6 March 2019 Volume 15 Issue 3

# The Column

the interactive e-publication for the global separation science industry

# Breath-Taking Analysis

## Tuberculosis diagnosis using GC×GC–TOF-MS

## Cover Story



## Features



## Regulars


# Tuberculosis Diagnosis Using GC×GC–TOF-MS

***The Column*** **spoke to Jane Hill from Dartmouth College, USA, about her group's development of a breath analysis method for tuberculosis (TB) diagnosis using multidimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS).**

***—Interview by Kate Jones***

**Q. Your group has developed a breath analysis method for tuberculosis (TB) diagnosis using multidimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS) (1). How did this research come about and why did you think that GC×GC would be the best technique to use?**

**A:** Several years ago, my team decided to emphasize infectious diseases of the lung. According to the World Health Organization, tuberculosis kills about 1.7 million people each year and infects over 10.5 million annually, making it the biggest infectious disease killer in the world. Diagnosing TB is challenging, particularly in patients with an HIV co-infection and in children, because the population cannot easily cough up the lower lung sputum sample needed for current diagnostic systems. We realized we could deploy our analytical, microbiological, and clinical experience to this global health epidemic.

**Q. How do you collect the samples for breath analysis? Are there any precautions you need to take when collecting samples for breath analysis?**

**A:** Our group and others have published protocols regarding how to collect breath samples (2). Details found there can guide the reader on which approaches might be most applicable to their application. In general, breath is collected onto thermal desorption tubes and then hermetically sealed and stored at 4 °C before being run on an instrument. Personal protective gear is required when sampling potentially infectious patients, though typically no more than what is required working in a biological safety level 2 (or equivalent) laboratory. When working with patients who might have tuberculosis, face masks are worn. We also have to be

careful not to potentially infect patients who are immunocompromised, so often our personal protective gear is worn just as much to protect the patient as the sampler.

**Q. What does this method offer over existing methods?**

**A:** Untargeted analysis demands the best peak capacity possible, which GC×GC–TOF-MS provides. Thus, it became necessary for me to invest in this instrumentation. Along with the instrument and the associated software came the internal development of software tools to manage the immense data produced. So, on the one hand, the instrument reveals 2–10 times more features compared to one-dimensional systems, however, the bioinformatics required to process the data is, accordingly, at least an order of magnitude higher.

**Q. What advice can you offer to other scientists thinking of implementing GC×GC in their research?**

**A:** Comprehensive chromatography is superior to single-dimension analysis for biomarker discovery. It is well worth ascending the learning curve so that you can comfortably use such a helpful tool. Study design and becoming proficient at bioinformatics is essential to generate the best data and make the best use of your datasets, respectively.

**Q. Are there any particular challenges associated with untargeted analysis?**

**A:** Untargeted analyses present specific technical challenges. The alignment of two-dimensional data can be substantially more tricky to do because molecule retention time shifts do not always co-vary. If you are trying to align thousands of molecules, that can be a bit of a nightmare, requiring the creation of code or working through each chromatogram by hand (and you probably always want to do the latter). Reducing the dimensionality of data is the other major challenge to overcome. Statistical evaluation coupled with the use of machine-learning tools is one way to navigate that issue.

**Q. Were there any results that surprised you?**

**A:** Absolutely! Comprehensive chromatography has revealed new compounds, some of which have become putative biomarkers for various infectious diseases. Reverse engineering their possible origin—a slower process than biomarker discovery—has led to remarkable insights into infectious disease pathogenesis, even though we have only just touched the surface of that part of our scientific enterprise.

**Q. Is breath analysis using chromatography currently being used in clinical practice?**

**A:** Not much. The field is still somewhat young in terms of clinical translation. Nitric oxide in breath is used to diagnose asthma, but there are instruments that cater specifically to that molecule. *Helicobacter pyroli* can also be diagnosed by a breath test, but again, with an instrument specifically measuring carbon dioxide. There are some ongoing clinical trials for breath analysis in the infectious diseases area as well as various cancers, however, the results of these are not yet known.

**Q. What is your group working on at the moment?**

**A:** Mostly we are targeting the dominant lung infections caused by bacteria, viruses, and fungi, including tuberculosis, of course. We are currently conducting four clinical validation studies of candidate breath biomarkers.

## References


1. J.E. Hill *et al.*, *Journal of Chromatography B* **1074–1075**, 46–50 (2018).
2. M. Beccaria, C. Bobak, B. Maitshotlo, T. Mellors, G. Purcaro, F. Franchina, C. Rees, M. Nasir, A. Black, and J.E. Hill, *Journal of Breath Research* **13**(1), 016005 (2019).



**Jane Hill is an Associate Professor at Dartmouth College. She has a Ph.D. from Yale University.**

E-mail: **jane.hill@dartmouth.edu**

# News

**Agilent Announces Thought Leader Award Winners**

Agilent Technologies (Santa Clara, California, USA) has awarded Paul Bonnington and Kimbal Marriott an Agilent Thought Leader Award in support of their innovative research into data analytics, machine learning, artificial intelligence, and information visualization.

Based at Monash University, Melbourne, Australia, Bonnington and Marriott will receive funding and Agilent instrumentation to facilitate and accelerate their research. Drawing upon Monash University's vast knowledge and experience, Bonnington and Marriott are developing new, innovative, and smarter analytical products that will increase laboratory efficiency, as well as support mechanisms that will significantly reduce customer downtime.

"There are exciting new discoveries awaiting us when we bring advanced scientific instrumentation together with the advanced data processing capabilities of the Faculty of IT and the eResearch Centre," stated Paul Bonnington, Director eResearch Centre, Monash University.

"The recent great advances in artificial intelligence, data science, and human-computer interaction allow us to reimagine the scientific research laboratory. For the first time we can build a lab that puts the scientists first, not the scientific instruments," stated Kim Marriott, Director Computer-Human Interaction and Creativity Group, Faculty of IT, Monash University.

The Agilent Thought Leader Programme promotes fundamental scientific advances by contributing financial support, products, and expertise to the research of influential thought leaders in the life sciences, diagnostics, and chemical analysis space.

For further information, please visit: **www.agilent.com**

# Pulse Flow Modulation for Multidimensional GC

Researchers from the University of Washington, USA, have developed an ultrafast separation using pulse flow valve modulation to enable high peak capacity in comprehensive two-dimensional gas chromatography (GC×GC) and three-dimensional gas chromatography (GC×GC×GC) (1).

With the advent of multidimensional GC, increased peak capacity and chemical selectivity became available to researchers who required significantly more analytical clout in the laboratory. However, the cost of this extra power came in the form of a high likelihood of unresolved analytes. While this could be overcome by adjusting the sample throughput cycle, in cases where multiple samples required analysis, it was not ideal to have increased run times and cool down times.

The problem is further exacerbated when researchers seek out increased selectivity from higher order instruments, such as GC×GC×GC systems; the trade-off between increased selectivity provided by the three separation dimensions and peak capacity reaching new heights.

One promising area of research to address these challenges is modulation technology, with a recent report using pulse flow valve modulation to produce more optimal separation conditions (2).

Building upon this report, researchers used pulse flow valve modulation with GC×GC and utilized the results to advise the use of pulse flow valve modulation in GC×GC×GC.

The results of the research suggest that using a high temperature diaphragm valve modulator in conjunction with a recently introduced pulse flow valve modulator enables researchers to take advantage of the increased selectivity of GC×GC×GC instruments while achieving peak capacity production approaching 1000 peaks/min.

Researchers also indicated that future studies will be focused on applying these instrumental improvements with mass spectral data to create an ultra-fast four-dimensional data structure to address complex separation mixtures.—L.B.

**References**


1. H.D. Bahaghighat *et al., J. Chroma. A* **1573**, 115–124 (2018).
2. C.E. Freye *et al., Talanta* **117**, 142–149 (2018).

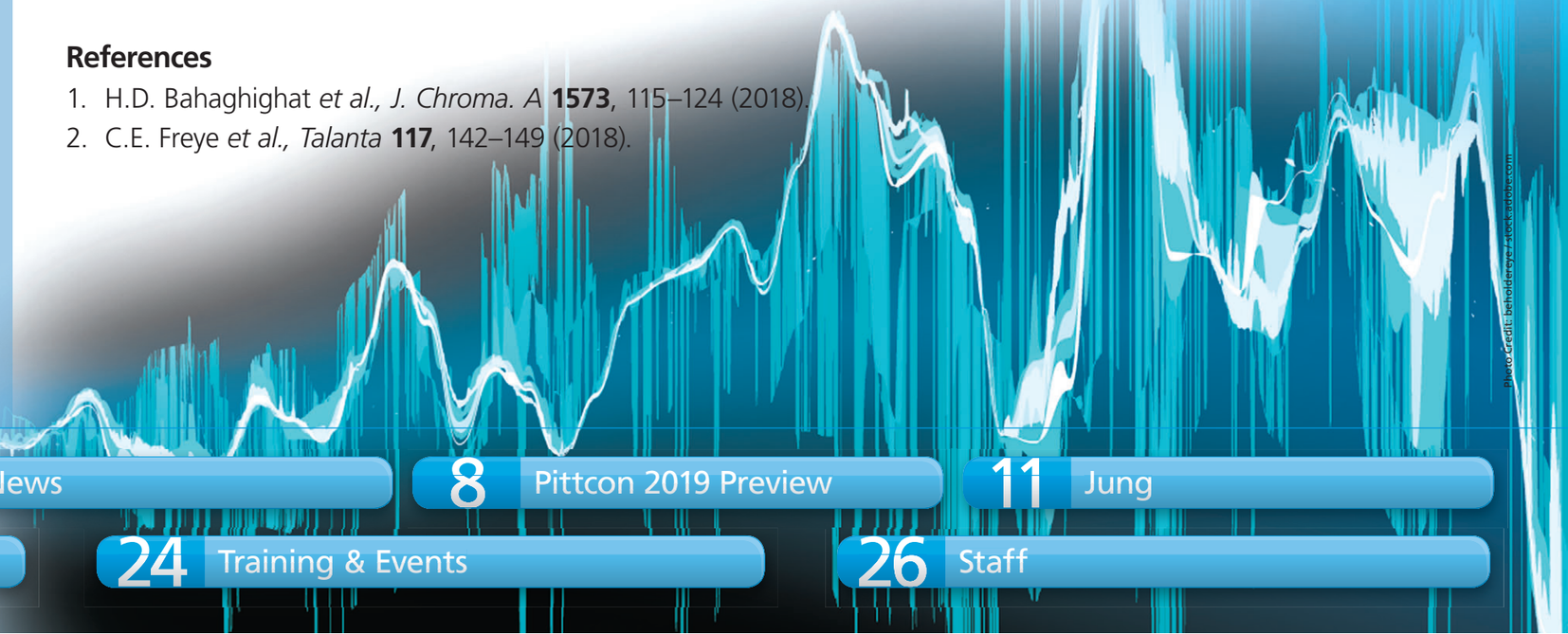



Photo Credit: beholdereye / stock.adobe.com

### Knauer Wins Employer Award

Knauer (Berlin, Germany) has been ranked as one of the top employers in Germany for 2019, according to a study by 3.works GmbH, a Düsseldorf-based employment research institute.

This is the second time Knauer has been honoured as one of the best employers in Germany. The study is based on a comprehensive employer evaluation system in Germany, which has evaluated the data of more than 70,000 companies. Criteria included independent certifications, ranking and awards, employee satisfaction ratings, student surveys, as well as activities in the areas of corporate social responsibility, diversity, and family friendliness.

"We are very pleased to have made it among the top one percent of employers this year. This award confirms that we are on the right track with respect to our human resources strategy and our activities for sustainability and social responsibility," said Alexandra Knauer, CEO of Knauer.

For more information about Knauer, please visit: **www.knauer.net**

For more information about 3.works GmbH, please visit: **www.leading-employers.de**

# Investigating the Effect of Kynurenic Acid in Alcoholic Beverages

Researchers from the Medical University of Lublin, Poland, have investigated the presence of kynurenic acid (KYNA) in alcoholic beverages and the significance of its presence using high performance liquid chromatography (HPLC) (1).

A metabolite of tryptophan, KYNA has many pharmacological properties, and its potential role in human physiology is a continued topic of interest for researchers. The discovery of its neuroprotective activity sparked imaginations in the scientific world before the discovery of its inability to penetrate the blood-brain barrier scuppered hopes of a pharmacological dream (2–6). Recent research has pointed to KYNA's interaction with receptors located on the outside of brain tissue with studies indicating anti-ulcerative (7), anti-inflammatory (8), antioxidant (9), analgesic (10), antimigraine (6), and metabolic activities (11).

The further discovery of KYNA's presence in food and its ability to be absorbed along the digestive tract has led to the possibility that KYNA may affect other bodily processes. One such process KYNA could impact is alcohol degradation because it is known to interact with alcohol metabolism through the inhibition of aldehyde dehydrogenase activity *in vivo* (12). This fact is particularly pertinent because of KYNA's presence within alcoholic beverages because it may influence alcohol degradation rate and relate to the intensity of alcohol's negative post-consumption attributes.

To investigate this premise, researchers used HPLC and fluorometric detection to determine the content of KYNA in a wide range of alcoholic beverages. Further tests determined the absorption and elimination rate of KYNA following beverage consumption in humans.

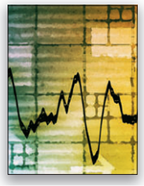
Researchers found KYNA in all studied beverages at varying rates with mead containing the most at 9.4 µg/100 mL and spirits containing the least at 0.01–0.1 µg/100 mL. Furthermore, it was found that KYNA was rapidly absorbed from the digestive tract with the highest blood concentration being identified 30 min after alcohol consumption. This confirmed the possibility of KYNA and alcohol interaction occurring within the human body following alcohol ingestion.—L.B.

## References


1. M. Turska *et al., Med. Hypotheses* **122**, 200–205 (2019).
2. C. Hilmas *et al., J. Neurosci* **21**, 7463–73 (2001).
3. A.H. Ganong and C.W. Cotman, *J. Pharmacol. Exp. Ther.* **236**, 293–299 (1986).
4. M.H. Mok *et al., Neuropharmacology* **57**, 242–249 (2009).
5. S. Fukui *et al., J. Neurochem* **56**, 2007–2017 (1991).
6. G. Olah *et al., Drug Des. Devel. Ther.* **7**, 981–987 (2013).
7. G.B. Glavin and C. Pinsky, *Res. Commun. Chem. Pathol. Pharmacol.* **64**, 111–119 (1989).
8. J. Kaszaki *et al., Neurogastroentrol Motil* **21**, 53–62 (2008).
9. R. Lugo-Huitron *et al., Neurotoxicol. Teratol.* **33**, 538–547 (2011).
10. F. Resta *et al., Neuropharmacology* **108**, 136–143 (2016).
11. L.Z. Agudelo *et al., Cell Metab.* **27**, 378–392 (2018).
12. A.A. Badawy *et al., Alcohol Alcohol.* **46**, 651–660 (2011).


# Peaks of the Month


- **The *LCGC* Blog: HPLC Diagnostic Skills–Noisy Baselines**—Just as medical practitioners are able to discern worrying features from a variety of medical physics devices (electrocardiogram, electroencephalogram, and ultrasound, for example), we need to develop the skill to identify worrying symptoms from our HPLC instrument output. **Read Here>>**




- **What's Trending in LC Troubleshooting?**—The challenges we face in troubleshooting problems with liquid chromatography (LC) separations are highly diverse. This month we take a closer look at topics that have garnered more attention recently. **Read Here>>**

- **Is Headspace Sampling Quantitative?**—Confusion exists on the quantitative nature of headspace sampling, because it is an equilibrium-based technique when done in the static mode, but not necessarily in the dynamic mode. To aggravate matters further, the concentrations of headspace compounds in common applications, such as foods, flavours, or petroleum distillates, can easily vary by an order of magnitude or more. Thus, what defines quantitative may depend largely on the goals of the analysis. This month we'll take a look at headspace sampling and its quantitative nature. **Read Here>>**


- **Tips & Tricks GPC/SEC: System Peaks or Ghost Peaks in GPC/SEC**—Extraneous peaks, unrelated to the solute to be characterized, are quite common in gel permeation chromatography/size-exclusion chromatography (GPC/SEC), especially when refractive index (RI) detection is used. This instalment of Tips & Tricks explains why system or ghost peaks appear and how to minimize their appearance. **Read Here>>**


- **The Quest for Greener Sample Preparation in Food Analysis**—When analytical chemists apply green chemistry approaches, which seek to minimize negative environmental effects, an important area of focus is reducing the consumption of toxic solvents, such as those used in extraction steps in sample preparation. Developing and testing greener extraction processes for food analysis is a major focus of Elena Ibáñez, a research professor at the Institute of Food Science Research in Madrid, Spain. **Read Here>>**

Like us Join us Follow Us

# News In Brief

Phenomenex has announced the opening of a new direct sales and support office in Taiwan. The direct presence in Taiwan will allow Phenomenex to provide immediate technical support locally, improved product availability, and faster delivery of chromatography products to scientists throughout the country. With the corporation's recent expansions into the Asia Pacific regions and the acquisition by Danaher Life Sciences in 2016, Phenomenex is now able to provide more support and solutions to its customers throughout Taiwan. For more information, please visit: **www.phenomenex.com**

Researchers have developed a high-performance thin-layer chromatography (HPTLC)-based method for natural deep eutectic solvents (NADES) extracts analysis. The method is capable of targeted and nontargeted analysis. Made mainly with abundant primary metabolites, NADES are being increasingly applied in green chemistry. One of the main difficulties encountered in the development of novel products and their quality control arises from their low vapour pressure and high viscosity. These features create the need for the development of new analytical methods.
DOI: 10.1016/j.chroma.2017.12.009

# Pittcon 2019 Preview

**Kimberly Palastro,** The Pittsburgh Conference, Pennsylvania, USA

**Pittcon celebrates its 70th year with thousands of chromatographers from around the globe and a range of industries taking over the Pennsylvania Convention Center in Philadelphia, Pennsylvania, USA, from 17–21 March 2019 for a week of education, research, instrumentation, and networking.**

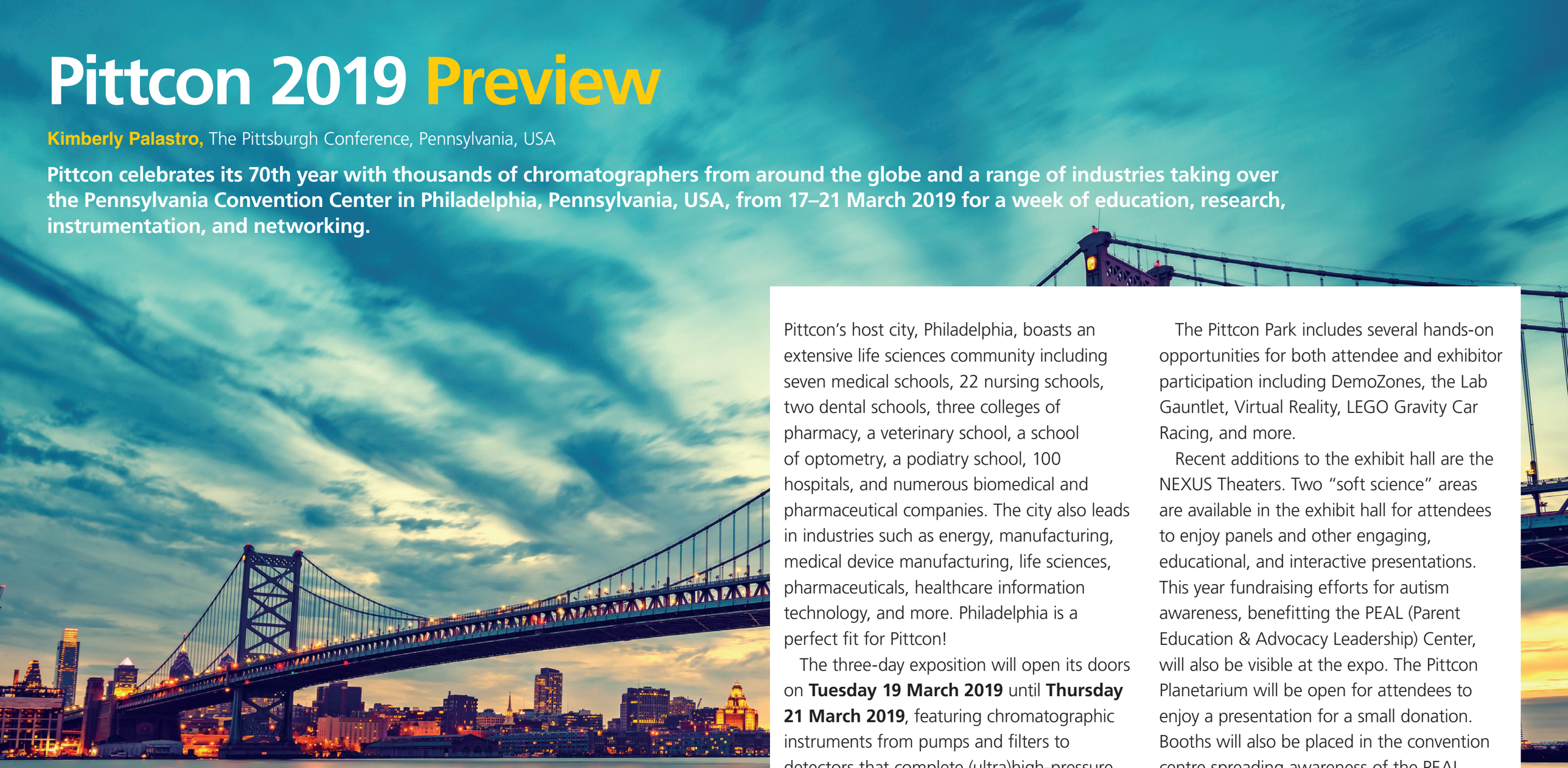


Photo Credit: sborisov/stock.adobe.com

Pittcon's host city, Philadelphia, boasts an extensive life sciences community including seven medical schools, 22 nursing schools, two dental schools, three colleges of pharmacy, a veterinary school, a school of optometry, a podiatry school, 100 hospitals, and numerous biomedical and pharmaceutical companies. The city also leads in industries such as energy, manufacturing, medical device manufacturing, life sciences, pharmaceuticals, healthcare information technology, and more. Philadelphia is a perfect fit for Pittcon!

The three-day exposition will open its doors on **Tuesday 19 March 2019** until **Thursday 21 March 2019**, featuring chromatographic instruments from pumps and filters to detectors that complete (ultra)high-pressure liquid chromatography (U)HPLC, solid-phase extraction (SPE), gas chromatography (GC), and ion chromatography (IC) systems. To allow attendees ample time to visit the exhibit hall, the organizers have created a technical programme- and short course-free afternoon on **Wednesday 20 March 2019**.

The Pittcon Park includes several hands-on opportunities for both attendee and exhibitor participation including DemoZones, the Lab Gauntlet, Virtual Reality, LEGO Gravity Car Racing, and more.

Recent additions to the exhibit hall are the NEXUS Theaters. Two "soft science" areas are available in the exhibit hall for attendees to enjoy panels and other engaging, educational, and interactive presentations. This year fundraising efforts for autism awareness, benefitting the PEAL (Parent Education & Advocacy Leadership) Center, will also be visible at the expo. The Pittcon Planetarium will be open for attendees to enjoy a presentation for a small donation. Booths will also be placed in the convention centre spreading awareness of the PEAL Center's mission, collecting donations, and selling items.

The technical programme will kick off on Monday 18 March at the Wallace H. Coulter Lecture with Nobel Laureate, Dr. Fraser Stoddart. He will present "Serendipity Stokes Discovery: Disrupting Established Industries".

On Tuesday evening, Dr. Fenella France from the Library of Congress will take the stage at the Plenary Lecture to speak about "Preserving and Revealing History—Challenges of a Cultural Heritage Scientist."

Pittcon's technical programme features a line-up of high-calibre speakers and hot topics in a range of industries and methodologies. Chromatographers may want to attend the following symposia:

- *Recent Advances in Two-Dimensional Liquid Chromatography—Theory and Practice*, organized by Dwight Stoll, will bring together experts in 2D-LC from both academic and industrial laboratories. Presentations will cover a wide range of topics, including polymer separations, separations of natural products, and separations of both small and large pharmaceutical molecules.
- *The Application of Analytical Chemistry in Biofuel Study*, organized by Yanhong Zhang, will showcase some novel approaches on how to characterize the feedstock, evaluate the conversion process, quantify the production yield, and provide specification of the biofuels. This is a great challenge for analytical chemists currently working in the USA because the main feedstock for the biofuel industry is starch and oil from agriculture production, and biomass is likely to play a more dominant role soon.
- *Advances in Capillary LC Separations*, organized by Stephen Weber, brings together researchers using capillary liquid chromatography in very creative ways to accomplish analytical goals. In these pursuits, capillary liquid chromatography becomes part of a larger analytical system. Such analytical systems, while requiring more attention to detail than analytical-scale systems, reward the analyst with better mass detection limits and increased capabilities because of their size and low volume requirements.
- *Automation in the Research and Development Laboratory: From Academia to Industry*, organized by Kaitlin Grinias and Marcelo Filgueira, will provide a concentrated forum for scientists from industry, pharmaceuticals, and academia to share and discuss the role of robotics in chemical research. It will also showcase how recent advances in automation are paving the way to exploit the benefits of machine learning.

The *LCGC* Lifetime Achievement and Emerging Leader in Chromatography Awards Symposia is another must-attend session. Milos Novotny, the 2019 Lifetime Achievement in Chromatography Award winner, was a pioneer in virtually all capillary separation techniques, including capillary GC, LC and supercritical fluid chromatography (SFC), and capillary electrophoresis (CE) and capillary electrochromatography (CEC) of peptides and carbohydrates. He also developed important LC–MS and CE methodologies in glycomics and glycoproteomics. Ken Broeckhoven, the 2019 Emerging Leader in Chromatography Award recipient, has advanced chromatography fundamentals in areas such as applying the kinetic plot method to gradient LC, addressing viscous heating effects in UHPLC, using very high operating pressures, and researching turbulent flow in SFC.

Editorial director of *LCGC*, Laura Bush, will moderate a special session of talks presented by major figures in the separation science sector, including the two award winners, on Tuesday 19 March at 13:30 in Room 125.

In addition to the technical programme and exposition, the continuing education short course programme is an integral part of the conference for chromatographers looking for courses to increase their job productivity or to improve their skill set. Pittcon has a variety to choose from. The classes are taught by industry experts and the class sizes are small to allow a more

personalized educational experience. Chromatographers may find value in the following:

- *GC & GC–MS Troubleshooting & Maintenance*, instructed by Diane Turner. The course has been approved by the Royal Society of Chemistry for purposes of Continuing Professional Development (CPD). The course will cover parts of the system requiring maintenance, how often and how to identify when maintenance is required, preparing for problems, how to identify problems, and the troubleshooting process. This knowledge is then put to the test in troubleshooting chromatograms.
- *Cannabis Analytics*, instructed by Ganesh Moorthy. This course will cover a brief history of cannabinoids, isolation and identification of cannabinoids, analysis of cannabinoids in cannabis, analysis of cannabinoids in biological samples (blood, plasma, and urine), and recent developments and future directions of cannabinoid and terpene analysis.
- *Analytical Sampling and Sample Preparation for Chromatography*, instructed by Doug Raynie. This short course is designed to provide participants with an in-depth understanding of the role of sampling and sample preparation in analytical chemistry, particularly related to chromatography. Upon successful completion of the course, the participant will have an understanding of sampling consideration and approaches, and sample preparation strategies.
- *Mastering Gas Chromatography*, instructed by Lee Polite. In this class, a firm understanding of the fundamentals of GC will be gained and the fundamentals applied to daily analyses. Students will learn how to develop a method from scratch, cut analysis time at least in half, maybe even by a factor of 10 (without affecting the resolution), improve sensitivity by 10–1000-fold, improve separations, and explain what GC is and how it works.

Another stop on an attendee's agenda should be a networking session. These moderated, roundtable discussions on a particular topic offer a relaxed environment to brainstorm with peers and ask questions in an open forum-type environment. Consider stopping by one of these discussions:

- *Hot Topics and Adoption of Comprehensive GC (GC×GC)*, facilitated by Lorne Fell and Joe Binkley, will focus on success stories of GC×GC, as well as sharing information on best practices for success. The discussion will cover the types of GC×GC (thermal or flow) and the choices for detection that are used today.
- *Smarter and More Automated LC Systems?* facilitated by Thomas Swann and Sebastian Rakus. LC systems are powerful analytical tools, but should they be smarter and more automated? This session will discuss what is needed in smarter and more automated LC systems, how they should be interacted with, and how they might help us do our jobs better.
- *Mobile Phase Selection for LC–MS Analysis* facilitated by Subhra Bhattacharya and Stephen Roemer. With the advances in instrument sensitivity, demand for interference-free mobile phase is increasing. Selection and optimization of a mobile phase is critical for the overall performance of LC–MS. A limited number of mobile phases are compatible with LC–MS. It is challenging to select the correct mobile phase to achieve the separation of a complex mixture and identify the separated components.
- *Novel Approaches to Undergraduate Chromatography Education*, facilitated by Amber Hupp and Michelle Kovarik, will bring together users and educators from the separations community. Share ideas and help to build connections among academic, industry, and government sectors.

Pittcon can be a bit overwhelming to newcomers, and even the seasoned attendee! In order to make the planning process as simple as possible, there are an array of tools for attendee use. The most convenient is the Pittcon 2019 mobile app. The app can be downloaded for free and can search the programme, short courses, and exhibitors, view the exhibit hall and convention centre maps, add sessions to the user agenda, favourite and take notes, and much more. There is also an online search feature available that syncs with the mobile app on the Pittcon website.

Registration for Pittcon 2019 is now open to celebrate 70 years of bringing the analytical chemistry community together.

**Contact:** Kimberly Palastro
**E-mail:** publicity@pittcon.org
**Location:** Philadelphia Convention Center, Philadelphia, Pennsylvania, USA
**Registration:** www.pittcon.org/register

**Stay Connected with *LCGC***

The *LCGC* team will be at Pittcon 2019 (Booth 1307), bringing you the latest news and updates from around the event. Tweet us at @LC_GC to keep up-to-date or to send us your personal conference highlights.

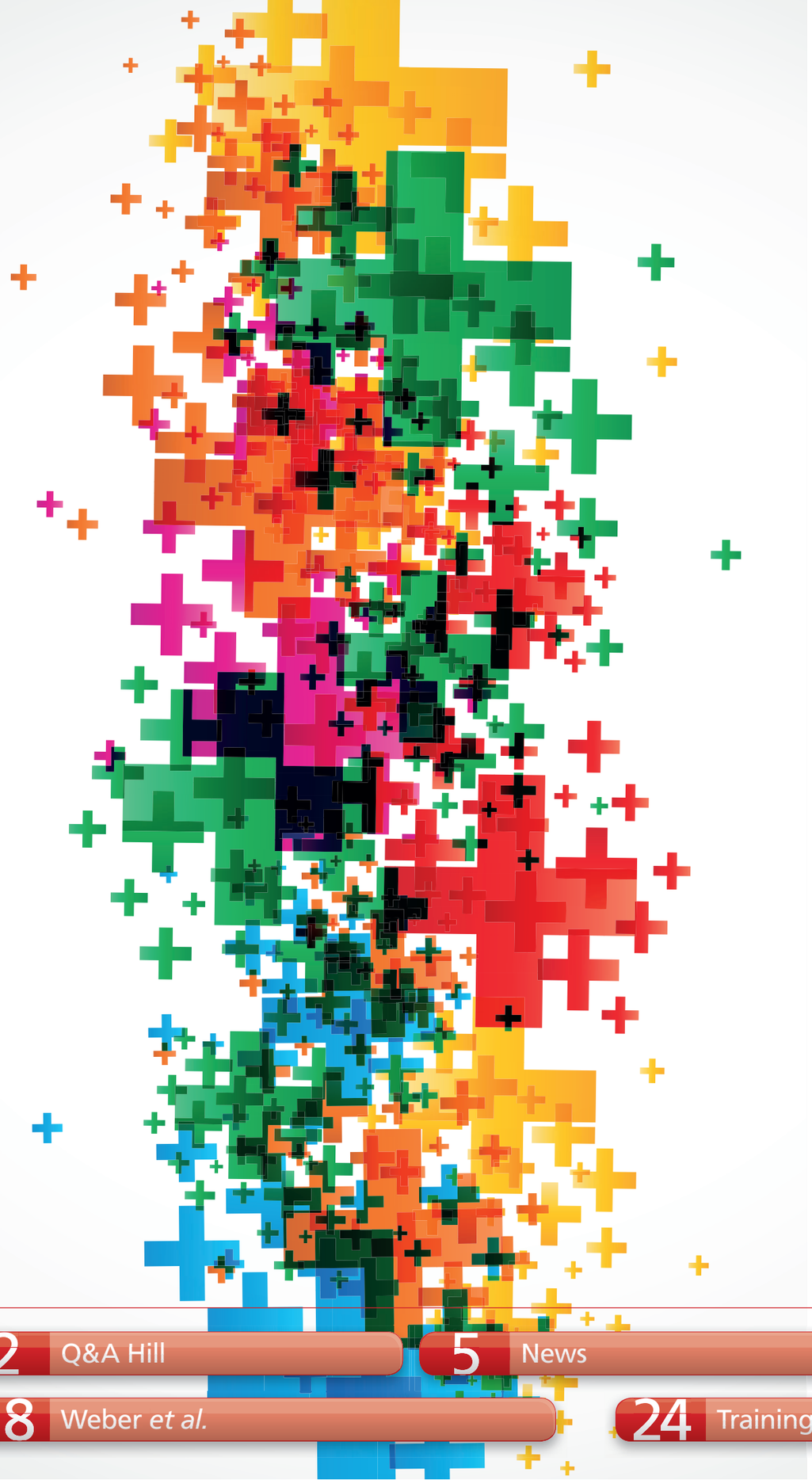


Photo Credit: burak çakmak/stock.adobe.com

# Strategies to Improve Recoveries of Proteins and Peptides from Sample Containers Before LC–MS Analyses

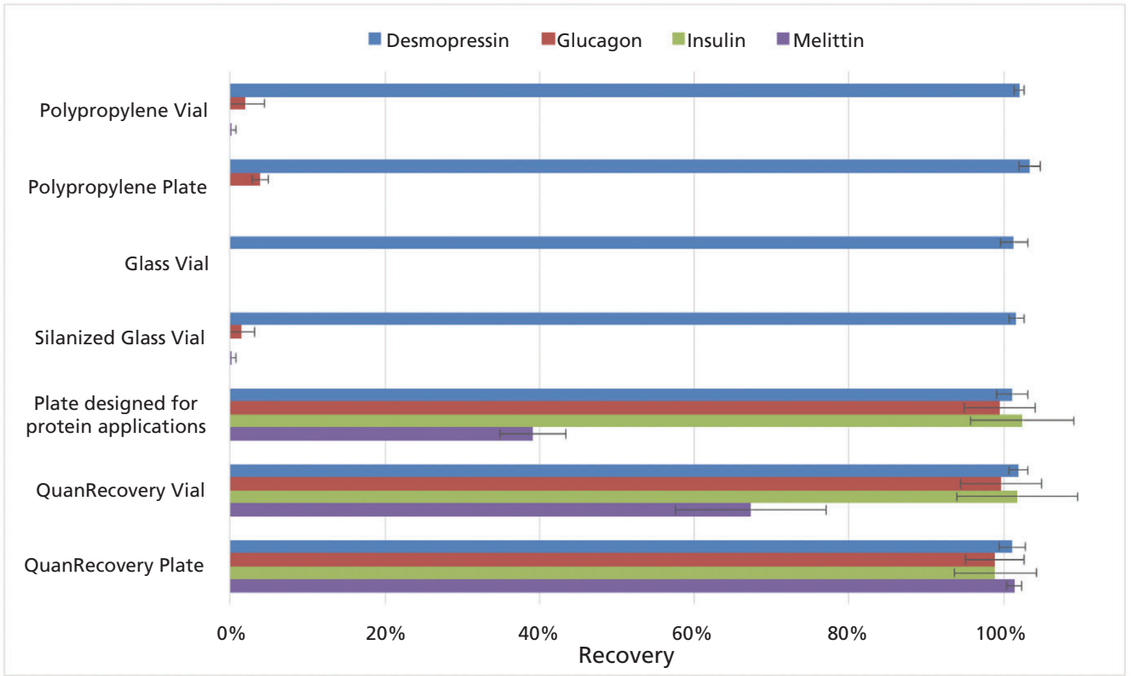
**Moon Chul Jung,** Waters Corporation, Milford, Massachusetts, USA

**This article describes how to prevent the loss of sample analytes by understanding the factors responsible for poor recoveries.**

Successfully quantitating proteins and peptides with liquid chromatography–mass spectrometry (LC–MS) is often more challenging than a similar task for small molecules. Their large and complex structure not only requires a different approach to LC–MS method development, but may also cause other unique problems such as nonspecific adsorption (or nonspecific binding) (1). Proteins and peptides in solution tend to stick to the surfaces they encounter, such as the LC–MS fluidic path, LC column packings, and even sample containers (2,3). Such losses do not just negatively impact the assay sensitivity, but also compromise the reproducibility, precision, and accuracy of the analysis.

Small molecules may stick to these surfaces as well, but their surface adhesion is typically driven by a single attraction mechanism and can be dealt with in a straightforward manner. For example, one can choose a polymeric container for

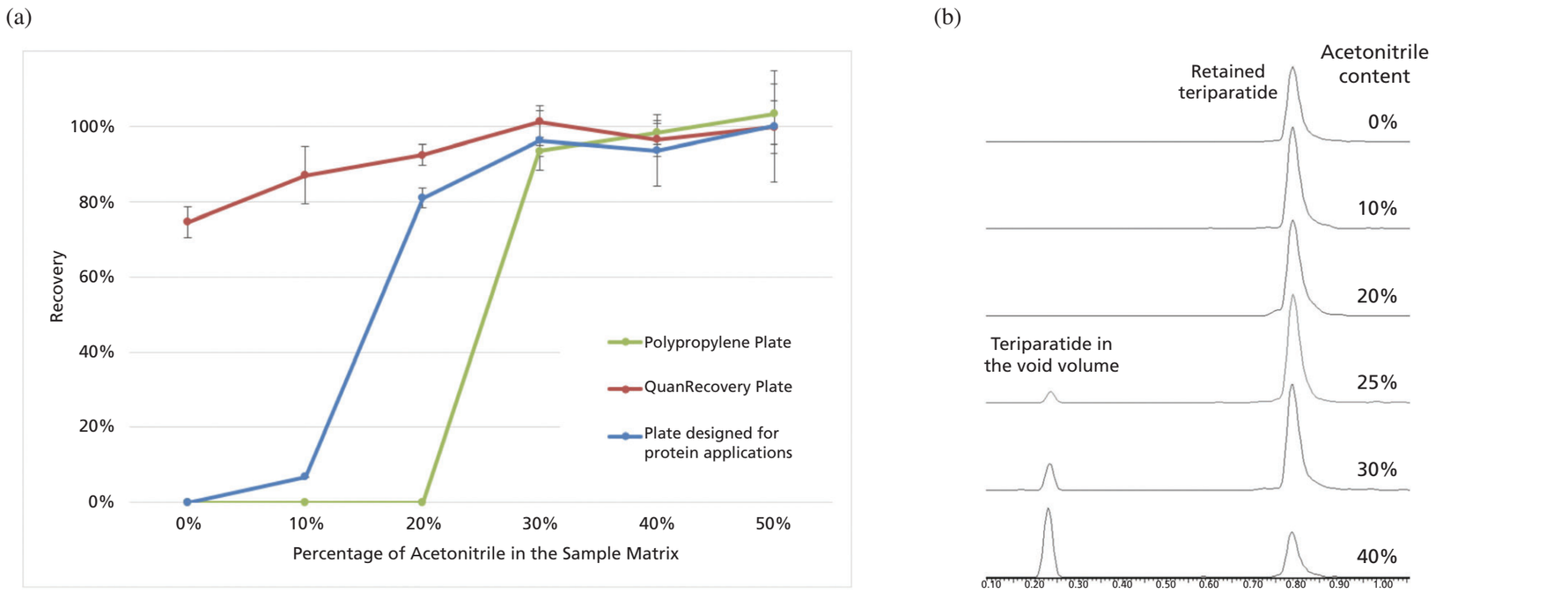
**Figure 1:** Average recovery (n = 4) of four peptides (1 ng/mL per peptide) after 24 h of storage at 4 °C. The error bars show the standard deviations. Peptides solutions were prepared in 80:20 water–acetonitrile solution acidified with 0.2% trifluoroacetic acid (TFA).




a highly basic analyte, instead of a glass container that bears acidic silanol groups on the surface. For proteins and peptides, however, the picture is more complicated. They may form multiple interactions with exposed surfaces through various chemical attraction mechanisms. The more proteins or peptides the sample contains, the bigger the challenge is. It is therefore not trivial to find a single sample storage condition that prevents the loss of all proteins or peptides of interest.

A widely used alternative approach to avoid these losses is to add blocking agents, such as surfactants or carrier proteins (4,5), to the sample, or preconditioning the surfaces (2). Preconditioning is an extra step that

**Figure 2:** (a) Average recovery (n = 4) of 1 ng/mL teriparatide after 24 h of storage at 4 °C. The error bars show the standard deviations. The peptide solutions were prepared in various water–acetonitrile mixtures and acidified with 0.2% TFA. After 24 h of storage, the samples were diluted with appropriate water–acetonitrile mixtures to adjust the acetonitrile content to 20% before injection. The teriparatide peak quantitation, and thus the recovery calculation, was not compromised by poor retention. (b) Example chromatograms of teriparatide. Teriparatide samples were prepared in sample matrices with varied water–acetonitrile ratio, and were injected without dilution.




often produces irreproducible results. Although highly effective in general, adding blocking agents to the sample creates more complexity in downstream LC–MS analyses by presenting extra peaks in chromatograms or MS spectra or by inducing ion suppression or enhancement. Therefore, using blocking agents is not always the best tactic unless it is absolutely necessary. This article will review the experimental parameters that affect peptide recovery the most and offer advice for maximizing recovery without using blocking agents.

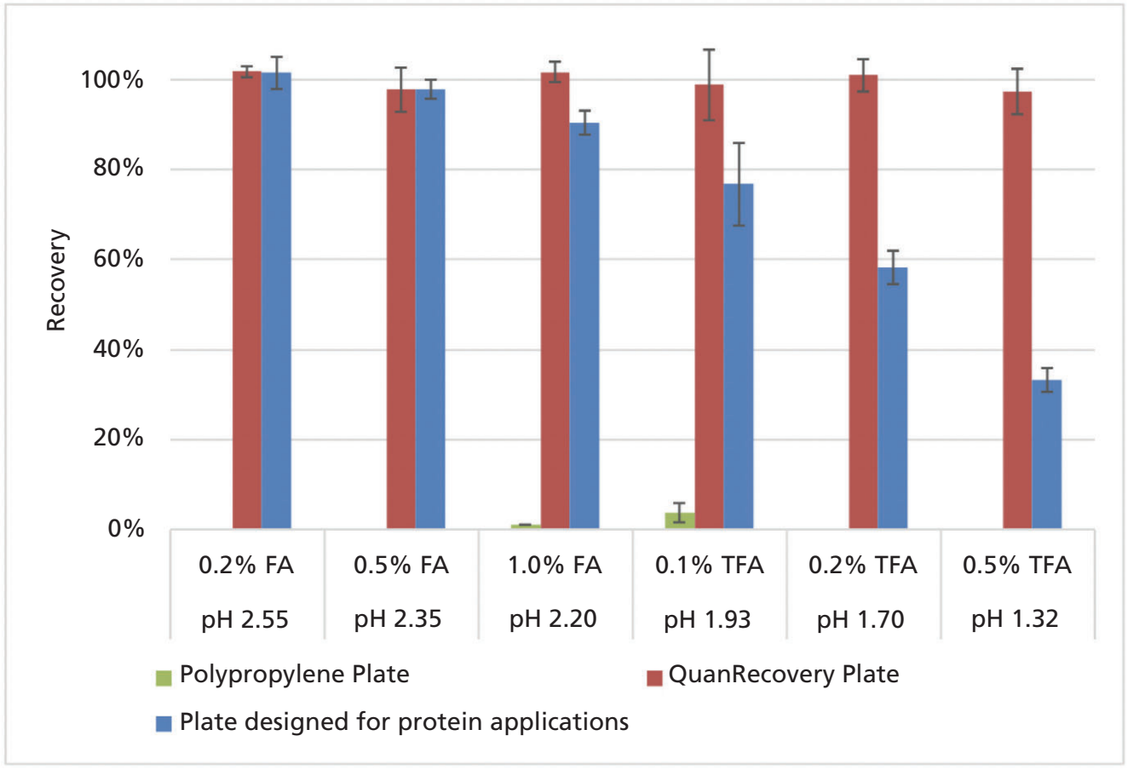
## Methods

Peptide solution standards in the concentration range of 20 pg/mL to 100 ng/mL were prepared in various sample matrices and stored in several commercially available sample containers prior to LC–MS analysis. To accurately determine the recovery of challenging peptides, solutions containing carrier proteins were used as recovery reference solutions: the solutions were prepared in groups, with and without 0.1% rat plasma, and the peptide recovery was calculated by comparing the peptide peak area from the solution that did not contain the blocking agent to the reference peak area. Peptides in each sample were separated using a 2.1 × 50 mm, 1.6-µm charged surface solid core $C_{18}$ column (Waters) on a low dispersion LC system (Acquity I-Class System, Waters) with a water–acetonitrile linear gradient, each with 0.1% formic acid, and detected using a tandem quadrupole MS (Xevo TQ-S, Waters) in the selective reaction monitoring (SRM) mode. To understand the role of the container's surface properties on peptide recovery, polypropylene vials and 96-well plates, QuanRecovery polypropylene vials and plates (all Waters), and commercially available plates designed for protein applications were evaluated. Other experimental conditions, such as the composition of the peptide sample and peptide concentration, were varied to clearly highlight how these experimental factors affected peptide recoveries.

## Results and Discussion

Polypropylene sample containers are commonly used for various LC–MS applications because of their good chemical compatibility and a

**Figure 3:** Average recovery (n = 4) of melittin after 75 h of storage at 10 °C. The error bars show the standard deviations. The peptide solutions were prepared in an 80:20 water–acetonitrile mixture and acidified with formic acid (FA) or trifluoroacetic acid (TFA) while varying the volume (*v/v*). The pH of the solutions were experimentally measured.

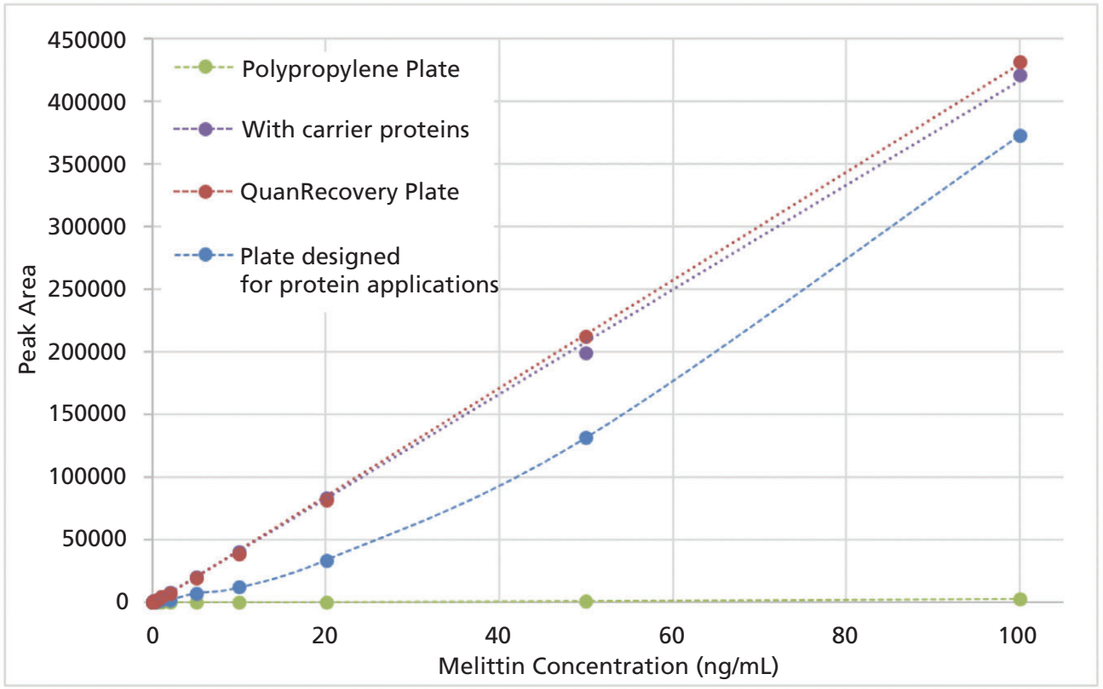



wide selection of shapes and sizes. Polypropylene 96-well plates have been particularly popular in high-throughput applications with the availability of multichannel pipettes and robots. Another reason they are used frequently with protein or peptide samples is that they are less likely to induce analyte loss from ionic attractions than glass containers. Any analyte lost on the polypropylene containers, when it occurs, is likely a result of hydrophobic interactions.

## The Influence of Materials of Construction on Peptide Recovery

Figure 1 compares the recovery of four peptides that were prepared in a typical LC–MS diluent and stored in various sample containers. The recoveries of the peptides correlated well with their relative hydrophobicity. The least hydrophobic peptides produced the highest recovery values while the most hydrophobic peptides showed the lowest recovery values. All containers showed complete recovery of desmopressin, the least hydrophobic peptide (MW 1069, HPLC index [6] 16.8). Polypropylene containers, glass, and deactivated (silanized) glass showed little or no recovery for three hydrophobic peptides: glucagon (MW 3482, HPLC index 86), bovine insulin (MW 5734, HPLC index >120), and melittin (MW 2846, HPLC index 124.4). Of the three peptides, melittin showed the most drastic recovery difference among the tested containers. A commercially available plate for protein applications showed higher recovery than the polypropylene and glass surfaces, but with some mixed results. From the results shown, the loss of peptides in the polypropylene containers depended on their relative hydrophobicity. Next, how much recovery is influenced by the elution strength of the sample matrix was examined.

**Figure 4:** Calibration curves for melittin in the concentration range of 20 pg/mL to 100 ng/mL. The calibration standards were prepared with 80:20 water–acetonitrile + 0.2% TFA in each sample container by serial dilution. To obtain the "true" calibration curve without suffering from the analyte loss, one set of calibration standards were prepared with 0.1% rat plasma as carrier proteins. All other standards were prepared without carrier proteins.




### The Effect of Sample Solvent on Recovery

Figure 2(a) shows the recovery of teriparatide, another hydrophobic peptide (MW 4118, HPLC index 90.4), in sample matrices with varied acetonitrile content. In general, recoveries improved as the sample matrix contained more acetonitrile, while the minimum acetonitrile concentration that led to full recovery was not the same for different sample containers. Using a highly organic sample matrix appeared to be the easiest and most effective way to completely recover hydrophobic peptides. Teriparatide solutions prepared with 30% or more acetonitrile could be stored in any of the three containers without the risk of analyte loss. While quite effective, this approach doesn't always work. For LC–MS, analytes in samples prepared in highly organic injection solutions may not retain well on the chromatographic column.

Figure 2(b) shows the disrupted retention of teriparatide as the acetonitrile concentration in the sample matrix was increased. When the acetonitrile percentage in the sample matrix was equal to or greater than 25%, teriparatide breakthrough peaks were observed in the void volume. It was thus necessary to prepare teriparatide samples with less than 25% acetonitrile to achieve good chromatography. However, limiting the concentration of acetonitrile to less than 25% with the polypropylene plate or the plate designed for protein applications causes the teriparatide to be lost on the container surface (Figure 2[a]). The same sample can be stored in a plate with a proprietary surface designed for minimizing hydrophobic nonspecific adsorption without the risk of analyte loss.

### How Acidic Additives Affect Peptide Recovery

The choice of the acidic additive in the sample matrix also affects the peptide recovery. Figure 3 shows the recovery of melittin after 75 hours of storage. Using a weak acid such as formic acid at a low concentration helped increase the melittin recovery, but the increase was not as drastic as changing the acetonitrile concentration in the sample matrix. Melittin was never recovered from the polypropylene plate by changing the additive only, whereas it was completely recovered from plates with proprietary surfaces designed for minimizing hydrophobic nonspecific adsorption regardless of the additive type and concentration. When using a plate that may have an intermediate surface binding activity, it is advised to monitor the recovery using various additives in different concentrations during method development, as highlighted by the recovery changes shown in Figure 3. It should also be noted that the choice of the acidic additives has an influence on the peak shape in the downstream LC–MS analyses (7), although the effect was not as drastic as the additives in the mobile phase. Formic acid in the sample, being a volatile additive, gave a stronger MS signal, but led to poor chromatographic peak shapes compared to trifluoroacetic acid.

### The Influence of Sample Volume, Amount of Time in Storage, and Peptide Concentration on Peptide Recovery

To complicate matters further, factors such as sample volume and storage time also influence the peptide recovery. In

general, the smaller the sample volume and the longer the sample was stored in the container, the poorer the recovery (data not shown). In practice, however, these factors may be difficult to control. For example, the length of time a sample spends in storage is often dictated by instrument availability, the number of samples in the queue, and the LC–MS run time. On the other hand, sample volume is limited by sample availability and the sample prep protocol. Therefore, it is advised that these factors should be used only as guidelines during method development, but not as the experimental parameters to increase peptide recovery.

Peptide concentration is another variable that affects recovery but cannot be independently controlled. It is particularly problematic when constructing a calibration curve. Figure 4 shows the calibration curves for melittin using various sample containers. To illustrate the ideal calibration curve that was not affected by the peptide loss, one sample set was prepared with 0.1% rat plasma as carrier proteins. Peptide loss may be easily identified from the shape of the calibration curve. Linear calibration curves were obtained when no peptide loss occurred on the sample container. However, in some cases, the peptide loss was so severe that the calibration curve could not be constructed at all. Even when peptide loss was not so severe, it was impossible to construct a calibration curve: the trace formed a concave curve of which the curvature depended loosely on the severity of the peptide loss. This curve showed the nonlinear relationship between the peptide loss and the concentration and would not fit a linear model.

## Conclusions

Analyte losses in sample containers is a significant problem in peptide quantitation and is often not recognized early enough. Failure to mitigate it can lead to hours of wasted time during method development, or even worse, to suboptimal methods that are limited by poor sensitivity and reproducibility. Peptide losses can be reduced by optimizing the sample matrix based on the chemical properties of the analyte of interest and the surface of the container. It was observed that the most effective way to reduce the loss of hydrophobic peptides was to change the organic solvent content of the sample. However, there were limits to this approach. When the organic solvent content was above a certain level, there was an increased risk of poor chromatography, while below a certain level there was an increased risk of low recovery. We found that the types and concentrations of the acidic additive in

the sample matrix also influenced peptide recovery.

Using a container with a low surface binding property proved to be a useful complementary option. Unlike standard containers, the inert surface of low bind containers protects against peptide loss. The use of low bind containers and an LC–MS-friendly sample matrix greatly simplifies the process of selecting the optimal storage conditions without compromising peptide recovery.

## References


1. B. Bobaly, E. Sipko, and J. Fekete, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **1032**, 3 (2016).
2. K. Kristensen, J.R. Henriksen, and T.L. Andresen, *PLoS One* **10**, e0122419 (2015).
3. M. Goebel-Stengel, A. Stengel, Y. Tache, and J.R. Reeve, Jr., *Anal. Biochem.* **414**, 38 (2011).
4. K. Stejskal, D. Potesil, and Z. Zdrahal, *J. Proteome Res.* **12**, 3057 (2013).
5. K. Maes, I. Smolders, Y. Michotte, and A. Van Eeckhaut, *J. Chromatogr. A* **1358**, 1 (2014).
6. C.A. Browne, H.P.J. Bennett, and S. Solomon, *Anal. Biochem.* **124**, 201 (1982): cited HPLC index values were calculated using Waters BioLynx software.
7. D.V. McCalley, *J. Chromatogr. A* **1038**, 77 (2004).



**Moon Chul (Moon) Jung is Principal Scientist at Waters Corporation, Chemistry R&D. After receiving his Ph.D. in analytical chemistry from the University of Pittsburgh, Moon joined Waters to conduct various research and development projects including sample prep and handling devices, microfluidic columns, and novel separation media.**

E-mail: moon_chul_jung@waters.com
Website: www.waters.com/quanrecovery

# Analysis of Dioxins in Foods and Feeds Using Gas Chromatography Tandem Mass Spectrometry

**Waldemar Weber[1], Masato Takakura[1], Thomas Lehardy[2], and Philippe Marchand[2],** [1]Shimadzu Europa GmbH, Duisburg, Germany, [2]LABERCA, Nantes, France

**In the early days of dioxin analysis, applied methods were laboratory- and time-consuming. Only gas chromatography–high resolution mass spectrometry (GC–HRMS), which is complicated, was used. Nowadays, GC–MS/MS is suitable for control proposes. Using GC–MS/MS means that solvent consumption for sample preparation can be reduced by a factor of 10 and the purity of the obtained fraction can be enhanced, indicating that GC–MS/MS is appropriate for dioxin analysis.**

Photo Credit: Piman Khrutmuang/stock.adobe.com

Persistent organic pollutants (POPs) are semivolatile and can be detected worldwide, even in remote regions. They bioaccumulate with potential negative influence on the environment and human health. To a varying degree, these organic compounds resist photolytic, biological, and chemical degradation.

POPs in foods and feeds can be analyzed with several methods. Dioxins are particularly toxic, even for POPs, so quantitative analysis is required down to low concentrations. Until recently, the analysis of dioxins was performed using gas chromatography–high resolution mass spectrometry (GC–HRMS), which provides highly accurate quantitation. However, triple quadrupole GC–MS/MS is less expensive and easier to handle than GC–HRMS, so its use is increasingly being investigated.

In recent years, the quantitative accuracy of GC–MS/MS has improved significantly. Accordingly, the use of this analysis method has been officially recognized in the EU (EU589/2014, 644/2017). However, in order to change from GC–HRMS to GC–MS/MS, it is necessary to compare their respective quantitative abilities.

In this article, dioxins (polychlorinated dibenzo-p-dioxin [PCDD] and polychlorinated dibenzofuran [PCDF] only) were analyzed in 44 types and 200 samples of foods and feeds using GC–MS/MS. Additionally, GC–MS/MS analysis results were compared with those from GC–HRMS in order to evaluate the quantitative capabilities of both techniques.

## Experiment

For the various food samples, pretreatment was performed using an automatic

pretreatment unit (extraction: Speed Extractor [Buchi]; purification: GO-xHT [Miura Co., Ltd.]).

**System Configuration:** Autosampler: AOC-20i/S; GC–MS/MS system: GCMS-TQ-8050 NX; software: GCMSsolution Ver 4.45 SP1, LabSolutions Insight Ver. 3.2 SP1, GC–MS/MS method package for dioxins in food (all Shimadzu).

**Analytical Conditions (Autosampler):** No. of rinses with solvent (pre-run): 3; no. of rinses with solvent (post-run): 3; no. of rinses with sample: 0; washing volume: 6 µL; injection volume: 2 µL; viscosity comp. time: 0.2 s.

A 10-µL measure of nonane was used as final solvent for the samples. For the standard, a mixture of DF-ST and DF-LCS, commercially available standard mixtures, from Wellington Laboratories was used. In terms of the analytical conditions for GC–MS/MS, the conditions registered in the method package were used.

**Analytical Conditions (GC):** Insert liner: Topaz single gooseneck liner with wool; column: 60 m × 0.25 mm, 0.25-µm SH-Rxi-5Sil MS (Shimadzu); injection mode: splitless; sampling time: 1.00 min; injection temperature: 280 °C; column oven temperature: 150 °C (1 min) > (20 °C/min) > 220 °C > (2 °C/min) > 260 °C (3 min) > (5 °C/min) > 320 °C (3.5 min); HP injection: 450 kPa (1.5 min); flow control mode: linear velocity (45.6 cm/s); purge flow: 20 mL/min; carrier gas: helium.

**Analytical Conditions (MS):** Ion source temperature: 230 °C; interface temperature: 300 °C; detector voltage: 1.8 kV (absolute).

## Results

**Analysis Results for the Standards:** In the analysis of dioxins in foods, maximum levels (MLs) are prescribed for each sample. With the food and feed samples in this investigation, MLs for pig fat and pig meat were the lowest at 1 pg/g of fat. In addition, the limit of quantitation (LOQ) required for each compound in the analysis depends on the sample's ML, the pretreatment method, and the toxic equivalence factor (TEF) of each compound. The compounds 2,3,7,8-Tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-Pentachlorodibenzo-p-dioxin have the highest TEF (TEF = 1), requiring lower LOQs than other compounds. In this investigation, the LOQ for both dioxins in pig fat and pig meat was 0.060 pg/µL at the concentration in the final vial.

**Signal-to-Noise (*S/N*) Ratio ("Method 1"):** The concentration of an analyte in the extract of a sample that produces an instrumental response at two different ions

**Figure 1:** GC–MS chromatograms for a concentration of 0.050 pg/µL.

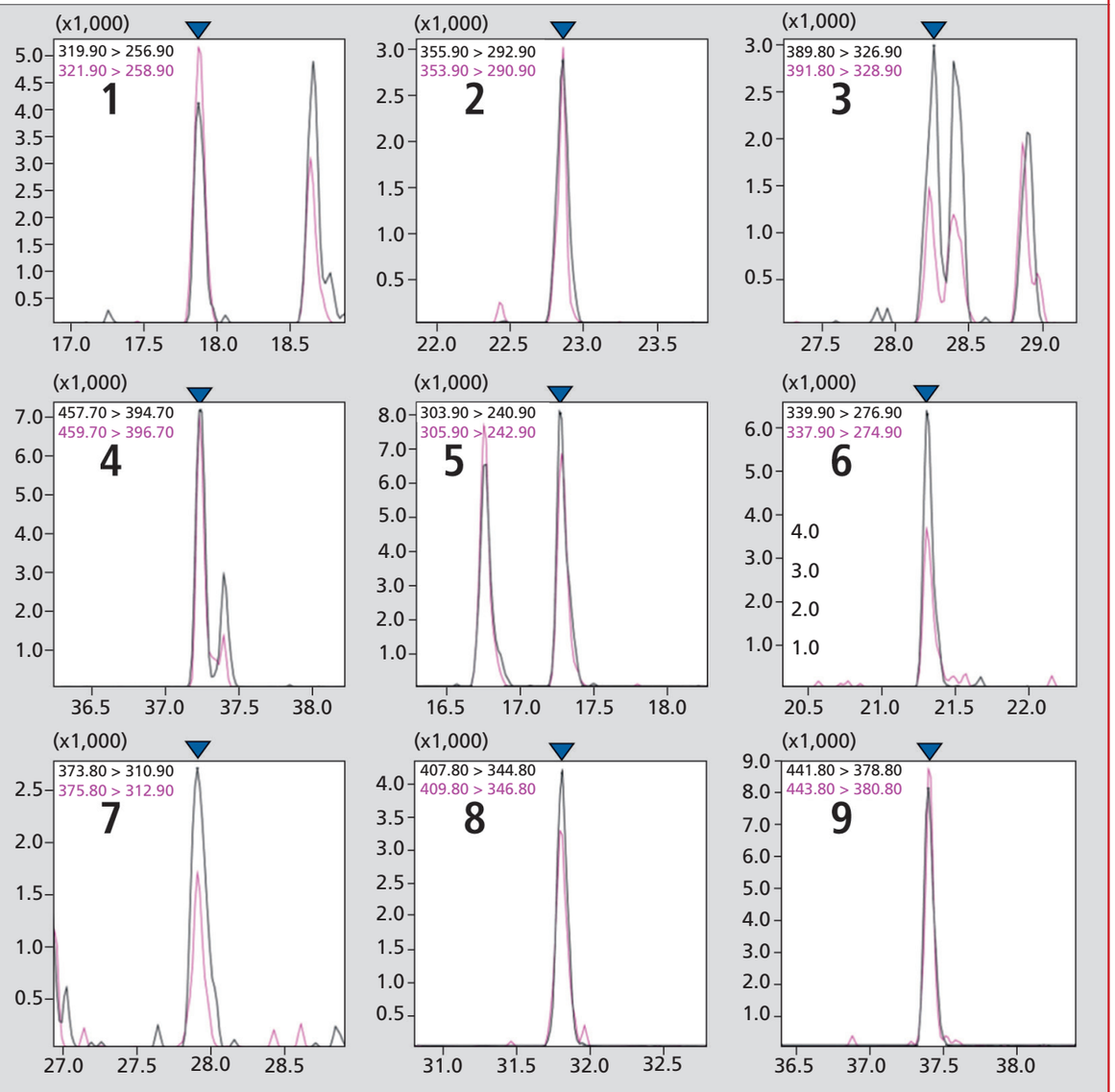



**Table 1:** *S/N* results for standards according to Method 1

| No. of Compounds in Figure 1 | Compound Name | Calculated *S/N* |
|---|---|---|
| 1 | 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 285 |
| 2 | 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 1658 |
| 3 | 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin | 396 |
| 4 | Octachlorodibenzo-p-dioxin | 2518 |
| 5 | 2,3,7,8-Tetrachlorodibenzofuran | 2117 |
| 6 | 1,2,3,7,8-Pentachlorodibenzofuran | 1882 |
| 7 | 1,2,3,7,8,9-Hexachlorodibenzofuran | 546 |
| 8 | 1,2,3,4,6,7,8-Heptachlorodibenzofuran | 1784 |
| 9 | Octachlorodibenzofuran | 4282 |

to be monitored with a *S/N* ratio of 3:1 for the less intensive raw data signal.

**Lowest Concentration Point on the Calibration Curve ("Method 2"):** The lowest concentration point on a calibration curve that gives an acceptable (≤ 30%) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation from the average relative response factor calculated for all points on the calibration curve in each series of samples. In this article, for the purposes of confirmation, an evaluation was performed using both criteria.

As noted above, for 2,3,7,8-Tetrachlorodibenzo-p-dioxin it is necessary to set the LOQ to 0.060 pg/µL or less. Accordingly, the prepared standard solution (STD) was prepared so that the concentration of each compound was 0.050 pg/µL (for Octachlorodibenzo-p-dioxin and Octachlorodibenzofuran 0.100 pg/µL). From the results of the analysis, it was evident that the criteria for Method 1 were satisfied for all compounds. *S/N* ratios for each compound are shown in Figure 1 and Table 1.

With Method 2, a calibration curve was created with all six levels used, including 0.025 pg/µL, 0.050 pg/µL, 0.100 pg/µL, 0.250 pg/µL, 0.500 pg/µL, and 1.000 pg/µL. The concentrations for each compound

**Figure 2:** Comparison of the TEQ results for each food and feed.

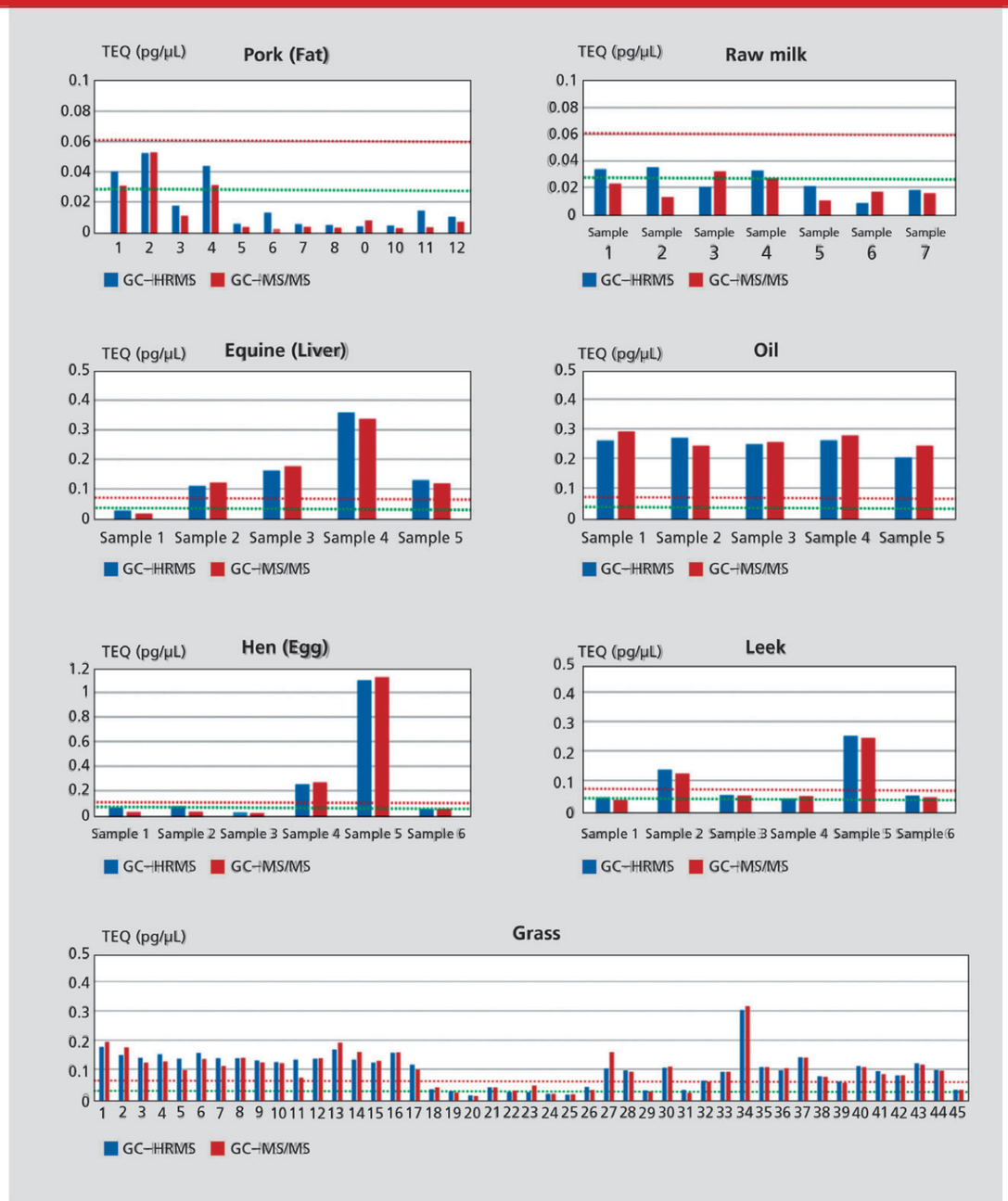


**Figure 3:** Summarized comparison of the TEQ results for triple quadrupole (TQ) and HRMS.

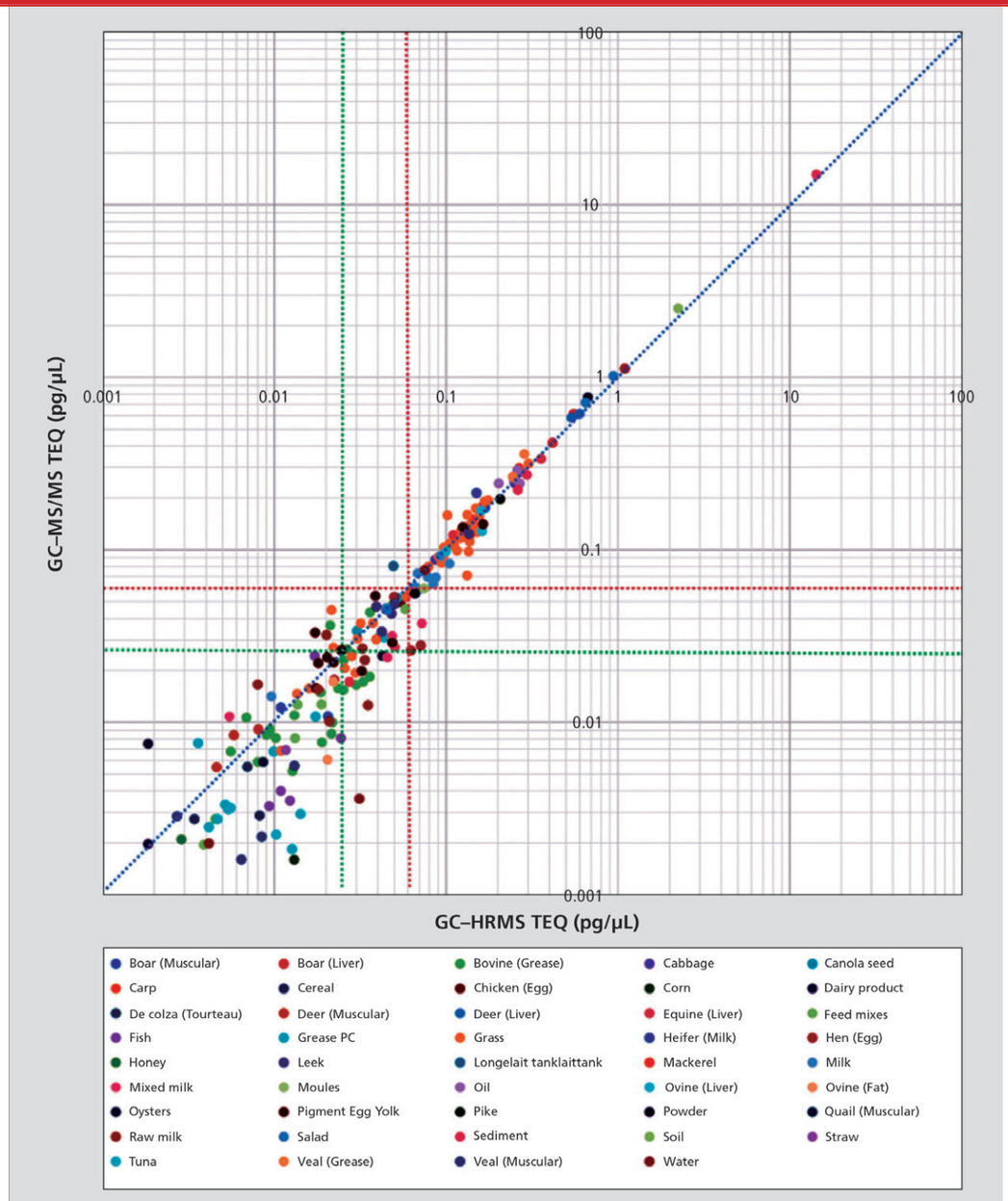



**Table 2:** Each calibration point concentration and RRF for the measured compounds

| Compound Name | TEF | Avg RRF | RRF (lvl 1) | RRF Dev (%) lvl 1 |
|---|---|---|---|---|
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 1 | 1.07 | 1.15 | 8.1 |
| 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 1 | 1.09 | 0.97 | 10.56 |
| 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin | 0.1 | 1.14 | 1.39 | 22.26 |
| 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin | 0.1 | 0.95 | 0.92 | 2.72 |
| 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin | 0.1 | 1.03 | 1.25 | 21.44 |
| 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin | 0.01 | 0.92 | 0.82 | 11.46 |
| Octachlorodibenzo-p-dioxin | 0.0003 | 1.19 | 1.04 | 12.21 |
| 2,3,7,8-Tetrachlorodibenzofuran | 0.1 | 1.10 | 1.05 | 4.66 |
| 1,2,3,7,8-Pentachlorodibenzofuran | 0.03 | 1.04 | 1.00 | 3.23 |
| 2,3,4,7,8-Pentachlorodibenzofuran | 0.3 | 0.97 | 0.89 | 7.59 |
| 1,2,3,4,7,8-Hexachlorodibenzofuran | 0.1 | 1.03 | 0.82 | 20.72 |
| 1,2,3,6,7,8-Hexachlorodibenzofuran | 0.1 | 1.09 | 1.36 | 24.62 |
| 2,3,4,6,7,8-Hexachlorodibenzofuran | 0.1 | 1.09 | 1.39 | 27.83 |
| 1,2,3,7,8,9-Hexachlorodibenzofuran | 0.1 | 1.06 | 1.23 | 16.10 |
| 1,2,3,4,6,7,8-Heptachlorodibenzofuran | 0.01 | 1.17 | 1.05 | 10.37 |
| 1,2,3,4,7,8,9-Heptachlorodibenzofuran | 0.01 | 1.02 | 0.97 | 4.97 |
| Octachlorodibenzofuran | 0.0003 | 1.00 | 0.84 | 15.80 |

at each calibration curve point (level) are shown in Table 2. For each compound, when the level 1 RRF and average RRF were compared, it was found that all compounds satisfied the criteria for Method 2. From the above-mentioned results, it was evident that at the LOQ, the criteria were satisfied for all compounds.

**Analysis Results for the Test Samples:** As previously noted, the level of toxicity differs for each dioxin compound. The TEF, calculated for each compound by taking the toxicity of 2,3,7,8-Tetrachlorodibenzo-

p-dioxin as 1, is used as an index of strength. The TEF values for each compound are shown in Table 2. The ML for the dioxins in foods and feeds are prescribed by their toxic equivalents (TEQ). The TEQ is calculated by multiplying the concentration of each compound by the TEF and then calculating the total TEQ for all compounds.

Figure 2 and Figure 3 show a comparison of the TEQ values for GC–MS/MS and GC–HRMS for food samples. A TEQ of 0.060 pg/µL (red line) and a TEQ of 0.025 pg/µL (green line) are marked as indicators for the samples.

## Conclusion

In this article, dioxins were analyzed in 44 types and at least 200 samples of foods and feeds using GC–MS/MS. In addition, the GC–MS/MS analysis results were compared with the analysis results from GC–HRMS in order to assess the quantitative capabilities of both methods. Before analyzing, a STD was measured using GC–MS/MS, and it was confirmed that the criteria were satisfied at the LOQ.

From the above-mentioned results, it is evident that GC–MS/MS analysis provides a quantitative capability equivalent to that of GC–HRMS for samples at the concentration levels required for analysis. However, at concentrations below the required level, differences in quantitative capability could arise. For this reason, it is necessary to confirm quantitative capability at the LOQ, and evaluate whether there has been a decrease in sensitivity.

**Waldemar Weber started his study as a chemist in 2005 at the University of Münster, Germany. After graduation as a chemist, he did his Ph.D. at the University of Münster in 2011, and finished as a postdoc in 2013 at the Research Center "MEET" in Münster, Germany. From 2012 to 2013 he was employed as an Application Specialist at JAS in Moers, Germany. In November 2018 he joined Shimadzu Europa as Product Manager for GC–MS.**

**Masato Takakura works at Shimadzu Europa GmbH.**

**Thomas Lehardy works at LABERCA in Nantes, France.**

**Philippe Marchand works at LABERCA in Nantes, France.**

E-mail: **shimadzu@shimadzu.eu**
Website: **www.shimadzu.eu**

# Training Courses

## GC

**The Theory of GC**
**Website:** www.chromacademy.com/gc-training.html

**Practical Essentials of GC and GC–MS**
**29 April–1 May 2019**
The Open University, Milton Keynes, UK
**Website:** www.anthias.co.uk/training-courses/PE-GC

**Advanced GC**
**30 April 2019**
Chicago, Illinois, USA
**Website:** www.axionlabs.com/courses/advanced-gc/

## HPLC/LC–MS

**The Theory of HPLC**
On-line training from CHROMacademy
**Website:** www.chromacademy.com/hplc-training.html

**Fundamental LC–MS**
On-line training from CHROMacademy
**Website:** www.chromacademy.com/mass-spec-training.html

**HPLC Troubleshooter**
On-line training from CHROMacademy
**Website:** www.chromacademy.com/hplc_troubleshooting.html

**HPLC Method Development**
Onsite training
**Website:** www.crawfordscientific.com/training-consultancy/hplc-training/hplc-method-development

## SAMPLE PREPARATION

**Overview of Solid-Phase Extraction**
On-line training from CHROMacademy
**Website:** www.chromacademy.com/sample-prep-training.html

**Hands-on Sample Preparation for GC and GC–MS**
**18–22 March 2019**
The Open University, Milton Keynes, UK
**Website:** www.anthias.co.uk/training-courses/hands-on-sample-preparation

## MISCELLANEOUS

**Basic Lab Skill Training**
**Website:** www.chromacademy.com/basic-lab-skills-training.html

**Introduction to IR Spectroscopy**
**Website:** www.chromacademy.com/infrared-training.html

**DryLab4 Masterclass for UHPLC Method Development**
**8–10 April 2019**
Molnár-Institute, Berlin, Germany
**Website:** http://molnar-institute.com/fileadmin/user_upload/Training/SeminarRegistrationForm.pdf

**Method Development for the Separation of Therapeutic Proteins (Biopolymers)**
**6–7 May 2019**
Molnár-Institute, Berlin, Germany
**Website:** http://molnar-institute.com/fileadmin/user_upload/Training/SeminarRegistrationForm.pdf

**Please send your event and training course information to Kate Jones kate.jones@ubm.com**

# Event News

## 12–17 May 2019

**43rd International Symposium on Capillary Chromatography (ISCC) and the 16th GC×GC Symposium**

Fort Worth, Texas, USA

**E-mail:** info@isccgcxgc.com

**Website:** www.isccgcxgc.com

## 16–20 June 2019

**48th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2019)**

Milano-Bicocca University, Milan, Italy

**E-mail:** hplc2019@effetti.it

**Website:** www.hplc2019-milan.org

## 18–20 June 2019

**LABWorld China 2019**

Shanghai New International Exhibition Center (SNIEC), Shanghai, China

**E-mail:** salesoperations@ubm.com

**Website:** www.pmecchina.com/labworld/en

## 7–10 July 2019

**International Symposium on Preparative and Process Chromatography (PREP 2019)**

Baltimore, USA

**E-mail:** janet@barrconferences.com

**Website:** www.prepsymposium.org/

# Contact Information


**Europe**

**Vice President/Group Publisher**
Michael J. Tessalone
michael.tessalone@ubm.com

**Associate Publisher**
Oliver Waters
oliver.waters@ubm.com

**Sales Executive**
Liz Mclean
liz.mclean@ubm.com

**Sales Operations Executive**
Sarah Darcy
sarah.darcy@ubm.com

**Editor-in-Chief**
Alasdair Matheson
alasdair.matheson@ubm.com

**Managing Editor**
Kate Jones
kate.jones@ubm.com

**Associate Editor**
Lewis Botcherby
lewis.botcherby@ubm.com

UBM Americas
Hinderton Point, Lloyd Drive,
Ellesmere Port, CH65 9HQ, UK
Tel: +44 (0)151 353 3621
Fax: +44 (0)151 353 3601

**North America**

**Vice President/Group Publisher**
Michael J. Tessalone
michael.tessalone@ubm.com

**Publisher**
Edward Fantuzzi
edward.fantuzzi@ubm.com

**Sales Manager**
Stephanie Shaffer
stephanie.shaffer@ubm.com

**Sales Manager**
Brianne Molnar
brianne.molnar@ubm.com

**Editorial Director, Analytical Sciences**
Laura Bush
laura.bush@ubm.com

**Senior Technical Editor**
Jerome Workman
jerome.workman@ubm.com

**Managing Editor**
John Chasse
john.chasse@ubm.com

**Associate Editor**
Cindy Delonas
cindy.delonas@ubm.com

Administation and Sales Offices
Woodbridge Corporate Plaza,
485 Route 1 South,
Building F, First floor, Iselin,
NJ 08830, USA
Tel: +1 732 596 0276
Fax: +1 732 225 0211

Corporate Office,
641 Lexington Ave., 8th Floor,
New York, NY 10022-4503, USA


**Mission Statement**

The Column (ISSN 2050-280X) is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating, and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.

*Whilst every effort is made to ensure the accuracy of the information supplied, MultiMedia Healthcare LLC accepts no responsibility for the opinions and statements expressed.*

Custom Reprints: Contact Brian Kolb at Wright's Media, 2407 Timberloch Place, The Woodlands, TX 77380. Telephone: 877-652-5295 ext. 121. Email: bkolb@wrightsmedia.com.