ABSTRACT BOOK

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CONTENT

Plenary session

Temperature-responsive Liquid Chromatography for Enhanced Quantitative Analysis with Refractive Index Detector

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Refractive Index Detection (RID) stands out as a liquid chromatography detector with characteristics resembling universal detection offering the potential for standard-independent analysis. Its advantage lies in the bulk property nature of the refractive index fostering a more uniform response across various analytes compared to detectors like UV or mass spectrometry [1]. However the practical use of RID is confined to isocratic High-Performance Liquid Chromatography (HPLC) due to its heightened sensitivity to changes in solvent composition.

Addressing this limitation this study explores the application of temperature-responsive stationary phases to overcome challenges associated with RID and solvent gradients. These phases involve water-soluble smart polymers particularly those exhibiting a Lower Critical Solution Temperature. Such polymers undergo conformational changes in response to temperature variations. At elevated temperatures the polymers dehydrate forming a more hydrophobic phase while lower temperatures result in increased hydration and the creation of an extended hydrophilic phase. This gradual transition with temperature allows for precise control of analyte retention as they traverse the column using water as the sole mobile phase. Consequently TRLC eliminates the need for solvent gradients replacing them with a temperature profile throughout the analysis [2].

The current work demonstrates the compatibility of temperature gradient TRLC with RID by analysing a series of non-UV visible molecules. Notably this platform yields overlapping calibration curves for chemically similar compounds indicating virtually identical detector responses within a negligible margin of error [3]. The efficacy of this approach is showcased in the analysis of challenging-to-detect molecules such as free fatty acids and long-chain alcohols expanding its application to more complex samples containing compounds difficult to elute with pure water. Moreover the TRLC responsive behaviour persists even when incorporating a moderate fraction of green organic modifiers into the mobile phase allowing for analysing more apolar solutes with a specific focus on longer chain functionalized hydrocarbons [4].

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Plenary session

Greening preparative liquid chromatography for the purification of biopharmaceuticals

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Therapeutic peptides are considered one of the most promising class of biopharmaceuticals. Their industrial production (upstream processing) has exceptionally advanced in the last ten years especially for what regards solid-phase synthesis. However these advancements have not been matched by equivalent improvements in purification procedures (downstream processing) which still represents the bottleneck in terms of both cost time and sustainability in the entire production process.

Single-column preparative liquid chromatography in reversed-phase (RPLC) conditions is the most widely used approach to purify the target peptide from its impurities. Solid-phase synthesis indeed do not lead only to the target peptide but also to a series of unwanted product-related impurities which have very similar chemo-physical properties as well as chromatographic behavior to that of the target. This means that very often more than one chromatographic step is required thus leading to a relevant volume of waste solvent mainly composed by acetonitrile (ACN) which need to be disposed. This solvent is toxic for humans and the environment; therefore (bio)pharmaceutical industries are claiming for the development of greener approaches to purify peptides as well as other biopharmaceuticals.

This lecture will show the latest results obtained by our group in this field. In the last years we have worked on two approaches to increase the sustainability of biopharmaceutical purification. The first one involves the replacement of ACN with greener alternatives. We have tested not only alcohols (ethanol and isopropanol) but also dimethyl carbonate (DMC) which is ranked among the greenest solvents by Solvent Selection Guides but it has been barely applied as organic modifier in liquid chromatography [12].

The second approach relies on the use of advanced multicolumn countercurrent preparative liquid chromatography platforms which allows for the recycle of overlapping regions of the chromatogram inside the system. This mechanism is beneficial not only for automating the purification process but also for reducing the solvent consumption. It will be shown that by employing Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) for the purification of Icatibant (a small therapeutic peptide composed of 10 amino acids) it can be reduced by more than 80% with respect to the correspondent single-column process [3].

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Plenary session

Boosting comprehensive two-dimensional gas chromatography with Artificial Intelligence: Computer Vision helps to see what we smell

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Artificial intelligence (AI) is an exponentially expanding field poised to transform the landscape of analytical chemistry [1]. As an intriguing test bench for AI tools and concepts food applications offer many challenges due to the compositional complexity of samples (e.g. primary metabolites secondary/specialized metabolites processing derivatives exogenous compounds from microbial communities presence of xenobiotics and contaminants etc) and the properties connected to specific yet unique chemical patterns. Multidimensional analysis systems combining physicochemical discrimination of individual components by chromatography with the structure-elucidation potential of MS are the ultimate solutions for comprehensive investigations. However the analytical data generated by MDA platforms have to be fully exploited by applying non-conventional approaches supported by the AI potential to take a further step ahead and generate new knowledge on food properties going beyond current quality indexes.

The contribution aims to illustrate how AI techniques can efficiently explore the complex datasets derived from comprehensive two-dimensional gas chromatography (GC×GC) in the application area of food-omic. By harnessing the power of AI we highlight the potential for streamlined and insightful data processing based on different kinds of features. Through this exploration we showcase the transformative impact of AI in deciphering the complexities of GC×GC data emphasizing efficiency and precision in food markers discovery.

Thanks to the multidimensional nature of GC×GC data arrays Computer Vision and Augmented Visualization open new opportunities for decision-making strategies. By these advanced yet peculiar AI tools retention-time shifts that cause image distortion are efficiently compensated thereby facilitating comparative visualization and related strategies. Moreover by actively exploiting mass spectral signatures in the presence of co-elutions and misalignments unknown-knowns can be specifically targeted and by identifying key food odorants in the presence of interferents aroma signatures can be predicted by an AI-smelling machine[23].

Practical examples showcase the challenges and potentials of the application of AI to GC×GC-MS data obtained in studies related to food volatilomics[4] nutrimetabolomics[5] and sensomics[6].

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Plenary session

The rise of MS based structure identification: a Star Mass Story.

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Due to its selectivity throughput and sensitivity as well as its structure identification capabilities liquid chromatography - mass spectrometry is the analytical method of choice for the identification of drug metabolites in complex biological matrices. Full structure elucidation can however often be challenging. In these cases NMR is the technique of choice but the relatively large amounts of compound in very high purity that is required can be time consuming and sometimes challenging to obtain.

The ability to arrive at a final structure based on MS related methods alone has steadily increased over the years and has improved greatly in recent years. Fragmentation is still the basis for MS structure elucidation. In addition to classical CID fragmentation alternative fragmentation tools that also work for small molecule structure ID such as electron activation dissociation and UVPD have emerged. H/D exchange and derivatizations are other distinctive tools used to expose more specifically the functional groups in a molecule. Infrared ion spectroscopy (IR-IS) is another emerging technique that can provide structural information (IR spectra) that is orthogonal to MS fragmentation. Thanks to the mass spectrometer part of IR-IS this information can be obtained with the sensitivity and selectivity of the mass spectrometer. IR spectra obtained on the first quadrupole ion trap - table top infrared laser combination installed in the pharmaceutical industry will be shown.

Quantum chemical calculations and more recently also machine learning are also taking a more prominent place in structure identification for instance by predicting ion mobility MS measured collision cross sections IR spectra most likely sites of metabolism etc.

The application of the combination of these techniques and methodologies will be illustrated in the field of drug metabolite structure identification e.g. distinguishing positional isomers of oxidative metabolism primary secondary and tertiary amines acylglucuronides from other glucuronides N-oxides from other oxidations etc.

Plenary session

Mass Spectrometry Imaging in peri-operative diagnostics – The tip of the iceberg on the possibilities

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Mass spectrometry imaging (MSI) offers a revolutionary approach by capturing intricate molecular and spatial data simultaneously heralding a new era of research opportunities. While it's rapid advancement enables widespread use across various domains such as food analysis environmental monitoring and forensic investigations its potential in biological contexts promises to revolutionize clinical diagnostics.

The presentation will focuses on the development and translation of Mass Spectrometry Imaging-based methods towards clinical applications. This includes new fast and objective detection methods of disease markers in biological samples but also intra-operative diagnostics & prognostics and 'on the spot' visualization & identification of the disease type tumour border or cancer cells and their hypoxic state.

From Single cell research …

The full chemical composition of cancer cell lines including lipids and protein identification is generated on a single cell level. Thanks to the applied imaging technique lipid distributions within a single cell can be visualized. Based on statistical analysis of the single cell profiles we can generate models that are able to distinguish different cell types and receptor status in a tissue context in a matter of seconds.

Our direct identification and visualization method of biomolecules on a single cell level opens new possibilities in cancer research and diagnostics. Cellular differences can be detected possibly leading to the identification of specific molecular pathways and biomarkers. Moreover visualization of compound distributions within a single cell is a major step in understanding intracellular pathways.

…to intra-operative diagnosis and prognosis

Real-time Rapid Evaporative Ionisation Mass Spectrometry (REIMS) enables in-situ tissue characterization based on the mass spectrometric analysis of electrosurgical vapours generated during dissection. We demonstrate the use of REIMS for the molecular characterization of brain tumour tissue (including glioblastomas) and patient survival prediction thereby providing quasi real-time assessment of tumour type. Method sensitivity and discriminating molecular biomarkers are determined leading to new understandings for in situ tumour detection.

All shown research projects have a direct and highly translatable purpose keeping the end-users mostly clinicians in mind. The main research focus is translating molecular insights into clinical solutions by developing methods and products that will have added value in the strive towards personalized medicine. Showing these examples the presentation will discuss the needs and hurdles in the journey of MSI towards routine diagnostic techniques.

Plenary session

How mature is GC? State-of-the-art and a look into the future.

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In this presentation an overview will be given of the developments in capillary gas chromatography that we have witnessed during the past decades. These developments in column technology GC instrumentation detection and sample preparation have brought GC to a mature state in the sense that the technique is able to deliver accurate qualitative and quantitative data that can be exploited in a very broad range of application areas from chemical and petrochemical industry to life sciences.

State of the art GC-MS equipment for instance allows to reach detection limits orders of magnitude below the limits of detection and quantification that could be reached at the beginning of the century. Based on that sample preparation methods could be miniaturized and automated. Examples of such sample preparation method such as sorptive extraction micro-liquid-liquid extraction miniaturized clean-up and just-in-time derivatization will be presented and their potential reduction on environmental impact will be discussed.

A “mature state” clearly does not mean that there is no room for further development. As stated by Grob many years ago: “In a field such as GC there is no standstill – either progress or degradation”. Therefore we are looking forward to future developments such as the introduction of new formats for GC columns and the application of artificial intelligence in data analysis.

Plenary session

New Generation Micro-Pillar Array Columns and Their Intrinsic Performance

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The past decades have witnessed an enormous progress in separation efficiency and peak capacity that can be achieved in 1D liquid chromatography (LC) columns in a practically affordable time. However there are still areas where the need for more efficiency and speed is imminent. One such area is that of proteomics. Given the typical small size of proteomics samples and the need for sensitivity micro- and nano-flow LC is the current LC method of choice in proteomics research.

Nano-flow LC is also the area wherein recently the concept of micro-pillar array columns (µPACs) has been introduced. µPACs are produced by silicon micromachining and were developed to benefit from the advantages of perfect order in chromatographic separations. Another advantage of pillar arrays is their low flow resistance which is owed to the fact that the pillars are freestanding and do not touch each other as is the case in a sphere packing.

In contrast to packed bed columns which seem to have reached the limit of what is possible in terms of a further reduction of the particle size µPACs are only at the start of their descent of the feature size ladder. which Indeed considering that silicon micromachining offers a spatial resolution well below the 1 µm mark it is clear that the 1st generation of µPACs (introduced with a pillar diameter of 5 µm and an inter-pillar distance of 2.5 µm) is still amenable to a considerable further reduction.

In pursuit of higher efficiencies and speeds a 2nd generation µPAC is now commercially available. In this new format both pillar size and the inter-pillar distance are halved compared to the 1st generation. The present contribution compares the performance of Gen1 and Gen 2 µPACs under identical conditions using the trans-parent cover wall to follow the band broadening process on-chip measurements of the evolution of the band broadening of injected tracer dyes. In this way the true intrinsic performance is measured devoid of any extra-column band broadening.

Next to this the present contribution will also report on the performance of a µPAC wherein the pillars are stretched out radially to obtain rectangular pillars. This concept builds further upon earlier work where the use of radially-elongated pillars was pro-posed as a means to (1) increase the radial mixing (2) obtain a more uniform flow field and (3) quasi completely eliminate the side-wall effect. Both formats will be compared in terms of plate height curves as well as by using kinetic plots.

Plenary session

Analysis of emerging environmental contaminants using ion mobility mass spectrometry

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Beyond well-known pollutants such as legacy persistent organic pollutants (POPs) our environment likely contains various emerging and unknown contaminants. POPs are ubiquitous in our environment and they bioaccumulate when they are passed from species to species through the trophic chain ending up and ultimately to human. Targeted gas chromatography (or liquid chromatography) coupled with high-resolution mass spectrometry (GC-MS or LC-MS) is the most common methods currently used to test for the presence of POPs. This technique is powerful and effective but produces limited results on regulated contaminants.

In this context this presentation demonstrates the advantages of adding ion mobility spectrometry separation to existing GC-MS and LC-MS workflows for historical and emerging halogenated contaminants analysis. Specifically the integration of Trapped Ion Mobility Spectrometry (TIMS) coupled with time-of-flight mass spectrometer (TIMS TOFpro II Bruker Bremen) equipped with a GC-APCI source for sample separation and ionization (GC-APCI II Bruker Bremen) provides a powerful tool to monitor a broad range of suspected and non-targeted contaminants. TIMS separations are performed using the new concept of Sliding Window in Ion Mobility (SWIM) mode to enhance the ion mobility resolving power [1].

The proposed non-targeted workflow encompasses feature detection (retention time drift time m/z and isotopic patterns) feature filtering (Kendrick mass defect filtering CCS trends filtering and homologous series) and feature characterization (exact mass and molecular formula assignment open-source database queries and ultimately structural elucidation). Through different examples of complex matrix samples the methodology is conducted for various classes of legacy (e.g. PCBs PBDEs OCPs) as well as emerging (e.g. PXBs) persistent organic pollutants (POPs). We show that among the features developed drift time filtering is of great help in obtaining clean mass spectra in order to get rid of most matrix background signal as well as other coeluting isobaric interferences greatly improving the quality of the spectra and the identification process especially for low signal intensity features.

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Plenary session

Targeted Blood Lipidomics of Colorectal Cancer

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Being the third most frequently diagnosed cancer colorectal cancer (CRC) is also the third leading cause of cancer-related deaths. Additionally CRC early-onset incidence has been rising at a significant rate for the last 25 years further urging the need for population-based screening not only for individuals presenting red flag signs and symptoms. Current invasive and resource-demanding diagnosis methods such as colonoscopy need to be supported by a triage strategy that would allow to rule out non-CRC patients. Fecal immunochemical tests have high potential but lack sufficient sensitivity and would benefit from additional rapid chemical tests. In that context developing chemical methods to identify CRC markers with high specificity sensitivity robustness low level of invasiveness and cost effectiveness is of high interest.

We conducted a study on 64 human serum samples from different groups (adenocarcinoma adenoma and control) using cutting-edge GC×GC–LR/HR-TOFMS techniques. We analyzed samples with two different sample preparation approaches for targeted lipidomics12 (fatty acids) (25 µL serum) and metabolomics34 (50 µL serum). Samples were randomized with a QC sample (pooled human plasma) and NIST SRM 1950 for QA/QC requirements.

Highly structured 2D chromatograms facilitated the identification of chemical families and structures (e.g. structuration of fatty acid methyl esters (FAMEs) based on C numbers and number of double bonds). Before applying the statistical tools median normalization cubic root transformation and autoscaling of the data were applied. A chemometric screening including unsupervised (PCA HCA) and supervised analysis (PLS-DA) univariate analysis (volcano plot) and random forest (RF) classification algorithm was performed on both metabolomics and targeted lipidomics data sets. Out of the 354 compounds isolated in the metabolomics data set 52 were identified: 20 were amino acid derivatives 14 were lipids 8 were organic acids and 10 other compounds not belonging to a specific family.

In the targeted lipidomics data set 36 compounds were identified: 13 saturated fatty acids (SFA) 8 monounsaturated fatty acids (MUFA) 14 polyunsaturated fatty acids (PUFA; 6 ω-3 7 ω-6 1 ω-9) and 1 cholesterol derivative. Amongst them 8 features (MSI confidence levels of 1 or 2) were identified as significant (VIP score >1 MDA cut-off >0008). It revealed that specific PUFA (ω-3) molecules were inversely associated with increased odds of CRC while some PUFA (ω-6) analytes shown a positive correlation. Additionally a tendency to sub-categorization of the adenocarcinoma samples based of cancer stages appeared and is currently under deeper investigation. Random forest cross-validation also demonstrated the ability of the approach to predict sample classes with low-class error rates (OOB error 0015).

The preliminary finding suggests that despite the lower number of compounds identified the lipidomics data set results in a more efficient sample differentiation. The targeted lipidomics method is also advantageous compared to metabolomics approach as it simpler faster and more easily automatable to large-scale studies.

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Plenary session

Innovative chromatographic strategies for 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 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 while 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.

Plenary session

Data processing and other challenges in LC-MS and GC-MS multi-compound target-analysis in the area of food flavor and taste

Hans-Gerd Janssen* (12) Boudewijn Hollebrands (12) Oscar Dofferhoff (1) Herrald Steenbergen (1)

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With modern hyphenated- and comprehensive analytical instruments hundreds -if not thousands- of compounds can be analysed in minutes to hours. Driven by modern -omics strategies the quest for even more peaks and even more detailed data continues. Generation of data peaks features etc. however no longer is the bottleneck in modern analysis. The biggest concern in our experience is the translation of these massive data sets into meaningful information and knowledge.

In our use of advanced chromatography and mass spectrometry methods in food analysis we see two problems. Firstly it is absolutely impossible to check the accuracy of peak integration for every single peak in a sample. Massive data tables are obtained in a fully automated manner but how reliable are the numbers in the table? And how reliable are automated peak identifications? Secondly finding meaningful information in the ever expanding data sets is becoming increasingly difficult. The more data you get the more samples are needed to find the relevant information. However in case of food-quality issues there are often just two samples: a good one and a bad one. Natural variation between foods is large further complicating marker detection. Additionally human sensory panels are expensive and notoriously unreliable.

In the current presentation we will discuss the various tools we apply for food flavour- and taste analysis. The above-mentioned problems will be discussed in more detail. We exploit the high resolution offered by modern methods but more and more move to knowledge-based targeted analysis. The core of our strategy is the analysis of a long target list of food flavour and taste species. As an example for food flavour analysis we focus on 226 key food odorants amidst the circa 10 000 food volatiles. High resolution is needed not to detect all species present but to separate the targets from the ‘noise’. Peak areas are translated to flavour or taste intensities using either experimental information or based on flavour and taste thresholds predicted using Artificial Intelligence.

Plenary session

Embracing the Hyphen - A Dash to the Answer

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Hyphenation has been with us since the fifties the work of McLafferty and Gohlke joining GC with MS on followed by LC-MS developments in the sixties and seventies. The eighties delivered new ionisation techniques and atmospheric pressure ionisation interfaces that now make hyphenation techniques ubiquitous across all areas of science.

Choosing the correct hyphen to answer the analytical question is more of a key step today than ever; often the question asked now requires the use of several hyphenated approaches to deal with the complexity of samples presented for analysis. This presentation will discuss the need and use of different hyphenations to answer questions of complexity and specific problems presented by a range of different application areas.

One example that requires modern hyphenation(s) is the introduction of renewable fuels into the already complex petrochemical fuel matrix. First generation biodiesels FAMES in themselves are complex materials added to which their chemical/structural diversity means that these materials could be a problem. The provenance and quality of these FAMES therefore demanded good analytics e.g. initially GC-MS and then SFC-MS.

Recently hydrotreated vegetable oils have been introduced to the fuel chain again these present new challenges that require different hyphenated solutions to determine fuel quality of the newly processed material and subsequent identification within a diesel fuel matrix. For this GC-MS 2D GC-MS SFC-MS and SFC-FID have been employed to characterise these new materials.

Only by understanding the materials in hand and appreciating the strengths (and weaknesses) either side of the hyphen and across different hyphens can the appropriate results be determined.

Plenary session

Effective optimization of SFC-MS coupling using prediction equations and artificial neural networks

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Supercritical fluid chromatography coupled with mass spectrometry (SFC-MS) has recently gained increase in its popularity due to its analytical capabilities such as high separation efficiency speed of analysis interesting selectivity and complementarity to other chromatographic techniques. However the coupling of supercritical fluid chromatography and mass spectrometry is not straightforward compared to LC-MS due to the inherent properties of CO2 used as a main component of the SFC mobile phase. Indeed a density drop responsible for the decrease in solvating power and precipitation of analytes is observed after decompression in API ion sources resulting eventually in compromised chromatographic performance and method sensitivity. Therefore dedicated interfaces implementing a sheat pump delivering a make-up liquid are needed in SFC-MS coupling.

This study investigates the effect of make-up solvent composition in SFC-MS/MS using electrospray ionization (ESI) Unispray (US) and atmospheric pressure chemical ionization (APCI) ionization techniques in both positive and negative modes. Sixty target analytes were subjected to a generic

SFC-MS/MS method featuring a diol column and a generic gradient elution with methanol and

10 mmol/L ammonia in methanol using state-of-the-art SFC-MS instrumentation with a triple quadrupole analyzer. Employing statistical analyses and artificial neural networks (ANN) we unravel the dependencies among analyte physicochemical properties eluent volume entering MS make-up solvent composition and ionization source. 25 make-up solvents including methanol ethanol and isopropanol and methanol with additives including ammonia ammonium formate ammonium acetate formic acid acetic acid and water all at different concentrations were examined. A total of 207 molecular descriptors were computed for each of the 60 target analytes to characterize their physicochemical properties. This was undertaken to facilitate the identification of key molecular features influencing SFC-MS responses. The impact of each molecular descriptor on the observed MS response under different conditions was assessed using ANN. The descriptors with the most significant effects on MS responses were identified and compared for each make-up solvent and ionization source. Subsequently these influential parameters were utilized to construct prediction models through multi-linear regression allowing for a swift and straightforward determination of optimal conditions for various target compounds. Indeed by employing the proposed prediction model and optimization processes based on the outlined behavioral trends the optimal makeup solvent composition for a given target analyte can be readily estimated. This not only streamlines the optimization procedure but also reduces solvent consumption and enhances the environmental friendliness of the SFC technique.

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Plenary session

Recent Developments in Needle-Trap Technology: An Effective Exhaustive Microextraction Technology Facilitating Hyphenation of Sampling/Sample Preparation to Gas Chromatography.

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The advent of microextraction techniques revolutionized sample preparation by combining clean-up sampling and sample preparation into a single step. Further adding to their appeal microextraction methods are also sensitive environmentally friendly fast portable and rather inexpensive. Of the various available microextraction techniques needle-based methods are among the most intriguing. In needle-based methods an extraction phase is immobilized/packed inside of a needle which is then used for extraction and the subsequent desorption of analytes via direct injection into chromatographic instruments. The talk will focus on needle-trap devices (NTD) which consist of a small tube or a needle that is packed with an extraction phase for the active or passive extraction of volatile compounds. The main difference between NTDs and other microextraction techniques is that NTDs enable exhaustive extraction as long as the breakthrough volume (BTV) is not reached [1]. This is significant as it means that NTD extractions are less sensitive to kinetic and thermodynamic conditions and do not require the extraction conditions to be strictly controlled (i.e. temperature) compared to non-exhaustive versions. Over time various configurations and sampling modes have been introduced to simplify the construction of the device and the extraction procedure reduce the cost and improve desorption efficiency. The lecture will provide an overview of the evolution of the structure of NTDs followed by a detailed description of the filter containing NTD technology and its application to the investigation of aerosol samples [2 3 4]. The importance of incorporating the particle filter as an additional packing material will be emphasized. This modification results in a Filter-Incorporated NTD (FI-NTD) which expands the potential areas of this technology including a free and total concentration measurement when combined with non-exhaustive SPME measurement as the active sampling mode of the NTDs are also suitable for trapping particle- and droplet-bound compounds. The various NTD designs can be coupled with benchtop or portable GC and automated [4-8]. NTDs are robust green and fast sampling devices that are ideal for on-site sampling due to their simple calibration and their ability to eliminate analyte loss during storage [5]. Furthermore NTDs are flexible as their capacity and selectivity can be optimized by changing the packing type packing length and sampling volume. Recent applications of the NTD technology to environmental monitoring breath determinations exposure and food investigations will be covered during the presentation.

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Plenary session

Aptamer-based sorbents for the selective extraction of molecules and ions at trace levels in complex samples

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The evolution of instrumentation in terms of separation and detection has allowed a real improvement in sensitivity and analysis time. However the analysis of ultra-traces from complex samples often requires a purification and pre-concentration step before the analysis of the target analytes (organic molecules ions) by powerful conventional instruments such as mass spectrometry coupled or not with chromatographic systems. In this context extraction sorbents based on a molecular recognition mechanism appear as powerful tools for the selective extraction of a target analyte in order to obtain a more reliable and sensitive quantitative analysis.

One possible approach is the use of immunosorbents (ISs) based on the use of antibodies specific to the molecule of interest. Indeed the high specificity and affinity of the antigen-antibody interactions allow selective cleaning of complex samples or extracts with high enrichment factors. This molecular recognition mechanism can also be generated by molecularly imprinted polymers (MIPs) whose synthesis leads to the formation of specific cavities mimicking the antibody recognition site. They have the advantage that they can be synthesized in a few days. On the other hand their application to real samples requires a careful optimization of the extraction procedure to reach the expected selectivity.

An alternative is to use single strands of DNA or RNA i.e. a specific oligonucleotide sequence capable of binding to a given target analyte with the same affinity as antibodies. This sequence called aptamer can be grafted onto a solid sorbent and the resulting oligosorbent (OS) can be used for the selective extraction of different types of target analytes (toxins pesticides drugs...) from complex samples such as biological fluids and food samples. Once the sequence is available the development of an oligosorbent is less expensive than that of an IS and the associated extraction procedure is almost as easy to develop on OS as on IS unlike MIPs.

Disposable SPE cartridges containing OS can be prepared and used as conventional SPE sorbents for processing various types of samples or extracts. The high selectivity provided by the extraction procedure may make their use particularly necessary when developing miniaturized devices due to the decrease in resolution that results from the use of shorter separation devices. In this context a fully miniaturized analytical system has been developed for the quantitative analysis of target molecules in complex samples. Furthermore if the potential of OS has been so far widely demonstrated for the extraction of organic compounds their potential for the extraction of metal ions has been recently explored and applied to the purification of samples prior to ICP-MS analysis.

Plenary session

Wastewater Analysis for Narcotics Detection by direct-injection LC-MS/MS and LC-QTOF-MS: a spatiotemporal investigation in England

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Wastewater-based epidemiology (WBE) has been successfully used over the past two decades for the estimation of community-scale consumption of drugs by analysing a pooled sample of sewage taken from a wastewater treatment plant (WWTP). In 2021 4859 deaths related to drug poisoning were registered in England and Wales the highest number since 1993. Therefore there is a constant need for reliable analytical solutions to identify and monitor illicit drugs. Now the challenge lies in scaling up analytical platforms to cope with thousands of samples collected at high spatiotemporal resolution. The aim of this study was to quantitively monitor selected illicit drugs and other contaminants of emerging concern including pharmaceuticals pesticides and personal care products in influent wastewater samples in a high spatiotemporal manner. High throughput direct-injection analysis methods were used for targeted analysis and suspect screening using tandem- and high resolution liquid chromatography-mass spectrometry (LC-MS/MS and LC-HRMS respectively). For quantitative analysis 20 illicit drug consumption markers were monitored across >2000 samples. A spatiotemporal analysis of the drugs across all sites will be presented using estimated consumption data (mg/1000 people/day). Moreover correlations between compounds such as cocaine cocaethylene and ketamine are shown. Suspect screening was performed in 96 samples based on a fully curated library of 1219 compounds. Several points of confirmation were used: retention time ±0.5 min of the curated library value mass accuracy ≤5 ppm isotopic distribution score >20 and a library identification similarity index score >45; signal-to-noise ratio of 3:1 and a minimum 2000 peak intensity signal. Suspect screening resulted in the detection of 278 unique compounds (~30 compounds per sample). Pharmaceuticals represented the highest proportion of compounds detected across all sites The frequency of detection of compounds per site will be also presented as a means to shortlist and prioritise compounds for further analysis/monitoring for WBE. This work represents the first and largest investigation of a high spatiotemporal WBE using direct-injection LC-MS methods for quantitative and suspect screening data across England.

Plenary session

Atmospheric Analytical Chemistry - Advances and Challenges for Hyphenated Techniques in Chromatography and Separation technology

Marja-Liisa Riekkola* Kari Hartonen Jose Ruiz-Jimenez Eka Dian Pusfitasari Matti Jussila Tuukka Petäjä Markku Kulmala

University of Helsinki Department of Chemistry and INAR – Institute for Atmospheric and Earth System Research Finland

Multidisciplinary approaches are needed to tackle the Grand Challenges the Earth's system is facing on a global scale and especially to tackle those related to climate change and air quality. The combined effects of complex mixtures of gaseous and particulate pollutants their interactions and feedbacks more specifically need to be resolved before we are able to understand the changing climate to solve air pollution problems in the most polluted environments or to quantify air quality-climate interactions. Poor understanding of the atmospheric interactions may lead to unwanted climate impacts and various negative health effects. The Intergovernmental Panel on Climate Change (IPCC) recognizes atmospheric aerosols as the single largest source of uncertainty in human-driven climate change (IPCC 2013 2021). The effects of these aerosols on climate include their direct effect on radiative forcing their impact on cloud formation and properties and their feedbacks in the natural carbon cycle. Airborne particulate matter or aerosols have also a profound effect on air quality. In addition to their ability to limit visibility and cause haze in many parts of the world the inhalable particles can transport toxic chemical species into the vulnerable deeper regions of the lungs causing health problems. Along with aerosol particles the substances that need to be studied include volatile organic compounds (VOCs) emitted from anthropogenic and biogenic sources. They have major effects on atmospheric chemistry and new aerosol particle formation since when they are oxidized by atmospheric oxidants they form lower volatility condensable products and further secondary organic aerosols that are major components of fine particulate matter. Therefore the identification and quantification of VOCs in the atmosphere is essential for the understanding of the mechanisms of aerosol formation and global climate change.

Advanced on-line coupled and hyphenated analytical techniques and systems give us sensitivity and selectivity often needed to understand the atmospheric chemistry and its impact on both human health and the environment. However requirements for fast automated and reliable multi-targeted detection of analytes in the atmospheric samples still call for new analytical approaches.

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Plenary session

Advancements in mRNA structural characterization – The coming-of-age of the analytical toolbox

Koen Sandra* Kris Morreel Jelle De Vos Helena Vanluchene Piotr Alvarez Porebski Gerd Vanhoenacker Griet Debyser Stefanie Jonckheere Nathan Cardon Emmie Dumont Ruben T'Kindt Pat Sandra

RIC group Belgium

Worldwide successes in confining the COVID pandemic can largely be ascribed to the development of vaccines based on messenger ribonucleic acid (mRNA). The approval of these landmark mRNA vaccines has paved the way for the full exploration of this 'novel' technology both for prophylactic and therapeutic purposes. This evolution has been facilitated by 30 years of research with key discoveries being (1) the incorporation of naturally occurring modified nucleotides into mRNA to suppress innate immune activity and (2) the packaging in lipid nanoparticles (LNPs) to protect the mRNA upon administration and to enhance cellular uptake. The sudden rise of the latter medicines is accompanied by the need for proper analytical methods to study various structural properties such as amongst others mRNA sequence 5' capping efficiency 3' poly-A tail length post transcriptional modifications mRNA integrity fragmentation double-stranded RNA (dsRNA) and aggregation. The current talk will reflect on various methodologies based on liquid chromatography (LC) mass spectrometry (MS) multi-angle light scattering (MALS) detection and mass photometry for the in-depth study of the latter attributes.

Plenary session

Ultra-high resolution MS and ever more powerful ion mobility-mass spectrometers and we are talking about chromatography: Why?

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Two opposing trends can currently be observed in the instrumental analysis of small organic molecules (up to around 2000 Da). Firstly faster analytical methods need to be developed in order to cope with the constantly growing number of samples. Promising current developments here include increasingly powerful ultra-high-resolution mass spectrometers and ion mobility mass spectrometry. Secondly the demands on modern instrumental analysis are becoming ever greater. The desire for decreasing detection limits and the comprehensive analysis of more complex samples (all possible components of a sample should be determined qualitatively and

quantitatively) are leading to ever more sophisticated analysis platforms and immense challenges in data analysis.

To save time it raises the question of whether it is possible to dispense with time-consuming and cost-intensive chromatography and simply inject the samples directly into the mass spectrometric analysis system after sample preparation. This presentation will discuss the advantages and disadvantages of chromatographic pre-separation and ultra-high resolution mass spectrometry or ion mobility - mass spectrometry without chromatography. To further optimise the analysis in terms of separation performance new developments in LCxLC and ion mobility mass spectrometry will also be presented and new software developments shown.

Plenary session

Integrated multidimensional assessment of multiple antibody-CQAs from single sample injections

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Therapeutic monoclonal antibodies (mAbs) comprise a large structural variability with respect to charge size and post-translational modifications (PTMs). These critical quality attributes (CQAs) need to be assessed during and after the production of mAbs. This normally requires off-line purification and sample preparation as well as several chromatographic modes making the process time-consuming and error-prone. We developed an integrated and automated multi-dimensional analytical platform for the simultaneous assessment of multiple CQAs of mAbs in cell culture fluid (CCF) from upstream processes [1]. The platform allows mAb characterization at the intact subunit and peptide level. The system combines protein A affinity chromatography (ProtA) with immobilized enzyme reactors (IMERs) and/or size-exclusion ion-exchange and reversed-phase liquid chromatographic modes with UV absorbance and high-resolution mass spectrometric detection. Multiple heart cuts from a single mAb elution band from ProtA are stored in loops and successively sent to the multimethod options in the second dimension. When desired online IMERs holding either the protease IdeS or trypsin provide efficient digestion of the isolated mAb. The applicability of the developed workflows will be demonstrated by the direct analysis (i.e. not requiring off-line sample preparation) of a therapeutic mAb in CCF obtaining comprehensive information on accurate molecular mass amino acid sequence charge and size variants glycosylation and PTMs of the studied mAb product. The performance of the system is comparable to established off-line methods fully compatible with upstream process samples and provides a significant time-reduction of the characterization procedure.

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Plenary session

Analytical Lab of the Future: Trends and Challenges in Industrial Analytical Labs

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The first laboratories were already around for several centuries when alchemy was a popular discipline. As the chemistry knowledge evolved so did the laboratory–becoming a place for mainly distillation towards the investigation of chemical reactions. However chemical experiments were carried out wherever there was space such as a barn or a kitchen. The modern chemical laboratory emerged in the 1800s. First with wooden benches with bottle racks above them and cabinets underneath them for storage. Also first steps were taken towards ventilation as well as a drainage system for the liquid waste to be transported to the main sewage was installed. Over the years the materials for benches racks storage cabinets ventilation and drainage systems were optimized but the of the design of the labs did not change a lot. The introduction of computers completely changed the design of the analytical lab. The fast evolution in computers computing power miniaturization and automation has currently impact on the location itself as well as on the people working in the laboratory. But more changes to the analytical labs are forthcoming. The evolution in data science and artificial intelligence (AI) we are witnessing for the moment will lead to some significant changes in the analytical lab design and laboratory operations. In this lecture new trends impacts and challenges will be presented.

Plenary session

Chromatographic big data analysis using Bayesian statistics: finding alternative ways to automation

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All techniques of data analysis methods applied to chromatographic data including base-line correction peak detection alignment peak tracking calibration and/or classification are a routine part of most modern analytical workflows. With the emergence of hyphenation (especially high resolution mass spectrometry) and two-dimensional methods (e.g. GCxGC and LCxLC) we can truly talk about a “Big data” era in analytical chemistry. Analysing these enormous and complex quantities of data becomes a tremendous challenge especially because of the need of automation.

Automation is always a challenging task. In most of cases the scientist has to “rely” on the algorithm taking (automated) decisions on both qualitative (e.g. peak identification) or quantitative (e.g. calibration) processes. However Bayesian statistics really offers a paradigm shift on the automation process. Contrary to classical methods it is not the algorithm but the scientist the one who takes the decisions and the role of the algorithm is to calculate the probabilities of the variables of interest. Such probabilities are modified as long as new observations are introduced into the equation. In the end dealing with (propagated) probabilities allows the scientist to take final (informed) decisions: the role of the algorithm is limited to “inform” the user instead of taking decisions itself.

This way of thinking has been applied to a broad range of situations in chromatography. One example concerns peak detection in 2D chromatography in which several configurations may be possible concerning the beginning and the end of a peak affecting the quantitative results. Another example covers screening techniques in which the probabilities of a list of compounds being present in the sample are considered analysed with LC-MS. Another example is a Bayesian deconvolution of chromatographic data in which the classical multivariate curve resolution (and hence library search if possible) is applied probabilistically so the question of parsimony of the model (i.e. how many compounds are present) is solved using Bayesian model averaging. Finally an application for peak assignment and alignment in LC-MS will be presented which is tackled from a combinatorial optimization perspective. Surprisingly innovative situations may arise specially in those cases in which multimodal probability distributions appear which are in principle counter-intuitive but after some reflection become the best picture of the current knowledge (regarding the scientific question) that the experimental data is able to offer.

All in all the use of Bayesian statistics to deal with massive data treatment requires a shift in the way of the chromatographer thinks about dealing with data. Basically we are proposing to work with probability distributions (opposed to fixed parameters) and update them as long as more information/data is taken into account. This is opposed to deliver the final answer to the user.

Plenary session

Challenges and opportunities in supercritical fluid hyphenated systems

Caroline West* (1) Clément De Saint Jores (1) Benjamin Caux (12) Laurine Réset (1) Isabelle François (3)

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Supercritical fluid extraction (SFE) and chromatography (SFC) have long been established as independent techniques. Recently commercial instruments hyphenating these two separation steps as SFE-SFC have been made available. In addition hyphenation of SFC to mass spectrometry (MS) is routinely used although the best way to hyphenate SFC to MS takes different forms and is still a matter of debate.

On another hand two-dimensional chromatographic systems including supercritical fluids are attracting interest but are not yet ready for routine use. In particular 2D-SFC systems with an SFC separation in both dimensions would be of great interest as there would be no problems with fluid compatibility between the first and second dimensions. As SFC can be carried out in numerous modes (e.g. chiral or achiral normal-phase reversed-phase ion-exchange etc.) complementary separations can be obtained simply by using different stationary phases in the two dimensions. Therefore there would be a great advantage in developing 2D-SFC hyphenated systems.

Whether for combining SFE and SFC or for 2D-SFC systems the compressible supercritical fluid is causing specific difficulties in hyphenating the different parts of the instruments.

In this presentation the difficulties that should be overcome when hyphenating SFE SFC and MS steps will be discussed while the interest in developing such instruments will be exemplified with sample applications.

Keynote

Quantitative non-target screening of environmental samples - endless possibilities but with caution

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More than 100000 industrial chemicals are used in Europe today and over 1000 new compounds are added every year. The rate that relevant authorities assess new chemicals is much slower than the rate of introduction thus only a limited number of chemicals have satisfactory assessment of environmental fate and toxicity. Currently more than a third of the chemicals registered in Europe are multi-constituent substances and substances of unknown or variable composition complex reaction products or biological materials. Thus targeted screening methods do not reveal the true chemical composition of many of these substances.

The combination of speed of introduction of new chemicals and chemical complexity of a large proportion of substances leaves the majority of new chemicals undetected in the environment and untested for toxic potential. New studies show that only a fraction of the toxicity of a given environmental sample be it surface water or drinking water can be explained by toxicity of the standard monitored chemicals. Thus there is an unmet need to develop tools that can identify the chemicals causing the remaining 95-99% of toxicity [1].

In addition to target screening where analytical standards are available and the concentration can be determined by the analysis of calibration standards other less targeted methods have been developed in recent years [2]. These methods are covered by the term non-target screening where all ionizable chemicals in a specific molecular mass range are recorded prioritized and finally identified [3]. More and more public and industry sectors are demanding non-target screening to provide a more comprehensive assessment of the chemical composition of their samples. However non-target screening analysis is hardly standardized across different laboratories and seldom used in routine analysis [4].

Within this presentation I will present workflows for quantitative non-target screening analysis using a combination of complementary chromatography and mass spectrometry methods such as reversed phase liquid chromatography supercritical fluid chromatography and comprehensive two-dimensional liquid and gas chromatography with high resolution mass spectrometry detection. The presentation will discuss what to consider to perform reliable target- suspect- and non-target screening analysis of contaminants of emerging concern (CECs) in waste- surface- and drinking water.

The presentation will have a focus on signal processing and data analysis (how to go from raw data to the identity and quantity of a chemical substance) and on the visualization of results. Finally I will show how chemical data from target- and non-target screening can be correlated to toxicological fingerprints to identify potential sources events and pinpoint potential hazardous chemicals which are the driver for effects in the wastewater effluent samples.

Keynote

Detailed investigation of cannabis phenolics using 1- and 2-dimensional liquid chromatography hyphenated to ion mobility spectrometry and high resolution MS

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Stellenbosch University South Africa

The cannabis industry has experienced significant global growth in recent years making this an important commercial product. While the phytochemistry of Cannabis has been extensively studied particularly in terms of the cannabinoid and terpenoid composition relatively limited emphasis has been placed on the phenolic composition of Cannabis. Phenolics may play a role in the so-called 'entourage effect' and Cannabis may potentially be a source of valuable flavonoids in particular. However less than 50 flavonoids have been reported in Cannabis to date and little information is available regarding their variation between strains and as a function of plant development.

This contribution reports results for a detailed investigation of Cannabis phenolic constituents. 13 strains of Cannabis were grown in-house from commercial seeds and leaf samples were collected at three points during plant growth while bud samples were collected after flowering. Phenolic extracts of the collected plant material were analysed by 1-dimensional reversed phase LC (RP-LC) hyphenated to cyclic ion mobility spectrometry (cIMS)-high resolution MS (HR-MS) as well as comprehensive two-dimensional hydrophilic interaction chromatography (HILIC) × RP-LC-HR-MS. Annotation of phenolic constituents based on UV spectra low- and high collision energy HR-MS data as well as IMS collisional cross sections (CCSs where available) will be discussed in detail. The presentation will highlight new information on the phenolic composition of Cannabis including a large number of flavonoids reported in Cannabis for the first time and how the phenolic composition varies as a function of strain growth stage and plant material (leaf vs. bud). The benefits of cIMS-HR-MS detection and HILIC×RP-LC separation for the detailed characterisation of plant phenolics will also be discussed.

Keynote

Sustainability in separation science – focussing on the 'How?'

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The concept of sustainability has come sharply into view as the anticipated effects of climate change on the planet become a reality.

Albeit a small part of the issue decreasing the impact of chemistry on the environment is something analytical scientists can proactively do to help address this. As separation science is the most prevalent of all analytical techniques (producing the greatest carbon dioxide (instrument energy use as well as organic solvent synthesis and disposal) highest water usage and largest waste footprint) it should be investigated as a priority.

There are many approaches that may be implemented to address the environmental impact of separation science. The first step is to assess how polluting a chromatographic method (typically) may be. The premiss of 'reduce replace remove and recycle' (the '4R's') should then be considered to reduce carbon dioxide and waste footprint. In this framework reducing the volume of a chromatographic separation the use of more benign organic solvents and mobile phase additives switching to less waste intensive alternative analytical approaches and recovery of solvents can all be considered. A greater focus on sample preparation appropriate detector selection and the use of digital approaches within separation science are also very important to reduce the number of experiments undertaken when developing or modifying methods. More holistically the concept of 'right-first-time every time' is important in reducing waste - and efforts should be taken within organisations to provide the training and infrastructure to enable this.

This presentation will touch on many of these points and offer ideas and practical approaches to 'green' separation science.

Keynote

Novel approaches based on electro-driven separation for the control of emerging risks in food and environmental samples

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Emerging risk is defined by the European Food Safety Authority (EFSA) as a risk to human animal and/or plant health resulting from a newly identified hazard to which a significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard. Its successful identification in food and waters is one of the main objectives to protect public health. Therefore advanced analytical tools are required that provide sensitive selective and accurate results.

Capillary electrophoresis coupled with mass spectrometry (CE-MS) can be considered an alternative to chromatography applied in food analysis because of its high separation efficiency short analysis time low sample and reagent consumption reduced cost of capillaries and sustainability. In the last decade another electro-driven technique ion mobility spectrometry (IMS) has reemerged due to its coupling with high resolution mass spectrometry (HRMS). Several advantages are attributed to IMS when integrated in traditional LC-MS workflows in food safety including improved method selectivity by providing an additional separation dimension that allows the separation of isobaric and isomeric compounds; increased method sensitivity by isolating the compounds of interest from background noise; and it provides complementary information to mass spectra and retention time using the collision cross-section (CCS) parameter.

In this sense we have proposed the study of some emerging risks in different food and environmental samples using CE-MS or LC-IMS-HRMS. The main difficulties are the low concentrations of the analytes the complexity of the samples and the need to simultaneously determine compounds with different chemical properties. Different proposals have been developed for the determination of pesticides or emerging mycotoxins using micellar electrokinetic chromatography (MEKC) with a volatile surfactant compatible with MS or non-aqueous CE (NACE)-HRMS. Also CE-MS has shown to be an excellent choice to determine highly polar compounds. This is the case of multiclass determination of cyanotoxins which are not reverse-phase LC amenable due to the large differences in the polarity of the analytes. Finally for the first time LC-IMS-HRMS has been evaluated to determine ergot alkaloids (EAs) and their epimers or cyanotoxins showing the advantages provided by the integration of travelling wave ion mobility spectrometry (TWIMS) into a LC-MS workflow in terms of greater separation resolution and concentration sensitivity. In all these methods efficient sample treatments have been optimized and validation has been carried out according to EU regulations demonstrating satisfactory sensitivity and accuracy for the analysis of real samples.

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Keynote

Peak forecasting: the power of predictive models in analytics

Lennart Martens*

Universiteit Gent Belgium

TBC

Keynote

Comprehensive Analysis of Organic Micropollutants in Industrial Wastewater: a Tool Towards Net Zero Emissions

Torsten C. Schmidt* Reyhaneh Armin Max Reuschenbach Felix Drees Lucie K. Tintrop Gerrit Renner Amir Salemi Maryam Vosough

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There is a great deal of environmental concern associated with industrial wastewater since it is a source of multiple organic micropollutants like pharmaceuticals pesticides and industrial chemicals that can negatively impact water quality and public health. Qualitative and quantitative analysis of these micropollutants is crucial for developing effective strategies and management protocols to achieve net zero emissions. Central to this exploration is the role of non-target screening (NTS) as a pivotal approach to the comprehensive screening of micropollutants. With undefined chemicals being detected NTS allows one to comprehensively study chemical contaminants in different aquatic samples regardless of their fate transport and occurrence [1].

NTS has been facilitated by advanced hyphenated chromatographic systems such as liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) and novel data acquisition and handling techniques. The latter i.e. data evaluation is essential as the measurement data of such non-targeted analyses is often extensive multi-dimensional and complex. This presentation gives an overview of our latest studies for high throughput monitoring of influent and effluent industrial wastewater samples using different NTS workflows evaluating pollutant trends and detecting anomalous or unusual events (retrospective analysis) aligning with net zero emission goals. To this end by emphasizing the critical importance of using diverse feature detection approaches through HRMS data mining (mainly open-source software) the presentation compares these tools and explains the limitations and challenges inherent in NTS workflows. Further we will explore how multivariate chemometric/machine learning tools can be used in various research scenarios including wastewater group comparison monitoring time series data and prioritizing chemical features to reveal unknown micropollutants among others and their associated profile shapes [2-3].

By combining state-of-the-art analytical techniques with comprehensive data analysis strategies this presentation aims to highlight the indispensable role of NTS in managing organic micropollutants in industrial wastewater systems. Ultimately this knowledge will help formulate effective strategies to mitigate organic micropollutant release and move towards close to zero emission of micropollutants from industrial wastewater.

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Keynote

Experiments with Online Pyrolysis GC-HR/AM Orbitrap MS to assist in the identification of Biological Warfare Agents

Cedric Wissel (2) Eileen van 't Zelfde (2) Joeri Vercammen* (1)

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Over the past decade CBRN threats that originate deliberately have persisted or even increased. These include repeated chemical attacks both by state and non-state actors during the Syrian conflict (since 2012) the alleged North Korean chemical weapons program and the execution of the North Korean leader's half-brother with VX nerve agent in 2017 as well as the Salisbury Novichok poisonings committed by individuals associated with a State security service and the breakdown of two ricin terror plots in Germany and France in 2018. This sudden and extreme attention to CBRN threats raised even greater concern in the European Union that malicious actors may be resuming their interest in using CBRN agents in future plots given the huge impact on society. Gas chromatography combined with mass spectrometry (GC-MS) or ion mobility spectrometry (IMS) are well established analytical technologies in the arms forces for detection and identification of volatile organic compounds and microbial biomarkers both in laboratories and in the field.

In the framework of EDF project TeChBioT (ProjectID: 101103176 funded by the European Union) the feasibility of direct pyrolysis of bacterial moieties in combination with HR/AM Orbitrap MS has been assessed. Most appropriate experimental conditions have been identified to process minute sample amounts accurately and reproducibly. In-line derivatization is used increase the abundancy of GC amenable components and allow identification of particular biomarkers in each pyrogram. Chemical structures of unknown unknowns are proposed by fully exploiting the high resolution and accurate mass capabilities of the Orbitrap detector.

In a final phase of the project which runs until Dec 2025 the methodology as well as the obtained results will be applied to support the development of a miniature GC-IMS prototype for application by armed forces and first responders thereof.

Please note that views and opinions are those of the author(s) only and do not necessarily reflect those of the European Union or the European Defence Fund. Neither the European Union nor the granting authority can be held responsible for them.

Oral

Use of Selected-Ion Flow Tube Mass Spectrometry (SIFT-MS) to monitor industrially relevant Volatile Organic Compounds in air

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Monitoring of Volatile Organic Compounds (VOCs) in air is critical as specific VOCs present potential hazards discomfort and/or odor nuisance. Due to the increased awareness and concern for indoor and outdoor air quality specifications for VOC concentrations in air are becoming more and more stringent. This creates analytical challenges to obtain reliable data in a sensitive and fast manner.

In this respect Selected-Ion Flow Tube Mass Spectrometry (SIFT-MS) was evaluated to complement existing gas chromatography (GC) techniques where a combination of speed of analysis and sensitivity is required. SIFT-MS is a direct-injection mass spectrometric technique based on soft chemical ionization and enables the real-time quantification of target volatile compounds in air at trace levels.

SIFT-MS was evaluated for the analysis of a series of industrially relevant target compounds with different hazard classifications and thus varying specification limits. These include aliphatic hydrocarbons aromatic compounds aldehydes oxides etc. Linearity repeatability sensitivity and accuracy were determined. Advantages and challenges related with the use of SIFT-MS for these applications will be discussed and compared with traditional GC-based analysis.

Oral

Analysis of albumin adducts biomarkers of exposure to vesicant agents by LC-MS/MS

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Vesicants are strong alkylating agents used as chemical warfare agents (CWA) (e.g. sulfur mustard)1. The synthesis storage and use of CWA have been forbidden since 1997 with the entry into force of the Chemical Weapons Convention (CWC). However the threat of the use of such molecules remains then robust and sensitive analytical tools have to be developed to monitor any use in order to rapidly diagnose those exposed and prove it unambiguously.

Vesicants show similar reactivity quickly reacting in vivo forming by nucleophile substitution reactions with episulfonium ions intermediate for sulfur based vesicants and with aziridine ions intermediate for nitrogen-based ones. These ions can then bind to macromolecules such as albumin on nucleophile sites like Cysteine34 ’s thiol function (not involved in a disulfide bond). This adduction does not alter the functions of the protein hence it does not cause any decrease in the lifetime of the protein. The monitoring of protein adducts can allow a retrospective exposure to vesicants up to 20-25 days for human albumin2.

These adductions are best monitored by tracking adducted small peptides rather than intact adducted protein as intact protein analysis is not trivial3. Here tripeptides and dipeptides including the adducted Cys34 issued of a proteolytic digest (respectively proteinase K and pronase) were studied.

Analyses using two separations RPLC-MS/MS and HILIC-MS/MS were developed to retain di- and tri-peptide adducts of sulfur mustard (SM) sesquimustard (Q) and nitrogen mustards (HN-1/2/3) based on different chemo-physical parameters. The developed analytical method exhibited higher sensitivity for adducted tripeptides compared to their dipeptide homologues. The limits of quantification (LOQs) for adducted tripeptides ranged from 4.5 to 308.8 ng/mL in the RPLC-MS/MS method and from 0.4 to 26.2 ng/mL in the HILIC-MS/MS method.

Monitoring albumin adducts requires several time-consuming sample preparation steps. For this reason Immobilized Enzyme Reactor (IMER) could offer valuable advantages as digestion time can be greatly reduced4. Following the online digestion a refocusing of the analytes prior to the LC-MS/MS analysis is necessary to maintain good sensitivity. To that extent an online SPE will be implemented to the method. Method development is still in progress.

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2: Von Stedingk et al. Issues in Toxicol 2011 2 1-22

3: Camperi et al. J. Pharma Biomed Analysis 2020 178 112921

4: Hallez et al. J Chromatogr B 2021 1186:123031

Oral

The Analysis of Pesticide Products using Ultra-High-Performance Supercritical Fluid Chromatography-Mass Spectrometry

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This talk highlights several key examples of where SFC-MS has advantages over reversed-phase UHPLC in the analysis of pesticides and their formulations.

The versatility of RP-UHPLC and GC allows them to cover most of the chemical space causing us to sometimes default to these techniques even when another could outperform them. We can move to NP-UHPLC or HILIC for compounds outside the polarity range of RP-UHPLC but these suffer with challenging MS hyphenation and long equilibration times. SFC can provide fast methods coupled to API-MS for quantitation. The orthogonal selectivity of SFC can also allow resolution of analytes that co-elute using RP-UHPLC mechanisms. It is also becoming increasingly important where the two techniques are equivalent to move towards SFC for its sustainability particularly at preparative scale where it should be the technique of choice for its low solvent consumption and less energy intensive solvent evaporation post-separation.

Achiral UHPSFC-MS methods have been developed for pesticide formulation materials using a Waters UPC2 coupled to a Xevo TQD mass spectrometer with positive and negative ion ESI. Several key examples will be discussed including an achiral impurity separation and the analysis of pesticide components in complex formulation matrices. Method development has been performed to optimise the stationary phase co-solvent gradient column temperature and backpressure. Discussion on the performance of each method will be made in terms of resolution speed and cost compared to the current RP-UHPLC/HPLC methodology. The sustainability of the current methodology and the newly developed UHPSFC-MS methods will be compared utilising the Analytical GREEnness Metric Approach and Software (AGREE) tool for a quantitative assessment.

Oral

Quantifying Phospholipids in Organic Samples Using a Hydrophilic Interaction Liquid Chromatography - Inductively Coupled Plasma High Resolution Mass Spectrometry (HILIC-ICP-HRMS) Method

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In this study a novel method using hydrophilic interaction liquid chromatography (HILIC) coupled with inductively coupled plasma high resolution mass spectrometry (ICP-HRMS) is introduced for the quantification of phospholipids in oil samples. The method employs a bridged ethyl hybrid (BEH) stationary phase HILIC column with a tetrahydrofuran (THF)/water mobile phase enhancing the solubility and detection of phospholipids. During the study a gradient/matrix effect on sensitivity was observed and successfully compensated for experimentally ensuring reliable quantification results. This approach proves effective in various oil samples including vegetable oils animal fats and phospholipid supplements. Notably this method quantifies phospholipids directly in oil samples bypassing the need for prior sample processing such as solid phase extraction (SPE) thereby streamlining the analytical process. The precision accuracy and reduced need for extensive sample preparation offered by this method mark a significant advancement in lipid analysis. Its robustness and broad applicability have substantial implications for industries such as food and renewable energy production where efficient and accurate lipid analysis is crucial.

Oral

Advancements in bioanalytical extraction: a journey through 3D-printed sorbents for custom targeted and untargeted analysis

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In the field of sample extraction for bioanalysis 3D printing introduces exciting possibilities [1]. When 3D-printed objects possess sorption properties it significantly transforms the landscape of designing extraction tools. This implies the rapid creation of custom tools and the direct fabrication of advanced sorbents. Our research demonstrates how these 3D-printed sorbents with their special ability to extract analytes can find applications in pharmaceutical and bioanalytical studies. This presentation introduces the notion that 3D printing can streamline sample preparation preceding LC- or GC-MS analysis.

In general our research delves into the possibilities offered by Fused Deposition Modeling (FDM) and Digital Light Processing (DLP) 3D printing techniques utilizing both commercially available and self-designed materials. We investigate how the geometry of sorbents can influence their performance and explore potential applications. In this presentation we'll highlight the design and application of our custom-made composite incorporating polypropylene ABS and C18-modified silica particles.

In 2023 we introduced a novel composite material containing silica particles embedded in the porous polymer matrix intended to use as filament for FDM printers. This material underwent systematic evaluation for its extraction efficiency presenting a promising avenue for advancing the capabilities of bioanalytical procedures [2].

In a subsequent phase of our research we explored the application of sorbents for a comprehensive panel of 15 benzodiazepines with varied structures. This expanded investigation aimed to showcase the versatility of 3D-printed sorbents across a broader range of analytes. Additionally we extended our reach to untargeted lipidomic analysis applying 3D-printed sorbents to human milk human plasma and bacterial cells. This novel application highlights the adaptability of 3D-printed sorbents beyond traditional analytes demonstrating their efficacy in untargeted lipidomic studies.

These findings collectively contribute to the evolving landscape of bioanalysis emphasizing the pivotal role of 3D printing in designing tailored solutions for diverse analytical challenges.

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Oral

Ion-mobility high-resolution mass spectrometry for the annotation of novel emerging contaminants and their metabolites in indoor dust and human urine samples.

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Ion mobility high-resolution mass spectrometry (IM-HRMS) can provide an additional separation dimension in the suspect and non-target screening analysis (SSA/NTS) of environmental samples. Thereby IM-MS derived collision cross section (CCS) values serve as an additional identification parameter in the correct annotation of contaminants of emerging concern (CECs) and their metabolites1. Indoor dust has been characterized as a relevant matrix for human exposure to CECs especially for toddlers due to crawling behavior and frequent hand-to-mouth contact.

The presented work focused on IM-HRMS based suspect screening analysis of CECs in indoor dust and human urine samples. Indoor dust samples (n=46) were collected at 40 different locations in Flanders Belgium. After extraction and analysis by liquid chromatography IM-HRMS the obtained data were matched with a suspect list containing > 4000 compounds covering various classes of expected indoor contaminants. IM derived CCS values obtained for the assigned suspects were matched against previously established reference values1 and m/z-CCS trendlines to increase identification confidence. More than 60 contaminants were identified with confidence levels (CLs) ranging between 1 and 3 according to the system of Schymanski et al.2 whereby experimental CCS values added identification confidence especially for compounds assigned with CL33. Known contaminants were identified at CL1 by matching with reference standards comparing retention time exact mass fragmentation spectrum and CCS values whereby the reproducibility of the latter was independent of the analyzed matrix.

Several novel contaminants were identified such as decyl nonyl phthalate and decyl undecyl phthalate which showed DFs > 80%. Their partially uneven numbered and different substituents were confirmed by characteristic fragments and their clear match with the m/z-CCS trendline previously established for known phthalate homologues1. These findings highlight the added value of the additional separation dimension provided by IM in the identification of CECs and provide a valuable tool for the application of this analytical approach in other environmental matrices.

Another advantage of IM-HRMS was shown within its application for the analysis of human urine samples collected from workers highly exposed to electronical waste and as a result thereof to numerous known and novel plasticizers. Implementation of IM-HRMS allowed to identify and partially annotate isomeric structures of plasticizer metabolites which remain undetected or unresolved with common LC-HRMS methods. This application shows also the added value of IM-HRMS for isomer separation revealing novel parts of the structural variability of metabolites present in human samples.

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Oral

Optimization of the LC-MS analysis of intact glycoproteins

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Glycosylation is one of the common protein modifications that regulates their biological properties. For certain diseases its characterization enables diagnosis and prognosis. The conventional approach for the glycosylation characterization is based on the analysis of the glycopeptides obtained after the enzymatic digestion of the glycoforms of a given glycoprotein. It is also possible to analyze the intact glycoforms using liquid chromatography coupled with mass spectrometry1. This approach preserves crucial information namely the link between the structure and the activity or the number of glycoforms.

For several years our focus has been on human chorionic gonadotropin (hCG) better known as the pregnancy hormone. hCG consists of two non-covalently linked subunits hCGα and hCGβ. It has eight glycosylation sites resulting in numerous glycoforms some of which could serve as biomarkers of pregnancy-related pathologies. We demonstrated that the analysis of hCG glycoforms is possible keeping the protein intact using nano liquid chromatography (nanoLC) coupled with high-resolution mass spectrometry (HRMS) featuring an Orbitrap analyzer2. NanoLC enhanced sensitivity and its coupling with HRMS led to the detection of more than seventy different glycoforms of hCGα and hCGβ in concentrated hCG samples (fertility drugs containing recombinant hCG or purified from women's urine). The impact of adding two different acidic additives namely formic acid (FA) and trifluoroacetic acid (TFA) into the LC mobile phase was investigated. It was found that only TFA enabled the separation of hCGα and hCGβ glycoforms in both reversed-phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC). However even at low concentrations TFA induced a strong signal suppression and led to the formation of numerous adducts which considerably complicates the interpretation of MS data3.

Hence it became necessary to study the effect of other additives on the separation (resolution efficiency) and detection (intensity adduct formation) of the hCG glycoforms in nanoLC-HRMS in both RPLC and HILIC modes. With the aim of maximizing the glycoform ionization while maintaining the separation of the two subunits difluoroacetic acid (DFA) was used as a substitute for FA and TFA. To overcome the ion suppression effect the performance of adducts fragmentation in mass spectrometry was investigated. Two types of mode have been studied: source fragmentation of the mass spectrometer (isCID) and fragmentation within the collision cell (AIF mode). Furthermore the "On/Off" elution properties of proteins have been highlighted for monoclonal antibodies (mAb)4. Through a multiple isocratic step gradient an improvement in separation was observed. Therefore we wanted to assess the performance of such a gradient on the studied glycoprotein. Thus an optimization of the elution gradient was carried out by introducing multiple isocratic steps enhancing the chromatographic separation of the two subunits of hCG.

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Oral

Automated tuning of denoising algorithms for noise removal in chromatograms

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Different algorithms such as the Savitzky-Golay filter and Whittaker smoother have been proposed to enhance the quality of experimental chromatograms. These signal processing approaches prevent excessive noise from interfering with down-stream data analysis thereby facilitating precise analyte detection and quantification. However effective implementation of these algorithms requires meticulous fine-tuning of their hyperparameters to regulate their smoothness and flexibility. Traditionally this fine-tuning is done manually until an optimal signal is obtained that removes the noise efficiently while preserving crucial peak information. Despite the availability of more objective and automated approaches these so-called parameter tuning methods are usually method specific and/or require previous knowledge which in most cases is not readily available.

This work introduces alternative parameter tuning methods namely the L-and V-curve k-fold cross-validation autocorrelation function and residual variance estimation approach [1-4] that are easily automatable and universally applicable eliminating the need for any previous information and the compatibility constraints when applied with different denoising techniques. Additionally a novel robust implementation based on median estimators is introduced specifically tailored to handle the distinctive shape of chromatograms typically composed of alternating flat baselines and sharp peaks. The tuning methods are investigated in combination with four established denoising methods; the Savitsky-Golay filter Whittaker smoother sparsity assisted signal smoother and baseline estimation and denoising using sparsity approach [5-8].

The results demonstrate that the use of median estimators significantly improves the denoising and information conservation performance of all smoother-tuner combinations for both simulated datasets and experimental chromatograms. Moreover the parameter tuning methods based on residual variance estimation k-fold cross-validation and autocorrelation function consistently yield small root-mean-squared errors across diverse simulated datasets and experimental chromatograms surpassing the performance of the L- and V-curve approach. Furthermore the two denoising methods relying on the use of sparsity namely the sparsity assisted signal smoother and the baseline estimation and denoising using sparsity approach systematically outperform the other methods in this study and are hence most appropriate for chromatograms. In conclusion the introduced tuning methods can be applied to several different smoothing methods and allow an automated and robust determination of optimal hyperparameters for any chromatogram resulting in optimally denoised signals.

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Oral

Analytical method lifecycle of SFC methods from development to QC and commercialization in pharmaceutical development.

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Oral

An oligonucleotide analysis method target on high chromatographic separation using bio-inert column followed by with and without ion pair agents on MS detection

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Oligonucleotides (ONs) are single or double-stranded polymers of nucleotides that can be modified in various ways to enhance biotherapeutic efficacy in the treatment of genetic diseases. Ion-paring reversed-phase liquid chromatography coupled to mass spectrometry (IP-RPLC-MS) has emerged as the gold standard for ON quality control. The role of IP agents added to the mobile phase is to enhance ON retention but generally MS detection sensitivity is compromised and MS fouling leads to low method robustness. The goal of this study was two-fold:
i) We systematically investigated instrument configuration and operating conditions including organic modifier effects of IP agents (TEA DIPEA and HA) and counter ions (HFIP HFMIP and NFTBA) on resulting chromatographic resolving power and MS sensitivity applying emerging bioinert column technologies. Stationary-phase considerations included particle type (core-shell vs. fully porous particles) particle diameter and pore size. The incorporation of an active column preheater mitigated thermal mismatches yielded narrower peaks overcome peak splitting and led to a reduction of the column pressure. Use of HFIP led to high detection sensitivity albeit the chromatographic resolution was slightly enhanced when applying HFMIP. Sub-min analysis for 15-35 polyT ladders was achieved operating a 50 mm long core-shell column at the kinetic performance limits. Operating a coupled-column system at the kinetic-performance limits allowed for the separation of a full-length product (FLP; 21 mer) from its n-1 (20-mer) and n-2 (19-mer) impurities and from phosphorylated oligomer (PO) impurities.
ii) In the next step a membrane-based microfluidic stripper was developed aimed at selective post-column removal of the IP agent (TEA) prior to MS detection. Suppression of TEA is induced by selective ion transport across a Nafion anion-exchange membrane where TEA is exchanged for positively-charged ions. TEA removal was determined by off-line GC-FID

analysis. Design aspects of the multiple parallel channel membrane-based microfluidic stripper and performance evaluation will be discussed. Different regenerant composition including organic content exchanged ion concentration and thickness of membrane were optimized. The applicability of the miniaturized suppressor is demonstrated by ON intensity on MS direct infusion experiment.

Oral

GC-HRMS Orbitrap for non-targeted screening of environmental matrices

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Environment quality requires periodic updates of the environmental contamination regarding the presence of conventional and emergent pollutants. Therefore the evaluation of environmental exposure is an important step in establishing a list of pollutants in order to propose appropriate protection measures. Most of the known priority pollutants and emerging contaminants are semi-volatile compounds and the non-targeted analysis using thermodesorption gas chromatography coupled to high resolution mass spectrometry (TD- GC-HRMS) with Orbitrap can reveal novel classes of organic contaminants increasing the number of identified compounds with a high reliability. In this study in order to expand the range of the detected contaminants and improve the quality of non-targeted screening using a TD-GC-HRMS Orbitrap stir bar sorptive extraction (SBSE) method using homemade stir bars with ionic liquids (ILs) as sorptive phases was developed. The homemade SBSE coupled to TD-GC-HRMS Orbitrap method was applied on naturel waters collected from the Robec river in France. The comparison between the home-made stir bar and a commercial stir bar with PDMS as sorptive phase showed a significant increase of the number of the detected compounds furthermore a real increase of peak areas was noticed. Overall more than 1072 compounds were detected and then classified according to the identification scale proposed by Miller [1] using different attributed scores (RSI RHRMF and ΔRI). Moreover the identification scale was also applied to the identification of compounds emitted after an accidental industrial fire and a fire simulation in a laboratory combustion chamber.

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Oral

Approaches to unravelling the complexity of dense product ion spectra and relating measurement to structure

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Ticagrelor is an active pharmaceutical ingredient which when studied by tandem mass spectrometry produces a highly dense product ion spectrum with multiple product ions at the same nominal mass. To fully understand the structure of these product ions it is important to know which parts of the molecule they have come from. High resolution tandem mass spectrometry combined with ion mobility separation has been used to provide gas-phase isolation of the isobaric product ions and enable a further stage of MS/MS.

Ticagrelor at 1 mg/ml in methanol:water 1:1 plus 0.1% formic acid was directly infused at a rate of between 2-10 ml/min. Electrospray ionisation was used to produce positive ions with all operational parameters tuned to deliver the optimal peak intensity for [M+H]+ at m/z 523.

Quadrupole collision-induced dissociation using a 9.4T SolariX FT ICR mass spectrometer (Bruker GmBH) tuned to show the hyperfine structure at each of the nominal mass windows of interest (> 300000 FWHH) enabled molecular formulae to be proposed for each of the multiple product ions observed within 0.03 Da windows at m/z 355 363 and 373 respectively. Single frequency ejection FT ICR infrared multiphoton dissociation was able to further isolate some of the individual product ions within each 0.03 Da window in turn and deliver MS/MS. These results support the proposal that the some of the product ions have largely similar structures and therefore come from the same part of the precursor ion.

Using ion mobility separation as an alternative means to separate these gas-phase product ions a Cyclic IMS (Waters Corp.) and a timsTOF (Bruker GmBH) both revealed only partial mobility separation. Data from both systems is highly comparable. Taking m/z 363 as a case example the mobility separation of the 3 product ions at this nominal mass indicates an amalgam of shapes with one product ion having one major shape and another product ion having 3 or 4 shapes. This amalgam of shapes all resides within a 8 Å2 window. Whilst the mobility resolution is not yet optimal it was sufficient to provide reasonably clean MS/MS data that show visible differences which have been assigned to support proposed structures.

This work highlights the challenges which will need to be overcome and understood before CCS and ion mobility data should be used as a robust metric for unknown small molecule analysis.

Oral

Development of set-ups for real-time analysis of the effluent of a microreactor by mass spectrometry

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In organic chemistry the synthesis of a product often involves the synthesis of reaction intermediates before the synthesis of the final product. The detection of these intermediates is essential for elucidating and understanding the mechanism of a synthesis reaction.[1] Usually such intermediates are analyzed by spectroscopic methods but could face major limitations especially when analyzing complex mixtures. In addition spectroscopic methods could require the use of chromophore moiety fluorophores radiotracers or nuclei with proper spin characteristics. The use of mass spectrometry (MS) could be an alternative tool since it can reach the accuracy and the sensitivity required for an efficient analysis of species produced during the synthesis not only because it's able to give the mass of molecules but also because structural characterization analysis (MS/MS) could be performed.

The hyphenation of a chip-based microreactor with MS has already allowed to monitor synthesis processes.[23] However this coupling concerns most of the time reactions performed in solvents compatible with MS restricting thus the monitoring to specific compounds or synthesis reactions. To overcome this major limitation we have developed an universal set-up hyphenating a microreactor to a mass spectrometer and based on two switching valves.[4] It allowed the analysis of several molecules of different natures and polarities solubilized in various solvents including DMSO and DMF known to be the worst solvents for MS analysis (ion suppression effect and high loss of sensitivity). A dilution step was included in the analytical set-up to allow MS monitoring of compounds synthesized at different concentrations to avoid the MS signal saturation and to limit the ionization source dirtying. Our two-valve set-up evidenced good repeatability and a linear response for the detection of the studied compounds. To totally validate our set-up an amination reaction synthesized in real time into a micro-reactor hyphenated to our two-valve set-up was successfully monitored allowing the detection of the reaction products in 4 min and the identification of new byproducts.

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Oral

Aroma profiling as a tool to support innovation in the food industry

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Nowadays there is an increasing demand for animal source proteins which leads to an enhanced pressure on the global climate health food security and biodiversity. This triggered research into development of more sustainable ways of producing foods and food ingredients. As a result alternative ways of delivering proteins to consumers were developed including plant-based products cultivated meat and proteins produced via microbial fermentation (such as biomass and precision fermentation). While the availability of these alternative proteins has greatly expanded over the past years there is still significant room for innovation especially on acceptance by the consumers. Extensive research is ongoing on understanding how to make the alternative foods taste and smell more like the conventional foods. This is a real challenge and aroma analysis is of paramount importance for this industry. Accurate aroma profiling can help manufacturers to ensure quality through proper selection of the raw materials and to provide product consistency. On the other hand it can support the development of new products and the understanding of the influence of the different ingredients on the aroma of the final product. Also detection of off-flavor compounds which occasionally can be present at ultra-trace levels need to be carefully monitored. Additionally to this investigation of the influence of processing and the shelf-life on the final product is mandatory.

A specific product odor can consist of a few up to hundreds of different aroma molecules of which key aroma compounds are often present at very low levels. During food processing and storage many chemical reactions involving sugars amino acids and lipids are occurring e.g. Maillard reactions oxidations etc. These chemical reactions lead to the formation of additional volatile aroma compounds and potentially to the formation of important off-flavors. In general the analysis of aroma compounds in foods is challenging especially because of their presence at a broad concentration range (ppt to ppm) broad polarity range volatility (high vapor pressures) and instability of some important classes of aroma compounds. Therefore advanced analytical techniques are required to address these challenges by providing improved extraction separation identification and comparison of aroma profiles of different food batches.

In this presentation an overview of different untargeted aroma profiling approaches will be presented. These are based on the implementation of green robust efficient cost-effective and sensitive analysis methodologies. The right combination of automated sample preparation techniques such as solid phase microextraction (SPME) dynamic headspace sampling (DHS) in its different modes and stir bar sorptive extraction (SBSE) with separation either via the classical one-dimensional gas chromatography or for complex samples for enhanced separation via comprehensive two-dimensional gas chromatography (GC×GC) is critical. Detection using Single Quadrupole mass detectors is often used but Time-of-Flight (TOF) mass detectors allow to further extend the range of aroma compounds detected in a sample. This is due to the high sensitivity of the TOF detectors in full scan mode which is ideal for untargeted screening spectral continuity and increased acquisition speed allowing deconvolution of even chromatographically coeluting aroma

compounds. The information obtained by combining such techniques can allow the alternative food industry to routinely optimize selection of ingredients processes and preservation (optimal conditions packaging etc.) and help tackling any possible occurring off-odor issues. The potential and performance of the applied aroma profiling approaches are illustrated with different conventional and alternative food samples.

Oral

Supercritical fluid chromatography hyphenated to ultra-high resolution mass spectrometry and comprehensive two-dimensional chromatography coupled with qTOF (RPLC x SFC-qTOF) for the characterization of advanced bio-oils

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Microalgae bio-oils are a promising source of energy that could complement fossil fuels in the future. However their high content of heteroatoms makes their use as biofuel impossible without upgrading treatments. To optimize these treatments a detailed knowledge of the samples is required. The characterization of such complex mixtures is challenging due to the high number of compounds covering a wide range of polarity and molecular weight. Their characterization is often performed by direct introduction into a Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) allowing the assignment of one molecular formula to each m/z ratio. However two major issues arise with a direct introduction: (1) the impossibility to differentiate the numerous isomers present in bio-oil samples and (2) the possible matrix effects that prevent the detection of low concentration compounds.

To overcome such issues chromatography is often hyphenated to a mass spectrometer. In particular it has been shown that supercritical fluid chromatography (SFC) coupled to time-of-flight mass (TOF) spectrometry is of particular interest for bio-oils characterization [1]. However the relatively low resolving power of TOF yields to isobaric interferences especially in microalgae based bio-oils.

In this work we developed a SFC-FTICR method by optimizing the stationary phase the organic modifier and the make-up solvent with quality descriptors including the retention space coverage and the peak shapes and intensities. The best results were obtained with an unbounded silica stationary phase and a mixture of methanol and water with formic acid as a make-up. Quadrupolar detection was used in on-line SFC-FTICR to maximize the acquisition rate and the coupling allowed to combine the large separation power in SFC with the very high resolution of FTICR. Thousands of compounds were identified by their molecular formulas many of which being found throughout the chromatogram showing the isomeric complexity of microalgae bio-oils. The second technique used is comprehensive two-dimensional chromatography coupled with qTOF (RPLC x SFC-qTOF). The SFC method has been adapted to enable ultra-fast second-dimensional analyses. The orthogonality of the RPLC and SFC techniques is highlighted by the very wide occupation of the separation space. The isomeric profiles of families of compounds were obtained in RPLC x SFC-qTOF from the identifications obtained in SFC-FTICR. Many isomers not separated by SFC alone were separated by RPLC and vice versa demonstrating the complementary nature of the two chromatographic techniques [2].

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Oral

Development of a Standard Reference Mixture for GCxGC Method Performance Evaluation

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Oral

Identification and quantification of unsaturated components and aromatics in plastic pyrolysis oils

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Sustainability and circularity are relevant and exciting topics. In the Petrochemical Industry one area of focus has been placed on closing the loop and creating a plastics' circular economy. A possible route includes the conversion of waste plastics into an oil through pyrolysis which has been gaining attention as a potential cracker feedstock. The evaluation of these plastic pyrolysis oils (PPOs) is of extreme importance to understand both the physical and chemical properties especially as it has been accepted that these materials require upgrading to improve the hydrocarbon composition and remove impurities.

In order to evaluate these materials new and advanced analytical techniques are required. The introduction of the vacuum ultraviolet detector coupled to gas chromatography (GC-VUV) has shown much promise for the identification and quantification of the hydrocarbon composition (paraffins iso-paraffins olefins naphthenes and aromatics – PIONA) and has been applied to both fossil-based feeds as well as PPOs. However to better understand the undesired components (e.g. olefins di-olefins heteroatom impurities) more detailed characterization is required. For example a deeper insight into olefins characterization would help a researcher determine the hydrogen depletion degree for potential upgrading options. Such information could shed light on two different directions: 1) molecules with lower hydrogen to carbon content could lead to oligomerization or 2) to a higher heat of reaction upon hydrogenation. Additionally better segregation of the naphthenes and cyclic olefins is also desired to determine more accurate estimations for hydrogenation reactions. In order to obtain such detailed characterization of the PPOs other separation and detection methods are required including comprehensive GC (GC×GC) and detectors such as mass spectrometry and even element specific detection.

Current focus has been placed on the identification and quantification of the unsaturated components (olefins and di-olefins) and aromatic hydrocarbons present in PPOs to assess the impact of these molecules on steam cracking and down-stream operations. For this purpose GC-MS and GC-VUV were utilized. A discussion will be given on the components identified and how they can be mitigated to avoid process upsets.

Oral

Accessing the higher order structures of biomolecules in solution from the gas phase ? The coupling of capillary electrophoresis and ion mobility mass spectrometry to the rescue

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The fine characterization of samples requires identification and quantification of the content the determination of the stoichiometry and connectivities (primary structures) and interaction domains (higher order structures) of (supra)molecular assembly. The elucidation of structure-activity relationships holds paramount significance in evaluating and comprehending the underlying processes involved in various applications such as therapeutic oligonucleotides (ONs) aptamers functional foods and bio-active compounds including pre and probiotics. Mass spectrometry plays a pivotal role in the comprehensive analysis of organic samples even at minute trace levels. The exploration of higher order structures relies on numerous analytical methods tailored for relatively pure and concentrated samples typically utilizing spectroscopy methods in solution. However the utilization of mass spectrometry (MS) and ion mobility mass spectrometry (IM-MS) both of which are vacuum-based methods is increasingly important primarily due to its fast screening capabilities for trace amounts in non-pure samples. It is highly tempting to extrapolate data obtained from mass spectrometry (MS) and ion mobility mass spectrometry (IM-MS) to deduce structural information in solution based on the assumptions of native mass spectrometry. However it is imperative to validate these assumptions for each investigated model to ensure their applicability and accuracy. Nonetheless there is still a way to circumvent these problems and create a shortcut. Here we propose the concomitant use of the different modes of Capillary Electrophoresis (CE) on-line coupled with IM-MS. CE is a separation method in solution presenting different operation modes which separates analytes according to the shapes averaged charge states and hydrodynamic radii (capillary zone electrophoresis CZE) the relative electrophoretic mobilities (μe) in solution (transient isotachophoresis t-ITP) or even on the affinity and binding constant of host-guest systems (kinetic capillary electrophoresis KCE). Different models e.g. peptides naturally occurring and chemically modified oligonucleotides and G-quadruplexes were investigated by CZE t-ITP and KCE coupled with ion mobility mass spectrometry energy-resolved collision induced dissociation (breakdown curve and V50) and collision induced unfolding (CIU). Advantages and drawbacks of CE-IM-MS coupling will be addressed from the analytical point of view. The adequacy of parameters extracted from CE experiments and the ion mobility constant (K) obtained from IM-MS will be also addressed from the physical chemistry point of view.

Additionally we also propose the used of matrix assisted laser desorption ionization and in-source decay (MALDI-ISD) to solve issues concerning the sequencing of (chemically modified) therapeutics ONs frequently observed using the classical collision induced dissociation (CID) fragmentation method. The use of thin layer chromatography (TLC) for the separation of these ONs in mixtures

coupled to the use of MALDI-ISD and mass spectrometry imaging detection of these TLC plates is also proposed as innovative impurities profiling methods.

Oral

Multidimensional LCxSFC-MS for the separation of isomers and structurally similar compounds

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The separation of highly complex samples (100-1000 components) is commonly addressed with comprehensive two-dimensional liquid chromatography (LC×LC). One of the obstacles to the proliferation of LC×LC is the difficulty to select separation dimensions with orthogonal selectivity especially when analytes are neutral compounds. The polar interactions that SFC promotes offer the perfect complementarity to RPLC mechanism. The multidimensional LCxSFC drastically improves the separation of neutral compounds in complex mixture1. Thus far the potential of LCxSFC for the separation of structurally close compounds has not been fully investigated although especially in the case of isomers this combination could offer significant benefits to the full characterization of complex mixtures.

In this work we demonstrate using an off-line setup coupled to MS/MS detection that LCxSFC can achieve the separation of structurally similar compounds and isomers inducing a spatial distribution of chemical structures that helps in the identification process. Three case studies will be discussed to exemplify this specific ability namely biolubricant polymers phytosterols from algae and depolymerized lignin oligomers (over 400 detected molecules per sample). Advanced data analysis methods (MS-DIAL SIRIUS and Feature-Based Molecular Network) were integrated into the non-target workflow to rapidly visualise and study the detected compounds. The comparison of the technique to one-dimensional separation showed that the LCxSFC method is superior for the separation of isomers which is especially beneficial for lignin as 77% of the detected compounds had at least one isomer2.

In the second part we will present the latest instrumental developments on the online coupling of LC with SFC. Doing so has been challenging historically because of the mismatch between the relatively high mobile phase concentrations of water required for the RPLC separations and the CO2-rich environment of the SFC gradient. We will show how different valve configurations can overcome this challenge as well as injection in partial fill mode and split without CO2 depressurization. When coupled online with mass spectrometric detection the resulting online RPLCxSFC exhibit both high peak capacity and short analysis time opening new opportunities in the field of 2D separations.

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Oral

The emerging application of liquid chromatography-based metallomics techniques to the bioinorganic chemistry of toxic metal(loid)s

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In 2015 9 million premature deaths were attributed to the exposure to natural and increasingly anthropogenic pollutants released into the biosphere.1 Since this ongoing global contamination of air food and water also adversely affects reproductive health pollution has effectively become a planetary threat.2 Potentially toxic arsenic mercury and cadmium species represent an important pollutant class as they are recalcitrant and therefore tend to accumulate in the very ecosytems which are used for food production. While the accurate quantification of inorganic pollutants in the bloodstream of humans of different ages gender and populations represents an important public health initiative to identify worrisome environmental chemicals and elements this 'biomonitoring approach' is inherently incapable of establishing causal links between exposure to specific pollutants and the onset of adverse health effects and potentially also of diseases (e.g. type 2 diabetes autism). Since classic toxicology approaches are no longer sufficient to assess the effect of low doses of potentially toxic metal(loid) species on humans mechanism-based approaches that focus on their biochemical pathways emerge as an alternative approach to more accurately assess the risk of real exposure szenarios. In this context the bioinorganic chemistry of potentially toxic arsenic mercury and cadmium species that unfolds in the bloodstream has not received the attention that it deserves.3 This may at first appear surprising given that the multitude of biomolecules that these metal(loid) species can interact with in blood plasma and red blood cells will collectively determine which molecular metal(loid) species impinges on any given target organ to initiate damage therein. The main reason why we still know comparatively little about the bioinorganic chemistry of potentially toxic metal(loid) species that unfolds in the bloodstream and within organs is the underlying biological complexity as blood plasma and red blood cell cytosol contain thousands of proteins. To overcome this fundamental bottleneck however hyphenated techniques which involve the on-line coupling of liquid chromatographic techniques with a variety of element-specific detectors can provide much needed insight. By employing different separation mechanisms (size-exclusion chromatography reversed-phase chromatography anion-exchange chromatography) and using mobile phases that resemble biological fluids (e.g. phosphate buffered saline buffer) we have been able to obtain fundamentally new insight into a) the biomolecular mechanisms that unfold in blood plasma and deliver toxic metal species to their target organs b) the binding of toxic metal species to metalloproteins within in red blood cells and c) the potential mobilization of toxic metal species from the liver. Since it is important to employ atomic spectroscopic techniques that are compatible with the employed high salt containing mobile phases we have used inductively coupled plasma-atomic emission spectroscopy (ICP-AES) flame atomic absorption spectroscopy (FAAS) and graphite furnace atomic absorption spectroscopy (GFAAS) to detect metal(loid) species in the column effluent. Relevant metal(loid) species were then structurally characterized by X-ray absorption spectroscopy (XAS) and/or electrospray ionization mass spectroscopy (ESI-MS). In this presentation I will provide an overview of the various hyphenated techniques that we have developed and

employed to better understand the toxicological chemistry of arsenic cadmium and mercury species in the blood plasma-red blood cell-organ system which is a pre-requisite to better understand the mechansims of their chronic toxicity in humans and to eventually establish causal exposure-disease relationships.4

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Oral

Second Dimension Temperature Programming in GC×GC: from the Idea to Implementation

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In chromatography the inability to effectively separate all components of a sample under a single set of conditions when the properties of those components differ widely is referred to as the general elution problem. In gas chromatography it is dealt with by increasing the oven temperature during the separation. However this temperature programming does little to alleviate the general elution problem in the second dimension (^{2}D) of comprehensive two-dimensional gas chromatography (GC×GC) because the modulation period is typically very short (a few seconds) and during this time the main GC oven temperature changes very little at typical GC temperature programming rates. As a result the ^{2}D separation is carried out under practically isothermal conditions. Even though the analytes entering the ^{2}D column are pre-separated based on their volatility they can still be retained too strongly to elute during a single modulation period leading to undesirable consequences including peak wraparound. Thus far the only practical way to deal with this problem has been the use of an additional oven which keeps the 2D column temperature above the main oven temperature. While this reduces peak wraparound it leads to deterioration of the separation of components eluting early in 2D. In addition ^{2}D peak widths increase rapidly under the practically isothermal separation conditions which limits the attainable ^{2}D peak capacity. To overcome this limitation we have proposed to introduce 2D temperature programming to GC×GC. Early proof-of-concept experiments involving ballistic heating of the column demonstrated the feasibility of the idea and its effectiveness at improving ^{2}D separations. Following that several systems were designed and built with increasingly more refined control over the ^{2}D separation conditions including the heating rate heating duration and temperature offsets. Initially a fine thermocouple attached to the ^{2}D column was used to monitor its temperature and an Arduino-based unit with in-house written software was used to control it. This system demonstrated that peak capacity of the ^{2}D can be increased by nearly 50% with temperature programming but it was not very robust or user-friendly. In the most recent designs the ^{2}D column itself was used for analyte separation temperature measurement and column heating. This system proved to be very robust and simple to use. Further refinements focused on the optimization of the Arduino code to broaden the range of conditions a given implementation of the system is suitable for. This version of the system can be incorporated into any GC×GC instrument. Tests on various samples demonstrated that the temperature programming of the ^{2}D in GC×GC leads to significantly better selectivity and higher peak capacity compared to currently used solutions.

Oral

Quantitative Assessment of Retention Mechanisms in Hydrophilic Interaction Chromatography (HILIC)

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Retention mechanisms in HILIC have been investigated and reported in literature. However the current understanding of the retention mechanisms is qualitative and lacks quantitative details. Previously mechanism elucidation was based on indirect evidence and unambiguous assignment of retention mechanisms has not been reported based on direct data. This study aims to quantitatively determine the contributions of two major retention mechanisms in HILIC hydrophilic partitioning and surface adsorption to the overall retention of neutral compounds. Using the methodologies we developed previously the phase ratio for adsorbed water layer and distribution coefficients were measured and used to calculate the retention factors attributed to hydrophilic partitioning. The methodology allows the determination of the contribution of surface adsorption simultaneously. The evaluation of five test compounds demonstrates that the retention may be controlled by hydrophilic partitioning surface adsorption or both depending on compound characteristics. Quantitative assessment of retention mechanisms also makes it possible to better understand the effect of acetonitrile on retention in HILIC.

Oral

The use of robotic automation as a tool to certify multi-analyte reference materials fully traceable to the SI

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As the UK's designated institute for chemical and bio-measurement the National Measurement Laboratory (NML hosted at LGC) provides certified reference materials (CRMs) traceable to the International System of Units (SI unit – the kilogram). CRMs are needed to support standardisation of clinical diagnostics as required by ISO 15189 but typically take several months to certify and usually only consist of one or a few measurands. To increase both the number of measurands and speed of CRMs being made available a novel process is required that has the capability of traceably measuring multiple measurands with high accuracy and low uncertainty. Therefore the automation of the certification process by use of liquid handling robots with integrated balances has been trialled.

Double exact matching isotope dilution mass spectrometry (DEM-IDMS) is used to certify measurands traceable to the SI with high accuracy and low uncertainty to develop higher order reference methods and CRMs. A higher order reference method for manually certifying amino acids has been previously developed at the NML with a standard uncertainty below 2% using DEM-ID-GC-MS/MS. This method was translated onto two liquid handling robots with integrated balances the Agilent 7696A Sample Prep Workbench and the GERSTEL Multipurpose Sampler. Comparisons were made between mixed standards gravimetrically prepared by an analyst and preparations by the two liquid handling robots. Both robots' initial ability to make gravimetric standards was unexpectedly variable.

Liquid handling settings such as draw speed dispense speed washes dispense washes dispense pumps syringe airgap and overfill were optimised. Initially blending the natural working solution with the labelled working solution in equal parts was repeatable within the low uncertainty of the method. However when including a dilution step before the blending step there were inconsistencies and bias. A major difference between analyst preparation and robot preparation was identified as the use of a pipette as opposed to a syringe piercing a closed vessel with a septum. Further experiments were conducted by an analyst mimicking the robot by preparing the solutions using a syringe both piercing a septum and not going through a septum. It was concluded that the variability was likely due to solvent droplets depositing on the septum during removal of the syringe which is adding to the weight but not incorporated into the solution affecting the calculated concentration. Therefore we will discuss issues around optimising settings air gaps contamination considerations and cleaning metal syringes to overcome this issue.

The use of an automated decapping device was assessed to prevent these piercing errors and further challenges with evaporation from decapping vessels was explored. Disposable pipette tips were also trialled on the automated system as an alternative to syringes although initial work has demonstrated issues with dripping and therefore they currently work better with more viscous solutions.

Both robotic systems are now optimised for amino acid solution preparation and a standard comparison between an amino acid mixture prepared by the robot was compared successfully to the NIST amino acid CRM 2389a. Using the precise DEM-IDMS workflow the accuracy and precision of the robotic technique have been assessed and a measurement uncertainty calculated.

Oral

An automated quality-by-design (QbD) method development approach for the separation of pharmaceutical impurities

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Highlights:

DOE allows flexibility in customizing and combining pH organic modifiers pump flow rates injection volumes column temperatures and gradient times/slopes.

Resolution heat map allows to tweak the desired separation for the obtained peaks.

The methods with the desired separation could be achieved in a short time frame.

More modeling options than the other competitive method development software.

An automated and efficient quality by design (QbD) reversed-phase HPLC method screening and optimization strategy has been implemented to separate pharmaceutical impurities. The applicability of Fusion QbD software has been demonstrated with an active pharmaceutical ingredient (API) and its seven potential process impurities/degradants. Unlike the traditional chromatographic method development approach the design of experiments (DOE) methodology has been used which allowed an automated creation of multiple method parameters based on given pump flow rates injection volumes column temperatures gradient times/slopes and stationary phase types. The DOE further assisted in generating a buffer system of desired pH in broader ranges e.g. by online mixing of formic acid/ammonium formate duo buffer system (pH 2.9-6.45). The chromatographic data system (CDS) e.g. Empower was then populated in the form of a sample sequence by exporting the generated design combinations (containing defined method parameters). The obtained (imported) chromatographic raw data from CDS was then employed for the data analysis to perform the best overall answer search. The quality of this search was enhanced by applying a set of USP tailing and resolution parameters which predicted the chromatographic peaks within a range of desired peak tailing and resolution. Furthermore a Resolution heat map allowed the best predicted conditions by considering the combinatorial impact of stationary phase type gradient time and organic modifiers. Compared to these point predictions each peak in the real chromatogram showed a baseline separation within the %RSD of 0.4% - 9.0%. Further enhancement of separation and prediction quality was accomplished by deliberate optimization of the most impactful method parameters. Thus a baseline separation of an API and its seven potential process impurities/degradants was achieved. Hence an automated quality-by-design (QbD) method development strategy could be a solution to tackle the separation problems arising both in the early and late development of an API.

Oral

Reconsidering the Cm and Cs-terms of van Deemter's equation: are the two mass transfer resistances independent?

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Mass transfer resistances between the mobile and stationary zones are main sources of band broadening in chromatographic columns. The increase of this band broadening as a function of the velocity poses a limit to the kinetic performance of chromatographic separations. Within the framework of the van Deemter equation the mass transfer resistances within the mobile and stationary zones are respectively represented by the Cm- and Cs-terms of the plate height. Whether or not the two mass transfer resistances are mutually independent – and hence whether or not the Cm- and Cs-terms are additive – was first put into question by Giddings in the 1960's. However only a few studies have since then followed up on this critical remark.

To scrutinise the generally assumed additivity of the Cm- and Cs-terms we have recently applied Brenner's macrotransport theory to model the band broadening in packed beds as well as micropillar array columns. This mathematical framework allows to compute the plate heights of chromatographic columns both accurately and efficiently. The data acquired from this computational fluid dynamics study clearly show the mass transfer resistances in mobile and stationary zones are not mutually independent since diffusion through one zone can affect the other zone. Therefore assuming the Cm- and Cs-terms are additive typically overestimates their combined effect by more than 10% causing errors in the modelling of band broadening. Furthermore the acquired data provide the basis for an alternative expression for the C-term.

Based on the conclusions drawn from our fundamental study this presentation aims to shed new light on the true behaviour of the mass transfer resistances in chromatographic columns and furthermore demonstrate the potential of Brenner's macrotransport theory for the modelling of band broadening.

Oral

Novel epitope-mimetic peptide recognition technology for enrichment of antibody drugs in biological samples

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Efficient enrichment is a vital prerequisite for the production clinical research and in vivo analysis of antibody drug. However current enrichment technologies suffer from the prominent dependence for support materials complex preparation and unstable ligand coupling. As a high-affinity precipitating agent peptide based supramolecules exhibited great potentials in antibody enrichment without support materials. But they still face numerous challenges for antibody enrichment including poor self-assembly ability undesirable ligand-antibody binding rates and formidable target binding barriers caused by ligand crowding. To tackle these issues a “polyvalent recognition” strategy employing CD20 mimotope peptide derivative NBD-FFVLR-GS-WPRWLEN (act on the CDR domains of rituximab) was proposed to develop supramolecular nanofibers for target antibody recognition. These nanofibers exhibited rapid self-assembly within only 1 min and robust stability. Their binding affinity (179 nM) for rituximab surpassed that of monomeric peptide (7 μM) by over 39 folds highlighting that high ligand density and potential polyvalent recognition can efficiently overcome the target binding barriers of traditional supramolecules. Moreover these nanofibers exhibited amazing “instantaneous capture” rate (within 15 s) high recovery (93% ± 3%) and good specificity for target antibody. High-efficiency enrichment of rituximab was achieved from cell culture medium with good recovery and reproducibility. Intriguingly these peptide nanofibers combined with bottom-up proteomics were successful to track the deamidation of asparagine 55 (from 10% to 16%) on the rituximab heavy chain after 21-day incubation in human serum. In summary this study may open up a new avenue for the development of versatile mimotope peptide supramolecules for bio-recognition and bioanalysis of biopharmaceuticals.

Oral

An in-depth evaluation of the advantages and limitations of HILIC RPLC RPLC×HILIC and HILIC×RPLC coupled to UV (- cyclic IMS) - HRMS for phenolic and flavonoid analysis in non-target screening

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In recent years multidimensional liquid chromatography and ion mobility spectrometry (IMS) have been given increasing attention due to the desire to better resolve isomers and compounds of interest in complex samples. Both one- and two-dimensional liquid chromatographic platforms are extensively applied to plant extracts. Reversed phase liquid chromatography (RPLC) and hydrophilic interaction chromatography (HILIC) coupled to ultraviolet (UV) and high-resolution mass spectrometry (HRMS) detection is often used for phenolic and flavonoid analysis. A limited number of studies have used IMS for these compound classes as an additional separation dimension. While the hyphenation of multiple dimensions offers better separation of compounds it also adds additional instrumental complexity and extra emphasis on choosing a suitable hyphenation of analytical platforms. Therefore it is paramount for the analyst to have a detailed understanding of how the choice of analytical platform will affect the final separation and data quality.

In this study we present an in-depth evaluation of the following analytical platforms: RPLC HILIC RPLC×HILIC and HILIC×RPLC coupled to UV-HRMS and UV-cyclic IMS-HRMS to better understand what advantages and limitations each of these platforms offer. All the analytical platforms were applied to a wheat flag leaf extract. The comparison is based on quantitative diagnostics such as mass spectral purity the number of identified compounds use of the separation space peak capacity and peak production rate in addition qualitative parameters such as the degree of structure in the chromatograms.

Each of the analytical platforms was optimized individually. Two-dimensional liquid chromatographic conditions were optimized in-silico using a predictive kinetic optimization program previously described [1] while one-dimensional chromatographic methods were optimized manually to maximize gradient coverage. Separate cyclic IMS methods were used for the one- and two-dimensional chromatographic methods to allow for a higher mass spectral scan speed in the LC×LC methods.

The findings of this study further strengthen our understanding of what potential advantages and limitations each of these platforms offer for phenolic and flavonoid analysis thereby guide the choice of analytical platform for a given application.

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Oral

Higher order structure promotes oligonucleotide diastereomer separation in hydrophilic interaction chromatography under high ionic strength and low-temperature conditions

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Therapeutic oligonucleotides (ONs) have emerged as promising option for addressing "undruggable" diseases through gene expression regulation. However various challenges arise due to the inherent limitations of unmodified ONs. This includes enzymatic degradation potential immune activation and limited cellular uptake. In this context chemical modifications particularly phosphorothioate (PS) modifications have become essential to improve the stability specificity and efficacy of ON therapeutics.

However the incorporation of PS linkages introduces new chiral centers to the phosphorus atom of the ON backbone resulting in a large number of diastereomers with distinct pharmacological properties. Therefore monitoring the diastereomeric distribution is crucial to ensure batch-to-batch consistency and thus drug safety and efficacy.

While anion exchange chromatography and ion-pairing reversed-phase liquid chromatography have shown potential for partially separating diastereomers [1] the overall resolution remains limited and peak are often very broad. Hydrophilic interaction chromatography (HILIC) a chromatographic technique which is more and more widely used for ON analysis has recently shown promising diastereomers selectivity while applications to representative therapeutic ONs are still lacking [2].

In this study we investigated the applicability of HILIC for the separation of diastereomers in full-length single- and double-stranded PS-modified ONs. We have uncovered that specific HILIC parameters including column close to freezing temperatures and relatively high mobile phase ionic strength (e.g. 100 mM) strongly enhance diastereomer selectivity. More importantly the intrinsic higher order structure of ONs was found to play a pivotal role in diastereomeric separation paving the way for a better understanding of the mechanism driving diastereomeric separation under HILIC conditions.

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Oral

Biosolvents in Chromatography: Application of the Biodegradable Biosolvent 'CyreneTM' in the Quantitative Analysis of Residual Production Solvents in Pharmaceuticals using HS-GC-MS

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The utilization of renewable resources and biomass in the sustainable production of chemical feedstocks is crucial to meet the growing societal demand for a greener and more sustainable chemical industry and one which is far less dependent upon fossil fuels. Thus the conversion of bioderived renewable compounds such as sugars and their polymeric derivatives into valuable precursor chemicals has progressed rapidly from laboratory-scale experimentation to industrial implementation. This is particularly true for bioderived solvents a development driven in part by increasing restrictions on toxic petroleum-based solvents commonly used in industrial-scale chemical synthesis. The development of safer solvents is one of the core tenets of the Twelve Principles of Green Chemistry [1].

CyreneTM sourced from sawdust is a bioderived sustainable biosolvent and potential substitute for aprotic dipolar solvents such as N-methylpyrrolidone (NMP) and NN-dimethylformamide (DMF) both of which are of concern due to their toxicological profiles and regulatory limitations2. CyreneTM has already been successfully applied as a direct substitute for these solvents in organic synthesis functioning not only as a solvent but also as a reactant. Furthermore its applicability extends to nanotechnology and sample preparation. However to-date its application in separation science and chromatography has been rather limited to extraction [23] and as mobile phase additive in liquid chromatography [4].

Given the routine application of solvents in the synthesis and processing of pharmaceuticals it is important to monitor and control their presence in the final products. To fulfil this objective solvents characterized by elevated boiling points such as dimethyl sulfoxide (DMSO) dimethyl formamide (DMF) and 13-dimethyl-2-imidazolidinone are commonly employed for the preparation dilution and dissolution of pharmaceuticals for the analysis using gas chromatography-mass spectrometry. However the biosolvent CyreneTM also possesses a high boiling point but is simultaneously biodegradable and a sustainable alternative with reduced toxicity.

In this study CyreneTM has been employed as a novel solvent for sample preparation in gas chromatography-mass spectrometry marking the first instance of its application in such a context. Utilizing the AGREE software to assess the environmental impact of the analytical method the substitution of CyreneTM for DMSO or 13-dimethyl-2-imidazolidinone has resulted in an increase in the AGREE greenness coefficient from 0.58 to 0.73. The elevated boiling point of CyreneTM at 227 °C

has facilitated the analysis of solvents with diverse boiling points enabling the concurrent analysis of DMSO ethylene glycol methanol octane ethanol among others.

The impact of CyreneTM on chromatographic and method parameters including peak shape theoretical number of plates and sensitivity has undergone thorough evaluation. Comparative analysis with DMSO revealed that the residual solvent mix prepared in CyreneTM exhibited comparable peak symmetry for all analysed residual solvents while maintaining satisfactory sensitivity levels. Limits of detection and quantification achieved using CyreneTM as a solvent were consistent with those obtained with DMSO. Furthermore owing to its effective solvation properties CyreneTM facilitated the facile solvation of various drugs enabling the detection and quantification of diverse residual solvents.

This study showcases the capacity of CyreneTM to serve as a viable substitute for alternative solvents in residual solvent analysis. Additionally the research highlights the substantial potential of CyreneTM in chromatographic applications particularly as its purity is enhanced.

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Oral

Analytical Strategies for Safety Evaluation of (Recycled) Food Contact Materials - Critical Discussion of the Current Situation

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The European Union faces a massive turnaround currently: the European Green Deal aims to transform Europe to the first climate-neutral continent with economic growth being sustainable and independent from resources use [1]. One powerful tool in the transformation is the Circular Economy Action Plan which sets actions along the entire life cycle of products to reduce waste. Recycling of resources shall assure that they stay in the circular economy as long as possible [2]. In more detail 65% of all packaging materials shall be reusable or effectively recyclable by 2025 and 75% by 2030. This means that 55% of plastic packaging will need to be recycled by 2025 and by 2030 all plastic packaging produced in the EU market will need to be reusable or recycled in a cost-effective manner. This will also apply for food packaging.

To adopt to these changes also new developments new technologies and – of course- new regulations are needed. One of those is the new Commission Regulation (EU) 2022/1616 on recycled plastic materials and articles intended to come into contact with food [3 4]. It is in force since October 2022. The regulation lead to huge concerns because - to accelerated the change - it allows to place products made of recycled plastics on the market which had been produced by newly developed non-authorized technologies and approaches (“novel technologies”). This can lead to a lack of safety and an increased risk of exposure to hazardous chemicals for the general public because the regulation does not give appropriate details on how to proof suitability of the novel technology and the safety of the material. “Methods” appropriate for the intended purpose shall be used to determine the decontamination efficiency of the technology and the contamination level of the final product for every single batch produced.

This talk shall discuss the challenges for proper selection of analytical methods for safety evaluation. These methods need to cover various materials (polymers and cellulose based materials) and various analytes as well. Beside various analytical methods mainly based on (multidimensional) chromatographic solutions additional bio tests should deliver additional information on the safety of these materials intended to use as a direct food contact material.

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Oral

Comprehensive simulation toolbox for gas chromatography

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This presentation introduces a comprehensive simulation toolbox designed for advancing gas chromatography (GC) methodologies in the face of evolving techniques. The toolbox accommodates novel GC approaches such as temperature and film thickness gradients and multidimensional complex systems like GC×GC or GC×GC×GC significantly expanding the optimization parameter space.

Built upon an open-source GC simulation [1] the toolbox facilitates the creation of diverse GC systems using modular components. The sequential simulation of analyte movement through these modules provides retention time and peak width data which are used as initial values for following modules. As a by-product the toolbox is a flow calculator for complex GC systems by mathematically abstracting the system as a graph network and using the conservation of mass principle.

Beyond system simulation the toolbox addresses the need for understanding analyte retention factors and their temperature dependencies. Traditional methods involve time-consuming isothermal GC runs but the toolbox offers an efficient alternative. Utilizing optimization algorithms it estimates parameters for a retention model using a minimal number of temperature-programmed measurements [2].

The toolbox's versatility is shown through examples of complex GC systems. The three main components (flow calculator simulation engine and retention parameter estimation) are presented. By offering a unified solution for understanding simulating and optimizing complex GC systems this toolbox emerges as a valuable asset.

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Oral

Compositional analysis for prescreening of sustainable aviation fuels (SAF) using a multidetector comprehensive two-dimensional gas chromatograph (GCxGC-FID/ qMS).

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The sustainable transformation of aviation will require a variety of approaches such as the application of alternative hydrogen or battery-powered propulsion systems. However improving the climate impact of long-haul flights and the currently existing fleet will mainly rely on the use of sustainable aviation fuels (SAF). SAF provide the most technically mature alternative to conventional kerosene at the moment[1]. They are hydrocarbon based alternative fuels compatible with conventional aircraft and provide multiple possible approaches to improve sustainability of aviation. The net carbon-emissions of SAF are indirectly reduced prior to the utilization in the aircraft through implementation of carbon from direct air capture or the respective biomass feedstock serving as renewable carbon storage[1–3]. Furthermore SAFs offer opportunities for fuel design strategies to actively improve aviation's non-CO2 effects. For example through reduction of the sooting tendency and subsequently the contrail formation which has a significant impact on radiative climate forcing[4–8]. At the moment many projects are underway to develop new ways to synthesize and produce novel sustainable fuels that meet the standards in aviation. The various pathways lead to diverse compositions which subsequently is reflected in the alteration to a range of properties. To analyze the complex fuel samples non-targeted comprehensive two-dimensional gas chromatography (GCxGC) is required as it is capable of significantly higher peak capacities than standard GC[9–13]. Based on the detailed compositional data in-house probabilistic property models are able to estimate a number of key properties relevant for the fuel approval process allowing for preliminary feedback on those novel fuels[14–16]. Since GCxGC requires only microliters of product the technique is also ideally suited as a monitoring technique during the different stages of fuel production.

The utilized chromatographic instrumentation is equipped with a reversed phase column configuration. After the separation in the second column a four-port splitter enables concurrent detection with a flame ionization detector (FID) and a quadrupole mass spectrometer (qMS). This way sensitive quantification can be conducted while simultaneously group-type structural and elemental information within those novel fuels could be evaluated[9]. Since structural diversity increases with the carbon number compound-by-compound specific identification is often not feasible even with comprehensive MS reference data. Therefore this procedure usually generates compositional results categorized by carbon number and group-type (n- iso cycloalkanes aromatics etc.).

Structural differences between isomeric compounds can result in significant differences in properties e.g. ignition properties. This consequently presents a challenge to the precision of property predictions when the compound structures within individual groups are undefined[817]. To identify smaller subgroups and address the lack of detail within individual group-types an investigation on the influencing parameters to the retention time was carried out. The current research focusses on

the isomeric alkane group. Initially only structurally derivable molecular descriptors were included and analyzed on their respective correlation to avoid limitations from the extent of reference data of correlating properties. Particular interest was placed on the varying performance of different approaches for describing the degree of branching. The influence on predictions of different properties is also discussed.

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Oral

Speciation of oxygen- and nitrogen-containing molecules in plastic pyrolysis oils

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Plastic includes a large family of materials having different characteristics properties and uses. These compounds are widely used around the world and after use they need appropriate waste management. In this context the use of feedstock derived from plastic waste in existing industrial processes is a key point to improve environmental performance. Pyrolysis is a thermochemical process in which plastic waste is converted into an oil named plastic pyrolysis oil (PPO). This oil can be used as a feedstock for steam crackers to produce virgin-grade polymer products. However due to the diversity of plastic materials and their application PPOs contain a wide range of molecules that are not present in fossil-based feedstocks such as oxygenates nitrogenates halogen-species and metals. Therefore the speciation of these molecules is crucial to define a proper upgrading process such as hydrotreatment before feeding PPO into steam crackers.

In this study PPOs from different sources and their distilled fractions were characterized via a comprehensive gas chromatography system coupled to a high-resolution time-of-flight mass spectrometry (GCxGC-HR-TOFMS) for speciation of oxygen - and nitrogen species. Additionally a GC coupled to a nitrogen chemiluminescence detector (GC-NCD) was used for quantification of the nitrogen-containing molecules. Split and on-column injection was employed and the nitrogen concentration obtained via both injection types was compared with the total nitrogen content from the elemental analysis. The oxygen and nitrogen species found were different depending on the PPO feed. For the nitrogen-containing molecules quantification on-column injection showed better nitrogen recovery compared to split injection which can be attributed to the presence of heavy nitrogen-containing molecules as observed in the analysis of the PPOs distilled fractions.

Oral

Tackling the analytical challenges to establish a routine workflow for breathomics research

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Breathomics an emerging field investigates the intricate relationship between volatile organic compounds (VOCs) contained in exhaled breath and human health offering a non-invasive approach to disease monitoring and detection. Analytical chemistry stands as a crucial component in the comprehensive characterization of the complex molecular composition of exhaled breath. However challenges related to sampling or quality control still need to be addressed to establish routine clinical protocols. In this context several community initiatives aim to establish guidelines for exhaled breath analysis[1]. A typical breathomics workflow includes critical steps such as sampling protocols characterization methods and data processing.

In this study we present a comprehensive analytical workflow utilizing bag-based sampling combined with thermal desorption coupled with comprehensive two-dimensional gas chromatography and high-resolution mass spectrometry (TD-GCxGC-HRTOFMS). We developed sampling kits and standard operating procedures (SOPs) to reduce sampling variability. Additionally we established common GCxGC-HRMS conditions compatible with all breathomics studies and worked on SOPs for data processing for this type of dataset.

Once the workflow fully developed we conducted a multicentric clinical study to test the robustness of our approach focusing on patients with systemic sclerosis (SSc) and investigating the potential of exhaled breath to monitor the development of interstitial lung disease (ILD) a major clinical complication in SSc. Forty-two patients (21 SSc 21 SSc-ILD) were prospectively recruited from University Hospital of Liège (CHU) Belgium and Maastricht University Medical Center (MUMC+) the Netherlands. Sampling kits were sent to both centers and analyses were performed using a single instrumentation.

This study demonstrated that ready-to-use kits has helped to reduce cross-contamination while reducing the workload of care staff and thus potential errors. This process also increased the number of patients sampled in a defined period reducing variability caused by longitudinal study. TD-GCxGC-HRTOFMS enabled the detection of around 700 features with high inter-day repeatability confirmed using a mixture containing 21 standards covering a wide range of the chromatograph. Each of these standards was carefully selected to mimic exhaled breath composition based on previous studies[2]. This confirmed the identification of features but also allowed us to proceed to instrumental maintenance or intensities correction when needed. Finally a breath-based model based on nine markers was developed. This model achieved an AUC (Area Under the Curve) of 0.82 accuracy of 85%

sensitivity of 77% and a specificity of 100% for identifying ILD phenotype. Among them four markers were also previously highlighted in our previous study[3]. In addition correlation between functional respiratory parameters and the VOCs confirmed the model's ability to classify patients accurately. QC and cross-validation were also conducted to exclude overfitting problems or data structure modification. Random feature selection and class assignment permutations were performed to rule out overfitting.

In conclusion this work represents a major step in demonstrating the potential of breathomics in a clinical setting. The implementation of SOPs in medical centers located in two different countries facilitated a comparative analysis of breath from patients with SSc and SSc-ILD. The discovery of potential markers contributes to a better understanding of the disease and metabolic pathways involved offering the prospect of rapid targeted treatments for ILD patients. The multicentric design provided valuable insights into the robustness of our analytical methods contributing to the development of guidelines and SOPs for larger exhaled breath studies. This marks a significant stride towards integrating breathomics into clinical settings.

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Oral

Per aqueous liquid chromatography-a green alternative for the separation of polar and ionogenic solutes.

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The consumption of acetonitrile in hydrophilic interaction chromatography is relatively low when used with short small internal diameter columns (10 x0.21 cm) of sub 2 micron particle diameter. Thus with isocratic analysis of peaks with retention factor up to 5 700-1000 samples can be analysed with 1 litre of acetonitrile. In addition acetonitrile is generally placed in a category of lower environmental toxicity than most normal phase solvents. Nevertheless many scientists wish to limit the consumption of acetonitrile still further or to eliminate it altogether. We have explored the low organic solvent end of the HILIC retention curve using concentrations of 10 % or less in the separation mode named peraqueous liquid chromatography (PALC) to obtain separation of polar and/ or ionised solutes with acceptable peak shape. The technique was shown to be viable with a number of different stationary phase and mobile phase combinations.

PALC generally involves some sacrifice of column efficiency compared with HILIC for the same solutes and stationary phases. Nevertheless we were still able to achieve up to 11000 plates for some solutes using a 10 cm column. The separation mechanism of PALC appears to be quite complex. There certainly seems to be a contribution of ionic retention of (protonated) basic solutes on silanol groups of silica based columns. There is also undoubtably some hydrophobic retention on column groups such as siloxanes on bare silica. This process leads to appreciable retention of some non-polar solutes. Other mechanisms such as polar neutral retention effects may also contribute to retention in some situations. It would seem that the relative strength of these interactions within the mixed retention process contribute to the peak shape. The low solubility of hydrophobic compounds in a highly aqueous mobile phase environment may contribute to retention . The best peak shapes were observed from polar neutral compounds or organic acids.

Oral

High Throughput Untargeted Lipidomics on multiple matrices: Breaking the 5-Minute Barrier with HT-4D-TIMS

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Lipidomics has rapidly become a fundamental discipline within the omics sciences essential for elucidating the role of lipids in biochemical processes and various life science domains including biomedical clinical and drug discovery applications.

LC-MS represents the golden standard in untargeted lipidomics but the low throughput still represents one of the major shortcomings of UHPLC-HRMS approaches. Indeed the possibility to rapidly analyze large sample cohorts is essential for the identification of disease progression biomarkers therapy monitoring and phenotypic drug discovery campaigns.

To tackle this challenge we evaluate the application of a sub-5-min high-throughput four-dimensional trapped ion mobility mass spectrometry (HT-4D-TIMS) platform [1] for the fast profiling of multiple complex biological matrices. Human AC-16 cells mouse brain liver sclera and stool were used as samples. By using a fast 4-min RP gradient the subsequent implementation of TIMS allowed to differentiate coeluting isomeric and isobaric lipids with correct precursor ion isolation avoiding co-fragmentation and chimeric MS/MS spectra. Globally the HT-4D-TIMS allowed us to annotate 1910 different lipid species 1308 at the molecular level and 602 at the sum composition level covering 58 lipid subclasses together with quantitation capability covering more than three orders of magnitude. Notably TIMS values were highly comparable with respect to longer LC gradients (CV% = 0.39%). These results highlight how HT-4D-TIMS-based untargeted lipidomics possess high coverage reducing approximately 5 times the analysis time respect to conventional UHPLC methods and can be used for fast and accurate untargeted analysis of complex matrices to rapidly evaluate changes of lipid metabolism in disease models or drug discovery campaigns.

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Oral

Untargeted analysis of contaminants in river water samples: Development of a Comprehensive SPE Method for Polarity-Based Extraction of Organic Contaminants followed by Liquid and Gas Chromatography hyphenated to High-Resolution Mass Spectrometry.

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The non-targeted study of organic pollutants in environmental media has become essential in order to gain a better understanding of the origins of pollutants their fate in the environment and their degradation products and to study their effects on ecosystems and health. In particular in 2019 a big fire came from two industries Lubrizol and Normandie Logistique in Rouen and was one of the biggest industrial accident of the 21st century in France. 9511 tons of products were burned mostly mineral oils producing a 22 km long black cloud of smoke. Fallout of soot was reported up to 100 km away from the plant. Samples were taken at different periods from 2019 to 2022 on 5 rivers in Normandy (Robec Nounice Cailly Crevon and Andelle). These 15 river water samples were processed by solid phase extraction (SPE) using two successive cartridges: a StrataX reverse phase (6mL 200 mg) and an Oasis MCX mixed cation exchange phase (6mL 150 mg). The method was optimised (elution solvents elution volume sample pH adjustment) to partition the sample into 5 fractions of different polarities to cover the widest possible range of organic compounds. Developed on the basis of a set of 43 molecules the final method enabled 34 of them to be recovered with yields of over 70% in most cases. Real water samples were then treated with the optimized SPE method and the fractions were anayzed by LC-HRMS and/or GC-HRMS. The chromatograms were reprocessed on Compound Discoverer 3.3 to deconvolve and align the data then compared with Compound Discoverer's mzCloud database. The data was then filtered of all pollutant ions from the extraction blanks and solvent blanks and sent to R software for comparison with an in-house database of possible fire markers. The remaining results were then classified into different confidence levels using the Schymanski scale [1] for LC-MS data and using a modified confidence scale from Koelmel et al. [2] for GC-MS data. This data processing method was used to annotate organic compounds in order to identify potential markers of the fire.

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Oral

Understanding the chemical composition of tyres and their propensity to off-gas volatile organics

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Tyres are complex products with sophisticated physical design and chemical composition. They must deliver a range of often conflicting performance requirements including slow wear long life low rolling resistance (to reduce carbon dioxide emissions and fuel consumption) wet grip and low noise. There exist regulatory and labelling requirements around some but not all of these. Current discussions around the proposed 'Euro 7' regulation envisage establishing a limit value for tyre wear emissions. What remains less well understood is the chemical composition of the tyres and how those chemicals affect the environment.

This presentation will focus on the chemical composition of hundreds of different models of tyres from both European and US markets It will draw on Emissions Analytics' database of its reverse - engineering of these tyres using its optimised process of thermal desorption and pyrolysis coupled with two-dimensional gas chromatography and time-of-flight mass spectrometry. It will look at the concentrations of the preservative 6PPD which is an essential component in tyres but which has been linked to the mass die-off of coho salmon in the USA The California Department of Toxic Substances Control has instigated a regulatory rule to force the industry to consider alternatives to 6PPD.

In addition to looking at the effects on aquatic life from the tyre particles and their leachate potential the impact on air quality will also be considered through the off-gassing of volatile organics from the surface of the tyres. Key dependencies such as the effect of ambient or tyre surface temperature on the rate of off-gassing will be studied. Potential for forming secondary organic aerosols and ozone formation will be estimated.

Oral

Exploring new fuels using advanced hyphenated techniques

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The adoption and implementation of renewable biodiesels for use in the transportation sector have presented unintended consequences such as marked increases in engine and fuel filter problems. The complexity of the materials and their addition to already complex fuels required new analytical approaches to be developed. The same is true for hydrotreated (or hydrogenated) vegetable oils (HVOs) as the adoption into the fuel chain increases.

New methods for the analysis of HVOs are required firstly to identify the chemical makeup of these new materials secondly compare different HVOs to understand the similarities and differences and relate this to physical properties. The final part of the analytical challenge is to be able to analyse these materials when blended with petrodiesel and petrodiesel biodiesel mixes.

To address the complexity and sample diversity challenges presented a top-down strategy was applied to the investigation using different instrumentation and new methods developed to provide a comprehensive overview of the HVOs e.g. SFC-FID SFC-MS and GCxGC-MS. The data from these different hyphenated approaches are then brought together to build a comprehensive picture for each of the fuels. Some of the key initial results will be presented to illustrate the need for this multi-instrumentation approach.

Oral

Application of capillary electrophoresis combined with inductively coupled plasma tandem mass spectrometry (CE-ICP-MS/MS) for studying systems comprising liposomes and cosmetically active compounds

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The cosmetics industry is constantly looking for ways to increase the ability of active compounds to penetrate the epidermis and increase their bioavailability. Nanometric liposomes can be considered a solution. Liposomes are vesicles built from a double phospholipid layer. The ability of these structures to encapsulate active compounds inside them release them in a controlled manner and build into the membranes of skin cells makes them promising carriers of cosmetically active compounds. Encapsulation of a cosmetically active substance inside liposomes which resemble cell membranes in their structure may affect its more effective delivery to the appropriate layers of the skin and thereby enable a reduction of the concentration used while maintaining the same level of action.

The research aims to develop a method for the effective formation of liposomes and encapsulation of cosmetically active compounds inside them: the GHK tripeptide complex with copper (which has an anti-aging effect) and selenium sulfide (anti-dandruff and antifungal effect).

The studies proved the usefulness of the ethanol injection method for the simple and effective formation of liposome-GHK-Cu and liposome-selenium sulfide systems. Also they allowed us to determine the influence of the total lipid content the lipid composition of the formed liposomes and the presence of additional steps in the forming procedure on the encapsulation efficiency of the tested active compounds and the physicochemical properties of probed systems.

Capillary electrophoresis combined with inductively coupled plasma tandem mass spectrometry (CE-ICP-MS/MS) was used for the first time to verify liposome formation and encapsulation of the cosmetically active compound and to determine the encapsulation efficiency. ICP-MS/MS enables selective detection of the liposome marker (phosphorus isotope). Combining this technique with capillary electrophoresis has allowed the direct analysis of samples containing liposomes and cosmetically active compounds. The described combination of techniques enabled the elimination of organic solvents from the separation procedure and made it possible to imitate the physiological environment during the analyses.

Upcoming research plans include studies of transport systems comprising liposomes and cosmetically active compounds from simple cosmetic formulations into the skin. Models imitating human skin will be used for this purpose. Studies of the stability of described systems under conditions simulating transport through the stratum corneum will be conducted using

also the ICP-MS/MS-based techniques.

Oral

Primary factors driving improvement of chromatographic performance in microfluidic LC devices.

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In the quest for enhanced separation capabilities microfluidic pillar array columns are being proposed as an alternative to packed bed and monolithic column technologies. The utilization of photolithographic techniques and Deep Reactive Ion Etching enables the precise formation of features in a high-purity solid silicon wafer. This results in microfluidic separation beds where the bed geometry can be finely controlled with nanometer-scale precision.

By creating perfect order in the separation bed the impact of Eddy (A-term) dispersion on total column dispersion can be minimized leading to an increase in column permeability. Various pillar array column formats have been developed featuring cylindrical pillars arranged in an equilateral triangular grid. Typically these columns require lengths ranging from 50 to 200 cm to achieve efficiencies ranging from 100000 to 400000 theoretical plates.

In this contribution we will investigate possible strategies to augment chromatographic performance concentrating on three crucial elements: (1) the effect of pillar shape (2) the influence of flow-through pore dimensions and (3) the impact of fluidic connection quality and extra-column volume. Theoretical principles and practical benefits for low-flow LC-MS applications will be explored elucidated by concrete real-life examples.

Oral

Comprehensive Analysis of Polyphenols in Wine Grape Pomace Using Two-Dimensional Liquid Chromatography (2D-LC)

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Wine grape pomace a byproduct of the winemaking process consists of seeds skins pulp and stems creating a complex matrix that contains a wide range of bioactive compounds. Grape pomace represents a rich source of polyphenolic compounds with potential health benefits. This study employs a comprehensive two-dimensional liquid chromatography (2D-LC) approach to analyze and characterize the polyphenolic profile of different wine grape pomaces. The methodology combines in the first dimension a pentafluorophenyl (PFP) column (150 x 2.1 mm x 1.7 µm) and a short C18 column (50 x 4.6 mm x 2.6 µm) which were hyphenated through an eight-port two-position-switching valve with two trapping loops of the same volume. The online 2D-LC setup was coupled to a diode array detector (DAD) and a QToF mass spectrometry for the identification of the bioactive compounds.

The 2D-LC approach provides increased resolution allowing for the simultaneous analysis of diverse polyphenolic classes including flavonoids phenolic acids and tannins. High-resolution mass spectrometry is employed for the accurate identification of individual polyphenolic compounds contributing to a detailed understanding of the complex polyphenolic composition of wine grape pomace.

This detailed polyphenolic profile enhances our understanding of the potential applications of wine grape pomace in the food and pharmaceutical industries emphasizing its role as a valuable and sustainable resource in the context of circular economy practices.

Oral

A detailed comparison of in-house developed and commercial two-dimensional liquid chromatography systems in heart-cutting and selective comprehensive modes

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Two-dimensional liquid chromatography (2D-LC) is becoming increasingly popular to analyze complex samples partly due to the recent introduction of commercial 2D-LC systems. Before these commercial systems were available 2D-LC was mostly carried out on in-house developed setups typically consisting of several switching valves and sample loops as the interface between both dimensions. The main advantage of such in-house developed systems compared to commercial options is their lower cost. Commercial systems on the other hand usually offer different 2D-LC modes in combination with specialized software to operate the instrument and analyze the data making them highly user-friendly.

This study compares an in-house developed and commercial 2D-LC instrument based on experimental differences in addition to more general differences including cost flexibility and ease of operation. An important aspect in the comparison is the different strategy to deal with the mobile phase incompatibility between the highly orthogonal separation mechanisms considered in this work: hydrophilic interaction liquid chromatography (HILIC) and reversed-phase LC (RPLC). For the in-house developed setup this is done using a combination of restriction capillaries and a trap column. For the commercial 2D-LC instrument active solvent modulation (ASM) is used which is a valve-based approach allowing the on-line dilution of the effluent from the first dimension column before transfer to the second dimension (2D) column. To compare both setups a sample of 28 compounds with a large polarity range is used to evaluate peak shapes and recoveries of the 2D-chromatograms obtained in single heart-cutting and selective comprehensive mode.

It is demonstrated that the selective comprehensive approach currently only possible on the commercial 2D-LC instrument results in the best peak shapes and recoveries for early eluting compounds (gradient retention factor < 5) in the second dimension however at the cost of an increased analysis time (almost double the analysis time of the single heart-cutting approach). For later eluting compounds there are no significant differences between setups and 2D-LC approaches in terms of peak shapes and recoveries. In this case the most advantageous setup in terms of analysis time is the single heart-cutting approach on the commercial instrument (total analysis time of 30 min versus 45 min on the in-house developed system) since the 1D- and 2D-separation can be carried out simultaneously. On the other hand in terms of total cost the in-house developed setup is the least expensive with a list price that is almost half that of the commercial setup.

In conclusion an in-house developed setup can be advantageous for the analysis of specific target compounds/samples depending on the analytical goal (single heart-cutting versus comprehensive 2D-LC). To deal with more complex problems it can be interesting to use a more specialized commercial 2D-LC instrument. This comparison study provides advice for analytical scientists

considering to use 2D-LC on the type of equipment to use depending on the needs of their particular applications.

Oral

Analytical Performance and Disruptive Potential of Fully Portable and Field-Deployable LC-MS Technology

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Bringing analytical chemistry out of the lab and into the field has been a trend within the discipline for many decades now. With recent innovations and developments in advanced manufacturing the capability to miniaturise and 'make portable' conventional lab-based systems is now much more widespread and amenable. Today instrument manufacturers are increasingly looking towards system miniaturisation and portability with several having commercially released new fully portable instruments including variety of gas and liquid chromatographs and mass spectrometers which have their origins in standard laboratory analytical technologies.

However miniaturisation of hyphenated technologies adds considerable complexity to be considered and practical challenges to be overcome particularly so when deploying such systems to real-world field-based use cases. Demands on size portability and connectivity are matched with more fundamental requirements of stable power mobile gas supplies system robustness and environmental protection. Further to all these physical considerations actual analytical applications as is also the case in lab-based systems depend heavily upon the sample and sample preparation which is also far more complicated to get right when working out in the field.

Work at the University of Tasmania in collaboration with our partners in Trajan Scientific and Medical (Melbourne Vic) has for the past decade been focussed upon the development of portable and field deployable liquid chromatography-based analysers [1-4] together with portable sample preparation technologies and more recently hyphenating these developed technologies with mass spectrometric detection. Within this presentation we will detail the evolution of this technology culminating in practical demonstrations of on-site environmental analysis using our combined mobile LC-MS solution. Herein we present a full and detailed evaluation of the analytical performance and capability of the hyphenated and fully portable system comparing performance when applied within the laboratory with that delivered outside of the laboratory and in the field demonstrating all the associated practicalities of doing so. We will demonstrate system performance with a number of on-site environmental applications of current and emerging concern focussing on soil/sediment extracts and natural waters applying a variety of field-based sample preparation procedures.

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Oral

Exploring molecular composition of upgraded pyrolysis bio-oil by using GC×GC-(EI/PI)-TOF MS

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Bio-oils produced from the pyrolysis of lignocellulosic biomass have proven to be a promising renewable energy source. These bio-oils are very complex organic mixtures consisting of thousands of compounds covering a wide range of mass and polarity. However the liquid obtained after pyrolysis is rich in oxygen which causes instability and corrosion problems. It therefore requires dedicated post-pyrolysis treatments such as hydrotreatment to increase the H/C ratio of the products to be used as fuels or chemicals. Here the bi-dimensional gas chromatography-mass spectrometry (GC×GC MS) was used to characterize a bio-oil and its hydrotreated effluents allowing a molecular characterization of volatile compounds. The GC×GC MS was interfaced with an electron ionization (EI) source coupled to a quadrupole time-of-flight (QTOF) MS. With this system two column sets were investigated: a normal phase (nonpolar × mid-polar) and a reverse phase (mid-polar × non-polar). The results revealed a great potential to trace bio-oil molecular composition as a function of hydrotreatment time on stream and to understand the chemistry of recalcitrant difficult-to-process species. A clear change in composition was observed. Regarding hydrocarbon species polyaromatics increased significantly while cycloalkanes and monoaromatics decreased. Concerning the oxygen compounds their quantity and diversity also increased significantly over time. Results highlighted the occurrence of catalyst deactivation. From an industrial point of view these observations will help to adapt bio-oil hydrotreatment processes.

Oral

LC-GC×GC: A POWERFUL HYPHENATED TECHNIQUE TO ENHANCE CHROMATOGRAPHIC SEPARATION AND SIMPLIFY SAMPLE PREPARATION FOR ROUTINE APPLICATIONS

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The introduction of hyphenated chromatographic techniques has unveiled the complexity of many apparently simple samples pushing the development of multidimensional techniques to boost the separation power and unravel such complexity. In 1980 the first attempt to hyphenate LC and GC was presented. Since then the technique has followed a winding path never gaining the attention it deserved and even facing commercial discontinuation. Contrarily comprehensive two-dimensional GC (GC×GC) was introduced in 1991 and quickly garnered attention and spread across various research fields. Despite its popularity in research and its undeniable benefits the technique has not found the routine application it merits. The resistance to accepting multidimensional techniques on a routine basis primarily stems from concerns regarding the initial investment cost and the perception that it is neither robust nor reliable. Since 2008 the issue related to the determination of mineral oil hydrocarbons (MOH) in food has revitalized the LC-GC technique giving it the leading place as a routine method. GC×GC was proposed as a confirmatory method but it still faced resistance.

More recently a completely hyphenated LC- GC×GC-TOFMS/FID system has been developed and validated proving its robustness for routine applications. The platform has been extensively used to produce highly informative data but also to reduce the uncertainty associated with the overall analytical workflow playing a fundamental role in simplifying the sample preparation providing an alternative method to the time-consuming less reliable and error-prone epoxidation procedure used for MOAH purification. Specifically it achieves a much lower quantification bias of around 5% instead of over 40% when using epoxidation.

Oral

A Novel Approach to Chiral Compound Screening: Combination of Reversed HILIC and Temperature Responsive Liquid Chromatography with Chiral Chromatography in Comprehensive Two-Dimensional Liquid Chromatography

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The separation of chiral solutes in mixtures of unrelated species is important in life sciences and pharmaceutical analysis. Although theoretically achievable through comprehensive two-dimensional liquid chromatography (LC × LC) challenges arise when the solvent introduced to the second dimension (2D) is too strong or incompatible leading to peak broadening and loss of resolution. Achieving chiral separation in the second dimension is particularly challenging because of the slower mass transfer processes involved in chiral retention which hinders the speed of analysis especially under the overloading conditions to with 2D is subjected in LC × LC and because of the super-optimal flow rate necessarily involved. These drawbacks are particularly evident when the technique is applied to a range of chemically discrepant chiral compounds where both a chiral column and a broad gradient solution in the second dimension are required.1 In this study we developed a novel achiral × chiral platform allowing fully automated screening of complex mixtures comprising chiral compounds. In the first dimension (1D) of this platform chromatographic techniques with water-rich mobile phases are employed. These techniques include either purely aqueous temperature-responsive liquid chromatography (TRLC) or reversed HILIC (or per-aqueous liquid chromatography (PALC))2 which is similarly characterized by requiring only very small fractions of organic modifiers in the 1D mobile phase. The aqueous mobile phase in 1D thereby facilitates the complete refocusing of organic solutes before entering the second dimension preventing the loss of resolution in the 2D. Furthermore it enables the application of various mobile phase compositions. The tolerance of the chiral 2D separations depending on the percentage of modifier in 1D was thereby also studied in detail. Moreover in 2D fast chiral chromatography with a broad range of gradients was investigated. These features make the full comprehensive achiral × chiral technique promising for analyzing diverse natural and synthetic mixtures compounds particularly those containing stereoisomers.

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Oral

An original dereplication strategy based on iterative molecular networking and nuclear magnetic resonance: application to lignocellulosic biomass samples.

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Considering the current environmental challenges transforming lignocellulosic biomass into bioethanol offers a sustainable alternative to petroleum fuels [1]. Some bioconversion processes imply a pretreatment step which deconstructs the native biomass' polymeric backbone and produces cellulose-rich solids soaked by aqueous phases called hydrolysates. These complex mixtures contain a wide range of oxygenated compounds that could be used as bio-based building blocks (i.e. platform molecules) [2 3]. Therefore a comprehensive molecular description of these biomass by-products is essential for the complete valorization of the conversion process. In this context dereplication strategies which imply the initial identification of previously referenced species prior to new compound discovery could provide a valuable initial chemical overview of the hydrolysate samples [4].

Dereplication approaches based on nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography hyphenated to tandem high-resolution mass spectrometry (LC-HRMS/MS) data exploitation have shown significant potential [5–8]. Yet direct characterization of complete hydrolysates by NMR would prove challenging with the considerable number of constituents of these intricate samples and their limited overall concentration [9]. The feature-based molecular networking (FBMN) workflow [10] which uses spectral similarity to group compounds has been largely used in untargeted LC-HRMS/MS approaches. However multiple processing parameters require fine-tuning before generating informative networks [11]. The selection of the minimum matched peaks (MMP parameter) which specifies the minimum number of similar fragmentation peaks for compound grouping was particularly important. In the case of small molecules (50 – 1200 Da) setting a high value would increase the feature matching confidence but also increase the number of ungrouped species (i.e. solitary nodes) showing limited fragmentation. Conversely selecting a low value would favor grouping but markedly decrease confidence in node matching. In this study liquid-liquid extractions (LLE) and centrifugal partition chromatography (CPC) [12] were initially used to fractionate the biomass hydrolysates and provide chemically simplified analyzable samples. An original iterative strategy based on Python was then used to split the LC-HRMS/MS dataset to select the optimal MMP parameter for the FBMN workflow. The resulting molecular networks representing varying confidence levels were subjected to a combined HRMS/MS and NMR dereplication approach to profile the studied samples and highlight constituent chemical families.

Twenty industrial hydrolysates from heterogeneous biomass feedstocks were initially pooled and concentrated to produce a model sample for this study. The latter was then subjected to consecutive LLE and CPC steps to fractionate the constituent chemical families into 41 concentrated samples. Those simplified fractions were then analyzed by LC-ESI(±)-HRMS/MS in data-dependent acquisition

mode by one-dimensional (1H & 13C) and by two-dimensional NMR experiments (COSY HSQC & HMBC). Initial mascot generic format (mgf) files containing detected precursors and their associated fragments were obtained after preprocessing positive and negative LC-HRMS/MS data. A Python code was then employed to iteratively split the mgf files into sub-files containing only precursors with selected minimum fragment counts. For ESI (+) 9 sub-files were generated with minimum fragment counts (MFC) ranging from 4 to 12 while for ESI (-) 13 sub-files were generated with MFC ranging from 4 to 16. All mgf files were processed through the FBMN workflow to generate 2 global MN and 22 sub-MN. The sub-molecular networks were all generated using a "MMP = MFC" strategy (e.g. for the mgf file containing only precursors with more than 7 fragments 7 was selected as the MMP parameter). As a result 9 and 13 MN with varying match confidence levels were obtained for ESI (+) and ESI (-) respectively. Higher node-matching confidence was associated to clusters in MN generated from higher MMP values as they intrinsically implied higher molecular similarity between matched features. Subsequently the SIRIUS v5.8.5 HRMS/MS workflow was applied to the nodes and clusters present in the MN. Multiple possible compounds were assigned to each feature after the query of molecular databases. The most probable candidates were then selected and subjected to cross-validation using NMR by comparing simulated and experimental spectra. Additionally the CaraMel NMR workflow which consists of 13C data alignment pattern recognition through hierarchical clustering analysis and chemical shift database query was used to validate the cross-validated candidate choice and confirm structural details [13]. Finally the combined HRMS/MS and NMR identification was manually propagated to the neighboring nodes in the MN to ensure thorough molecular characterization.

This study showcases the use of a combined iterative molecular networking and NMR dereplication strategy for enhanced chemical identification in complex samples. The first part of the presentation will focus on developing the iterative split strategy and its application to the biomass sample's LC-HRMS/MS dataset. The propagation of confident chemical family identification across the 22 MN will then be discussed. Ultimately the interest of our approach will be highlighted through the identification multiple compounds from oligosaccharides coumaric acid derivatives carbohydrate-derived coumaric acid esters and lignan phenolic derivatives chemical families.

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Oral

Development and challenges of an SFC-SFC configuration in multiple heart-cutting to analyze chiral flavonoids from bitter orange (Citrus aurantium L.)

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The rapid development of analytical technologies has opened up new perspectives in chromatography with significant advances in multidimensional chromatography. The latter provides higher resolution than one-dimensional chromatography1 notably through the possibility of combining different selectivities in the two dimensions.

Compared with LC SFC offers a number of advantages such as the high efficiency at high flow rate environmentally-friendly and cost-effective solvents (principally CO2). Considering two-dimensional chromatography using the same chromatographic mode in both dimensions avoids problems associated with mobile phase incompatibilities. For instance achiral and chiral SFC methods are operated with the same mobile phases (usually CO2 and an organic co-solvent).

However setting up a two-dimensional SFC system in (multiple) heart-cutting mode is complex. It involves modifying a one-dimensional system by adding valves and loops: valves to bypass the first or second dimension and loops to store the fractions harvested from the first dimension before sending them to the second. This additional dimension can create multiple effects such as peak distortion or repeatability problems in the second dimension due in particular to the variability of loop volumes or valve switch times. It is therefore important to understand these effects in order to ensure the repeatability and conformity of analyses.

In this presentation we will demonstrate the setting of a multiple-heart-cut SFC-SFC system and exemplify its use with samples of bitter orange (Citrus aurantium L.). The latter has many soothing and relaxing properties related to its flavonoid content which is used in cosmetics and pharmaceuticals3. In particular it contains chiral flavonoids (flavanones)2. In this study samples of Citrus aurantium L. were analyzed with the SCF-SFC system with an achiral separation in first dimension to isolate the target flavonoids and a chiral separation in second dimension to quantify the enantiomeric or diastereomeric purity.

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Oral

Implementation of a QA/QC system for untargeted GC×GC analysis.

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Over the past few decades the field of analytical metabolomics has witnessed continuous growth particularly in the realm of investigating human health through untargeted analyses. The primary goal has been to detect and identify a broad spectrum of small molecules [1]. The integration of comprehensive two-dimensional gas chromatography (GC×GC) has been a pivotal advancement in facilitating these untargeted analyses [2]. However as this technology has become an indispensable part of research in this domain the pressing need for enhancing and standardizing quality assurance (QA) and quality control (QC) practices in untargeted metabolomics has come to the forefront. The metabolomics quality assurance and quality control consortium (mQACC) have played a significant role in developing various QA/QC systems [3]. Despite these efforts the field continues to face challenges with a noticeable lack of documentation and standardization highlighting the importance of addressing these gaps for the advancement of untargeted metabolomics research [4].

In our research we delved into the establishment of a robust QA/QC system tailored for untargeted analysis employing two distinct GC×GC-TOFMS systems. Initially we conducted a thorough comparison of the analytical capabilities of two GC×GC instruments each equipped with a different modulator system—a cryogenic and a Peltier system. Our investigation involved scrutinizing the analytical performance through the analysis of alkanes (C8-C20) standard solutions and QC solution mix comprising 37 compounds with diverse chemical properties derived from high-quality analytical grade standards.

Subsequently we devised a comprehensive method for monitoring the behavior of 37 different compounds utilizing both systems. Indeed the comparison between the two systems has revealed that despite having similar analytical parameters and systems (including the same column set injection and chromatographic methods as well as mass spectrometry (MS) systems) some variations on compounds detectability can be observed. This underscores the importance of implementing a robust QA/QC system.

The analytical method developed for monitoring the 37 compounds each exhibiting varying volatility and response due to the diverse chemical functions represented in the panel has proven effective. Compounds are distributed across the entire 2D chromatogram demonstrating good distribution and separation in both the first and second dimensions over a 35-minute run.

In examining the control charts generated for both instruments over the last few months no deviations for retention times were observed for all compounds indicating the system's suitability for extended run periods. Additionally the areas remained stable over months with an average deviation of 7.73% ± 4.85 for all 37 compounds. This method not only showcased the versatility of the systems but also highlighted its efficacy in handling a diverse range of compounds.

Finally to ensure the reliability and stability of our analytical processes we implemented a sophisticated QA/QC system featuring control charts. This system meticulously tracked the evolution of retention time and response (area values) for all 37 compounds over six months. Our final goal is to extend this systematic follow-up to encompass all four GC×GC systems within our laboratory. By doing so we aim to establish a standardized and robust QA/QC framework that will contribute to the precision and reproducibility of our analytical endeavors in the long run for untargeted metabolomics.

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Oral

FAST GAS CHROMATOGRAPHY USING A DIRECTLY RESISTIVELY HEATED GC COLUMN – ENABLING NOVEL MODES OF OPERATION AND A BETTER THEORETICAL UNDERSTANDING OF THE SEPARATION PROCESS

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Improving the speed of chromatographic separation while maintaining chromatographic resolution is one of the challenges in the development of advanced chromatographic techniques and instrumentation. The interrelation of the parameters separation time capacity of the separation system and separation efficiency requires working under compromise conditions that are typically well-accepted for gas chromatography. Accelerating the separation beyond optimum conditions inevitably compromises the other two aspects.

Innovative approaches have been proposed over the past decades to overcome this limitation including multiplexing GC [1] vacuum-outlet GC [2] or thermal gradient GC [3]. The latter technique employs a short chromatographic column which is exposed to a negative thermal gradient along the column length. Separation time is reduced due to the fact that the column length is typically only 3-5 m compared to the 30 m of a regular GC column while separation efficiency is maintained as the peaks are focused by the negative thermal gradient in longitudinal direction. Very narrow peak widths can thus be achieved which together with the fast temperature gradient allow completing separations in less than one minute that would typically have taken a half hour on a conventional GC system.

We have introduced a new twist to the concept of thermal gradient GC as implemented by Boeker et al. [4] by using an innovative approach for controlling the temperature along the chromatographic column: Instead of applying constant heating and gradient cooling (by a forced air flow) to achieve the thermal gradient we are controlling the temperature along the column segment-wise by individual heating only segments of the separation column which offers a much greater flexibility of controlling temperatures or producing thermal gradients along the (short) GC column. Thus isothermal temperature-programmed as well as thermal gradient operation can be realised. Moreover the temperature profile can be controlled to move in waves through the column providing thus separations of exceptional speed and good resolution.

We will demonstrate the applicability of this system called by us the “Wavemaker” for fast (less than 1 minute) separations of hydrocarbon mixtures and other relevant applications. The theory underlying to this novel mode of separation will be presented and used to discuss possibilities to even further improve / accelerate gas chromatographic separation by this moving thermal gradient.

Acknowledgements

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Oral

In vivo solid-phase microextraction for therapeutic monitoring and pharmacometabolomic fingerprinting of lung during in vivo lung perfusion of FOLFOX

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In vivo lung perfusion (IVLP) is a novel isolated lung technique developed to enable the local in situ administration of high-dose chemotherapy to treat metastatic lung cancer. Combination therapy using folinic acid (FOL) 5-fluorouracil (F) and oxaliplatin (OX) (FOLFOX) is routinely employed to treat several types of solid tumours in various tissues [1]. However F is characterized by large interpatient variability with respect to plasma concentration which necessitates close monitoring during treatments using of this compound. Since plasma drug concentrations often do not reflect tissue drug concentrations it is essential to utilize sample-preparation methods specifically suited to monitoring drug levels in target organs. In this study in vivo solid-phase microextraction (in vivo SPME) is proposed as an effective tool for quantitative therapeutic drug monitoring of FOLFOX in porcine lungs during pre-clinical IVLP and intravenous (IV) trials [2]. In vivo SPME facilitates simultaneous isolation of FOLFOX and its metabolites in pig lungs during the pre-clinical IVLP and IV administration of this drug combination. The chemotherapeutic efficacy of these administration routes was subsequently assessed via pharmacometabolic fingerprinting via liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) to illustrate SPME's ability to characterize changes in lung tissue over the course of the IVLP and IV procedures. Furthermore a supplementary study was conducted to assess the metabolomic profile's stability under the standard storage conditions (-80 °C) currently employed in metabolomics to determine whether these conditions were conducive to reliable sample preservation. The concomitant extraction of endogenous and exogenous small molecules from the lung via in vivo SPME and their detection using LC-HRMS enabled an assessment of FOLFOX's impact on the metabolomic profile of the lung and revealed the metabolic pathways associated with the route of administration (IVLP vs. IV) and the therapy itself. This study also shows that the immediate instrumental analysis of metabolomic samples is ideal as long-term storage at -80 °C results in changes in the metabolite content in the sample extracts.

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Oral

Ion Chromatography-Mass Spectrometry: A Critical Tool for Environmental Industrial and Natural Products Research

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Since its development by Small in 1971 [1] and following 52 years of continuous innovation ion chromatography (IC) has earned its status as the preferred technique for ion analysis [2]. Today ion chromatography finds applications in various fields including water quality environmental studies food analysis nutraceuticals forensics industrial processes and pharmaceutical research [345]. Significant developments achieved in IC technology specifically eluent suppressors have facilitated the hyphenation of IC with mass spectrometry (MS) examples of which date back to the early 90's [6]. Many ions that are important in solution chemistry but could not be produced in the gas phase back then could be analysed in the gas phase with the introduction of electrospray ionization (ESI) in the late 90's [7]. Since then these applications have continued to expand benefiting from IC's unique selectivity and the sensitivity specificity and structural elucidation capabilities of MS [8]. Nonetheless despite its numerous advantages and extensive applications IC-MS continues to retain comparatively limited visibility and recognition within the broader domain of mass spectrometric hyphenated techniques.

In this context we aim to underscore the versatility of this technique by presenting several case studies conducted at the Australian Centre for Research on Separation Science (ACROSS) over the past five years employing IC-MS. These studies include the analysis of inorganic anions and oxyhalide disinfection by-products in potable water and aquaculture marine systems the assessment of biomass burning markers in atmospheric aerosols filters and lake sediments anion analysis in complex samples from sustainable chemical manufacturing processes the analysis of xylooligosaccharides produced from enzymatic biomass hydrolysis and the quantification of boron in preservative-treated timber.

Throughout these case studies we will discuss the advantages of employing IC-MS the instrumental setup requirements key parameters in method development and challenges encountered. The results achieved in each unique scenario validate the applicability of IC-MS for monitoring of environmental contaminants industrial processing monitoring and research on green and sustainable biomass products.

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Oral

Aflatoxin determination in food: a greener DES-based extraction followed by SPE-UHPLC-FLD analysis

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Since their discovery in the 1960s aflatoxins have become a significant public health concern presenting a formidable obstacle to ensuring food quality. Due to the carcinogenic nature of these substances and their widespread presence in various food types regulatory authorities have established maximum allowable levels for these contaminants in such products. Traditional official methods have traditionally employed substantial quantities of methanol/water or acetonitrile/water solutions for extraction given the slightly polar characteristics of aflatoxins. Food matrices high in lipid content such as pistachios (constituting approximately 40% of the total lipid fraction) typically require multiple sample preparation steps before analysis. Specifically a de-fatting step using hexane is commonly used to reduce the presence of lipid-based interferents from the matrix.

A novel approach has been developed utilizing choline chloride–urea (molar ration 1:2) deep eutectic solvent (DES) + 20% H2O (w/w) as the extraction medium. Given the complex nature of extracts obtained from aflatoxin-contaminated pistachios a subsequent purification and concentration step becomes crucial. The DES extracts are diluted with water and passed through a C18 solid-phase extraction cartridge. A minimal amount of methanol is used to elute the trapped aflatoxins which are then injected into a UHPLC system coupled with a fluorescence detector. A 2-µm partially porous C18 column was employed to effectively separate aflatoxins. The mobile phase consisted of a gradient elution system using methanol and water ranging from 30% to 60% MeOH over a period of 7.5 minutes.

The developed methodology demonstrated robustness and reliability for each analyte (CV% lower than 6.03%) with limits of quantification (LOQs) below 0.41 ng/g without using MS detectors and/or derivatization agents.

The environmental friendliness of the developed procedure was assessed through two relevant metrics namely AgreePrep and WhiteChemistry – RGB indicating superior performance compared to official methods.

Oral

Bringing machine learning into tile-based processing for complex GC×GC-TOFMS datasets

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In the quest of making multidimensional gas chromatography (MDGC) a robust method for untargeted screening of small molecules one of the key remaining challenges is the data processing meaning transforming raw data into pertinent information [1]. To reach this objective important analytical aspects such as data integrity workflow transferability and comparability need to be tackled. For MDGC the tile-based approach developed by the Synovec Group has gained popularity to conduct chromatogram comparison [2]. The success of this method relies on its large applicability its commercial availability and its lower computing power requirements.

The tile-based approach is mostly used in combination with Fisher Ratio Analysis (FRA). This analysis of variance (ANOVA) is performed in order to identify the tiles which display a significant difference between classes. FRA is a highly efficient univariate statistical test. The statistical background is easy to grasp and the link with the chemical information is easy to establish. Nevertheless FRA suffers for some limitations. The variance within the different classes should be equivalent the univariate dimension could underestimate the importance of correlated variables and it is not suitable for unbalance data set. Over the years several complementary methods have been tested to overpass the first limitation [3]. In this study we aim to tackle the two others. We have developed and evaluated a new processing approach combining tile-based image comparison and machine learning-based feature selection. Our approach is using the four-grid tiling scheme with random forest (RF) algorithm. RF was selected for its capacity to work with unbalanced data and its low dependency on the data pre-processing methods [4].

Nevertheless several challenges needed to be overcome for the implementation of such tool. The used of RF required the development of new feature selection metrics and the set-up of cross-validation protocols for the training and the testing of the workflow. We have combined out-of-bag error m/z purity and fold change calculation to evaluate our workflow output.

The study was conducted on a data set based on whole stool research grade test materials (RGTMs) prepared by NIST for interlaboratory studies. The RGTMs contain two diets vegan and omnivore and two sample formats liquid vs lyophilized. In this study we have focused on two groups: omnivore liquid and omnivore lyophilized. This data set was processed using the classical FRA approach and our RF workflow.

Our findings suggest that the RF approach is a promising method for identifying class-distinguishing analytes in settings characterized by both high between-class variance and high within-class variance making it an advantageous method in the study of complex biological matrices.

These promising results open the door to future investigations where other machine learning algorithms could be used for the feature selection. Moreover these RGTM data set will also be

processed using other exciting and emerging data processing workflows to evaluate comparability and transferability of these workflow. Finally the combination with retention prediction tools could improve the way we compare the tiles across chromatogram.

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Oral

Capillary electrophoresis with inductively coupled plasma-tandem mass spectrometry as a technique for investigation of anticancer drug-loaded liposomes

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Tumors are one of the leading causes of death worldwide and still nowadays chemotherapeutics indicate many side effects. To reduce them and increase treatment efficiency the application of drug carriers e.g. liposomes seems crucial. Liposomes are biocompatible biodegradable and non-toxic carriers. A few analytical techniques are used to investigate the fate of drug-loaded liposome systems in vitro and in vivo. Still most of them have some shortcomings e.g. the presence of numerous interferences. In response to these drawbacks a hyphenated technique capillary electrophoresis (CE) with inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) was proposed here. It is a comprehensive technique for drug–loaded liposome characterization and allows information about liposome stability size distribution and drug encapsulation efficiency (EE%) to be obtained.

During our study we have been changing the lipid composition initial drug (cisplatin) amount and applying different post-formulate modifications to get the most suitable liposome preparation procedure characterized by the highest encapsulation efficiency narrow size distribution of formed nanomaterial and stability of carriers. CE-ICP-MS/MS and dynamic light scaterring techniques were used to monitor these parameters.

In the first step of the investigation the method through which the sample components will be separated with high signal resolution and low reagent consumption has been optimized. Using a tandem mass spectrometer it was possible to obtain the quantitative monitoring of both phosphorus (liposome marker) and platinum (drug marker) signals. Then the influence of lipid composition drug amount and (post)formation liposome conditions on the drug encapsulation efficiency was tested. Moreover another advantage of CE-ICP-MS/MS was the possibility of monitoring liposome interactions with proteins (due to sulfur monitoring). After incubating carriers with human serum which mimics in vivo conditions we can determine if proteins had interacted with liposomes predict carriers' behavior in blood and assess their stability and drug leakage.

Oral

Recent advances in retention time prediction: Impact of structural similarity and practical applications

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In this contribution we provide a systematic study of the relationship between the accuracy of the retention model (Quantitative Structure-Retention Relationships QSRR) the size of the training set and its structural similarity to the predicted compound. The results presented indicate that accurate and predictive models can be built from a small dataset containing as few as 25 compounds provided that the training set meets certain similarity criteria. These findings potentially bring the prediction of retention times within the practical reach of pharmaceutical analysts involved in chromatographic method development and enable QSRR to be used for de-risking chromatographic methods in relation to newly formed or hypothetical compounds arising from synthetic processes or formulation activities. They can also be used to identify optimal separation conditions or in support of structural elucidation.

Oral

Online coupling of size exclusion chromatography to Raman spectroscopy for the analysis of therapeutic proteins and disaccharides

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Biopharmaceuticals i.e. therapeutic proteins are a steadily growing business area of the pharmaceutical industry. The therapeutic effect of proteins is determined by their secondary and tertiary molecular structure. Any variations in this structure can lead to a loss of their pharmacological activity. Compared to small molecules as active pharmaceutical ingredients (APIs) therapeutic proteins are much more complex and vulnerable to external influences. Therefore techniques that enable for a direct verification of structural integrity are of major interest.

Raman spectroscopy (RS) is a powerful technique for structural analysis of proteins and other compounds. In combination with size exclusion chromatography (SEC) proteins can be separated from matrix components without compromising their native structure. Thus online coupling of SEC and capillary enhanced Raman spectroscopy (CERS) enables structural assessment and identification of proteins in biological samples or biopharmaceutical formulations. Separated proteins are excited coaxially while flowing through the pathway of a liquid core waveguide (LCW). The Raman scattered light is then detected in backscattering assembly.

The online coupled SEC-CERS enables the acquisition of Raman spectra of proteins such as bovine serum albumin hen egg white lysozyme and β-lactoglobulin and of the monoclonal antibody rituximab in a medicinal product[1]. Furthermore serum albumin and IgG antibody can be identified in a highly complex sample such as porcine blood serum according to their Raman spectra obtained by SEC-CERS.

Additionally the CERS detector can be applied to quantitate the excipient sucrose in a biopharmaceutical drug product. Since disaccharides have no chromophores UV/vis absorption detection is not applicable for these analytes and the CERS detector offers a suitable alternative. A limit of detection (LOD) of 120 µg and a limit of quantification (LOQ) of 363 µg injected on the column can be achieved for sucrose by CERS detection[1].

Yet online coupling of SEC to CERS requires advanced data post-processing which poses new challenges compared to offline Raman spectroscopic applications. Therefore a software platform has been developed to support and automate the multi-step processing of Raman spectra acquired online by CERS.

In conclusion online coupled SEC-CERS can be a promising technique for the biopharmaceutical development or quality control since it enables the simultaneous acquisition different characteristics

of biopharmaceutical products i.e. the elucidation of the higher-order structure of proteins and a quantitation of excipients.

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Oral

Overcoming the challenge of large-volume modulations in coupling organic SEC with RPLC to empower forensic characterization of explosives

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In recent years much of the research in improving the applicability of two-dimensional liquid chromatography (2D-LC) has focused on solving one of its biggest challenges: solvent incompatibility. Solvent incompatibility occurs when two complementary separation modes are coupled where the solvent of the first dimension (1D) is a strong eluting solvent for the second-dimension (2D) separation mode. Several strategies have emerged to tackle this problem including stationary-phase-assisted modulation (SPAM) active solvent modulation and at-column dilution. However some of these state-of-the-art methods are not as straightforward to use in an extreme scenario where a large modulation volume is required to maintain sensitivity in heart-cut 2D-LC applications.

Smokeless powders (SPs) are a type of sample where the application of 2D-LC will likely result in such a challenge. SPs are used as propellants for ammunition but can also be exploited as energetic material in improvised explosive devices such as pipe bombs. SPs comprise of mainly nitrocellulose (NC) a polymeric material and also contain additives including stabilizers and small-molecule explosives like nitroglycerin. Depending on the manufacturer and the type of rifle or rocket that the propellant is designed for a different additive composition or quality of NC may be used. For this reason the in-depth characterization of SPs is of significant importance to aid forensic investigations for both pre- and post-blast explosive casework as well as any shooting investigations. In this study a two-dimensional liquid chromatography (2D-LC) method was developed to obtain information regarding the NC component and simultaneous chemical profiling of the additives.

The system combined size-exclusion chromatography (SEC) and reversed-phase liquid chromatography (RPLC) in an online heart-cut 2D-LC configuration. In the first dimension the SP was separated into its two main components NC and additives and the polymeric NC was characterized by the molecular-weight distribution (MWD) and its nitration degree. The small-molecule additives eluted near the void volume of the SEC column. However due to the variation of molecular weight and polarity of the additives they did not elute as one sharp peak. On the contrary the elution volume containing all additives was roughly 600 µL of 100% THF. To obtain a quantitative additive profile and maintain 2D sensitivity this entire fraction should be transferred to the 2D RPLC column in a single modulation. To overcome this challenge published modulation strategies provided insufficient dilution or focusing of the relatively polar additives to maintain good 2D peak shapes.

Therefore a combination of several modulation strategies was required to create a tailored modulation approach. Ultimately multiple parameters of smokeless powders could simultaneously be determined in one system. High discrimination powers were obtained for these parameters individually resulting in high combined evidential value. This novel combination enables a detailed forensic comparison of SPs. Additionally as opposed to conventional SP analysis methods no extensive sample preparation is required making the developed method less labor intensive and less sensitive to human errors during sample preparation.

Oral

Determination of unknown concentrations without calibration curve by modelling molecules ionisation in LC-MS through machine learning

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In LC-MS unknown concentrations are usually determined by drawing a calibration curve. However this method requires an available sample of known concentration to be applied. Other methods are also available for concentration determination such as MRN or spectrophotometry. Although being well known and currently used these methods are poorly suited for impure samples synthesized in the laboratory or for unknown mixture. Machine learning has already been used in the literature to predict the ionization efficiency of molecules [12]. More precisely RandomForest algorithm is frequently used in this context because of its simplicity and good results when compared to frequently used techniques such as lasso or linear models [2]. In this project we developed a method to estimate an unknown concentration by modelling the relation between a molecule physical and chemical properties and its ionisation in LC-MS. We then applied this method for the quantification of seven modified nucleosides synthesised at the laboratory.

For the training part of the machine learning we used 30 molecules chemically close to our unknown samples: nitrogenous base ribonucleoside deoxyribonucleoside and nucleotide modified or bot. All these compounds were injected on our LC-MS system with the same column in isocratic condition. They were injected individually at known concentration to obtain similar peak area. More than 16 000 physical and chemical parameters were then computed for each molecule with OCHEM web platform [3] using 5 different algorithms 2 of them computing 2D parameters : OEState ALogPS and 3 of them computing 2D and 3D parameters: alvaDesc V.2.0.16 Dragon V.7 PaDEL2. Some parameters were redundant between different algorithm they were removed from the model. CHEMAXON structure preprocessing was used with standardization neutralize and clean structure and 3D structure were optimized with OpenBabel tool. The parameters obtained with OCHEM were then used to construct a Random Forest model which was able to associated computed and experimental parameters to the concentration of the sample. Optimization of the model was then made by selecting the 8 more relevant parameters. As per usual 90% of the data were used to build the model and 10% were used to test it.

Using this method we obtain a final explained variance for our model of 65%. The model was able to estimate the concentration of our test samples with a deviation of 17% for the highest concentration molecule (1ppm) 5% for the medium range concentration molecule (0.5ppm) and 60% for the lowest concentration tested (0.05 ppm). Thanks to this method we were able to estimate unknown concentration of 7 laboratory synthesised molecules with only a few injections in LC-MS.

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Oral

Elevating Odorants Analysis in Alcoholic Beverages: Advancements in SPME Extraction Strategies to Tackle Low Sensitivity and Displacement Challenges

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It is estimated that approximately 10000 volatiles are present in food yet only a small fraction significantly contributes to the final aroma of food products. A major challenge arises from the fact that many of these aroma-active compounds exist in extremely low quantities often at the ppt (part per trillion) level and are characterized by high polarity (logP < 1). The inherent difficulties in analyzing such compounds stem from the low sensitivity and affinity of sorbents or solvents towards polar analytes rendering odorants analysis a complex and demanding process. Alcoholic beverages serve as an ideal model for investigating odorants due to their rich composition of volatiles with diverse physicochemical properties coupled with the frequent occurrence of off-flavors in these products. Various methods advocate for developing Solid-Phase Microextraction (SPME) citing its efficiency robustness reliability automation potential and eco-friendly nature. However a notable challenge in SPME for food samples particularly those with a complex matrix composition or a high concentration of hydrophobic compounds is the displacement effect. This effect can impede the quantitation of analytes with low affinity towards the extraction phase.

In addressing this issue novel strategies will be presented and thoroughly discussed. These innovative approaches include sequential TF-SPME extraction the fully automated integration of TF-SPME with conventional SPME fibers and the addition of C18 particles before the extraction process to the sample. These procedures aim to reduce the presence of highly non-polar compounds potentially enhancing the efficiency of extracting polar compounds.

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Oral

Heart-Cut 2D LC-MS as Tool for the Analysis of Cholesterol Biosynthesis in Pancreatic and Melanoma Cancer

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Recent studies have highlighted the role of cholesterol in various types of cancers highlighting the importance of understanding the corresponding biosynthetic pathways. However characterizing the complex biosynthesis of cholesterol poses different analytical challenges arising from structural similarities matrix interferences and significant concentration differences among precursor molecules and cholesterol itself.

This study presents a novel heart-cut 2D LC-MS method designed to address these challenges. Therefore a one-dimensional separation utilizing a pentafluorophenyl (PFP) phase was developed to achieve baseline separation of sterols with identical mass-to-charge ratios. Using a trap-based modulation the cholesterol peak was selectively transferred for separate analysis to reduce matrix interferences caused by the high µM concentration of cholesterol. A short C18 column served as the trap facilitating the transfer of a 240 µL fraction from the first dimension. The cholesterol and coeluting dimethylcholestadienol were focused followed by a second separation using an EC-C18 column. Further integration of a six-port valve allowed the simultaneous detection of both substances using APCI-QqQ-MS in scheduled-reaction-monitoring (SRM) mode ensuring high sensitivity for low-abundance sterols.

The developed method characterized according to ICH guidelines on bioanalytical method validation exhibited low detection limits (10 - 300 nM) and high robustness. Long-term measurements were conducted and matrix effects on quantification were investigated demonstrating the reproducibility of the method. The fully characterized method is applied to analyze pancreatic and melanoma cancer samples and provides valuable insights into biosynthetic changes at the metabolic level.

Acknowledgment

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Poster

Theoretical and practical guidelines for solvent dilution between the two dimensions in online comprehensive two-dimensional liquid chromatography

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With the increasing complexity of samples in diverse application areas such as pharmaceuticals environmental studies energy research and food science conventional one-dimensional liquid chromatography (1D-LC) can be limited in terms of speed sensitivity and ability to resolve multiple components. To address these challenges comprehensive two-dimensional liquid chromatography (LC x LC) has emerged as an attractive solution particularly in the online mode (online LC x LC) which has grown significantly in popularity in recent years.

Among the different chromatographic modes that can be combined hydrophilic interaction chromatography (HILIC) and reversed-phase liquid chromatography (RPLC) are particularly interesting because they offer a high degree of orthogonality. However the combination of these two modes remains complex to implement due to the opposite solvent strength in both dimensions. The first dimension (1D) effluent may adversely affect the 2D-separation leading to strongly distorted peaks. To avoid this problem one solution is to dilute the 1D-effluent with (zdilution -1) volumes of a weaker solvent added to one volume of the 1D-effluent where zdilution represents the factor by which the fraction volume has been multiplied. This can be achieved either by using active solvent modulation technology or by using an additional pump prior to the second dimension analysis.

The aim of this study 1 was to develop theoretical models to predict whether or not dilution can be effective and if so what is the minimum zdilution value required. Our approach is based on the calculation of the ratio (called xdilution) between the peak standard deviation due to the injection process and the peak standard deviation in the absence of extra-column dispersion. Xdilution is calculated for any compound of interest on the basis of theoretical relationships and plotted as a function of zdilution to predict the dilution required to obtain a good peak shape for that compound bearing in mind that the maximum xdilution value was found experimentally to be equal to about 1. The proposed theoretical approach was experimentally validated on a number of representative small molecules and peptides. The agreement between experimental results and theoretical models was very good especially for small molecules. Finally it is shown that this approach combined with previously developed prediction tools 2 can predict for given compounds the most appropriate set of conditions in HILIC x RPLC. This predictive capability is significant because it is expected to make the method development process simpler and more systematic thereby accelerating the progress of 2DLC method development in many application areas.

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Poster

Altering the supporting material and coupling chemistry of temperature responsive liquid chromatography (TRLC) for enhanced column life-time and performance.

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Temperature-responsive liquid chromatography (TRLC) has been further developed as an alternative to conventional reversed-phase liquid chromatography by allowing for tuning both retention and selectivity solely through temperature. In recent years the applicability of this unconventional separation approach has been demonstrated through a variety of semi-polar to polar compound classes. Additionally TRLC offers unique benefits in terms of detection whereby e.g. the isocratic character of the approach now allows the application refractive index detection while obtaining enhanced control of the retention through the use of temperature gradients.1 The use of TRLC in the first dimension of LCxLC analyses has also been demonstrated to allow avoiding issues regarding modulation.2 Despite these promising applications some issues still linger regarding limited column lifetimes as a loss of retention is observed over time when operating the columns extensively under mostly aqueous conditions at elevated temperatures (>30°C).

Since the acrylamide-based polymers should be stable under these conditions this loss of retention likely originates from loss of polymer from the supporting material. For which there are currently two distinct sources. The first being the use of aminopropyl silica which is sensitive to hydrolysis due to use of elevated temperatures under aqueous conditions. This is facilitated through backbiting of unreacted aminopropyl groups on the silica particle.3 The second possibility is potential cleavage of the amide-bond which is currently used to chemically couple the polymer to the silica particles. A combination of elevated temperatures together with low pH might in this case also result in hydrolysis.4

To further increase column lifetimes while identifying the root cause leading to loss of modification over time two new types of temperature-responsive stationary phases were developed. In the first approach the existing coupling chemistry is replaced by a thiol-epoxide based click chemistry to investigate the relative stability in comparison with the original amine-ester coupling yielding an amide. Since this methodology also immediately avoids the presence of free amines on the silica particle a second approach was investigated in which commercially available polystyrene-divinylbenzene (PS-DVB) particles were modified through amine-ester type coupling. The column lifetimes and overall performance using of these two approaches were compared extensively with current column technology. This with series of small organic test compounds and with more complex natural or synthetic sample mixtures.

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Poster

Evaluating transferability of GC methods across carrier gases for improved sustainability

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As an industry we are making strides towards greater laboratory sustainability. In addition to the fact that there are increasing pressures from global supply shortages of important resources replacing non-renewable and expensive resources is becoming more important by the day. Within GC this can be seen by the shift from helium carrier gas to more sustainable options such as nitrogen and hydrogen. This poster details the work that has been done to demonstrate the options for transferring methods between carrier gases using a simple solvent mixture. It discusses two approaches investigated including one which maintains linear velocity vs one which maintains efficiency and the pros and cons of each. The overall goal is to make method transitions easier for all by breaking down the method development barrier to sustainability improvements in chromatography.

Poster

Novel Photoionization Source with Carbon Fluorescence

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To enable application in many areas ionization-based detectors such as ion mobility spectrometers (IMS) must be cost-effective and compact devices. In particular the use of a non-radioactive ionization source reduces costs and effort on the user side as it is not subject to legal restrictions. In this article we report on a compact low-energy photoionization (LEPI) source. A carbon-based transmission electrode is used avoiding toxic beryllium. The achieved low photon penetration depth of 0.5 cm allows for small detector volumes. In the example of an IMS it enables field-switching ion shutters required for highest sensitivity. Furthermore low voltage of only 500 V is needed for LEPI operation enabling a very compact power supply and high-speed switching.

Low energy photo ionization

The LEPI source consists of a TO housing in which a thermal electron emitter is operated and the electrons are accelerated onto a carbon membrane with a thickness of 1 µm [1]. We will present measurements of the emitted photon spectra for various acceleration voltages showing that the emission is dominated by the carbon fluorescence line at 277 eV. Due to the low thickness of the membrane a high integral intensity is achieved even at low acceleration voltages. Furthermore using an anode at 200 V in 1 cm distance the ion current is compared for different voltages and different anode materials. We compare the 1 µm thick pure carbon membrane to a carbon membrane with a 50 nm gold target and a standard 125 µm beryllium membrane with a 750 nm silver target. At low acceleration voltages the pure carbon membrane achieves the highest intensity even though the higher energy content is reduced due to the decreased bremsstrahlung efficiency caused by the absence of the metal target. Even compared to higher voltages the increase in ion current is only about one order of magnitude for the metal target membranes. This difference can be mitigated by a higher emission current.

Operation in an IMS

A first proof of principle measurement in a field-switching IMS is presented. The reactive ion peak generated by the LEPI source is compared to a standard tritium source setup (mean energy of about 5.7 keV). At an acceleration voltage of 500 V and 5 µA emission current the LEPI source reaches a significantly higher ion intensity. Furthermore the thermionic source can be operated at currents up to 200 µA giving much headroom for even higher ion intensities and thus detector sensitivities.

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Poster

Novel non-radioactive Electron Capture Detector based on a Hot Electron Emitter

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Translator

In this work we present a novel type of electron capture detector (ECD) based on a hot electron emitter as a non-radioactive electron source. ECDs are typically used as gas chromatography (GC) detectors for analyzing complex trace gas mixtures containing electron-affine substances. They are characterized by their fast response high linearity of up to four orders of magnitude and detection limits in the lower pptv range especially for polyhalogenated compounds. This makes GC-ECD particularly suitable for analyzing pesticides or other environmental pollutants. However due to the radioactive sources used in most commercial ECDs their operation results in high costs and effort due to legal requirements which has led to a decreasing number of ECDs being used in analytical laboratories. Non-radioactive commercial alternatives do exist but have not yet achieved a broad acceptance for various reasons. Therefore we introduce a new type of non-radioactive ECD using a hot electron emitter as a small sized source of free electrons at ambient pressure.

Electron emission of this hot electron emitter is based on Fowler-Nordheim tunneling which is facilitated by the stacked structure of a non-conductive oxide layer and a thin conductive carbon layer on a highly n-doped silicon substrate. This structure allows electrons to tunnel at low operating voltage (< 20 V) into the conduction band of the oxide where they are accelerated by the electric field. As the carbon layer is thin and has a low electron scattering cross section a portion of the tunneling electrons are available as free electrons in the gas phase.

In the current generation of such hot electron emitters the emitting electrons do not have enough energy yet to further ionize the neutral gas which leads to a fundamental difference between the here presented ECD and a common radioactive ECD. Radioactive ECDs can only achieve their high sensitivity by secondary ionization of the carrier gas or any dopants by the high-energetic radiation. Nevertheless the high electron emission of the hot electron emitter can also be used to achieve a significant measuring effect through electron capture reactions between electron-affine substances and the free electrons. The high amount of low energy electrons forms a space charge region close the emitter surface which inhibits the emission of further electrons due to the coulombic repulsion. Even if some of these electrons are attracted by an external electric field to a near detector plate the space charge region remains mostly intact due to the high electron emission rate. As already mentioned for photoemission ECDs the additional ion formation in the presence of an electron-affine substance inhibits the transportation of the charge out of the space charge region to the detector causing a decrease in the measurable detector current. As a result the measured current difference is

directly related to the substance concentration. Preliminary measurements of chloroform in a parallel plate arrangement of the hot electron emitter and a faraday detector already show a linear decrease of the detector current for analyte concentrations in the lower ppbv range. Due to the small size of the emitting area a small internal volume for fast response times was already possible in a first design. Thus even with an emitter area of only 3 mm x 3 mm an electron current of up to 10 nA could be achieved in argon. Due to its fabrication process the emitter technology also has an inherent high temperature durability allowing for temperatures of up to 350 °C as commonly used with ECDs. Besides its use in ECDs this emitter also offers potential as an electron or ion source in other areas of analytical application such as ion mobility spectrometry or mass spectrometry. Especially if the emission energy of future emitter generations becomes sufficient to ionize neutral gas or a tunability of the emission energy becomes possible.

Poster

Comprehensive Physicochemical Modeling of Analyte Retention in Temperature-Responsive Polymeric Columns for High-Performance Liquid Chromatography

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Temperature-responsive polymers characterized as water-soluble smart polymers undergo conformational changes in response to external stimuli particularly temperature. Among these polymers featuring a Lower Critical Solution Temperature (LCST) have been exclusively applied in temperature-responsive liquid chromatography (TRLC) to date. In this process elevated temperatures induce polymer dehydration due to dominant monomer-monomer forces while lower temperatures promote interaction with water molecules resulting in the formation of a homogeneous phase. When coupled with silica this mechanism enables the controlled modulation of analyte retention as they traverse the column utilizing water as the sole mobile phase [1].

Despite the correlation between TRLC retention and hydrophobicity resembling reversed-phase liquid chromatography (RPLC) significant variations in retention behaviour exist between the two techniques. While ongoing research aims to expand the utility of TRLC a comprehensive understanding of the retention mechanism remains elusive. To enhance control over column manufacturing and selectivity it is crucial to identify the key physicochemical parameters influencing separation at both high and low temperatures. This awareness will facilitate easier and faster method development for future applications by determining optimal conditions for retaining specific molecules with various polymer types.

The proposed modelling approach initiates with the construction of a retention factor (k) model at two temperatures (above and below the polymer LCST 45 and 5 °C) followed by elucidating the critical features influencing the model. The experimental dataset based on poly(N-isopropyl acrylamide) (PNIPAAm) columns employs identical isothermal methods at each temperature. This dataset is then combined with 5666 in silico molecular descriptors. 139 compounds across different molecular classes were analysed to ensure dataset diversity allowing for facile comparative analysis with RPLC [2]. Various algorithms including Linear Regression with different regularization modes and machine learning techniques such as Support Vector Regression and tree-based ensembles were tested for the prediction model [3]. Algorithms with selection methods identified key features influencing variable predictability. Analysis of molecular descriptors at 45 °C suggested reversed-phase column behaviour driven by logP while at 5 °C retention mechanisms were complex influenced by negative and lipophilic descriptors.

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Poster

Maximizing Sensitivity in Multiresidue Pesticide Analysis via comprehensive 2-D per-aqueous liquid chromatography coupled to high-resolution mass spectrometry

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Accurate monitoring of pesticide residues at minimal concentrations is imperative for adherence to stringent regulatory standards in numerous countries [1]. This study presents an innovative methodology employing comprehensive two-dimensional liquid chromatography coupled with high-resolution mass spectrometry (PALCxRPLC-HRMS). The approach ensures both high sensitivity and selectivity in the detection of targeted compounds.

A pivotal component of this methodology is the utilization of per-aqueous liquid chromatography (PALC) facilitating the use of water-based mobile phases [2]. PALC proves instrumental in refocusing and enables the practical application of narrow-diameter columns (1.5 mm I.D.). This column design permits a direct split-free connection of LCxLC to an electrospray-based mass spectrometer (ESI-MS) contributing to heightened sensitivity. The MS acquisition is performed in targeted SIM mode ensuring reliable quantification and identification of the pesticide compounds.

A comprehensive evaluation of key performance metrics including signal-to-noise ratio limit of detection and response linearity is conducted. Comparative analysis with a state-of-the-art 1D method demonstrates superior results. The methodology achieves sub-parts per billion (ppb) level limit of detection and exhibits response linearity within the concentration range of 1-100 ng/mL. The robustness of the approach is further demonstrated through intraday and interday repeatability validations.

This study not only introduces an advanced analytical methodology for pesticide multi-residue analysis but also underscores the significance of PALC in enhancing sensitivity by facilitating the use of smaller-diameter columns and water-based mobile phases. The proposed approach showcases promising results in achieving stringent detection limits and reliable quantification for effective pesticide residue monitoring.

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Poster

A Comprehensive Metabolomic-Machine Learning Approach for Precision Medicine in Early Breast Cancer

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Introduction

Breast cancer represents a heterogeneous disease in terms of biology outcome and response to treatments. The use of neoadjuvant therapy for early breast cancer has progressively increased over time. Patients who achieve a pathological complete response have better long-term outcome. Conversely patients with residual disease after neoadjuvant therapy have a worst prognosis.

Today the analysis of molecular components in serum using mass spectrometry can be used to improve the knowledge of host related factors and for precision health care.

In this research we aim to evaluate the use metabolomics analysis coupled to artificial intelligence to predict the treatment outcome for breast cancer patients undergoing neoadjuvant therapy.

Experimental

Untargeted metabolomics analysis was performed on serum samples from 84 breast cancer patients responder and non-responder to neoadjuvant therapy. Small molecules were extracted from serum derivatized and then analyzed using bi-dimensional gas chromatography/time-of-flight mass spectrometer (GCxGC-TOFMS). The metabolomics profiling between the two groups were then compared. A machine learning approaches was used with several features selection algorithm combined with classifier algorithms. 70% of samples were used for training the model while the remaining 30% were employed for external validation.

Results

We identified the presence of a metabolic signature associated with the response to the neoadjuvant therapy. Untargeted metabolomic analysis identified and quantified the relative abundances of 445 reliable compounds correlated to clinical outcome including several fatty acids amino acids and small molecules that could be used as predictive biomarkers but also as dietary supplements. In addition by combing the metabolomic profiles with artificial intelligence we were able to correctly predict the response to the therapy for the 98% of the patients.

Conclusions

The data reported in this study showed that metabolomics analysis combined with artificial intelligence is a valid non-invasive approach to improve prognostic stratification and response to therapy in breast cancer.

Poster

Evaluation of GC-Orbitrap for urine volatilome investigation

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Volatilomics is a field of research that focuses on analyzing volatile organic compounds (VOCs) released by biological systems1. These VOCs present different classes with some properties such as boiling point less than 300 °C and low molecular weight. After being produced by different metabolic processes they enter into the blood system to be released later through breath urine feces and skin. Compared to other types of metabolites which must be extracted from tissues before analysis a part of VOCs is directly accessible in the gaseous phase (headspace) at room temperature (25 °C) thus requiring minimal sample preparation and allowing non-invasive and real-time monitoring. Among biofluids urine is considered the most convenient as it is easily accessible and available through non-invasive sampling and it can be stored for long periods. Additionally urine samples provide a comprehensive metabolic profile with VOCs ranging in polarity and complexity. Nevertheless volatilome investigation was based on untargeted compound strategy and so need to optimize the operating condition to detect a large variety of compounds and to limit the instrumentation variability. Optimization of the conditions for extracting VOCs analysis were performed to increase the number of VOCs extracted by HS-SPME-GCMS from urine. A design of the experiment was built using Azurad software and it allowed us to allow us to evaluate different parameters and their factors in a limited number of experiments and obtain the optimum parameters. The different parameters optimized were the amount of added salt (Salting-out effect) extraction time and extraction temperature. Moreover the impact on the result variability of some operating conditions like tune frequency resolution source temperature was evaluated.

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Poster

A new strategy to identify compounds released by plant roots by GC-HRMS

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Our knowledge of the metabolites released by plant roots via root exudation is a major factor in understanding biotic interactions. The lack of knowledge about the composition of root exudates is due to the difficulty of sampling and the complexity of these matrices [1]. Nowadays most of studies were based on soilless experiments [23] which did not allow access to the processes resulting from interactions with the soil and surrounding species. To consider interactions with the soil matrix fauna and to not modify exudation the growth method selected for our study was a rhizobox system and an original collection method using sorption filters in contact with Flax roots. This offers an environment as close as possible to the one found naturally.

After estimating the sorption capacities of the collection systems and optimizing sample preparation a process of 13C labelling was performed using 13CO2 for few hours during Flax growth. Sorption filters were placed on roots before and after the process to be able to isolate compounds coming from root exudation. To do so non-targeted analyses by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) were performed. The comparison of carbon isotopic pattern before and after labelling allowed to identify various labelled compounds released during exudation. In addition a strategy based on comparison with spectral databases the use of retention indices and high-resolution mass filters was employed to propose a list of annotated molecules.

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Poster

Different strategies to increase the greenness of downstream processing of biopharmaceuticals

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Biopharmaceuticals are essential for the treatment of diseases including cancer symptoms due to their unique properties that traditional drugs cannot replicate. However synthesis of impurities alongside with the target are inevitable during synthesis thus rigorous purification processes are necessary to ensure high purity for pharmaceutical scopes.

Biopharmaceuticals are usually purified in reversed-phase preparative liquid chromatography (RPLC) which involves the use of an apolar stationary phase and a more polar mobile phase usually made of a mixture of water and an organic modifier. Acetonitrile (ACN) has traditionally been preferred as organic solvent for its good properties such as good miscibility in water and excellent strength elution despite its environmental and health hazards. Current trends in analytical chemistry emphasize enhancing the sustainability of every process including purification ones with approaches focusing on reducing toxicity [12]. Our research group has actively worked on this by either employing continuous purification processes which allows to reduce solvent consumption or by replacing toxic solvents with greener ones. In particular this last case can be very challenging since many green solvents have been never or barely used in liquid chromatography.

This contribution will show through a series of case studies how it is possible to increase the greenness of the purification process by means of these approaches [23]. Both fundamental and practical aspects will be disclosed.

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Poster

Online combination of supercritical fluid extraction coupled with chromatography and mass spectrometry (SFE-SFC-MS) to analyze plastic additives in medical devices

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Plastics are some of the most used materials in our society. Among their possible applications are medical devices used in hospitals such as infusions blood or nutrition bags tubing syringes gloves etc. Plastics are principally composed of organic polymers. In the field of medical devices the most used polymer is polyvinylchloride (PVC) thanks to its safety durability chemical resistance and low production cost. However to obtain the required plastic properties plastic additives are added to the polymer in order to modify physico-chemical properties as flexibility durability color or to increase lifetime [1]. Depending on their purpose the additives may then belong to the class of plasticizers antioxidants lubricants and slip agents etc. However one of the major problems is the migration of these possibly harmful compounds from the plastic container to the contents which in the case of medical devices may be blood nutritive liquid water or skin.

Different extraction methods have already been developed to extract plastic additives from PVC [2]. After extraction a wide range of analysis methods can be used to analyze plastic additives such as spectroscopic methods chromatographic methods or some physical analysis methods [3]. However one of the main issues of analysis of plastic additives is the laboratory contaminations. Contaminants can come from plastic laboratory consumables during the different steps of sample preparation such as filtration or evaporation [4]. Some of these contaminants like phthalates are so abundant that they may also be present in the air and may thereby deposit in samples or instruments.

The online combination of extraction separation and identification avoids laboratory contamination. We have thus investigated the interest of hyphenating supercritical fluid extraction to supercritical fluid chromatography and mass spectrometry (SFE-SFC-MS) for this purpose. To hyphenate SFE and SFC various methods are possible such as the use of a selective trap column a cryofocusing jacket or a sample loop [5]. The latter allows independent development of the SFE and SFC steps and proved better analysis repeatability in this case. In order to manage the quantity injected into the SFC after the SFE step the use of two pressure regulators allows splitting the flow through a pressure difference. Plastic additives are composed of a wide variety of molecules so using CO2 with or without a polar co-solvent allows selective extraction of the target compounds. Online combination with SFC also enables to keep the same eluent for analysis while avoiding tedious sample preparation steps. In this presentation we will present the challenges and advantages of SFE-SFC-MS to quantify plastic additives from medical devices.

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Poster

Rapid and non-separative determination of organophosphate flame retardants metabolites in urine by means of a restricted access material coupled to tandem mass spectrometry

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Organophosphate flame retardants (OPFRs) are used to reduce the flammability of various materials such as furniture electronics and textiles. Triphenyl phosphate (TPhP) and tris(13-dichloro-2-propyl) phosphate (TDCIPP) are the most abundant OPFRs which have been associated with reproductive effects in animal studies raising concerns about potential impacts on human health associated with endocrine disruption and neurotoxicity. The determination of their respective urinary dialkylated metabolites (DPhP and BDCIPP) is essential to evaluate bioaccumulation persistence and toxicity in living organisms. This information is crucial for regulatory agencies to make informed decisions about safety and usage of these OPFRs.

Herein we describe a novel rapid and non-separative method to determine DPhP and BDCIPP simultaneously in human urine. These metabolites were extracted from 500 µL of urine by means of an on-line restricted access material (RAM) and quantified using a triple quadrupole mass spectrometer in less than 5 minutes with no need for chromatographic separation. A conventional clean-up pretreatment based on SPE was also necessary in order to reduce matrix effect (≃20%) and to achieve limits of quantification (≃0.1 ng/mL) well below the usual range of urinary concentrations. The whole methodology was validated by analyzing unspiked and spiked urine samples from healthy individuals. Recoveries were found to be satisfactory with values ranging from 98% to 109%. Good reproducibility was also demonstrated. Nonetheless the method was also validated by the analysis of a certified reference material (SRM 3673). In summary our approach exhibited outstanding reliability precision robustness and sensitivity making it well-suited for regular application in routine laboratories. It should be highlighted that in contrast to earlier literature and for the first time to the best of our knowledge the chromatographic separation is here avoided resulting in a remarkably short analysis time of less than five minutes per sample. Therefore we conclude that the method here developed could be a rapid non-intrusive and efficient tool for population studies and any other future research regarding OPFRs.

Poster

Utilization of Kendrick's diagram and Molecular networking for identifying compound families on lip balm MOAH fractions

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Mineral oils were largely used in cosmetics due to their interesting physicals properties like good protective properties good dermatological tolerance a wide range of viscosity and they have nonexistent smell and taste. Lip balms can contain ingredients like paraffins squalane vegetal oils and/or beeswax. Paraffins used in their compositions comes from petroleum and contains MOSH as alkanes between 20 and 35 carbons and they could contain MOAH. MOSH are considered bio-accumulable and are stored as micronodules inside liver meanwhile MOAH are considered as carcinogen for these reasons the presence of these oils in the composition of lips balm may be disturbing because they are considerate completely ingested.

A standardized method [1] for the analysis of these compounds was developed initially for the food industry [2] due to the pollution of foods by MOSH-MOAH after the recycling process of cardboard packaging. This method consists into a separation of these two families in NPLC with a LiChrospher Si 60 column with a hexane/dichloromethane gradient then an online analysis in GC-FID. Samples are dopped with internal standard in order to control separation of both families in LC-UV.

The objective of this study is to use tools such as Kendrick's diagrams and molecular networks in order to attempt identification of compounds families on MOAH fractions analyzed by GC-Orbitrap and GCxGC-Orbitrap.

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Poster

Multi-omics workflow to characterise oxidative stress at the molecular level using in vitro models

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Oxidative stress is a pathological condition that arises when there is an imbalance between reactive oxygen species (ROS) production and cellular detoxification ability [1]. This condition has been linked to various diseases such as asthma and cancer making it an important area of research for better diagnosis and treatment of inflammatory diseases. In vitro cell cultures have become an essential tool to comprehend the intricate mechanisms of oxidative stress involved in inflammatory reactions. The use of in vitro cell cultures provides an ethical and controlled environment where the effects of oxidative stress can be studied independently of other confounding factors.

Volatolomics the analysis of volatile organic compounds (VOCs) in biological samples represents a promising approach for the non-invasive fast and cost-effective diagnosis of diseases [2]. The objective of this study is to gain a better understanding of oxidative stress at the molecular level by inducing chemical stress on epithelial cells in vitro to mimic in vivo stress and characterize the VOCs released in the process. Additionally the study aims to develop standard operational procedures (SOPs) for stress induction and cell analysis to transpose these SOPs to lung organoids in a near future.

Specifically A549 lung epithelial cells were subjected to hydrogen peroxide (H_2O_2) to induce chemical stress. To the best of our knowledge no consensus currently exists regarding an ideal H_2O_2 concentration and exposition time to induce this stress. The concentration of H_2O_2 was controlled by a titration with $KMnO_4$ previously calibrated with oxalic acid. At the final step of the process the cellular pellet was separated from the medium. The cellular pellet was analysed using a derivatization step while the VOCs released by the medium were directly characterized using solid-phase micro-extraction (SPME). Both conditions were conducted using comprehensive two-dimensional gas chromatography coupled with a time-of-flight mass spectrometer (GC×GC-TOFMS) [3]. For the cellular pellet two different derivatization protocols previously developed in the lab on serum samples were tested to extract the lipidomic and the metabolomic information [4].

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Poster

Investigating the Metabolomic Profile of Biological Samples Using Bidimensional Gas Chromatography–Mass Spectrometry

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Introduction

The two dimensional gas chromatography (GCxGC) in combination with time-of-flight mass spectrometry (TOF/MS) are widely used to improve accurate molecule identification in the chemical industry and for the analysis of very complex samples such as oils environmental samples food contaminants and residues. Here we presented and validated the performances of bidimensional GC×GC-TOF/MS against that of GC-TOF/MS for metabolomic analysis which offers a great opportunity to study disease mechanisms biomarkers drug discovery and precision medicine.

Experimental

Clinical samples such as urine blood cells feces saliva and tissues are rich sources of small molecules but are highly complex and very difficult to analyze. To overcome this issue comprehensive two-dimensional GC-TOF/MS has increasingly been applied to improve separation performance by using GC×GC a system consists of two capillary GC columns connected serially with different stationary phases. GC×GC-TOF/MS offers the advantages of high sensitivity robustness reveals an excellent chromatographic separation and outperformed traditional GC-TOF/MS in terms of separation performance and identification coverage.

Results

The performance of GC×GC-TOF/MS was assessed from few case studies applied on different matrices. Several metabolites found in saliva stool exhaled breath condensed plasma/serum tissues and cells were exclusively detected using GC×GC-TOF/MS and we showed the potential of the technique in the biomedical context. The results obtained have great relevance for the discovery of new diagnostic and prognostic biomarkers; the prediction of the response to the therapy; the investigation of metabolisms in vitro and ex-vivo and for the investigation of tumor microenvironment.

Conclusions

Any advancement in technology is a challenge and strong initial efforts are necessary. Here we demonstrated how a GC×GC approach is convenient in a research laboratory setting as well as the great power of GC×GC-TOF/MS compared to the traditional ones. Using a GC×GC system can result in time savings in sample preparation instrument analysis and non-target data assessment. A competitive advantage can be gained by having the capacity to simultaneously recognize targets and non-targets while analyzing a variety of complex sample. In conclusion the comprehensive two-

dimensional gas chromatography coupled to mass spectrometry can play an important role in the separation science and in the future of biomedical research.

Poster

Method Development and Validation of an Aerosol Sampling Technique for the Analysis of Nicotine in Electronic Cigarettes: A Comprehensive Approach

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E-cigarettes are a popular alternative to smoking and a tool for smoking cessation.1 However because of the fast-growing nature of the market regulation and risk assessments doesn't always keep up with changes on the market. The Tobacco Products Directive (2014/40/EU) mandates the disclosure of all ingredients in e-liquid and forthcoming emissions.2 However there are currently no standardized methods defined for analyzing constituents in e-cigarette vapors. Although there have been several investigations on e-liquid aerosols most researchers focus on validating the quantitative analysis step only and tend to overlook the validation of the aerosol sample preparation method. In this study several steps in the process of e-cigarette aerosol analysis were investigated as well as the different parameters that might influence these. As an application a method for the determination of nicotine in aerosols using a vaping robot was developed and validated. The sample preparation step of e-cigarette aerosol testing can be a complex process that strongly depends on the compound of interest. First aerosols must be generated in a realistic and consistent manner for which a commercial vaping robot was used. This also involved testing the durability of the coil used in e-cigarette devices over a prolonged usage time. Next the aerosol should be collected in such a way that the compound of interest is captured in a precise and accurate manner. For this preliminary tests were done to assess the ideal puff number per sample. Cambridge filter pads successfully collected repeatable amounts of aerosol after collecting 20 puffs per puffing session. Additionally aerosol masses were monitored as a function of the puff count to screen for filter saturation effects. Finally the collected aerosol must be extracted so that it is compatible with the analytical instrument used for analysis. The extraction performance of ammonium borate buffer methanol and acetonitrile for nicotine was investigated. The extraction test showed that after 20 minutes all three solvents had the same extraction capacity. Based on the findings of these preliminary tests a method was formulated which was validated using the total error approach. Method validation was successfully achieved by generating accuracy profiles showing that the tolerance intervals for β-expectation did not exceed the acceptance limits of ± 20%. Next several factors such as power puff volume puff duration filter positioning and stability were used to evaluate the robustness of the method. The stability of nicotine in the extraction solvent and on the filters was deemed acceptable within the specified time range. Robustness tests indicated significant influences of power and puff duration on nicotine yields. After validation the method was applied to 15 commercial e-liquids. Overall a thorough methodology for developing and validating analytical methods to study e-cigarette aerosols was described in this study. In this case it was done in the context of nicotine quantification in the aerosols. This approach can be applied to any developed method using a commercial vaping robot to assure reliable methods and results for emissions testing of e-cigarette.

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Poster

Investigation of the impact of the SBR/BR ratio on the quantification of tire wear particles by pyrolysis-gas chromatography/mass spectrometry

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Micro- and nanoplastic (MNP) pollution is increasingly raising the public concern as they are ubiquitously present in the environment. Among the different sources of MNPs the abrasion of the tire tread on the road surface producing tire wear particles is one the most common. Consequently tire wear particles are found in soil as well as in surface water and can end up to 100 meters from the roads.

The International Organization for Standardization (ISO) released the procedure ISO/TS 20593:2017 suggesting a pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) method for the analysis of tire wear particles in soil sediment and air. In order to detect tire wear particles a pyrolysis marker compound is suggested being 4-vinylcyclohexene (4-VCH) which is used for the quantification of styrene butadiene rubber (SBR) and butadiene rubber (BR). However each tire has a different composition including a different proportion of SBR and BR depending on the brand and the type of tire (e.g. summer winter four season car or truck tire). Therefore the importance of assessing the impact of the different SBR/BR ratios on the abundance of the pyrolysis marker is crucial to minimize the quantification error of the tire wear particles in the environment.

In the scope of this study the impact of the different proportions of SBR and BR on the abundance of the pyrolysis marker (i.e. 4-vinylcyclohexene) obtained and on the quantification of tire wear particles was investigated. For this purpose several samples containing a mix of known SBR/BR ratios have been analyzed as well as 5 different random tire samples. In addition alternative pyrolysis fragments to quantify tire wear particles were explored. During the measurements no internal standard was used but poly(4-fluororstyrene) was used as control.

Based on the obtained results the pyrolysis profile appeared to deviate depending on the SBR/BR ratio leading to a challenging the quantification of these polymers in tire samples.

Poster

Steroid hormones: Context Challenges and Metrological Needs in human serum.

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Steroid hormones are blood biomarkers used for the diagnosis and therapeutic monitoring of patients. A slight variation of these hormones in the range of ng/mL or even pg/mL can either indicate the development of diseases (e.g. tumors reproductive disorders Down syndrome during pregnancy) or exposure to endocrine disruptors1 or cause significant health problems. Therefore it is crucial to have robust specific and accurate methods at these concentration levels. However external quality assessment programs have revealed bias and variability in the measurement results at low concentration levels for some hormones2. Thus there is a need to improve the quality and standardization of steroid hormone assays as highlighted by the CDC (Center for Disease Control and Prevention) program aiming to standardize measurement of 17β-estradiol and testosterone. One way to guarantee the reliability of diagnostic test results is to establish metrological traceability of results to the International System of Units (SI) through the development of reference methods and reference materials. In a previous work a method for the quantification of a panel of 23 steroid hormones using isotopic dilution analyzed by LC-MS in human serum was developed2. However the performances of this method in terms of limits of quantification (LOQ) (e.g. 1 ng/mL for testosterone and 0.05 ng/mL for dansylated 17β-estradiol) and uncertainties (about 50% at LOQ) were high and needs to be further improved to achieve the goal of developing a reference measurement procedure and relevant new reference materials.

In the present work we first performed a literature review to update the list of steroid hormones of clinical interest for which the quality of measurement strongly needs to be improved. The number of annual biological tests was assessed to determine which steroid hormones are most commonly monitored. In addition an evaluation of existing metrological tools (reference methods and materials) and external quality assessments was carried out to identify molecules for which measurement improvement is necessary. Based on this review three steroid hormones have been selected: aldosterone testosterone and 17β-estradiol and the targeted LOQs in the serum matrix have been set at 10 pg/mL for testosterone and 5 pg/mL for aldosterone and 17β-estradiol with uncertainties below 10% at these concentration levels.

Then the LC-MS method developed previously was optimized by transferring the analysis to a new instrument (XEVO TQ-S micro Waters) changing the column from a biphenyl to a C18 phase modifying the additive of the mobile phase from acetic acid to ammonium fluoride or adapting the gradient. These first optimizations have resulted in a 30-fold improvement for testosterone's LOQ.

In order to achieve the LOQ objectives the existing sample preparation method was also modified to improve the extraction and the reconcentration factor specifically for the three molecules of interest.

Finally interferences were investigated to assess the method's specificity. Following the literature review that provided a list of potentially interfering molecules the gradient was further optimized to

separate the testosterone from its isomers the epitestosterone and the DHEA and the 17β-estradiol from the 17α-estradiol. Subsequently analyses on a high-resolution Orbitrap mass spectrometer (QExactive Focus Thermo Scientific) using Selected Ion Monitoring (SIM) and Parallel Reaction Monitoring (PRM) have been performed on several matrix samples to demonstrate that no additional interference is observed at the retention times of the three molecules of interest.

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Poster

Native anion exchange chromatography coupled to mass spectrometry for the analysis of charge variants of IgG4-bases monoclonal antibodies

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Monoclonal antibodies (mAbs) are immunologically active proteins which have a defined specificity for certain antigens e.g. on cancer cells. Thereby an immune response against the target cell is induced. This makes mAbs not only an efficient tool in cancer therapy but also for the treatment of different haematological immunological and infectious diseases. Advances in biotechnology and protein biochemistry have resulted in the formation of various novel recombinant variants. A large number of therapeutic antibodies are already available on the market and several hundred more are currently under investigation. Efficacy and/or safety of mAbs are ensured by tightly controlled critical quality attributes (CQA). One CQA is the charge heterogeneity which is caused by post-translational modifications.

Besides capillary electrophoresis (CE) ion exchange chromatography (IEX) is a common method to determine the overall charge heterogeneity. Since most commercially available mAbs are IgG1-based and possess a high isoelectric point (pI) of usually ≥8 cation exchange chromatography (CEX) is the most suitable method for analysis. Also coupling to mass spectrometry (MS) has been successfully described [1].

However the importance of mAbs based on IgG4 as human therapeutics is currently increasing. In contrast to IgG1 mAbs they possess a pI <8 so that CEX is less suitable for their analysis. Therefore anion exchange chromatography (AEX) may be an alternative approach. In this study the successful application of an AEX method for charge heterogeneity analysis of IgG4-based mAbs coupled to MS was achieved [2]. Five different IgG4-based mAbs with different pIs (between 6.1 and 7.3) as well as the NISTmAb (pI=9.2) were analysed using BioPro IEX QF a strong anion exchange (SAX) column with non-porous particles. To enable MS analysis under native conditions which require high salt concentrations a special setup combined with nanoelectrospray ionisation mass spectrometry (NSI-MS) is needed.

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Poster

Comparative evaluation of Microwave-assisted processes for fatty acid methyl esters analysis in food matrices

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The analysis of fatty acids can yield meaningful insights in many field of applications such as clinical forensic and biological. In the particular case of food analysis profiling the fatty acids in relation or not to the total fat content can provide useful information regarding their impact on nutrition and health.

This kind of analysis may involve a first extraction step of the lipids from the sample matrix and subsequently transforming the fatty acids into fatty acid methyl esters (FAMEs) to profile them in the following gas chromatographic analysis. Many of the commonly used techniques may need long processing times and involve the use of hazardous chemicals usually BF3 for derivatization. Recently we developed a method known as microwave-assisted extraction and derivatization (MAED) with the aim of reducing the sample preparation time. This approach uses acidic methanol a less hazardous solvent and has been shown to be equivalent to the official AOCS Ce2b-11 which involves a simultaneous alkali hydrolysis and methylation procedure.

In this study we extended the comparison to the results obtained with the AOCS Ce2c-11 official method which involves prior acid digestion followed by alkali hydrolysis and finally methylation (the last part as for AOCS Ce2b-11). These data were also compared with the common procedure in routine laboratories to perform an extraction to determine the total fat content and then continue with the derivatization for FAMEs profiling. Specifically two different kinds of extractions supported by microwave technology were used: solvent extraction and extraction + hydrolysis. After that both the extracts were derivatized using i) BF3 and ii) an acidic methanol solution in the microwave device. Overall seven distinct procedures were applied to seven distinct food samples —including dairy meat and ready-to-eat foods.

In the end a reversed fill/flush flow modulation comprehensive multidimensional gas chromatography (GC×GC-FID) was used to identify and quantify the FAMEs and AGREEPrep measures were used to evaluate all the single methods from both greenness and FAMEs profile perspectives.

Poster

Analytical Method Development for Synthetic Opioids in Wastewater to Monitor Community Consumption Activity

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Opioid misuse in England and Wales accounted for over 2261 deaths (36.7 deaths per million people) in 2022: ~46 % of drug-related registered deaths for that year [1]. The number of opioid-related registered deaths has also been increasing yearly for over 30 years. Although the majority of opioid-related deaths are due to heroin consumption newer synthetic opioids have also led to an increased number of deaths. For example the potency of fentanyl is 50 to 100 times higher than morphine and some nitazenes are even ten times more potent than fentanyl leading to concerns about a public health crisis such as that observed in the USA. There 87.8 % (70601 deaths) of opioid deaths involved synthetic opioids primarily fentanyl [2]. Estimating community consumption of illicit drugs is a challenging task. One emerging means to do this in near real time is through wastewater-based epidemiology (WBE). The aim of this work was to develop analytical methods for quantitative monitoring of 17 fentanyls and 10 nitazenes in influent wastewater samples. The extraction method involved 1300-fold analyte pre-concentration and sample cleanup using solid-phase extraction followed by analysis with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with at least two transitions monitored for each compound. Two fast 8-min analytical methods were developed (one for each compound class) using short biphenyl columns. This was to ensure sufficient analyte separation and to reduce the effect of matrix interference at this concentration factor. Performance of both methods (limits of detection and quantification accuracy precision recoveries and linearity) was excellent with sensitive quantification possible to pg/L concentration levels in most cases. To our knowledge this is the first quantitative analytical method capable of routine detection and monitoring of consumption of so many of these compounds via WBE.

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Poster

Comparison of sample preparation for determination of mycotoxins in cannabis and cannabis derived products using LC-FLD and LC-MS/MS

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Due to the potential healthcare benefits of cannabis and hemp the market for this plant and its products have increased dramatically in the last years. In several states cannabis is already legal for medicinal and/or recreational use. Quality control before human consumption e.g. the determination of pesticide residues and mycotoxins in cannabis biomass and its derived products is therefore mandatory. The number of regulated pesticides varies dependent on state/country/region. The U.S. Food & Drug Administration (FDA) [1] sets a limit value of 20 ppb for mycotoxins in human food and animal feed. Similar but also lower values depending on the matrix are set by Commission Regulation (EU) 2023/915 [2]. Organizations like AOAC are developing method requirements for mycotoxin determination in cannabis matrices. AOAC SMPR® 2021.010 [3] defines aflatoxins B1/B2 aflatoxins G1/G2 and ochratoxin A as analytes of interests and specifies limits of quantification and qualification for cannabis biomass and cannabis derived products. Real samples were mixed with a standard containing the mandatory aflatoxins (B1 B2 G1 G2) ochratoxin A and zearalenone. Sample preparation was carried out and mycotoxins were separated under RP-HPLC conditions.

Four different samples: hemp pellets hemp seeds commercially available hemp flour and hemp oil were investigated using different sample preparation procedures: solid liquid extraction (SLE)/liquid-liquid extraction (LLE); a standard QuEChERS extraction with dispersive cleaning; SLE/LLE with following CrossTOX cleanup; SLE/LLE and solid phase extraction using immunoaffinity columns (IAC SPE). The results are investigated and evaluated in terms of time costs per sample solvent consumption and achievement of limit values.

The most chosen detector for mycotoxin determination is the mass spectrometer (MS). The regulations are met easily but due to the complexity of an LC-MS system the operation can be challenging. Therefore fluorescence detection (FLD) is investigated as an alternative detection method. The results of LC-FLD and LC-MS/MS measurements are compared regarding the achievement of valid limit values as well as handling/user-friendliness of the detectors.

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Poster

Development of isocratic and gradient methods for high-throughput separation of lipids used in LNP formulations

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Lipid nanoparticles (LNPs) became famous for the rapid development and large-scale production of vaccines during the Covid-19 pandemic. Theses vaccines consist of mRNA coding for an antigen encapsulated in LNPs. More recently LNPs gained interest as delivery systems for RNA as a therapeutic drug beyond vaccines e.g. for cancer treatment or the treatment of inherited disorders [1].

In research as well as in pharmaceutical production LNPs are subjected to careful quality control. The identity quality and purity of the raw materials especially the mRNA and lipids as well as the quality and composition of the product must be verified. Two of the quality criteria of LNPs are the identity of lipids and lipid content [2] for which the development of an HPLC method is shown here.

For method development a quaternary low pressure gradient pump and an evaporative light scattering detector (ELSD) are used. Mixtures of the lipids used in two LNP formulations for mRNA vaccines were chosen as exemplary samples. For the separation of the mixtures a core-shell and a fully porous phenyl-hexyl UHPLC column are considered. The need to combine acetonitrile and methanol as eluents and to add ammonium acetate as a modifier for good peak shape and complete elution is shown. Two method options are established: a gradient method which replaces methanol by acetonitrile during the gradient run and an isocratic method which is equally suitable for analysis of LNP lipid content within four minutes. Both methods are transferred to the fully porous HPLC column. After optimization of the ELSD evaporation temperature a limit of detection between 5 and 15 ng is reached for all tested lipids. All methods can be transferred to a binary pump easily and are compatible with MS detection.

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Poster

Trace Quantification of Micro- / Nanoplastics by Pyrolysis-GC/MS and Preparative Ultracentrifugation

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Today nano- / micro-plastics are ubiquitous in the environment. Originating from diverse sources such as littering of packaging materials tire abrasion or paint degradation they are a topic of public concern as well as rising academic industrial and regulatory attention.1 Mechanisms such as the mechanical-oxidative fragmentation of larger microplastics may lead to the formation of nano-plastics characterized by particle sizes below 1 µm. Concerns have been raised about nano-plastics as due to their colloidal character they might be able to cross biological barriers.23

In addition to microscopical techniques including IR- and Raman microscopy that have a detection limit of 1 to 10 µm Pyrolysis-GC/MS a thermal analytical method offers outstanding sensitivity and specificity for the trace detection and quantification of nano-plastics down to the ng level.4 However the method can suffer from strong matrix effects and appropriate sample clean-up and enrichment is crucial to circumvent these effects and allow robust quantification at trace levels.

In the current study tissue models (EpiIntestinalTM) were employed in conjunction with Pyrolysis-GC/MS as detection method enabling the in vitro monitoring of possible transport of nano-plastics across the human intestinal barrier. The goal of the test was to detect whether any material passes the barrier under the chosen test conditions. To alleviate matrix effects from the medium a novel sample work-up procedure was established for the receptor medium by repetitive washing steps using preparative ultracentrifugation (PUC).

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Poster

Higher order structures of oligonucleotides investigated by ion mobility mass spectrometry breakdown curve experiments and collision induced unfolding

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The last few years have seen a remarkable rise in the development of oligonucleotide-based therapeutics [1]. Oligonucleotides (ON) can adopt various conformations known as higher-order structures (HOS) such as double helices or G-quadruplexes. The characterization of the HOS is crucial notably for the rationalization of their physicochemical properties for biological or chemical applications. Within this framework innovative HOS characterization approaches based on ion mobility mass spectrometry are proposed. The concept is to use ion mobility hyphenated with mass spectrometry to separate and monitor stable conformers in gas phase based on their collision-cross section (CCS) value allowing rapid screening and quality control of HOS. An increase of the internal energy provided by soft ion-neutral collision with helium can influence the conformation distribution leading to an energy landscape fingerprint of the HOS (called CIU experiments). Additionally the resilience of HOS induced by the coordination of cations was also evaluated by combining CIU and energy-resolved collision induced dissociation (CID) experiments.

Our results revealed that the CIU heatmaps (evolution of the CCS as a function of acceleration energy) and CID breakdown curve plots (survival yield of the precursor and apparition of the product ions) provided insights into the conformational changes cation ejections and fragmentation pathways upon activation. Various oligonucleotides were studied including three G-quadruplexes with different sequences. Additionally chemically modified RNA duplexes bearing conjugates were investigated.

As examples the breakdown curve experiments performed on the z=-5 precursor ion required higher collision for the [(TG4T)4+3NH4+]5- quadruplex than the human telomeric sequence HTS [(TAG3T2AG3T)2+2NH4+)5- and the [(G4T4G4)2+3NH4+]5-quadruplex to dissociate. Furthermore these 3 G-quadruplexes experienced different fragmentation pathways which could be explained by their differences in terms of sequences and number of coordinating cations. No significant arrival time distribution (ATD) change was observed for the 3 structures during CIU experiments. Nevertheless a thinner ATD was observed for the “TG4T” quadruplex than for HTS and “G4T4G4” suggesting the presence of a lower conformers population diversity linked to a more rigid structure. The comparison of CIU and breakdown curve experiments of RNA duplexes and their conjugates was not straightforward due to the different charge state distribution. However when comparing with the unconjugated duplex a similar CIU heatmap was observed for the palmitate conjugate at z=-6 but rather different for the tocopherol and palmitate species at z=-9 supporting an influence of the coulomb repulsion but no significant contribution of the conjugates on the behavior of these RNA duplexes in the gas phase

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Poster

Ion mobility spectrometer equipped with a novel hot electron emitter as the ionization source

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Miniaturized ionization sources that provide high-speed switching localized control and/or tunable energy of emission are highly sought after for ionization-based detectors. Graphene-oxide-semiconductor (GOS) hot electron emitters can potentially replace x-ray or radioactive ionization sources such as H3 and Ni63 and furthermore provide additional variable parameters for improved or novel detection schemes. In this work we present GOS emitters that can operate at ambient pressure in e.g. nitrogen and air. Furthermore we demonstrate the applicability of the GOS hot electron emitter as an ionization source in a drift-tube ion-mobility-spectrometer (DT-IMS) for negative mode measurement on 12 Dichloroethane.

The emitters under investigation consist of rectangular windows etched into a 300 nm thick wet oxide in which a 13 nm dry tunnel oxide is grown. A graphenic carbon sheet with a thickness of 8 nm is then deposited as the gate material. Subsequently the gate is structured by an O2 plasma etch and aluminum is used for the contacts.

Electrical characterization was carried out in a vacuum needle prober which allowed to control the pressures of nitrogen or ambient air. An anode was placed above the emission area at a distance of approximately 1 mm to collect the emitted electrons and negatively ionized molecules. Three consecutive gate current sweeps are conducted prior and after a constant current measurement. This allows monitoring of changes in the characteristics due to different gases and residual gas pressures at the interface. Subsequently constant current lifetime testing is performed to determine the charge to breakdown value.

IV-characteristics of the GOS-emitter in vacuum exhibit a maximum transfer ratio of 0.27%. The transfer ratio is defined as the ratio between the emission current measured at the anode and the total tunnel current through the device. By introducing nitrogen and air at ambient pressure the transfer ratio decreases to 0.046% and 0.084% respectively. These reduced values at ambient pressure may be attributed either to capturing and removal of electrons by the present gas or an increase of the work function caused by adsorbates. The evolution of the gate current is similar for all measurements and has a nearly linear behavior in a Millikan-Lauritsen representation. Hence the GOS-emitter does not show any significant changes in the buried tunneling behavior due to varying nitrogen or ambient air pressure conditions. Lifetime measurements in vacuum for several current densities result in charge to breakdown (CTB) values between 5.3 C/cm2 and 31.6 C/cm2. In nitrogen and ambient air CTB values from 19.1 C/cm2 to 80.3 C/cm2 and from 12.4 C/cm2 to 28.5 C/cm2 were measured respectively. The vacuum and nitrogen measurements failed due to oxide breakdown

while the emission into air failed due to destruction of the gate material. This indicates the formation of oxygen radicals at the GOS surface and the subsequent etching of the graphenic carbon. In general the given measurements show a sufficient lifetime of the GOS hot electron emitter for low current density applications (<100 nA/cm2).

For utilization in a DT-IMS the GOS-emitter is mounted inside the reaction volume near the analyte gas inlet and opposing to the ion gate which leads to the drift region. Only negative ionization by electron capturing will be considered as the electron energy is expected to be well below 10 eV. Hence the electron affine 12 Dichloroethane is used as the test substance. Without the emission of electrons from the GOS-emitter no signal was detected in the DT-IMS. While operating the ionization source 12 Dichloroethane could be clearly detected. This provides a proof of concept that the GOS-hot electron emitter can be used as an ionization source in DT-IMS.

Poster

Analysis of Extracellular Vesicle Lipid Content: Comparing Direct Injection LC-MS to Traditional Extraction Methods

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Extracellular vesicles (EVs) are membranous structures produced by both eukaryotic and prokaryotic cells encapsulating a diverse array of bioactive molecules including proteins lipids and nucleic acids. These vesicles play pivotal roles in intercellular communication epigenetic regulation and hold promise for diagnostic and therapeutic applications in various diseases.

Lipids constitute essential components of EVs forming the structural framework of their membranes. Despite their significance understanding the lipid composition and function within EVs remains a burgeoning area of investigation. Lipidomics a powerful analytical tool enables comprehensive profiling of lipid species within EVs. However challenges arise due to the limited sample volumes low lipid concentrations and methodological complexities associated with lipid extraction prior to mass spectrometric analysis.

This study investigates the feasibility and efficacy of employing direct injection LC-MS (DI-LC-MS) for EV lipidomic profiling circumventing the prerequisite of the lipid extraction step. Utilizing reverse-phase liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (RP-LC-Q-TOF-MS) EVs isolated from diverse sources including human plasma and cultured cells were subjected to lipidomic profiling. Additionally column lifetime and impact on MS signal were evaluated during the study.

Results demonstrate that DI-LC-MS offers comparable lipidome coverage and repeatability to conventional extraction techniques such as LLE. Notably DI-LC-MS reduces chemical background noise and eliminates the need for sample evaporation. Furthermore the streamlined procedure of DI-LC-MS requiring smaller injection volumes and simplifying the workflow enhances the efficiency of EV lipidomic analysis without compromising analytical precision.

The obtained results underscore the utility of DI-LC-MS as a promising alternative for expedited and simplified lipidomic analysis of EVs offering insights into their lipid composition and facilitating further exploration of their biological functions and potential clinical applications.

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Poster

Monitoring degradation of polymer nanoparticles by hydrodynamic and size-exclusion chromatography

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Polymeric nanoparticles (NPs) are nanometer-sized materials that find numerous applications such as for coatings and drug-delivery systems. NPs can degrade over time for instance by exposure to ambient conditions or by bio-degradation in medicinal NPs. Monitoring NP degradation over time is important for quality assurance and helps understanding the release of degradation products or change in material properties. The predominant quality attributes for polymeric NPs include the particle-size distribution (PSD) and the molecular-weight distribution (MWD) of the constituting polymer. Standard analytical methods often are limited to the analysis of one of these properties rather than analysing multiple properties in the same method. Our ultimate aim is to develop a two-dimensional liquid chromatographic (2D-LC) system capable of assessing both PSD and MWD simultaneously. For this purpose we first established suitable hydrodynamic chromatography (HDC) and size-exclusion chromatography (SEC) methods.

Our research focused on monitoring the (changes in) PSD and MWD of polymeric NPs upon temperature-induced degradation. For this commercial and in-house formulated dye-loaded poly(lactic-co-glycolic acid) (PLGA) NPs were used for testing. PLGA can be hydrolysed and is biodegradable. HDC was used to assess the PSD of the intact PLGA NPs and monitor potential particle degradation and aggregation over time. Common particle sizing techniques such as dynamic light scattering would have provided average particle size only. After temperature exposure the PLGA NPs were disassembled using acetonitrile releasing the PLGA and encapsulated-dye molecules. Subsequent SEC with three detection methods (UV/ELSD/MS) was used to study the MWD of the constituting polymer. Additionally the SEC method allowed for the quantitative analysis of the dyes alongside with the polymer.

The developed HDC and SEC workflows enable the assessment of PSD MWD and encapsulated compounds of biodegradable polymeric NPs. In a next step we aim to develop a comprehensive HDC-SEC method allowing determination of the PLGA MWD as function of NP PSD during the NP degradation process.

Poster

EFFECT OF CO-EXTRACTED TRITON X-100 ON THE INSTRUMENTAL MEASUREMENTS OF PESTICIDE ANALYSIS BY GC-MS/MS WITH CLOUD POINT EXTRACTION AS SAMPLE PREPARATION TECHNIQUE

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Introduction

Cloud point extraction (CPE) has many advantages as a green surfactant-based extraction procedure which can be combined with different instrumental techniques. However the compatibility of CPE with gas chromatography (GC) is highly questionable since it is often believed that the usage of surfactants (such as Triton X-100) can lead to different problems in the GC analysis e.g. the surfactant can be adsorbed onto the stationary phase leading to modification of its properties or can elute as a series of peaks over a wide range of times from the GC column affecting the quantification of the target analytes. The present study aims to evaluate the influence of the residual Triton X-100 in the sample solutions obtained after cloud point extraction followed by back-extraction into hexane on the instrumental analysis of organochlorine and organophosphorus pesticides by GC-MS/MS.

Methods

GC-MS/MS TSQ-9000 Thermo Fisher Scientific was used in SRM mode for the purpose of the study. 1 µl of sample was injected into the PTV injector at a split ratio 5:1. All the measurements were carried out alternatively with glass and metal liners pre-conditioned by injection of a matrix-matched medium (hexane containing 0.09 % w w-1 Triton X-100). To evaluate the influence of Triton X-100 on the sensitivity two series of standard solutions of the target analytes (concentrations up to 15 ng mL-1) were prepared in hexane and matrix-matched media and the slopes of the two calibration lines were compared. Additional studies to establish the GC-MS/MS system stability in the presence of surfactant were performed by assessing the repeatability (n=20) of the peak area and retention times of the analytes.

Results

SRM mode of the mass spectrometer allowed us to selectively determine 19 target pesticides. No significant signal drift was observed and no statistically significant difference in the retention time was evident for any analyte in the presence of Triton X-100 when glass or metal liner was used. It was found that in the presence of surfactant compared to pure solvent hexane a significant increase in the sensitivity was achieved if a Silcosteel metal liner was used (enhancement in the range 126 % - 230 %). The same effect but less pronounced was also observed for several compounds: chlorpyrifos endrin pp-DDE op-DDD pp-DDD & op-DDT pp-DDT (enhancement ~10 %) when a glass liner was applied. Only for dieldrin a 10% decrease in the sensitivity was detected. However it can be noticed that the measurement precision deteriorated when the metal liner was used compared to the application of the glass liner. The last can be explained by the retention or/and interaction of the

pesticides occurring on the bare metal surface of the liner. So it could be assumed that the observed induced increase in the signals of the target analytes in the presence of Triton X-100 is likely due to an in-situ passivation of the liner.

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Poster

Automatic ligand fishing platform for active compounds screening

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Many affinity screening methods have been developed for rapid and accurate discovery of bioactive compounds from complex natural products. Among these methods ligand fishing based on ultrafiltration has been widely used because of the easy preparation and simple process. However most of the processes are manually operated. In order to decrease the labor and increase the efficiency of ligand fishing in this study an automatic ligand fishing platform was established to perform ultrafiltration-based screening from complex matrices. 3D printing was applied to produce the necessary components. Two approaches were set up for application in different conditions. Approach A is more flexible consisting of a pressure-conducted screening process with syringe pumps and stepper motors and automatic computer control of the system through Python programming. On the other hand approach B does not require programming and it can utilize liquid chromatography equipment to perform the pressure-conducted screening process with an air compressor and normal motor. It can be automatically controlled by a designed circuit. All apparatus and tools of the platform are commercially available. As a proof of concept ligand fishing was demonstrated successfully through the automatic platform to screen ligands to albumin. Future studies will focus on a further and wider application to screen bioactive compounds from complex natural products. This automatic ligand fishing platform can be easily adapted as well to other fishing modes for screening.

Poster

Chemical fingerprinting using target- and suspect screening analysis to track urban runoff pollution sources

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Urban runoff is a ubiquitous source of environmental pollutants from multiple sources such as building materials vehicle traffic biological pest control and more [1]. With increasingly frequent extreme weather events and many pollutants occurring at environmentally relevant concentrations there is an urgent need for solutions to manage urban runoff and mitigate the discharge of pollutants into recipient ecosystems. For this it is essential to understand the chemical composition of different types of urban runoff. Although previous studies been done to characterize urban runoff pollution (see for example [2] [3]) more research is needed to determine spatial and temporal trends in urban runoff pollutants particularly for chemicals of emerging concern (CECs) that are excluded in routine analysis despite contributing significantly to environmental toxicity.

In this study we present an ongoing project to determine the chemical fingerprint of urban runoff from across Europe. Runoff samples are prepared with solid-phase extraction (SPE) and pressurized liquid extraction (PLE) on liquid and solid phase respectively. Following sample preparation chemical analysis is done with Liquid Chromatography- and Comprehensive Two-Dimensional Gas Chromatography High-Resolution Mass Spectrometry (LC-HRMS and GC×GC-HRMS) to cover a broad range of compounds. Quantification of more than 150 target analytes is done using external calibration and suspect screening is performed in MSDial for LC-HRMS data and GC-Image for GC×GC-HRMS to determine the occurrences and signal intensities of suspected pollutants.

Preliminary results reveal clustering of runoff samples based on compounds associated with wastewater (such as Caffeine and Desvenlafaxine) and industry compounds including 13-Diphenylguanidine and Bis(2-ethylhexyl) phthalate. Concentrations of 6PPD-Quinone up to 0.49 µg/L exceed toxic levels [4] which shows the potential impacts from releasing urban runoff into nearby ecosystems. Further analysis will be done to determine chemical fingerprints and environmental risks associated with different runoff sources.

The study is part of the Horizon Europe project D4RunOff (www.d4runoff.eu). Here methods and results from our study will be used to support stakeholders and policymakers in mitigating the impacts of urban runoff by developing concrete tools to assist urban water management.

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Poster

New valve setup for increased sample loading capacity for online SPE-HPLC

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The introduction of a sample into an LC system is typically carried out with an injection valve. But valves are capable of a lot more than only injecting samples. The clever use of switching valves and the availability of a broad variety of valve types can boost the performance of every HPLC system and a complex workflow can be automated easily. A very good example for the application of valves is sample preparation like solid phase extraction (SPE). This type of sample preparation procedure is used to concentrate or clean samples before injection to the column. Due to current regulations the limits of detection (LOD) and limits of quantification (LOQ) for many many compounds are very low which makes SPE mandatory. By implementing valves you can turn manual SPE into online SPE and create an automated workflow.

With the exemplary separation of polycyclic aromatic hydrocarbons (PAHs) the advantages of adding up to three valves to a standard system configuration are shown in terms of automation time saving in sample preparation and improvement of detection limits.

The core of this solution is the use of a new special multi-injection valve an 8-port 2-position valve and an 8-port multiposition valve. The simple change of injection options without replumbing the system enables users to apply a broad variety of different volumes from µl to ml scale. A sample can be injected onto the SPE column via the autosampler. Depending on the used syringe and sample loop different volumes can be loaded. Alternatively a feed pump is connected to the SPE column for automated conditioning sample loading and cleaning with the solutions from the multiposition valve. The developed method is suitable for the determination of 16 PAHs according to EPA 610 method [1].

The recovery of the SPE injection was in range from 80 %–120 % and all calibrations showed excellent linearity. The approach of injecting via the feed pump enhanced the detection limits in comparison to direct injection still using a relatively low injection volume.

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Poster

Radius of gyration of full and empty adeno-associated viruses determined by SEC-MALS

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Adeno-associated viruses (AAV) have emerged as a powerful delivery tool for gene therapy. Currently five therapies have been approved by the FDA/EMA and with a pipeline of up to five novel products in 2024 the potential of AAV-based products is extremely promising. AAVs consist of a protein-based capsid and a genomic payload in a form of ssDNA. The manufacturing process results in not only the intended fully packed vector but also product-related impurities such as empty or partially filled vectors that compromise the safety and efficacy of the product. Various methods are currently used to determine the filling status such as spectrometric determination at Absorbance 260/280 nm analytical ultracentrifugation and separation of empty and full vectors by anion exchange chromatography. However these methods are either associated with long analysis times or would otherwise benefit from other orthogonal biophysical methods to corroborate determination of the AAV packaging status. Here size exclusion chromatography (SEC) in combination with multi-angle light scattering (MALS) was used to determine the radius of gyration (Rg) of empty and full AAV5 and their mixtures in various ratios. As expected The Rg value decreased with higher filling ratio due to a higher density centre in full vectors. The results of this study suggest a correlation between Rg and the filling status that can be used to characterize the packaging status of AAVs.

Poster

Enhancing sensitivity and robustness in untargeted metabolomics by microbore UHPLC-HRMS

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Untargeted metabolomics is invaluable for understanding disease mechanisms and treatment responses. While narrowbore-based (2.1 mm I.D. columns) LC-HRMS is commonly employed higher sensitivity is still essential to enhance metabolome coverage as well as to deal with low-volume and limited amount samples. Microbore columns (1.0 mm I.D.) offer potential but are still underutilized in metabolomics.

In this contribution we optimized and tested a microbore UHPLC-HRMS method. The analytical setup consisted in a microflow UHPLC coupled to Orbitrap Exploris 120 MS and 1.0 mm I.D. columns employing sub-2 µm fully porous particles were used in both RPLC and HILIC mode. Human plasma and cells were tested. Comparative analysis with 2.1 mm I.D. columns was also performed.

Several aspects were investigated such as optimal flow rate gradient injection volume and column temperature by injecting a mixture of polar endogenous metabolites covering a large retention time window. Microbore UHPLC-HRMS showed excellent reproducibility in retention time (≤0.5% CV) and peak area (≤2.3% CV) better than narrowbore. Additionally it exhibited higher sensitivity in Full Scan-Data Dependent Acquisition (FS-DDA) with LOD and LOQ values of 0.95 and 3.18 ng/mL respectively.

Concerning metabolome coverage the 1.0 mm I.D. method yielded nearly twice MS1 spectral features and metabolite annotations (at MSI level 2) compared to narrowbore. Robustness was confirmed with over 300 consecutive injections over 24 hours.

Lastly solvent consumption was significantly reduced enhancing environmental sustainability. This study showcases the robustness sensitivity and versatility of microbore UHPLC-HRMS for untargeted metabolomics applications.

Poster

Development of a comprehensive 2D-LC method for the analysis of pharmaceuticals as emerging organic micropollutants in hospital wastewater

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The occurrence of organic micropollutants (OMPs) in the environment has recently become a topic of concern. OMPs are hard to degrade in conventional wastewater treatment plants (WWTP) and are therefore still present at trace levels (ng/L - µg/L) in WWTP effluents. Examples of OMPs include pharmaceuticals hormones contrasting agents personal care products and pesticides which can accumulate over time potentially harbouring toxic effects affecting the aquatic and public health. One of the major sources of OMPs is hospital wastewater. Due to the complex composition of the samples and the wide array of physicochemical characteristics 1D-LC the current method of choice falls short in terms of separating power. Therefore new and innovative analysis techniques are needed to monitor OMP levels. For this purpose two-dimensional LC (2D-LC) might be a more viable approach. By carefully selecting orthogonal separation mechanisms much higher peak capacities can be acquired thus increasing the resolving power of the system significantly. Sample preparation is moreover crucial to enhance the sensitivity of the analysis. Solid phase-extraction (SPE) can be used to pre-concentrate samples and remove matrix components that can interfere with MS-detection.

In this study we developed a comprehensive 2D-LC (LC x LC) MS method capable of fully separating pre-selected target OMPs typically occurring in hospital wastewater and thus allowing a comprehensive characterization of these samples. First various HILIC and RPLC stationary phases with different bonding chemistries and capping technologies in combination with different mobile phases were tested and compared. Suitable orthogonal separation mechanisms were identified via a combination of the convex-hull and the bin-box method resulting in an orthogonality factor. To counteract potential solvent strength mismatch problems between separation dimensions active solvent modulation was applied. Other compatibility issues were addressed by adjusting flow rates column dimensions and temperatures. Sample preparation consisted of SPE with Oasis HLB sorbents suitable for a broad range of polarities. For most compounds typical recoveries were found to be in the range of 60-80% with acceptable RSD values. The optimized method was applied to real hospital wastewater samples. Target analytes were detected by Q-TOF MS in positive electrospray ionization (ESI) mode. Results from the optimal LC x LC method were compared with their 1D-LC counterparts performed on the same hospital wastewater samples in order to assess the benefits of the increased peak capacity.

This study highlights the potential benefits of LC x LC methods in the field of environmental analysis.

Poster

Expanding a traditional SFC chiral screening platform to enable high-throughput analysis of "polar" biomolecules through the addition of EFLC

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Polar analytes are often used as building blocks for Active Pharmaceutical Ingredients (APIs) especially with increased interest in therapeutic peptides and biologics as potential API's. Determining the achiral and chiral composition of such polar molecules (e.g. amino acids small amines peptides etc.) is often challenging mainly because they will not elute or very late in standard SFC methods whereas in reversed-phase UPLC the opposite is seen and peaks will elute very early.

An SFC chiral method screening setup was already available within Process Analytical Research but mainly focused on "classical" apolar to mid-polar compounds. Herein we describe the expansion of that screening setup to a much wider polarity- range by combining different approaches: addition of water to modifiers use of specific columns and running gradients up to 90% of modifier (Enhanced Fluidity Liquid Chromatography EFLC). We will also present our tiered screening approach for SFC–EFLC in the form of a flowchart.

Poster

A novel non-destructive identification method of prehistoric hafting adhesives with DHS-GC×GC-TOFMS

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It is well-known that prehistoric people were gifted stone workers making most of their tools from stone. Moreover they invented ways to attach a handle to those stone tools transforming the stone tools into powerful knives spears and arrows etc. this skill is called hafting. The handle is often made from wood or antler and can be attached with an adhesive (resin birch tar) or with bindings (animal or vegetal) or a combination. Evidence is scarce as the handles were made from organic materials which perish over time. Luckily hafting leaves traces on the stone tools which are preserved under optimal conditions and providing us with indirect evidence. The first evidence of hafting without adhesive is found from about 250000 years ago[1] and from 80000 years onwards presence of adhesive is more prevalent[2].

Chemical identification of those adhesives is challenging but important. Challenging because the sample size is tiny and each artefact is unique due to degradation. Important as it might reveal the technical expertise of prehistoric man and to what natural resources they had access to[3]. Currently the most accurate identification technique is gas chromatography coupled to mass spectrometry (GC-MS). Unfortunately GC-MS requires solvent extraction and derivatisation destroying (most of) the adhesive. As a result only a few artefacts with enough adhesive preserved have been identified with GC-MS[4]. Therefore research for a new powerful but non-destructive technique is crucial.

In this study a new non-destructive identification technique is investigated; dynamic headspace coupled to a two-dimensional comprehensive gas chromatography-time-of-flight mass spectrometer (DHS-GC×GC-TOFMS). Headspace techniques are known to be clean non-destructive and fast. Generally DHS is one of the most sensitive headspaces techniques. Several different adhesives are measured with DHS to test the versatility of the method. Subsequently a library including these adhesives is made to assist with the identification of unknown residues on archaeological artefacts.

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Poster

A pyrolysis-GC/MS study of volatile compounds generated during the pyrolysis of wood-based material used in construction

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To further understand the production of compounds that may become airborne and available for respiratory and dermal exposure the pyrolysis byproducts of five wood-based materials commonly used in construction were studied using pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Previous studies have successfully sampled a wide range of combustion by-products but obtained results with high variability due to environmental conditions of the test.12 The materials studied in this work include low-density wood fiberboard oriented strand board pine particle board and plywood.

The instrument is consisted of a GC/MS coupled to a robotic arm sample handler and a pyrolysis module. A small sample from each material was heated to 800 °C under helium gas in the pyrolysis chamber and the resulting compounds were separated for further analysis using GC/MS. Volatile organic compounds (VOCs) namely benzene toluene ethylbenzene p-xylene styrene and a polycyclic aromatic hydrocarbon (PAH) naphthalene were selectively monitored.These compounds are associated with human health concerns. Non-targeted analysis was also conducted to identify byproducts other than VOCs and PAHs.

The results indicate that plywood and particle board generated the largest variety in results. Samples from solid pine board generated the smallest number of volatile compounds followed by low-density wood fiberboard that is manufactured with wood fibers starch and wax. Compound that are identifiers of wood such as furfural and p-cresol were identified in the non-targeted analysis. This study provides insight into how different types of wood-based fuels can generate various byproducts under anaerobic conditions and high temperatures. Pyrolysates constitute a portion of the chemical compounds released during combustion of wood-based materials. Future studies are aimed to sample the smoke from reproducible combustion events using sorbent tubes and analyzed with thermal desorption-GC/MS.

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Poster

Highly Efficient LC-MS/MS Analysis of Multiple Mycotoxins Utilizing Biphenyl Column Selectivity with Inert Column Technology

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As more mycotoxins come under regulatory purview new methods are needed to help food safety labs continue to operate efficiently. Comprehensive multi-mycotoxin methods are an attractive alternative to separate methods for different analyte lists but they can be difficult to develop due to the wide range of chemical characteristics among mycotoxin classes. In particular the Alternaria toxins and ergot alkaloids create additional challenges for method developers. These emerging mycotoxin food contaminants are unique in that when analyzed on a C18 column high pH conditions must be used to obtain acceptable peak shape for the Alternaria toxins and adequate separation of the ergot alkaloid epimers. Use of high pH conditions is stressful for LC columns and not suitable for analyzing other classes of mycotoxins so another approach is required for a truly comprehensive method.

Analyzing compounds that have nonspecific adsorption (NSA) or nonspecific binding (NSB) to metal surfaces in LC columns have historically been a challenge. Poor peak shape and sensitivity are key indicators that polar usually acidic compounds are interacting with the metal surfaces in the column causing poor data quality.

Mycotoxins analysis can be challenging and often requires a great deal of column conditioning and equilibration to achieve acceptable peaks. This is due to the reactive nature of the compounds which contain acidic polar or otherwise metal chelating groups. Inert column hardware combined with a Biphenyl stationary phase helps simplify methods and improve the response and peak shape of these compounds.

In this talk I will describe how a simple sample preparation procedure the unique selectivity of a Biphenyl stationary phase run under acidic conditions and inert column hardware technology combine to provide sensitive and efficient simultaneous analysis of Alternaria toxins Ergot Alkaloid Epimers and other major mycotoxins.

Poster

Flash chromatography as a fractionation tool for characterizing bio-oils

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As a renewable energy source biomass is now seen as one of the key solutions to the energy transition. It can be converted by pyrolysis into bio-oil which can be used to produce biofuels or valuable chemicals. However bio-oils produced by pyrolysis are highly complex mixtures containing thousands of organic compounds covering a wide range of mass and polarity. Their processing into greener fuels or value-added chemicals requires in-depth knowledge of their molecular composition which can be obtained using various analytical techniques. Here flash chromatography was used as an efficient method to fractionate bio-oils into four distinct fractions reducing the complexity of the sample and allowing a more accurate chemical description. The fractions obtained were analyzed using different analytical techniques. Two-dimensional 'comprehensive' gas chromatography (GC×GC) coupled with mass spectrometry (MS) and flame ionization detection (FID) was used to characterize the composition of the volatile fraction of bio-oils. High-performance thin-layer liquid chromatography (HPTLC) was used to identify and quantify the main sugars. Finally Fourier Transform Ion Cyclotron Resonance (FTICR) MS was used to identify the molecules present in each fraction. This approach was used to compare three bio-oils produced by different pyrolysis processes: fast pyrolysis (FP) catalytic fast pyrolysis (CFP) and reactive catalytic fast pyrolysis (RCFP). The results show that the bio-oils produced by FP and CFP are chemically very similar. The RCFP bio-oil has less oxygenated products and more unsaturated hydrocarbon species. Therefore the bio-oil produced by the RCFP process was found to be the best process for producing oils with a low O/C ratio.

Poster

Exploring the flavour profiles of plant-based milk using sorptive extraction and GC×GC–TOF MS

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The rapid growth in popularity of plant-based milk alternatives (derived from sources such as oats almond soy and coconut) has sparked heightened interest in understanding their flavour profiles. This is a challenging prospect due to the diverse range of plant sources processing methods and sensory attributes that contribute to the overall taste experience and its influence on consumer preference.

Traditionally solid-phase micro-extraction (SPME) has been used for flavour profiling of foods and beverages and although fast and simple it can suffer from limited sensitivity and reproducibility. In addition immersive sampling is often avoided because it can reduce the fibre lifetime or cause matrix interference (resulting from capillary effects).

High-capacity sorptive extraction probes can tackle these issues by providing a relatively large volume of PDMS stationary phase that results in higher sample loadings. The probes are also robust and easily rinsed free of matrix making them well-suited to immersive sampling of beverages such as milk. Used in conjunction with secondary refocussing this approach offers excellent sensitivity as well as improved chromatographic resolution and enhanced water management.

Nevertheless the resulting flavour profiles are often extremely complex covering a wide range of chemical classes. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF MS) provides the enhanced separation required to tackle this sample complexity by coupling two columns of different selectivity enabling separation of the entire sample based on two different chemical properties (e.g volatility and polarity).

Here we show how sorptive extraction and GC×GC–TOF MS can be leveraged for the comparison of flavour profiles for different brands and sources of plant-based milk to help innovate refine and tailor products to meet consumer demands.

Poster

Detailed chemical characterisation of pyrolysis oils from mixed plastic waste using GC×GC–TOF MS

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Globally we produce about 400 million tons of plastic waste annually but it is estimated that less than 9% of this plastic waste is recycled. Chemical recycling specifically pyrolysis offers a promising avenue for sustainable waste management and energy production. By converting plastic waste into valuable resources we can reduce the burden on landfills minimize environmental pollution and promote the transition towards a circular economy.

Pyrolysis is a thermal decomposition process that converts plastic waste into liquid and gaseous products in the absence of oxygen and often in the presence of a catalyst. The resulting pyrolysis oils are considered a form of renewable energy and can serve as an alternative for fossil fuels in various industrial processes including power generation heating and transportation or as a feedstock for the petrochemical industry.

However the composition of pyrolysis oils can vary depending on the type of plastic feedstock used and the pyrolysis process parameters. Typically pyrolysis oils contain a complex mixture of n-paraffins iso-paraffins olefins diolefins iso-olefins naphthenes and aromatics but the presence of trace impurities such as sulfur and nitrogen compounds can affect the quality of the oil and its applicability. Unfortunately the unknown chemical composition and extreme complexity of pyrolysis oils means complete characterisation is a challenge using conventional 1D GC–MS particularly in terms of individual compound identification and distinguishing naphthenic and olefin molecules.

Comprehensive two-dimensional GC coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is an advanced separation technique ideal for the characterisation of such complex mixtures. Here we use a TOF MS capable of acquiring both hard and soft electron ionisation in a single analysis. This so-called Tandem Ionisation provides complimentary spectra to improve confidence in the identification of isomeric species that are too similar to identify based on their 70 eV spectra alone.

In this study we demonstrate how GC×GC–TOF MS with Tandem Ionisation can provide detailed non-target characterisation of pyrolysis oils to identify possible contaminants and help to refine process design.

Poster

A holistic view of river water quality using passive sampling and GC×GC–TOF MS

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Routine monitoring of water quality is now a requirement of environmental legislation but the cause of a poor water quality status is often unknown and extensive investigative monitoring is needed to determine what chemical may be responsible.

Sampling of water has historically been carried out using grab-samples but passive samplers (e.g. silicone rubber semi-permeable membrane devices (SPMD and LDPE) are now available that indirectly lower detection limits by concentrating pollutants over time. Here silicone rubber samplers were deployed for several weeks in a UK river course to sequester large volumes of water and provide a concentrated representative extract for analysis. However the sample complexity resulting from passive sampling calls for enhanced separation.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) with its vastly expanded separation space offers significant advantages over conventional single-column chromatography in such cases as well as highly-sensitive detection for confident non-target screening.The combination of passive sampling and GC×GC–TOF MS allows for the detection and identification of a wide range of organic pollutants including pesticides pharmaceuticals and emerging contaminants at trace levels. The high sensitivity and selectivity of this approach provides a more comprehensive assessment of the chemical composition of river water and a better understanding of pollution sources and trends.

As the need for efficient and informative monitoring techniques grows the adoption of this integrated approach holds significant promise in advancing our understanding of pollutant dynamics in river ecosystems ultimately contributing to effective water resource management and protection. Here we show the potential of passive sampling coupled with GC×GC–TOF MS as a powerful tool for environmental monitoring offering a holistic view of river water quality.

Poster

Towards new approaches in purification and isolation of extracellular vesicles using hydrophobic interaction chromatography

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Extracellular vesicles (EVs) represent a diverse group of lipid bilayer membrane vesicles ubiquitously produced and released by various cell types into the extracellular matrix. They function as mediators of intercellular communication by facilitating the transportation of biomolecules such as proteins lipids and nucleic acids (e.g. DNA mRNA and miRNA) to recipient cells.

The isolation and separation of EVs typically involve intricate and multi-step procedures resulting in a heterogeneous fraction of EVs with uncertain origins. Conventional techniques such as ultracentrifugation (UC) often fail to effectively eliminate numerous interfering compounds such as proteins or lipoproteins due to their similar density or size. Therefore the development of novel analytical methods for the isolation separation and characterization of EVs is imperative to elucidate their structural diversity physiological and pathological roles and potential diagnostic and therapeutic applications. Notably there is a growing interest in the application of chromatographic techniques for EV separation.

In this study we employed hydrophobic interaction chromatography (HIC) as a tool for the purification of native EVs based on differences in membrane hydrophobicity as well as isolation from other sample components such as lipoproteins. We investigated the effect of both stationary-phase chemistry and mobile-phase composition (salt concentration and type) on the retention and separation of EVs. The EVs applied in this study were mainly microvesicles apoptotic bodies and small EVs which originated from cyanobacterial cells human and bovine fetal serum as well as human milk. Fractions collected during the HIC analysis were examined using transmission electron microscopy nanoparticle tracking analysis (NTA) and SDS-polyacrylamide gel analyses to confirm and identify the EVs. Additionally collected fractions were profiled for their lipid content.

We observed that EV fractions obtained through conventional ultracentrifugation particularly those derived from biofluids contain substantial contamination that could be effectively separated from EVs using HIC. The EVs exhibited differential retention behavior when varying the stationary-phase chemistry suggesting variations in hydrophobicity and a greater heterogeneity of EV fractions obtained through conventional techniques than previously anticipated. Importantly the integrity of

the EV remained largely intact with limited changes in shape and size. Our results demonstrate the potential utility of HIC for EV separation and isolation.

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Poster

Incorporating automation to improve the efficiency and data quality of nitrosamine analysis.

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Drug product recall due to the presence of trace nitrosamines has highlighted the need for pharmaceutical companies to rapidly develop their understanding of potential nitrosamine formation pathways and how to effectively monitor and control them. Some of the notable pathways for formation have been artificial formation during sample preparation formation caused by interactions with combined excipients in the drug product and the risk of formation in packaging. The strict regulatory safety criteria and ever-expanding complexity of sample preparation to overcome sample matrix effects and in situ artificial formation has presented ample opportunity for innovation.

At AstraZeneca the trace analysis capability continues to expand to overcome the technical challenges of nitrosamine analysis. We have a standardised Thermo Fisher Scientific Orbitrap platform that allows us to develop robust and transferrable high-resolution LC-MS/MS and GC-MS methods. These methods have demonstrated LOQs and LODs of 4 ppb and 2 ppb respectively. Numerous methods have been analytical technology transferred to internal operations quality control (QC) laboratories and external contract research organisations (CROs) ensuring on-going testing of drug products and guaranteed supply of medicines to patients.

The drive for high-throughput analysis in the monitoring of nitrosamines and the common issues relating to method robustness sample preparation unexpected sources of nitrosamine contamination and the risk of false positives have been addressed through automation. A recent example at AstraZeneca was the successful automation of a novel approach to sample preparation required for the analysis of metformin extended-release products.1 The automation of the effective but laborious dispersant-first dispersive liquid-liquid microextraction (DF-DLLME) method provided valuable benefits. The main impact of automation was taking a 7-hour day for sample preparation with complete analyst attention to 2 hours without the need for an analyst's presence. There is also a reduced chemical exposure to the analyst reduced human error costs and an overall improved method ruggedness and robustness.

The next generation automated workflow being developed at AstraZeneca is the grouping of LC and GC compatible sample preparation from one parent sample. Working with the Thermo Fisher Scientific TriPlus RSH autosampler the automated workflow will follow the DF-DLLME approach.

With each new generation of medicines being scrutinised for their nitrosamine risk there is an ever-expanding complexity to trace nitrosamine analysis; this is in conjunction with increased time restraints for pharmaceutical companies to develop effective methods. The use of automation can further address the challenges in relation to nitrosamine and mutagenic impurity analysis by focussing on efficiency by creating self-optimising sample preparation procedures.

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Poster

Analysis of carbohydrates in bio-oils by HPTLC-UV and HPTLC-MS

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The depletion of fossil oil resources and their impact on the environment have become the main reason for developing sustainable energy sources. Pyrolysis oil is attracting considerable interest as a renewable fuel source. Wood is composed mainly of cellulose hemicellulose (a polymer of sugars) and lignin. After pyrolysis cellulose and hemicellulose produce a wide variety of sugars with one or two cycles and lignin mainly produces phenols.

The oil can be analyzed directly by mass spectrometry techniques but between a complex matrix and therefore an ionization preference for certain compounds and a whole series of sugar isomers present in the sample it is preferable to first pass through a chromatography stage. In this work high-performance thin-layer liquid chromatography (HTPLC) is applied to the determination of sugars in wood pyrolysis oil. This technique enables 20 samples to be analyzed with a single disposable plate without the need for sample preparation. The samples on the plate can then be analyzed by Matrix Assisted Laser Desorption Ionization - Fourier Transformation - Ion Cyclotron - Mass Spectrometry (MALDI-FT-ICR-MS) in the presence of graphene oxide [1] to identify the sugars present as a function of elution distance.

Cross-checking data between UV and FT-ICR-MS confirms the presence of the main sugars suspected by UV. The proposed procedure enables UV separation and quantification of the anhydrosugars levoglucosan cellobiosan glucose arabinose xylose and cellobiose [2]. Calibration results gave an R^2> 0.98 for 5 standards and separation enabled them to be quantified in the samples. Levoglucosan appears to be the main sugar present in these samples accounting for around 3% of the mass content. In addition MALDI-FT-ICR-MS analysis revealed the presence of other sugars that had previously been detected but not identified by UV.

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Poster

Advancing in-depth analysis and characterization of clinically relevant monoclonal antibodies by gradient recycling hydrophobic interaction chromatography

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Monoclonal antibodies (mAbs) play a vital role as modern biopharmaceuticals serving as critical therapeutic agents for various diseases including autoimmune disorder cancer and infectious diseases. As the number of mAbs as therapeutic proteins are increasing a particular focus is put on characterization and monitoring of critical quality attributes (CQA) that impact product safety efficacy and quality.

Analytical chromatography emerged as an irreplaceable tool offering insights into mAbs structural integrity heterogeneity and chemical and biological modifications. Unlike other chromatographic methods that rely on charge or size differences hydrophobic interaction chromatography (HIC) separates biomolecules based on their hydrophobicity. Analytes are applied to the column under high salt conditions favouring hydrophobic interactions between the nonpolar regions of the protein and the hydrophobic stationary phase followed by elution under low salt conditions to release the bound mAbs. With that HIC offers the potential to discriminate closely related mAb variants including oxidized variants isomers aggregates deamidated variants or charge variants. However while HIC offers distinct advantages in mAb analysis sufficient separation of variants is a difficult task due to the complex folding states of mAb.

To enhance the resolving power and improve peak resolution of closely eluting analytes increasing the column length is a common strategy in chromatography especially when other optimization parameters such as stationary phase or mobile phase composition alone do not result in sufficient separation. Nevertheless increasing the column length has certain restrictions like cost commercial availability and most importantly increased back pressure. To overcome this limitation recycling chromatography has emerged as a promising approach. By repeatedly passing the sample alternatively through the chromatographic columns the column bed length is virtually increased depending on the number of cycles. This technique efficiently utilizes the chromatographic system by increasing the resolution without raising material costs or back pressure concerns.

Here our work focuses on the fundamental principles of recycling chromatography for the analysis of clinically relevant mAbs of different complexity. By applying long columns and scaling elution conditions resolution enhanced targeting the separation of co-eluting variants. We aim to highlight its potential applications revealing in-depth knowledge of complex biopharmaceuticals which could not only guide downstream product formulation but also upstream expression and purification of recombinant mAbs. Furthermore we discuss recent advancements challenges and future prospects emphasizing the role of gradient recycling chromatography in advancing both the characterization and validation of mAbs.

Poster

Suspect and non-targeted screening of halogenated contaminants in a stranded killer whale (Orcinus orca) using GC-HRMS hyphenated with TIMS

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The killer whale (Orcinus orca) is likely the most contaminated marine mammal globally facing extremely high levels of contaminants1. This contamination combined with reduced prey availability and vessel impacts poses a significant conservation threat to the species worldwide2. Beyond well-known pollutants like legacy persistent organic pollutants (POPs) killer whale tissues likely contain various emerging and unknown contaminants potentially leading to additional toxic impacts. In this context this study aims to assess the advantages of using high-resolution ion mobility for the suspect and non-targeted screening of halogenated contaminants in the blubber of a stranded killer whale. Approximately 70 mg of blubber were collected from a killer whale that stranded on the beach of De Panne Belgium on October 29 2023. The sample preparation involved a simple extraction in n-hexane at room temperature and atmospheric pressure followed by a limited clean-up on an acid silica column to remove lipids. The resulting solution was analyzed using a TIMS TOFpro II mass spectrometer (Bruker Bremen) equipped with a GC-APCI source for sample separation and ionization (GC-APCI II Bruker Bremen) before IM-MS analysis. TIMS separations were performed in SWIM mode to enhance the IM resolving power. Suspect and non-targeted screening were conducted using the software DataAnalysis 5.3 (Bruker). Suspect screening was conducted for various classes of legacy (e.g. PCBs PBDEs OCPs) as well as emerging (e.g. PXBs) persistent organic pollutants (POPs). Features were confirmed based on four criteria: exact mass isotopic pattern retention time and CCS. Overall approximately 150 suspected POPs were tentatively confirmed with the vast majority being PCBs (104 congeners identified). Comparison of experimental CCS with those available in our in-house database greatly contributed to the confident confirmation of these features since CCS deviations were typically below 1%. For those features whose CCS was not present in the database confident confirmation of the contaminant class could still be obtained by checking whether these belonged to the CCS vs m/z trendline of a specific class of halogenated POP. For the non-targeted analysis a Kendrick mass defect (KMD) plot was used to identify unknown classes of halogenated contaminants. Several compounds were identified as probable halogenated compounds based on their mass defect and isotopic pattern which are characteristic for halogen-bearing molecules. Here the additional dimension of separation provided by the ion mobility was of great help in obtaining clean mass spectra. Indeed filtering the data according to the ion mobility unable to get rid of most matrix background signal as well as other coeluting isobaric interferences greatly improving the quality of the spectra and the identification process especially for low signal intensity features. Up to this point we were able to tentatively identify two other OCPs: Toxaphene and Tris(chlorophenyl)methane (TCPMe). Taken together these preliminary results demonstrate the potential of using IM to enhance both suspect and non-targeted screening of halogenated contaminants in complex matrices.

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Poster

Determination of effective diffusion coefficients of proteins at elevated pressure in columns packed with wide-pore core-shell particles

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To determine the efficiency that can be obtained in a packed-bed liquid-chromatography column for a particular analyte a correct determination of the molecular and effective diffusion coefficients (Dm and Deff) of the analyte is required. The latter is usually obtained via peak parking experiments wherein the flow is stopped. As a result the column pressure rapidly dissipates and the measurement is essentially conducted at ambient pressure. This is problematic for analytes whose retention depends on pressure such as proteins and potentially other large (dipolar) molecules. In that case a conventional peak parking experiment is expected to lead to large errors in Deff. To obtain a better estimate of Deff the present study reports on the use of a set-up enabling peak parking measurements under pressurized conditions. This approach allowed us to report for the first time Deff for proteins at elevated pressure under retained conditions. First Deff was determined on a 400Å-pore column at a pressure of 120 bar for a set of proteins with varying size namely: bradykinin insulin lysozyme β-lactoglobulin and carbonic anhydrase in a column packed with 400Å core-shell particles. The obtained data were then compared to those of several small analytes: acetophenone propiophenone benzophenone valerophenone and hexanophenone. A clear trend between Deff and analyte size was observed. The set-up was then used to determine Deff of bradykinin and lysozyme at variable pressures ranging from 45 bar to 450 bar. These experiments showed a decrease in intra-particle and surface diffusion with pressure which was larger for lysozyme than bradykinin. The data show that pressurized peak parking experiments are vital to correctly determine Deff when the analyte retention varies significantly with pressure.

Poster

Molecular characterization of new renewable feedstocks by multi-scale analysis using gas chromatography mass spectrometry and an oxygen selective detector

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Alternatives to fossil fuels have been at the forefront of scientific research for many years. Detailed molecular characterization of new feedstocks such as pyrolysis oil from biomass recycled plastics or from used wind blades is therefore of prime importance for better understanding and predicting the macroscopic behavior of these new types of materials. It is necessary to understand their faith in the part of the energy transition. These feedstocks are made of complex chemical mixtures that require multi-scale analysis to be properly characterized at the molecular scale. Several analytical techniques are currently employed for this very purpose. The volatile fraction of the samples can be analyzed by gas chromatography (GC) hyphenated to mass spectrometry to identify the unknown compounds.

In comparison to classical fossil fuels the diversity of the chemical classes present in such samples is very large and comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GCxGC-MS) was successfully used in an earlier study [12]. A GCxGC system operating with a reverse column set has been investigated in the present study to separate identify and quantify compounds present in hydrotreated vegetable oil to be used to produce sustainable aviation fuel (SAF).

Next to MS heteroatom-specific detectors such as NCD and SCD can also be coupled to the chromatographic separation to gain additional valuable informations. Because some compounds like oxygenated molecules can significantly impact the macromolecular properties of the alternative fuel with a direct impact on its properties and usability the need for clear understanding of oxygen-bearing compounds present in pyrolysis oil is a major goal [3]. However quantifying and identifying oxygenated molecules is a major limitation due to the lack of oxygen-specific detectors suitable for this purpose.

To address this a new type of oxygen-specific detector capable to identify and quantify molecules without the use of any standards was recently developed [4] and used in this study. In addition to the characterization of oxygen-containing molecules this new GC detector can also detect and quantify carbon nitrogen and sulfur by considering their volatile forms: CO2 NOx and SOx [5].

This multi-scale analytical approach involving GCxGC and various type of detectors appears to be valuable for the characterization of new feedstocks and to enhance our ability to adapt the related industrial processes in order to produce high-value products.

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Poster

New method for acrylamide determination in foods applying High-Performance Thin-Layer Chromatography (HPTLC)

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Acrylamide (AA) is an organic compound with low molecular weight and highly soluble in water. It is known for its neurotoxic and genotoxic properties[1] and it has been classified by the International Agency for Research on Cancer as a probable human carcinogen (Group 2A)[2]. This compound is present in various starchy foods particularly French fries bread breakfast cereals pizza and coffee[3]. Its formation results from a complex chemical process that occurs during the conventional thermal processing (>120°C) of starchy products in the presence of the amino acid asparagine through a series of chemical reactions known as Maillard Reaction[4 5]. This reaction occurs between a carbonyl compound often from a reducing sugar and an amino compound from free of amino acids peptides or proteins. Maillard Reaction are responsible for sensory characteristics such as aroma flavor and color in baked fried roasted extruded and toasted products[6]. Due to the population chronic dietary exposure to acrylamide from a variety of foods some regulations have been established like the European Commission (EC) N° 2017/2158 which established mitigation measures and reference levels to reduce the presence of AA in food[7]. Additionally the World Health Organization (WHO) the European Food Safety Authority (EFSA) and the Food and Agriculture Organization (FAO) consider a priority the control of AA levels in foods[8].

Chromatography is the technique of choice for AA detection. Due to the low detection limits required (µg/kg) liquid chromatography (LC) and gas chromatography (GC) coupled with one or more mass analyzers (tandem) are the most used technique[9]. GC achieves a lower detection limit than LC but requires a laborious and complex derivatization step. For this reason recent studies have preferred the combination of LC with mass spectrometry (MS) avoiding the need of derivatization. In addition LC has the advantage of easily analyze aqueous samples which is relevant considering AA solubility and polarity[10]. Both techniques have advantages mainly associated with analytical resolution and sensitivity and some disadvantages including high costs complex sample pretreatment and high operational requirements[11 12]. The objective of this work was to develop an affordable selective and simple planar chromatography method for determining and quantifying AA in foods. Since fluorescence detection show increased sensitivity compared to absorption measurements and better selectivity[13] AA detection was based on derivatization reaction with a fluorescent coumarin reagent (7-mercapto-4-methylcoumarin) through a Michael addition. Triethylamine was added to increase the efficiency of the reaction due to its catalytic properties over thiol-Michael addition. Critical reaction factors i.e. temperature and time were optimized using a design of experiment to improve the efficiency of AA derivatization. An optimal derivation reaction was established at 58°C and 8 minutes using and AA concentration of 5 mg/mL and 10% triethylamine.

Derivatized standards and samples were applied though a CAMAG (Muttenz Switzerland) fully automated sample application device (ATS4) to high-performance thin layer chromatography (HPTLC) Silica gel F254 plates. Chromatography was performed in a 10 x10 cm twin trough chamber using a mobile phase composed of dichloromethane acetic acid and ethanol (90:7:3 v/v/v) up to a migration distance of 85 mm. After development the plates were dried at 80°C for 20 minutes in a CAMAG plate heater. Quantitative analysis was carried out using a CAMAG spectrophotodensitometer Scanner 3 in fluorescence mode at 366/>400 nm. Data was acquired and process using WinCATS software. This methodology was validated according to the International Conference on Harmonization (ICH). Calibration data fit a polynomial model in the range of 10 to 500 ng per band showing a determination coefficient of 0.999. Precision was determined by repeatability (n=6) showing RSD <5%. Selectivity was evaluated by mass spectrometric observing a signal at m/z: 286 which corresponds to a sodium adduct [M+Na]+ of the derivation product (7-mercapto-4-methylcoumarin + acrylamide). Accuracy was determined via recovery analysis spiking bread samples observing a recovery of 70±195%. According to the results this new thin-layer chromatography method is a proper alternative to determine AA in bread samples.

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Poster

Effect of several sterilization techniques on the stability of peptides and oligonucleotides for the production of sterile dosage forms

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Biologicals such as proteins peptides and oligonucleotides are increasingly utilized to treat illnesses. They are primarily administered parenterally due to their instability within the gastrointestinal tract. Consequently their administration must be pyrogen-free to prevent severe infections. To ensure pyrogen-free conditions biologicals need to be sterilized. However the sterilization of biologicals is challenging due to their susceptibility to degradation at high temperatures. As a result biologicals are primarily sterilized through membrane filtration followed by aseptic filling into a sterilized container. However this is not a reliable sterilization technique making it impossible to guarantee complete elimination of pyrogens.

This project therefore aims to explore alternative sterilization techniques to ensure adequate sterilization of biologicals. Techniques such as gamma irradiation E-beam nitrogen dioxide (NO2) and vaporized hydrogen peroxide (VHP) are investigated for this purpose. Since these sterilization methods can cause damage to biologicals the addition of stabilizers such as glycerine histidine trehalose and ascorbate and the effect of dry ice and inert conditions are investigated to protect them. As representative biologicals bradykinin and homo-oligonucleotides with varying base lengths are considered. The impact of the sterilization techniques on bradykinin is evaluated in both aqueous and solid samples. Oligonucleotides are only considered in aqueous solution.

To quantify the extent of degradation ultra-high-performance liquid chromatography (UHPLC) with a diode array detector is employed. Analytical methods allowing accurate quantitation of both types of molecules are developed. For this purpose a HALO C18 column with a particle size of 2.7 µm and a pore size of 90 Å is considered. For bradykinin a mobile phase consisting of 0.1% trifluoroacetic acid in water and acetonitrile is used in gradient elution mode. For the oligonucleotides the ion pairing reagent triethylamine acetate is added to the aqueous phase (0.1 M) while ACN is used as the organic modifier. Both methods allow an accurate quantification of the compounds of interest to a level of 10 ppm for bradykinin and 0.1564 to 0.2700 nmol/ml for the oligonucleotides which is sufficient to quantify up to 96-99% degradation.

Poster

Optimizing Chiral HPLC with L-Ethyl Lactate: Enhancing Selectivity and Green Solvent Compatibility

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Numerous studies have demonstrated that the composition of the mobile phase can influence the configuration of polysaccharide-based chiral columns 1 thereby impacting enantiomer separation factors. Additionally the direct interaction between the mobile phase and enantiomers themselves can lead to either enhancements or compromises in the separation process. Consequently the development of novel mobile phases can significantly affect separation factors in chiral chromatography providing a potential solution to the challenges associated with the difficult enantioseparation of specific chiral compounds. However the potential of optically active solvents as modifiers in chiral HPLC has not been extensively explored to date. Therefore this study investigates the potential of L-Ethyl lactate (LEL) a bio-based solvent for its unique interactions with both the mobile phase and analytes as anticipated from its chiral nature 2. The results demonstrate that LEL exhibits distinct selectivity compared to commonly used modifiers in chiral HPLC. Through comparisons in reversed-phase LC chiral separations under various conditions LEL emerges as a promising modifier showing an increase in chiral resolution for 8 out of 16 test compounds. However for 5 compounds a decrease in resolution was observed while 3 test solutes failed to yield satisfactory results under any tested conditions on polysaccharide columns. When LEL was combined with methanol instead of acetonitrile worse results were obtained presumably due to its protic nature. Despite challenges such as its higher UV absorbance L-Ethyl lactate demonstrates excellent compatibility with salt additives and complete miscibility with aqueous phases. Interestingly a more pronounced increase in chiral resolution is observed for LEL compared to acetonitrile at lower temperatures. While LEL is somewhat hindered by its higher UV absorbance it holds promise for streamlined chiral screening platforms potentially requiring fewer columns and incorporating greener solvents.

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Poster

Advancements in Chiral Separation through Temperature-Responsive and Reversed-Phase Chiral Chromatography Integration

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The resolution of chiral solutes within mixtures of unrelated species holds significant importance in both life sciences and pharmaceutical analysis. While this goal can be theoretically achieved through comprehensive two-dimensional liquid chromatography (LC × LC) the incorporation of chiral separation in the second dimension (2D) remains relatively limited. However coupling this with conventional reversed-phase separation in the initial dimension extends the capabilities of traditional achiral analysis methods. Nonetheless the practical implementation of rapid chiral analyses in the 2D has been hindered by the challenging transfer of the mobile phase from the first dimension (1D) while maintaining effective chiral separation. This study assesses the combination of temperature-responsive and reversed-phase chiral liquid chromatography for the enantioselective separation of a wide range of pharmaceutical compounds. By employing temperature-responsive liquid chromatography (TRLC) in the 1D analyses can be conducted under purely aqueous conditions facilitating comprehensive and more generic solute refocusing before entering the 2D. This enhanced capability allows for the utilization of rapid and broad compositional gradients across the chiral dimension thereby expanding the technique's applicability. The proposed platform involves screening seven chiral columns including both superficially porous and fully porous columns with polysaccharide and macrocyclic antibiotic phases. Additionally four mobile phase gradients were examined using a pharmaceutical test mixture. The platform demonstrated the necessary resolving power for the targeted molecules offering a novel approach to chiral screening in mixtures of unrelated compounds. 1

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Poster

2D-LC-IRMS – The future of stable isotope analysis?

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Compound specific stable isotope analysis (CSIA) can be applied in many fields including the evaluation of sources and transformation processes of micropollutants or the verification of food authenticity. When coupling liquid chromatography (LC) to an isotope ratio mass spectrometer (IRMS) the stable carbon isotope signature of analytes in water samples can be assessed. Limitations of this method occur due to the wet chemical oxidation of carbon in the LC-IRMS interface where all oxidisable carbon is transformed to carbon dioxide (CO_2). Because of this the use of organic substances as eluents buffers or modifiers can lead to falsifications in the determination of the carbon isotope signatures of the analytes and has to be avoided. This limits the use of already established chromatographic separations based on the use of organic eluents which make up over 90 % of applied LC-methods. Only inorganic buffers and temperature can be used for method development which makes it difficult and time consuming especially for more complex samples.

A possibility to overcome this limitation is offered by combining two dimensional-LC (2D-LC) with LC-IRMS. Conventional 2D-LC works with two chromatographic columns with different separation mechanisms. In this way peak capacity can be significantly increased allowing chromatographic separation of compounds that coelute in only one dimension. Adaptation of this system to LC-IRMS allows LC-methods taken from the literature to be used with organic additives in the first dimension without the need for any method development. The analyte of interest is then transferred via so called heart-cut modulation using a sample loop transfer onto the second dimension where the organic solvents are separated from the analyte prior to oxidation.

In this study we aim to investigate the potential of heart-cut-2D-LC coupled with IRMS to make CSIA more accessible for a broader spectrum of applications. Here we present the initial development of an 2D-LC-IRMS system. Caffeine which is well studied in CSIA was used for method development and validation. Parameters such as peak shape retention time reproducibility and carbon isotope signatures were considered for the method validation. A real sample containing caffeine which cannot be sufficiently separated by conventional LC-IRMS methods was measured in the 2D-LC-IRMS system to demonstrate its applicability.

Poster

Prediction of Kovats Retention Indices for the Gas Chromatographic Separation of Jet Fuel Components

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The aviation industry accounted for about 10% of worldwide greenhouse gas emissions in 2019 [1]. In an effort to reduce anthropogenic CO2 emissions sustainable aviation fuels (SAF) have become increasingly important. SAFs are produced for example by the Fischer-Tropsch process with syngas derived from the conversion of biomass. However before being considered as an alternative aviation fuel the products must undergo comprehensive very detailed and thus extremely cost-intensive testing of their physical and chemical properties. For this reason there is great interest to predict different fuel properties in order to quickly evaluate potential candidates for SAF production [23]. This work attempts to develop a quantitative structure property relationship (QSPR) model to predict relevant physical properties in this case Kovats retention indices.

The data set used in this study consisted of almost 400 compounds from different classes (alkanes alkenes cycloalkanes aromatics alcohols acids aldehydes ketones and esters). The retention indices (RIs) have been mainly collected from the PubChem database for standard non-polar (DB-1) and semi-standard non-polar (DB-5) columns. Furthermore 266 different molecular descriptors (MD) were initially obtained from the online chemical database that describe the structure and shape of molecules of which finally 51 descriptors were retained for the model.

To build the model the data set was split into a training and a test set and was pre-processed by Pareto Scaling. The training set was used to train the model with the following regression methods: Partial Least Square (PLS) and Support Vector Machine Regression (SVM-R). Venetian Blinds were used as a cross validation method and tested on the test set.

We were able to accurately predict RIs of the different compound classes on both thee DB-1 and DB-5 columns with slightly better performance of the model fort the prediction of RI values for the DB-5 column. For DB-1 no pre-processing of the data was done resulting in root-mean-standard error for calibration RMSEC = 26.3 and root-mean-standard error for cross-validation RMSECV = 38.8 and coefficient of determination R2 = 0.99 and for the predicted test data RMSEP = 47.1 R2 = 0.93.

For the DB-5 phase the same pre-processing of the data resulted in RMSEC = 27.4 RMSECV = 37.2 R2 = 0.99 and RMSEP = 28 R2 = 0.99.

The model is thus considered suitable for the prediction of retention index on standard and semi-standard non-polar columns and hence also boiling point. It is thus a suitable starting point for the prediction of further physico-chemical properties merely based on molecular descriptors.

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Poster

Method Development for Simulation of Emissions Formed during the High-Temperature Curing of Stone Wool Products Using Thermal Desorption Gas Chromatography-Mass Spectrometry

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The research focuses on developing and optimizing a method to simulate emissions formed while curing stone wool products produced with a new lignin-based binder using Thermal Desorption Gas Chromatography-Mass Spectrometry (TD GC-MS). The TD unit allows the thermal treatment of a sample under the flow of air and He simulating the curing oven conditions. Moreover due to the direct connection of the TD unit to the GC-MS system any sample losses are minimized.

Stone wool products are commonly used materials for thermal and acoustic insulation plant growth and filtering. Stone wool fibres are produced from a variety of different materials such as basalt anorthosite and dolomite as well as from recycled materials from other industries. During production an organic binder is applied to create the necessary integrity between the fibres mechanical strength and shape of the final product after thermal curing. Typically a thermosetting resin such as phenol-urea-formaldehyde is applied. During the thermal curing of the material a certain amount of ammonia formaldehyde and phenols are emitted. The continuous reduction in allowed emission levels of these chemicals in production and especially in products together with the market shift towards more sustainable products are becoming a driving force for the development of new non-added phenol-formaldehyde binders [1]. The application of such binders will reduce the environmental impact and improve the sustainability of the products.

The aim was to create a procedure to investigate the emission profile of a novel binder. It should not only mirror the chemical composition of the emissions but also be sensitive to the different process parameters such as curing temperature duration and the composition of the raw materials. The study explores various thermal desorption parameters to optimize the analytical method for capturing a wide range of volatile organic compounds (VOCs) emitted during the curing of the new lignin-based binder. Desorption temperature (230 – 300 °C) desorption time (10-30 min) flow rates (10 – 100 mL/min) and reactive media gas composition (He and synthetic air) are systematically varied to enhance the sensitivity and selectivity of the TD GC-MS analysis. Also different liner materials such as Tenax-TA and Carbotrap B were tested. The developed method can be used for the screening of new binders to provide information regarding their environmental impact to help in the process of decision-making in the launching of new products. Moreover it can also be used to better understand the chemistry of the curing process and provide information for preparation for the industrial trial measurements at the factory.

Recently a new type of lignin-based binder was developed at ROCKWOOL A/S and its emission profile was analyzed. Deconvolution of the GC-MS data revealed the presence of the large number of compounds belonging to different compound classes namely ketones aldehydes carboxylic acids phenolics compounds alcohols sugars glycols N S-compounds aromatic hydrocarbons and furan derivatives. Principal component analysis separated the samples corresponding to the applied

reaction gas in PC1. The abundances of ketones N S – compounds alcohols sugars glycols aromatics and hydrocarbons were higher for curing simulation in an air atmosphere while the abundances of furan derivatives and phenolics were higher in a He atmosphere. Evaluation of the different adsorption materials such as Tenax-TA and Carbotrap B for capturing VOCs showed that smaller oxygenated compounds have a higher affinity toward Carbotrap B material while Tenax-TA is more suitable for capturing different hydrocarbons.

The simulation results were validated by comparing them with actual emissions collected during the industrial trials at the factory. It was found that the emission simulation in the He atmosphere has higher similarity with the results of the factory measurements regarding the number of observed compounds and their classes than in the air; however the pool of oxygenated compounds is underrepresented under He conditions meaning that the combination of He and air would provide the closest results to factory samples.

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Poster

Application of a Semi-Quantitative Machine-Learning Model to Plant Root Metabolomics Data

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Lotus japonicus is a key model plant for studying beneficial plant-microbe interactions such as root-nodule symbiosis (specific to the leguminous family) or arbuscular mycorrhizae fungi [1]. Plants exude up to 40% of their assimilated carbon into the rhizosphere where the root exudates constitute a vital part in plant-microbe interactions [2]. Comprehensive characterization of root exudates will allow for a better understanding of the rhizosphere processes and has been identified as an important step in developing more sustainable agricultural practices [3].

Liquid chromatography coupled with electrospray ionization (ESI) mass spectrometry is an important tool in non-targeted metabolomics. The development of high-resolution mass spectrometers mass-spectral databases and in-silico annotation tools have facilitated putative identification of the detected features. However the ionization efficiency within the ESI source can differ by several orders of magnitude even between compounds with similar functional groups necessitating the use of analytical reference standards for quantitative analysis [4]. Liigand et al. presented a machine-learning model for ionization efficiency prediction covering a wide range of compounds eluent compositions and instruments. The quantification model was validated on pesticides and mycotoxins in cereal and the model was recently extended by Lauria et al. to include PFAS compounds [5].

Most studies on semi-quantification are done in the context of toxicology where risk assessment necessitates quantitative knowledge [4]. Extending this strategy of semi-quantification to include biological samples and metabolites could aid in elucidating the dynamics of plant metabolism and discovery of new biomarkers within the rhizosphere. In this study we include natural products to the previously published training data to provide more accurate quantification of the plant root metabolome which also considers the difference in ionization efficiency between plant metabolites. Root exudates and root extracts of Lotus japonicus were analyzed using an ultrahigh-performance liquid chromatograph coupled to a quadrupole time-of-flight mass spectrometer through an ESI inlet in negative mode. Putative identification was performed using in-silico annotation tools (SIRIUS + CSI:FingerID) [6].

Preliminary results from the experiments indicate clear differences in the overall chemical composition of root extracts and root exudate when accounting for the difference in ionization efficiency across all the detected features.

This work was funded by the Novo Nordisk Foundation.

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Poster

In-depth analysis of pistachio aroma compounds utilizing high-capacity concentration tools coupled with GC×GC-MS

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The quality and consumer acceptance of pistachio are heavily influenced by its key organoleptic characteristics and aroma is one of these. This attribute is connected to various factors like cultivar geographical origins harvesting conditions and storage as is common with natural products. This study focused on evaluating the volatile organic compounds (VOCs) of pistachios from diverse geographic locations. The evaluation was conducted using a high-capacity concentration tool called HiSorb. Various HiSorb probes were tested to optimize results considering factors such as temperature and extraction time by means of a design of experiment. The extracted VOCs of 18 pistachio samples belonging to different geographic areas were subsequently analyzed using two-dimensional comprehensive gas chromatography - triple quadrupole mass spectrometry (GC×GC-QQQ-MS) equipped with a reversed fill/flush flow modulator. For the GC´GC separation a conventional (non-polar/polar) set of columns was used. The first dimension (1D) column used was a 20m ´ 0.18 mm i.d. ´ 0.18 µm film thickness; equivalent in polarity to 5% phenyl / 95% dimethyl polysiloxane while the (2D) column was a 50ms 3m ´ 0.25 mm i.d. ´ 0.25 film thickness equivalent in polarity to 50% phenyl / 50% dimethyl polysiloxane. The MS was operated in single-Q mode using electron-impact ionization (EI) at 70 eV. The ion source and transfer line temperatures were 200 °C and 250 °C respectively. The scan range was set to 35–350 m/z with an acquisition frequency of 50 Hz. A core of 15 volatile molecules able to discriminate the different geographic origins of pistachio samples was also established using classification algorithms (random forest). The use of GC×GC enhances separation power maximizing the extraction of information and providing a chromatographic fingerprint of the VOCs in pistachios.

Poster

Comparison of CCS values of isobaric and asymmetric dimers of PFCA to assess the gas-phase conformation of PFCA dimers.

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Per- and polyfluoroalkyl substances (PFASs) are emerging pollutants of great concern with over 5000 compounds currently reported. Hyphenated techniques such as ion mobility spectrometry (IMS) coupled to liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) hold promise for non-targeted screening of these substances. Among the many advantages of IMS a descriptor related to molecular shape is obtained through the collision cross-section (CCS) which can be calculated from the measured ion mobility and provides an additional identification point. However when trapped IMS (TIMS) is coupled to LC-MS to analyze legacy perfluoroalkyl carboxylic acids (PFCAs) multiple mobility peaks are observed at the mass-to-charge ratio (m/z) of a single deprotonated ion ([M-H]-) preventing the determination of an unambiguous CCS as an identifier. We determined that one of the unexpected peaks was due to a homodimeric PFCA ion ([2M-H]-) that existed prior to ion mobility separation and could dissociated after mobility separation into the corresponding deprotonated ion ([M-H]-). As CCS-m/z trendlines could be obtained for the multiple monomeric PFCA homologues and their corresponding homodimeric ions a plausible structural conformation was hypothesized. All the PFCA monomers detected shared the same linear relationship between CCS and m/z suggesting that the addition of CF2 units induces a growth of the ion in a cylindric shape with a constant diameter [1]. For the homodimeric ions the CCS-m/z trendline deviated from linearity and was best fitted with a power regression model. This suggests that the proton-bound PFCA homodimer ([2M-H]-) more likely adopts a V-shape with the proton bridging the carboxylate extremities rather than a cylindric shape. To support this hypothesis we performed IMS-MS measurements of asymmetric but isobaric (i.e. sharing the same m/z ratio and number of CF2 units) proton-bound PFCA dimers. If PFCA dimers do indeed adopt a V-shape the CCS values of these asymmetric dimers can be expected to differ. To this end several mixtures of two PFCA homologues capable of forming isobaric dimers e.g. C4+C14 and C8+C10 were analyzed in direct injection (i.e. without prior LC separation) to promote the formation of the corresponding heterodimers (e.g. C4-C14). The results show that the more asymmetric the dimer is the higher is its CCS value. Consequently the CCS value is influenced by the longer fluorinated chain of the two coordinated monomers suggesting that the two chains have not folded over each other which tends to confirm the V-shape hypothesis. To further support this theoretical calculations will be performed to determine whether it is possible to predict the observed CCS trend. Finally the influence of monocharged cations other than the proton (H+) on the CCS trendlines of the PFCA heterodimers was investigated to gain insight into their influence on the overall shape of these dimers. For this purpose four alkali metal cations (Li+ Na+ K+ and Cs+) were added to the injected PFCA solutions to promote the formation of cation-bound dimers and the resulting solutions were analyzed by IMS.

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Poster

Investigation of the higher order structure of small oligopeptides in solution and gas phase by capillary electrophoresis coupled with ion mobility mass spectrometry.

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Bioactive Peptides obtained from hydrolyzed proteins are nowadays of great interest in both the food and pharmaceutical industries for their wide range of therapeutical application [1]. These peptides like proteins derive their biological activity from their specific structure including their three-dimensional conformation.

Capillary electrophoresis (CE) separates compounds by (averaged) charges and in-solution shape according to thedescribed in terms of hydrodynamic radius. CE offers a powerful alternative to liquid chromatography (LC) for the separation of peptides potentially in non-denaturing condition improving the detection of both highly hydrophilic and hydrophobic compounds while providing insights onto the structure of the analyzed peptides inanalytes in solution [2].

Ion mobility spectrometry (IMS) is somewhat similar to CE but operates in the gas phase at moderate pressure. The ions are also separated by charge this time induced by the electrospray ion source as well as the ion shape described in terms of collision cross section (CCS). Ion mobility coupled to mass spectrometry (IM-MS) improve the peak capacity of any separation methods coupled to MS depending ofon the degree of orthogonality between the separation technique and ion mobility. The recent development of interfaces allows nowadays for robust hyphenation of CE and ESI-MS and ESI-IM-MS instruments.

Peptides generated from BSA tryptic digest were separated by capillary zone electrophoresis (CZE) at different pH and detected on-line by ESI-IM-MS using a homemade sheath liquid microfluidic interface. We observed that for some peptides the conformation in solution and in the gas phase of unique peptide sharing the same charge state were not strictly correlated. Additionally several conformers of the same peptide could be detected either in the gas phase either in solution either both i.e. solution and gas phase These results suggest that the structure conservation hypothesis which states that the structure of a specie is preconserved by being kinetically trapped during the ESI process might not be true for oligopeptides containing less than 20 residues.

CZE coupled with IM-MS and collision induced unfolding (on helium) experiments were performed on some peptides. Our data suggest that conformation of oligopeptides could indeed be kinetically trapped conformations under our experimental conditions.

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Poster

Determination of anticancer arginase inhibitor using high performance liquid chromatography with fluorescence detection.

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The analysis of new active pharmaceutical ingredient (API) are of great importance during drug development process. New drug candidate must meet criteria set by regulatory agencies. Optimized and validated analytical methods should provide information about safety and purity of API which has to be manufactured under controlled GMP (Good Manufacturing Practices) standards [1].

The new drug candidate in cancer immunotherapy is an active arginase inhibitor which restore immune cell activity. OATD-02 boronic acid derivative is the highly potent and selective small-molecule arginase inhibitor involved in both tumour immunity and metabolism [2]. The OATD-02 compound poses difficulties in chromatographic analysis because of its polarity zwitterionic nature and poor solubility in most organic solvents. Additionally there are also detection problems due to weak chromophores in OATD-02 molecule. Its structure contains chromophore groups with low molar absorption coefficient in the UV and visible light range. High-performance liquid chromatography (HPLC) methods with UV-Vis detection are the most widespread analytical procedures in pharmaceutical applications but it suffers from limitations for molecules that do not possess UV chromophores.

It was necessary to develop a sensitive and precise analytical method capable of monitoring related substances in API. For detection of boronic acid groups in molecules high-performance liquid chromatography with a post-column reaction with alizarin were used [3]. OATD-02 molecule contains boronic acid which can react with the alizarine dye and forms fluorescent complex. To obtain the best separation of analysed molecule different of eluent compositions stationary phases derivatization reagent flow rate and fluorescence wavelength were optimized. The developed method has been successfully validated and used as a routine method to control the quality of the active pharmaceutical ingredient a new boronic acid derivative clinical development candidate for cancer immunotherapy [4].

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Poster

Characterization of the New PAL Micro-SPE Cartridge for Pesticides Extract Clean-up

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Since more than ten years micro-SPE (µSPE) emerged as micromethod for sample preparation and clean-up in forensic environmental and food safety analysis. Applications are SPE typical wide-ranging and can cover drugs different environmental contaminants and in particular the QuEChERS extract clean-up in pesticide analysis. At the same time the automation of the µSPE sample preparation steps led to the desired increase in sample throughput and the potential for online hyphenation with GC-MS and LC-MS instrumentation.

With the increasing demand and use of automated µSPE workflows on PAL Systems mechanical and also analytical limitations of the so far employed µSPE cartridge (ITSP Solutions Inc. Hartwell GA USA) the requirement for extended functionality evolved. With the new PAL µSPE cartridge CTC Analytics introduced a novel septumless cartridge design dedicated to reliable high throughput automation and extended application range. The evaluation of the new cartridge design is presented with results for pesticides analysis from leading laboratories.

Design of the new PAL µSPE cartridge

The new PAL µSPE cartridge consists of two parts only. The polymer material used is chemically inert and free from leachables. The outer part provides a higher capacity and flexible volume for filter disks and sorbent materials. The bottom outlet is designed to penetrate pre-slit septa and also to deliver directly to LC injection ports. The inner part provides critical functionality with the compression of the sorbent/filter layers and a precise needle guide for safe and always upright transport. Of key importance here is the resulting high pressure resistant and leakfree seal against a syringe needle for increased load speeds and volumes.

Workflow optimization and first results

The publication by Nicolas Michlig and Steven J. Lehotay evaluated the novel septumless cartridge with high extract load flow rates in the range of up to 10 µL/s using extract volumes of up 600 µL. More than 250 pesticides were tested using the QuEChERSER methodology with LPGC-MS/MS analysis achieving recoveries of 80 to 120% for more than 260 pesticides. Optimization experiments led to a routine extract load volume of 500 µL at 5 µL/s flow rate.

A poster and oral was presented by Ederina Ninga and coworkers during the EPRW 2022 in Bologna Italy. This work presents a recovery study using several alternative sorbent materials using the novel PAL cartridge design with focus on fatty matrices from insects and feed materials using GC-MS/MS.

The evaluation of the automated clean-up using the novel PAL cartridge design for multiresidue pesticides analysis by LC-MS/MS was very recently reported by Lorena Manzano Sanchez Florencia

Jesus and coworkers. Improved recoveries were achieved for acidic compounds and sulfonylureas. A significant increase in sample throughput and reliability of the automated method is reported.

Conclusion

The novel septumless PAL µSPE cartridge design showed high reliability in automated high throughput workflows with excellent clean-up results and improved recoveries. The cartridge design successfully showed flexibility for different types and volumes of sorbent materials.

Poster

A powerful LC×SFC-HRMS/MS method for the non-target analysis of depolymerised lignin

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Lignin is an abundant natural polymer obtained mainly from the paper industry by the fractionation of cellulose from the overall lignocellulosic biomass. It is by far the largest natural resource for aromatic compounds; however approximately only 2% of it is commercialised while the rest is mainly burned to power the same paper industry. Thus in the name of circular economy lignin must be valorised more by isolating valuable aromatic compounds incorporating into novel materials (e.g. coatings resins thermoplastics) or converting into biofuel. For making these valorisation processes as efficient as possible thorough structural characterisation via a powerful chromatographic technique can be highly beneficial. However this is an arduous task as lignin is a complex natural material whose composition also depends on the botanical origin isolation and depolymerisation processes.1

Therefore in this study an off-line comprehensive two-dimensional (2D) chromatography method combining liquid chromatography supercritical fluid chromatography and high-resolution mass spectrometry with fragmentation (LC×SFC-HRMS/MS) was developed. The implementation of a 1-aminoanthracene column in the second dimension enabled a class separation of potential lignin monomers dimers trimers and tetramers with additional separation based on the number of hydroxyl groups and steric effects. The pentafluorophenyl column in the first dimension additionally improved the separation based on hydrophobicity; thus the first 2D LC plot demonstrating the classification of lignin compounds was obtained. The comparison of the technique to 1D SFC showed that the LC×SFC method was superior for the separation of the lignin compounds including isomers. The improved separation of isomers is especially beneficial for lignin as 77% of the detected compounds had at least one isomer detected in the same sample. Advanced data analysis methods (MS-DIAL SIRIUS and Feature-Based Molecular Network) were integrated into the non-target workflow to rapidly visualise and study the detected compounds.2

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Poster

Doubly Hyphenated Method (LC-MS/MS-Fluorometric) for Determination of Nucleoside Triphosphates and Analogs in Peripheral Blood Mononuclear Cells (PBMCs)

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We report a validated doubly hyphenated technique (LC-MS/MS-fluorometry) for determination of nucleoside triphosphates and analogs (TPs) in peripheral blood mononuclear cells (PBMCs). The analytical process involves sequential lysis of a PBMC sample extraction and selective determination of TPs in the total extract volume by LC-MS/MS and subsequent determination of sample cell counts by use of a DNA-specific fluorogenic intercalant to flourometrically quantify DNA in the residual pellet of the extracted sample. The amount of DNA is calibrated by standards prepared from commercially available purified reference human genomic DNA. Since a known constant amount of DNA is present in every human PBMC (and within ±1.5% between males and females) the amount of DNA in a PBMC sample can be used to determine the number of PBMCs in the sample. The overall amount of TPs in a PBMC sample can then be presented as mass of TPs per million cells which can be further converted to an intracellular molar concentration of TPs by use of the known mean volume of a PBMC and the TP molecular weight. Although the initial PBMC sample lysis for TP extraction and the corresponding TP quantification method need to be developed and validated for the specific TP(s) of interest the subsequent determination of PBMC number in the residual pellet sample applies to any PBMC sample regardless of drug analyte; therefore validation and application of the cell count method is independent of drug analysis and can be performed once.

As an example we present a hyphenated method validated for simultaneous determination of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) in a PBMC sample by LC-MS/MS and determination of the PBMC count by fluorometric detection. Both TFV-DP and FTC-TP are TP metabolites of dosed prodrugs (tenofovir alafenamide fumarate and emtricitabine respectively) that are administered in combination as treatment for HIV. Validation results for both the analyte and PBMC components of the LC-MS/MS-fluorometric hyphenated method are summarized and meet relevant expectations of regulatory guidances for bioanalytical methods.

Poster

StreamFind: Data processing workflow designer for non-target screening and highly hyphenated analytical systems

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Technological advances in instrumental analysis have increased the complexity and size of datasets requiring efficient processing solutions. In response vendors have taken two approaches: monolithic software with overwhelming user interfaces and micro-services resulting in lower user adoption. Challenges arise in applications such as non-target screening (NTS) for environmental analysis and quality evaluation of pharmaceuticals where proprietary software lacks flexibility for different use cases.

StreamFind aims to become an overarching platform for assembly of data processing workflows focusing on transparency reproducibility routine use flexibility and expandability. Also StreamFind aspires to improve users' data literacy by providing a flexible and understandable solution for assembling data processing workflows. Independently of the programming language data processing workflows are assembled for a given data processing engine (used as a metaphor) via harmonized processing settings which link diverse native and open source tools and ensures interface conformity for streaming data within the data processing workflow. The processing settings can be saved e.g. to reproduce the workflow with new data and share it within inter-laboratory studies. Furthermore the processing settings also hold information from the origin (i.e. developer and link to further information) of a given processing algorithm to support the utilization by the user. The data processing engines are fundamentally reference classes dedicated to specific data types (e.g. mass spectrometry (MS) and Raman spectroscopy data) serving as a framework for parsing managing inspecting and reporting raw and processed data. Currently StreamFind can be used via the R library but python and C++ variants are planned where other data types (e.g. sensor X-Ray diffraction (XRD) and DNA and RNA Sequencing data) will be supported. Data processing workflows for MS and Raman data are supported by the StreamFind R library. MS data processing workflows for NTS are assembled via integration of processing modules using patRoon[1] R functions R and C++ native algorithms as well as external algorithms such as the C++ qAlgorithms library[2]. Algorithms for feature extraction grouping annotation curation prioritization and identification are available for NTS. Installation instructions for the R library can be found in the GitHub repository[3]. Detailed documentation and demonstration articles can be found in the GitHub web page[4].

Data processing workflows for NTS of known and unknown micropollutants in the environment deconvolution of intact antibodies via MS data for identification as well as quality evaluation of pharmaceuticals by fusion of MS and Raman data are application case scenarios to be presented in the proposed poster for the StreamFind platform.

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Poster

Anti-influenza inhibitors screening from natural products based on magnetic beads-based ligand fishing

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Influenza is an acute respiratory infectious disease caused by the influenza virus which has the characteristics of high pathogenicity high morbidity and high mortality. As the core target of the RNA-dependent RNA polymerase (RdRp) enzyme PAN endonuclease has been used to develop RdRp inhibitors for anti-influenza virus activity because it is highly conserved and less prone to the development of resistance. Magnetic beads-based ligand fishing is very appealing for the screening of enzyme inhibitors from complex matrices due to the advantages of easy magnetic separation easy preparation and modification as well as large specific surface area[1]. This project aims at developing PAN endonuclease modified magnetic beads for screening potential RdRp inhibitors from Artemisiae argyi Folium. PAN endonuclease was immobilized on the magnetic beads with both covalent binding and affinity binding immobilization methods. When comparing both immobilization methods it is proposed that the affinity binding method can directly immobilize enzyme from bacterial lysates and the resulting beads show a high efficiency for ligand fishing. The excellent ligand fishing efficiency indicates that the proposed method possesses great potential for screening active ingredients from traditional Chinese medicine.

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Poster

A new approach to MOSH/MOAH analysis – application of GCxGC-MS for determination of mineral oil hydrocarbons in vegetable oils

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Mineral oils are complex mixtures of compounds of petroleum origin consisting mainly of mineral oil saturated hydrocarbons (MOSH; represented by n-alkanes isoalkanes and cycloalkanes) and mineral oil aromatic hydrocarbons (MOAH; represented by highly alkylated aromatic hydrocarbons). The toxicity of MOAH is of a greater concern as they are associated with mutagenicity and genotoxicity whereas MOSH may be retained in the human tissues but with possibly low/no adverse effects at exposure levels[1].

The analysis of MOSH/MOAH is a very challenging task due to the difficult separation required for their proper characterization and the lack of suitable (well-characterized) standard mixtures. When analysed by gas chromatography (GC) MOSH/MOAH form long humps of unresolved compounds providing no information about what is actually integrated. Furthermore the coeluting matrix components such as terpenes (monoterpenes and squalene) carotenes naturally occurring olefins and n-alkanes with odd-numbered carbons[2] can lead to overestimation of the contamination or even false positive results.

The main purpose of this pilot study is a prove of concept of a novel approach using comprehensive gas chromatography coupled with mass spectrometry (GCxGC-MS) method for the determination of MOSH/MOAH in vegetable oils. An off-line solid phase extraction (SPE) technique was used to isolate MOSH/MOAH from vegetable oil samples. The quantification of MOSH/MOAH was based on identification of peaks using a classification by their retention times on both columns and on the most abundant masses shared by several MOSH/MOAH in the deconvoluted mass spectra. The principle of this classification was proposed by Welthagen[3]. For the separation of the analytes a mid-polar (Rxi-17Sil MS column 15 m x 0.25 mm i.d. x 0.25 µm film thickness Restek Bellefonte USA) column was used in the first dimension and a non-polar (Rxi-1HT column 1.5 m x 0.25 mm i.d. x 0.10 µm film thickness Restek Bellefonte USA) column was used in the second dimension. Peak classification was verified by comparison of peak spectra with the NIST mass spectra library (v2.3 NIST Gaithersburg USA). Final quantification was done by the standard addition method.

The method was validated by analysis of in-house spiked vegetable oils at three different concentration levels (each in six parallels). The recoveries were 77 – 92% and the repeatabilities were <19%. In addition the results obtained by this method were verified by analysis of reference materials of olive oils (n=2) obtained by participation in an interlaboratory study organised by the Joint Research Centre (JRC). The obtained z-scores ranged from -2.0 to 1.1 giving satisfactory results.

The proposed GCxGC-MS method exploits the advantages of MS (peak confirmation/identification) and could be an alternative to established approaches. Taking advantage of the superior resolving power of GCxGC both MOSH and MOAH were analysed in one GC run thus simplifying the sample preparation step.

Acknowledgements

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Poster

MULTIPLE LINEAR REGRESSION MODELS FOR PREDICTING LINEAR RETENTION INDICES OF VOLATILE COMPOUNDS IN ESSENTIAL OILS ANALYZED BY GAS CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY - DEVELOPMENT AND VALIDATION

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Introduction

Due to their relative independence from the operational parameters linear retention indices (LRIs) are useful tools in gas chromatography-mass spectrometry (GC-MS) to support qualitative analysis. The current study aims to develop a multiple linear regression (MLR) model for the prediction of LRIs as a function of selected molecular descriptors.

Methods

Liquid phase injection GC-MS/MS was used for the analysis of essential oils (rose lavender and peppermint) using a semi-standard non-polar stationary phase. NIST mass spectral library was used for the identification of the volatile compounds (acceptance criteria: ±20 RI units and a similarity value higher than 80%). A standard mixture of n-alkanes (from C8 to C20) was measured in order to calculate the LRIs for the experimentally identified compounds using the van den Dool and Kratz equation [1]. The PaDEL-Descriptor software [2] was used for generation of molecular descriptors. A stepwise MLR was applied for the selection of the significant descriptors (variables). A split experimental design was applied for deriving and validating the MLR model by applying Enter and Stepwise algorithms. An external validation was performed for further testing the developed MLR model based on HS-SPME-GC-MS/MS measurements.

Results

By liquid injection GC-MS/MS analysis a sum of 103 compounds were identified in the three essential oils and the LRIs were calculated. By applying a stepwise MLR only 16 variables were found to be significant and independent from a tested set of over 2000 molecular descriptors. At this point split design validation was applied: the identified compounds were randomly divided into training (85%) and validation (15%) sets. The training set (87 compounds) was used to derive two MLR models by applying i) the ‘enter’ algorithm (R2 = 0.9960 RMSE = 17) and ii) the ‘stepwise’ one (R2 = 0.9958 RMSE = 17). The predictive power was assessed by the validation set (16 compounds) as follows i) q2F1 = 0.9896 RMSE = 25 and ii) q2F1 = 0.9886 RMSE = 26 respectively.

The adequateness of the two regression approaches was further evaluated. HS-SPME in combination with GC-MS/MS was used for an alternative analysis of the tested essential oils. The values of the significant descriptors were within the model working range for 12 additional compounds not previously detected by the liquid sample introduction mode of analysis. For the last compounds the LRIs were calculated and the experimental data was used as an external set. The predictive power for

both regression approaches was assessed as follows: Enter RMSE = 41 q2F2 = 0.9503 and Stepwise RMSE = 40 q2F2 = 0.9521.

Acknowledgements

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Poster

Sorbent immobilization using DLP 3D-printing and its use in producing Instant Extraction and Headspace devices.

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The popularity of 3D printing technology has been on the rise for several years now. Analytical chemistry and separation science have embraced this technology utilizing it extensively for creating customized laboratory equipment and enhancing advanced analytical machines. This not only improves the quality of usage but also enables analyses that were previously impossible. While FDM printers dominate the 3D printing landscape Digital Light Projection (DLP) a technique based on the polymerization of photo-curable resin is also widely employed. It is particularly prevalent in the production of custom microfluidic devices and intricate precise shapes. [1].

Presented study expands the idea of using DLP 3D printing as a useful and cheap way of sorbent immobilization as well as custom-shape sorbent production. In our previous findings octadecyl-modified silica particles were suspended in printing resin and used for printing a volumetric lattice shape. This provided a method to produce effective direct-immersion sorption devices on-demand in a cheap and efficient way. Presented evolution of this idea tests two approaches. First one is based on how DLP 3D printing can handle producing instant extraction devices eliminating sorption and desorption times from the original approach. Second one was to test how 3D printed sorption devices can handle extraction of volatile compounds (dioxins and polychlorinated biphenyls) using it as a headspace device instead of direct immersion. For instant-extraction devices (based around printing a complex shape inside a channel similar to SPE column) various shape types for the filling were tested and compared in terms of extraction efficiency and repeatability. Headspace devices were also compared using the same criteria.

Presented study further expands the idea of using DLP 3D printing as a sorbent immobilization method showing it as a valid way to produce not only direct-immersion sorbent devices but also much more complex devices where shape intricacy eliminates the waiting time required for standard extraction protocols. Furthermore using this approach to produce headspace devices broadens the knowledge on both its usefulness and limitations.

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Poster

Optimizing gradient profiles for liquid chromatography: a comparison of optimization algorithms

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Liquid chromatography (LC) is the single largest analytical field in terms of people involved and money spent. LC is crucial for almost all public and private sectors and the technique has seen tremendous technological advancements. However despite the enormous size and importance of the field separations are often performed under sub-optimal conditions and technological capabilities remain unused. Exploiting the full technological capabilities of liquid-phase separation technology (e.g. complex gradient assemblies two-dimensional LC etc.) requires deep knowledge and great time investments. Method optimization strategies that can optimize the large number of parameters involved simultaneously are thus of great interest to the field of chromatography [1].

To address this we recently proposed the "AutoLC" workflow where (i) retention models are established based on tracked retention data from a sample of interest (ii) chromatographic separations can be simulated using the retention models so that (iii) a suitable candidate method is selected and programmed into the LC system after which (iv) the new data is then evaluated and added to the retention models [2]. This process loops unsupervised until a suitable optimum is found. Choosing the correct optimization algorithm to find the best suitable method parameters is challenging due to the vastly different samples (both in nature and complexity) and optimization goals for which the algorithms are applied. Therefore a comparison of the effectiveness of the algorithms is crucial to improve method-development workflows.

In this poster we discuss the framework we constructed to investigate the efficiency of various optimization algorithms in arriving at an optimum in chromatography method-development workflows. Specifically we evaluated the data- and time-efficiency of optimization algorithms across different computational samples varying gradient-segment designs and a variety of chromatographic response functions.

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Poster

Efficient Human Plasma Proteome Profiling at up to 300 Samples Per Day

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Introduction

LC-MS-based proteomics stands as a vital tool for the analysis of intricate biological and clinical samples. Within the separation core the LC column assumes a pivotal role by reducing sample complexity before its injection into the MS. The micro pillar array column (µPAC™) emerges as an innovative LC column featuring uniformly arranged pillars within a microfluidic channel offering both highly efficient separation and minimal back pressure. In this study we used a 5.5 cm long µPAC Neo High Throughput column that has channels filled with rectangular-shaped silicon pillars coupled with HRAM mass spectrometry. Our goal was to expedite bottom-up proteomics profiling of neat human plasma samples while leveraging pillar arrays' benefits such as high reproducibility and minimal sample carry-over.

Methods

Pooled human blood plasma underwent S-Trap™ mini spin column digestion followed by resuspension in 0.1% TFA to 1000 ng/µL. Neat plasma digests were analyzed using an Orbitrap™ Exploris 240 mass spectrometer coupled to a Vanquish™ Neo UHPLC system employing a data independent acquisition strategy. Separation occurred on a µPAC Neo High Throughput column in either trap-and-elute or direct injection mode. A dilution series from 10 to 2000 ng of neat plasma digests was separated using various flow rates. Methods accommodating 100 180170 and 300240 samples per day (SPD) were explored for optimal throughput. The isolation window was 10 Th with MS1 and MS2 resolutions of 30K and 15K respectively. LC-MS data were analyzed using Proteome Discoverer 3.1 and Spectronaut® 18.

Preliminary data

Using a 3-minute gradient 300240 SPD method the amount of protein groups that could be identified from neat human plasma steadily increased according to the sample load up to a load of 200 ng plasma on column where a plateau of about 250 protein groups was achieved. Increasing beyond 200 ng didn't significantly improve protein identification suggesting 200 ng is sufficient for favorable outcomes in this timeframe. This was also confirmed by checking the base peak chromatograms and overlaying the different sample loads where 200 ng was identified as the onset of overloading. Given the extremely high throughput of this method fair quantitation could be achieved resulting in up to 205 protein groups quantified at CV below 20% for 500 ng of plasma analyzed in triplicate.

Similar observations were done across different SPD settings (180170 100) where increasing sample loading beyond 500 ng did not increase proteome depth. By extending the gradient length to respectively 5.5 and 11 min increased depth and especially an increase in number of quantifyablequantifiable proteins could be obtained. The µPAC Neo High Throughput column achieved a pragmatic compromise at 180170 SPD quantifiyingquantifying 248 protein groups (below

20% CV). The impact of extending gradients beyond the 5.5 min used for the 180170 SPD method appeared to be very limited as barely any depth could be gained. Ongoing efforts are focusing on extended performance testing and comparative analysis of the susceptibility to sample related carry-over related to different LC and injection configurations.

Poster

Characterization and Price Prediction Modeling of Whisky Using GC×GC-QTOF Analysis and PLS Regression

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The total whisky revenue in 2024 is expected to amount to €92.84 billion. Due to the often-high prices of commercial whiskies whiskies on the cheaper end of the spectrum are introduced. Similarly smaller distilleries may struggle with pricing their whisky in such a competitive market. Likewise extremely high prices are not unheard of increasing the risk of counterfeit whiskies. Besides tests like visual inspection of the bottle seal and label and qualitative analysis describing the taste and smell the complexity and variability of these whiskies may increase the need for comprehensive characterization techniques that can distinguish real and counterfeit – as well as cheap and expensive – whisky.

One such technique that caters to this need is comprehensive two-dimensional gas chromatography coupled to a quadrupole-time-of-flight mass spectrometer (GC×GC-QTOF). By separating and analyzing the constituents that make up a whisky identification of the individual compounds can be ensured. Prior work has mainly focused on whisky authentication and provenance using chemometric techniques such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) while the relationship – if there exists one to begin with – between the compounds in a whisky and its price is yet to be established.

This recent work focuses on the GC×GC-QTOF analysis of 59 different whiskies containing a wide price range (€7.00 to €115.00) seven countries of origin and several cask types and maturation years. Samples were injected ‘as is’ without any preparation on a thermally modulated system. Data analysis was in part performed using the GC Image software. Besides exploratory PCA of the data an in-house PLS-regression model was developed using the (corrected) intensities or volumes of the detected peaks to predict the price of the whiskies.

Poster

Rapid analysis of chromium species using µLC-ICP-MS

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Chromium (Cr) is a transition element that exists in oxidation states ranging from - 2 to +6. The common stable ones in the environment are trivalent Cr(III) and hexavalent Cr(VI) chromium. Cr(III) is an important micronutrient for the human body while Cr(VI) is highly toxic and carcinogenic. The environmental concentrations of both oxidation states are low. Due to the differences in toxicity between Cr(VI) and Cr(III) compounds speciation of Cr is very important. Therefore an improved sensitive and robust method for the simultaneous determination of Cr(III) and Cr(VI) in water samples (saliva waste water and nutritional solution) has been developed. The method uses a hyphenated micro liquid chromatography (µLC) system coupled to inductively coupled plasma mass spectrometry (ICP-MS). The optimised method incorporates a pH adjusted EDTA complexation step to stabilise Cr(VI) and Cr(III). The µLC system uses an anion exchange micro-sized column to separate the Cr species. Cr(III) and Cr(VI) were separated with different retention times at 170 and 230 sec respectively. The method was optimized and validated by spiking Cr(III) and Cr(VI) in various water samples. Furthermore the method was validated using a drinking water proficiency testing material sample. The developed method can be used for rapid routine determination of chromium species with high precision and reliability.

Poster

Novel Platform for Purity Control of Highly Reactive Starting Materials

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Because of their intrinsic reactivity Starting Materials (SM) are often one of the most valuable compounds in the API's synthesis train perfect examples being acid chlorides and heteroaromatics. In this regard an important prerequisite is the strict control of the SM's purity profile to avoid the creation of unwanted impurities at API level. Conventional GC methods for purity determination however rely on direct injection without prior protection of the reactive group which causes this process to be greatly complicated by the possibility of artefact creation during analysis. Such inadequate identification and quantification of impurities must be avoided at all costs as it could adversely impact final product characteristics.

Since its discovery in the nineties Full Evaporation Static Headspace Sampling (FESHS) has been exclusively applied in the pharmaceutical industry for the identification and determination of residual solvents in solid materials i.e. to reduce available matrix effects[1-2-3] as well as to improve the sensitivity of high boiling solvents[4]. Careful examination within our team at J&J however revealed the opportunity to also apply this technique as a more suitable alternative to direct injection when evaluating the purity of highly reactive Starting Materials. For multiple compounds we demonstrated that under the right conditions e.g. by combing a preceding derivatization procedure with an optimal subset of sample preparation and instrument parameters the use of FESHS offers unprecedented baseline separation and impurity quantification. Even more after effective validation against J&J standards it was shown that this novel platform can be successfully transferred to multiple QC labs around the world illustrating its tremendous potential.

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Poster

Modeling Software for Retention Prediction in Gradient Reverse Phase Liquid Chromatography (RPLC) – A Diagnostic Tool

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Computer-assisted modeling of retention time is a powerful tool to accelerate method development for analytical and preparative separations. Building retention models using RPLC is dependent upon peak tracking and component identification. Dwell volume is an important variable in predicting analyte retention. Here it has shown that computer-assisted modeling can also be of great diagnostic value. We present the ability to switch the order of elution of analytes based on organic modifier and stationary phase combinations and the ability to optimize the final separation considering the % initial final and gradient time.

In wave 1 various stationary phases are combined with different organic modifiers in gradient RPLC at a fixed gradient slope and column temperature. In wave 2 a two-dimensional gradient time versus temperature resolution model is created with the LC Simulator module in ACD/Method Selection Suite software. This constructs a resolution response and predicts optimum separation conditions with 9 experiments from 3 temperatures and 3 gradient times.

In Wave 1 using both PDA and MS detection all components in the mixture could be peak tracked in Method Selection Suite. The ability to switch the order of elution of analytes based on organic modifier and stationary phase combinations was also observed.

In Wave 2 peak movement as a function of temperature and gradient time is observed. The resolution map shows that the accuracy of the retention model is $\%\Delta tR < 0.3\%$ with improved separation observed at temperatures below 35°C. Switches in peak elution are indicated by the resolution map. LC simulator was used to investigate the behavior of the analytes in the test mixture (composed of 8 analytes of varying hydrophobicity). Excellent retention time predictions were achieved when the initial %B was kept the same as that used to generate the retention model input data. When there were larger differences in the initial %B optimized and %B used to create the model anomalous retention predictions were observed. This was caused by analytes with low to medium retentivity that migrated isocratically down the column here small errors in dwell volume can cause larger inaccuracies in retention time prediction. More accurate retention time predictions were obtained using Method Selection Suite software to try different dwell volumes that fit the retention model compare the residuals obtained and select the dwell volume that gave the lowest residual. It was also seen that analytes that were more polar exhibited this inaccuracy to a greater degree compared to analytes that were more non-polar. This observation was tested further with an additional test mixture of phenone standards which showed the same behavior.

Poster

Fully automated workflow for Sample Preparation and Measurement of Dioxin in Food and Environmental samples

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Dioxins are a class of very toxic compounds found throughout the world in the environment. Equipment sensitivity is of great importance for the analysis of low concentrations of these highly-toxic compounds. Historically analysis and detection of dioxins was done with magnetic sector-type high-resolution mass spectrometers (HRMS). However in recent years the performance of triple quadrupole mass spectrometers (MS/MS) has improved significantly. In addition the development of the Boosted Efficiency Ion Source (BEIS) offers compound- specific sensitivity up to 4 times greater than previous ion sources and provides accurate quantitation of dioxins at levels comparable to HRMS. Detection limits as low as 20 fg for Tetrachlorodibenzo-p-dioxin (TCDD) were achieved. In this study we analyzed dioxins in about 250 samples of approximately 40 types of food and animal feed products using a GC-MS/MS with BEIS. We also evaluated the number of analyses possible while maintaining sensitivity at low concentrations in order to verify the durability of the GC-MS/MS instrument.

Poster

Development of a UHPLC-MS assay to evaluate beta-hematin polymerization inhibition for the identification of new potential antimalarial candidates

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Malaria is among the deadliest parasitic diseases infecting humans worldwide. In 2022 the World Health Organization (WHO) reported 249 million cases and approximately 608000 deaths. These figures have risen since 2019 partly due to the various disruptions caused by the COVID-19 pandemic. Other factors have contributed to this rise along the years climate change being a major contributor. The fight against this disease is also rendered more difficult by the emergence and spread of drug-resistant strains [1]. All endemic regions are impacted by this phenomenon. The need for new therapeutic candidates is now greater than ever. The disease is caused in humans by several species of Plasmodium protozoans. During the intraerythrocytic stage of the infection the parasite consumes hemoglobin which releases free heme and globin. The latter is further digested to release amino acids that can be used by the parasite. However free heme can cause oxidative damage to the parasite. To avoid this Plasmodium parasites detoxify free heme through a process called biocrystallization during which heme (Fe(II)PPIX) is oxidized into hemin (Fe(III)PPIX). Hemin molecules then bind together which leads to the formation of a non-toxic by-product hemozoin. The inhibition of this pathway is among the main targets for antimalarial drugs [2].

The present work aims to develop and optimize a LC-MS method that allows to detect and quantify remaining free hemin after incubation with growing concentrations of test compounds [3]. This will allow us to calculate the inhibition rate of polymerization. The overall objective being to identify potential antimalarial compounds that block the hemozoin formation pathway.

The reaction medium was optimized. Hemin is dissolved in dimethylsulfoxide and incubated at 60°C for 1h in acidic conditions. This leads to the formation of a precipitate of beta-hematin often referred to as synthetic hemozoin. It is structurally and chemically similar to the native form. This was confirmed by infra-red spectroscopy. Chloroquine a known inhibitor of heme detoxification was selected as the reference compound for our tests. After incubation the supernatants are analyzed by LC-MS. The initial chromatographic conditions have been selected but require further optimization especially regarding the carry-over problem. For this purpose several washing solvents will be tested. The molecular ion peak corresponding to hemin at 616.18 m/z will be monitored. A negative control (no test compound with incubation) and a control without incubation will also be analyzed to allow the determination of the inhibition rate (%).

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Poster

Chemical characterization of European medicinal plants by multi-2D LC x LC

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The growth of antibiotic-resistant bacteria and strong side- or patient-dependent effects of existing drugs encourages the expansion of knowledge about herbal remedies that have been used as alternative until now [1 2]. Despite the expertise about the therapeutic effects of traditional plants there is a lack of understanding about how these effects are achieved and which compounds are responsible for such beneficial effects. Consequently further information about their chemical composition by powerful separation techniques that improve the identification of the compounds as well as the bioactivity of these plants and their compounds is needed.

Four European medicinal plants (Agrimonia eupatoria Angelica archangelica Sambucus nigra and Sambucus ebulus) known for their hepatoprotective activity were chosen and extracted using an optimized green microwave-assisted extraction (MAE) method. In order to unveil the metabolic profiles of these extracts a comprehensive multi-2D liquid chromatography (multi-2D LC x LC-DAD) method was developed. Multi-2D LC x LC is a novel multidimensional technique that uses not only two orthogonal columns in the first (1D) and second dimension (2D) but also two orthogonal 2D columns which are switched according to the polarity of the analytes [3]. Using a PFP (Kinetex 150 x 2.1 mm 1.7 µm) column in the 1D and combining a ZIC-HILIC (50 x 4.6 mm 5 µm) and a C18 (Kinetex 50 x 4.6 mm 2.6 µm) in the 2D the developed method is capable of separating complex plant extracts derived from various plant parts and species providing a maximized separation power. For the identification of the separated compounds a non-target approach based method was followed after coupling the multi-2D LC x LC to high resolution mass spectrometry (HRMS/MS).

Among the different plant parts such as flowers berries leaves seeds roots and barks the most promising extracts were derived from the leaves of S. nigra A. archangelica and A. eupatoria as well as the flowers of S. nigra according to their total phenolic content and antioxidant activity. Variances in their chemical profiles or bioactivity were found even within the same plant part and species. Thus a deep monitoring of the herbal medicine formulations is undoubtedly necessary to uncover counterfeits adulterations and plant material of deficient quality.

Acknowledgements

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Poster

Comprehensive Analysis of Sterols and Oxysterols by Heart-Cut 2D-LC-MS

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Oxysterols the oxidation products of sterols crucially regulate inflammation cholesterol metabolism and cellular homeostasis. Additionally their association with tumor development and metastasis underscores their importance in cancer research. However understanding their molecular mechanisms remains limited necessitating new analytical methods capable of simultaneously analyzing sterols involved in cholesterol biosynthesis and oxysterols. This is an analytical challenge due to the substantial concentration differences between cholesterol (in high µM concentration) and sterols/ oxysterols (typically in nM concentrations) compounded by their structural similarity differing only in the position of a double bond or hydroxy group.

To address this we developed a heart-cut 2D-LC-MS approach customized for comprehensive sterol and oxysterol analysis. Utilization of the concentration disparity our method selectively transfers the cholesterol peak from the first to the second dimension for enhanced separation using a trap-based system which allows to transfer large volumes (~250 µL) to the second dimension.

In the first dimension baseline resolution of critical sterols and oxysterols is achieved with a PFP column while an isocratic step separates oxysterols and a slowly gradient the cholesterol biosynthesis precursors. Scheduled SRM mode on a triple quadrupole MS enhances sensitivity and specificity with APCI offering superior performance for the analysis with LODs in the low nM concentrations range. Also APCI showed little to no ion suppression and higher robustness compared to other ionization sources such as ESI. Applied to primary tumors and metastases of melanoma and pancreatic cancer the method allows to characterize the molecular mechanism of the sterol metabolism in these cancer types.

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Poster

Multiple headspace extraction approach applied to thermal desorption prior to gas chromatography

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In its native mode thermal desorption (TD) collects volatiles from the environment to be analyzed on a sorbent immobilized in a sample tube. Subsequently these tubes are loaded in the TD for desorption and refocusing followed by gas chromatographic (GC) analysis. It is quite evident that all process parameters need to be optimized. Here focus will be on the desorption process. After selecting the sorbent the remaining desorption parameters are time flow and temperature. Although there is a relationship between time and flow (double the flow halve the time) it remains a time consuming process since for each combination a complete run has to be executed. A TD can also be used in direct desorption mode. In this case no sorbent is used but the sample is placed directly in the TD sample tube. As a consequence the sample is subjected to the heating cycle of the desorption process which often results in thermal degradation. This may lead to extra peaks and a less stable baseline in the chromatogram. Moreover altered interactions between the analytes and the matrix are possible. In this way finding the optimum desorption conditions becomes even harder.

In the standard workflow a newly loaded TD tube is used for every parameter combination. However it is possible to program the TD-GC combination in such a way that a TD tube is processed repeatedly without being removed from the desorption stage. When this is performed using abnormally (for TD) short desorption times a data set is created showing sample depletion. This can be used to determine the optimal tube desorption time in a shorter time frame. On the other hand the data can also be treated in a similar way as data obtained from multiple headspace extraction to deal with the effects induced by thermal decomposition of the analytes/matrix. This will be illustrated with some examples.

Poster

Impact of diluents on the calibration of direct dynamic thermal desorption prior to gas chromatography

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A thermal desorber (TD) can be used in different ways to introduce samples in a gas chromatographic (GC) system. Besides its conventional use where the collected analytes are released from the adsorbent in the sample tube direct dynamic desorption is an interesting option where the sample is put directly in the TD tube. However since no adsorbent is used for the sample proper calibration is not straightforward. This issue was investigated in the present work using offline liquid calibration (OLC) and inline liquid calibration (ILC). ILC avoids the use of a sorbent in the sample tube since the reference solution is injected in de desorption gas stream of the TD just before the desorption stage. This way a complete transfer of reference compounds to the cold trap of the TD is assumed.

In this work the effect of two diluents (water and toluene) on ILC and OLC prior to TD-GC has been investigated for the determination of methanol ethanol and tertiary butanol. Unexpectedly ILC yielded a lower response than OLC. This could be related to the adsorption kinetics of the analytes and water on the cold trap of the TD. More insight was gained performing double injection ILC experiments with toluene as diluent for the analytes and injecting water before or after the toluene solution. This revealed a clear influence of the diluent. The influence of water was further explored applying two cold trap temperatures (4 °C and -30 °C). Inserting a LiCl trap in the TD tube to capture the water was found to be an effective solution for the problem and quantitative aspects of this approach were demonstrated.

Poster

Ultra-low dispersion microfluidic cell design for UHPLC with online radio-activity detection

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UHPLC with high-resolution mass spectrometry (HRMS) and MS/MS detection is the key technology for identification and structure elucidation in drug metabolism studies. Entering the development stage of a drug this is often complemented with radioactivity detection which allows for a targeted approach and absolute quantitation given its unique selectivity.

Radioactivity detection is based on detection of photons via photomultiplier tubes (PMT) which are emitted by a scintillant a mixture of chemicals which will react to the beta-decay of the radiolabelled analytes. The concentration is proportional to the number of disintegrations per minute (DPM) hence the longer the residence time in the detection cell the higher signal intensity. For this reason the typical detection cell for online radio-LC consists of a transparent tubing coiled in a plane between two PMT occupying the entire surface of the PMT. A large ID tubing results in a long residence time and increased signal this however at the cost of dispersion affecting peak shape and influencing the limit of detection. For this reason the evolution of radio-HPLC towards radio-UHPLC led to the reduction in cell volume to maintain LC resolution. The peak shape is positively affected by the necessary addition of a flow of scintillation liquid in a 2:1 to 4:1 ratio [1]. Nevertheless the detected peaks are much broader compared to those observed in the prior UV detector with a 2 µL flow cell.

We present the development and evaluation of a novel cell design making use of a microfluidic chip. Two chip designs are tested both etched with a serpentine channel of 500 µm i.d. which has a path length of 160 to 200 cm depending on the bend radius applied. The flow is continuously forced to change directions disrupting the flow profile which would typically be observed in a tubular pipe. This results in much more favorable dispersion characteristics as well as a mixing functionality compared to a classical tubing. Moreover the negative effect on peak dispersion coming from a gradient in bend radius which is the case in a spiral coiled tubing is absent. The increase of peak width or volume during passage in the cell was determined to be 55 % lower compared to the classical spiral-tubing cell effectively maintaining a much narrower peak.

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Poster

Analytical method advances in measuring nitrites in pharmaceutical excipients

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Regulatory authorities like the U.S. Food and Drug Administration (FDA) have set maximum daily intake levels for N-nitrosamines in finished drug products. In an acidic environment nitrite is a potential precursor for the formation of N-nitrosamines when secondary or tertiary amines are also present in the active pharmaceutical ingredient (API) synthesis or formulated drug product. An accurate and sensitive determination of nitrite will be useful when a drug product manufacturer chooses to investigate the reaction kinetics between nitrite and amines or to select appropriate excipients for its drug formulation. Pharmaceutical excipient manufacturers may also benefit from an accurate nitrite measurement to investigate the nitrite content in their excipients. In this talk we will give an overview on a variety of nitrite testing methods for analysis in excipients. We will discuss in detail an ion chromatography mass spectrometry (IC-MS) method for trace nitrite determination in microcrystalline cellulose (MCC). MS operated under selected ion monitoring mode was used to solve the commonly encountered interference issue with conductivity detection. Nitrite isotope internal standard was employed to address the ion suppression with MS detection. The optimized method was successfully validated with excellent linearity precision and recovery. Technical tips and tricks for operating IC-MS system will also be provided. Furthermore we will share the learnings on developing a more advanced technology - two-dimensional ion chromatography with mass spectrometry detection (2D-ICMS) for nitrite quantitation at parts per trillion level in hydroxypropyl methylcellulose (HPMC) solution.

Poster

Minimize Pre-Column Band Broadening with Immiscible Solvent Sandwich Injection

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We investigated the possibility to reduce the effect of pre-column band broadening (PreCBB) by sandwiching the sample between two small plugs of an immiscible liquid. It has been found that in cases of severe PreCBB improvements in peak efficiency can amount up to 20x for the early eluting compounds. For smaller degrees of PreCBB the gain on the efficiency of early eluting compounds is smaller (order of 50%) yet still significant. It has been verified that the presence of the immiscible fluids sandwich does not affect the repeatability of the analysis nor the linearity of the calibration curves used for analyte quantitation. It is also shown that the main effect of the two sandwich plugs is the minimization of the dispersion in the pre-column transfer tubing itself which makes the method fundamentally different from pure on-column focusing methods such as the Performance Optimizing Injection Sequence (POISe) method. It is further demonstrated that both halves of the sandwich are needed for the beneficial effect is clearly much smaller when only one plug is present. A drawback of the method is that some of the later eluting peaks are slightly adversely affected by the presence of the sandwich liquid in case of 127 µm i.d. tubing was used. The mechanism for this peak deterioration effect is at present still unclear but only occurs under gradient conditions and is clearly linked to the size of the sandwich plugs (the smaller the plugs the smaller the adverse effect) and the internal diameter or the tubing used between injection valve and column.

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