### Cover Story

**High-Throughput Peptide Mapping of Monoclonal Antibodies Using Tandem Liquid Chromatography–Mass Spectrometry**  
*Sara Carillo, Silvia Millan Martin, and Jonathan Bones, NIBRT*

The benefits of tandem LC–MS are revealed.

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While women may be entering more degrees and careers in science than ever before, there is still a need to advocate for gender parity in many sectors. |
High-Throughput Peptide Mapping of Monoclonal Antibodies Using Tandem Liquid Chromatography–Mass Spectrometry

Sara Carillo, Silvia Millan Martin, and Jonathan Bones, NIBRT – The National Institute for Bioprocessing Research and Training, Dublin, Ireland

Analytical methods for quality control (QC) of monoclonal antibodies (mAbs) rely on peptide mapping workflows with liquid chromatography–mass spectrometry (LC–MS) systems. Whilst LC–MS is effective, peptide mapping with LC–MS can involve long analysis times that reduce the throughput of QC testing. Modern tandem LC methods can increase analysis efficiency and are designed to reduce the downtime of MS sampling to increase the efficiency of QC pipelines. The approach presented here improves the efficiency of mAb analysis peptide mapping, whilst returning high-quality data.

The development and production of therapeutics, such as monoclonal antibodies (mAbs), require high-throughput sample analysis and high-quality data. For example, post-translational modifications (PTMs) can have a major impact on the quality and
efficacy of the final product and, therefore, need to be monitored. Orthogonal analyses of protein PTMs are required to ensure certain critical quality attributes (CQAs) are within the established ranges to maintain lot-to-lot consistency in line with regulatory requirements and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (1).

Peptide mapping is considered a gold standard for mAb analysis because it delivers information on many attributes at the peptide level within a single analysis, providing a strong method for an analytical control strategy. One type of peptide mapping approach using liquid chromatography–mass spectrometry (LC–MS) analysis is the multi-attribute method (MAM), which allows high-quality, confident PTM identification in research and at later stages in quality control (QC) laboratories (2–4). However, peptide mapping with LC–MS methods can involve long analysis times due to the lengthy LC gradient needed to resolve peptides in the complex sample resulting from tryptic digestion. This creates challenges for high-throughput laboratories where efficiency is key. Moreover, long downtimes are also created between analyses to allow for column washing and re-equilibration steps, and when eluents are diverted to waste. During these periods, no separation is taking place. This reduces the time available to perform MS testing and reduces efficiency in research and QC pipelines.

Innovations in ultrahigh-performance liquid chromatography (UHPLC) instrumentation have led to the development of a tandem LC system that can be leveraged for high-throughput “tandem-mode” LC–MS analysis. This article demonstrates the applicability of a tandem LC–MS workflow for peptide mapping analysis of trastuzumab in a high-throughput manner. With a tandem LC–MS approach, a greater number of sample injections were performed in the same timeframe, increasing analytical throughput and significantly reducing MS downtime. Chromatographic results were found to be equivalent across the two chromatographic channels. Additionally, the reproducibility of the obtained PTM values during a continuous 16-h period of operation were tested, demonstrating the stability of MS performance under continuous acquisition.

Materials and Methods

Sample Preparation: Samples were prepared following a published method (5) to obtain peptides from tryptic digestion of trastuzumab. Trastuzumab samples were diluted to 2 mg/mL in water. For each sample digest, sample, digestion buffer (buffer 1, pH 6.5 or buffer 2, pH 7.2), and 5 mM TCEP (final concentration) were added to each lane of a Thermo Scientific KingFisher deep well 96-well plate. Trypsin
The Column www.chromatographyonline.com

bead “wash buffer” was prepared by diluting digestion buffer 1:4 (v/v) in water. Bead buffer was neat digestion buffer. Digestion was performed using a Thermo Scientific KingFisher Duo Prime Purification System with Thermo Scientific BindIt software (version 4.0).

Samples were incubated for 5 to 40 min at 70 °C on medium mixing speed to prevent sedimentation of beads for the digestion time course study, and beads were removed at each time point. Following digestion, 100-μL samples were transferred to 300-μL Thermo Scientific PP Screw vials and cap and 1 μL of 10% trifluoroacetic acid (TFA) was added (final concentration 0.1% TFA) and immediately analyzed by high-resolution accurate mass LC–MS.

**Chromatography Step:** The resulted peptides were separated on a 2.1 × 250 mm, 2.2-μm Acclaim Vanquish C18 column (Thermo Scientific) using the Thermo Scientific Vanquish Horizon Duo UHPLC System for tandem LC–MS workflows. Analysis was performed using a binary gradient of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B) with a flow rate of 0.3 mL/min. The gradient started at 2% B and increased to 40% B over 45 min on the analytical pump, while the reconditioning pump performed two cycles from 2% to 80% B in 20 min and equilibrated back to 2% B for 25 min.

Mass spectrometry data acquisition was performed with the Thermo Scientific Orbitrap Exploris 240 MS system with biopharma option at resolution 120,000 FWHM. Application-specific MS tune and acquisition settings are templated and provided within the Thermo Scientific Chromeleon Chromatography Data System (CDS) software, which makes this approach directly and easily transferable from instrument to instrument.

**Tune Settings:** Spray voltage: 3.8 kV; sheath gas: 25; auxiliary gas: 10; ion transfer tube temperature: 320 °C; vaporizer temperature: 150 °C; pressure mode: peptide.

**MS Scan Settings:** Resolution: 120,000 (at m/z 200); scan range: 200–2000 m/z; RF lens (%): 60; AGC target: 3 × 10^6; max injection time: 100 ms.

**MS/MS Settings:** Resolution: 15,000; normalized collision energy: 30; injection time: 200 ms; AGC target: 1 × 10^6; TopN: 5; microscan: 1; isolation window: 2 m/z.

Data were processed using the Thermo Scientific BioPharma Finder Software 4.0.

**Results and Discussion**

Peptide mapping is a routine analysis in biopharmaceutical assessment and is gaining more interest as part of the MAM workflow. It is driving the wider adoption of MS methods for later stages of biopharmaceutical product development and, ultimately, allowing its use in the QC laboratory. Method transfer onto the QC stage requires improved robustness and high-throughput due to the high number of samples that must be processed and the required reliability of the methods assuring product quality.
In this study, trastuzumab monoclonal antibody was digested using a trypsin protein digestion kit and the generation of tryptic peptides was performed automatically on the purification system. The digested monoclonal antibody was then analyzed via tandem LC–MS/MS analysis. CDS software automatically split the gradient across the two columns and pumps, managing when the analytical gradient was complete and the wash step started. Twenty-one sample injections were performed in this study, alternating between the two columns to obtain a perfect overlap of runs coming from the two channels (Figure 1). This qualitative evaluation demonstrates the reproducibility of LC performance not only across the two channels but also when using two different columns. Chromatographic reproducibility was evaluated across the gradient by plotting the RSD for individual charge states for VEIKR, FNWYDGEVHNAK, TVAAPSVFIPPSDEQLKSGTASVVCLLNNFYPR, ALPAPIEK, and VVSVLTVLHQDWLNGK tryptic peptides across 21 injections performed in tandem mode on the UHPLC system. By evaluating the shift of retention times across the length of the gradient, it was possible to assess the reproducibility of the tandem workflow, which matched the performance of an experiment performed on a single column, while reducing analysis time.

![Figure 2](image_url)
However, it would be possible to allow higher values of %RSD for the retention times when using high-resolution MS, as this level of resolution gives a confident identification of retention times for five different peptides along with the 21 injections. No difference was observed between the two data sets, returning overall relative standard deviation (RSD) values lower than 1.5% (Figure 2). Excellent retention time reproducibility is important for correct identification of components to evaluate and quantify drug product CQAs using MS. However, it would be possible to allow higher values of %RSD for the retention times when using high-resolution MS, as this level of resolution gives a confident identification of correct...
components even when using a wider retention time window.

Peptide mapping analysis is mainly performed to evaluate PTMs in therapeutic proteins that need to be monitored during bioprocessing and batch release to guarantee drug product quality. For this reason, we evaluated product quality attributes for trastuzumab across the 21 injections performed in tandem mode (Table 1). Each modification was calculated considering up to one missed cleavage, and without considering Na+ and K+ adducts or non-specific cleavages, using a confidence score ≥ 95% and within ± 5 ppm. Detection of most PTMs resulted in very low RSD values, which exceeded 15% only for some low abundant modifications (N328 deamidation = 0.20%). This proved excellent chromatographic equivalence of the two channels for PTM evaluation, as well as the suitability of the tandem LC–MS workflow for PTMs assessment and quantitation.

Conclusion
In this study, we have demonstrated the suitability of a tandem LC system for tandem LC–MS analysis in peptide mapping and the MAM workflow. This tandem LC approach significantly increased analytical throughput by reducing the time when MS was not fully utilized for valuable data generation; this was achieved by minimizing the time window where LC was diverted to waste and the MS not utilized. This workflow proved to be a more time-efficient alternative to traditional methods, while also maintaining or improving the performance and reproducibility required for a method to be considered for adoption in a QC laboratory.

Twenty-one samples of trastuzumab tryptic digest were analyzed using LC–MS/MS analysis in tandem mode. System performance was evaluated by monitoring retention time reproducibility and identifying PTMs, with both evaluations yielding excellent data and low %RSD values. Tandem analysis of peptide mapping replicates did not affect the accuracy of the results and the correct identification, but it had the benefit of saving at least 6 h of instrument time when compared to single mode during 24 h. The use of automated digest kits versus in-solution digestion has been shown to save between 2–20 h preparation time for each sample (6), providing significant sample preparation time savings, which, when combined with the efficiency benefits of the tandem analysis, can dramatically increase throughput.

In conclusion, tandem LC–MS represents a powerful way to increase laboratory productivity without compromising data quality, especially when combined with automated sample preparation of the tryptic digest. The operational simplicity of tandem LC–MS methods means that little training is required for high-throughput peptide mapping of biopharmaceuticals, enabling straightforward compliance-ready enterprise acquisition of LC–MS data for current good manufacturing practice (cGMP) environments.

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Jonathan Bones obtained his B.Sc. in analytical science (chemistry) and Ph.D.
Sara Carillo completed her Ph.D. in chemical sciences in 2013 at the University of Naples “Federico II”. Under the guidance of Professor Corsaro, she focused on the structural characterization of polysaccharides and glyco-conjugates from Gram-negative bacteria via nuclear magnetic resonance (NMR) and mass spectrometry techniques, focusing on the immunological properties and potentials of extremophiles endotoxins. After a period at the University College of Dublin, she joined Jonathan Bones’s research group in NIBRT in 2015, working on the understanding of the effects of extractables and leachables from single-use bioreactors on CHO cells N-glycome and produced monoclonal antibodies. She is now working at NIBRT as Applications Development Team Leader for the development of new analytical approaches in biopharma.

Silvia Millán Martin is Applications Scientist in the Characterization and Comparability Laboratory in NIBRT, Ireland. She obtained her degree in pharmacy and Ph.D. in analytical chemistry from the University of the Basque country in North Spain, where her work focused on the development of LC–MS/MS- and GC–MS-based analytical techniques for the identification and quantitation of environmental, pharmaceutical, and nutritional significant compounds. She also worked for four years in a small contract research organization in the analytical services unit, and focused on the determination of bioactive compounds in natural sources and the application of metabolomics/proteomics approaches with the aim of evaluating their health benefits to potentially be used as functional ingredients in the food industry. In September 2012, she joined NIBRT, becoming part of the GlycoScience Group as a postdoctoral researcher in the mass spec lab and working on academic and contract research projects with biopharma companies. Her work focused on full detailed glycan analysis by MS and MS/MS and other orthogonal techniques, and on the development of advanced LC–MS-based platforms for quantitative glycomics, with applications in bioprocessing and clinical biomarker discovery. In September 2016, she joined the Characterization and Comparability Laboratory, which is led by Jonathan Bones and collaborates with Thermo Fisher Scientific, to focus on the development of new applications for the characterization of biopharmaceuticals, and the associated processes used for their production.

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Thermo Fisher Scientific Continues Expansion

Thermo Fisher Scientific (Massachusetts, USA) celebrated the opening of its new single-use technology manufacturing site in Ogden, Utah, USA, and announced a $97 million expansion to its bioanalytical laboratory operations across three new locations in Virginia’s Greater Richmond region. The state-of-the-art facility in Utah creates additional capacity for the development of new vaccines and therapies.

The Ogden site is a 55,000-square-ft facility that will manufacture customizable bioprocess container (BPC) systems. These BPCs are used for the delivery, processing, separation, storage, and transportation of critical liquids used in the production of biologics, vaccines, and cell and gene therapies. The site has hired more than 300 employees and has the potential to employ up to 450 people, adding to the 2000 strong workforce the company has across the state of Utah and establishing a critical hub for bioprocessing.

“The Ogden facility further strengthens our global manufacturing network, which has been growing to meet increasing demand for single-use consumables and critical raw materials,” said Mitch Kennedy, President, Single-Use Technologies, Thermo Fisher Scientific. “This new facility expands our presence in Utah and locating here gives us the ability to work closely with our Logan site, providing access to a talented workforce that is ideal for manufacturing.”

In Virginia, the existing laboratory services operation there comprises more than 300,000-square-ft, making it one of the largest laboratories of its kind in the world; it employs more than 1200 professionals. Thermo Fisher acquired the laboratories with the purchase of PPD, Inc., in December 2021. The expansion is part of Thermo Fisher’s $650 million multi-year investment, announced last year, to help ensure flexible, scalable, and reliable bioprocessing production capacity exists for critical materials used in developing new and existing biologics and vaccines, including for COVID-19. Longer term, the company plans to continue to invest in and connect the capabilities across the combined company. The bulk of the expansion will occur in immunochemistry and chromatography functions, as well as subsequent enhancements in biomarker and vaccine sciences. Two new bioanalytical laboratories will be established in Virginia’s Henrico County, and a third will be established at the VA Bio+Tech Park in the city of Richmond.

"Worldwide, we continue to expand our lab capacity to provide customers with high-quality bioanalytical lab services to advance their clinical research programmes," said David M. Johnston, Ph.D., Senior VP and President, Clinical Research, Thermo Fisher Scientific. “These services are vital for drug development, as approximately 70% of data generated in clinical trials comes from analytical laboratory testing. We’re pleased to extend our partnership with Virginia, Henrico County, and the city of Richmond to continue our efforts to help customers deliver life-changing therapies to patients in need.”

For more information, please visit: https://corporate.thermofisher.com
Researchers from Japan have used liquid chromatography tandem mass spectrometry (LC–MS/MS) to investigate the ciguatoxins (CTXs) responsible for ciguatera fish poisoning (CFP), commonly known as *ciguatera* (1).

Common within the tropics and subtropical Indo-Pacific region as well as the Caribbean Sea, CFP affects people when they have ingested seafood infected with CTXs, which are produced by the algae dinoflagellates. These algae are found in shallow coastal waters on the surface of seaweed and other marine plants such as corals and are subsequently consumed by herbivorous fish. Carnivorous fish then go on to consume these smaller species, and this continues up the food chain with the toxins becoming more and more concentrated in a process known as *biomagnification*.

Once the toxins have been consumed a wide range of symptoms are possible, with acute symptoms appearing within 48 h and affecting almost all organ systems. These include fatigue, generalized pain, nausea, vomiting, diarrhoea, low blood pressure, and heart rhythm disorder. Unfortunately, most of these symptoms are quite general and could hint towards other food poisonings, which is one of the main issues when attempting to estimate the global burden of the disease, as there is widespread under-reporting and misdiagnosis. However, in regions where the poison-producing dinoflagellates grow, such as the Pacific Islands, estimates are that up to 10% of the local population is affected each year. Furthermore, at least 20% of affected persons can continue to have symptoms for months or even years after the initial poisoning, with neurological, neuropsychiatric, and memory disturbances. Recurrence can be triggered by certain foods, by behavioural situations, such as fatigue, or even external factors, such as sun exposure (2).

While CFP is endemic to the regions where the dinoflagellates grow, it is also increasingly a global problem because of the international seafood trade, with the European Rapid Alert System for Food and Feed issuing several alerts for ciguatoxins in the past few years, all stemming from chilled and frozen fish imported from other continents.

The causative toxins of ciguatera pose several analytical challenges. They are ladder-shaped cyclic polyethers, classified into four groups based on their skeletal structures and the place of occurrence (1). However, they are oxidized in fish while moving up the food chain, thereby further diversifying their structure (3,4,5). Furthermore, their lipophilic features and vast diversity in polarity further increases their analytical challenge.

In this study, researchers focused on specimens collected from the Ryukyu Islands, an area of around 1000 km extending from a region near Taiwan to Kyushu Island, Japan. They targeted the grouper fish, *Variola louti*, as it is frequently implicated in CFP cases in this region. Using LC–MS/MS they analyzed the flesh of 154 individuals across various locations, detecting CTX in 99 (64%) specimens. In 65 specimens (43%) the toxin levels exceeded the FDA guidance level of 0.01 µg/kg. Researchers also confirmed the results of a study performed around a decade ago that indicated three analogues as the primary toxins in this fish species, namely CTX1B, 52-epi-54-deoxyCTX1B, and 54-deoxyCTX1B, indicating a consistent toxin profile in this species. Interestingly, the study found that skinnier fish contained more toxins than fattier fish, confirming fishermen folklore in the Ryukyu Islands that claims exactly that.

In summary, the researchers believed that the current FDA recommendations were too strict based on their data relating to toxin levels and the number of CFP cases. However, they acknowledged that further investigation and more data accumulation related to human CFP cases and fish toxicities are both needed.

**References**

Analyzing Road-Associated Microplastics Using Py-GC–MS

News of microplastic particles in the environment has become more and more common, however, there is still a significant deficit of data on one of the largest sources of these particles, as well as a lack of harmonization on how to analyze, quantify, and report study findings. Tyre and road wear particles could well constitute the largest source of microplastic particles into the environment, but without adequate analytical methodologies there will continue to be huge uncertainties surrounding their impact. As such, researchers have presented a new methodology utilizing pyrolysis gas chromatography–mass spectrometry (Py-GC–MS), which aims to improve the analysis of tyre and road wear particles (1).

Currently, visual analysis coupled to a chemical analysis step, such as Fourier-transform infrared spectrometry (FT-IR), is the most commonly applied method for microplastics. Unfortunately, the black pigment (carbon black) within tyre particles prevents the use of FT-IR, as the infrared light is absorbed, thereby preventing the identification of the rubber content (2).

Thermal methods such as Py-GC–MS or thermal extraction desorption (TED-) GC–MS are instead being favoured. These methods use the products of thermal decomposition as markers to identify and quantify polymers and rubbers that can be potentially used to identify specific tyre and asphalt markers, as well as assess the amount of rubber released into the environment.

The composition of the tyre and road asphalt adds a further layer of complexity to this analytical challenge, with a wide range of ingredients, production variability, and other factors combining to present a conundrum to analysts. Which compounds represent the best markers for analysis? Researchers chose to analyze styrene-butadiene rubber (SBR), butadiene rubber (BR), and styrene-butadiene styrene (SBS), with SBR and SBS having never been explored as potential markers using pyrolysis. The study aimed to quantify the total mass of these products in environmental samples, combining multiple pyrolysis products for the quantification to compensate for the individual differences between tyre manufacturers. Furthermore, the method also explored the ability to calculate the total mass of tyre and polymer-modified bitumen (PMB)—the latter being used in asphalt—using different calculation approaches based upon available traffic data, sample locations, and the sample time.

The results indicated that the method provided high recoveries of 83–92% for a tyre matrix and from 88% to 104% for road sediment. When the validated method was applied to urban snow, roadside soil, and gully-pot sediment samples, the concentrations of tyre particles in these samples ranged from 0.1 mg/mL to 17.7 mg/mL in snow and from 0.6 mg/g to 68.3 mg/g in soil/sediment. The concentration of PMB ranged from 0.03 mg/mL to 0.42 mg/mL in snow and from 1.3 mg/g to 18.1 mg/g in soil/sediment.

By combining large datasets of tyres with a prediction simulation, researchers believe that this method is an improvement on current methodologies. Predicting the possible tyre values based on the variation in rubber content offers a mean value and a standard deviation of that value. This in turn decreases the uncertainty found in methodologies that use fixed rubber concentrations to calculate the mass of tyres in the environmental samples. The team highlight the importance of clearly communicating the difficulties in analyzing tyre matrices but also how the use of locally adapted values could improve data and provide relevant information to environmental researchers.

References
Peaks of the Month

- **The LCGC Blog: Not Another Helium Crisis**—As chromatographers, our thoughts turn to the use of hydrogen as an alternative carrier gas, to ensure continuity of supply and reduce long-term costs. [Read Here]

- **Supercritical Fluid Chromatography for Chiral Analysis, Part 1: Theoretical Background**—In the first part of this review article, the theoretical advantages, technological developments, and common practices in chiral SFC are discussed. This is followed by a contribution discussing recent applications in pharmaceutical, clinical, forensic, and environmental analysis. [Read Here]

- **Modern Nontargeted Screening (NTS) Methods for Environmental Analysis**—*LCGC Europe* spoke to Selina Tisler and Jan H. Christensen from the University of Copenhagen in Denmark, about recent research projects focusing on nontargeted screening (NTS) approaches for important environmental monitoring applications, including the analysis of chemical leaching from plastic bottles using liquid chromatography tandem mass spectrometry (LC–MS/MS) and sediment analysis using comprehensive two-dimensional gas chromatography–high-resolution MS (GC×GC–HRMS). [Read Here]

- **How Tube-Based Thermal Desorption Can be Used to Monitor Chemical Warfare Agents at Ultratrace Levels**—This study demonstrates the analysis of live chemical warfare agents and simulants at trace and higher levels. [Read Here]

- **The Sisters in Science: Separating Stereotypes**—The Column interviewed Lotte Schreuders, Noor Abdulhussain, and Mimi Den Uijl, the “Sisters in Science”, about challenging common stereotypes associated with studying chemistry, and their mission to put women front and centre in science. [Read Here]

- **Identifying Novel Ebola Therapeutics**—Researchers searched for inhibitors of the Ebola virus from over 500 medicinal plant extracts, utilizing SEC and HPLC alongside cell-based assays with replication-incompetent pseudotyped viral particles to identify antiviral lead compounds. [Read Here]

More News

**Agilent Joins AMBIC to Advance Biomanufacturing**

Agilent Technologies Inc. (California, USA) has announced that it has become a member of the Advanced Mammalian Biomanufacturing Innovation Center (AMBIC), to demonstrate the company’s commitment to partnering to support the provision of the next generation of analytical tools and comprehensive solutions needed by academic, pharmaceutical, and clinical researchers.

“Our customers recognize the need to improve biotherapeutic manufacturing,” said Darlene Solomon, Senior VP and CTO at Agilent. “Working with AMBIC we look forward to advancing technologies.” There is a growing need to rapidly assess critical process and product quality attributes to support biotherapeutics manufacturing and facilitate adaptive process controls. AMBIC brings together leading academic and industrial biotechnologists, who are focused on mammalian cell culture manufacturing. Its mission is to develop enabling technologies, knowledge, design tools, and methods that apply and integrate high-throughput and genome-based technologies to fast-track advanced biomanufacturing processes. Through systems-level biology analysis, novel cell-line development, bioreactor optimization, and advanced analytics, AMBIC aims to provide transformative solutions that can lower biomanufacturing costs and improve bioprocessing efficiency. For more information, please visit: [www.ambic.org](http://www.ambic.org)
Accelerating the understanding of metabolomics in disease is increasingly high on the agenda for many researchers looking to develop their drug discovery efforts into clinical trials. With personalized medicine the goal that many are striving to achieve, metabolomic technologies must continue to develop improved sensitivity if they are to make a real difference to the drug research of the future.

Lucy Woods, Bruker Daltonics, Bremen, Germany

Accelerating the understanding of metabolomics in disease is increasingly high on the agenda for many researchers looking to develop their drug discovery efforts into clinical trials. With personalized medicine the goal that many are striving to achieve, metabolomic technologies must continue to develop improved sensitivity if they are to make a real difference to the drug research of the future.

Metabolites are the chemical entities that are transformed during metabolism in the body, and they provide a functional readout of cellular biochemistry. Metabolites are the products of metabolic pathways and include small molecules such as lipids, sugars, nucleotides, and amino acids. With mass spectrometry (MS)-based metabolomics techniques, thousands of metabolites can be quantitatively measured from minimal amounts of biological material, which has subsequently enabled systems-level analyses. Global or untargeted metabolite profiling is revealing new discoveries linking cellular pathways to biological...
mechanisms, shaping the understanding of cell biology, physiology, and medicine. Metabolomics plays a major role in clinical practice, as it represents more than 95% of the workload in clinical laboratories worldwide (1). Although relatively new compared with its genomic and proteomic predecessors, research into metabolomics has already led to the discovery of biomarkers for disease diagnosis, fundamental insights into cellular biochemistry, and observations related to disease pathogenesis.

**The Metabolomic Challenge**

One of the biggest challenges facing untargeted metabolomics research is reliable compound identification to ensure accurate interpretation of data. Due to the chemical similarity of metabolites (isobars and isomers), identification by MS or chromatography alone can often be difficult for researchers looking into the behaviour of metabolites. The challenge is that isomers can have the same mass, charge, and physical properties, but can vary widely in their bioactivity, making

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**Figure 1:** Lipid identification annotation capability on different dilution levels of the reference serum SRM1950. VIP-HESI reveals on all concentration levels a higher number of rule-based lipid annotations as shown in (a). The lower the concentration level, the higher this effect can be observed. The higher performance can be explained with the improved ionization capability of VIP-HESI as shown in (b), in which a lipid shows an unprecedented intensity increase by a factor of five.

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**A higher Lipid ID annotation capability**

(a) VIP-HESI vs. ESI Lipid species annotation comparison Negative mode

(b) SRM 1950 plasma extract equivalent on column

VIP-HESI Rule base annotation  ESI Rule base annotation

HESI ESI ~5 fold peak intensity increase
both separation and identification a time- and resource-intensive process.

Another challenge is that the metabolome is extremely sensitive, meaning a person’s metabolic phenotype is influenced by environmental factors. Data can also be more ambiguous because metabolites are the products of upstream biological transformations of molecules in a similar mass range, which in turn presents the need for higher sensitivity to obtain broad metabolite coverage and relative concentrations. The field of metabolomics is much newer than proteomics and researchers also experience significant challenges in the interpretation of data, for example, in the interpretation of fragmentation data.

Ion mobility spectrometry–mass spectrometry (IMS-MS) has emerged as a powerful technology to address such issues, and the use of IMS-MS in untargeted metabolomics has improved the separation of metabolite isomers and supports metabolite identification through generation of orthogonal data, such as collision cross section values.

New technology is continually being developed to realize the biological relevance of these metabolites, to delve deeper into their behaviours, and to look further into new metabolomic possibilities.

Metabolomics and the Study of Lipidomics

Alterations at the metabolome level reflect disturbances in preceding biological cascades, bridging the gap between the genome and phenotype. Changes at this level can lead to the onset of disease symptoms, making metabolomics an essential diagnostic and prognostic tool in investigating the mode of action of chemical compounds and obtaining an in-depth understanding of the impact of infection, for example (2). Current metabolomics research focuses on a range of challenging diseases, including Alzheimer’s disease and cancer.

Scientists are looking further into metabolomic research to examine how the cells in our body behave and what this could mean as we look towards a future of personalized medicine. The acceleration of this research has driven new developments in lipid biochemistry. Lipids are fatty small molecules that share common physical and chemical properties, and the abundance of lipids and different lipid classes is key to metabolic regulation. Lipids studied using metabolomic approaches—lipidomics—are an ideal subject for metabolomic measurements. Lipids also allow scientists to gain further insight into genomes and genetic polymorphisms (3).
As metabolomic possibilities progress, the study of lipidomics has become a prominent source for collecting and measuring data within the metabolome. Because the abundance of lipids in typical samples, such as plasma extracts, can vary considerably, analytical methods with high sensitivity and dynamic range are required. One novel method focuses on coupling a vacuum insulated probe heated ion source (VIP-HESI) with a mass spectrometer. This technology looks at enhancing the limit of detection (LOD) and the dynamic range, as well as examining the lipid identification annotation capability. It enables sensitivity gains of up to 16-fold by efficient desolvation, enabling new applications across all small molecules (4).

Looking further into lipids is crucial to further understanding challenging diseases. Lipids are involved in biological processes such as blood clotting, mediating inflammation, or as signalling molecules, which makes them an interesting target for the discovery of new biomarkers. Insights such as this are essential for developing new treatments and for personalized medicine (5). The discovery of new biomarkers increases the likelihood of finding real-life outcomes for patients as this data progresses into the clinic.

It is key that research progresses in the field of metabolomics, and, within that, lipidomics, to transform insights and cellular findings into new drugs. In turn, personalized medicine becomes a brighter prospect to combat a range of diseases.

Data from an experiment using VIP-HESI is highlighted in Figure 1, showing the difference that it makes to the analysis and sensitivity of lipids across different sample concentrations (4). This technology allows data from lipids to be acquired through a deeper and broader analysis than in previous lipidomic workflows, with the figure showing a higher number of lipid identification annotations for untargeted workflows (4).

Looking Towards the Future of Personalized Medicine
As new technologies evolve and researchers look to optimize equipment to accelerate their studies, it is important that software and analytics keep pace to support the abundance of new data. Looking to a future in which personalized medicine is perceived to be within reach, advances in metabolomics research are significant in discovering new biomarkers to guide insight into our genetic make-up. With the advancement of MS, as well as technologies such as nuclear magnetic resonance (NMR), further strides can be made in clinical practice.

References

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Rising Stars of Separation Science: Valentina D’Atri

This month we interview Valentina D’Atri, a Research and Teaching Fellow in the Analytical Sciences Department of the School of Pharmaceutical Sciences at the University of Geneva in Switzerland, about her work to couple cation-exchange chromatography (CEC) to mass spectrometry (MS), and the challenges of analyzing increasingly complex biopharmaceuticals, such as therapeutic Fc-fusion proteins and bispecific antibodies.

—Interview by Lewis Botcherby

Q. When did you first encounter chromatography and what attracted you to the subject?

A: It was in February 2016, when I was recruited by Jean-Luc Veuthey and Davy Guillarme as a postdoctoral researcher at the University of Geneva. The goal of the project was the characterization of biopharmaceutical proteins by coupling several chromatographic methods to mass spectrometry (MS). Although I had no previous experience with chromatography, I was motivated to challenge myself in the field, and it seems that I was motivated enough for them to accept me into the group. Szabolcs Fekete gave me my first “practical lessons” in front of a high performance liquid chromatography (HPLC) system and introduced me to the elegance and the beauty of chromatographic separations. It was only after a while that I realized that there would be no better place to debut with this technique! What attracted me most about the subject was its versatility; it is simply marvellous how the results achieved with different chromatographic modes can be combined to understand the specific features of the molecules being analyzed. Although everything may seem...
predictable it is not just about a mobile phase passing through a column, it is about interactions, partitioning, adsorption, and the deep understanding of all these mechanisms.

Q. Can you tell us more about your Ph.D. thesis?
A: My Ph.D. thesis was focused on G-quadruplexes, some particular DNA secondary structures that have a natural propensity to self-associate in three-dimensional scaffolds. These higher-ordered DNA structures exhibit a dramatic thermal stability and represent a suitable nucleic acid scaffold for DNA-based nanostructures and therapeutic oligonucleotides (aptamers). During my Ph.D. research studies, I focused on both applications. I identified and characterized some DNA-based nanostructures with a fixed and tailored length, in addition to potentially therapeutic aptamers endowed with anti-HIV activity. In the latter case, the aim was to identify...
the structural features required for the aptamer biological activity and succeed in improving them. It was therefore necessary to exploit the synthesis and the structural characterization of quadruplex-forming oligonucleotides, the analysis of the sequence-specific thermodynamic stability, the physical-chemical properties and the structural features of resulting G-quadruplexes, and evaluate the binding properties to the selected proteins. I really enjoyed my Ph.D. thesis; I had the opportunity to learn different techniques and be supported by highly competent mentors.

**Q. What chromatographic techniques have you worked with?**

**A:** I started with hydrophilic interaction liquid chromatography (HILIC) and the comparison of its performance with reversed-phase LC for the analysis of biopharmaceutical proteins performed at the subunit level. Then, I moved to non-denaturing techniques, such as size-exclusion chromatography (SEC) and ion-exchange chromatography (IEC), for performing the analysis at the intact protein level. In all cases, my goal was the direct coupling to MS for the comprehensive characterization of post-translational modifications (PTMs) and critical quality attributes (CQAs) associated with biopharmaceutical proteins.

**Q. The success of therapeutic monoclonal antibodies (mAbs) has led to the development of many new antibody-based drug formats. What are some of the challenges involved in ensuring that the final product produced is the intended drug?**

**A:** The field of antibody-based drug formats has practically exploded in recent years, with biosimilars, antibody-drug conjugates, multi-specific antibodies, and Fc-fusion proteins filling the pipelines of the pharmaceutical industries to get into the market. To ensure that the final product is the intended drug, the challenges are virtually the same: a complete characterization of the structural features associated with the micro-heterogeneity of these formats. Unfortunately, there is no single LC mode that allows for this characterization. The use of different chromatographic methods is necessary and the complementarity between the modes is the key to overcoming these challenges.

**Q. Post-translational modifications of the drug product can result in changes to the molecular surface charge distribution, with IEC and cation-exchange chromatography (CEC) being key techniques to monitor this. In your recent paper you mention**

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“salt-mediated pH gradients” as a powerful tool to achieve better separation efficiency (1). Could you explain in more detail what this entails?

A: In CEC, the separation is performed with a negatively charged stationary phase that retains ionic compounds, allowing their elution through two different mechanisms: i) via salt-gradient mode, by increasing the salt concentration to weaken the ionic interactions between the protein and the stationary phase; or ii) via pH-gradient mode, by increasing the mobile phase pH while keeping a constant ionic strength to change the charge of the proteins (based on their isoelectric point [pI]). A “salt-mediated pH gradient” can be considered as a boosted pH-gradient mode that is basically obtained by using a mild salt gradient in combination with a pH-gradient mode. This approach allows the cover of a wider range of protein pI and therefore improves the separation of proteins that can be simultaneously analyzed.

Q. When attempting to discover the cause of the changes to molecular surface charge distribution your paper mentions that MS would be the go-to detection method, and in the past would be paired with reversed-phase LC and HILIC, what has changed in this regard and why?

A: The mobile phase composition of denaturing chromatographic techniques such as HILIC and reversed-phase LC allows their direct coupling to MS, although at the expense of losing the native conformation of proteins. On the other hand, CEC is a non-denaturing technique that allows the therapeutic proteins to keep in their native folded state while separating charge protein variants. Historically, CEC was performed with a high concentration of nonvolatile salts in the mobile phases and was therefore incompatible with MS. In these conditions, apart from being able to assign a given variant as acidic or basic by reference to its elution time in relation to the main peak, it was not possible to identify which variation was responsible for the change in charge. MS analysis is essential for such identification and was obtained after performing the desalting of the CEC-collected peaks. Among the first attempts at obtaining the coupling of CEC to MS was multidimensional chromatography (mD-LC). This could have been a solution, but the approach was not straightforward to implement and not well adapted for routine applications. For these reasons, MS-compatible mobile phases for CEC applications have emerged with the aim of obtaining a direct coupling of CEC to MS for allowing unbiased and straightforward characterization of charge variants. Different solutions have been proposed by several research groups and it is now clear that CEC–MS is a real possibility.

Q. Your recent publication details a straightforward and rapid CEC–MS method that allows the separation and identification of several charge variants (1). What challenges did you face in creating this method and what benefits does it offer over alternatives?

A: Our goal was to develop a generic CEC–MS method by using a compact benchtop time-of-flight mass spectrometer (TOF-MS). The first challenge to overcome was to find a compromise between the LC performance and the MS sensitivity. Indeed, when using mobile phases consisting exclusively of volatile salts, it should be considered that they may have a lower buffering capacity compared to mobile phases containing nonvolatile salts. In addition, the LC setup should be compatible, with a proper MS ionization process to avoid the risk of obtaining low signal intensities. Therefore, we optimized the chromatographic conditions by testing various buffers and column dimensions to select the most suitable mobile phase in terms of pH response, buffer stability over time, MS compatibility, and in terms of efficiency versus MS compatibility for the column. Then, we took care of the second challenge: the CEC–MS coupling. Honestly, we expected that it would work on the first try and that a slight optimization of the MS source conditions would be enough to achieve a good ionization of the proteins, but we had to change our minds. The first attempts at direct CEC–MS coupling were not at all satisfactory; the MS signal had very low values of intensity and resolution, and it took a moment to realize that everything depended on the way we prepared the mobile phases. Indeed, metal contaminants from the water source and the glassware, including all the laboratory glassware, mobile phase bottles, and vials, were mainly responsible for the loss of MS resolution and sensitivity. It seemed to be a small detail, but it was not obvious. We then switched to trace metal certified thermoplastics, avoided glass-bottled water, and we managed to overcome the problem of the MS sensitivity. Having solved this problem, everything was smooth and easy to perform. The optimized CEC–MS setup allowed CEC analysis in less than 10 min, and in particular the simultaneous separation and identification of several charge variants by MS.
Therefore, the therapeutic potential of the pharmacologically active moiety would be increased, together with the general stability and solubility of the construct directly linked to the presence of the Fc-domain. Ideally, any active domain suffering from low circulation time could be fused to an Fc-fragment to generate an Fc-fusion protein. For this reason, the therapeutic uses can be really different, from providing a replacement therapy for haemophilia A/B to the treatment of rheumatoid arthritis, plaque psoriasis, or colorectal cancer.

Q. What are the analytical challenges that therapeutic Fc-fusion proteins present?

A: Given the diverse nature of the biologically active domain constituting the Fc-fusion proteins, the analytical challenges can indeed be sample-dependent, even though the main goal is generally to obtain a complete characterization of the PTMs. Among the PTMs, I think that the most challenging one would be the characterization of the glycan profile. Indeed, some Fc-fusion proteins, such as etanercept, abatacept, and belatacept, contain up to six N-glycosylation sites, afilbercept can have up to 10, and conbercept up to 14. In addition, O-glycans may also be present, as in the...
case of etanercept, which can have up to 26 O-glycans. It is therefore easy to imagine the complexity of these glycosylation profiles and how they can be tricky to analyze compared to mAbs, which generally contain only two N-glycosylation sites and no O-glycans.

Q. You recently published a paper on bispecific antibodies (bsAbs) (3). What exactly are bsAbs and what do they offer that other biopharmaceuticals do not?
A: Bispecific antibodies (bsAbs) combine the antigen recognition sites of two (or more) antibodies in a single protein construct, and therefore allow the targeting of two (or more) different epitopes either on the same or on different antigens. As an example, emicizumab is a bsAb with a mAb-shaped structure consisting of two identical light chains (LCs) and two different heavy chains (HCs) able to recognize two different targets, namely the activated coagulation factor IX (FIXa) and the coagulation factor X (FX). By using this double recognition, emicizumab can mimic the function of the plasma clotting factor VIII (FVIII) that is generally missing in patients with haemophilia A. So, without having any homology to the native FVIII, emicizumab acts as cofactor mimetic and is used for the routine prophylaxis of patients missing FVIII.

Q. What unique analytical challenges do bsAbs present?
A: The correct chain-association is the most critical challenge to monitor during bsAbs development and production. The higher the number of antigen recognition sites, the higher the probability of mismatched chains. Therefore, beyond the canonical analytical characterization that is applied to mAb-related formats, the correct chain-association is the dedicated challenge related to bsAbs.

Q. What are you currently working on?
A: I am currently having fun with “exotic” mAb-related formats including multi-specific antibodies and immunocytokines. In parallel, drug formats as therapeutic oligonucleotides and viral vectors for gene therapy have attracted our attention because they represent the pharmaceutical applications of a future that is already here. From an analytical point of view, these new research fields come with completely different and exciting challenges compared to the field of biopharmaceutical proteins. Therefore, beyond mAb-related formats, I am also currently working on the development of LC–MS analytical methods for the characterization of DNA/RNA oligonucleotides and viral vectors, and given the speed at which these fields are evolving, I imagine that I will not have time to get bored!

References
Effectiveness of High-Resolution Ion Mobility for Complex Analyses

Jody C. May\textsuperscript{1}, Katrina L. Leaptrot\textsuperscript{1}, Bailey S. Rose\textsuperscript{1}, Kelly L. Wormwood Moser\textsuperscript{1}, Liulin Deng\textsuperscript{2}, Daniel DeBord\textsuperscript{2}, and John A. McLean\textsuperscript{1}, \textsuperscript{1}Center for Innovative Technology, Department of Chemistry, Vanderbilt Institute of Chemical Biology, Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt-Ingram Cancer Center, Vanderbilt University, United States, \textsuperscript{2}Mobilion Systems, Chadds Ford, United States

Ion mobility is a structurally selective separation strategy that is now routinely integrated with mass spectrometry (IM-MS) to improve peak capacity while simultaneously aiding in the separation of isobaric signals such as those encountered in complex chemical samples. Structures for lossless ion manipulation (SLIM) technology provides a modular architecture for developing tailored ion processing systems, including ion mobility spectrometers. This article evaluates the separation capabilities of a SLIM-based high-resolution ion mobility spectrometer (HRIM) incorporating an extended (~13 m) separation path length design in order to determine benefits for scientists working with complex and challenging analyte classes such as glycans, peptides, and lipids.

While conventional ion mobility (IM) does not perform at the same level of selectivity and resolution as liquid and gas chromatography (LC and GC), IM separations are significantly faster than LC and GC (100s of ms vs. minutes), and IM can be coupled online with chromatography techniques or mass spectrometry (MS) imaging to further increase peak capacity (1). Concomitant with chemical separations, IM provides an additional molecular
measurement in the form of gas-phase collision cross sections (CCSs), which can provide further structural insight and an additional molecular descriptor useful for compound identifications (2). Many of the technical hurdles associated with integrating IM with MS, such as poor sensitivity and limited throughput, have largely been addressed in the latest generation technologies. However, one contemporary challenge of IM has been the limited resolution imposed by the conventionally short, sub-metre path lengths used.

High-resolution ion mobility-mass spectrometry (HRIM-MS) based on structures for lossless ion manipulation (SLIM) technology has emerged as a promising separation strategy due to the ease at which this architecture can be scaled up to longer separation path lengths. HRIM-MS uses printed circuit boards (PCBs) held in a chamber maintained at a constant, reduced pressure (2–4 Torr). The PCBs have a series of radio frequency (RF), direct current (DC), and travelling wave electrodes printed on them that provide an ion conduit through which the analytes traverse along a separation path. The electric fields that propel the ions also prevent them from striking surfaces while moving, therefore preventing any losses along their way. Depending upon the speed of the travelling wave, ions either “surf” and are not separated (as in a total ion transmission mode), or they undergo enough collisions with the gas molecules present that they roll over the travelling wave peaks and IM separation occurs (3,4).

In HRIM, as with other IM technologies, an ion’s migration time is determined by its gas-phase size-to-charge ratio, which is representative of the analyte size and shape. As ions are driven along the separation path, collision with an inert buffer gas slows them down to a degree proportional to their size. The length of the ion separation path is crucial to achieving the high degree of separation required to gain adequate resolution of compounds with challenging structural diversity. In this HRIM arrangement, the ion path is 13 m (roughly half the length of a tennis court), allowing for high resolution separation power in a compact design (Figure 1).

Experimental

Data were acquired using a high-resolution ion mobility spectrometer (Mobie, Mobilion Systems) integrated with a commercial quadrupole time-of-flight mass spectrometer (6545, Agilent Technologies). An LC system (1290 Infinity II, Agilent) was used to introduce samples to the HRIM-MS system via flow injection analysis (20 µL injection volume; 100 µL/min flow rate). Samples were ionized via electrospray (Jet Stream, Agilent) operated at 4.0 kV on
Figure 2: Four isomer systems that are resolved by SLIM-based HRIM. Chemical structures are shown in the grey boxes (top row), conventional drift tube measurements (DTIMS) and HRIM results are given in the middle and lower rows, respectively. The same isomer samples are used in both examples, which include individual overlays (blue traces) as well as the corresponding isomeric mixtures (purple traces). Isomer systems include (a) reverse sequence peptides, (b) triglycerides exhibiting double bond position isomerism, (c) trisaccharide isomers with various monosaccharide subunits and linkages (blue circle = glucose, yellow circle = galactose, and green pentagon = fructose), and (d) ganglioside glycosphingolipids with different sialic acid linkages at the headgroup (yellow square = N-acetylgalactosamine and purple diamond = N-acetylneuraminate). Adapted and reprinted with permission from J.C. May et al., Resolving Power and Collision Cross Section Measurement Accuracy of a Prototype High-Resolution Ion Mobility Platform Incorporating Structures for Lossless Ion Manipulation, JASMS, Copyright 2021, American Chemical Society (4).

Results and Discussion

HRIM resolving power (CCS/ΔCCS) was found to be greater than 200 across a range of ion masses and instrument parameters (travelling wave speeds and amplitudes). In many cases, resolving powers greater than 300 were achieved, most notably when analytes were measured at the threshold between nonselective “ion surfing” to mobility-selective ion drift, which corresponds to ion speeds approximately 30–70% greater than the travelling wave speed.

The ability of HRIM to resolve analytes that are isobaric in mass was evaluated using a series of isomers from different biomolecular classes (peptides, lipids, and carbohydrates), including reversed sequence peptides (SDGRG and GRGDS), triglyceride double bond positional isomers (TG 3,6,9 and TG 6,9,12), trisaccharides (melezitose, raffinose, isomaltotriose, and maltotriose), and ganglioside lipids (GD1a/GD1b). HRIM was able to resolve the corresponding isomeric mixtures (Figure 2), which were found to be unresolvable.
using the standard resolution drift tube IM instrument (see the middle row of Figure 2) (4).

These results indicated that HRIM-MS can resolve peaks with CCS differences as small as ~0.6% without the need for targeting a specific separation window or extending the analysis time. Furthermore, under these high-resolution conditions, the measured CCS values were found to exhibit low biases (<0.5%) compared to CCS measurements obtained on a conventional drift tube instrument. All ions were separated by HRIM in less than one second, which was approximately three orders of magnitude faster than LC. Importantly, all analytes could access high-resolution conditions (>200) within the same HRIM spectrum simultaneously, which indicated that HRIM was well-suited for untargeted, discovery applications.

**Conclusion**

HRIM-MS utilizing SLIM to enable the transfer and mobility separation of ions across a large distance (~13 m) was critically evaluated in terms of upper resolution and the ability to resolve isomeric mixtures. The HRIM resolving power (CCS/ΔCCS) was benchmarked between 230 and 315 for a commonly used MS tuning mixture. Notably, all analytes within this mixture were transmitted within a short dispersion time frame (<700 ms) and all exhibited resolving powers in excess of 230, indicating that HRIM-MS was well-suited for high-throughput broadband separations, which are important analytical figures-of-merit for untargeted studies. For the separations of several biochemical isomers (peptides, lipids, and carbohydrates), HRIM-MS achieved near or full baseline resolution for the corresponding isomeric mixtures, with measured peak spacings as little as 0.6% difference in CCS.

As a result, it was concluded that HRIM provided improved resolution over conventional IM without trade-offs, meaning that HRIM-MS not only increased throughput but also provided enhanced separation capabilities and additional structural information in a shorter time, with an easily transferable method. Because HRIM methods are analyte-agnostic, the benefits of speed, reproducibility, and resolution can be applied to assays of different biomolecule classes—proteins, peptides, lipids, glycans, and other metabolites—without having to change out hardware or perform extensive method development to achieve optimal results. Compared to other ion mobility separation techniques, HRIM-MS provided high resolution separation across a broad m/z range.

HRIM-MS provides fast, efficient, high-resolution critical quality attribute analysis of biologic therapeutics—capabilities beneficial for scientists working with complex and challenging analyte classes, such as glycans, peptides, and lipids.

**References**


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**Daniel DeBord** is Vice President of R&D at Mobillion Systems. Daniel has extensive experience in the field of mass spectrometry. After receiving his Ph.D. in analytical chemistry from Texas A&M University, Daniel served as Associate Director of the Advanced Mass Spectrometry Facility at Florida International University. His industry experience includes positions at BASF and 1st Detect Corporation, where he worked to design and develop...
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**Bailey S. Rose** is a recent graduate from the McLean Research Group at Vanderbilt University with a Ph.D. in chemistry. She received her B.S. in chemistry from Belmont University in 2017. Bailey’s research focuses on the application of ion mobility-mass spectrometry and novel informatics approaches to untargeted metabolomics and lipidomics in support of high-confidence molecular annotation.

**LiuLin Deng** is Principal Scientist at Mobilion Systems Inc. and is currently responsible for designing, constructing, validating, and developing the next generation ion mobility spectrometry based upon SLIM technology.

**Kelly Wormwood Moser** is leading Mobilion’s application development, working with industry collaborators to develop methods to demonstrate HRIM separation capabilities with biological samples. Kelly is leveraging her Ph.D. research experience, where, under the direction of Dr. Costel Darie, she used mass spectrometry to identify potential protein biomarker signatures for autism spectrum disorders.

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**Bailey S. Rose** is a recent graduate from the McLean Research Group at Vanderbilt University with a Ph.D. in chemistry. She received her B.S. in chemistry from Belmont University in 2017. Bailey’s research focuses on the application of ion mobility-mass spectrometry and novel informatics approaches to untargeted metabolomics and lipidomics in support of high-confidence molecular annotation.

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Improved Characterization of Malodours in Recycled Plastics

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Thermal desorption (TD) with two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS) can be used for comprehensive characterization of the volatile organic compounds emitted by recycled plastics. This article presents a method that overcomes the limitations of current malodour detection techniques and is easy to translate to routine quality control (QC) screening.

A push towards a circular economy, in which materials are reused or recycled for as long as possible, has led to plastics manufacturers being urged to produce or use more post-consumer recycled (PCR) plastics, especially for food and beverage packaging. However, PCR plastics require more rigorous quality control (QC) measures than new plastics to ensure that they will not produce volatile emissions that could be considered harmful or have a negative impact on the packaged product (for example, malodours). Unfortunately, existing methods for the detection of odours from plastics have several limitations.

A human sensory panel is a sensitive approach, but it is also subjective, time-consuming, and requires skilled individuals. In addition, it is restricted to sensory information: no chemical identities are provided, so sensory panels cannot identify a possible source or clean-up process to eliminate the malodour.

The electronic nose (eNose) is faster and simpler to use as it is a handheld device with...
sensor technology. However, the technique is not specific, meaning that samples that fail QC testing must undergo further analytical investigation.

Gas chromatography coupled with mass spectrometry (GC–MS) can provide a more quantitative approach, but it may struggle to fully resolve all the volatile organic compounds (VOCs). Typically, the odour profiles are dominated by aliphatics from the polymer itself, which easily mask the trace-level odorants (for example, oxygenated species). Traditional quadrupole MS must operate in scan mode to find these nontarget components, but this limits the sensitivity of the instrument. Additionally, common sample introduction techniques, such as headspace injection and solid-phase microextraction (SPME), may lack the necessary sensitivity to capture the trace odors.

With these approaches, it is often not possible to identify the precise compounds responsible for high odour in recycled plastics, which means that the recycling process cannot be improved and QC failures continue to occur.

Trace odours can cause the end users’ products to fail QC further down the production chain, which passes further cost onto the customers and can cause losses in returned product or compensation claims.

Thermal desorption coupled with comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry (TD–GC×GC–TOF-MS) can address these challenges by providing high sensitivity and improved separation of the odour profiles prior to confident identification of the individual analytes.

In this article, we demonstrate how TD–GC×GC–TOF-MS can provide confident characterization of complex odour profiles from recycled plastics for fast and simple identification of the compounds causing QC failures. Once the key odorants are known, methods can be easily translated to TD–GC×GC–flame ionization detection (FID) for routine screening in QC laboratories at production sites.

**Experimental**

**Samples:** An empty TD tube (Markes International) was filled with 0.3 g of plastic pellets. TD: Instrument: TD100-xr (Markes International); focusing trap: Materials Emissions (Markes International); trap low: -20 °C; desorption temperature: 100 °C. GC×GC: Insight flow modulator (SepSolve Analytical); modulation period (P_M) = 2.5 s. TOF-MS system: BenchTOF2 time-of-flight mass spectrometer (SepSolve Analytical); mass range: m/z 30–600. Software: ChromSpace software (SepSolve Analytical) for full instrument control and data processing, with chemometric comparisons by ChromCompare+ (SepSolve Analytical).

**Method:** First, the VOCs were sampled using direct desorption in which a small number of plastic pellets were placed directly into an empty TD tube, which was heated to release the VOCs. Optimal sensitivity was achieved by preconcentrating the analytes on an electrically cooled focusing trap before they were sent to the GC system in a narrow band of vapour. Some TD instruments can enable automated analysis of up to 100 tubes and operate solvent-free and cryogen-free, making them ideal for high-throughput screening of plastics.

Next, GC×GC using a consumable-free flow modulator provided the separation necessary to resolve such complex odour profiles, to obtain clean spectra and confident identification of analytes by the TOF mass spectrometer. TOF-MS provided the acquisition speeds necessary to cope with the narrow peak widths generated by GC×GC, as well as improved sensitivity and selectivity compared to single quadrupole MS, making it the ideal technique for untargeted “discovery” applications.

Finally, software compared the resulting chromatograms, enabling the important
The differences between high- and low-odor plastics to be identified. This automated, untargeted workflow used all the raw data to minimize the risk of missing any compounds of importance.

Results and Discussion
In one-dimensional (1D) GC separations, the trace odorants in plastics are often masked by the high-loading aliphatics and may be overlooked entirely. Figure 1 provides the TD–GC×GC-TOF-MS chromatogram for the direct desorption of plastic pellets. The aliphatics were retained longer in the second-dimension column, thus eluting in a band of intense peaks along the top of the color plot (as annotated in Figure 1). On the other hand, the odor-active compounds (such as oxygenated species) eluted earlier in the second dimension, separating these compounds from the aliphatics.

For example, Figure 2 shows a zoomed region of the chromatogram in which a trace peak is physically separated from the high-loading aliphatics and could be confidently identified as nonanal, which is known to impart a waxy, aldehydic odor (1).
It is important to note that the rapid secondary separations in GC×GC frequently result in peak widths less than 100 ms, so detector speeds of 100 Hz are essential to maintain at least 10 datapoints across a peak. A TOF-MS system is inherently well-suited to handling such narrow GC peaks, since they are dispersive (not scanning) instruments, and so effectively monitor all masses at once with fast acquisition rates. The ability to record full-range mass spectral information to extremely high densities enables TOF-MS to handle the narrowest peaks encountered in well-optimized GC×GC couplings.

To prove the performance of TD–GC×GC–TOF-MS to identify differences between plastics that have passed and failed QC tests, a selection of “high”-odour and “low”-odour plastics were analyzed. It is clear from the example chromatograms in Figure 3 that there are additional peaks in the odour-active region of the high-odour plastic. However, for such high-throughput applications, automated comparison of samples is essential to deliver fast results.

Here, software was used to automatically compare all the raw TD–GC×GC–TOF-MS data for three high-odour plastics and three low-odour plastics to identify the key odorants responsible. The resulting principal components analysis (PCA) score plot (Figure 4) shows the clustering of the low- and high-odour plastics, indicating there are compositional differences between their odour profiles.
The software could identify the components found in increased amounts in the high-odour pellets. For example, Figure 5 shows one of the top differentiators, which was identified as n-octanol. It was found in increased abundance in the high-odour pellets and is known to impart a waxy odour (1).

Once the key odorants were identified, a class prediction model was developed in the software to instantly classify future unknown samples. This is a key aspect for QC labs, where instrumentation and workflows must be as simple and cost-effective as possible. With this in mind, TD–GC×GC methods can also be easily translated to FID, for routine use in quality control laboratories.

Conclusions
This study has shown that TD–GC×GC–TOF-MS provided automated cryogen-free sampling of VOCs from plastics using direct desorption in TD tubes. Detection of trace odorants was improved using TD preconcentration and high-sensitivity TOF-MS, while flow-modulated GC×GC provided enhanced separation and discovery of odorants that could not be identified using 1D GC–MS. Reference-quality spectra generated by TOF-MS enabled key odorants to be confidently identified. The workflow was streamlined using a single software platform for full instrument control and data processing, with chemometrics to automatically find differences between complex chromatograms. Flexible configurations with simple translation from TD–GC×GC–TOF-MS to TD–GC×GC–FID mean that the setup could be used in QC laboratories.

References

Laura McGregor received an M.Chem. in chemistry from the University of St Andrews, UK, followed by an M.Sc. in forensic science at the University of Strathclyde, UK. Her Ph.D. in environmental forensics, also at the University of Strathclyde, focused on the chemical fingerprinting of environmental contamination using advanced techniques such as GC×GC–TOF-MS. In her current role at SepSolve Analytical, she specializes in the application of GC×GC and TOF-MS to challenging applications.

Elinor Hughes obtained her B.Sc. in chemistry and Ph.D. in organic chemistry at Bangor University, UK. After working for a chemical manufacturing company for three years, she moved to the Royal Society of Chemistry where she worked in journals publishing for six years and on Chemistry World magazine for four years. Her current role is technical copywriter at Markes International.
ChromSoc Meeting Preview: Challenges in Small Molecule Analysis in the Pharmaceutical Industry

The Chromatography Society (ChromSoc) Spring Symposium will be held in person from 19 to 20 May 2022 in the historic city of Chester. Here’s a glimpse of what to look forward to.

The Chromatography Society (ChromSoc) Spring Symposium will be held in person from 19 to 20 May 2022 at the oldest racecourse still in operation in the world, in the historic city of Chester, UK. The two-day symposium will consider the developments and challenges in the field of small molecule work with an emphasis on pharmaceutical applications. It will look at a range of topics including: challenges and approaches to impurity profiling, including nitrosamine and counterfeit testing; applying quality-by-design (QbD) to method development; high-throughput screening (HTS) and its role in finding new drug candidates; the challenges in automating analytical data batch-processing; chiral screening methodology; and challenges within environmental analysis. There will be discussions on the latest developments in instrumentation/column technology and on the future of small molecule analysis in general. Leading specialists from industry and academia will explain how they apply theory and the latest developments to
solve very difficult challenges in these areas. Each session will be followed by a Q&A session with the experts.

Key speakers include Dr Anthony Edge (Avantor), Dr Lewis Couchman (ASI), Professor John Dean (Northumbria University), Tim Underwood (GSK), Sylvain Demanze (AZ), Dr Andrew Feilden (Hall Analytical Laboratories), Keira Bailey (Reach Separations), Dr Simon Hird (Waters), Lewis Jones (Sensient), Dr Brian Montgomery (Avantor), and Professor Peter Myers (Liverpool University).

The conference will facilitate abundant networking opportunities and includes a mixer event in the evening at Chester racecourse, which will be supplemented by an outdoor barbecue and refreshments. There will be representation from the key manufacturers in the industry, and instrumentation and consumables will be displayed in a comprehensive supporting exhibition.

During recent times, where meetings and networking have been very restricted, the organizers hope that you can join them in Chester!

For more information and to register, please visit the Chromatography Society webpage at: https://chromsoc.com or via the registration link here: https://na.eventscloud.com/ereg/index.php?eventid=663387&

Email: steph@sasevents.co.uk
The 26th ISSS 2022 and 25th HPTLC 2022 will be held from 28 June–1 July 2022 in Ljubljana, Slovenia. This preview offers a flavour of what to expect.

The 26th International Symposium on Separation Sciences (ISSS 2022) and The 25th International Symposium for High-Performance Thin-Layer Chromatography (HPTLC 2022) will be held from 28 June–1 July 2022 in Ljubljana, Slovenia. ISSS 2022 is organized jointly with HPTLC 2022 to allow scientists to attend both symposia. This will be a special opportunity to join the two symposia, meet more speakers and participants, and gain access to more workshops and content. Several renowned keynote speakers from 23 countries have already confirmed their participation.

ISSS 2022 will cover new advances and challenges in all fields of separation sciences, and includes a scientific programme that will encompass everything from the fundamentals of separation science to metabolomics and biomarker discovery, as well as applications in all fields. A special scientific section will be dedicated to Professors Pavel Jandera and Milan Hutta to memorialize their contributions to the field of separation sciences.

HPTLC 2022 will cover new advances and challenges in the field of high-performance thin-layer chromatography (HPTLC). Scientific contributions will include all areas of HPTLC, such as fundamentals, advances in instrumentation, hyphenated techniques, and their applications in all fields. A special scientific section will be dedicated to Professors Rudolf E. Kaiser and Joseph Sherma to memorialize their contributions to the field of HPTLC.

The programme of both symposia will consist of invited lectures, oral and
poster presentations, young scientist oral presentations, and workshops. Each symposium will also conduct a Best Poster Award competition that all poster presentations will enter. The presenting author of the winning poster at ISSS 2022 will be awarded with free participation at ISSS 2023. Additionally, in recognition of important contributions to the development of separation science, the Central European Group for Separation Sciences Awards will be presented at ISSS 2022. The events will also feature a comprehensive exhibition of key sponsors and exhibitors.

For the first time all participants will have the opportunity to take part in a special creative competition called CHROM-ART. Interesting HPTLC plate images, chromatograms of unusual shapes, and other images created during chemical analysis will be presented in a CHROM-ART gallery from an artistic point of view. One creative CHROM-ARTWORK will receive the CHROM-ART Award.

The series of ISSS and HPTLC symposia have historically been an excellent means for young researchers to expand their knowledge about state-of-the-art methods and approaches used in separation sciences, as well as build vital social connections. An attractive and rich social programme will create the perfect atmosphere to connect with old acquaintances and build long-lasting friendships that may blossom into fruitful joint adventures. We hope to inspire future research, innovation, and scientific collaboration, as well as offer participants a taste of Slovenia’s natural beauty, intriguing history, culture, and cuisine.

We invite researchers from all relevant areas to submit abstracts to both ISSS 2022 and HPTLC 2022.

For more information and registration, visit the ISSS 2022 and HPTLC 2022 webpage at: https://isss2020.si and https://hptlc2020.si • E-mail: info@isss2020.si and info@hptlc2020.si
The LCGC Blog: She Separates—The Female Voices of Separation Science

Katelynn A. Perrault, Chaminade University of Honolulu, Honolulu, Hawaii

While women may be entering more degrees and careers in science than ever before, there is still a need to advocate for gender parity in many sectors.

This time of year always makes me particularly proud to have become a scientist. In February we are surrounded by a multitude of materials advertising the great accomplishments of women in science, due to UNESCO’s International Day of Women and Girls in Science. Shortly after, in March, we celebrate accomplishments of women in a variety of fields that impact societal advances with International Women’s Day. Often, we are reminded of women who were overlooked in science history for discovering great knowledge that we use today. I am reminded of a great blog article written by Michelle Misselwitz that explains the history of Dr. Erika Cremer’s contributions to the invention of gas chromatography (GC) (1). The inaccessibility of Cremer’s work during the period in which it was performed has largely been attributed to the gender and political biases of the time. If you are a chromatographer, and you are unaware of Cremer’s contributions to GC, I highly encourage you to read this article (or other historical accounts) about the early days of GC in Cremer’s laboratory.

My Experience as a Woman in Separation Science

I was fortunate to be raised in an
environment where I was never told that I couldn’t do or study something just because I was a girl. I have, however, dealt with gender bias many times in my career, and know that it is something that I will continue to face in the future to some extent. From each experience, I try to learn and grow. As a graduate student, I told myself I had to work extra hard to get my work published, be noticed, and make connections with prominent scientists. As a postdoctoral scientist, I struggled with taking ownership of my science, often being perceived as an assistant to male scientists with whom I worked closely. As a faculty member, I have made it my mission to share my passion for chemical separations with my students, and get them to dream big. I am a “cheerleader” for separation science, hoping to convince students how exciting this field can be. The Column www.chromatographyonline.com

While the programmes I teach are heavily dominated by female students, I am very cognizant that many of my students don’t believe they could go to graduate school, or do other things well within their reach. They tell me they aren’t sure they can do it, and it breaks my heart every time. We host large events to expose them to women scientists; I try to connect with every student in the classroom about what makes them excited; I meet one-on-one with students as much as possible and remind them of their potential; I teach a class dedicated to the transition to life after college; I share my stories and emotions. Most of all, I believe fiercely in them until they are able to do so themselves.

In 2019 I was awarded the American Chemical Society’s Satinder Ahuja Award for Young Investigators in Separation Science. This award is given annually by the Subdivision for Chromatography and Separations Chemistry (SCSC), the subdivision responsible for this blog article (and where I now serve as an Executive Committee member). I was shocked and thrilled about the award and quickly went to the website to view the list of past winners. To my surprise, I was the first woman to receive this award since its inception in 2004. I am proud to be joined by another female on the list, Dr. Robbyn K. Anand, in 2020, and to know that this landscape is changing. The list will one day be populated by many women who become our future separation science leaders. We are fortunate that the activities of the SCSC have helped to unite many women who are doing work in the field of separations, and to grow our networks. We are all extremely willing to network with others who may feel the benefit of this type of professional community, and hope you will join us at some of our future events.

Come to think of it, I’m not aware of any awards in my field of work that are named after a female scientist. It is not because they have not made meaningful and celebrated advances in the field of separations chemistry. Perhaps this comes from historical, political, or geographical factors. I hope this landscape will also change some day.

I am fortunate to be connected with a network of powerhouse scientists (who also just happen to share the same sex as me). I have benefitted from interactions with this amazing network in my career and do not take it for granted, because I know many women before me did not have such a privilege. I have always had colleagues to turn to when I had questions uniquely suited to women scientists. Today I recognize these women for their valuable contributions to the field of separation science by sharing with you some of their responses to questions I asked them. I hope you will enjoy hearing from them as much as I enjoyed recording their responses.

What got you interested in separation science?
• “I had access to well-equipped undergraduate labs, where the students

...
could make their own injections on a GC–flame ionization detector (FID) or GC–mass spectrometer (MS), or load their sample into a nuclear magnetic resonance (NMR) spectrometer. The first class where I remember having that experience was in the organic chemistry lab. After college, when I went to interview for positions working in analytical labs, my hands-on experience with instrumentation was appreciated by the potential employers.”—Dr. Heather Bean, Assistant Professor, Arizona State University

• “I actually had a class in separation science in my third year at university that changed all my plans (I wanted to work on DNA analysis). I think it’s the closeness to application and ‘real things’ that I really enjoy.”—Dr. Caroline Gauchotte-Lindsay, Senior Lecturer, University of Glasgow

What has been a major challenge you have experienced in your career?

• “Overcoming impostor syndrome and truly believing that I belong, and that I still belong even when I don’t know every answer. I still work on this every day, but I do think it has given me the ability to better recognize when others may be struggling with the same thing. I love when I am able to mentor younger scientists and connect with them on that level. We help each other.”—Dr. Sarah Prebihalo, ORISE Postdoctoral Fellow, U.S. Food & Drug Administration

• “I thought I had to choose between career ambition and motherhood. I decided I would put a pause on my career to have children, but then I found a fantastic opportunity to work part-time from home consulting with Chemistry Matters. It was the best of both worlds, and I realized that career ambition and motherhood do not need to be mutually exclusive.”—Michelle Misselwitz, Ph.D. student, Duke University

• “Convincing the academic world that forensic science is based on ‘real’ science. I am trained as a chemist first and forensic scientist second, but many think that forensic science has no scientific foundation.”—Dr. Shari Forbes, Professor and Canada 150 Research Chair, University of Quebec at Trois-Rivieres

What are some of the activities you’d suggest students get involved in if they are looking to become separation scientists?

• “Summer programmes, co-ops, and working in the laboratories of researchers are excellent ways to learn and be inspired”—Dr. Jane Hill, Associate Professor, The University of British Columbia

• “Attending conferences and workshops—especially those that offer courses on the technology. There are some great free events out there.”—Dr. Laura McGregor, Product Marketing Manager, SepSolve Analytical

• “Try to get involved in research as soon as you can. Getting hands-on experience not only gives you an idea of whether this is something you could enjoy doing every day but also helps gives context to what you may be learning in class.”—Dr. Sarah Prebihalo, ORISE Postdoctoral Fellow, U.S. Food & Drug Administration

Is there anything you wish you knew or did when establishing your career as a separations chemist?

• “You absolutely do not have to put up with environments that don’t work for you. If something makes you uncomfortable, bring it up to management. If you bring it up and nothing gets done or you get dismissed, look for something different. You will spend about eight hours of your day or more in that environment, so I cannot stress how important it is that you feel comfortable in it.”—Nadin Boegelsack, Applications Chemist, SepSolve Analytical

• “My career path would not be a straight line. I could never have predicted at the end of high school, end of college, or the beginning of my Ph.D. (after some time working in industry) where I would have ended up. Even after finishing my Ph.D., I couldn’t have predicted the field of study I ended up specializing in.”—Dr. Heather Bean, Assistant Professor, Arizona State University

What has surprised you most about the work you do in separation science?

• “I am surprised about the evolution that has and still is taking place. From software to hardware and everything in between, separation science is constantly changing and improving. I am excited to see where we will be in another 10–20 years.”—Dr. Melissa Dunkle, Research Scientist, Dow Benelux, BV

• “How much I enjoy it! Chemistry was never my favourite subject at school, even though I enjoyed science generally. Had I realized the kind of career I could have using chemistry, I probably would have been more excited about it. I am lucky that my interest in forensic science meant that I established a foundation in chemistry first, which led me into separation science.”—Dr. Shari Forbes, Professor and Canada 150 Research Chair, University of Quebec at Trois-Rivieres

What other advice would you give to students that are unsure about their career path?

• “I like to tell my students that charting
your career is more like sailing a boat than choosing one of ‘two roads diverged in a yellow wood’. There’s more room for course changes and tweaks than you might expect. So don’t stress too much about making the wrong decision or getting every choice right on the first try. It’s okay to check something out and change your mind, as long as you’re being reflective along the way.”—Dr. Michelle Kovarik, Associate Professor of Chemistry, Trinity College

• “Spend time with training (3–6 months) opportunities in the field in or outside university to get hands-on experience with what a specific job or task requires and how you feel when you get involved in something. Theory is nothing without feelings.”—Dr. Chiara Cordero, Full Professor of Food Chemistry, University of Turin

What do you still dream about for the future of separation science?
• “Handheld systems with great sensitivity and specificity that have a low cost... one can dream!”—Dr. Jane Hill, Associate Professor, The University of British Columbia

• “So many! The biggest dream for me, however, is to close the gap between academia and industry.”—Nadin Boegelsack, Applications Chemist, SepSolve Analytical

• “For scientists who are not practicing separation science to really understand how it helps in the understanding of many phenomena. I like to say that integration (like the approach applied in systems biology) has its most powerful application in separation science.”—Dr. Chiara Cordero, Full Professor of Food Chemistry, University of Turin

A Final Thought
And there you have it—advice from some amazing separation scientists. Thank you to everyone who responded to my survey and shared their hearts and souls to demonstrate how powerful separation science can be in the eyes of women in the field. Maybe one day we will all be celebrating together the inaugural awardee for an “Erika Cremer Prize in Gas Chromatography”. Maybe blogs and events and discussions like this will help us to achieve some of our dreams for the future of separation science with a diverse and inclusive workforce. One thing is for sure: Women have always been, and will continue to be, a major driving force behind advancement in the field of separation science.

Reference

This blog is a collaboration between LCGC and the American Chemical Society Analytical Division Subdivision on Chromatography and Separations Chemistry.

Katelynn A. Perrault is an associate professor of forensic sciences and chemistry at Chaminade University of Honolulu. She specializes in the application of comprehensive two-dimensional gas chromatography for odour analysis applications, and mentors numerous undergraduate researchers as part of her integrated teaching and research programme. Her current interests include odour production from post-mortem microbes, development of GC×GC data processing workflows for dual-channel detection, promoting the adoption of GC×GC in the forensic sciences, and establishing GC×GC curriculum to be taught in undergraduate chemistry classes. Perrault was recently named the 2019 American Chemical Society Division of Analytical Chemistry Satinder Ahuja Young Investigator in Separation Science.

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## Training Courses

### GC
- **GC Introduction**
  - **Website:** www.chromacademy.com/channels/gc-training-courses/principles/gc-introduction

- **GC Troubleshooter**
  - **Website:** www.chromacademy.com/channels/gc-training-courses/troubleshooting/gc-troubleshooter

### Operating and Understanding GC
- **Website:** www.crawfordscientific.com/training-consultancy/gc-training/gc-fundamentals

### GC Headspace
- **Website:** www.crawfordscientific.com/training-consultancy/gc-training/gc-headspace

### Applied Interpretation of GC–MS
- **Mass Spectra**
  - **20–22 July 2022**
  - **Website:** www.anthias.co.uk/training-courses/interpretation-mass-spectra-gcms

- **Practical Essentials of HPLC and LC–MS**
  - **27–29 June 2022**
  - **Online—virtual**
  - **Website:** www.anthias.co.uk/training-courses/PE-LC

- **How to Develop HPLC Methods**
  - **22–23 September 2022**
  - **Online—virtual**
  - **Website:** https://mournetrainingservices.com/develop-hplc-course/

### HPLC/LC–MS
- **Understanding HPLC**
  - **Website:** www.crawfordscientific.com/training-consultancy/hplc-training/hplc-fundamentals

- **HPLC Troubleshooter**
  - **Website:** www.chromacademy.com/channels/hplc-training-courses/troubleshooting/hplc-troubleshooter

- **Fundamentals of LC–MS**
  - **Website:** www.chromacademy.com/channels/lc-ms/principles/fundamentals-of-lc-ms-video-training-course

- **LC–MS Introduction**
  - **Onsite training**
  - **Website:** www.chromacademy.com/channels/lc-ms/principles/lc-ms-introduction

### SAMPLE PREPARATION
- **Fundamentals of Solid-Phase Extraction (SPE) Mechanisms**
  - **Online training**
  - **Website:** www.chromacademy.com/channels/sample-preparation/technique/fundamentals-of-spe-mechanisms

### MISCELLANEOUS
- **Introduction to Infrared (IR) Spectroscopy**

### Website:
- **www.chromacademy.com**

### Training & Events

Please send your event and training course information to Kate Jones kjones@mjhlifesciences.com
**Event News**

**18–20 May 2022**

The 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-17)

Ghent University - Aula, Ghent, Belgium

**E-mail:** htc17@kuleuven.be

**Website:** https://htc-17.com/

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**21–24 June 2022**

Analytica 2022

Messe München, Munich, Germany

**E-mail:** info@analytica.de

**Website:** www.analytica.de/en/

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**6–9 September 2022**

The 10th International Symposium on Recent Advances in Food Analysis (RAFA 2022)

Prague, Czech Republic

**E-mail:** RAFA2022@vscht.cz

**Website:** www.rafa2022.eu

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**18–22 September 2022**

The 33rd International Symposium on Chromatography (ISC 2022)

Budapest, Hungary

**Website:** https://isc2022.hu