16TH Multidimensional Chromatography Workshop

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Workshop Guidebook

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KL01

INTRODUCING SUPERCRITICAL FLUID CHROMATOGRAPHY IN THE COMMUNITY OF MULTIDIMENSIONAL CHROMATOGRAPHY

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In recent years, multidimensional liquid chromatography has received increasing attention due to the desire to better resolve non-volatile compounds in complex samples. The selection of chromatographic modes in each dimension is of paramount importance to ensure the orthogonality of the overall 2D separation. To overcome the limitations encountered in the analysis of non-ionisable molecules, the use of supercritical fluid chromatography (SFC) combined with polar stationary phases is considered as a potential dimension of interest. This contribution reports on the advantages of comprehensive RPLC×SFC separation for the characterisation of valuable bio-wastes (wood lignin, spent ground coffee) and the incorporation of renewable materials into industrial products. Recent advances in modulation interfaces for on-line coupling of LC and SFC are described.

KL02

GC×GC-MS - FRAGRANCE ALLERGENS - THE OLYMPIC GOLD STANDARD

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Keywords: Comprehensive two-dimensional chromatography, Allergens, Regulated substances.

On July 26, 2023, the European Commission (EC) published amendment 2023/1545[1] to Annex III of the Cosmetic Products Regulation, significantly expanding the number of fragrance allergens from 24 to 57 organic compounds for which presence should be indicated on the product's label when above the established thresholds for leave-on and rinse off products (0.001 and 0.01%). The labelling of cosmetic products placed on the EU market will therefore be impacted over a period of 3 to 5 years.

While the percentage of consumers developing allergic contact dermatitis remains low, the extended list of 57 fragrance allergens corresponds to typical perfume notes commonly found in fragrance raw materials (more than 99% of natural ingredients contain at least one allergen).

Accurate determination of fragrance allergens levels is a crucial activity for the fragrance industry to comply with legislation and to provide robust data to customers, together with perfumers during the creation step. However, this remains an analytical challenge regarding the quantification of a wide range of different chemical structures (i), across a large concentration range from ppm to high percentage levels (ii), and in complex natural substances potentially containing more than 10'000 different molecules (iii).

The fragrance industry has developed technical recommendations[2,3] to address this target quantitation analysis: GC-MS technology, two different stationary phases, SIM/Scan acquisition and a decision tree for data processing.

Although the classical GC-MS approach, involving two different devices, can provide sufficient data confidence for simple raw materials, it becomes a daunting task when dealing with natural substances owing to multiple co-elutions with matrix peaks. For instance, for a complex essential oil, up to 16 GC-MS runs may be required to cover the full concentration range of allergens. Advanced MS technologies, such as MS-MS or HR-MS, have been recently proposed but cannot differentiate molecules having the same elemental formula or MS fragments, like terpenes or terpenoids.

Comprehensive two-dimensional gas chromatography (GC×GC-TOFMS) is the technology of choice to fully overcome co-elution issues in natural samples. Dedicated GC×GC conditions have been developed through a patented method[4] that helps select the best optimal parameters to maximize the separation between targets and the separation capacity. As a result, a unique normal hybrid configuration (Mid-polar × Polar) has proven to separate all fragrance allergens, even the discrete isomers, in a single run while providing a very high observed peak capacity of 5000. The developed methodology, based on GC×GC-MS for content level up to 3% and calibrated FID data for higher percentages, provides excellent performance: no compromise in separation, very high data confidence for unambiguous identification and quantification, a maximum of three data sets for complex substances and a streamlined semi-automated data process.

With more than 10,000 analyses and a daily use over the past 10 years, this state-of-the-art method is at the heart of a monitoring program for regulated substances, in new or existing ingredients, and for customer questions.

GC×GC-TOFMS has become the gold standard for quantifying fragrance allergens due to its unsurpassed performance and user-friendly technology.

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KL03

HOW TO DESIGN MICROCOLUMNS FOR COMPREHENSIVE GC

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The miniaturization of gas chromatographic (GC) systems is a major challenge for on-site analyses and space exploration in the solar system. Most of the systems developed are mono-dimensional GC system but the miniaturization of comprehensive two-dimensional GC system will be necessary to enhance the peak capacity. In recent years, Nanoelectromechanical System-type (NEMS) chromatography columns have demonstrated their high potential for column miniaturization. Various designs and geometries have been studied to obtain similar or better performances than conventional columns. Moreover, GC columns with radially-elongated pillars (REP) presented an innovative design [1]. A particularly promising feature of the REP columns is their capability to combine high efficiency with high flow rate which is important to obtain an efficient re-injection using microfluidic modulators. However, the stationary phase coating particularly polar ones, remains a challenge. An overview of the two-dimensional GC systems using µ-columns will be discussed, along with their limitations [2].

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KL04

APPLICATION OF 2D-LC-MS FOR ANALYSIS OF PHARMACEUTICAL PEPTIDES

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This contribution focus on peptide purity methods, *i.e.* RPC methods for the determination of peptide related impurities present in pharmaceutical peptides. There is a common misperception that peptides should be analyzed with TFA based eluents only. Salt based eluents, however, often provide both better peak shape and better selectivity something that we will exemplify. 2D-LC-MS allow almost real time MS data for salt-based eluents. Consequently, 2D-LC-MS is an important tool for the identification of peptide related impurities.
Irrespective if volatile or salt-based eluents are used 2D-LC-MS probably is the best technique for assessing if impurities are co-eluting with the main peak, so-called peak purity analysis. A strategy for 2D-LC-MS-based peak purity analysis of pharmaceutical peptides will be presented. Our focus is on 2D-LC separations using reversed-phase separations in both dimensions, and particularly isomer selectivity, since isobaric compounds are not readily distinguished by MS and therefore must be separated chromatographically.
One problem with peptide analysis is that very shallow gradients typically are required and that peptides respond strongly to small fluctuations in organic modifier and temperature resulting. This may result in fluctuating 1D retention times and difficulties to capture peaks of interest for 2D analysis – a moving target. A solution to this problem will be presented. We will also show how 2D-LC-MS analysis can result in false identification of oxidation products and how this can be addressed.

OL01

DEVELOPMENT OF A MULTIPLE HEART-CUT SFC-SFC SETUP

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Abstract

SFC offers a number of advantages, compared with LC, such as the high efficiency at high flow rate, environmentally-friendly and cost-effective solvents (principally CO_2). 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 CO_2 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. Furthermore, storing and transferring a fraction of a compressible fluid is setting different issues from liquid fractions, but can also be advantageous as it favors high efficiency.

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.). In this study, samples of Citrus aurantium L., which contains chiral flavonoids (flavanones); were analyzed with the SFC-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.

OL02

TOWARD UNRIVALED CHROMATOGRAPHIC RESOLVING POWER IN PROTEOMICS: DESIGN AND DEVELOPMENT OF COMPREHENSIVE SPATIAL THREE-DIMENSIONAL LIQUID-PHASE SEPARATION TECHNOLOGY

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Abstract

Spatial comprehensive three-dimensional chromatography (3D-LC) offers an innovative approach to achieve unprecedented resolving power in terms of peak capacity and sample throughput. This advanced technique separates components within a three-dimensional separation space, where orthogonal retention mechanisms are incorporated. The parallel development of the second- and third-dimension stages effectively overcomes the inherent limitation of conventional multi-dimensional approaches, where sampled fractions are analyzed sequentially. This contribution focuses on the design aspects of the microchip for spatial 3D-LC and the selection of orthogonal separation modes to enable the analysis of intact proteins. The design considerations for the flow distributor and channel layout are discussed, along with various approaches to confine the flow during the subsequent development stages. Additionally, the integration of stationary phases into the microchip is addressed, and interfacing to mass-spectrometry detection is discussed. According to Pareto-optimality, the integration of isoelectric focusing, size-exclusion chromatography, and reversed-phase chromatography in a spatial 3D-LC approach is predicted to achieve an exceptional peak capacity of over 30,000 within a 1-hour analysis, setting a new benchmark in chromatographic performance.

OL03

ENHANCED CHIRAL SCREENING OF COMPLEX SAMPLES VIA AQUEOUS ACHIRAL × CHIRAL COMPREHENSIVE LIQUID CHROMATOGRAPHY

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Abstract

Chiral resolution of solutes occurring in mixtures of unrelated species is of relevance in life sciences and in pharmaceutical analysis. While this is conceptually achievable by comprehensive two-dimensional liquid chromatography (LC×LC), few approaches are available whereby the second dimension comprises the chiral separation. This is ideally preferable in combination with a conventional separation mode in the first dimension (^{1}D) as it then offers an additional layer of chiral information through the second dimension (^{2}D), on top of the conventional achiral ^{1}D analysis. In this work, the potential of combining aqueous separation modes in ^{1}D with chiral liquid chromatography, operated in the reversed phase in ^{2}D, is studied. The proposed ^{1}D approaches include temperature-responsive liquid chromatography (TRLC) and reversed-HILIC (or per-aqueous liquid chromatography (PALC)). These allow the use of mobile phases composed entirely or mostly of water. The use of such aqueous mobile phase in ^{1}D facilitates the complete refocusing of organic solutes on the ^{2}D column, when the latter is operated with reversed phase mobile phase gradients. While improving chiral separations, this also makes the approach more broadly applicable. The influence of the composition of the ^{1}D column effluent and of the ^{2}D gradient profile on the chiral resolution is studied. In this way full comprehensive achiral × chiral platforms are developed allowing the analysis of diverse natural and synthetic mixtures compounds, particularly those containing mixtures of enantiomeric or diastereoisomeric pairs.

OL04

APPLICATION OF 2D-LC TO THE ANALYSIS OF CHIRAL AND OTHER ISOMERIC MOLECULES IN BIOSCIENCES

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Abstract

The analysis of isomeric molecules in complex mixtures in bioanalytical applications may be challenging. Mass spectrometry has its Achilles‘ heel in distinguishing betwen isomeric structures. Ion mobility mass spectrometry can distinguish some constitutional isomers and even diastereomers. However, the selectivity is often modest. Liquid chromatography benefits from an number of mobile-stationary phase combinations (i.e. phase systems) and allows to separate all kind of isomers. Yet, one dimension may be often not enough to separate all isomers of a complex mixture.

For this reason, 2D-LC is a favorable solution for the separation of isomers in complex mixtures such as in pharmaceuticals, biopharmaceuticals and metabolomics samples. In one example, 2D-LC will be employed for identification of impurities such as conjugated PUFAs from a lipid emulsion. The problem could be finally solved by comprehensive 2D-LC in which a chiral column in the ^{1}D separated most of the isomers while the RP column in the ^{2}D could resolve oxidized PUFAs from non-oxidized ones. A multiple heart cutting 2D-HPLC method with achiral RP in the ^{1}D and a chiral tandem column in the ^{2}D connected via an interface of 2 loop decks each equipped with 6 sampling loops enabled to acquire a full enantioselective amino acid profile within 45 min. A multi-column 2D-LC screening platform consisting of 6 achiral columns in the ^{1}D and 6 chiral columns in the ^{2}D, both installed via column selection valves in the column compartments of the two dimensions allowed to resolve the 16 stereoisomers of a bioactive non-ribosomal tetrapeptide.

OL05

ONE-STEP-MICROWAVE-ASSISTED EXTRACTION AND DERIVATIZATION FOLLOWED BY COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY COUPLED WITH FLAME IONIZATION DETECTOR TO ANALYZE FATTY ACID METHYL ESTERS (FAMEs) IN COMPLEX FOOD MATRICES.

Donatella Ferrara[1,2], Giulia Giacoppo[3], Alberto Cavazzini[3], Luisa Pasti[3], Marco Beccaria[3], Chiara Emilia Irma Cordero[2], Giorgia Purcaro[1]

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Abstract

The analysis of fatty acids (FAs) is crucial from different viewpoints, as it can provide valuable insights into the composition of fats for instance for industrial processes or their impact on nutrition and health. Typically, the analysis of FAs involves extracting lipids from the matrix and subsequently undergoing a derivatization process to convert them into FAMEs before gas chromatography (GC) analysis. This work aims to characterize the FAs profile of complex matrices using a one-step microwave-assisted extraction and derivatization (MAED) method used to simultaneously extract and derivatize the lipid fractions in FAMEs, followed by a flow-modulated (FM) two-dimensional comprehensive gas chromatography – flame ionization detector (GC×GC-FID) analysis. The use of FM-GC×GC enhances interpretation capabilities, and the structured chemical patterns generated in the 2D plot allow for precise characterization of the FAMEs profile based on specific chromatogram positions, ensuring reliable identification without the need for MS. Moreover, the use of FM allowed to obtain the same profile as a cryogenic modulator. FAMEs were tentatively identified through standards, literature data, and the 2D-GC plot position. The MAED method proved to be a robust, greener, and rapid alternative to the more time-consuming routine methods normally in use.

OL06

PROFILING PHENOLIC COMPOUNDS IN SHEA BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY HYPHENATED TO ION MOBILITY SPECTROMETRY AND HIGH-RESOLUTION MASS SPECTROMETRY

Nikoline J. Nielsen[1], Oskar M. Kronik[1], Romina A. F. Neran[1], Jan H. Christensen[1], André de Villiers[2]

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Abstract

The shea tree (*Vitellaria paradoxa*) is native to sub-Saharan Africa, and distributed over 5000 km from Senegal to Ethiopia. Shea fruits play an important role in the local diet, being a source of carbohydrates, vitamins, and minerals. The shea kernel contains up to 60% triglycerides. This fraction is extracted by local women to produce shea butter, which is used for food and skincare. Industrially, shea butter is commercialized as a cocoa butter replacement and as an ingredient in personal care products. Being a commodity obtained from wild-growing trees, shea represents a sustainable alternative to palm oil, and global demand for shea kernels far exceeds supply. Shea trade contributes to the livelihood of local West-African shea collectors and processors. More recently, focus has been directed towards producing high-quality kernels, which usually refers to kernels with high triglyceride and low free fatty acids content. However, shea also contains secondary metabolites that may convey beneficial properties and, in that way, further valorize shea products in the future. Phenolic compounds are secondary metabolites known to exhibit a range of desirable biological activities. Until now, only four studies of phenolic compounds in shea have been published. Recently, we profiled 32 phenolic compounds, 16 of which were reported in shea for the first time, by RPLC-HRMS. In this presentation, we will discuss the outcome of applying RPLC × HILIC hyphenated to UV, IMS and HRMS, to support the compound identification, and to enhance separation to further characterize phenolic compounds previously unknown to shea.

OL07

INVESTIGATING THE IMPACT OF PACKAGING ON OAT VOLATILES USING GC✕GC–TOF MS

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Abstract

Investigating the migration of volatiles from food packaging is essential for ensuring the safety, quality, and sensory integrity of food products.. Packaging materials can release volatiles that may migrate into the food over time, significantly affecting the sensory characteristics of food, altering its taste, aroma, and overall consumer appeal. Understanding the extent and mechanisms of this migration is vital not only for meeting stringent food safety regulations but also for developing packaging solutions that minimise these interactions.

However, the study of volatiles in complex food matrices requires advanced analytical techniques due to the low concentrations and diverse nature of the compounds. Traditional methods, such as SPME–GC–MS, often lack the sensitivity and resolution needed to fully characterise the volatile composition.

Here, we employ headspace sorptive extraction and GC×GC-TOF MS to investigate the volatile profiles of oats. The high-capacity probes enable efficient extraction and pre-concentration of a wide range of trace-level volatiles, while GC×GC-TOF MS provides exceptional separation and identification capabilities. This powerful combination allows for a detailed investigation of the volatile profile of foods and the potential impact of packaging materials on these profiles.

We apply this approach to analyse volatiles from six brands of packaged oats. Using sophisticated chemometrics workflows, we identify key differentiators among the packaging materials—cardboard, paper, and plastic—and analyse the volatiles emitted from these sources to confirm and understand migration patterns.

OL08

NON-TARGETED ANALYSIS OF PFAS USING TWO DIMENSIONAL GAS CHROMATOGRAPHY

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Abstract

Analysis of per- and polyfluoroalkyl substances (PFAS) has become necessary due to their presence in the environment and associated health risks. PFAS detection is usually accomplished using a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology focusing on the 40 EPA regulated PFAS compounds. While LC-MS/MS is sensitive (part-per-trillion, ppt), it lacks the ability to measure all (EPA and non-EPA regulated) PFAS compounds that are present. Using two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS), it is demonstrated that it is possible to reach sub-ppt levels of sensitivity while also performing non-targeted analyses, allowing for detection of all PFAS compounds that are present. The GC×GC-TOFMS methodology was applied to a class of fluoropolymers (chlorotrifluoroethylene-co-vinylidene difluoride) for fingerprinting the numerous PFAS compounds present allowing for lot-to-lot differentiation. Furthermore, the application of high-resolution mass spectrometry was investigated for its ability to discover unknown PFAS compounds.

OL09

MULTI-DIMENSIONAL LC-MS PLATFORMS FOR STRUCTURE-FUNCTION CHARACTERIZATION OF THERAPEUTIC ANTIBODIES

Jelle De Vos[1], Liesa Verscheure[1,2], Shauni Detremmerie[1], Isabel Vandenheede[1], Mabelle Meersseman[1], Frederic Lynen[2], Pat Sandra[1,2], Koen Sandra[1,2]

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Abstract

The biopharmaceutical industry has undergone a remarkable transformation in recent decades, with monoclonal antibodies (mAbs) becoming one of the most prominent therapeutics for the treatment of life-threatening diseases including cancer and autoimmune diseases. This success has resulted in the development of multiple formats of antibodies, designed to further enhance the therapeutic potential. With sales values exceeding $220 billion in 2023, which is expected to reach $500 billion by 2030, mAbs still rule the charts of most sold pharmaceutical.

Antibody therapeutics not only boast an enormous therapeutic potential, but at the same time also have an immense structural complexity as a result of the biosynthetic process and subsequent manufacturing and storage. These heterogeneous molecular giants exert different functions, facilitated by the Fab or Fc part, including antigen, neonatal fragment crystallizable receptor (FcRn) and Fc-gamma receptor (FcγR) binding. The latter two, are crucial in, respectively, regulating half-life of mAbs in circulation and triggering the immune system through antibody-dependent cellular cytotoxicity. mAb binding to the latter is highly sensitive to amino acid substitutions and post-translational modifications such as glycosylation, oxidation, deamidation and isomerization and it is primordial to understand the impact of these structural attributes on functionality. As such, a thorough analysis of their structure and function is essential.

This talk will highlight the advances made in multidimensional liquid chromatography (MD-LC) in hyphenation to mass spectrometry (MS) to elegantly study structure/function relationships of mAbs in detail. Set-ups with FcRn and FcγRIIIa affinity chromatography implemented will be discussed.

OL10

THE CENTURY MIX AS QC FOR UNTARGETED METABOLOMICS USING TWO-DIMENSIONAL GAS CHROMATGRAPHY

Anaïs Rodrigues[1], Djulia Bensaada[1], Jean-Marie Dimandja[2], Pierre-Hugues Stefanuto[1], Jean-François Focant[1]

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Abstract

Over the past decade, metabolomics has gained significant importance in the life sciences, offering insights into metabolic pathways involved in various pathological processes. As this technology becomes crucial in research, the need to enhance and standardize quality assurance (QA) and quality control (QC) practices in untargeted metabolomics has intensified. Despite efforts to develop QA/QC systems, such as those of the Metabolomics Quality Assurance and Quality Control Consortium (mQACC), challenges remain, particularly in terms of documentation and standardization, which are essential for advancing non-targeted metabolomics.

In our research, we focused on establishing a robust QA/QC system for untargeted analysis using GC×GC-TOFMS systems. We analyzed the Century Mix (CM), a QC solution mix developed by the FDA comprising 100 diverse compounds, to assess analytical performance. The method effectively monitored compounds with varying volatility and chemical functions, demonstrating good distribution and separation across the 2D chromatogram during a 60-minute run on different instruments with varying column sets.

To ensure the reliability of our analytical processes, we implemented a sophisticated QA/QC system featuring control charts that tracked retention time and response (area values) for all compounds over six months. Our goal is to extend this systematic approach to all four GC×GC systems in our laboratory, across various sample types and studies, to establish a standardized and robust QA/QC framework using the CM, thereby enhancing the precision and reproducibility of untargeted metabolomics research.

OL11

OBSERVATION OF CHROMATOGRAPHIC DIFFERENCES BY NON-SPECIALIST VIEWERS FOR ONE-DIMENSIONAL GAS CHROMATOGRAPHY AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY OUTPUT

Clarissa Camara[1], Cynthia Cheung[1], Katelynn Perrault Uptmor[1,2]

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Abstract

In the context of forensic investigations, chromatography is used to characterize a sample's components, providing a chemical pattern to compare with known references which is often presented to individuals without specialized training in analytical chemistry. Comprehensive two-dimensional gas chromatography (GC×GC) has recently become popular in forensic research for analyzing samples such as fire debris, drugs, chemical threats, human remains detection, and more. New methods are developed in forensic research regularly, which challenge our view of what may be increasingly complex to convey through scientific communication. This study investigated individuals' ability to observe differences in images for photographs, one-dimensional gas chromatography (GC) chromatograms, and comprehensive two-dimensional gas chromatography (GC×GC) contour plots. The goal was to identify whether comparative observations between two outputs were facilitated or hindered when observing GC chromatograms compared to GC×GC contour plots, using photographs as a control. Participants indicated low difficulty in finding differences between pairs of images in all categories. They scored highly at indicating when two images were distinguishable or indistinguishable, with no significant difference between control images and each category. These results support that GC×GC output can be implemented in expert testimony without challenges over traditional one-dimensional techniques. Statements should be avoided that GC×GC may facilitate or hinder juror comprehension, as the results currently indicate no significant benefit or drawback. Additional research is needed to improve understanding of how explanation could aid expert witness testimony to better evaluate how this increasingly common technique will fit into future forensic casework opportunities.

OL12

ADVANCED DATA PROCESSING TECHNIQUES IN GC×GC-TOFMS FOR BIO-OIL ANALYSIS

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Abstract

The Two-dimensional Gas Chromatography (GC×GC) with Time-of-Flight Mass Spectrometry (TOFMS) is increasingly utilized to identify and quantify the numerous compounds in bio-oil samples. Their detailed compositional analysis via GC×GC-TOFMS can optimize biofuel production. However, managing and interpreting the large volume of data, often focused on a few key components, may overlook other significant compounds.

In response to this challenge, this presentation will outline effective strategies for analyzing, processing, and reporting GC×GC-TOFMS data, starting with creating a semiquantitative table of identified compounds, their areas, fragmentation patterns, and other data from ChromaTOF software. The data is enhanced by constructing a comprehensive database of identified compounds using the PubChem database. Compounds are broken down into functional groups, and bulk properties like density and boiling point are calculated using a group contribution method. The qualitative analysis is refined further by using the concept of entropy maximization. A key feature of the proposed approach is the incorporation of a 'chemometric detector model' to ensures compound identity. All procedures are programmed in Matlab, streamlining workflow and enabling detailed reports on functional group mass fractions, useful for assessing the chemical diversity and potential yield of bio-oil constituents.

This developed algorithm has undergone preliminary testing on several bio-oil samples derived from diverse biomass sources and processes. This presentation will demonstrate how advanced data handling techniques can drastically reduce human error and enhance the analytical accuracy and comprehensiveness of bio-oil analyses, paving the way for more effective biofuel production and process optimization.

OL13

DEVELOPMENT OF UNKNOWN COMPOUNDS ANALYSIS METHOD COMBINING HIGH_RESOLUTION MASS SPECTROMETRY, SOFT IONIZATION TECHNIQUE, AND AI TECHNOLOGY FOR COMPREHENSIVE 2-DIMENSIONAL GAS CHROMATOGRAPHY

Masaaki Ubukata

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Abstract

GC-MS plays an important role in a lot of application fields and is widely used for qualitative and quantitative analysis, especially for volatile organic compounds. Most of the qualitative analysis in GC-MS is compound identification by comparison with commercially available library databases for the EI mass spectra. The EI mass spectral pattern, which is commonly used in GC-MS, is known to be highly reproducible and independent of the instrument. Therefore, a large number of EI mass spectra are available in commercial databases, and it becomes an advantage comparing with qualitative analysis in LC-MS. However, there are still many compounds that are not registered in commercial EI mass spectral databases. For this qualitative analysis issue, we have created an AI model to predict EI mass spectra from structural formulas by machine learning. We prepared approximately 100 million compounds structures from PubChem, 10 million TMS compounds and 5.5 million pyrolyzates compounds using *in-silico*. Totally, we have created a new predictive EI mass spectral database for 120 million compounds that are not registered in commercial databases. In this study, we report the details of the development for the predicted EI mass spectral database and applying to comprehensive 2-dimentional gas chromatography data.

OL14

LEVERAGING CHROMATOGRAPHIC AND STATISTICAL APPROACHES FOR ENHANCED GC×GC-MS DATA PROCESSING

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Abstract

Gas Chromatography coupled with Mass Spectrometry (GC-MS) is a powerful analytical technique for the separation and identification of complex mixtures. Two-dimensional Gas Chromatography coupled with Mass Spectrometry (GC×GC-MS) enhances the analytical capabilities providing superior separation and increased sensitivity. However, the vast amount of data generated by GC×GC-MS requires sophisticated data processing methods for meaningful interpretation.

The advantages of a combined chromatographic and statistical approach for GC×GC-MS data processing are presented. The chromatographic approach allows for improved peak resolution and better separation of co-eluting compounds. The use of multiple columns in GC×GC facilitates the separation of complex mixtures.

Statistical methods play a crucial role in data analysis. Multivariate statistical techniques, such as Principal Component Analysis (PCA), enable the extraction of relevant information from large datasets. These methods help identify patterns, outliers, and correlations, ultimately leading to a deeper understanding of the sample data.

The combination of chromatographic and statistical approaches enhances the reliability of data interpretation. This may reduce false positives/negatives and enhance the robustness data interpretation. This is particularly valuable in fields such as environmental monitoring, food analysis, and metabolomics, where complex sample matrices and trace-level compounds are common.

In conclusion, the integration of chromatographic and statistical approaches for GC×GC-MS data processing offers several benefits, including improved compound separation, enhanced data interpretation, and increased analytical reliability. This synergistic approach is essential for extracting meaningful insights from complex GC×GC-MS datasets, contributing to advancements in various scientific disciplines and applications.

OL15

EVALUATION OF THE RELATIONSHIP BETWEEN PEAK AND SIGNAL CHARACTERISTICS AND THE PERFORMANCE OF COMMON PEAK-DETECTION METHODS IN COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY

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Abstract

The success of any (two-dimensional) chromatographic separation does not depend exclusively on the ability of the chromatographer to achieve a separation, but also on the computational tools used to extract information. For users, the selection of the appropriate computational tools method may be a daunting task, but this is also true for automated workflows where chromatograms must be processed for use in unsupervised closed-loop method development. Arguably, the most critical step is peak detection as – ultimately – computational efforts such as library searches, peak integration, peak tracking, and retention modeling all hinge on the ability of peak detection methods to quantify, identify, or characterize sample constituents.

In this presentation, we present the results of our most recent study, where we have studied the effect of different peak and signal characteristics on the success of different peak detection (and, by extension, peak integration) methods in comprehensive two-dimensional chromatography (i.e. GC×GC and LC×LC). The peak properties studied included peak width in both dimensions, peak ratios, peak shape characteristics, and the impact of two types of modulation shifting (e.g., diagonal elution patterns as seen with shifted gradients in 2D-LC) on peak detection. We hope that our work contributes to (i) the much-needed further development of peak detection tools, (ii) aiding researchers in the selection of appropriate detection techniques, and (iii) raising awareness of the strengths and weaknesses.

OL16

APPLYING STATISTICAL DATA PROCESSING TOOLS FOR GC×GC DIFFERENTIATION OF ALTERNATIVE AVIATION FUELS

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Abstract

As an increasing number of pathways for producing alternative aviation fuels are accepted, with some synthetic fuels being described as "more like what we want from jet fuel than what we pull from the ground," more robust and easier-to-interpret GC×GC data processing comparing batches of samples becomes of greater importance for workflows that monitor fuel quality. Hydrocarbon group-type composition is often the main focus for routine GC×GC analysis of aviation fuels. However, the distribution of aromatics and other heteroatomically-substituted compounds that are usually present in much smaller concentrations than the predominant paraffins and naphthenes can supply additional information. These sulfur-, nitrogen-, and oxygen-containing or substituted multi-ringed aromatic analytes can provide further insight into the processes used to generate these fuels and their potential impacts on fuel performance. This presentation explores the different types of results that are possible when approaching diverse samples using several statistical methods to highlight and identify significant variation between aviation fuel samples. Multiple aviation fuels from production processes including Fischer-Tropsch reactions and hydroprocessed esters and fatty acids (HEFA) are compared to traditional petroleum-based fuels.

OL17

MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE ELUCIDATION OF INDOOR AIR QUALITY IMPROVEMENTS ARISING FROM PLANNED INTERVENTIONS

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Abstract

Indoor air quality has a direct impact on human health, and encompasses exposure from both domestic and occupational environments. Combustion is a known source of semi-volatile organic compounds, including polycyclic aromatic hydrocarbons (PAHs), many of which may negatively impact human health with particular reference to respiratory illness and disease. Governments and industries may therefore undertake interventions to reduce combustion emissions and their associated impacts. A robust analytical methodology is required to correctly assess any improvements in the resulting air quality. Multidimensional gas chromatography-mass spectrometry (GC×GC-MS) is an indispensable tool in this regard, as the enhanced separation which it provides enables correct peak identification and quantification.

The results of two studies are presented which aimed to improve air quality by the implementation of combustion-related interventions. The first involved an occupational setting, whereby biodiesel was tested as an alternative to diesel in fueling a heavy duty vehicle in a South African underground platinum mine. Sampling of gaseous emissions onto multichannel polydimethylsiloxane traps was conducted in this confined environment (akin to indoor air) followed by GC×GC-MS analysis, focusing on PAHs. In the second study, the impact on domestic air quality resulting from the replacement of coal/wood stoves with gas stoves was assessed in an informal settlement in South Africa, by similarly sampling and analyzing the gaseous combustion emissions pre- and post intervention in 25 households and at eight ambient locations. The importance of utilizing multidimensional chromatography for these applications will be discussed in the context of the assessment of air quality improvements.

OL18

WHAT'S IN THE DUST? GC×GC-MS BASED NON-TARGET SCREENING OF HOUSE DUST

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Abstract

Air pollution is a global health concern, leading to ca. 9 million premature deaths annually. The WHO reported that ca. 99% of individuals are exposed to polluted air. Furthermore, studies have shown that European citizens spend up to 90% of their time indoors being in contact with household chemical residues. Therefore, it is crucial to determine which chemicals are being emitted and at what levels they exist within indoor media to which residents are exposed, such as house dust.

For this, a non-target screening (NTS) workflow was developed. Samples included dust from Umeå (Sweden), Munich (Germany), Lviv (Ukraine), a collaborative trial from the NORMAN Network, and NIST Standard Reference Material 2585. A two-step ultrasonic extraction using dichloromethane (DCM) and acetone was applied, followed by fractionation using aminopropyl solid-phase extraction cartridges. Two fractions were collected with DCM and DCM:methanol (1:1, v/v), respectively, and analyzed using LECO Pegasus BT 4D GC×GC-TOFMS. The acquired data were matched against NIST23 library, filtered, and aligned.

Over 2500 compounds were tentatively identified and classified. Samples showed a significant presence of plastic additives, particularly phthalates, and tertiary amines, including DIMLA 1214, whose increased use was reported during the COVID-19 pandemic. Principal component analysis illustrated that samples from Munich, Lviv, and NORMAN displayed similarity, while samples from Umeå and NIST differed from those and each other, suggesting the presence of unique contaminant sets. The work is ongoing, with the aims of creating an easy-to-use NTS workflow and identifying as many as possible contaminants in dust.

OL19

TD-GC-MS/O AND TD-GC×GC-HRTOFMS FOR THE CHARACTERIZATION OF ODOROUS COMPOUNDS IN RECYCLED MATERIALS

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Abstract

Gas chromatography coupled to mass spectrometry and olfactometry (GC-MS/O) is a methodology widely applied in the material industry to establish correlation between the chemical nature and concentration of specific odorous compounds. However, some materials may produce very complex VOC profiles and make it difficult to identify odor active compounds. This phenomenon is accentuated with the emergence of recycled materials.

Comprehensive two-dimensional gas chromatography coupled to high resolution time-of-flight mass spectrometry (GC×GC-HRTOFMS) has been identified as a powerful tool to characterize complex VOC profiles. The HRMS is required not only for the identification of unknowns but also as a third dimension to increase sensitivity and selectivity and allow detection of odorous compounds at trace levels, specific from materials.

Practical examples will be presented to demonstrate the useful complementarity of this advanced technique for the characterization of material emissions and the evaluation of processes for odor reduction.

OL20

AN ALIQUOT PUSH-PULL INTERFACE FOR COUPLING THE FIRST AND SECOND DIMENSION SEPARATIONS IN TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

Dwight Stoll[1], Daniel Meston[1], Thomas Lauer[1], Stephan Buckenmaier[2], Konstantin Shoykhet[2], Iwan Bouzid[2]

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Abstract

Two-dimensional liquid chromatography (2D-LC) separations are increasing in popularity, both for the analysis of relatively simple, but hard-to-separate mixtures, and the analysis of highly complex mixtures such as those encountered in the analysis of biological samples. Despite advances in many aspects of the instrument hardware and software needed for routine use of 2D-LC, there are some aspects of the technology that have hardly changed since the first online 2D separations were demonstrated several decades ago. In this presentation we will describe a novel approach to interfacing the first and second dimensions of separation that addresses some of the shortcomings of existing technology. The approach – which we descriptively refer to as a "push-pull" interface – lets go of the conventional fixed loop feature of existing interfaces, and uses a precisely controlled, high pressure syringe to first "pull" in an aliquot of first dimension effluent that we desire to transfer to a second dimension for further separation. Then, it is "pushed" into the mobile phase stream of the second dimension and carried to the second dimension column. There are several advantages of this approach over existing ones, including software control of both the aliquot volume, and the rate at which the aliquot is fed into the second dimension mobile phase stream. In addition to an explanation of the modulation cycle, we will share results of preliminary work to illustrate the basic features of the interface/approach and show application examples that highlight the advantages of the push-pull interface over existing interfaces for 2D-LC.

OL21

DEVELOPMENT OF A MULTI-2D LC×LC-ESI/TPI-DUAL SOURCE-QTOF-MS FOR THE ANALYSIS OF COMPLEX SAMPLES

Marvin Haessler[1], Katharina Wetzel[1], Lidia Montero[2], Juan Cabrera[3], Florian Uteschil[1], Oliver Schmitz[1]

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Abstract

Even though mass spectrometers, especially in combination with ion mobility spectroscopy, are becoming increasingly powerful and are characterized by an impressive separation performance in a very short analysis time, ion suppression poses a serious problem with these analysis systems.

Here we show the influence of ion suppression using 1D and 2D LC methods and present an LC×LC method with two stationary phases in the 2nd dimension that can be freely selected during the analysis to increase the chromatographic separation power and reduce the ion suppression. For the subsequent mass spectrometric detection, it is also possible to switch between two different ion sources during the measurement in order to always be able to use the optimum ionization method. The construction and optimization of this dual ion source, consisting of electrospray ion source (ESI) and tube plasma temperature ion source (TPI) is also discussed.

Finally, first results of the coupling of 2D-LC and GC methods with ion mobility mass spectrometry are presented.

OL22

CHARACTERIZATION OF CHEMICAL EXPOSURES FROM CANNABIS AND VAPE DEVICES USING GC×GC-MS

James Harynuk, Andrea Velasco Suarez, Ritu Mann-Nuttel, Paul Forsythe

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Abstract

In Canada, more than one third of Canadians aged 18-44 reported using cannabis at least once in 2023 with about 10% of Canadians in this age range reporting daily or almost daily use. While there are many ways in which cannabis can be consumed, the most popular modes of consumption for Canadians remain the smoking of dried cannabis (65% of legal sales), followed by inhaled extracts (~25% of legal sales). Vaping (not necessarily of cannabis extracts) is particularly popular among younger Canadians, with 14% of youth aged 15-19, and 20% of adults aged 20-24 reporting past-30-day vaping. Consequently, there is a clear need to characterize these exposures and investigate the impacts of these exposures on humans. Recent work in our laboratory has focused on the characterization of mainstream particle and volatile composition of vape and cannabis smoke as well as exposure studies to explore impacts of smoke on lung cells. In addition to presenting results studying the impacts of these direct exposures to cannabis and vaping, some preliminary results exploring the accumulation and retention of smoke by fabrics, representing potential third-hand exposures will be presented

OL23

QUANTIFICATION OF HETEROCYCLIC AROMATIC COMPOUNDS (NSO-HET) IN UNFRACTIONATED AND FRACTIONATED FUEL SAMPLES BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRY

Pedro Victor Bomfim Bahia[1], Anaïs Rodrigues[2], Aleksandra Gorska[3], Paula Albendea[3], Pierre-Hugues Stefanuto[2], Jean-François Focant[2], Giorgia Purcaro[3], Maria Elisabete Machado[1]

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Abstract

The polycyclic aromatic sulphur, nitrogen, and oxygen heterocyclic (PASH, PANH and PAOH, respectively) have received increasing attention over the years because they are considered relevant recalcitrant compounds in fuels. However, the determination of NSO-HETs in these matrices (e.g., diesel and gasoline) is a challenge due to the complexity and coelutions problems. The comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-ToFMS) has already been successfully employed for qualitative analyses of individual NSO-HET classes, but a simultaneous and quantitative analysis has never been performed. The aim of this study was to, for the first time, apply the GC×GC-ToFMS for simultaneous and quantitative determination of NSO-HET in fuels. A preliminary liquid chromatography fractionation (LC) was performed to enrich the NSO fraction and remove interferences. After optimization of the chromatographic parameters, the method was validated in terms of matrix-matched calibration. The limits of detection and quantification ranged from 0.34 to 70.34 ng mL^{-1}. Correlation coefficients (R^2) ≥0.99 were obtained for all compounds within the linear region (10-1000 ng mL^{-1}). Addition/recovery tests were carried out at three levels (100, 300 and 600 ng mL^{-1}) and the results were within a suitable range (70-120%). The instrumental precision, both intraday and interday, was assessed at two concentration levels (100 and 600 ng mL^{-1}), and relative standard deviation (RSD) was <20%. Finally, the method optimized and validated was applied in fuels, priory and after offline LC fractionation, collected in Salvador, Bahia, Brazil and in Gembloux, Belgium. GC×GC-ToFMS method proved to be precise, accurate, and suitable.

OL24

POLYAROMATIC HYDROCARBON QUANTIFICATION IN PLASTIC PYROLYSIS OILS

Melissa Dunkle[1], Yannick Ureel[2], Tugce Sanliturk[1], Bruno da Costa Magalhaes[1], Pascal Pijcke[1], Niels Verhoosel[1], Matthijs Ruitenbeek[1], George Bellos[1,2], Marvin Kusenberg[2], Kevin Van Geem[2]

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Abstract

Industry is moving from a plastics linear economy to a plastics circular economy, where both mechanical and advanced (chemical) recycling will be required to close the loop and prevent plastics from being landfilled or incinerated. Advanced recycling is complementary to mechanical recycling and should only be considered for waste plastic that cannot be (easily) mechanically recycled. Advanced recycling consists of different technologies, including solvolysis, pyrolysis, and gasification. In this work, the oils obtained from the pyrolysis of waste plastic are considered as potential circular feedstocks to produce ethylene via steam cracking.

Compared to fossil-based cracker feedstocks, plastic pyrolysis oils (PPOs) contain not only an extremely different hydrocarbon composition, but also a wide range of impurities. It has already been established that PPOs will require upgrading to bring these materials into specification for the crackers. Focus here is placed on the investigation of the hydrocarbon composition of the PPOs, specifically aromatics and polyaromatic hydrocarbons (PAHs). Aromatics cause fouling and coke formation during steam cracking, and as such are not desirable at high quantities in the feed. Analytical methodology is required to identify and quantify the various aromatic species present in the PPOs.

For this work, a comprehensive gas chromatographic (GC×GC) method was developed where emphasis was placed on aromatics speciation (e.g., mono-, di-, tri-). ASTM D8519, a high temperature 1D GC separation coupled to vacuum ultraviolet detection (GC-VUV), was also evaluated for aromatics identification and quantification. PPOs were evaluated using both methods, and the comparison between the obtained results is presented.

OL26

CHARACTERIZATION OF UNTARGETED GC×GC TOFMS PYROLZED VEGETATION UTILIZING A PYRO PROBE

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Abstract

GC×GC TOFMS was used to characterize the pyrolyzed live leaves from vegetation foliage. The major components of these hazardous fuels are lignin, cellulose, lipids, hemicellulose, and protein. The secondary metabolites from the pyro probe were detected and separated into various chemical categories. The chemical compositions of the vegetation were monitored at two different temperatures. This study will investigate the differences in the chemicals identified at various temperatures monitored by pyro probe parameters. Chemical categories were identified, labeled, and compared using molecular chemical maps. These maps were used to identify trends and locations of chemicals in both temperatures. The characterization of these trends will be used to characterize unknown chemicals in the location of trending categories.

OL27

MICROWAVE-ASSISTED LIQUID EXTRACTION FOLLOWED BY GC×GC-MS ANALYSIS OF SOLVOLYSIS PRODUCTS OF NEW ENERGY MATERIAL WASTES

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Abstract

The goal of this project was to develop analytical solutions to understand better the recycling of chemicals and valuable materials from wind turbine blades, addressing the need for sustainable end-of-life solutions for renewable energy infrastructure.

Wind turbine blades underwent solvolysis treatments for 8 and 13 hours. The resulting solvolysis mixtures, referred to as solvolysis soups, with highly basic pH levels, were neutralized. Afterwards, while the solid phases were directly processed after neutralization, the liquid phases needed an additional filtration step to remove solid residues from them.

Microwave-assisted extraction (MAE) was performed on the products of these procedures using a hexane-methanol solvent mixture (10:3 ratio), followed by water addition (2.5 ratio) and centrifugation. Subsequently, the organic phase was collected, concentrated, and analyzed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS). The system was equipped with a non-polar column in the first dimension and a medium-polar column in the second dimension, connected with a cryogenic modulator.

Identification was achieved through mass spectral electron ionization (EI) database matching at 70 eV (≥800/1000) and the Linear Retention Index (LRI) window within ±20, based on non-polar 1D-GC LRI data from the NIST database and literature. The location of the investigated molecules on the 2D-GC plane was also considered.

Due to this MAE-GC×GC-MS technique, it was possible to identify approximately 60 molecules from various chemical classes, including aromatic compounds, and nitrogen- and oxygen-containing compounds.

FL00

THE LABRULEZ PORTALS – A UNIQUE SOURCE OF INFORMATION NOT ONLY IN THE FIELD OF GC×GC AND 2DLC

Ivo Novotny

LabRulez, Prague, Czech Republic

Abstract

LabRulez portals [1] are a unique global concept focused on quick, simple and effective access to information, especially in the field of analytical chemistry. State-of-the-art IT technologies thus enable visitors to search, filter, and find information in one place, which would otherwise be time-consuming and sometimes impossible to find.

Information from GC×GC and 2DLC area are heavily represented mainly on the LabRuleuGCMS and LabRulezLCMS [2] portals, providing visitors with an unique source of news, applications, instrumentation and consumables overview and e.g. expert webinars or career section focused on analytical chemistry jobs. Together we will see how to work with and search this information.

Stop looking and start finding. Fast, efficient and in one place. Anyone can create a profile on the LabRulez analytical portals in ENG language. Whether you are an individual, a research group, a university or a company. Build your brand and share information in the community of (not only) analytical chemists.

[1] https://labrulez.com/
[2] https://gcms.labrulez.com/, https://lcms.labrulez.com/

FL01

COUPLING OF VAC-HS-SPME AND GC×GC-QMS FOR SIMULTANEOUS 5-HMF QUANTIFICATION AND VOLATILE PROFILING IN HONEY

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Abstract

Faced with the growing complexity of the food industry's demands, analytical techniques are evolving towards increasingly sophisticated and multi-response analyses. In this respect, multidimensional techniques such as GC-MS and GC×GC-MS are ideally suited.

Regarding sample preparation, headspace-solid-phase microextraction (HS-SPME) is one of the most favoured techniques for food volatile analysis thanks to its simplicity and ability to concentrate a wide range of compounds without needing solvents. However, the extraction of low-volatile compounds can be limited. Among different available strategies to increase their extraction, vacuum-assisted (Vac)-HS-SPME is highly promising. The use of vacuum facilitates the volatilisation of less-volatile compounds by lowering the gas-phase resistance to the mass transfer while maintaining the same extraction efficiency for the more volatile compounds.

In this project, Vac-HS-SPME has been coupled with GC×GC-qMS to quantify the 5-HMF (storage and heat processing marker) and analyse the volatile profile of honey. 5-HMF is regulated by the EU (2001/110/EC) fixing the maximum limit level to 40 mg/kg in most cases. Validation of the proposed method has been realized using a matrix-matched calibration, reaching LOD and LOQ of 1.6 and 4.7 mg/kg, respectively; while a recovery of 98% and a RDS of 21% were achieved. The method was trailed with eight real-world samples against the official HPLC method showing, an average bias of 6%. In terms of greenness the proposed method gave better results using the AGREE metrics, while the practicality was similar, as calculated with the BAGI.

FL02

MOSH&MOAH IN FOOD INGREDIENTS AND ADDITIVES, AND THE ADVANTAGES OF USING LC/GC×GC(-FID/TOFMS) FOR THEIR ANALYSIS

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Abstract

Mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) are contaminants of increasing concern for the food industry due to their widespread occurrence and uncertain toxicity. The ISO 20122:2024 is currently the only official method for their analysis, and it has only been validated on several vegetable oils. The workflow consists of extensive sample preparation followed by LC-GC-FID analysis. However, many challenges remain with this analysis. Accurate quantification is often difficult, particularly when biogenic substances coelute with MOSH or MOAH unresolved complex mixtures (UCMs). In these cases, chromatogram integration is largely based on the operator's interpretation.

Switching to LC-GC×GC-FID has proven to be a useful approach to reduce the uncertainty associated with interpretation. The second GC dimension helps differentiate biogenic compounds from MOSH and MOAH. Adding mass spectrometry (MS) detection further enhances the ability to distinguish coeluting substances. These advantages make LC-GC×GC-FID/TOFMS particularly useful for investigating MOSH/MOAH contamination in new matrices, where the present interferences are not yet well understood.

This presentation will illustrate the advantages of LC-GC×GC-FID/TOFMS for analysing MOSH/MOAH in food ingredients and additives, for which limited data is available. Additionally, it will show how the LC step preceding GC analysis, which initially serves to separate MOSH from MOAH and retain more polar compounds, can also help reduce or eliminate certain interferences.

FL03

AUTOMATION AND CHALLENGES IN ONE- AND -DIMENSIONAL LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT: WHAT IS OPTIMAL?

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Abstract

High-resolution liquid chromatography (LC) and two-dimensional liquid chromatography (2D-LC) have significantly enhanced the resolving power of separation technologies. However, a major barrier to widespread adoption is the substantial investment required for method development, data processing, and validation.

Our vision is to make advanced separation technologies accessible across various sectors, and our mission is to develop the necessary tools to achieve this. The chemometrics and chromatography communities have introduced a wide range of techniques that, when integrated, can facilitate the automation of multiple aspects of method development. Nevertheless, realizing this innovation requires addressing several scientific challenges.

In this context, we have recently developed a comprehensive, modular, closed-loop, and interpretive algorithm for automated LC method development, specifically designed for complex samples with unknown compositions. This platform was designed to iteratively program the LC system with new method parameters derived from previous experimental data until a specified objective function converges. By utilizing peak tracking, multi-start regression, retention modeling, and Bayesian optimization, such algorithms can automate the selection of various method parameters.

This presentation will discuss the current challenges in scaling automated method development technology for 2D-LC separations. Both retention modeling and machine learning approaches rely heavily on the chromatographic response function to optimize separation methods. A similar challenge arises in 2D column selection as we have to define what is optimal.

FL05

DEVELOPMENT AND TESTING OF A NON-CONTACT SCENT COLLECTION DEVICE ON REAL HUMAN SCENT

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Abstract

Evidence collection is a crucial step defining the subsequent course of investigations conducted by law enforcement. Traditional evidence collection methods involve direct contact between evidence and collecting tools or forensic personnel, such as casting footprints, visualizing fingerprints, or simply retrieving weapons with which the crime was committed. This approach is employed in the collection of scent evidence as well. In general, contact-based collection methods, while effective, carry risks of alteration, transfer, contamination, and the potential destruction of both evidence to be collected and surrounding materials. However, the volatile nature of scent provides an opportunity to implement a non-contact collection approach on them, theoretically reducing the above-mentioned risks. At the same time, the complexity of scent compounds makes GC×GC an ideal tool for their subsequent analysis.

In this study, a collecting device was developed based on a commerce vacuum cleaner, enhanced with 3D-printed polylactic acid components, besides other things, to secure a sorbent on which scent samples were collected.

The real human scent was being left on a table by volunteers for various time ranges and then was being collected diversely long as well. Thus, the design of the experiment consisted of several combinations of the leaving and collection durations. The effect of these parameters was also investigated for the contact collection approach.

FL06

TOWARDS A BETTER UNDERSTANDING OF THE BODY VOLATOLOME: FOCUS ON ENDOGENOUS PARAMETERS INFLUENCING BODY VOLATOLOME COMPOSITION

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Abstract

The body volatolome describes the numerous volatile organic compounds (VOCs) that are constantly emitted by the skin. These VOCs mainly result from the interaction of compounds produced by the sweat glands with microorganisms present on the skin surface. However, this production pathway can be affected by factors such as pathologies. This is why body volatolome analysis can be a smart and non-invasive way to perform large-scale health screening and monitoring. To do so, sampling body VOCs on a solid sorbent followed by thermodesorption into comprehensive two-dimensional chromatography coupled with time-of-flight mass spectrometry (TD-GC×GC/ToFMS) is among the most relevant solutions for the analysis of body odor because it provides both high sensitivity and resolution.

The first step of this study was the development of a user-friendly and reliable sampling system called SkinVOCs®. Then, SkinVOCs® was used to explore both endogenous and exogenous factors that influence the composition of the body volatolome, a needed knowledge to perform more accurate health monitoring. The specific study that will be presented here was carried out on 19 individuals, sampled on different body locations (armpits, forearms and upper-back) in order to understand the effect of age and gender over the body volatolome. Thanks to chemometric tools such as partial least square discriminant analysis (PLS-DA), 3 molecules specific to gender were identified while age groups were discriminated using a wider set of molecules. It also revealed a right/left symmetry in the body volatolome, as well as certain molecules specific to certain body areas.

FL07

METHOD OPTIMIZATION OF FINGERMARK RESIDUE USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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William & Mary, Williamsburg, USA

Abstract

Optimization is a crucial step in method development of routine analytical techniques. Fingermark residue, consisting of sweat and oil from sebaceous glands, is a complex biological mixture with fatty acids, fatty alcohols, and steroid hormones. Fingermarks have been analyzed using techniques such as liquid chromatography, capillary electrophoresis, and gas chromatography (GC) all coupled to mass spectrometry. There has been little research however in full, nontargeted characterization of fingermark residue using advanced chromatographic methods such as comprehensive two-dimensional gas chromatography (GC×GC-TOFMS). The goal of this study was to optimize a method for the nontargeted analysis of fingermark residue using GC×GC-TOFMS. A starting method based on a one-dimensional GC and GC×GC comparison was used to analyze residue samples. Full method optimization included testing five parameters (modulation period, hot pulse time, hold time at oven start, hold time at oven end, and oven ramp rate) with three options each and one parameter (secondary oven offset) with two options. Parameter options were compared to each other as a group and the best option chosen for the optimized method. The optimized method was evaluated as a whole with all optimized parameters. Fingermark deposition, regeneration, and sample preparation were optimized with different extraction processes with the goal of quantitation of analytes. Method optimization using GC×GC fully resolved hidden peaks such as the steroid hormone allopregnane. A fully optimized method will be used in future studies to differentiate between endogenous fingermark compounds and exogenous contamination compounds from the environment.

FL08

SEX AND PERSON IDENTITY RECOGNITION FROM GC×GC ANALYSIS OF SCENT SAMPLES

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Abstract

We present a method for the recognition of the identity and sex given a GC×GC chromatogram of a scent sample. In all stages, the method is fully automatic. First, the chromatogram is aligned by identifying 2D peak locations of pre-determined compounds that have been found to be present in all samples. The peak location is the point of maximum correlation with a machine-learned prototype of its spectrogram within a pre-learned part of the chromatogram. The GCxGC space is triangulated and affine warped to canonical coordinates. In the second step, the aligned chromatogram is flattened to a 1D vector whose values are, depending on the experiment, the total mass recorded at a given time, or the output of a spectrometer. The vector is the input of the identity or sex classifier. Two types of classifiers were trained and evaluated - the SVM (polynomial and RBF kernels) and a shallow convolution neural network.

Experiments were carried out on data collected from the palms of 40 volunteers onto glass beads, extracted into ethanol, and analysed by a LECO GC×GC-ToF over a three year period (2019-2022) . Each volunteer provided at least 10 samples, each a week apart. In case of the gender identification, by finding an optimal linear projection of the Total Ion Chromatogram data, we achieve approximately 85% validation accuracy in sex identification using 10-fold cross-validation on a balanced dataset (50% male, 50% female) of 504 measurements from 40 identities. The top 5 validation accuracy for classifying 20 identities is approximately 60%.

FL09

THE IMPACT OF THE MENSTRUAL CYCLE ON SKIN VOLATILE PROFILES

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Abstract

Historically, women have been underrepresented in biological and medical research, leading to significant gaps in knowledge with adverse implications for women's health. This exclusion often aims to avoid potential temporal variation linked to the female reproductive cycle. In studies on human odors and disease vector attraction, such gaps can critically impact human health. Yet, sex-specific differences in odor cues influencing mosquito attraction remain largely unexplored. To address this gap, we conducted a longitudinal study investigating variations in female body odors throughout the menstrual cycle. We collected skin volatile samples from female subjects during the menstrual, fertile, and luteal phases of their cycle, as well as from male controls. These samples were processed via two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) and further analyzed using GCImage software. Our findings provide crucial insights into sex differences in body odor and the potential effects of the menstrual cycle. Identifying volatile markers specific to sex and menstrual phases could significantly enhance our understanding of vector attraction. This knowledge may inform targeted strategies for improved health interventions and disease prevention and control, ultimately contributing to better public health outcomes.

FL10

DATA PROCESSING WORKFLOWS FOR NON-TARGET SCREENING ON LC×LC-HRMS DATA: READY TO GO?

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Abstract

Comprehensive two-dimensional liquid chromatography (LC×LC) has over the years matured from home-build intricate systems to commercially available solutions allowing for its proliferation in a broader community. A large body of research on LC×LC has focused on reaching optimal chromatographic conditions through various optimization schemes. Whereas the chromatographic performance of LC×LC outcompetes one-dimensional LC, the subsequent data processing is often laborious. Therefore, the data from LC×LC hyphenated to high-resolution mass spectrometry (HRMS) is often vastly underexploited. Even routine data processing operations for LC-HRMS remain challenging in LC×LC-HRMS.

In this study, we explore open-source data processing workflows for LC×LC-HRMS and assess their potential for non-target screening. Key aspects include: 1) data compression while preserving mass spectral resolution, 2) leveraging high-dimensional data through curve-resolution methods, 3) feasibility for trace-level analysis, 4) compound grouping across different samples, and 5) using LC×LC-HRMS to detect chemical differences between samples. We benchmark these workflows using LC×LC-HRMS data from environmental and plant metabolomics samples.

In recent work (unpublished), we have investigated how to extract high-quality mass spectra for trace-level compounds in LC×LC-HRMS data using variable selection prior to multivariate curve-resolution (MCR). We found that variable selection is a key step for increasing the detection-rate of trace-level compounds when using MCR.

FL11

COMPARATIVE ANALYSIS OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME-OF-FLIGHT MASS SPECTROMETRY DATA INTIME AND FREQUENCY DOMAINS

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Abstract

Numerous methods for rank-deficient modelling of multidimensional chromatographic data with drift along the first and second dimensional retention times have been proposed, but rely on selecting appropriate component numbers for each predetermined region of interest (ROI) for deployment to entire datasets. Most algorithms for determining regions of interest rely on the assumption of consistent instrumental parameters to maintain relatively consistent retention times, with only minor drift - and so extensibility to heterogeneous data is limited.

ANOVA-Simultaneous Component Analysis (ASCA) offers a parsimonious solution for significance testing and interpretation of tabular data, but can be applied to raw chromatographic data in a way that handles drift through a transformation of the data into a N-dimensional tensors of complex Fourier coefficients.

In this presentation, parallel analyses of multidimensional chromatographic data in the time and frequency domains will be performed using open source tools. The results of each analysis will be compared at a high level to assess the relative benefits and drawbacks of each technique.

FL12

ANALYSIS OF THE HUMAN SCENT ON THE CARTRIDGE CASES USING GC×GC-MS/TOF

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Abstract

Two-dimensional gas chromatography offers various applications due to its higher separation efficiency. This GC system could also be valuable for forensic purposes. This pilot study focuses on the human scent analysis on the crime scene, particularly on the cartridge cases. Although dactyloscopic fingerprints can sometimes be found on cartridges, they are often partial, lacking sufficient minutiae for successful database comparisons. However, the perpetrator also leaves behind its scent on the cartridges, which could be useful for both the individual as well as class identifications.

In this study, cartridge cases were collected from a simulated crime scene from four different surfaces. These cases were extracted using ethanol and the extract was analyzed with GC×GC-MS/TOF using liquid injection. Additionally, four different volunteers were sampled to be compared their scents with the samples from the simulated crime scene.

The results showed that the surface from where the traces were collected did not play significant role for the identification. While the ability to distinguish each volunteer´s scent was observed, it was not yet possible to link the fired cartridge cases to any of the volunteers. This issue could be resolved in the future by target analysis focused on genetically determined substances or by solving present problems with peak processing in the commercial software that accompanies the two-dimensional gas chromatograph.

This study is supported by Ministry of The Interior of The Czech Republic by the project No. VK01010240.

FL13

COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY FOR THE CHARACTERIZATION OF PHARMACEUTICAL RESIDUES IN HOSPITAL WASTEWATER

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Abstract

The increasing occurrence of organic micropollutants (OMPs) in environmental waters has become a topic of concern. These pollutants, that include pharmaceuticals, personal care products and pesticides, can accumulate over time and pose a risk to the aquatic environment and public health. An important source of OMPs is hospital wastewater, containing a wide variety of pharmaceutically active compounds and metabolites at increased concentration levels. Due to the complex composition of these samples and the wide array of physicochemical characteristics of their constituting compounds, innovative analysis techniques, such as two-dimensional LC (2D-LC) are needed as a more effective approach to fully characterize the complex profiles of these samples.

In this study, various comprehensive 2D-LC (LC×LC) high-resolution mass spectrometry (MS) methods, capable of separating and identifying a wide range of target OMPs in hospital wastewater, were optimized and compared. Different sets of RPLC×RPLC and HILIC×RPLC combinations were predicted to be orthogonal, and optimized individually. To deal with solvent strength mismatch problems, active solvent modulation was applied. The optimized methods were compared in terms of peak capacity and coupling feasibility (with focus on the mobile phase incompatibility), and applied to real hospital wastewater samples from the University Hospital of Leuven (UZ Leuven). Overall, this study highlights the potential benefits of LC×LC methods in the field of environmental analysis, but also the difficulties regarding the complex method development process.

FL14

DEVELOPMENT OF AN ONLINE SEC-UV-RP-MS METHOD FOR MULTI-ATTRIBUTE CHARACTERIZATION OF ADENO-ASSOCIATED VIRUSES

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Adeno-associated virus (AAV) vectors have become one of the preferred choices for gene therapies, with several FDA-approved products and numerous clinical trials underway. This preference is attributed to AAV's broad tissue tropism, non-pathogenic nature, favorable safety profile, and ability to sustain long-term transgene expression.

Despite their relatively simple structure, AAV-based gene therapy products pose challenges for their characterization due to large size and heterogeneity. Various chromatographic methods are classically employed to measure critical quality attributes (CQAs), such as aggregation levels, viral protein composition, and post-translational modifications (PTMs). To address the industry's need for more efficient characterization methods, multidimensional liquid chromatography (2D-LC) techniques have been developed to analyze multiple CQAs in a single run.

In this study, we present a novel SEC-UV-RP-MS method for simultaneous measurement of high molecular weight species (HMWs), viral protein ratio, capsid identity, and PTMs. This simple 2D-LC-MS approach integrates size exclusion chromatography (SEC) for aggregate semi-quantification in the first dimension, with reversed-phase liquid chromatography (RP-LC) coupled to mass spectrometry (MS) for detailed analysis of viral proteins in the second dimension. The developed collecting interface is relatively simple and the 2D-LC method was successfully applied for the comprehensive, streamlined, and versatile characterization of several AAV serotypes (AAV2, AAV8 and AAV9). Forced degradation study was performed using this generic method to demonstrate the platform’s sensitivity in detecting changes in AAV stability, such as increased aggregation and deamidation at elevated temperatures. This 2D-LC-MS approach confirms the applicability of multidimensional liquid chromatography for AAV vector characterization, providing a powerful tool for comprehensive analysis of gene therapies vectors.

FL15

GC×GC-TOFMS METABOLOMICS AND EXPOSOMICS FOR STUDYING THE IMPACT OF FETAL AND NEONATAL CANNABIS EXPOSURES

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Abstract

With the 2018 legalization of cannabis in Canada, there is growing concern surrounding the impacts of fetal and neonatal cannabis exposures. The long-term outcomes of early cannabis exposures are still unknown; however, some teens who use cannabis have permanent brain changes and increased risk of mental health problems. Much less is known about second-hand cannabis exposure and whether this exposes children to biologically significant levels of cannabis. Our interdisciplinary study follows mothers from early pregnancy until their babies are 18 months old. Mothers complete surveys on cannabis and tobacco use, second-hand exposure, demographics, health, and home environment. We are collecting urine from the mothers and infants, and breastmilk shortly after birth and 3 months postpartum. Additionally, we are utilizing wristband-based passive samplers to profile the exposomes of participants. We are employing comprehensive two-dimensional gas-chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) for the untargeted metabolomics of urine and breastmilk samples to determine how cannabis is metabolized, identify cannabis metabolites and biomarkers of other exposure events including tobacco smoke, and for exposome profiling. Here, we present preliminary results from the initial batch of urine samples we analyzed from the first 100 participants enrolled. In this cohort, 72.8% reported some level of cannabis exposure (first or second-hand) in the prior 12 months, with 9.7% reporting daily or weekly use. In urine, we identified a number of exposure markers, including cannabinoid metabolites, dietary markers, and pharmaceutical metabolites. This research provides valuable information to assist families in making informed choices about cannabis use during pregnancy.

FL16

IN-SITU ACCELERATED AGING AND ANALYSIS OF HIGH EXPLOSIVES VIA GC×GC-TOFMS

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Abstract

Understanding the chemical changes that occur in high explosives as they age is of paramount importance to the safe employment and storage of these compounds. Traditional methods of aging explosives even under accelerated aging conditions is extremely time intensive, with time scales on the order of months to years. This time investment is required even before the explosives are able to be analyzed and involves specialized equipment. The nature of these analyses reduce each sample to a snapshot data point separated widely in time, requiring many assumptions as to how degradation products develop.
In order to address these shortcomings with existing methods, a new method of accelerated aging of high explosives using a GC×GC-HRMS instrument was developed. This in-situ automated method reduces the time scale of aging to a matter of hours. GC×GC separation allowed for the identification of explosives aging products with far greater certainty than previously applied methods. This technique has allowed for collection of both evolved gasses and other decomposition products produced during the entire aging process in real time. Additionally, this method allowed for far higher throughput of sample analyses with greatly simplified sample preparation.

FL17

DUAL PARALLEL DETECTION RAW DATA FUSION: CHALLENGES AND OPPORTUNITIES FOR ACCURATE FINGERPRINTING OVER LARGE TIME FRAMES

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Abstract

Volatilomics is a growing field focused on identifying volatile metabolites in various samples. A straightforward approach using comprehensive two-dimensional gas chromatography (GC×GC) with parallel flame ionization detector (FID) and mass spectrometry (MS) offers both compound identification (via MS) and precise quantification (via FID). Typically, FID and MS data are analyzed separately, but with dedicated software solutions it is possible to combine raw signals from parallel detection. The fused signal brings the original information from both detectors (i.e., MS fragmentation pattern and FID response at each data point) and enable reliable image pattern recognition by template matching.

The contribution discusses the workflow to obtain combined detector signals and challenges posed by: (a) dual parallel detection, (b) dual parallel second dimension (^{2}D) columns set up, and (c) impaired acquisition frequencies. As test bench for the new data fusion workflow food volatilomics and fragrance allergens profiling are considered.

With data fusion, template matching using MS spectral similarity reduces false negatives by 80% on comprehensive UT features covering the hazelnut detectable volatilome (450 UT features) and improves true positive matches in allergens recognition. In both applications, accurate quantification is made straightforward due to the readily available FID signal.

FL18

BOOSTING NON-TARGETED ANALYSIS WITH COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND HIGH-RESOLUTION MASS SPECTROMETRY

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Abstract

Modern society continuously produces, markets, and uses a growing variety of chemicals that can enter the environment through multiple pathways. The use of Non-Target Screening (NTS) workflows, combined with high-resolution mass spectrometry (HRMS), has gained significant traction in detecting these chemicals in environmental samples and organisms. This study presents the integration of comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry (GC×GC-HR-TOFMS) as a powerful tool for NTS of highly complex samples.

A systematic workflow is outlined for screening both target and non-target contaminants across diverse, complex matrices. Enhanced separation is achieved using a combination of non-polar and polar stationary phases in the GC×GC method, resulting in better resolution between matrix components and target compounds, which led to cleaner MS spectra for persistent organic pollutants (POPs) such as PCBs, BDEs, and Toxaphenes. Identification and confirmation were facilitated by the injection of native standards.

The application of a novel Multi-Mode Source (MMS), capable of electron ionization (EI), positive chemical ionization (PCI), and electron capture negative ionization (ECNI), increased identification confidence by providing library-searchable spectra, accurate mass measurements, and molecular ion data for formula determination.

A list of identified target and non-target molecules is presented, alongside details on identification methods, including retention time, retention index, mass accuracy, and standard injections. EI high-resolution mass spectra and ECNI spectra are shown for further confirmation, and approaches for formula computation are highlighted.

FL19

PYROLYSIS AND GC×GC-MS. A HOT TOPIC!

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Abstract

Thermal desorption and pyrolysis (TD/PY) is a valuable sample introduction method for materials analysis with combined gas chromatography and mass spectrometry (GC-MS). Polymers can be identified by their characteristic pyrograms, and a database is available for the major pyrolysis products. However, pyrolysis produces much more complex mixtures than can be separated by one-dimensional gas chromatography alone!

Comprehensive two-dimensional gas chromatography combined with mass spectrometry (GC×GC-MS) is a useful approach to monitor pyrolysis reactions on a small scale and characterize the products of pilot-plant pyrolysis. High-resolution mass spectrometry and soft ionization methods (chemical ionization, photoionization, and field ionization) are essential tools for the identification of unknowns in these complex mixtures. To view things from a completely different perspective, soft ionization and pyrolysis with a deactivated fused silica column that provides _no_ GC separation shows high-molecular-weight pyrolysis products that are not detectable by GC-MS or GC×GC-MS.

FL20

SPECIATION OF CHLORINE-CONTANING MOLECULES IN PLASTIC PYROLYSIS OILS

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Abstract

Plastic pyrolysis oils (PPOs) can contain a wide range of impurities such as oxygenates, nitrogenates, organochlorides, and metals. Those contaminants can cause critical damage to steam crackers and related installations and affect the quality of end products.

In this study, PPOs were characterized via a comprehensive gas chromatography system coupled to a high-resolution time-of-flight mass spectrometry (GC×GC-HR-TOFMS). Scripting approaches and mass defects plots were used for the detection of chlorinated species at ppm levels in PPOs. Data screening scripts were developed based on exact mass differences and abundance ratios between chlorine isotopes using high resolution mass accuracy. Then, mass defect plots were used to validate the scripts and to visualize the datasets after filtering those chlorinated species out of the matrix. The Cl-filtering script was successfully applied to a sample dataset, allowing realistic manual investigations of peaks. The initial peak table contained approximately 1500 peaks. After applying the most selective Cl-filter, the table was reduced to a small number of peaks containing chlorine ranging from 0 to 10 peaks depending on the sample analyzed. Less selective Cl-scripts were also used to detect chlorine at low level; however, these scripts increased the number of false positives.

Therefore, this methodology, using scripting expressions and mass defect plots, showed to be a powerful tool for the identification of chlorine-containing species in PPOs. Future research should consider the evaluation of a halogen specific detector to validate this approach and to quantify the content of organochlorides.

FL21

APPLICATION OF PEARSON CORRELATION COEFFICIENT TO TWO-DIMENSIONAL GAS CHROMATOGRAPHY HIGH-RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY AS A COMPARISON AND DISCOVERY-BASED TECHNIQUE

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Abstract

Vacuum gas oils (VGOs) have long been heralded as an effective plasticizer due to their high boiling-points and lubricating properties. In this study, 18 VGOs, including HyVac® Oil 93050, were initially evaluated for density and viscosity. The oils were then ranked based on physical characteristics and analyzed for molecular composition using high-temperature comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry (HT-GC×GC-HRMS). Furthermore, because the datasets for the chromatograms being analyzed were large and complex, a simple metric was preferred when determining the similarities and differences between samples. This single metric concept led to the application of Pearson correlation coefficient. While Pearson correlation coefficient is well-known as a comparison technique, this study also utilizes it as a discovery-based method to determine significant chemical differences. Measurements of the covariances across the chromatograms utilizing the Pearson correlation coefficient equation were calculated, and visual representations of these differences were developed.

FL22

AMBIENT ULTRAFINE PARTICLES: CLASSIFICATION, CHEMICAL CHARACTERIZATION, AND QUANTIFICATION OF UBIQUITOUS PAHS VIA DTD-GC×GC-TOFMS

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Abstract

Ultrafine particles (UFP) have diameters below 100 nm and a high surface area. As they may enter the lung deeply, UFP pose a potential risk to human health. Efforts in sampling and analysing UFP are challenged by their low mass. Thus, to gain insights into their chemical composition, efficient sampling strategies and highly sensitive instrumentation are needed.

Ambient air UFP was sampled in two field campaigns in March and September 2023 in the city of Augsburg, Germany. Samples were collected on filters for 24 h and analysed via direct thermal desorption coupled to comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (DTD-GC×GC-TOFMS, LECO BT4D). Compound classes of known adverse health effects, e.g. polycyclic aromatic hydrocarbons (PAHs) including alkylated, oxygenated 4 and 5-ring PAHs and parent-4, 5 and 6-Ring PAHs were identified. Ten PAHs, including Benz[a]pyrene (BaP) - as one of the most ubiquitous and cancerogenic PAHs - were subsequently quantified. We found BaP concentrations of 6-14 pg/m^3 and 4-38 pg/m^3 in March and September, respectively. Moreover, the classification approach demonstrated relatively higher abundances of parent-4,5 and 6-ring PAHs as well as alkylated and oxygenated 4- and 5-ring PAHs during two days of high BaP concentration.

This study gives novel insights into the composition of ambient air UFP and highlights the importance of applying sophisticated analytical techniques such as GC×GC-MS to gain a comprehensive understanding of complex sample composition.

This project was financed by the Bavarian Ministry of the Environment and Consumer Protection.

P-1

APPLICATION OF SELECTIVE COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR THE SIMULTANEOUS ANALYSIS OF CONSTITUTIONAL ISOMERS AND ENANTIOMERS IN OOLONG TEA

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Abstract

The use of multi-dimensional liquid chromatography (MD-LC) in chiral analyses has become increasingly popular over the years due to its increased resolution and improved efficiency compared to traditional LC techniques. However, analyzing complex samples where constitutional and chiral isomers coelute remains challenging. In such situations, additional optimization steps are often required to ensure adequate resolution. Another common challenge for MD-LC analysis is achieving a balance between sensitivity, repeatability, and resolution, especially for trace analytes. One such group of analytes is *D*/*L*-Leu and Ile, where large differences in abundance in some food samples like tea further complicates their separation and quantification.

In this study, a method for simultaneously analyzing constitutional isomers and enantiomers using selective comprehensive 2D-LC was optimized, with *D*/*L*-Leu and Ile as an example. The loop filing percentage of the sample loop involved in the transfer of analytes from the first to the second dimension was identified as a key parameter that could affect the sensitivity, repeatability, and resolution of the targeted analytes. By investigating different loop-filling percentages, it was found that the sensitivity increased with higher loop-filling percentages while relative standard deviation (RSD%) and resolution decreased. A loop filling 100% was selected giving the best balance between the three factors. The versatility and robustness of the method were demonstrated on five oolong tea samples and *D*/*L*- Leu and Ile were successfully quantified. The optimized method showed excellent separation and identification of chiral isomers, highlighting the method's potential in other food and pharmaceutical applications.

P-2

OVERCOMING THE MODULATION CHALLENGES IN TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

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Abstract

To improve the applicability of two-dimensional liquid chromatography (2D-LC), much of the recent research aims to solve one of its biggest challenges: solvent incompatibility. Solvent incompatibility occurs when complementary separation modes are coupled whereby the solvent of the first dimension is a strong eluent in the second-dimension separation mode. Several modulation strategies have been developed to tackle this problem, including stationary-phase-assisted modulation (SPAM), active solvent modulation (ASM), and at-column dilution. These strategies aid in (partially) solving incompatibility for specific samples and selectivity combinations. However, they also further complicate the intricate method-development process for 2D-LC separations. During any 2D-LC method-development process, whether it be heart-cutting or comprehensive, the same question inevitably returns: Which modulation strategy should be used, if any?

Automated method-development workflows aim to answer such questions for the users. However, such automated workflows do not currently aid in selecting modulation strategies. To enable optimization algorithms to address this issue, we need to determine the exact nature of complications and the conditions under which they occur.

In this presentation, we present advancements in unifying existing theoretical and practical knowledge on modulation strategies with new findings to address challenges in the automated selection and design of modulation setups. We provide examples of common separation mechanism combinations for both small and large molecules, including reversed-phase liquid chromatography (RPLC), hydrophilic interaction chromatography, and organic size-exclusion chromatography paired with RPLC. Key parameters for consideration during the selection and design process are emphasized.

P-3

DUAL PARALLEL DETECTION RAW DATA FUSION: QUANTITATIVE FOOD VOLATILOMICS ON LARGE SAMPLE SETS

Andrea Caratti[1], Simone Squara[1], Angelica Fina[1], Fulvia Trapani[1], Erica Liberto[1], Stephen E. Reichenbach[2], Qingping Tao[3], Daniel Geschwender[3], Chiara Cordero[1]

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Abstract

Volatilomics, a rapidly expanding discipline, aims to characterize volatile metabolites in various matrices. Advances in two-dimensional gas chromatography (GC×GC) coupled with parallel flame ionization detection (FID) and mass spectrometry (MS) offer combined capabilities of compound identification (by MS) and precise quantification (by FID). Parallel detector signals are generally processed separately, this study integrates MS and FID chromatograms to enhance pattern recognition during template matching, enabling comprehensive quantitative volatilomics. Feature matching utilizes MS spectral similarity, reducing mismatches and facilitating accurate FID response extraction for quantification.

Applied to hazelnuts, a premium confectionery ingredient, GC×GC-FID/MS analyses identified volatiles distinguishing cultivars, geographic origins, post-harvest treatments, contamination, oxidative stability, and sensory quality. This methodology aligns with the Sensomics-Based Expert System (SEBES), an AI-driven platform predicting food aromas without human olfaction.

The dataset, encompassing raw hazelnut samples over four harvest years, required normalization of MS responses and correction with internal standards due to variability. Temporal chromatographic misalignments can cause 2D peak pattern inconsistencies. Data fusion, guided by MS spectral similarity, reduces false negatives by about 80% for 441 detectable features compared to FID alone and minimizes false positives, enhancing specificity and selectivity. Additionally, data fusion halves processing time and facilitates metadata transfer. After pattern recognition, FID signals are extracted for quantification based on calibration curves or predicted FID response factors.

This combined detection method for quantitative volatilomics reliably tracks aroma changes over crop production and shelf-life, enabling robust marker discovery for industrial quality assessment.

P-4

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY (GC×GC-TOF MS) AND IMAGE PATTERN RECOGNITION: VOLATILOMICS UNREVEAL METABOLIC SYNERGIES IN FECAL MICROBIOME

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Abstract

Comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOF MS) represents significant advancement in analytical techniques, providing exceptional resolution and sensitivity, especially for characterizing complex samples with high chemical dimensionality. Unlike one-dimensional gas chromatography, GC×GC-TOF MS offers a more precise analysis of volatile organic compounds (VOCs), effectively addressing issues of co-elution and covering a wide dynamic range of concentrations, a key feature in metabolomic studies. This study explores the application of GC×GC-TOF MS for analyzing fecal volatile metabolites, focusing on profiling the fecal volatilome, an underexplored area in metabolomics. Fifty individuals with suspected non-celiac gluten sensitivity (NCGS) underwent a double-blind placebo-controlled gluten challenge. Twenty-seven participants who were gluten responsive were randomized into two groups: one receiving daily probiotics (*L. plantarum, L. paracasei, L. salivarius*) and the other receiving a placebo, both under a gluten-free diet for 4 weeks, after which gluten was reintroduced for 2 weeks. Using combined untargeted and targeted fingerprinting (UT fingerprinting), the study identified over 830 volatile features, with around 200 being putatively identified and correlated with study variables using chemometric analyses. Results showed significant changes in the fecal volatilome after probiotic treatment, with Partial-Least Squares Discriminant Analysis (PLS-DA) providing classification models with 89% accuracy at 4 weeks and 90% at 6 weeks. Key features included butanoic and propanoic acid esters, alcohols, aldehydes and terpenoids. Despite the limited sample size, this study underscores the potential of GC×GC-TOF MS in advancing personalized nutrition and enhancing our understanding of fecal metabolomics.

P-5

COMPREHENSIVE SCREENING OF COMPLEX ENVIRONMENTAL SAMPLES FOR PFAS AND OTHER POLLUTANTS USING ENHANCED CHROMATOGRAPHY WITH HIGH-RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY AND SPECTRAL ANALYSIS TOOLS

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Abstract

Clean air, water, and soil are essential for the sustainability of life. There is an increasing interest in environmental screening for harmful substances in both outdoor and indoor settings. Frequent exposure to hazardous materials via inhalation or ingestion can be detrimental to health. Therefore, it is critically important to regularly screen for known and unknown toxic materials in the environment. Monitoring hazardous persistent organic pollutants (POPs) such as Polyfluorinated Alkyl Substances (PFAS) is challenging due to the number of different compounds, large concentration range, complexity of environmental matrices, and the fact that a great deal of these substances are not present in the commercially available mass spectral libraries. The goal of this study was to develop methodology for the untargeted and semi-targeted screening of samples for legacy pollutants and emerging (e.g. PFAS) related pollutants. Various environmental samples were analyzed (e.g., air particulate matter, water) by comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry (GC×GC-HRT-TOFMS) employing a multi-mode ionization source which accommodates electron ionization as well as both positive and negative chemical ionization. EI data were utilized to annotate compounds via spectral similarity searches of large databases and formula determinations using high-resolution accurate mass molecular and fragment ions. PCI and NCI spectra provided complementary molecular formula information and increased confidence in compound characterization. Advanced software tools (e.g., scaled mass defect & RDBE plots) simplified retrospective analysis of the comprehensive data for emerging pollutants. The analyses resulted in the annotation of polymeric markers, polymer additives, heterocyclics, aromatics, polyaromatics, PFAS, and bisphenols.

P-6

SYNERGIES AMONG DIFFERENT METABOLIC FRACTIONS IN GERMINATED PEANUTS: FLEXIBILITY AND INFORMATIVE POTENTIAL OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY – TIME-OF-FLIGHT MASS SPECTROMETRY

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Abstract

Peanuts (*Arachis hypogaea*) are consumed globally for their nutritional value, taste, and affordability. Peanut quality is assessed based on appearance, texture and flavor. Defining quality standards for peanuts involves understanding their chemical composition, stability, and sensory properties. This study investigates a germination defect known as splitting, where the peanut's cotyledons separate. Splitting, linked to early germination, triggers premature metabolism, negatively impacting peanut quality by causing dull flavors or risk of over-roasting. Different metabolite fractions were analyzed to understand the impact of germination. Primary metabolites, including free amino acids, organic acids, and sugars, were examined after defatting, extraction, and derivatization with GC×GC-TOF MS. The lipid fraction was analyzed for esterified and free fatty acids (FFAs) using lipid extraction, and transesterification of esterified fatty acids (EFAs), Fisher esterification for FFAs followed by GC×GC-MS/FID analysis. The volatile fraction was explored using headspace solid-phase microextraction (HS-SPME) followed by GC×GC-TOF MS. Results indicate that metabolic activation in split seeds leads to higher concentrations of monosaccharides such as mannitol and glucitol, while aroma precursors like valine, threonine, and sucrose are present in lower amounts compared to whole peanuts. Differences in lipid amount and distribution were evident between whole and split kernels. The volatilome confirmed further differentiation, with compounds such as 2-pentyl furan and dihydro-3-methyl 2(3H)-furanone showing higher responses in split peanuts. This study highlights the versatility and sensitivity of GC×GC, making it a platform of choice for food omics investigations due to its superior resolution and dynamic range coverage.

P-7

AROMA PROFILING OF COMMERCIAL POI PRODUCTS IN FRESH AND AGED STATES USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Abstract

Taro (Colocasia esculenta L.) is a plant originating from Southeast Asia. Now prevalent throughout the Pacific Islands, growing taro was and remains an important facet of Hawaiian culture. Poi is a food product prepared from steamed taro that has been macerated and allowed to ferment. While poi is commonly prepared at home, it can also be found in grocery stores from select Hawaiian brands. The general fermentation process of poi produces volatile organic compounds (VOCs) such as alcohols, aldehydes, ketones, organic acids, esters, and phenols, among others, through microbial and enzymatic reactions. The VOCs found in poi are traditionally analyzed through gas chromatography-mass spectrometry (GC-MS). This study aimed to observe changes in fresh to aged commercially purchased poi using comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry with flame ionization detection (GC×GC-qMS/FID). Samples were prepared according to package instructions, extracted via headspace SPME Arrow, and analyzed in replicates of 10. All brands showed a clear distinction between fresh and aged samples. Fresh samples across all brands contained acetoin while aged samples all contained 1-pentanol, acetic acid, and 2,5-dimethylfuran. Though Hanalei and Taro brand poi had similar profiles, He Mea Ono (HMO) brand poi differed. Aged HMO brand poi contained a greater variety of compounds than aged Hanalei or Taro brand poi. Visual distinctions in chromatographic plots were supplemented by principal component analysis (PCA) and volcano plots, identifying distinguishing compounds. Investigating the profile of poi via GC×GC-qMS/FID can enhance understanding of its unique qualities, historical context, and contemporary uses.

P-8

MOLECULAR CHARACTERIZATION OF NEW RENEWABLE FEEDSTOCKS BY MULTI-SCALE ANALYSIS USING GAS CHROMATOGRAPHY, MASS SPECTROMETRY AND AN OXYGEN SELECTIVE DETECTOR

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Abstract

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 used wind blades is therefore of paramount importance to better understand the macroscopic behavior of these new materials. These feedstocks are made of complex chemical mixtures that require multi-scale analysis to be properly characterized. Several analytical techniques are currently employed for this purpose. The volatile fraction of the samples can be analyzed by gas chromatography hyphenated to mass spectrometry to identify the unknown compounds.

However, the diversity of the chemical classes present in such samples is very large and comprehensive two-dimensional gas chromatography coupled to mass spectrometry is required.

Next to MS, heteroatom-specific detectors can also be coupled to the chromatographic separation to provide additional valuable information. Since some compounds, like oxygenated molecules, can affect the macromolecular properties of the alternative fuel, the need for clear understanding of oxygen-bearing compounds is a major goal. However, the quantification and identification of oxygenated molecules is severely limited by the lack of oxygen-specific detectors.

To address this, a new type of oxygen-specific detector capable to identify and quantify molecules without the use of any standards was used. In addition to the characterization of oxygen-containing molecules, this new detector can also detect and quantify carbon, nitrogen, and sulfur.

This multi-scale analytical approach using GC×GC and various type of detectors appears to enhance our ability to adapt the related industrial processes in order to produce high-value products.

P-9

HIGH-RESOLUTION GC×GC-TOFMS ANALYSIS OF CRUDE OIL AFTER GAMMA RAY RADIOLYSIS

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Abstract

Dating geological processes such as fluid charge and residence times in reservoirs has played a significant role in understanding the dynamics of petroleum systems. Recently, using in-reservoir crude oil radiolytic alterations as a chronometer for petroleum residence time was proposed, tracking radiolysis-induced compositional changes. When exposed to gamma radiation, virtually all compounds in the crude oil matrix are affected, forming dozens to hundreds of new products at trace concentrations from a single parent compound. Analytical platforms such as GC-MS, NMR, and FT-ICR-MS have been employed to study these alterations; however, advanced techniques with enhanced sensitivities, selectivity and non-targeted analysis capabilities are needed to overcome existing analytical challenges. In this study, we explore the potential of comprehensive two-dimensional gas chromatography coupled with high-resolution time-of-flight mass spectrometry (GC×GC-HRMS) to analyze gamma-irradiated crude oils at radiation doses up to 4 MGy, using a sample subset from DOI: 10.1016/j.gca.2019.07.020. The study's objective is to explore the effectiveness of GC×GC-HRMS for identifying radiolysis-induced molecular proxies, particularly newly formed species absent in non-irradiated oils, whose relative intensity correlates with the applied radiation dose. The superior analytical capabilities of GC×GC-HRMS make the technique well-suitable for untargeted analysis of GC-amenable compounds in irradiated crude oil, enabling the detection of subtle variations in oil composition following gamma irradiation. This work demonstrates the technique’s effectiveness in identifying new radiolysis proxies in complex organic mixtures, with immediate implications for understanding the evolution of radiolysis proxies in reservoired crude oils and its potential application in petroleum system dynamics.

P-10

GROUP-TYPE ANALYSIS OF HYDROCARBONS IN AVIATION FUEL USING DUAL-CHANNEL GC×GC–FID

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Abstract

Finished fuels, such as jet fuel (or aviation turbine fuel, AVTUR), are regulated to ensure that the total aromatic content does not exceed set limits. In general, aromatics burn slower than paraffins and create more soot and particulate matter, meaning that the hydrocarbon composition of jet fuel not only impacts combustion properties, but also environmental emissions.

Traditional jet fuel regulations dictate that the aromatic content must fall below 25% by volume. However, as the aviation industry works toward reducing its carbon footprint, new sustainable aviation fuels (or SAFs) are being developed which contain far reduced aromatic content. For this reason, further regulations were introduced to set a minimum value of 8% by volume for aromatics, since these compounds ensure proper swelling of certain O-rings and seals, thereby preventing leaks. SAFs are often blended with conventional jet fuels to meet the minimum requirements. These changes further drive the need for robust testing of hydrocarbon composition to ensure compliance and safety.

ASTM Method D8396 was established to deliver precise quantitative data on hydrocarbon composition using group-type analysis via flow-modulated GC×GC–FID. In this study, we showcase a dual-channel approach with automated group-type data processing, which doubles productivity and ensures robust quantitation, all while adhering to the guidelines specified in ASTM Method D8396. This approach ensures compliance with evolving regulations and supports the aviation industry's safety and sustainability goals.

P-11

MULTI-OMICS WORKFLOW TO DEFINE OXIDATIVE STRESS AT THE MOLECULAR LEVEL USING IN VITRO MODELS

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Abstract

Oxidative stress is a pathological condition that arises when there is an imbalance between reactive oxygen species (ROS) production and cellular detoxification ability. This condition has been linked to various diseases such as asthma, 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. The objective of this study is to gain a better understanding of oxidative stress at the molecular level by inducing chemical stress (hydrogen peroxide, $H_2O_{2)}$ on epithelial lung cells (A549) 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 future. The VOCs released by the medium were directly characterized using solid-phase micro-extraction (SPME) and analysed using comprehensive two-dimensional gas chromatography coupled with a time-of-flight mass spectrometer (GC×GC-TOFMS). For the cellular pellet, two different derivatization protocols, previously developed in the laboratory on serum samples, were tested to extract the lipidomic and the metabolomic information.

P-12

EVALUATION OF MINERAL OIL HYDROCARBONS IN VARIOUS TYPES OF UNPROCESSED MEAT USING LC-GC×GC-FID/MS

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Abstract

Mineral oil hydrocarbons (MOH) are a complex mixture of liposoluble environmental and processing contaminants of petrogenic origin. They may pose different toxicological risks to humans depending on their structure (i.e., saturated (MOSH) or aromatic (MOAH)) [1]. The increasing demand for more detailed information on MOH composition is driving a shift from LC-GC-FID, considered the routine technique, to more advanced techniques, notably LC-GC×GC-FID/MS [2]. In this study, a method to determine MOH in meat was optimized to provide a detailed characterization of the MOSH profile (i.e. linear and cyclic), as requested by the EFSA to evaluate whether animal food poses a risk due to MOSH accumulation [1]. A microwave-assisted saponification and extraction (MASE) procedure was optimized and validated based on recently published works [3, 4]. Meat samples (n=30) from various animals (mammals, poultry, and ruminants) were purchased from different local supermarkets in the Wallonia region of Belgium. They were subjected to a MASE before their analysis in LC-GC×GC-FID/MS. The use of GC×GC, together with the information obtained by the MS, enabled a detailed investigation and differentiation of linear from cyclic MOSH, thus giving some hints on the source of contamination.

Acknowledgment

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P-13

ACCELERATING WOOD METABOLITE EXTRACTION: OPTIMIZING PRESSURIZED LIQUID EXTRACTION (PLE) FOR ENHANCED WOOD METABOLOMIC PROFILING

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Abstract

Throughout human history, wood has been an essential resource, deeply embedded in cultures and industries. Alongside its value to human society, illegal logging has emerged as a serious issue impacting the environment, society and economy. To prevent environmental crimes and protect sustainable forestry, reliable wood authentication methods are needed to certify legal lumber and monitor the timber trade. Species-level identification is especially important for law enforcement. Current forensic wood identification methods are primarily based on anatomical observation, chemical profiling using direct analysis in real-time (DART) coupled to time-of-flight mass spectrometry (TOFMS), and DNA analysis. While these methods have advantages, they are not without limitations, especially with species-level identification and chemically treated wood products. Metabolite profiling using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) has been recently proposed as a promising wood authentication tool due to enhanced separation power capable of differentiating isomer species. A critical first step in this method is the efficient extraction of analytes. In this study, pressurized liquid extraction (PLE) was optimized for more efficient wood metabolite extraction. Key parameters such as sample mass, temperature, extraction time, and cycles were systematically examined using sawdust wood chips and acidified methanol as the solvent. The optimized PLE results were compared with the traditional extraction method. While the conventional method takes 18 hours at room temperature, PLE operates under elevated temperature and pressure, significantly reducing extraction time, a key advantage in the timber trade where speed and efficiency are important.

P14

UNRAVELING THE DISTRIBUTION AND ENANTIOMER RATIOS OF CAROTENOID-DERIVED AROMA COMPOUNDS IN OOLONG TEA USING MULTI-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

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Abstract

In recent years, the popularity of oolong tea has increased worldwide due to its distinctive and appealing flavour profile. Among the various volatile compounds found in oolong tea, carotenoid-derived compounds are especially important for their contribution to the characteristic flavour of oolong tea. However, the analysis of these compounds and determining their enantiomeric distributions in tea is challenging due to their low concentrations and wide variability. To address this, a comprehensive two-dimensional gas chromatography (GC×GC) method coupled with a quadrupole time-of-flight mass spectrometry detector was developed, optimising the modulation period and hot jet duration for untargeted fingerprinting of seven types of oolong tea. The use of a thermal modulator facilitated the transfer of compounds from the first to the second dimension, enabling the detection of trace carotenoid-derived compounds by refocusing them. Results showed that the second dimension significantly increased the number of identified compounds. Notably, 72 carotenoid-derived compounds that were undetectable by single-dimensional GC were identified in GC×GC across seven different oolong teas. Additionally, heart-cutting two-dimensional gas chromatography (GC-GC) was employed to separate enantiomers of *α*-ionone, *cis*-nerolidol, and dihydroactinidiolide without interference from co-eluting compounds by selectively transferring peaks of interest to the second dimension. The enantiomer ratios determined by GC-GC ranged from 0.23-0.89 for *R/S*-*α*-ionone, 0.17-0.77 for *S/R*-*cis*-nerolidol, and 0.79-1.57 for *S/R*-dihydroactinidiolide . The use of GC×GC and GC-GC techniques allow for more in depth studies of the enantiomeric compositions of key odorants such as carotenoid-derived compounds which are crucial for understanding the complex flavour of oolong tea.

P-15

DEVELOPING JET FUEL PROPERTY PREDICTION MODELS THROUGH COMPOSITION ANALYSIS USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Abstract

Sustainable aviation fuels (SAFs) are crucial to mitigate the carbon emissions associated with the aviation industry. The Fischer-Tropsch (FT) process offers a promising approach for SAF production from non-petroleum sources [1]. However, the complex composition of SAFs poses analytical challenges. Using two-dimensional gas chromatography with mass spectrometric detection (GC×GC/MS) proves highly effective in characterizing SAFs. Composition-property-based prediction models serve as a cost and time-efficient pre-screening strategy in SAF development [2]. This project aims to develop an analytical method for SAF characterization and prediction models using GC×GC/MS and machine learning. Surrogate and blend samples were analyzed using cryogenic modulation comprehensive GC×GC/MS with a reversed column configuration (mid-polar 1D column, nonpolar 2D column). The composition of the samples was characterized based on compound group class and carbon chain length. Training and test sets were created for prediction model development using multivariate regression techniques. Initial results show promising accuracy for fuel properties like density and heating value, but further exploration of other chemometric techniques is needed for additional properties. This approach leverages chemical information to optimize fuel formulations, meet regulatory standards, and enhance fuel performance.

Keywords: sustainable aviation fuel, two-dimensional gas chromatography, chemometrics

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P-16

CHARACTERISATION OF BIODEGRADABLE POLYMERS BY PYROLYSIS MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY (PY-GC×GC-MS)

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Abstract

Growing concerns over environmental plastic pollution has prompted the embrace of bio-based and biodegradable plastics as a solution to a global reliance on non-degradable petroleum-based plastics. However, the efficacy of bio-based and biodegradable plastics, hinges on understanding their environmental fate and degradation to non-toxic compounds.

Although significant advancements have been made in understanding the fate of conventional plastics in different environmental settings, there remains a notable gap in knowledge regarding the degradation pathways and fate of bio-based and biodegradable plastics. Furthermore, ambiguous end-of-life management and the misleading use of terms like 'biodegradable' or 'compostable' plastics based on industry standards, which do not align with consumer perceptions of natural degradation, mean plastics claiming to be ‘environmentally-friendly’ often persist for years in the environment.

Therefore, the aim of this project is to assess the performance and degradation of bio-based and biodegradable plastics under environmentally relevant conditions. Analytical characterisation was performed using pyrolysis (Py) coupled with comprehensive multidimensional gas chromatography with dual time-of-flight mass spectrometry and flame ionisation detection (GCGC-TOFMS/FID). Py-GCGC-TOFMS overcomes limitations such as lengthy sample extraction and preparation, surface analysis and library matching exhibited by traditional, widely adopted, spectroscopic methods such as Fourier Transform Infrared (FT-IR) and Raman.

Results from Py-GC-MS and Py-GC×GC-MS detected a range of products, indicative of intricate degradation pathways involving chain scission, oxidation, hydrolysis and rearrangement reactions.

Py-GCGC-MS afforded high sensitivity, increased peak capacity and enhanced resolution compared to Py-GC-MS, enabling unprecedented insights into the analysis and fingerprinting capabilities of complex polymers and degradation pathways.

P-17

IMMERSIVE INSIGHTS: TRANSFORMING GC×GC DATA VISUALISATION WITH VIRTUAL AND AUGMENTED REALITY

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Abstract

Data visualisation is a crucial part of chemometric data processing. In multidimensional chromatography, data visualisation is essential for understanding chemical complexity, assessing chromatographic performance, and educating those outside the field about advanced chemical concepts and the benefits and limitations of the measurement.

The data generated by multidimensional chromatography can be described as second-order tensors with univariate detectors (e.g. FID, UV) and third-order tensors with multivariate detectors (e.g. MS). Therefore, methods for visualising N-dimensional arrays of data are necessary for viewing the chromatograms. Chromatograms are typically represented as two-dimensional contour plots, colourised to show relative peak intensity. These contour plots can also be rendered as a three-dimensional (3D) projection. Although the 3D format is not widely used in data processing workflows (especially by chromatography purists), it represents an extremely powerful and engaging form of data visualisation that can be more interpretable than traditional 1D data and contour plots.

We demonstrate, as a proof-of-concept, how augmented reality (AR) and virtual reality (VR) can help transform complex analytical data into accessible and immersive insights. New methods for data processing and visualization were developed by leveraging a range of immersive technologies. Building on open-access protocols, this concept can be further developed to improve functionality and interactivity, aiming to create new tools for a wide range of applications including outreach and teaching, qualitative data analysis, cross-disciplinary engagement, and marketing.

P-18

IDENTIFICATION OF ANTIFUNGAL VOLATILE ORGANIC COMPOUNDS (VOCS) FROM *STREPTOMYCES SCABIEI* USING GC×GC-TOF-MS

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Abstract

Plant pathogens pose a serious global threat to food production, leading to significant crop diseases and economic losses. While synthetic fungicides have traditionally been used to combat these pathogens, their harmful impact on the environment and human health underlines the urgent need for alternative approaches. *Streptomyces* species, particularly *Streptomyces scabiei* 87-22, have shown great promise as microbial agents due to their production of volatile organic compounds (VOCs) with potent antifungal properties. This study aims to identify the specific VOCs responsible for antifungal activity against *Alternaria solani, Gibberella zeae*, and *Penicillium sp. NS1*.

Samples of *Streptomyces scabiei* 87-22 were co-cultured with three pathogens to study the ability of *Streptomyces* VOCs to inhibit fungal growth. The cultures were performed in five different culture media demonstrating different level of activities against the three different pathogens. To identify the VOCs responsible for the inhibition, the culture headspaces were analyzed using solid phase micro-extraction (SPME) and comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF-MS). Then, the chromatograms were compared using a Tile-based approach. Next, the data generated were processed using a targeted and non-targeted workflow. At the end, four promising VOCs were identified, and their antifungal activities were confirmed using in-vitro inhibition tests.

GC×GC-TOF-MS allowed us to effectively separate complex mixtures, and the results of this research provide valuable insights into the antifungal compounds of *Streptomyces* VOCs and open the door to developing environmentally friendly biofungicides.

P20

GC×GC-TOFMS AND GC/HRMS FOR THE DETAILED CHARACTERIZATION OF VOLATILE FRACTIONS FROM PYROLYSIS OILS OF WASTED TIRES AND HYDROCARBON RESINS

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Abstract

The human population growth and consequently the increased use of transportation are reflected in higher worldwide tire consumption and tire waste generation. Hence, several methods of tire waste management have been considered in the last few decades, particularly those involving material and energy recovery. Tire pyrolysis has become a well-established waste treatment method enabling to obtain value-added products such as pyrolysis oils. These raw materials can be reused in the tire manufacturing industry. For instance, hydrocarbon resins can be synthesized with monomers found in pyrolysis oils.

The first step of this study consisted in developing analytical methods to characterize pyrolysis oils and their distillation fractions. For this purpose, GC×GC-TOFMS and GC/HRMS methods were developed. GC×GC-TOFMS allowed for the organization of the chromatograms according to the structure of chemical compounds and a better separation of polar compounds from non-polar compounds whereas GC/HRMS provided the exact molecular mass of analytes, consequently their chemical formulae. Both methods highlighted the presence of monomers, which are typically used in the synthesis of hydrocarbon resins, in light distillation fractions of pyrolysis oils. However, several compounds were identified with GC×GC-TOFMS but not with GC/HRMS in pyrolysis oils, and vice versa. Therefore, the two methods appeared complementary. Additionally, the second step of this study was to propose analytical strategies to characterize hydrocarbon resins. SPME-GC×GC-TOFMS and SPME-GC/HRMS were implemented for analyzing the volatile compounds of resins: massifs were observed on chromatograms, which were likely to be dimers or trimers of building blocks of hydrocarbon resins.

P-21

ANALYSIS OF PERFUMES USING TWO-DIMENSIONAL GAS CHROMATOGRAPHY ON A QUADRUPOLE MASS SPECTROMETER

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Abstract

The global flavors and fragrances market has been estimated at 30 billion USD, so there is a high demand for chemical analysis products within the industry. Mass spectral analysis of these products is useful for formulation/deformulation, allergen testing, and quality control/counterfeit testing. Perfumes in particular benefit from GC-MS analysis due to high volatility of the samples, however, their complex nature can sometimes make chemical analysis difficult due to coeluting peaks in conventional GC. This study aims to use the separation power of GC×GC to look at the comprehensive chemical makeup of perfume samples and multivariate analysis to compare several brands of perfume.

Two-dimensionally GC benefits greatly from high-resolution mass spectrometry, however, this can often be costly, and may not be needed for certain applications. This application was tested on a quadrupole mass spectrometer, which offers better dynamic range and a lower price at the expense of accurate mass.

Perfumes from four different brands were measured using GC×GC-QMS. To help identify molecular ions, mass spectra were also measured using chemical ionization, which can help make up for the lack of accurate mass data.

Excellent separation was observed, far superior than using one-dimensional GC. Multivariate analysis showed clear separation of most samples, however, complete grouping of brands was not observed, suggesting a different perfume base for each product within a brand. Allergens were identified using targeted analysis based on the EU list of 26 allergens.

P-22

CHALLENGES IN DATA PROCESSING AND EVALUATION OF SCENT SAMPLES ANALYZED BY GC×GC-TOF

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Abstract

The aim of this contribution is to summarize the methods and procedures used in processing data obtained from the analysis of scent samples. An essential part of interpreting two-dimensional chromatographic data is the processing and evaluation of raw data. In the case of scent samples, thousands of peaks are detected in the measured data using the commercial software ChromaTOF. The subsequent processing of such a large amount of data requires precision in so-called data alignment. Unfortunately, in chromatography in general, we cannot ensure system stability over the long term (e.g., due to service interventions). This leads to shifts in retention times, in GC×GC in both dimensions, and the evaluation software does not always align the data correctly, i.e., it may not consistently identify the same compound across all chromatograms. The paper also defines problems with the ChromaTOF software (5.51.06.0) in processing raw data — such as the omission of sub-peaks, the choice of base peaks, and the combining of mismatched sub-peaks. These issues make target analysis (manual evaluation) impossible and make automatic and semi-automatic evaluation inaccurate.

This contribution summarizes the procedures by which we were at least partially able to solve this problem (automatic data alignment, alignment using anchor peaks (by RI), and target analysis).

P-23

GCDUO: GC×GC-MS ANALYSIS WITH OPEN-SOURCE SOFTWARE

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Abstract

Comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) has emerged as a powerful tool for the analysis of metabolites in complex matrices. The adoption of GC×GC-MS has been hindered by the inherent challenges associated with data analysis. In conjunction with new commercial systems that amplify resolution capabilities, the volume of data generated has grow and their manipulation has become an issue. Furthermore, the gold-standard tool, ChromaTOF (Leco corporation, USA) can be considered a black-box, making customizations difficult. Other alternatives exist, like Chromspace (SepSolve Analytical Ltd, UK) or Gc Image (Zoex Corp., USA), but none have developed a truly open-source workflow. We introduce GcDUO, a complete open-source tool tailored for GC×GC-MS data analysis. GcDUO leverages a unique approach by utilizing four-dimensional (4D) raw data applied to a parallel factor analysis (PARAFAC) algorithm. GcDUO software is implemented as an R statistics package, with friendly usage. Key features include data preprocessing, peak detection, identification, and visualization tools. The usefulness of GcDUO was tested with two mixtures (C8-C20 alkanes, and 12 reference standard representative of breath metabolites), at different concentrations. In both datasets, GcDUO successfully analyzed and detected the compounds in an untargeted analysis. Performance of GcDuo was evaluated comparing results to the gold-standard analysis tool, ChromaTOF. Notably, the areas exhibited strong correlations, with Spearman's rho coefficients of 0.856 for the alkane's dataset and 0.909 for the breath mixture dataset. GcDUO represents a new valuable asset in metabolomics toolbox, empowering researchers to create more controllable, automatic, transparent, and scalable workflows.

P-24

INVESTIGATING QUALITY TRAITS IN ARTISANAL CHEESE BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND QUANTITATIVE VOLATILOMICS

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Abstract

Assessing food quality is essential for consumer satisfaction and safety, yet traditional analytical methods often fall short in capturing the complex molecular and biochemical interactions within food matrices like cheese. This study utilizes advanced analytical techniques and Artificial Intelligence (AI) tools, such as Computer Vision (CV), to explore these intricate molecular interactions during artisanal cheese ripening. The research focuses on the benefits of comprehensive two-dimensional gas chromatography coupled with mass spectrometry and flame ionization detector (GC×GC-MS/FID), which offers superior resolution and sensitivity compared to conventional one-dimensional GC. Moreover, by image patter recognition algorithms that track and align features over many patterns, CV could be featured providing a prompt evidence of compositional differences among samples classes. Focusing on Valcasotto cheese, a Traditional Food Product, the sampling covered the entire production chain, including milk from two farms and harvest seasons (spring and summer), with curds ripened in controlled environments and Valcasotto caves for 30, 90, and 120 days. Utilizing multiple headspace solid phase microextraction (MHS-SPME), we optimized the capture of a wide range of volatiles and semi-volatiles produced during cheese production. Quantitative volatilomics precisely tracked marker volatiles and impactful odorants, including key-aroma compounds, across samples, identifying markers that define the unique traits of Valcasotto cheese. Significant compounds such as acetoin, phenylethyl alcohol, and sulfur derivatives were identified, highlighting the potential of GC×GC-MS in food quality assessment and flavor analysis.

P-25

BREATH SAMPLING AND PATIENT CONSIDERATIONS FOR CLINICAL IMPLEMENTATION: A COMPARATIVE STUDY

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Abstract

This study presents a comprehensive comparison of three exhaled breath sampling methods: ReCIVA, Tedlar bags, and BioVoc. To rigorously evaluate these methods, we employed the Peppermint Initiative, a standardized protocol designed to induce a temporary alteration in the exhaled breath profile. This approach allowed us to track the washout curves of volatile organic compounds (VOCs) following the ingestion of peppermint oil. The collected samples were analyzed using two-dimensional gas chromatography (GC×GC) coupled with high- and medium-resolution mass spectrometry, ensuring detailed and robust analysis.

In addition to determining the most effective and reliable sampling technique for clinical use, this study also provides an overview of the commonly used methods, highlighting their strengths and weaknesses in a clinical context as the breathomics community moves towards standardizing collection procedures.

This study is currently ongoing. 5 voluntary participants for each sampling method participated to the study. Samples were injected following strict QC protocols to ensure that variability was not instrument-related. Preliminary statistical analysis suggests that variability is induced by the sampling method, potentially linked to adsorption processes, although similar washout curves across all participants support the Peppermint Initiative as a reference for breath sampling assessments.

We also considered patient comfort and gathered feedback from hospital staff involved in sampling processes to ensure that the selected method is both analytically sound and clinically practical. The primary goal of this study is to deliver a detailed evaluation of these methods concerning their potential clinical application.

P-26

NON-DESTRUCTIVE IDENTIFICATION POSSIBILITIES OF PREHISTORIC HAFTING ADHESIVES WITH DHS-GC×GC-TOFMS

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Abstract

Hafting, the process of attaching a handle to a stone tool, leaves indirect traces on stone tools because the handles perish over time. The most important indirect evidence are traces of adhesives on the stone tool, as these can be chemically identified. Nonetheless, the finding of adhesives on stone tools is rare, not only are they subjected to degradation, not all hafted tools were hafted with adhesives. The first evidence of hafting without adhesive is from about 250,000 years ago and from 80,000 years onwards evidence of the use of adhesive is more prevalent.

Chemical analysis of such degraded adhesives is challenging, not only is the sample size tiny each sample is also unique due to the degradation processes. For this reason, most adhesives are investigated with multiple analysis techniques such as optical microscopy and infrared spectroscopy. In exceptionally cases there is enough adhesives left to perform gas chromatography coupled to mass spectrometry (GC-MS), which is the most accurate analysis to date. Due to the destructive nature of GC-MS, new minimal destructive but accurate analysis techniques are required.

In this study we explore the use of headspace analyse for the investigation of prehistoric adhesive. Headspace techniques are known to be clean, non-destructive, and fast. DHS is one of the most sensitive headspaces techniques and coupled with two-dimensional comprehensive gas chromatography-time-of-flight mass spectrometer (DHS-GC×GC-TOFMS) is it able to explore the headspace of severely degraded adhesives. Besides sensitive instrumentation a references database is required in order to identify the prehistoric adhesives.

P-27

APPLICATION OF ALTERATION ANALYSIS COUPLED WITH TWO-DIMENSIONAL CORRELATIONS ANALYSIS TO MULTIDIMENSIONAL GAS CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTRAL DATA

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Abstract

Two-dimensional gas chromatography (GC×GC) and other multidimensional techniques offer enhanced separation and identification of complex mixtures, ideal for the determination of minute chemical changes. However, the vast amount of data generated poses significant analytical challenges. The primary difficulty lies in managing and interpreting the high-dimensional data, typically constituting gigabytes of information. Typically, class-based chemometric data analysis techniques are used to analyze and interpret this chemical data, and rely on the use of distinct classes or groups to determine chemical difference. The use of classes or groups limits typical chemometric analysis techniques from discovering the nuances and relationships of how a chemical system changes across a series of experiments. However, Alteration Analysis (ALA) coupled with two-dimensional correlation analysis (2DCOR), an unsupervised chemometric technique capable of determining statistically significant chemical trends across a set of samples, has only recently been introduced to the field of chromatography. We will expand on our previously presented GC×GC workflow, through the incorporation of high-resolution mass spectrometry (HRMS) data, data pre-processing for ALA, and application of 2DCOR to these higher dimensional data sets. ALA will be shown as a robust tool for both determination of statistically significant chemical trends and a data reduction methodology, before the selective application of 2DCOR to determine relationships between chemical changes of interest.

P-28

SORBENTS FOR FORENSIC OLFACTRONIC

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Abstract

The main role in the identification of a person based on a scent trail currently play specially trained dogs. The currently used sorbent for scent sampling for this olfactory method in Czech Republic is a non-woven fabric with the trade name Aratex®. Due to the present impurities, this sorbent is unsuitable for olfactronic analysis of the human scent. Therefore, sorbents, which would be sufficiently clean (or cleanable) and at the same time it would also sufficiently absorb human scent, were searched for. In this study, Aratex®, was compared with other possible sorbents as teflon rollers, glass beads, rollers and tubes, nanotextiles and non-woven fabrics. All the materials were extracted with ethanol. The extracts were evaporated and the residue was dissolved in hexane and analysed using a GC-MS or GC×GC-MS. The glass tubes, rollers and beats were also analysed directly, using thermal desorption. The sorption ability was tested using the mixture of standard compounds. The ability of these sorbents to desorb the human body scent was also tested and compared. The liquid injection enables the use of sorbents, which decompose at higher temperatures (nanotextiles and non-woven textile materials). Of these materials, the Raucodrape appears to be the most promising. However, the cleanest materials with the lowest amount of impurities were glass and teflon. Both the materials are, unlike textile materials, reusable. On the contrary, nanotextiles were found to be unsuitable for the olfactronic analysis.

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P-29

Comparing GC×GC with GC-VUV in Polyaromatic Hydrocarbon Quantification & Qualification

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Abstract

Abstract:

Recycling is a major component in the transition from a plastics linear economy to a circular economy. Plastic recycling falls into two major categories: mechanical and chemical recycling. It has been observed that mechanical recycling alone is insufficient, necessitating the implementation of complementary chemical recycling techniques such as pyrolysis. This study focuses on the analytical aspects of pyrolysis.

It has been reported that the output from the pyrolysis of waste plastic could be considered as a potential steam-cracking feedstock. However, when compared to fossil-based feedstocks, the plastic pyrolysis oils show significant differences in hydrocarbon composition and impurities. These discrepancies can lead to substantial operational issues in production plants, such as fouling and coking. This observation underscores the necessity for advanced analytical techniques to characterize plastic pyrolysis oils fully.

This research focuses on the qualification and quantification of polyaromatic hydrocarbons in PPOs. Specifically, two analytical techniques will be compared: GC×GC and GC-VUV. GC×GC offers detailed analysis but is challenging to implement outside of an R&D environment, and GC-VUV, when applied with ASTM D8519, offers a 1D high-temperature GC separation with a broader wavelength range to facilitate PAH detection and quantification.

P-30

ELUCIDATION OF THE VOLATILE PROFILE OF WILD GARLIC BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND SOLID-PHASE MICROEXTRACTION

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Abstract

In this work, solid-phase microextraction (SPME) and comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) were employed for the elucidation of the volatile profile of wild garlic. Wild garlic, also known as Bärlauch or ramsons (*Allium ursinum*), is medicinal and dietary plant species with a long tradition of use. It contains a plethora of volatile organic and sulfur-containing compounds that give rise to its characteristic odor and flavor [1]. However, its volatilome has not been investigated in-depth yet. For the characterization of its volatile profile, an SPME method was optimized by evaluating different types of fiber, sample amounts, extraction timespans, and extraction temperatures. For the GC×GC-MS analysis, an RTX-5MS column (30 m × 0.25 mm, 0.25 μm) was used as the first dimension, and a StabilWAX (2 m × 0.15 mm, 0.15 μm) column was used as the second dimension. The proposed method was used for the analysis of eight wild garlic, ten onion, eight garlic, and eight spring onion samples. Overall, more than 100 VOCs were tentatively identified in the samples. Subsequently, multivariate chemometric tools were employed to assess their volatilome, provide a thorough comparison among the samples under study and establish characteristic markers or patterns for each sample type.

Literature:

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P-31

INVESTIGATION OF SPUTUM VOLATILES FOR CLASSIFICATION OF *M. TUBERCULOSIS* INFECTION BY MULTIDIMENSIONAL GAS CHROMATOGRAPHY - HIGH RESOLUTION MASS SPECTROMETRY

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Abstract

Tuberculosis (TB) is the world's deadliest infectious disease, claiming more than 1 million lives each year, largely in low- and middle-income countries. The lack of cheap, rapid diagnostics stands in the way between curbing transmission as well as between patients and treatment in many communities. Volatile analysis represents the potential for a rapid, non-invasive test, with the APOPO organization demonstrating rats can distinguish TB by sniffing human sputum (N = 24,000 patients[1]). To discover the biomarkers associated with this approach, the headspace of 100 sputum samples obtained from the Foundation for Innovative New Diagnostics (FIND) biobank were analyzed with SPME-Arrow – comprehensive two-dimensional gas chromatography to high resolution time of flight mass spectrometry. From the full set of volatile metabolites detected, two machine learning algorithms, random forest and partial least squares – discriminant analysis (PLS-DA), were applied to select a panel of 17 features which were optimal for distinguishing culture-positive sputum from negative. PLS-DA showed the best overall performance, with the cross-validated model yielding an AUC of 0.92 and a sensitivity of 0.9 and a specificity of 0.84. Within HIV+ patients only, the same selected variables yielded an AUC of 0.82 with a sensitivity of 0.77 and a specificity of 0.75. These results suggest that volatile biomarkers which distinguish tuberculosis are present in sputum and might contribute to a breathprint for tuberculosis that we are actively exploring.

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P-32

USING RESPONSE FACTORS TO IMPROVE QUANTIFICATION OF OXYGEN SPECIES IN WOOD PYROLYSIS OILS

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Abstract

Bio-oils produced by the pyrolysis of lignocellulosic biomass have proved to be a promising source of energy. To better assess the potential of these bio-oils to produce chemicals and fuels, advanced characterization methods are required. Two-dimensional gas chromatography (GC×GC) offers extensive detail for analyzing pyrolysis oils and hydrotreated products. Coupled with mass spectrometry (MS), it precisely identifies compounds, and with an FID detector, it allows for detailed quantification. However, bio-oils are rich in oxygenated compounds, and these compounds do not respond in the same way as hydrocarbon compounds, leading to a bias in their quantification. Specific quantification methods are therefore required for these new feedstocks. Current methods typically employ oxygenated and hydrocarbon internal standards for quantification, along with calibration curves to determine the response factor and apply it to the compounds. In this study, sixteen oxygenated standards with different functional groups, and one hydrocarbon standard, were used to establish response factors by chemical family. We analyzed these standards individually, as a mixture, and within a matrix. Response factors vary based on the analysis type and the matrix.

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INTERACTIVE ION PEAK ANALYSIS AND DIFFERENCING FOR COMPARING MULTIDIMENSIONAL CHROMATOGRAPHY DATA

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Abstract

Comprehensive two-dimensional chromatography offers superior separation capabilities for complex mixtures, but the resulting data complexity necessitates advanced comparative analysis methods. A common scenario involves comparing two samples to identify similarities and differences. Our comparative visualization methods are built upon conventional image comparison techniques. By aligning two chromatograms and generating a difference chromatogram with specialized color maps, these methods facilitate visual identification of discrepancies. In addition, to achieve a more comprehensive characterization of chemical variations, an interactive side-by-side differencing tool is developed to enable the matching of peaks across two chromatograms using chromatographic retention times, leading to both qualitative and quantitative analysis of sample differences at the individual peak level.

When coupled with mass spectrometry, we extend these methods further by leveraging spectral information for peak detection and matching across chromatograms. Peak detection is performed in individual ion chromatograms, followed by combining peaks corresponding to the same analyte across multiple ions. Peak matching is performed using two search techniques:

- A targeted peak detection is performed on one chromatogram using ion peaks detected in the other chromatogram.
- A bidirectional peak matching after ion peak detection is performed on both chromatograms, where the ion and the RT location are used as match criteria.

The method includes interactive analysis with side-by-side chromatogram views. The ion peak matching results are demonstrated for finding common and unique compounds from pairs of 2D chromatograms, and performing new peak detection (NPD) with LC-MS.

P-34

GC×GC-TOF-MS PROFILING OF ALLERGENS IN ESSENTIAL OILS

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Abstract

Essential oils (EOs) are a cornerstone of fragrance formulations in the cosmetics industry. While they are renowned for their therapeutic benefits, EOs often contain high concentrations of fragrance allergens, which can pose risks to sensitive individuals. As a result, careful monitoring and regulation are essential. The International Fragrance Association (IFRA) has established guidelines for fragrance allergens, recently expanding its list to include an additional 54 volatile allergens, bringing the total to 80. This update highlights the need for reliable analytical methods to detect both the old 24 allergens and the 56 newly introduced ones. In this study, we developed a comprehensive two-dimensional Gas Chromatography coupled with Time-of-Flight Mass Spectrometry (GC×GC-ToF-MS) method to profile these regulated compounds across a range of widely used EOs. By adding a second dimension of chromatographic separation, GC×GC enhances resolution and sensitivity compared to conventional one-dimensional gas chromatography (1D-GC), making it an ideal method for analyzing complex chemical mixtures, particularly fragrance allergens. Using GC×GC-ToF-MS, we can detect and quantify both old and newly regulated allergens, ensuring compliance with evolving regulations. Our findings provide a robust, high-throughput method for allergen profiling in essential oils, promoting safer product formulations and enhancing consumer safety in the rapidly growing fragrance market.

P-35

CONTRIBUTION OF LOW-ENERGY IONIZATION SOURCE FOR BIO-OILS' MOLECULAR CHARACTERIZATION BY GC×GC HIGH RESOLUTION MS

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Abstract

Bio-oils produced by the pyrolysis of lignocellulosic biomass have proved to be a promising source of renewable energy. These bio-oils are highly complex organic mixtures containing thousands of compounds covering a wide range of mass and polarity. However, the liquid obtained after pyrolysis is rich in oxygen, leading to problems of instability and corrosion.

To adapt the bio-oil processes, it is important to identify the oxygenated molecules present in these products. The volatile fraction has been analyzed using comprehensive two-dimensional gas chromatography (GC×GC) coupled to high resolution mass spectrometer (HRMS). For this purpose, the GC×GC has been applied successfully due to its significant gain in separation power, sensitivity, and selectivity compared to mono-dimensional GC techniques. So far, the coupling of the GC×GC with mass spectrometry (MS) using conventional electron ionization (EI) at + 70 eV is the main approach reported in the literature for obtaining structural information on the molecules detected.

The use of a low-energy source, called "Soft-EI" (10 or 12 eV), helps the identification thanks to the molecular ion conservation. Moreover, the use of high-resolution time-of-flight mass spectrometer detector allows to obtain precise raw formulas of the molecular and fragment ions detected. Indeed, HRMS enables us to differentiate between two ions with very close masses.

Using the two 70 eV and 10 eV ionization energies we can accurately obtain and identify many of the molecules present in bio-oils, such as tert-butylpyrogallol $C_{10}H_{14}O$ and desaspidinol $C_{11}H_{14}O_3$.

P-36

STEROLS ANALYSIS IN OLIVE OIL BY MICROWAVE-ASSISTED SAPONIFICATION AND EXTRACTION FOLLOWED BY FLOW MODULATION COMPREHENSIVE TWO DIMENSIONAL CHROMATOGRAPHY

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Abstract

This project focused on the unsaponifiable component of olive oil, particularly the sterols fraction.

The official method for sterols involves a saponification process followed by a liquid-liquid extraction and further purification through thin-layer chromatography (TLC), while the instrumental analysis was conducted using ^{1}D GC-FID. This methodology requires extensive processing time and significant solvent consumption. For this reason, in this project, efforts were made to improve the official method. In particular, the saponification and extraction steps were merged into one single step assisted by microwaves (MASE) to achieve lower processing time, and the TLC purification was replaced by a faster solid-phase extraction (SPE), which also allowed for the minimization of solvent volumes. In the end, for the chromatographic analysis, a flow modulation two-dimensional gas chromatographic system coupled with an FID detector (GC×GC-FID) was used to enhance the separation of sterols from possible interferences and guarantee an accurate quantification.

Acknowledgment

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THE EVALUATION OF GC×GC-QTOF DATA BY PIXEL-BASED ANALYSIS: A TUTORIAL

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Abstract

Comprehensive two-dimensional gas chromatography coupled with high-resolution mass spectrometry (GC×GC-HRMS) provides high resolution and sensitivity, making it a powerful tool for non-target screening of complex environmental samples. However, GC×GC-HRMS produces vast amount of data with signal intensities that spans several orders of magnitudes. Emphasizing the most reliable parts of data and extracting chemical information from such data remains a challenge.

To address these challenges, different approaches are employed, including the extraction of retention time (t_r) and mass-to-charge (m/z) pairs, comparisons of small chromatographic regions (tiles), and direct analysis of individual data points (pixels). Here, we present a pixel-based workflow that highlights the importance of key pre-processing steps and data prioritization, applied to 42 wastewater effluent samples obtained from two wastewater treatment plants in Denmark.

Our workflow incorporates distinct pre-processing steps such as retention time alignment, baseline removal, blank subtraction or removal, sample normalization, and scaling pixels to their analytical uncertainty. The impact of these steps was evaluated using principal component analysis and their influence on data prioritization is critically assessed.

We also demonstrate the effective use of laboratory procedure blank samples for pixel prioritization, facilitator samples representative of the dataset for retention time alignment and drift correction, and independent quality control samples to ensure reliable parameterization of pre-processing steps.

P-38

OIL EXTRACTION ASSISTED BY MICROWAVE AND FATTY ACID CHARACTERIZATION OF RAW PISTACHIO BY MONO- AND MULTI-DIMENSIONAL GAS CHROMATOGRAPHY

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Abstract

The aim of this research was to develop a fast and more environmentally friendly lipid extraction method assisted by microwave using solvent based on methyl-tert-butyl ether (MTBE), as one-solvent extraction or in a mixture with methanol and water.

MAE-MTBE-based methods were compared in terms of extraction yields and fatty acid (FA) composition of pistachio oil to Soxhlet and Matyash methods [1], gold-standard methodologies normally used for lipid extraction. FAs, derivatized into methyl esters (FAMEs), were analyzed through a mono-dimensional gas chromatography coupled with flame ionization detector - (GC-FID) and comprehensive two-dimensional GC (GC×GC).

GC-FID was generally effective in quantifying the FAMEs composition of pistachio oil, while multi-dimensional comprehensive gas chromatography coupled with mass spectrometry (GC×GC-TOFMS) allowed a more exhaustive identification of FAMEs also resolving critical FAMEs pairs under non-polar stationary phase separation (e.g., Me.C18:1n9t *vs* Me.C18:3n3c)

Combining MAE and MTBE as single solvent extraction provided extraction yields and FAMEs profiles comparable to the established extraction methodologies. When evaluated based on the AGREEprep metrics [2], the MAE-MTBE method resulted significantly greener than the reference ones, highlighting its environmental advantages over conventional methods.

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P-39

ANALYSIS OF (MICRO-)PLASTIC-ASSOCIATED CHEMICALS RELEASED INTO MARINE ENVIRONMENTS BY COMPREHENSIVE MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract

Plastics and microplastics are pervasive in marine environments, releasing chemicals that pose risks to marine life (Cole *et al.*, 2011; Thompson *et al.*, 2004). These chemicals include functional additives (e.g., plasticisers, flame retardants, antioxidants, UV stabilisers, and pigments) and trace levels of non-intentionally added substances accumulated during the plastic lifecycle (Rung *et al.*, 2023). Trace concentrations, coupled with the chemical complexity of these mixtures, present significant challenges for chemical analysis, particularly when also introduced to environmental matrices. This complexity hinders attempts to identify priority chemicals of environmental concern. A crucial step to address this knowledge gap, is to identify and quantify compounds released from microplastics under environmentally relevant conditions.

This study investigated the leaching and extraction profiles of virgin polymers – low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET) and polyvinyl chloride (PVC)) and bio-polymers (polylactic acid (PLA), polybutylene succinate (PBS), poly(butylene adipate-co-terephthalate) (PBAT), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) – analysed using comprehensive multidimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-ToF-MS). Extracts were prepared via ultrasonicated assisted extractions in organic solvents. Leachates were prepared in seawater and the simulated gut fluid of the marine lugworm, where leaching time, temperature, agitation, and UV exposure were controlled to simulate marine conditions. Analysis of both extracts and leachates provides a clear understanding of the movement of plastic-associated chemicals within marine environments. The enhanced separation capacity of GC×GC provides greater insight into the chemical profile of plastics and their leachates, which aids in the correct identification of priority microplastic pollutants which pose risks to the environment.

P-40

SKINVOCS®: AN INNOVATIVE SAMPLING SYSTEM FOR BODY ODOR PRIOR TO TD-GC×GC-TOFMS ANALYSIS

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Abstract

The cutaneous body volatilome (CBV), also called body odor, is a complex matrix composed of hundreds of volatile organic compounds (VOCs). Analyzing CBV may allow the identification of biomarkers specific to certain diseases, enabling non-invasive and early disease diagnosis. The complexity of these samples requires advanced techniques for accurate separation and identification of molecules that make up the CBV. In this context, a highly sensitive and resolutive analytical method, i.e. thermodesorption followed by comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (TD-GC×GC-TOFMS), was employed.

The most critical step in the process is the sampling, for which no standard method exists in literature. Aiming to standardize, this poster outlines the development and performances of the SkinVOCs® sampling system, designed to be applicable on various skin areas, easy to use, and user-friendly. To ensure high analytical sensitivity, requirements for the blanks were higher, i.e., low VOC emissions from the sampling system. The system includes a dressing, a hermetically sealed metal chamber for safe transport, and a holder with uncontaminated adsorbents for effective VOC collection. Developed through extensive trials and validations, the system's blanks exhibit a markedly different profile from real samples, with a much cleaner chromatogram compared to real samples. While blanks presented limited number of peaks, real CBV samples showed significantly richer profiles, reflecting the system's analytical cleanliness. A detection limit of 0.05 ng was achieved when combined with TD-GC×GC/TOFMS. The system has been tested by over 20 untrained participants without handling issues, confirming its usability and reliability.

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DATA ANALYSIS SOFTWARE FOR COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY (GC×GC): TOOLS TO FACILITATE NON-TARGET DATA COMPARISONS FOR SAMPLE SETS

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Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) with Time-of-Flight Mass Spectrometry (TOFMS) is a powerful analytical tool for evaluating complex samples. GC×GC provides enhanced peak capacity and sensitivity compared to one-dimensional GC, which often allows more individual analytes to be separated and detected, revealing additional information about a sample. When coupled with TOFMS, the acquired full m/z range spectral data often leads to the identification of these separated analytes. These capabilities make GC×GC-TOFMS an attractive choice for the analysis of a wide range of complex sample types for a variety of analysis objectives. In many cases, the analysis goals involve the non-targeted characterization or comparisons of multiple samples together. This can be challenging, but advanced data processing tools and software, like ChromaTOF Tile and ChromaTOF Sync 2D, can help to streamline these workflows and tasks. ChromaTOF Tile provides multiple options for raw data comparison to quickly find differences in GC×GC sample sets, and ChromaTOF Sync 2D provides full peak finding and alignment for sample sets. These complementary software tools can improve non-targeted characterizations of sample sets. Here, we highlight several application examples with different analysis objectives to demonstrate these software tools and their versatility for comparing and characterizing GC×GC sample sets.

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DEVELOPMENT OF AN *IN VITRO* ANALYTICAL WORKFLOW TO STUDY THE INFLAMMATORY MECHANISMS OF ASTHMA

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Abstract

Asthma is a chronic respiratory disease characterized by inflammation and oxidative stress in the airways. This oxidative stress plays a key role in the progression of the disease, but the molecular mechanisms involved remain poorly understood. The development of an in vitro analytical workflow is essential to explore these mechanisms and identify potential biomarkers.

In this study, we used A549 cell line, a model of human pulmonary epithelial cells, to investigate the effects of oxidative stress in the context of asthma. The cells were cultured in different media (DMEM, PBS, EMEM) and exposed to hydrogen peroxide (H_2O_2) to induce oxidative stress. This treatment resulted in the production of volatile organic compounds (VOCs) by the cells, which were then collected and analyzed by two-dimensional gas chromatography (GC×GC) coupled with solid-phase microextraction (SPME). This method enabled to identify and quantify a wide range of markers with great sensitivity and high chromatographic resolution, providing valuable information on the biochemical changes induced by oxidative stress.

This workflow, combining cell biology and analytical chemistry, represents an innovative platform for identifying asthma-specific biomarkers and advancing the understanding of the interactions between oxidative stress and inflammation. However, several challenges remain in order to perfectly mimic complex biological processes in vitro.