8,200 proteins at 6-8 samples per day throughput. Such methods are not suitable for analysis of medium to large sample cohorts considerably adding sample to sample variation. Ultra-long gradient methods also have issues in consistent protein quantitation due to variation in peptide retention times and overall low throughput. Here, we present a single-shot LC-MS/MS workflow with identification of >10,000 proteins from human cell lines on a new Orbitrap Astral mass spectrometer.

MP-4 | Improved profiling of sialylated n-linked glycans by HPAE-PAD

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Profiling the N-linked oligosaccharides of a glycoprotein is one of the important analyses used for glycoprotein characterization. This is especially true when a glycoprotein is being produced as a human therapeutic. High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is one of the techniques commonly used for in-depth analyses of N-linked oligosaccharides in glycoproteins.[1,2] HPAE-PAD separates native carbohydrates (i.e., underivatized) at high pH (>12) and detects them by pulsed amperometry on a gold working electrode. This poster demonstrates improved resolution of sialylated N-glycans on Dionex CarboPac PA200 columns. Starting with a recently described method,[3] changes to elution conditions were tested to improve resolution of sialylated N-glycans enzymatically released from four different glycoproteins. Separations were first evaluated on the analytical format (3 × 250 mm column). Next, the possibility that a shorter column, such as a guard column, would allow significantly improved throughput was evaluated. Finally, a narrow bore format 1 × 250 mm column was tested. The use of this column not only allows reduced eluent consumption but also makes the method more amenable to mass spectrometric oligosaccharide analysis. Results from all three different formats are discussed.

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MP-5 | How do isotope fingerprinting support the detection of the origin of medicines and food adulteration?

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Nowadays, with the expansion of global markets and business competition, the frequency of food and medicine adulteration has increased exponentially. Food and medicine adulteration refers to the alteration of food and medicine quality that takes place intentionally. It includes the addition of ingredients and impurities to modify different properties of food and medicine products for economic advantage. Adulteration of these poses a serious health risk. Therefore, there is a growing need to detect the adulteration and the selection of the appropriate analytical method is essential for these investigations. Earlier, adulterated food and medicine products were detected based on of simple physical or physico-chemical parameters, such as refractive index, viscosity, saponification, iodine value etc. However, the increase in the number of cases and the development of adulteration methods have prompted the introduction of new and more efficient analytical methods. One such promising technique is stable isotope ratio mass spectrometry with gas or liquid chromatographic separation.

The essence of stable isotope analysis is that the sample to be analysed is burned at high temperatures or subjected to strong chemical oxidation, during which CO_2, N_2, H_2 and/or SO_2 gases are released. The gas sample is then fed into a mass spectrometer to determine the isotope ratios, which serves as a very unique chemical fingerprint in the origin and adulteration investigations. In our poster presentation, we present the isotope ratio mass spectrometric (IRMS) solutions of Thermo Scientific and compare the applicability of bulk material (BSIA) and compound-specific (CSIA) stable isotope ratio analysis during drug and food adulteration. We also describe the role of the Orbitrap MS developed by Thermo Scientific in the direct (i.e. not requiring combustion and chemical oxidation) stable isotope ratio measurement from direct injection of liquid samples.