MAY 2017 # 52

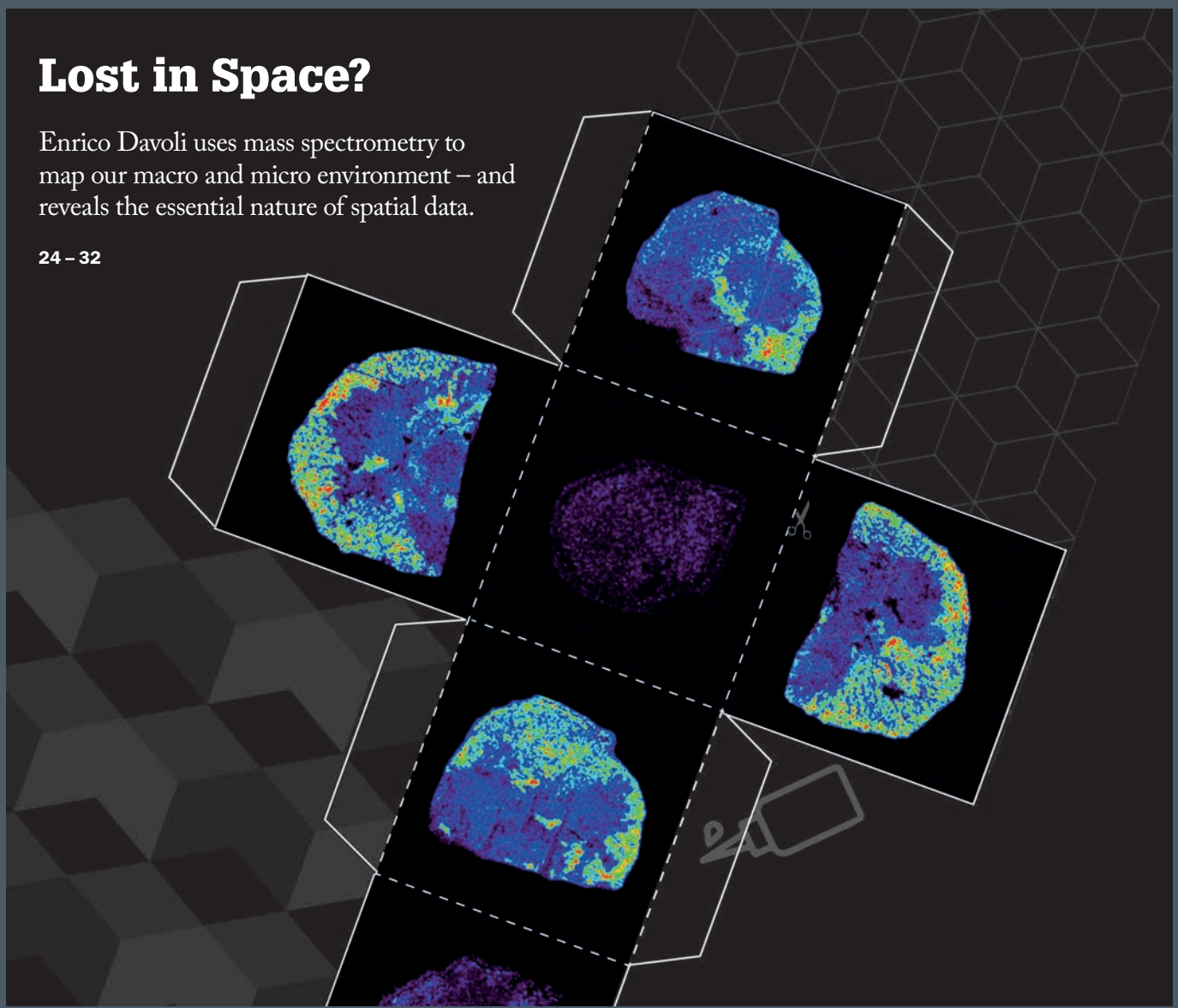
# the Analytical Scientist



## Lost in Space?

Enrico Davoli uses mass spectrometry to map our macro and micro environment – and reveals the essential nature of spatial data.





SHIMADZU
Excellence in Science


# Switch + Go

Nexera UC/s allows measurements by liquid chromatography (LC) as well as supercritical fluid chromatography (SFC) on a single system. An increased range of compounds can be analyzed as LC and SFC offer very different selectivities for analytes of interest. Switching between LC and SFC permits rapid screening for optimum separation conditions, resulting in improved analytical efficiency.

**Improved analytical results and efficiency**
using two different separation techniques

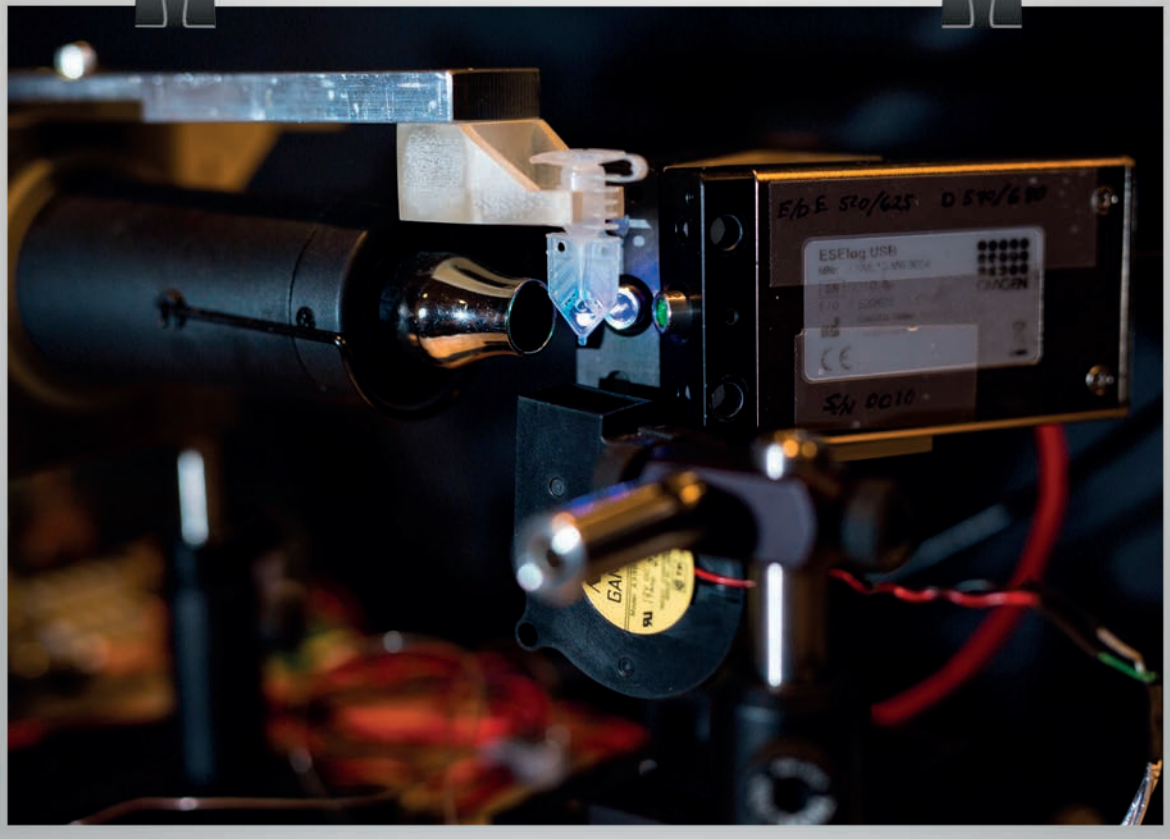
**Smaller footprint, reduced cost of acquisition**
while benefiting from a full SFC/UHPLC setup

**Automated workflow**
to create LC/SFC screening sequence

**Upgrade of existing LC to SFC functionality**
without the need to buy an additional instrument

**www.shimadzu.eu**/nexera-ucs

# Image of the Month




*DNA, Do You Copy?*

Biomedical engineers at Vanderbilt University have designed a new handheld device that uses left-handed DNA (L-DNA) to monitor and control the molecular reactions that take place in the polymerase chain reaction (PCR). The spectrophotometer on the left detects varying levels of fluorescence in the sample (center), illuminated by ultraviolet light, and uses them to control DNA duplication. The "adaptive PCR" device has the potential to identify bacteria or viruses by DNA signatures before symptoms appear – and its handheld size means it can be transferred easily from the lab to the clinic.

Credit: Anne Rayner, Vanderbilt University

*Reference: NM Adams et al., "Adaptive PCR based in hybridization sensing of mirror-image l-DNA", Anal Chem, 89, 728-735 (2017).*

Would you like your photo featured in Image of the Month? Send it to charlotte.barker@texerepublishing.com

# Contents




## On The Cover


*Constructing a 3D map of cancer. Mass spectrometry images of a tumor, courtesy of Enrico Davoli.*

## Upfront




the Analytical Scientist

ISSUE 52 - MAY 2017





Change of address
nina.duffissey@texerepublishing.com
Nina Duffissey, The Pathologist, Texere Publishing Ltd, Haig House, Haig Road, Knutsford, Cheshire, WA16 8DX, UK

General enquiries
www.texerepublishing.com | info@texerepublishing.com
+44 (0) 1565 745200 | sales@texerepublishing.com

Distribution:
The Analytical Scientist (ISSN 2051-4077), and The Analytical Scientist North America (ISSN 2514-7544), is published monthly by Texere Publishing Ltd and is distributed in the USA by UKP Worldwide, 1637 Stelton Road B2, Piscataway, NJ 08854. Periodicals Postage Paid at Piscataway, NJ and additional mailing offices
POSTMASTER: Send US address changes to The Analytical Scientist, Texere Publishing Ltd, C/o 1637 Stelton Road B2, Piscataway NJ 08854
Single copy sales £15 (plus postage, cost available on request tracey.nicholls@texerepublishing.com)
Annual subscription for non-qualified recipients £110

Scale 1:2,3


## Mira M-3 handheld Raman Spectrometer

# True single-handed operation


Barely larger than a smartphone, the Mira M-3 is one of the most convenient to use handheld Raman spectrometers in the market. The Mira M-3 is ...

- **Super compact** – 13 (h) x 8.5 (w) x 4 (d) cm
- **Super fast** – verify identity of materials in seconds
- **Fully compliant** with FDA 21 CFR Part 11

www.metrohm.com/mira

Metrohm

Use Promo Code TEXERE for 25% Off Registration!!!

CANNABIS
SCIENCE
CONFERENCE

*Hosted by*

jCanna.com

**August 28-30, 2017**

**Portland, Oregon**

OREGON Oregon Convention Center

**The World's Largest Cannabis Science Conference** returns to downtown Portland for an incredible gathering of analytical scientists, medical professionals, cannabis industry experts and novices interested in learning more about cannabis science!

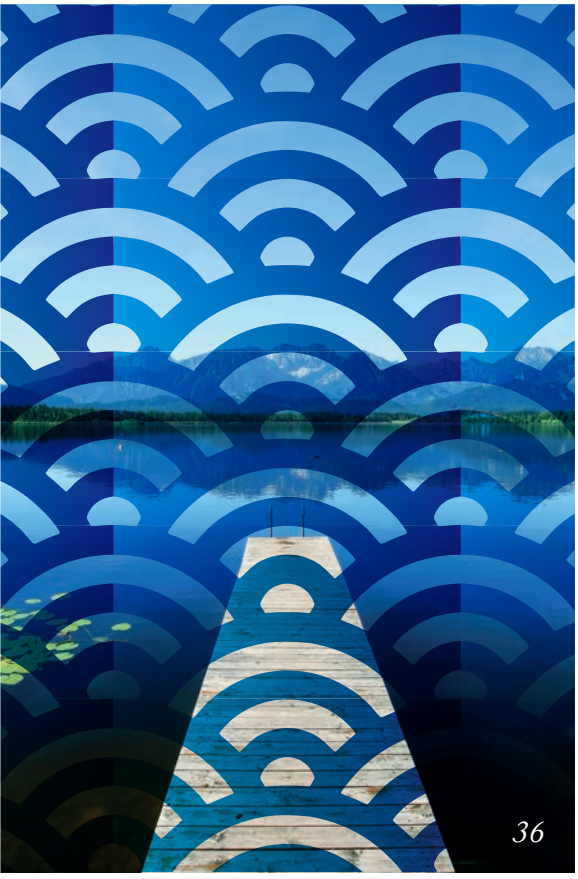
Contact Josh Crossney at josh@jcanna.com for more info.

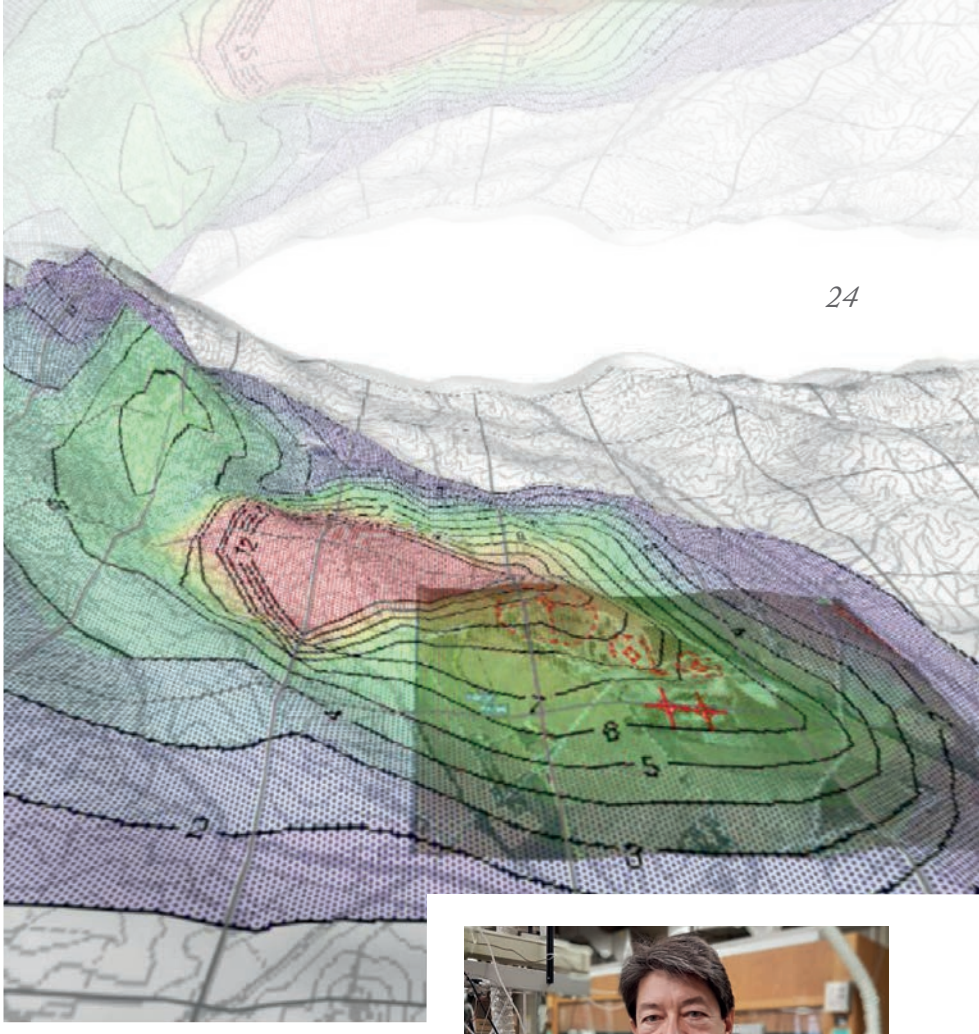
CannabisScienceConference.com

jCannaInc

jCanna_Inc

@jCanna_Inc





## In My View



## Features



## Reports



## Departments



## Sitting Down With

Raptor™
LC Columns

# SPP speed. USLC® resolution.
# A new species of column.

- Drastically faster analysis times.
- Substantially improved resolution.
- Increased sample throughput with existing instrumentation.
- Dependable reproducibility.

Choose Raptor™ SPP LC columns for all of your valued assays to experience *Selectivity Accelerated.*

**www.restek.com/raptor**


RESTEK®
Pure Chromatography

**www.restek.com/raptor**

# Marching for Sanity

*Reproducible measurement science has never been so vital.*



"The challenge is for scientists to be humble and acknowledge that in this day and age facts will not win the day." So says Michael Osterholm, Director of the University of Minnesota's Center for Infectious Disease Research.

A sobering thought. What surprised me most about the quote – taken from an article on the inevitable (and also sobering) outcome of "anti-vaxxer" campaigning (1) – was that it didn't surprise me at all.

Back in April, I was pleased to read, "How the March for Science Finally Found Its Voice" in Washington D.C. (2) – and in over 600 other cities around the world. With witty signs aplenty (do an Internet image search – "march for science signs" – to cheer yourself up with your morning beverage of choice), science fans young and old took to the streets to fight against "alternative facts" on Earth Day. And though humor and puns won the hour, the message was serious. "I can't believe I'm marching for facts," wrote one sign-maker.

"Science is not a liberal conspiracy," wrote another.

So we've finally reached the point where science must be defended. Political spin, "fake news" on social media, and perhaps more than a pinch of science illiteracy have all combined to create white noise capable of drowning out the purest sounds of science.

I don't want to dwell on the potential ramifications for humanity here – I'll save that for a lengthy book that I'll never write. I have a couple of other concerns that must be addressed, if science is to find its voice and regain power.

A year ago, I wrote "The Dogmatic Scientist" (3), which called for care in separating science from scientism – lest we become evangelist preachers on soapboxes in town squares (the March for Science had some of that vibe...). What we need is clear communication of current knowledge or thinking rather than hyperbole that attracts attention (but also, worryingly at times, funding). And, as my colleague Charlotte Barker noted in her editorial last month (4), transparency should not be limited to exchanges with the general public – "clarity over complexity" is essential when sharing our work with colleagues in other disciplines (and other parts of the world).

Finally, as I read a sign that shouted, "What do we want? Evidence based science. When do we want it? After peer review," I was reminded of Ira Krull's own March Against Irreproducibility in the literature (5). Ira concluded, "Don't be afraid to stand up and decry research or publications that fail to meet even the basic requirements for reproducibility. The whole of science is at stake."

And he's right. But as analytical scientists, you surely don't need me to tell you that.

**Rich Whitworth**
*Content Director*


*References*

1. *M Molteni, "Anti-vaxxers brought their war to Minnesota – then came measles", (2017). Available at: http://bit.ly/2qllKMp. Accessed May 9, 2017.*
2. *E Yong, "How the March for Science finally found its voice", (2017). Available at: http://theatln.tc/2p6mIsx. Accessed May 9, 2017.*
3. *R Whitworth, "The dogmatic scientist", The Analytical Scientist, 0516, 9 (2016). Available at: http://bit.ly/2pZfpFl.*
4. *C Barker, "Communicating or confusing?", The Analytical Scientist, 0417, 9 (2017). Available at: http://bit.ly/2pZ2xiF.*
5. *I Krull, "Care to Repeat That?" The Analytical Scientist, 0217, 32–35 (2017). Available at: http://bit.ly/2ndqBK8.*

# Upfront

*Reporting on research, personalities, policies and partnerships that are shaping analytical science.*

*We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker @texerepublishing.com*

## Making a Difference to Drug Discovery

**Differential mobility spectrometry aims to save R&D by making it cheaper, faster and more efficient**

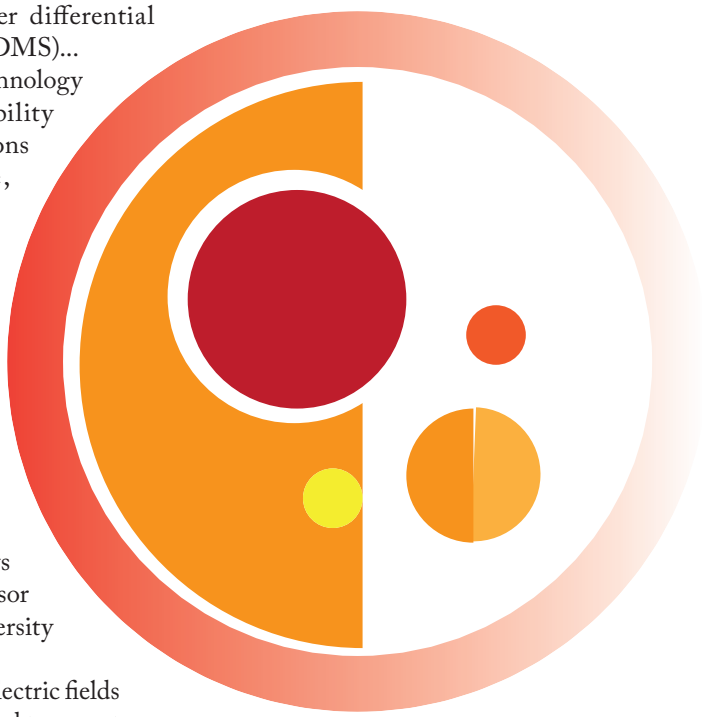
Early small molecule drug discovery uses cell permeability assays to determine the chemical structure and physiochemical properties of candidate molecules – but these in vitro studies are time-consuming, expensive, and lack the resolution to discriminate between similar molecules. Enter differential mobility spectrometry (DMS)...

Though DMS is a technology known more for its ability to separate analytical ions from chemical noise, researchers at the University of Waterloo were interested in probing how ions interact with solvent vapor. "We caught the attention of scientists at Pfizer, who were already collaborating with SCIEX, and so we decided to merge some of our efforts," says Scott Hopkins, a professor of chemistry at the University of Waterloo.

DMS uses oscillating electric fields to influence the motion and temperature of ions in the DMS cell. The researchers used this dynamic environment to drive rapid cycles of water condensation and evaporation (tens of thousands of times over the course of several milliseconds) and monitor the water vapor's interaction with drug molecules. The interaction, the researchers found, correlates well to properties like solubility, pKa and cell permeability (1).

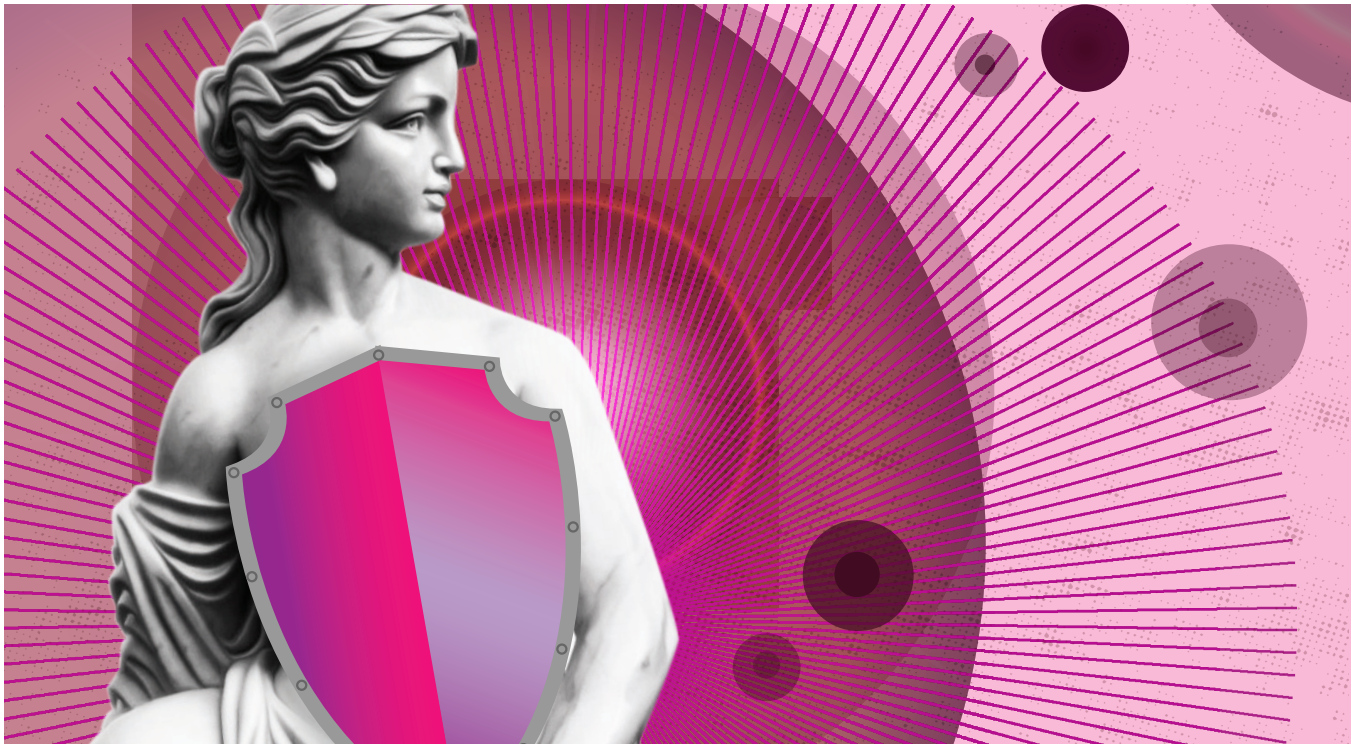
The technology could eliminate the need for experienced technicians to perform a battery of tests to ascertain the properties of a potential drug. Instead, a single DMS analysis could obtain the same information – plus the ability to differentiate between structurally similar molecules – in a fraction of the time.

"The key thing from the drug discovery perspective is that we can make property measurements in seconds, with only picograms to nanograms of sample. Consequently, with a little more work, DMS could be used for cost-effective, high-throughput assays," says Hopkins. *JS*



*Reference*

1. *C Liu et al., "Assessing physicochemical properties of drug molecules via microsolvation measurements with differential mobility spectrometry," ACS Cent Sci, 3, 101–109 (2017). PMID: 28280776.*



# Taking the EV Option

## LC-MS/MS helps open up new avenues in liquid biopsy-based cancer diagnostics

As the techniques behind liquid biopsy become more advanced, we should be able to diagnose and evaluate an increasing number of cancers using simple blood tests... in theory. Unfortunately, not all forms of the disease are cooperative. Breast cancer, for instance, has long eluded detection in this manner – but it may not remain hidden for much longer.

"Phosphorylation is one of the major regulation mechanisms in many diseases, including breast cancer," says W. Andy Tao, a biochemistry professor at Purdue University. "Phosphorylation and kinases have been the major target for cancer therapy, but unfortunately not for diagnosis." Why? Because the use of phosphorylated molecules as diagnostic biomarkers in blood is confounded by plasma-dwelling phosphatases, which remove the phosphate groups. The solution? Extracellular vesicles (EVs), according to a team of researchers led by Tao (1): "Our findings highlight that isolating EVs from plasma allows us to use blood or other liquid biopsy for potential cancer screening based on the status of protein phosphorylation – something that's not been done before."

The EVs found in blood plasma form a protective shell around the phosphoproteins, preventing dephosphorylation. To measure the cargo inside, the investigators broke apart the EVs and then used trypsin to create phosphopeptides ahead of analysis and identification using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Their study detected 144 phosphoproteins at higher concentration levels in breast cancer cases than in healthy controls.

The findings hold significance not only because of the improved ability to measure phosphoprotein biomarker levels in blood, but also because of the abundance of EVs in plasma, which could allow the detection of thousands of phosphopeptides from a single milliliter of sample.

Next, the team plan to investigate potential biomarkers in other cancers and diseases – a promising avenue, given that their initial investigation revealed over 10,000 unique plasma phosphoproteins. *WA*

*Reference*

1. *IH Chen et al., "Phosphoproteins in extracellular vesicles as candidate markers for breast cancer", Proc Natl Acad Sci USA, 114, 3175–3180 (2017). PMID: 28270605.*

# Hack Attack

## Cross-border "Hackathon" encourages young scientists to get entrepreneurial


The event? A programming and prototyping marathon. The participants? A group of students from France and Japan. The goal? To encourage creativity and entrepreneurship – as well as strengthen collaborative links between two institutions: École Polytechnique and the University of Tokyo. For this "Hackathon", teams of five students designed prototypes for lab equipment of the future, including a connected necklace to send and receive messages in a clean room, and a mini-robot to carry out mundane lab tasks or warn of impending danger. Representatives from École Polytechnique and event sponsor HORIBA tell us more about the bi-continental event.

How did the Hackathon come about?

*École Polytechnique:* The Hackathon took place during a two-week exchange at the École Polytechnique for students from the University of Tokyo. One of the activities was a 48-hour intercultural experience, mixing 10 students from Tokyo and 10 students from École Polytechnique. Hosted in our new building, fully dedicated to entrepreneurship and innovation, it was an opportunity for students from different cultures to collaborate.

*HORIBA:* Our headquarters in Japan has strong academic collaborations with top Japanese universities, and the HORIBA Europe Research Center has long-standing relationships with leading academic institutions, such as École Polytechnique. It seemed natural for us to become a partner in such an exciting educational event.

Why target the younger generation?

*EP:* For the students, it was an excellent opportunity to try working in a multicultural team towards a common and real goal. It was the first time many had faced a situation like this, which occurs frequently in real life – and is even more likely in an increasingly internationalized world.

*H:* HORIBA's scientific mission is to support R&D with cutting-edge technology products – so to work with younger scientists made sense to us. The challenge we proposed – to design "a device that you would like to have for future lab or fablab (digital fabrication laboratory) activities" – provided some clear messages: the value of gamification, the importance of a building a healthy and happy life, and teambuilding through sport, services to humanity and social activities.

How did the students respond?

*H:* They really enjoyed this experience – both because of cultural relations and because they had the chance to get familiar with some high-tech tools, including 3D prototyping, rapid electronic prototyping, and fast programming.

*EP:* All were impressed by what they had accomplished in 48 hours. It demonstrated to them the power of working as a team, rather than as individuals. All of them were praised by the jury.

What were the highlights for you?

*H:* For us, it was fantastic to see that in less than three days, students were able to produce prototypes of lab accessories and services supporting their vision.

*EP:* The highlight was seeing the extraordinary achievements made possible by people from different cultures and backgrounds working together. That is the way to foster innovation and creativity within an organization.

Will there be more Hackathons?

*EP:* Based on the success of the first Hackathon, yes! We would need to identify another theme that is as inspiring and has a potentially high impact.

*H:* To perform in a global market, innovation is a must – and for an international group, it is important to have a young vision that projects us into the future. This Hackathon fits within the ecosystem of the HORIBA group, which aims to create value through innovation. We are now considering a similar event with PhD students, to give them an initial experience of laboratory life.

# Testing for Toxins, Tracking Trace Gases

## What's new in business?

In our regular column, we partner with www.mass-spec-capital.com to let you know what's going on in the business world of analytical science. This month, we congratulate influential researcher and contributor to The Analytical Scientist Dwight Stoll (pictured), who received Agilent's Thought Leader Award, "in support of his research applying two-dimensional liquid chromatography (2D-LC) to biopharmaceutical analysis."


### Products

- bioMérieux receives 510(k) clearance for BacT/Alert VIRTUO system and announces CE mark for FilmArray Respiratory Panel 2 Plus
- Vicam introduces new method to detect ochratoxin A in coffee and wheat
- Bruker launches new clinical microbiology assays and kits at ECCMID
- Ionicon launches the PTR-TOF 6000 X2 Trace Gas Analyzer

### Investments & Acquisitions

- AMETEK acquires MOCON for $182m
- PerkinElmer completes sale of medical imaging business to Varex Imaging

### Collaborations

- Microsaic signs amended agreement with "a long-standing global partner."
- Thermo Fisher Scientific makes the Institute of Pathology at University Hospital Basel a Center of Excellence Partner
- Agilent Technologies presents Thought Leader Award to Professor Dwight Stoll for separation science research
- Lipotype announces results of two years of lipidomics services for VIB

*For links to original press releases and more business news, visit the online version of this article at: tas.txp.to/0517/BUSINESS*

# Summer of Science

**ASMS, HPLC, ISPPP... the next two months see some of the biggest conferences in analytical science**

## Mass Excitement

*What?* The Annual Conference on Mass Spectrometry and Allied Topics is celebrating its 65th year. Sponsored by the American Society of Mass Spectrometry, the conference has around 6,500 attendees, and boasts almost 400 presentations and over 2,700 poster sessions.

*Where and when?* June 4–8, 2017, at the Indiana Convention Center, Indianapolis, Indiana, USA.

*Why attend?* From bioinformatics to biopharmaceuticals, from forensic innovation to fundamentals, from art and cultural heritage to careers – if there are mass spec applications, ASMS covers it.

*Highlights?* The workshops at ASMS will have a focus on careers and collaboration, new trends and innovation in technology:

- Diversity and Outreach
- Biotherapeutics: Hot Topics
- Mass Spectrometry in the Developing World
- A Career in Mass Spec: Options and Where to Start?

*Final thoughts?* Kristen Marhaver will be giving the closing plenary lecture on "Saving the Great Coral Reefs" at 4.45pm Thursday 8th. Then head down to the closing event at the Indiana State Museum – which includes ASMS karaoke!

*http://www.asms.org*

## Wait and HPLC

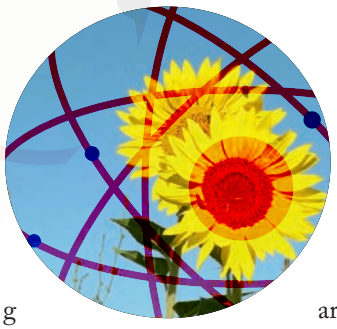
*What?* HPLC 2017, the 45th International Symposium on High Performance Liquid Phase Separations and Related Techniques is chaired by Michal Holčapek and František Foret. It covers fundamental and experimental approaches in liquid-phase separations, as well as column technologies, cutting-edge applications and innovative instrumentation.

*Where and when?* June 18–22, 2017, at the Prague Congress Center, Prague, Czech Republic.

*Why attend?* Speakers include some of the leading lights of liquid-phase separations and supercritical fluids. This year sees a strong focus on multi-dimensional separations and one program track devoted solely to hyphenated techniques and MS coupling.

*Highlights?* The scientific program includes four parallel tracks covering a vast array of topics:

- FUN (Fundamentals) – covering research from mechanisms of mass transport phenomena to data analysis, validation and chemometrics
- HYP (Hyphenations) – ranging from high resolution separations–mass spectrometry coupling to high-throughput data processing and bioinformatics
- APP (Applications and Instrumentation) – a diverse



portfolio of integrated -omics approaches and systems biology, clinical and environmental analysis, food analysis and/or (bio)pharmaceuticals and drug metabolism
- YOU (Young and Tutorials) – including presentations by young scientists.

*Final thoughts?* In addition to the future-facing array of topics at this year's conference, one of the main goals of the HPLC 2017 Prague symposium will be attracting the active participation of young researchers, with one of the four parallel program tracks aimed at young speakers, including tutorial lectures from top experts. Several awards will also be presented to young scientists in the field.

*http://www.hplc2017-prague.org*

## The Philadelphia Story

*What?* The 2017 International Symposium on Separations of Proteins, Peptides and Polynucleotides (ISPPP) was founded in 1981 by Fred Regnier and Milton Hearn, and brings together experts in the materials and methods of biomolecule separations, including developing applications of separations for discovery, analysis and structural characterization of biomolecules. This year's ISPPP will be co-chaired by Barry E. Boyes (Advanced Materials Technologies, Inc.) and Ron Orlando (University of Georgia).

*Where and when?* July 19–21, 2017, at Loews Hotel on Market Street, in downtown Philadelphia, Pennsylvania, USA.

*Why attend?* It's an exciting time for biochemists and process engineers using separation science. The field continues to grow and adapt, enjoying better materials, instruments and methods, and a seemingly never-ending list of interesting molecules to work on.

*Highlights?* High-resolution analytical applications are sure to be a hot topic, particularly LC and LC/MS methods for complex molecule analyses and applications of high-resolution methods during target discovery, validation, and product development. This is particularly relevant at a time when therapeutic and diagnostic humanized monoclonal antibodies are undergoing an explosive growth in clinical and research applications.

Themes include:

- Proteomics, glycoproteomics and phosphoproteomics
- Therapeutic biomolecules and biosimilars
- Monoclonal antibody analytics
- Novel protein structure analysis
- Novel separations materials and methods
- Metabolomics and enzyme analyses

*Final thoughts?* As with past ISPPPs, the meeting will encourage an atmosphere of collegial interactions and collaboration, with a variety of interactive sessions and social events. There will be ample opportunity to engage presenters and attendees. For the past four years ISPPP has been held jointly with the PREP Symposium (July 16–19), and we will continue that tradition this year.

*http://www.isppp.org.*

# In My View

*In this opinion section, experts from across the world share a single strongly-held view or key idea.*

*Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.*

*Contact the editors at edit@texerepublishing.com*

# Arsenic Determination: A Muddle of Methods

**Arsenite and arsenate contamination of rice is a global public health threat. If we cannot accurately report on our methodology, how on earth can we keep consumers safe?**

*By Julian Tyson, Professor Emeritus, Department of Chemistry, University of Massachusetts, Amherst, USA.*

The world's rice supply contains concentrations of toxic arsenic compounds that could put many consumers at risk of long-term adverse health effects (1). Regulatory and advisory agencies are introducing limits; for example, in 2015, the Commission Regulation for the EU adopted a cut-off of 200 µg kg-1 for inorganic arsenic in white rice and 100 µg kg-1 for rice destined for the production of infant foods. To understand the details of the contamination and how it might be evolving with time – and to enforce the regulations – we need reliable information about the concentrations of the various arsenic compounds in rice, notably arsenite and arsenate.

Reports of studies of arsenic and rice currently appear in the peer-reviewed literature at a rate of about 80 per year, having risen in an almost linear fashion from near-zero in 2001. Reports focusing on analytical methods for arsenic compounds in rice are a little harder to identify, but I would estimate that there are now 10–15 per year. I'm an active researcher in the field and write at least one review per year (2, 3), so I read large numbers of articles in which yet another procedure for the determination of arsenic compounds in rice is described. My conclusions? That there is no convergence on any aspect of the method – and that both writers and reviewers of manuscripts are falling down on the job.

*"Both writers and reviewers of manuscripts are falling down on the job."*

There are several important questions that readers ask: i) in what way is your method an improvement on previously published methods? ii) what, exactly, is new in your procedure? iii) what, precisely, did you do in each step of the method? and iv) why did you choose those particular steps? Many articles fail to answer these basic questions.

Authors describing HPLC-ICP-MS in particular omit a lot of important information. As a start, readers should know whether the chromatography (choice of column, mobile-phase

composition, temperature) is new. If not, there should be a citation to the article in which the method was originally described. If the chromatography is new, the researchers should include some explanation as to why this was necessary, together with some description of the optimization experiments and a clear statement of what figures of merit were being optimized. Some chromatographic performance parameters, such as resolution and plate numbers, should be provided and, if gradient elution was chosen, how long re-equilibration took. As isocratic elution is preferred, especially for ICP-MS detection, the decision to develop a method with gradient elution needs to be justified.

A chromatogram of standards – each of which contains the same concentration of arsenic – should be provided, together with a clear description of the calibration procedure. Details of possible compound-dependent responses is vital – if the researchers claim that they observed none, what is the evidence? If separate calibrations were constructed for each component, the slopes of the calibrations should be given. As rice contains enough chlorine to interfere if it co-elutes with an arsenic species, the fate of chloride in the chromatography should be clearly stated (and shown), or the detector operated in a mode that eliminates this problem. It should also be made clear whether the first peak represents chromatography or is the response to compounds running with the solvent front, and a "chromatogram" with an appropriate marker species should be shown. There are similar problems with the descriptions of the sample preparation procedures, but I'll save those discussions for another article.

I appreciate that journals have limited space, but much of this evidence could be provided in the "supplemental information" file. Without this crucial information, the convergence on methodology that is so sorely needed will remain a distant goal.

*References*

1. *MA Davis et al., "Assessment of human dietary exposure to arsenic through rice", Sci Tot Env, 586, 1237–1244 (2017).*
2. *J Tyson, "The determination of arsenic compounds: a critical review," ISRN Analytical Chemistry, Article ID 835371(2013).*
3. *R Clough et al., "Atomic Spectrometry Update: review of advances in elemental speciation", J Anal At Spectrom, 31, 1330–1373 (2016).*

# The Value of a Pragmatic Approach

**It's not enough for a method to be precise – it has to be practical for routine use.**

*By Cecilia Cagliero, Assistant Professor, Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino Via Pietro Giuria, Torino, Italy .*

People typically expect (or assume) that products that come into contact with their bodies (food, cosmetics, and so on) are free from potential health risks. To that end, international organizations study potentially toxic compounds and set limits of acceptability that industries and control organizations have to monitor. However, many foods and cosmetics include complex mixtures of natural products – so toxic compounds are typically present at trace levels, hiding amongst hundreds of other molecules. To detect trace toxins, analytical chemists must pay careful attention to both recovery and separation. Moreover, when developing a method, we must remember that many companies producing food and natural products do not have access to complex and expensive instrumentation – and high throughput (to control production) tops the list of priorities.

Our research group, led by Carlo Bicchi, is used to dealing with complex natural matrices, but even with optimized sample preparation and/or separation, the determination of possible contaminants in these samples can be challenging. What are the solutions? One route is to adopt highly sophisticated separation techniques (multidimensional chromatography, for example) and/or high-resolution mass spectrometry. Certainly, such techniques gain in popularity every day, but their complexity and cost puts them out of reach

*"Even with optimized sample prep and/or separation, determination of possible contaminants can be challenging."*

*"We propose a simpler approach: conventional instrumentation combined with one-step sample preparation methods."*

of many companies. We propose a simpler approach: conventional instrumentation combined with one-step sample preparation methods that make use of new materials tailored to the task.

In this respect, ionic liquids have great potential because of their unique selectivity. Even better, their chemical structures can be custom-designed to enhance extraction efficiencies of specific classes of analytes (when used to recover compounds of concern) or to improve critical separations (when used as stationary phases for their separation). In 2016, I visited Jared Anderson's laboratory at Iowa State University for 3 months and found that our experience of complex natural matrices, combined with his expansive knowledge of ionic liquids, was a winning combination when it came to dealing with challenging issues.

One result of this collaboration was a method for measuring acrylamide in coffee samples. Acrylamide is an unsaturated amide formed when carbohydrate-rich foods, such as potatoes, bread, biscuits and coffee, are subjected to high temperatures during cooking or thermal processing. Several studies have revealed that acrylamide is toxic, and a possible carcinogen, which is why regulatory agencies have indicated that regulation on acrylamide levels will be introduced. However, acrylamide analysis in food is a considerable challenge because of its chemical characteristics and trace concentration. Established ISO methods adopt SPE-HPLC-MS/MS, but this methodology is time consuming and cannot easily be automated. Instead, we applied direct immersion solid-phase microextraction (SPME) coupled to GC-MS with polymeric ionic liquid (PIL) sorbent fiber coatings to recover acrylamide with very high selectivity.

The method was immediately effective but there was one flaw – a neo-formation of acrylamide at the high temperatures of the GC inlet, formed by the asparagine and glucose co-extracted with PIL fibers. To address this issue, we added a very simple quenching reaction using ninhydrin to selectively degrade free asparagine and inhibit the formation of additional acrylamide during the desorption step. By tailoring the PIL structure, it was possible to reach a limit of quantitation for acrylamide in the ppt range, comparable to those of the ISO method. In contrast to the official method, our PIL-based SPME method is considerably quicker, does not require solvents, can be made fully automated, and requires only conventional and simple instrumentation.

Our research group has also studied ionic liquids in another interesting application: GC stationary phases for one-dimensional separations of complex samples in the essential oil, flavor and fragrance fields. But that's a story for another time.

I believe that the ultimate challenge for analytical scientists is not just to develop and study new technologies, but to make them simple enough for routine use.

# HPLC Will Survive and Thrive

**Those who thought HPLC had peaked in the 1990s were sorely mistaken – but can the technique continue to adapt to increasing demands?**

*By Attila Felinger, Professor, University of Pécs, Pécs, Hungary.*

High-performance liquid chromatography (HPLC) was born half a century ago, and has since become one of the most widely used analytical techniques in the world. The demand for higher separation efficiency, faster analyses and greater throughput has been the driving force for extraordinary advances in stationary phases. One of the secrets of HPLC's success is that column technology caters for all tastes, and it is capable of continuous renewal.

The versatility of stationary-phase chemistry, or surface modification, allows for the design of efficient and selective packing materials for the separation of nonpolar, polar, ionizable, or chiral analytes, small molecules or large biomolecules. Particle sizes have decreased from 50 µm to less than 2 µm. Core-shell particles have become the favorite packing materials for many users, and not only for the separation of macromolecules (even though those particles were originally developed for the efficient separation of biomolecules). Silica-based monolithic columns have become a popular alternative for efficient separations at moderate pressure drops. We are going to see many more exciting new developments in the field of monoliths, as the short columns developed for fast HPLC can be operated at higher pressures than the conventional silica-based monoliths.

It has become clear that column hardware has an essential impact on band broadening, and thus on separation efficiency. In a typical 50×2.1 mm column developed for fast liquid chromatography, the band broadening of an unretained analyte in the 50 mm-long chromatographic bed is about the same as in the two 1 mm-long frits (1). To produce higher-efficiency columns for fast HPLC, the

*"After the success of comprehensive multidimensional chromatography, we are anxious to see what spatial two-dimensional and three-dimensional HPLC will bring for us."*

*"One of the secrets of HPLC's success is that it is capable of continuous renewal."*

optimization of frit technology will be of utmost importance. Furthermore, instrumentation must develop so that the integration of the column in the instrument will not introduce dead volumes at the connecting elements, so that extra-column band broadening is kept to a minimum.

In the 1990s, many chromatographers thought that HPLC had matured and such a level that further development was possible only in incremental steps. But, soon after that, we witnessed the introduction of ultra-performance liquid chromatography, sub-2 µm and core-shell particles.

Now, we wait impatiently to find out how 3D-printing technology will impact on HPLC column technology. After the success of comprehensive multidimensional chromatography, we are anxious to see what spatial two-dimensional and three-dimensional HPLC will bring for us.

The perpetual development of column technology guarantees that HPLC will remain a success story. Some of the ideas being suggested for future developments may seem utopist, but given the huge and unforeseen developments seen recently in stationary-phase technology, we would be wise to keep our minds open.

*For more discussion on HPLC's place in modern analytical science, turn to page 36.*

*And to hear from HPLC 2017 Prague chairs, Michal Holčapek and František Foret, visit: tas.txp.to/0117/HPLC2017*

*HPLC 2017 Prague takes place June 18–22 at Prague Conference Centre. www.hplc2017-prague.org*

*Reference*

1. *N Lambert et al., "Comparison of the kinetic performance of different columns for fast liquid chromatography, emphasizing the contributions of column end structure", J Chromatogr A, 1473, 99–108 (2016).*

# Beyond Stone-Age Sample Prep

**Miniature detection technologies are evolving fast – but unevolved sample preparation is holding us back.**

*By Rodrigo Martinez-Duarte, Multiscale Manufacturing Laboratory, Department of Mechanical Engineering, Clemson University, South Carolina, USA.*

Miniature detection technologies have matured over the last decade thanks to significant investment from industry, funding agencies and investors. We can accurately identify target compounds using myriad technologies, including biosensors, spectrometers, PCR and sequencing. Highly abundant molecules, such as sodium and glucose, can now be monitored from a single blood drop using handheld systems, such as the i-STAT.

Unfortunately, when the target is of low abundance or contaminated with other substances, we're still struggling. Prevention of sepsis, food poisoning and water contamination, as well as the diagnosis and monitoring of cancer, all depend on the timely detection of rare targets – pathogens and circulating tumor cells. In these cases, we still rely on a series of cumbersome processes to convert the sample we gather into suitable fractions for analysis. Sample

*"Prevention of sepsis, food poisoning and water contamination, as well as the diagnosis and monitoring of cancer, all depend on the timely detection of rare targets."*

*"We still rely on a series of cumbersome processes to convert the sample for analysis."*

preparation currently relies on a suite of instruments for centrifugation, re-suspension, lysing, filtering and sorting; cue extensive labeling, wet chemistry and endless pipetting – all carried out manually so that reproducibility is too often dependent on experience...

Detection of one pathogen or tumor cell in a 10 ml sample is commonly required in clinical diagnostics; in environmental monitoring, there can be as little as one target per liter. To obtain statistically valid results in these applications, we have to process large samples. It is unrealistic to expect the accurate and precise detection of a low-abundance target when sampling only a few microliters of sample from a patient or water supply. Hence, preparation of large sample volumes is quite often a necessary step to enrich a target and enable analytical techniques. For example, lateral flow assays can only detect targets at a concentration of 100 nM. Even analytical technology with sensitivity of 1 attomolar would require at least one target per microliter of sample.

Is there a solution to this 'needle-in-the-haystack' problem? Well, transforming samples retrieved from a real-world scenario into ideal fractions for analysis is by no means a trivial task. But the reward is worth the effort, and a number of promising technologies for sample handling, particle and molecular sorting, and lysis are being developed. These include contained platforms such as centrifugal microfluidics and digital microfluidics, and label-free bioparticle sorting, such as dielectrophoresis, inertial microfluidics, deterministic lateral displacement, and acoustophoresis.

There is much work to do before these next-generation techniques can prepare a sample at the touch of a button. I foresee a combination of these techniques gradually enriching a target in a decreasing sample volume over time. Multi-scale fractionation of sample components will allow us to tailor the fraction depending on the analysis to be performed. Importantly, we will also need standards that enable the integration of modules developed by different companies; for example, standardizing the connector for transfer of a given sample type.

Improving throughput, efficiency and reproducibility of a technology, and its integration with others, are not incremental advances. They are enablers of a practical platform that can have tremendous impact on clinical diagnostics, as well as disease diagnostics in rural, space, battlefield and wilderness scenarios. Investors and funding agencies first need to understand the challenge of sample preparation – and then do more to reward our efforts.

# The Biopharma Multi-tool

## Size-exclusion chromatography – long a mainstay in routine biopharmaceutical analysis – receives an upgrade.

*By Judith Vajda, Senior Laboratory Specialist, TOSOH BIOSCIENCE GmbH, Germany.*

Quick and straightforward, size-exclusion chromatography (SEC) has become a Swiss Army knife for the analysis of protein aggregates. It is a "mild" technique that usually preserves biological activity and structural integrity – and could almost be considered a platform technology. Coupling of SEC with various advanced detectors, such as a mass spectrometry, light scattering or surface plasmon resonance, makes it a versatile tool for numerous applications beyond aggregates. With new biopharmaceutical formats in the pipeline – bispecific monoclonal antibodies, antibody–drug conjugates and virus-like particles, for example – rapid and thorough characterization will be even more important.

Timelines in analytical laboratories are increasingly tough – on the other hand, there is a strong drive to explore and understand biologics in more detail. In separation science, this means higher resolution and plate counts in shorter analysis times.

### Small is beautiful

Over the past 30 years, miniaturization has been the key trend both in stationary phases and instrumentation. Smaller particles provide better separation performance, so we can either shorten the required separation path lengths, or provide better performance at the same path length. The use of smaller particles also pushed the optimum column dimensions towards smaller values, with the welcome side-effect of saving solvent.

When it comes to biopharmaceuticals, HPLC and UHPLC cannot be as strictly segregated as in other disciplines. In this setting, UHPLC is defined as "ultra-high performance LC" rather than the more common definition – "ultra-high pressure LC". Especially in SEC, the small (2 µm) particles of UHPLC do increase the pressure – but rarely exceed the pressure limits of modern HPLC. Any higher, and the resulting frictional heating and shear forces would damage the macromolecules and lead to artefacts. Instead, bio-UHPLC is focused on optimization of valves, capillaries and flow cells to decrease dead volumes and subsequent Eddy diffusion – which also holds true for conventional HPLC columns.

### Upgrading biopharma's Swiss Army knife

UHPLC is already a standard technology in the analysis of small-molecule drugs so it is no surprise that biopharmaceutical manufacturers are now looking to follow suit. Our TSKgel 3000SWXL column is a standard tool for QC aggregate analysis by SE-HPLC, and we noticed an increasing demand for a 2 µm-particle column that enables easy method transfer to UHPLC.

There were many challenges in developing such a column. The minimum particle size for HPLC is believed to be 1 µm (1), so at 2 µm we are skirting the edge of what is possible – and that's never easy going. But chromatographers really

*"With new biopharmaceutical formats in the pipeline, rapid and thorough characterization will be even more important."*

appreciate the benefits in terms of speed and resolution.

Of course, UHPLC is not a magic bullet. Coupling a SE-UHPLC column to a non-optimized system with dead volumes is an unhappy marriage. The smaller internal diameters result in lower volumetric flow rates, which leave more room for undesirable Eddy diffusion. In some cases, this may result in worse separation performance than with a conventional SE-HPLC column, so it's important to consider the system as a whole.

Thorough characterization of every new drug is crucial for patient safety – and complex next-generation biopharmaceuticals present a challenge. I expect to see diversification of columns – columns capable of delivering high performance for fundamental research and columns dedicated to rapid in-process control and drug release. With regard to instrumentation, the use of LC-MS and other hyphenated techniques will continue to grow.


*Reference*

1. *I Halász et al., "Ultimate limits in high-pressure liquid chromatography" J Chromatogr A, 112, 37–60 (1975). Available at: http://bit.ly/2p4pVfh. Accessed May 9, 2017.*


# Aggregate Win

**Senior scientist Xianwen (Susan) Chen and her colleagues in the process analytical group at Boehringer Ingelheim Fremont, Inc. are making the switch from HPLC to UHPLC for some routine SEC analyses. We caught up with Chen to find out more.**

What is the focus of your work?

It's always been my goal to work in pharmaceuticals – and to help deliver drugs to patients. My PhD was on drug screening and afterwards I joined the pharmaceutical industry as a development scientist. I've been at Boehringer Ingelheim for two years, working in the process analytical group. We support process transfer, optimization and development, starting from the cell culture through purification, right up to formulation. We develop assays to test product quality; for example, if our purification group need to optimize their column conditions, they will try different buffers and processes, and send samples to us to test the product quality – aggregate level, charge variance, fragmentation, and so on – and we will provide feedback on the process that gives the best results.

What challenges do you face?

The samples we deal with are varied and often arrive in large numbers – if we're working with a team doing a Design of Experiments project, hundreds of samples might be submitted at once. So it's crucial that we use reliable instrumentation that allows us to carry out very robust, high-throughput assays.

How do you use size-exclusion chromatography?

SEC is one of the essential assays we run every day, particularly for monoclonal antibodies. It tells you aggregate and degradation levels of the product, which is absolutely key information that is required by the FDA. There is a safety concern, in that aggregates could trigger an immune response that could render the drug ineffective or even cause adverse events.

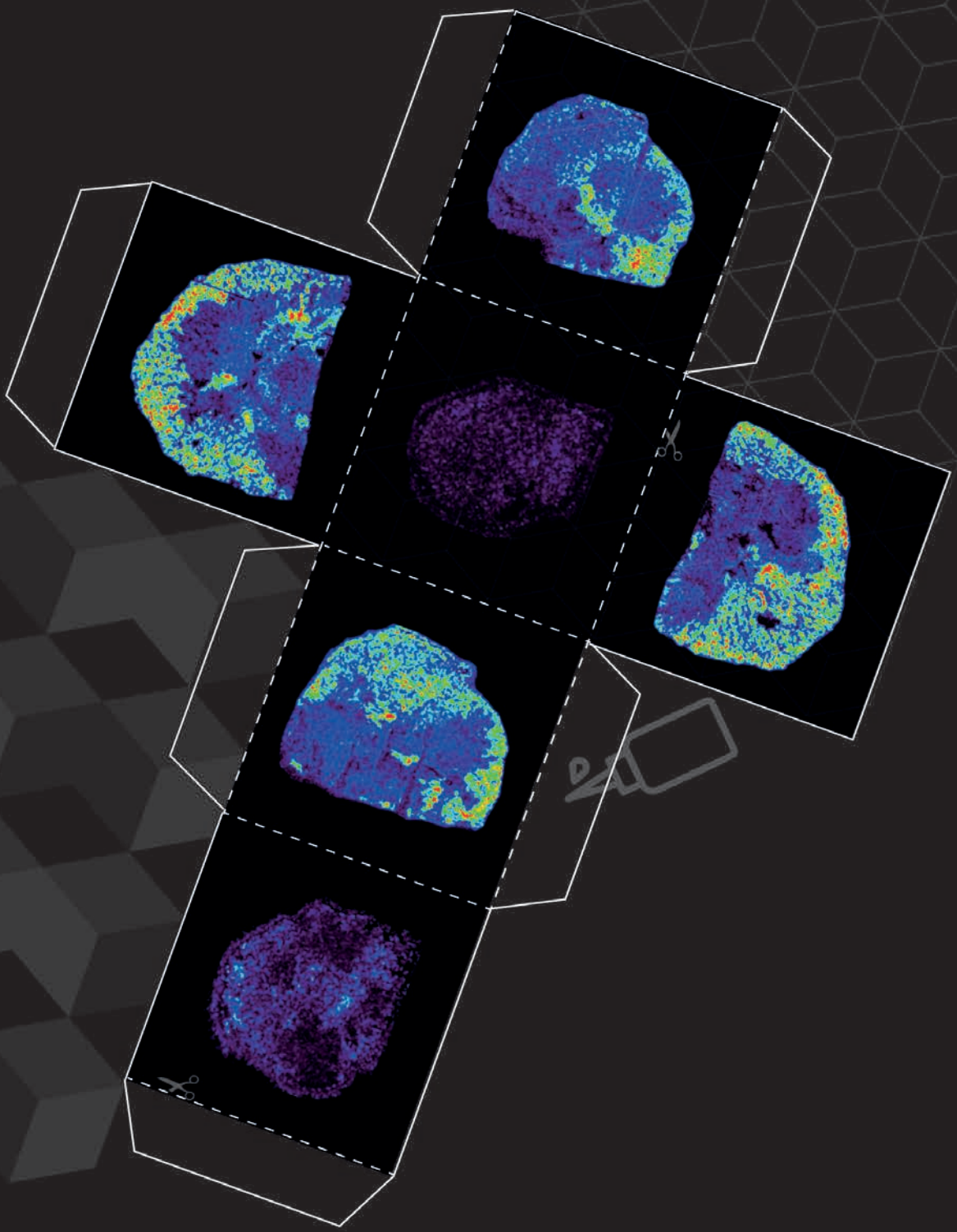
How has SEC technology advanced in recent years?

The main advance in the past decade has been the arrival of UHPLC with sub-2 µm-particle columns. The first UHPLC column for SEC was released in 2010; now, a number of new columns are coming onto the market, such as the Tosoh TSKgel UP-SW3000.

With existing UHPLC columns, we've noticed lot-to-lot variation and a tendency to lose resolution when analyzing low-molecular-weight species. To combat those problems, we had to spend a lot of time troubleshooting, trying different cleaning solutions to get the resolution back. So when the new sub-2 µm column was released from Tosoh, we were happy to try it out. In a poster presented at the HPLC 2016 conference, we showed that the column is robust, with minimal lot-to-lot variation.

Is UHPLC the future for SEC?

UHPLC brings many benefits – assays run faster and with higher resolution. Over time, I think it will replace some of the older HPLC assays, which have been around for 10–20 years. Many pharmaceutical companies are currently in the evaluation stage, deciding whether to switch. Changing assays can be hard work, especially for commercial drugs, and you want to make sure the new technology lives up to its promise. I think the two technologies will be used in parallel for a long time to come.



# CHEMICALS EVERYWHERE.

## BUT WHERE EXACTLY?

By exploring the space dimension at both the macro- and micro-scale, we uncover complex chemical heterogeneity and hidden patterns – and position analytical science as an essential tool in efforts to address the many deep issues facing society in the urban millennium.

*By Enrico Davoli*

*July 10, 1976.*

Chemical company ICMESA was manufacturing trichlorophenol (an active ingredient of herbicides) and an intermediate product for hexaclorophene (an antibiotic). The new synthetic pathway, developed by Givaudan chemists (1), was profitable but dangerous – it can result in an exothermic side reaction. Just after midday on an otherwise peaceful Saturday an accident occurred, releasing a six-tonne chemical "cloud". The cloud carried a miscellanea of toxic compounds, including dioxin (2,3,7,8 TCDD), which caused severe pollution in the densely populated Italian municipality – the so-called "Seveso disaster" (Figure 1).

Intensive monitoring plans were made to map the risk to the population – and assess the need for quarantines and decontamination. Back in 1976, mass spectrometers were available in Italy, but only in a limited number of laboratories, mainly in the petrochemical industry (2). The Mario Negri Institute cooperated in the analysis of dioxin congeners in thousands of samples to map soil and ecosystem contamination (3).

## Harnessing the space dimension

We are all familiar with meteorological maps – the space dimension of weather – but we don't typically think about (or even know of) the computational efforts at work behind them. Solving meteorological equations to predict the weather was made possible by polymath John von Neumann, who had access to the US Army's Electronic Numerical Integrator and Calculator – ENIAC. Von Neumann considered atmospheric processes to be one of the most complex modeling problems but nevertheless programmed ENIAC to perform the world's first "computerized" weather forecasts in the 1950s. Today, such modeling allows us to interact iteratively with reality; by continuously assessing the measurements used to build models against the accuracy of predictions, our models evolve and thrive. The results of mapping models – like meteorological maps – are widely used today and are essential in understanding reality.

When trying to understand the effects of the Seveso disaster, which affected up to 20 $km^2$ of the surrounding area, the importance of mapping and the need to conserve the space dimension of the environmental samples collected was self-evident. Soil and biological samples were brought into the laboratory, extracted, purified and analyzed by mass spectrometry (the gold standard for environment toxicologists) and the resulting data were used to draw contamination maps – the reality – to inform remediation plans. It was during those testing times that our laboratory developed the trace and ultra-trace environmental analytical culture that I learned when entering the Institute – and that remains strong within me today.

Air samples now play a significant role in monitoring "everyday" pollution, but present specific challenges when it comes to sampling and storage, especially for volatile analytes. Adsorbents and impingers suffer from a potential breakthrough of the analyte because of saturation; bags and canisters might adsorb or degrade analytes – or lose the sample via permeation through the polymeric walls. On-site analytical approaches are therefore becoming increasingly commonplace, especially with the advent of portable instrumentation. But there is a price. Reduced complexity (compared with laboratory-grade instrumentation) results in less sensitivity and higher data uncertainty – unacceptable when considering our trace and ultra-trace heritage.

Back in 1992, we overcame these problems by developing a transportable GC-MS with an on-line permeation tube, using deuterated analogs in the air sample as internal standards (4). The instrument was designed to accurately monitor benzene, toluene ethylbenzene and xylene (BTEX) air pollution (5). Fast gas chromatography allowed a relatively high sampling frequency, with a full analytical cycle time of 5 minutes. The instrument, derived from small quadrupole residual gas analyzer hardware,

Figure 1. Collecting samples after the Seveso disaster.

was installed in an early zero-emission, electric-vehicle prototype from Fiat (Figure 2) to avoid influencing the results with our own BTEX emissions (6). The instrument was capable of sampling and analyzing data while running in traffic – down at ppb levels – and allowed two dimensional (2D) mapping of carcinogens in urban traffic, demonstrating a highly variable concentration range of analytes, but also proving that the concentration of pollutants in the middle of the road where drivers are exposed, are 3–5 times higher than concentrations at the curbside, where air quality monitors are generally installed.

The same approach, developed on a more robust Agilent GC/MSD system, allowed us to measure airborne vinyl chloride monomer and dichloroethane inside production plants, both mapping diffuse pollution of these gases and pinpointing leaks in a large chemical plant. VCM data, along with meteorological data from an on-board monitoring station, were also used to feed an on-line Gaussian air diffusion model to describe air concentration levels outside the plant – at nearby hospitals, homes and schools (7, see Figure 3).

## Surviving the "urban millennium"

The space dimension in macro environments is used extensively in our laboratory in odor pollution studies. Our approach (now a suggested method in our Regional Guidelines: DGR Lombardia 16/4/2003, N. 7/12764) is based on a simple GC-MS analysis with isotopically-labeled internal standards and solid-phase microextraction pre-concentration – and allows us to identify

Figure 2. BTEX on-the-road monitor installed on a ZEV prototype from Fiat. Both vehicle and GC-MS instrument were powered with 12 V batteries, providing a maximum of two hours' operation time.

Figure 3. A mobile laboratory with a lab-made automatic chloride monomer and dichloroethane monitor, equipped with an air monitoring station and on-line diffusion model running in real time.

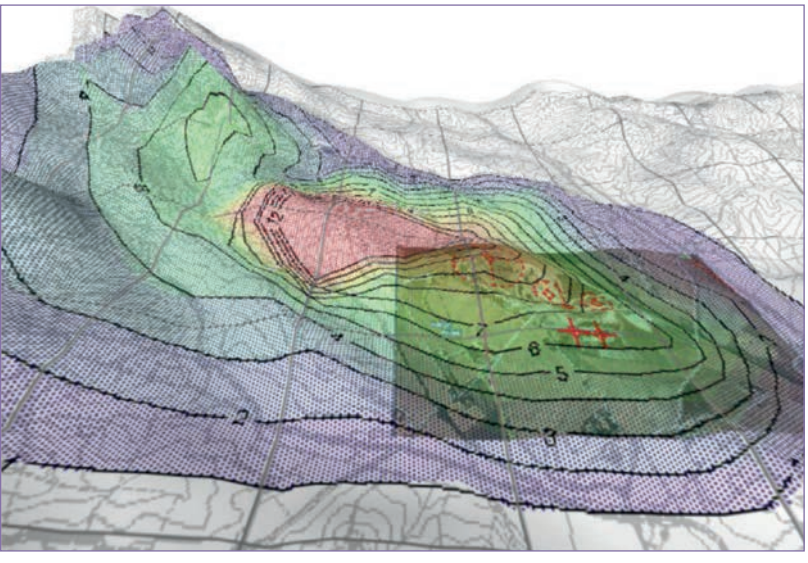


Figure 4. Environmental odor concentration mapping.

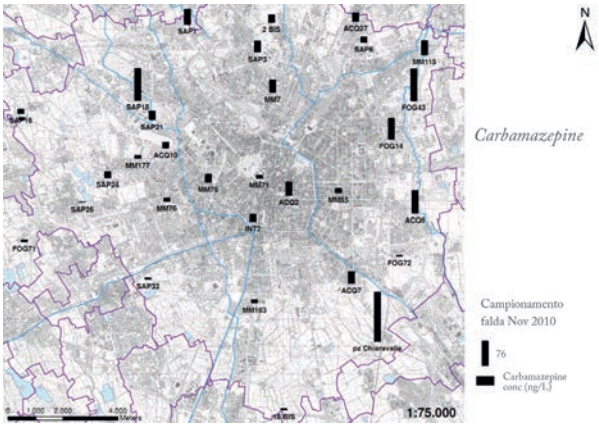



Figure 5. Concentrations of pharmaceutical residues in Milan's first water table (0-30 m); levels observed ranged from 1.5–152 ng/L.

odorants at low ppb/high ppt levels. Overlapping air diffusion model maps with ambient air grab samples makes it possible to identify sources of odor pollution (see Figure 4). Multivariate chemometrics are needed for odor source identification in complex situations where different odor sources (or different processes) are present in a confined space.

Water samples can also offer rich information when the space dimension is maintained. Trace analysis of contaminants can be used not only as an early warning system (8), but also as a tool to describe contemporary urban regions for regional planning. During the opening of the twenty-fifth session of the General UN Assembly (2001), Nobel laureate Kofi Annan described a world entering an "urban millennium" and called for the international community to rise to its many challenges

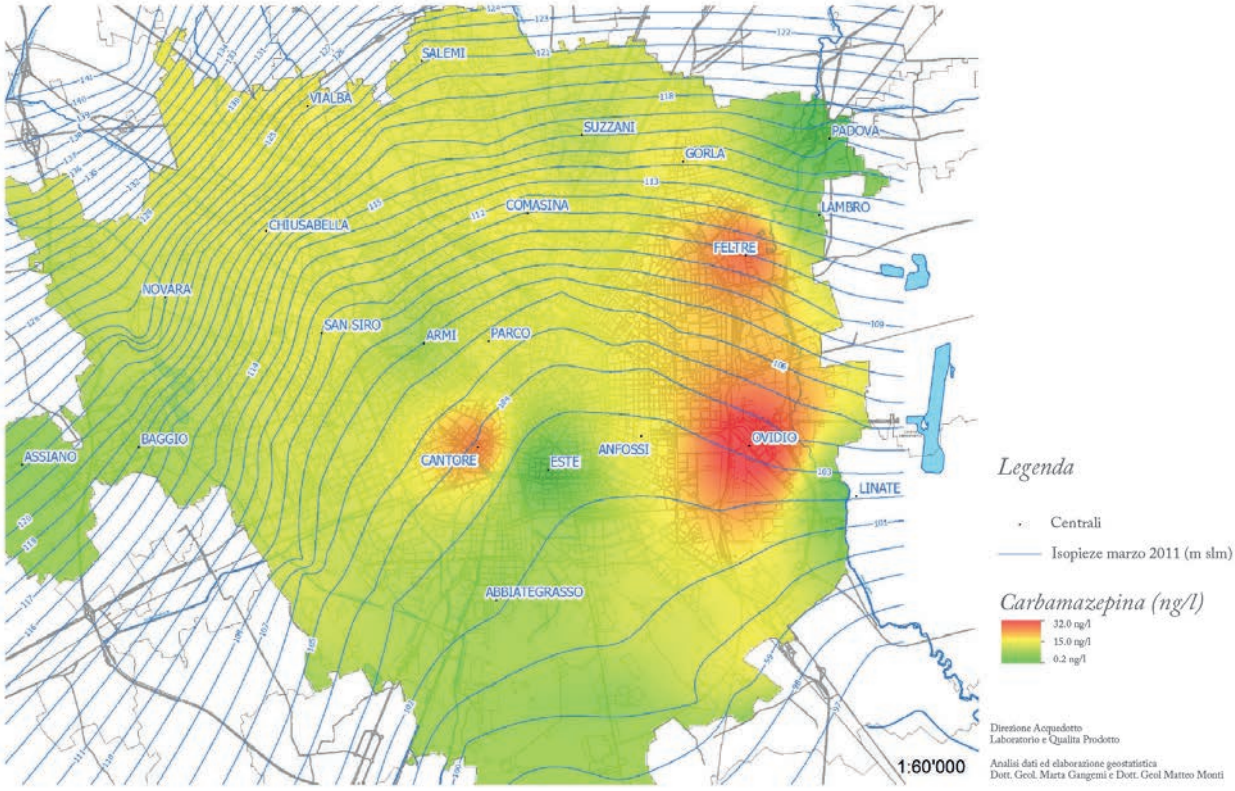



Figure 6. Concentrations of the same pharmaceutical residues in Milan's second and third water table (40-200 m). Concentrations are in the 0.2–32 ng/L range. Notably, the pattern of contamination is different from the surface layer at both the north-west and south-east borders, indicating alternative contamination paths. (Modeling of our analytical data was courtesy of Metropolitana Milanese Spa, Integrated Water Services for the City of Milano).

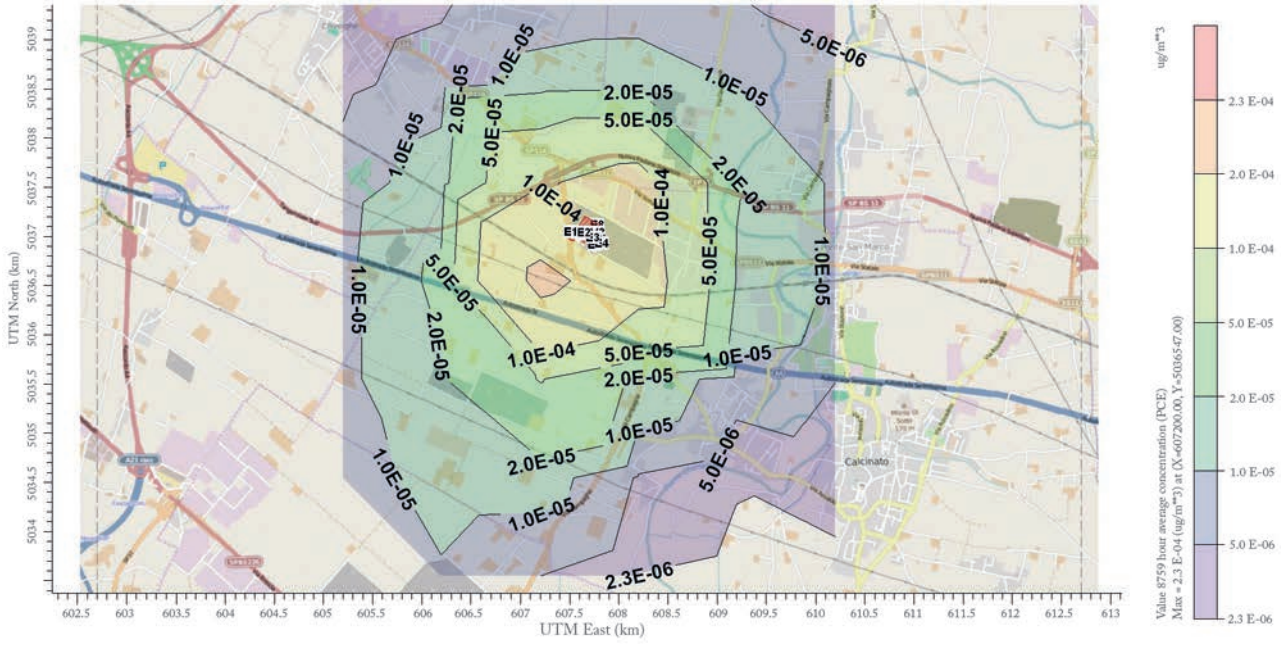



Figure 7. Lagrangian puff modeling of atmospheric perchloroethylene dispersion around a landfill area. Concentrations are one-year averages.

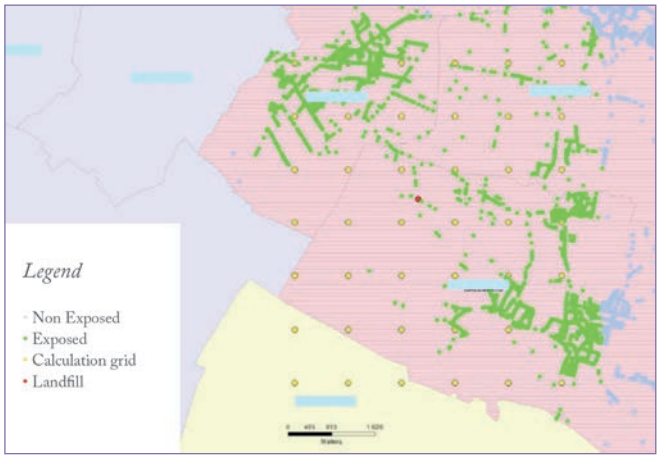



Figure 8. Geocoding of all exposed and non-exposed individual subjects to pollutants, as determined from dispersion maps in Figure 7.

(9). The UN's population division calculated that urban regions would house more than half of the world's population by 2016, and that issues such as outward urbanization would have significant implications not only for living conditions but also for the environment: "The aquifer will overwhelm sewage wastewater systems, threatening water supplies" (10).

It is now 2017 and though we are not yet in that situation, Cariplo Bank Foundation recently supported a large study of Milan's aquifer to accurately describe water supply status. To that end, we studied the presence and fate of emerging contaminants in the complex aquifer system of Milan's urban region, where the water-bearing deposits are more than 250 m deep and can be divided into four large-scale geological units separated by clay deposits, called aquitards. Samples were acquired – twice, in different campaigns – from seven points in the three rivers of Milan, from all 31 wells from the first water table, and twice from 21 wells of the second and third water table. Results showed that emerging pollutants from the rivers enter the first water table – but also, probably because of intense, increasing underground construction activities in recent years, surface water pollutants reached the second and third water tables, which should, in theory, be confined by the large clay deposits.

Compounds that directly derive from human activity, such as pharmaceuticals, are detected not only in surface waters, but in all aquitards. The spatial dimension of analytical data allows us to describe macro areas where water table confinement is less efficient (Figures 5 and 6), to help in regional planning. In Milan, the population density is becoming increasingly critical – an environmental policy that ensures proper water management is key for the benefit of all citizens.

## Exposure and response

Studies directly bound to human health can also benefit from spatial information. Typically, when environmental health impact assessments are made, health effect data are collected on an aggregate level (based on regional or urban administrative units) and health effects are estimated based on concentration–response functions – accurate exposure modeling needs to be performed. Overall health damage is derived from exposure–response functions, which are not aggregated into administrative units but on individual exposure. To further confound matters, health status data are not typically available at the individual level (in part due to concerns over confidentiality).

The upshot? The use of aggregated data could mask local hotspots and smooth out spatial variations in impact, meaning that health effects caused by personal exposure to pollutants is over- or underestimated. What if highly defined spatial data and disaggregated source data were used? We would be able to provide more accurate estimates of exposure and also highlight the effects of specific sources.

In a recent study, we collaborated with environmental epidemiologists in a retrospective cohort study to investigate health effects caused by landfill emissions. It was possible to estimate ambient air pollution at a relatively high resolution by modeling atmospheric dispersion of selected pollutants and validating the data in monitoring campaigns, but exposure assessment was much more complex.

In this case, obtaining health data was possible because of our collaboration with the local health service. Individual but anonymous data about hospital admissions for acute pathologies that are associated with exposure to landfill emissions (cardiovascular, reproductive and respiratory diseases) but also chronic diseases (endocrine disorders and child cancer) were collected over a ten-year period. It is fair to say that incorporating the space dimension into the study was costly in terms of the amount of data; even though the municipalities were relatively small, a total of approximately 30,000 residents over ten years results in the need to analyze 1.5 million health records.

After geocoding all subjects, residential history during the ten-year period was overlapped onto the map from the pollutant diffusion model (see Figure 7), and an exposure value was attributed to each individual subject (Figure 8). We could then accurately calculate relative risks using concentration–response functions with a proportional regression model showing a weak association hypothesis of local health effects due to the landfill. Spatial outcome data, used to assess risk within this context, did not show an association with distance and, more, was in counter-tendency with the expected effects of the period activities of the landfill.

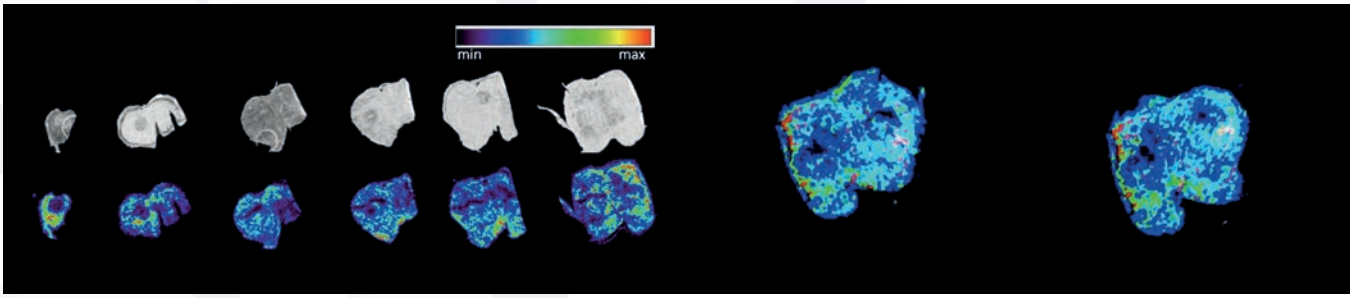



Figure 9. 3D reconstruction of anticancer drug in a ovarian tumor model (lower) from serial tissue slices (upper). Both optical serial slices images and MSI images of drug levels are reported. Spatial MSI resolution was 75µm.

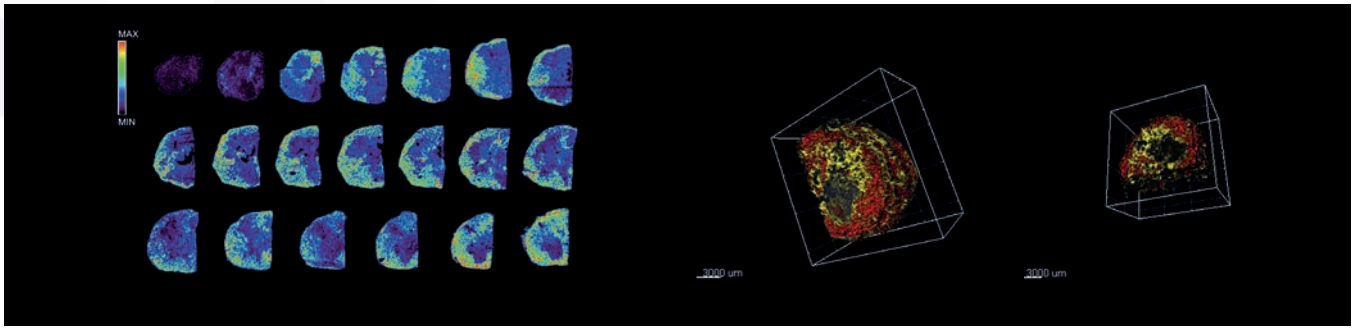



Figure 10. Left: Serial slices of a malignant pleural mesothelioma tumor xenograft. Upper lane are optical images, lower lane are ion intensities corresponding to paclitaxel concentration as from the color bar on top. Right: 3D reconstruction of the MSI serial images.

## From macro to micro

In pharmacology, many efforts have been made over the past decade to develop new analytical techniques aimed at localizing/mapping the relative distribution of drugs within micro environments, such as biological samples. Classical pharmacokinetic studies measure the average drug accumulation in tissues, assuming homogeneous distribution of the molecule through the different organs. It fails to give exact knowledge of how a compound localizes and distributes inside a tissue, which is critical if we are to ascertain whether it reaches the intended target site – and how. Mass spectrometry imaging (MSI) is ideal for this purpose, offering us the power to directly measure, point-by-point, the chemical composition of a sample – and so maintaining spatial information.

From 1997 onward – thanks largely to the works of Caprioli (11) – MSI gained popularity as a technique to investigate the distribution of large biomolecules in tissue sections. Specific ionization techniques allow direct measurements of low molecular weight compounds, like drugs and metabolites (12). We specifically designed nanostructured matrices that allow the imaging of different drugs with high specificity, sensitivity and reproducibility, based on metallic gold and titanium nanoparticles, in combination with inorganic halloysites and organic carbon nanotubes (13).

Two-dimensional (2D) data is particularly important in oncology, where the overall tissue data reveals very little about a drug's ability to penetrate a tumor. The local microenvironment greatly affects drug penetration, so that a tissue homogenate cannot tell us whether a chemotherapeutic drug reached an effective concentration in all tumor cells or not. Gathering 2D quantitative drug data inside tumors helps us to understand mechanisms of drug resistance, and to develop new strategies to improve penetration of chemotherapy into the tumor. To better describe the tumor microenvironment, three-dimensional (3D) studies made up of serial tissue slices were a logical next step (see Figure 9).

Quantitative imaging of drug penetration inside a tumor revealed a very heterogeneous drug distribution. In a model of malignant pleural mesothelioma (MPM, see Figure 10) – a very heterogeneous neoplasm – drug was detected in only 62 percent of the tumor volume, with concentrations ranging from 0.2 to >100 µg/g, while in the remaining 38 percent of the tumor, drug concentrations were lower than our limit of detection (0.2 µg/g). The result led us to "speculate that the in vivo resistance may be largely related to the inflammatory tumor microenvironment, possibly associated with the low and heterogeneous drug distribution" (14).

DataApex

# Clarity™

Chromatography Software

# TIME for CHANGE

Chosen by **THOUSANDS OF USERS**

Renowned for **EXCELLENT TECHNICAL SUPPORT**

**HUNDREDS** of **CONTROL DRIVERS**

Optional **EXTENSIONS** (PDA, MS, GPC, NGA...)

Supports all **RECENT OS**

**OEM** versions

**FREE DEMO VERSION**

**TRY IT**

DataApex Clarity™

advanced CHROMATOGRAPHY software for Windows

DataApex

www.dataapex.com

clarity@dataapex.com


DataApex www.dataapex.com

The importance of the space dimension has been underscored recently by studies that focus on tissue identification by mass spectrometry during cancer surgery. Direct MSI techniques are demonstrating great potential for identifying the margins of a tumor during surgery (15). The identification of cancer cells left behind after surgery is currently confirmed by time-consuming histopathological examination of tissue samples during the operation. MSI-based approaches are faster and more accurate, which could decrease local recurrence and increase overall survival.

## Chemicals everywhere?

Of course! Trace and ultra-trace analysis, historically developed in the analytical labs for the dioxins issue, are now widely used to describe our environment in terms of emerging contaminants, such as pharmaceuticals, drugs of abuse, and even for personal care products. Grab samples, single point or limited samples inform us about the potential presence of chemicals in the environment but do not answer our health-based questions. Here, a deep exposure assessment process is highly desirable for an accurate health impact assessment. Building contamination or concentration maps, using diffusion models, is a gold standard method that offers important quantitative information about chemical presence. Building contamination maps with analytical measurements, compared to model estimation, undoubtedly has a different value and impact. Understanding the distribution of data rewards scientists with new tools to probe environmental contamination issues or describe the presence of pharmaceuticals or drugs in our environment – and it pushes us towards prevention rather than cure.

The analysis of a tissue homogenate indicates drug presence, but has no useful information on treatment efficacy; it does not tell us if the drug reaches the target and, if it does, whether the concentration or dose is effective. With new instrumentation, it is possible to maintain spatial quantitative information even at the micro dimension of tissues, allowing us to go beyond simple descriptions. Here, understanding the distribution of data supports hypotheses, verifies pathologies, and measures treatment efficacy.

Whether in macro or micro environments, retaining analytical information in the space dimension offers flexibility and power. But it is not without challenges. Mass spectrometry has a well-deserved reputation for sensitivity and specificity, and managing mass spectrometry data has always required state-of-the art computing hardware. Adding the space dimension to the data flow takes the computing challenge out of the realm of today's personal computers; processing the additional data is likely to be a job for powerful remote machines (16). Most of the applications described in this article take advantage of several external software packages – some ready-to-use and some custom – to elaborate and present results.

As an analytical scientist, I hope that all vendors will eventually settle on a common, open-source data format for mass spectrometry that will set us free to use data in innovative ways and shed new light on our laboratory results – including the ability to take advantage of the space dimension.

*Enrico Davoli is Head of the Mass Spectrometry Laboratory in the Environmental Health Sciences Department at IRCCS Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy, and President of the Italian Mass Spectrometry Society.*

*Acknowledgements*

*These reflections come from many years of research in the environmental health field. So many beautiful students helped us with their innovative ideas and enthusiasm and are gratefully acknowledged. Financial support from sponsors, such as the CARIPLO bank foundation, has been the driving force for many studies, such as the large underground water studies in the water tables of Milan (conducted with Metropolitana Milanese Spa, Integrated Water Services for the City of Milan). Tumor heterogeneity studies are collaborations with our Oncology Department. Finally I need to thank the Environmental Epidemiology and the Cancer Registry Unit at Fondazione IRCCS Istituto Nazionale dei Tumori, and MB, in our Public Health Department, for infinite discussions that helped me in developing the idea of the space dimension and this manuscript.*

*References*

1. *T Margerison et al., New Scientist, 89, 334–336 (1981).*
2. *F Turco, "Archi e tracce. La spettroscopia di massa tra fisica e chimica", La Goliardica Pavese: 2004.*
3. *P Adamoli et al., Ecol Bull, 27, 31–38 (1978).*
4. *E Davoli et al., J High Res Chromat, 16, 626–628 (1993).*
5. *E Davoli et al., J Am Soc Mass Spectrom, 5, 1001–1007 (1994).*
6. *E Davoli et al., Int Arch Occup Environ Health, 68, 262–267 (1996).*
7. *E Davoli et al., Proceedings of the 45th ASMS Conference, June 1–5 1997, p 219.*
8. *E Davoli et al., Chemosphere, 16, 1425–1430 (1987).*
9. *United Nations (2001). Available at: http://bit.ly/2pqiNGe. Accessed May 8, 2017.*
10. *United Nations (2016). Available at: http://bit.ly/2qgI0am. Accessed May 8, 2017.*
11. *RM Caprioli et al., Anal Chem, 69, 4751–4760 (1997).*
12. *L Morosi et al., PLoS One, 8, e 72532 (2013).*
13. *S Giordano et al., Nanomaterials, 7, 71 (2017).*
14. *Giordano et al., Sci Rep, 6, 37027 (2016).*
15. *ER St John et al., Trends Anal Chem, 85, 2–9 (2016).*
16. *Metaspace. Available at: http://metaspace2020.eu. Accessed May 8, 2017.*

HUMANITY IN SCIENCE AWARD

the Analytical Scientist

*In partnership with* KNAUER


2016 Winner
*Waseem Asghar*

Nominations for the 2017 Humanity in Science Award are now open

**Wanted: Winning Project for 2017**

# Recognizing Altruism and Innovation

The Humanity in Science Award recognizes and rewards a scientific project that has the potential to make the world a better place.

Do you know of a project that is worthy of the prize?

*Nominate a project with a humanitarian impact now at www.humanityinscienceaward.com*

# From Research to Routine

**The needs of the pharma industry are always evolving – and analytical techniques must advance to remain relevant or adapt to fulfill new demands. Here, I describe how mass data is marching from R&D into more routine use: the search for genotoxic impurities.**

*By Moosung Kim, Senior Research fellow in R&D center of Daewoong Pharmaceuticals*


Daewoong Pharmaceuticals has seen countless analytical advances over the years. Founded in 1945 as a global healthcare company, it is one of the top ten largest drug companies in South Korea and regarded as a pharmaceutical "giant". In addition to its significant South Korean footprint, Daewoong's ongoing research into new drugs, generics and biosimilars is supported by its laboratories in the US, India and China.

As well as being essential for an increasingly global business, our laboratories around the world allow us to collaborate internationally on research that accelerates technological development. The president of the company, Jaechun Youn, readily highlights the core value of "open collaboration". In fact, he takes the concept a step further by stating that Daewoong's research facilities should be open to everyone – even external researchers. Accordingly, we also cooperate with professors from other institutes and also other laboratories.

Currently, liquid and gas chromatography represent our workhorse separation techniques – they've been in use for over 16 years – and we introduced mass spectrometry in around 2002. Over the years, we have invested heavily in the instrumentation necessary to create an environment where internal and external collaborators can flourish; keeping up to date with advances in chromatography, spectroscopy and mass spectrometry also ensures that we remain successful in a competitive market.

## Analytical stepping stones – and the power of mass detection

In the early days, our analytical work was closely tied to the development of quality generic raw material for export. In more recent years, we needed to develop analytical methods for new drugs and raw materials. Now, we are focusing on trace and structural analysis – a trend that demanded an expansion of my own scope of expertise into mass measurement.

Specifically, in my role of Senior Research Fellow in our new drug development center in R&D, I work for CMC (Chemistry, Manufacturing and Control), developing new drugs and managing CMC involvement in discovery, development, QA/QC and regulatory affairs.

Today, mass detection plays an essential role in our analytical workflows – and there are several general advantages that newcomers to the technology may be unaware of. Here, I present four points – some specific to the Waters ACQUITY QDa mass detector – that are particularly worth noting:

*"A special stand-out feature of the QDa is ease of use. After focused training for a day – or even less – almost anyone can operate the instrument. This is a game changer in terms of mass data accessibility."*

i. Perhaps the biggest advantage is that mass detection allows both qualitative and quantitative analysis. Qualitative experimental modes on ACQUITY QDa facilitate faster method development of API impurity methods – identification of co-elutions and method confidence, for example. And the quantitative experimental mode – single ion recording (SIR) – allows quantitation at lower limits of detection than typical optical detection, but comparable to legacy MS systems (1).
ii. The mass detector is more "universal" in operation – in other words, because detection is based on specific mass measurements, it can detect many substances – even at trace amounts – without concerns for the need of other attributes, such as the presence of chromophores or specific volatility.
iii. A special stand-out feature of the QDa is ease of use. After focused training for a day – or even less – almost anyone can operate the instrument. This is a game changer in terms of mass data accessibility. Though I should add a caveat: data analysis can still be a challenge when working with unknown or impure compounds.
iv. More specific to the QDa: the price point relative to legacy single quadrupole mass spectrometers has dropped considerably. Today, gaining access to the advantages highlighted in i) and ii) above no longer requires such a substantial investment in both capital equipment and resources.

## Making mass detection routine

It is important to note that regulations, such as the guidelines issued by ICH (2), have also driven our technology choices. Today, whenever new medicines are approved, compounds must undergo early inspection to detect foreign substances – and the data must be stored, even when submission of a report is not required. Therefore, the pharma industry requires sensitive instrumentation that can detect trace amounts of contaminating compounds in routine workflows, which is why, alongside the other benefits previously described, we switched from our legacy single quadrupole mass spectrometers to the QDa mass detector.

Previously, when mass spectrometry was primarily used in research, efficiency and ease of use were not high on the agenda. But the new demand for mass data in official documents for approval or quality control has really emphasized the need for rapid reporting and user-friendliness. In quality control, SIR mode is typically used – and that's where QDa comes into its own, offering fast turnaround of quantitative analyses.

When it comes to genotoxic impurity (GTI) analysis, for example, the ACQUITY QDa offers rapid, accurate – and reproducible – analyte quantitation. Here, the use of mass detection with SIR boosts specificity and sensitivity, both of which are essential given the low concentrations of impurities being quantified.

In the past, such analyses would have typically demanded triple quadrupole MS methods that could only be commissioned from a dedicated analytical laboratory – and would result in additional expense and necessitate the cumbersome process of checking equipment availability. Now, as the chromatographers have access to a mass detector, we can mostly perform this analysis ourselves. Direct analysis means time saved – and decisions can be made more efficiently.

## Mass detection in pharma's future

Accessibility to mass data is now at the point where it meets new demands in pharmaceutical analysis. By combining sensitivity, functionality, robustness, and chromatography data system (CDS) familiarity in such a user-friendly system, mass data can finally move out of research and specialist analytical laboratories and take on a quality control role in manufacturing.

I believe mass detectors like the ACQUITY QDa will continue to gain traction where the routine use of mass data complements more traditional methods – or where it boosts productivity by reducing the need to send samples to a dedicated mass spectrometry facility.

***Reference***


1. *M Maziarz, M Wrona, "Coupling mass detection with UV to improve method sensitivity for esters of benzenesulfonic acid in analysis of genotoxic impurities". Available at: http://bit.ly/2qYcdrV. Accessed May 9, 2017.*
2. *ICH, "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk", (2014). Available at: http://bit.ly/1vgSDCe. Accessed May 9, 2017.*

# LC on the Edge

In 2015, we posed a provocative question: have we reached the limits of liquid chromatography? Our experts returned a resounding, "no!" Two years on – and timed to coincide with HPLC 2017 in Prague – we reach out to gurus old and new to ask: are we still pushing the limits?

# Testing Boundaries

## Complex biological samples demand a new generation of LC systems

*By Davy Guillarme, Senior Lecturer, School of Pharmaceutical Sciences, University of Geneva/University of Lausanne, Geneva, Switzerland.*

A major breakthrough in LC over the past 10 years has been the commercialization of modern chromatographic systems able to work at pressures up to 1,000–1,500 bar, with low extra-column volume and dwell volume. In parallel, new columns packed with sub 2 µm fully porous particles and sub 3 µm superficially porous particles entered the market. Together, these two innovations have led to a resurgence of interest in HPLC.

### Good resolution

High-throughput separations are now easily attained. A HPLC separation that used to take 20–40 minutes can be replaced by a UHPLC separation of 2–5 minutes. The next hurdle is resolution. Our samples are increasingly complex – particularly in the fields of lipidomics, metabolomics, proteomics, natural products, and biopharmaceuticals. Such samples can contain thousands of compounds, outstripping the number that can be separated by HPLC.

How can we further increase resolution – and without sacrificing speed? It's clear to me that the solution is not to increase the upper pressure limit of current UHPLC systems. Working at pressures beyond 1,500 bar would create more problems than it solves (frictional heating). Instead, we need to develop innovative materials that allow excellent chromatographic performance without the need for extreme pressure, such as ordered chromatographic columns (pillar array), alternative particle designs (sphere-on-sphere) or monolithic supports. I suspect that monoliths, in particular, represent a very promising approach, and it's a shame that so little work is being done in this area.

As well as improved stationary phases, a major goal is to construct chromatographic systems with no tubing between the column, injection valve and detector. In modern HPLC, tubing remains the main source of band broadening when using narrow-bore or micro-bore columns, and narrow tubes could also generate a significant pressure drop at elevated flow rate. Tubing decreases the flexibility of HPLC systems and disrupts compatibility with a wide range of column widths. Dispensing with the tubing between injector and detector would allow use of a much wider range of columns – from capillary (0.3–0.5 mm ID) to micro-bore (1mm ID), narrow bore (2.1 mm ID) and conventional (4.6 mm ID).

2D-LC is another promising strategy for complex mixtures – it drastically improves peak capacity and overall resolving power, while maintaining a reasonable analysis time. The 2D-LC instruments on the market today remain somewhat difficult for non-specialists to use for comprehensive 2D-LC, but that's improving with efforts from providers. However, there is still no user-friendly software for data treatment and no calculator available to determine optimal conditions in the first and second dimensions of 2D-LC.

> "We need to develop innovative materials that allow excellent chromatographic performance without the need for extreme pressure."

In terms of chromatographic method development, I'm always surprised that so few chromatographers use modeling software, such as DryLab or ChromSword, to develop their methods. Instead, they rely on a time-consuming trial and error approach. It's a shame, since today's optimization software is extremely powerful, able to simultaneously optimize gradient profile, pH and temperature based on only 12 initial experiments. Plus, method robustness (important in a quality by design environment) can be assessed without additional experiments.

### Big molecule, little molecule

Today, one of the most important fields of application for liquid chromatography is the characterization of biopharmaceuticals. For this, we need columns that can analyze very large and complex molecules, such as monoclonal antibodies, which may have several hundred potential microvariants. Columns are available for performing size-exclusion chromatography, hydrophobic interaction chromatography and ion exchange chromatography, but the performance of these "historical" techniques will need to be enhanced with more innovative stationary phases; the resolving power is currently too limited compared to the complexity of the biopharmaceutical samples that need to be analyzed.

It's not just large molecules that pose challenges for separation

scientists; the analysis of small polar molecules is also difficult, as they cannot be retained under reversed-phase LC conditions and are not soluble in normal-phase LC mobile phases. Metabolomics regularly involves the analysis of polar substances, including sugars, amino acids, nucleosides, small organic acids or substances with several phosphate groups. Hydrophilic interaction chromatography (HILIC) is the best technique we have, but is still not as robust and versatile as reversed-phase LC. There is a strong demand for new analytical strategies able to deal with polar molecules, while maintaining MS compatibility. It's not yet clear what that will look like, but it could be either:

i. an evolution of HILIC involving new stationary-phase chemistry or mobile-phase components
ii. the use of purely aqueous liquid chromatography
iii. the use of hydride silica (Type C silica)
iv. something entirely new...

## Taking cues from MS

LC and MS are becoming increasingly inseparable. In the future, we need to exploit that relationship by developing the two techniques in tandem. The MS market is currently evolving in two different directions. In recent years, companies have commercialized easy-to-use, compact and relatively cheap MS detectors based on single-quadrupole technology. Such detectors are very well adapted to chromatographers with a limited background in LC-MS. On the other hand, there is also a need for high-performance MS systems able to deal with increasingly complex samples – devices that offer enhanced sensitivity, resolution, mass accuracy and overall performance, and are obviously dedicated to MS specialists. Obviously, these two types of MS devices can be easily hyphenated with LC and in the near future, I expect to see even more interactions between chromatographers and MS specialists, since they clearly have to learn from each other.

# New Wave

## Onwards and upwards – exciting new paths lie ahead for LC

*By Koen Sandra, Scientific Director, Research Institute for Chromatography, Kortrijk, Belgium.*

Progress in LC over the years has been steady, but much slower than advances seen in techniques like GC. The lag can be attributed to the fact that LC is the core analytical technique of the pharmaceutical industry, in which regulation and validation – rather than development – were the keywords until the end of the 20th century. However, the past decade has seen great advances, thanks to the creativity of scientists from both the academic and industrial world. Today, with LC-MS/MS, we can monitor more than 300 pesticides in our food in less than 20 minutes, and quickly determine the critical quality attributes of large biopharmaceuticals. Developments in LC and MS have led to an increased understanding of the human body in health and disease, which could ultimately improve and extend our lives.

## Perfect particles

The greatest impact has come from the development and commercialization of sub-2 µm porous particles and sub-3 µm core-shell particles. With these new columns, analyses are much faster, with at least twice the resolution and improved inertness. This, together with developments in mass spectrometry, has resulted in analytical tools that can characterize complex protein biopharmaceuticals in great detail, identify and quantify more proteins, metabolites and lipids in -omics research, quantify compounds at higher sensitivity in complex samples, identify more impurities in samples, and many other impressive feats. Columns packed with sub-3 µm core-shell particles are especially attractive for routine analysis, as they bypass the need for high-pressure instrumentation. These particles now come in many flavors with various chemistries, excellent lifetimes, higher pore sizes and improved pH stability.

## LC squared

Remarkable developments have also been made in 2D-LC (both heart-cutting and comprehensive). AJP Martin described the benefits of two-dimensional chromatography as early as 1944, writing "… but the two-dimensional chromatogram is especially convenient, in that it shows at a glance information that can be gained otherwise only as the result of numerous experiments" (1). What has, until recently, been developed mainly in universities is now commercially available in an improved and robust form, and is emerging as a very powerful technology in disciplines ranging from (bio)pharmaceutical analysis to petrochemistry. I expect to see 2D-LC gain momentum in the next five years and

"I expect to see 2D-LC gain momentum in the next five years and be widely applied."

be widely applied as a generic method for multiple samples from different fields.

Despite the enormous potential of 2D-LC and the gain in resolution typically achieved, it too can remain a compromised technique. Depending on how 2D-LC is performed, the technique suffers from issues with flow and mobile-phase incompatibilities, sensitivity, reproducibility, immature data analysis software, and others. Various groups are hard at work to make sure that these shortcomings will be challenged in the coming years.

## Better columns

We still need better chromatographic resolution for the analysis of bio- and synthetic macromolecules, and for the complex samples encountered in life sciences, there is never enough resolving power. One development I hope to see in the next five years is the advent of robust 1 mm bore columns with reduced plate heights – as low as for 4.6 mm ID columns – and instrumentation that can cope with these column characteristics, with no extra-column band broadening. I find it strange that this area has been neglected, while attention is lavished on chip-based and capillary columns with limited performance.

However, I would make an exception for the long micropillar array columns (µPAC) developed at the Free University of Brussels by Gert Desmet's group and now commercialized by PharmaFluidics. By using a lithographic etching process to create a perfectly ordered separation bed, the mobile-phase flow pattern through µPAC columns adds very little dispersion to the overall separation, giving rise to sharper peaks, better resolution and increased sensitivity. The freestanding nature of the pillars also leads to much lower back-pressure, allowing the use of very long column formats. Improvements to pillar density and surface chemistry are still needed to make µPAC generally applicable, but the principle is attractive and the results impressive. Exploiting the full potential of µPAC is hampered by the instrumentation available (nano- and capillary LC), so there is a clear need to rethink and redesign the current instrumentation.

## The next 10 years…

Looking further ahead, I foresee a number of changes:

1. I expect new developments in electro-driven separations. With renewed interest and research efforts it should be possible to profit more from electro-osmotic flow.
2. Mass spectrometers will become the standard LC detectors and in (bio)pharmaceutical routine analysis (GMP, QA/QC), mass spectrometers will be introduced and one single LC-MS method will replace various HPLC release methods.
3. 2D-LC will gain momentum, and more and more companies will enter this market.
4. Sub-3 µm core-shell columns and instrumentation up to 600 bars will become the standard in routine labs.
5. µPAC columns will be intensively evaluated for R&D in combination with state-of-the-art MS, and second-generation µPAC columns will become available.
6. For the happy few able to take advantage, sub-1 µm particles will be introduced, requiring adapted instrumentation.
7. Knowledge and know-how will further decline. As we've already seen in GC, LC systems will increasingly become black boxes.

The electric light did not come from the continuous improvement of candles; hence, someone might come up with a completely new separation principle, outperforming LC as we currently know it.

*Reference*

1. *R Consden et al., Biochem J, 38, 224–232 (1944).*

# Keep Pushing

## Tomorrow's instrumentation must match the skillset of the next generation of scientists

*By Fabrice Gritti, Waters Corporation, Milford, USA.*

During the last few years, the limits of liquid chromatography have been 'pushed', specifically in terms of:

1. UHPLC instrumentation: Recently, four 2.1 mm ID columns were coupled in series to give a total length of 60 cm and run at inlet pressures as high as 2,000 bar (1). This approach is very promising and should enable chromatographers to either reduce their analysis times or to deliver higher resolution by using longer columns. However, there may be some limitations regarding the stability of the packed beds, due to the high pressures involved.
2. Column packing: A recent breakthrough was the random packing of 1 m long x 75 µm ID capillary columns with sub-2 µm fully porous particles, halving current plate heights. This performance was achieved by combining high slurry concentrations to minimize heterogeneity in trans-column flow, and using ultrasound sonication to reduce large voids in the bed volume (2).
3. Analytical column imaging: For the first time, 3D image reconstruction of the actual bed of narrow-bore 2.1 mm ID columns was achieved using focused ion beam scanning electron microscopy (FIB-SEM) (3). This achievement opens new avenues for optimizing packing procedures.
4. Easy-to-use microfluidic LC-MS devices: 300 µm separation channels (straight or serpentine tubes with a rectangular cross-section area) can be packed with sub-2 µm particles as efficiently as standard UHPLC columns and connected to a mass spectrometer with minimum post-column dispersion. They provide a ten-fold increase in sensitivity (4).
5. UHPLC and SFC column performance: A new column technology is based on the suppression of heat transport between the column and its surroundings (5). Natural air convection and air conduction are eliminated by applying a high air-vacuum, and electromagnetic radiation is minimized by wrapping the column in a low–emissivity material. This approach allows users to bridge the gap between GC- and LC-like separations by using low-density supercritical fluids, while maintaining column efficiency (6).
6. 3D-printing: We are at a cornerstone for column technology: classical column slurry packing with silica-based particles is reaching its limit, so focus is turning to the design of ordered structures by 3D-printing technologies (7). Two-photon polymerization appears the most promising, because its feature size can be around 1 µm, while its build range remains of the order of a few centimeters.

## As for the future...

Chromatography is no longer considered a science in most academic institutions, and most chemistry students do not graduate with a deep fundamental knowledge of liquid chromatography. As this workforce faces complex analytical challenges in metabolomics, proteomics, genomics, and quality control in general, they will need instruments adapted to their qualifications, such as:

1. Easy-to-use, integrated, and self-control preparation/separation/detection systems. For instance, process analytical technology will enable the user to meet critical quality attributes rapidly and with a low rate of rejection by continuously controlling the critical process parameters.
2. More universal stationary phases (multi-mode chromatography), which allow the single-run separation of a large variety of compounds (hydrophobic, polar, ionizable, and so on).
3. New optimization software, enabling the user to rapidly develop an analytical method by accurately predicting retention and maximizing chromatographic resolution.
4. High-resolution (LC-MS → $LC^2$-MS → $LC^3$-MS?), high-throughput separation techniques to handle increasingly complex samples.
5. Efficient data processing, data storage, data handling, and data sharing in the cloud and via the internet.

*References:*

1. *R De Pauw, et al., J Chromatogr A, 1473, 48–55 (2016).*
2. *JM Godinho et al., J Chromatogr A, 1462, 165–169 (2016).*
3. *AE Reising et al., J Chromatogr A, [accepted for publication].*
4. *GT Roman, JP Murphy, Analyst, 7, 1073–1083 (2017).*
5. *F Gritti et al., J Chromatogr A, 1444, 86–98 (2016).*
6. *F Gritti et al., J Chromatogr A, 1472, 107–116 (2016).*
7. *C Fee et al., J Chromatogr A, 1333, 18–24 (2014).*

# Up the (R)Evolution!

## LC's recent incremental progress need not signify the end of innovation

*By Gert Desmet, Professor, Chemical Engineering, Vrije Universiteit Brussel, Belgium.*

Progress over the past few years in LC has been rather slow. But this does not mean we have reached the end of the journey. My personal favorite discovery in the past two years has been the work of Ta-Chen Wei, Xiaoli Wang and Bill Barber from Agilent Technologies (1). By making core-shell particles with mesopores that are purely radially oriented (instead of the conventional randomly connected mesoporous network) they could make the minimum of the van Deemter curve drop by 0.5 plate height units from roughly hmin=1.5 to hmin=1. The spectacular drop is caused by the fact that the radially-oriented pores strongly suppress the B-term, while the C-term is not altered at all. This discovery could be the next big revolution (given the other big drop from hmin=2 to hmin=1.5 when core-shell particles were introduced). It remains to be seen whether these particles have the same mechanical stability and mass loadability as conventional particles, but it does show how a small change in shape can have a remarkable impact.

As for the future, with new possibilities in materials engineering such as silicon micromachining and 3D printing becoming increasingly available, it seems inevitable that we will one day see columns that are no longer densely packed with particles, but rather filled with a perfectly ordered, low flow resistance material permeated by a sub-micron maze of flow-through channels. In the years to come, I expect more (r)evolutions in the shape of the supports, rather than further reduction of the size of our spherical particles. The latter only seems useful for ultra-rapid separations (a few seconds) needed in the final dimension of the best possible 3D-LC separations – but this is likely to remain a niche application for many years.

*Reference*

1. *T Wei et al., J Chromatogr A, 1440, 55–65 (2016).*

# Through the Maze

## A single separation mode is not enough for today's complex biologics

*By Monika Dittmann, Principal Scientist R&D, Agilent Technologies, Germany.*

Most developments in column and instrument technology over the last 10–15 years have been focused on achieving higher separation efficiencies in a shorter time (UHPLC), with smaller particles and higher pressures. Although further decreases in particle size and/or increases in operating pressure are theoretically feasible, the potential gain is limited. On the other hand, samples are becoming more and more complex in terms of number of components and chemical heterogeneity, particularly in metabolomics, lipidomics and the analysis of biopharmaceuticals. Often, a single method is not enough to fully separate all components in a mixture and the use of different (orthogonal) separation mechanisms is required.

The majority of future drug approvals will likely be biologics. In particular, monoclonal antibodies (mAbs) and antibody–drug conjugates (ADCs) have emerged as potent and specific anti-cancer agents. High molecular weights, numerous possible conformations, post-translational modifications and micro-heterogeneity make these therapeutic proteins challenging to analyze, and a single separation mode is often not enough to characterize all relevant properties. This increasing complexity in sample composition can ultimately only be addressed by multidimensional LC coupled to MS, a powerful separation technique that combines high separation efficiency with complementary selectivity and in addition enables coupling of non-compatible MS chromatographic methods (HIC or IEX as the first dimension) to MS instrumentation, by using reversed-phase LC in the second dimension (1).

Beyond the developments in column technology and instrument hardware, a prerequisite for the widespread success of complex separations schemes such as 2D-LC is the availability of software tools that support method development and data interpretation. Before these technologies can be applied in routine use, system robustness and ease of use have to reach the same level as in current 1D-UHPLC instruments.

"Increasing complexity in sample composition can ultimately only be addressed by multidimensional LC coupled to MS."

*Reference*

*1. DR Stoll et al., J Chromatogr B, 1032, 51–60 (2016).*

# 2D-LC Has Arrived

## Is 2D-LC finally hitting the sweet spot for routine use?

*By James Jorgenson, William Rand Kenan, Jr. Distinguished Professor of Chemistry, University of North Carolina, Chapel Hill, USA.*

In the last two years, I haven't seen any earth-shattering changes in LC separations. However, I have noticed an increasing willingness to embrace comprehensive two-dimensional liquid chromatography for separations of complex mixtures. Comprehensive 2D-LC has been around for almost 30 years, of course, but I wondered at one time whether it would ever catch on. People seemed to try it from time-to-time but couldn't stick with it, perhaps because of a perception that it was too slow or the lack of commercial equipment. But over the past decade a change has occurred. The development of commercial instrumentation designed for 2D-LC has been critical to making this technique feasible and more popular. This effort has been led most notably by Peter Carr, Dwight Stoll, Peter Schoenmakers, Luigi Mondello and Paola Dugo, although many others have also been involved. In fact, new demonstrations of its capabilities appear almost weekly. It appears 2D-LC has finally arrived as the viable and powerful separation technique that many knew it could and should be.

55 Years
**Science Together**

KNAUER


# Perfect fit for your analytical HPLC task

**AZURA® Analytical HPLC / UHPLC**

- Choose between a high pressure or low pressure gradient pump, up to 700 or 1000 bar
- Select detectors like UV / Vis or refractive index, featuring latest technologies, e.g. LightGuide flow cells
- Control your HPLC / UHPLC with your preferred chromatography data system: OpenLAB, ClarityChrom®, Chromeleon™ and more

Let your application define your system solution.
Learn more at: **www.knauer.net/azuraanalytical**

Solutions

*Real analytical problems*
*Collaborative expertise*
*Novel applications*

# Speccing Up Education

There's nothing quite like students to push even the most rugged instrument to breaking point. But regardless of their talent for destruction, the next generation of scientists need to learn how to use their instrumentation effectively if they are to avoid becoming "button pushers" in the future. Here we introduce two low-cost – and hardy - solutions: one, an easy-to-assemble spectrophotometer that is helping students look "beyond the box"; the other, a compact instrument that aims to democratize Raman spectroscopy.

## Thinking Outside the (Black) Box

*Patricia Forbes was interviewed by Joanna Cummings.*

### The Problem

Instruments are becoming more automated, robust and rugged. And though such advances are great in terms of measurement repeatability, from an educational point of view it's a bad thing – students have less interaction with the instruments, specifically with spectrophotometers. The opportunity to learn is limited, and this really impacts on their understanding and interpretation of their results. Considering the cross-cutting applicability of spectrophotometry, this lack of understanding needs to be addressed.

### Background

In my practical sessions for students, I noticed that little thought was given to how the instruments were actually working. Students would open the lid of the instrument, put in their cuvette, close the lid, press the button, and get a number. It was just a results-spewing "black box" to them, and the concept of what was actually going on inside the instrument was completely missing. I wanted to change that.

As educators, when we want to teach our students how a black box works, we break it down into smaller boxes on the whiteboard or in a PowerPoint presentation – we have the source, the detector, and so on. We decided we needed an educational instrument that followed the same approach – a spectrophotometer that allows students to touch all the components to really see how they work, as well as getting a feel for how changing one element can affect the results. We also wanted to encourage them to be more innovative and have a more enquiry-based approach to learning about spectrophotometry.

The University of York had already developed a DIY spectrophotometer some years ago, which I appreciated for its simplicity and circuit design, but I really wanted the components to move. I didn't want the cuvette to be in contact with the circuit board – because, inevitably, students will take a shortcut and pour their solutions directly into the cuvette on the circuit board, frying the most expensive component. We needed an alternative; thus, the SpecUP was born (1).

### The Solution

I had some funding available and a third-year mentorship student who was studying a co-major in physics and chemistry, and we worked together to develop the kit as it stands today.

We had several requirements when developing the instrument. It had to:

- Be available as a kit to be assembled by students
- Have accessible manual settings for component parts
- Be sufficiently rugged to withstand repeated use
- Be able to generate analytically spectroscopy-related applied experiments
- Be built so that the electronic component of the instrument was not the focus of the experiment
- Be significantly lower in cost than commercially available instruments.

The resulting instrument comprises a commercial telescopic drawer slide with three independently movable components: 1) LEDs for the light source, 2) the lens and grating, and 3) the slit, cuvette and light-dependent resistor

(LDR) – the latter of which serves as the detector. It is battery powered, and uses readily available components – both of which mean that, in theory, it is inexpensive to build and operate.

Initially, I just planned to use the SpecUP at my university; we'd seen such a rapid increase in class sizes that students were having very little hands-on time, even with the commercial instruments. We didn't have funds to buy more instruments, so this was a good alternative – especially as we designed it to generate analytically useful results; I wanted the students to get a feel for the spectra, and the SpecUP allows them to do that.

I have two questionnaires that I give to the students – one before the practical and one after – so I can get an idea of their perceptions of spectrophotometry and the SpecUP itself. I saw that it really helped their understanding, and makes them think more about what is involved in the whole process.

## Beyond the Solution

The funders saw that the SpecUP could be valuable beyond my own institution. They helped me apply to set up a workshop so that we could roll out the SpecUP to various institutions in Africa, and from there it's just taken off. There are now 20 countries with instruments, which is phenomenal. The last workshop we had, in September 2016, was part of a bigger "Teaching the Teachers" session, which focused on a range of different optics-

related experiments and instrumentation – expanding on the initial idea.

Naturally, some teachers prefer the ease of a commercial instrument, but others have really taken the SpecUP on board. In Stellenbosch, I was approached by teachers in the food science department to create a targeted workshop specifically for them. The Nelson Mandela Metropolitan University in South Africa is developing some new practical sessions using the instrument, and are even using it for their outreach to schools.

I would really like to get SpecUP into even more institutions. I have no experience in manufacturing or business-related activities, and it would be wonderful to have a company on board that could manufacture the parts and produce more kits. At the moment, the more we make, the more pressure there is on our department – and it's not really what our team is set up for. In addition, our current poor exchange rate is causing a real financial strain – the last set of kits that we prepared cost significantly more than the previous ones. They are still much cheaper than the commercial instruments, but we have a limited budget.

The South African government are keen to increase the number of graduates in all institutions throughout the country. We have two new universities being set up, but those expectations also put a lot of pressure on existing facilities. During the time I've been at the University of Pretoria, I've seen the number of students in the classes more than double, and although we received funds to upgrade our facilities, this was centred on infrastructure – we were able to buy new lab benches, but not the instruments to put on them! There's a bottleneck, and we need to be more creative about education, so that students aren't at a disadvantage because of a lack of commercial instrumentation. I think the SpecUP is the perfect tool to help fill that gap.

*Patricia Forbes is Associate Professor of Analytical Chemistry at the University of Pretoria, South Africa.*

*Reference*

1. *P Forbes, J Nöthling, "Shedding light on spectrophotometry: The SpecUP educational spectrophotometer", S Afr J Sci, 110, 2013-0096 (2014).*

## The Realities of Undergrad Raman

*By Brian Marquardt and Giora Proskurowski, MarqMetrix and Evan Friedman, Hellma*

### The Problem

Raman spectroscopy is a great tool for quality control, product verification and process monitoring – but a lack of staff with appropriate Raman experience often rules out its use. Undergraduate chemistry students rarely get exposure to – never mind experience with – Raman spectroscopy. Our research suggests that when it comes to teaching the technique, price is the primary barrier, so we set out to create a low-cost, capable Raman laboratory system that is suitable for both teaching and research at any level.

### Background

Strong Raman talent is simply not available below the PhD level. And though postdocs are a good source of team leaders, we also need experienced Raman users at the technician level. At MarqMetrix, we're doing some exciting things in the Raman space; we need people who understand the technology and are able to explain it to someone else, but we were having a very hard time finding the right people. We wondered if we could somehow help build the pool of talent.

We didn't see any commercial solutions that were designed for the academic market – many commercial solutions are pieced together, and therefore not "student-proof" or safe. We frequently hear from lecturers, "We have a Raman over there but 'X' component is broken, or piece 'Y' is missing, so we haven't taught Raman in three years."

MarqMetrix and Hellma decided to join forces to develop a moderate-cost Raman system that is specifically

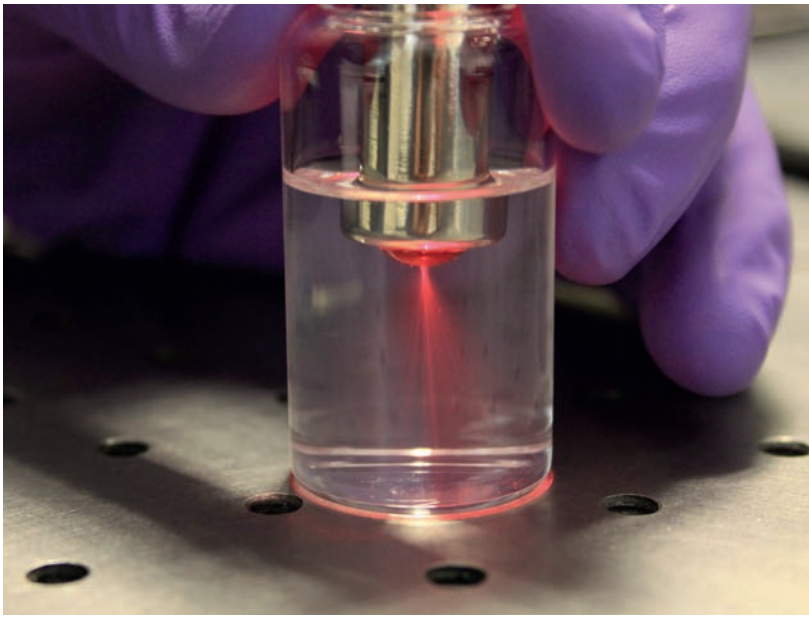
designed for training and academic use, but with a shallow learning curve. We were clear from the outset that we weren't designing an instrument for our usual scientist customers – we wanted to make it accessible to people who had no familiarity with Raman. Equally, we didn't want it to simply be a "black box" – we wanted it to be part of a curriculum. We also felt it was important to maintain a high level of performance that matched much of what we already do in the Raman world – in other words, we didn't want lower cost to mean lower performance.

### The Solution

In fall 2015, we mocked up the first prototype, dubbed "Project MOMO." The instrument makes use of our TouchRaman™ Ballprobe®, which only needs to touch the sample to make any measurement, removing a lot of method subjectivity and quashing concerns about how to effectively perform a Raman spectroscopy measurement – or what we call the "why can't I do this as well as my neighbor?" factor. The focus is very much on the value of the technique.

Crucially, the onus is not on teaching staff to bring themselves fully up to speed with Raman; the project is all about being able to cover the basics of Raman in a week – both in coursework and in the laboratory. To that end, we provide an accompanying curriculum, which Hellma made possible by partnering with Justin Shorb from Hope College in Michigan. The instrument and curriculum combination allows you to explain the concept in the classroom in the morning and by the afternoon you can be performing effective Raman experiments. Clearly, not all of the nuances of Raman can be covered in a single day, but we want students to be able to approach it immediately from a practical point of view.

Safety was a primary concern, as Raman spectroscopy is an optical technique that uses moderately powerful lasers. We knew from past experience that if students can break something, they will, so we designed a hardened instrument that was capable of withstanding the rigors of an undergrad teaching lab. To make it student (bomb) proof, we had some interesting and amusing discussions. For example, the distance between the hardened probe and the base is smaller than a human head – just in case a student thinks it might be



## Why MOMO?

Named after Momofuku Ando, the inventor of Cup Noodles, Project MOMO (now re-named REVA) operates on similar principles to Ando's quick and simple noodle-based concept. The Japanese inventor came up with the concept of instant noodles-in-a-cup in 1971, and the idea spread across the globe. The fact that anyone could make a tasty hot snack in just a few minutes gave it mass appeal. Hellma and MarqMetrix felt there were parallels with their own mission to increase accessibility of Raman; giving students easy access to low-cost instrumentation, and allowing them to perform Raman spectroscopic analysis in a matter of minutes – almost "instant Raman"...

funny to attempt some brain measurements. It's also housed as a full enclosure with the lid interlocked to the laser so that it can't be used with the cover open.

The idea of simplicity and accessibility is reflected throughout – there are no peripherals above and beyond the instrument. There are no fiber connections, no external probe... nothing "breakable". As the final design became reality, the MOMO was rechristened the REVA - the Raman Energy Vibrational Analyzer. The REVA includes a built-in PC and ships with a monitor, a mouse and a keyboard. It has one button, a USB port and an HDMI cable – and that's it. REVA is still a lab-grade instrument, capable of high-quality measurements in hundreds of milliseconds, spectral resolution under 9 $cm^{-1}$, and high signal-to-noise ratio.

In reality, not all applications need high resolution or high sensitivity; sometimes you get sold a Ferrari when what you actually need is a capable Sedan. One of our collaborators works with the Navy on fuel quality assessment and asked us if there was a way to move the capability "on ship". We began by trying to figure out how we could provide someone with limited expertise with the tool, but also make it simple, safe and highly capable. It would also need to be rugged – the Navy sailors were unlikely to show it any kindness. For the first iteration, we just added a lab jack on the bottom, built the frame, and enclosed it all in the head with the probe down. In fact, that instrument was one of the early inspirations for REVA.

### Beyond the Solution

We debuted the technology at an educational conference in late summer 2016, and feedback was really positive. People liked the idea of it being a complete package – that it could be placed in a lab and allow for Raman teaching with little preparation. Ongoing feedback will be essential as we further develop the concept.

The REVA is designed to be an early-stage teaching tool, but it has the capability and power of our processing instruments; it has just been packaged differently. We tested it internally by running samples for some of our commercial customers and verified the data with a higher-end unit. The result? A very close match.

# Routine Analysis of Ingredients in Wine

## A tool for every winegrower for standard control of wine quality and taste

*By Hagen Schlicke and Kate Monks*

The amount of sugar present in a wine determines its classification as a dry or sweet wine. Being a fruit product, wine can be expected to contain fructose and glucose and other additive sugars. Sorbic acid and citric acid are commonly used as acidulants and/or preservatives. Citric acids add a fresh taste, whereas succinic acid gives a more salty, bitter taste. The malic acid content gives wine its distinctive flavor and is also an indicator of the quality of the fermentation by the yeast in the wine. If the fermentation process is not properly controlled, any malic acid remaining can lead to spoilage from bacterial fermentation after bottling. Such wine is said to suffer from an imbalance of acid or to have spoiled. All listed compounds can be quickly analyzed by HPLC in combination with Eurokat H column, so that objective values related to sweetness, acidity, maturity and alcohol content can be obtained simultaneously with indication of deterioration or adulteration.

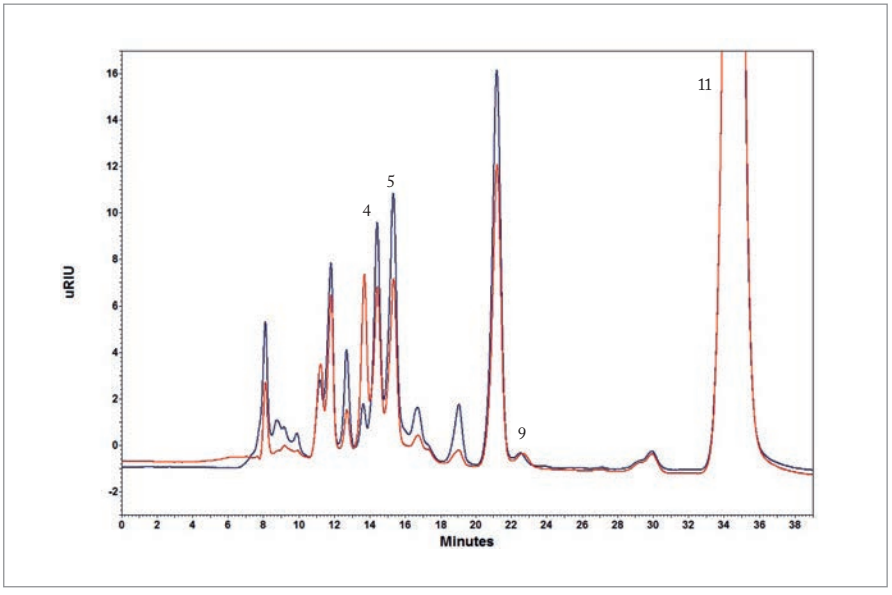



Figure 1. Overlay of a typical white wine sample (red) and red wine sample (blue).

| # | *Substance* | *Retention Time* | *Concentration (g/L)* | |
|---|---|---|---|---|
| | | | White Wine | Red Wine |
| 4 | Malic acid | 15.500 | 11.59 | 3.55 |
| 5 | Fructose | 16.417 | 12.92 | 5.73 |
| 9 | Acetic acid | 23.600 | 0.36 | 0.65 |
| 11 | Ethanol | 35.650 | 87.20 | 111.59 |

Table 1. Retention time and concentrations of a selection of substances in a typical white wine and red wine sample.

### Results

The substances citric acid, tartaric acid, glucose, malic acid, fructose, succinic acid, lactic acid, glycerol, acetic acid and ethanol were identified in different wines as being 12–13 percent of the sample weight (Figure 1). Interestingly, white wine contained a markedly higher amount of malic acid (11.585 g/L) and fructose (12.919 g/L) and less alcohol (87.202 g/L) in comparison to the red wine (Table 1) and all other wines analyzed. The wines analyzed gave an average total acid content of 7–8 g/L, with the exception of white wine. The concentration of volatile acetic acid was nearly 0.36 g/L in the white wine sample and, as expected, twice as high (0.65 g/L) in the red wine.

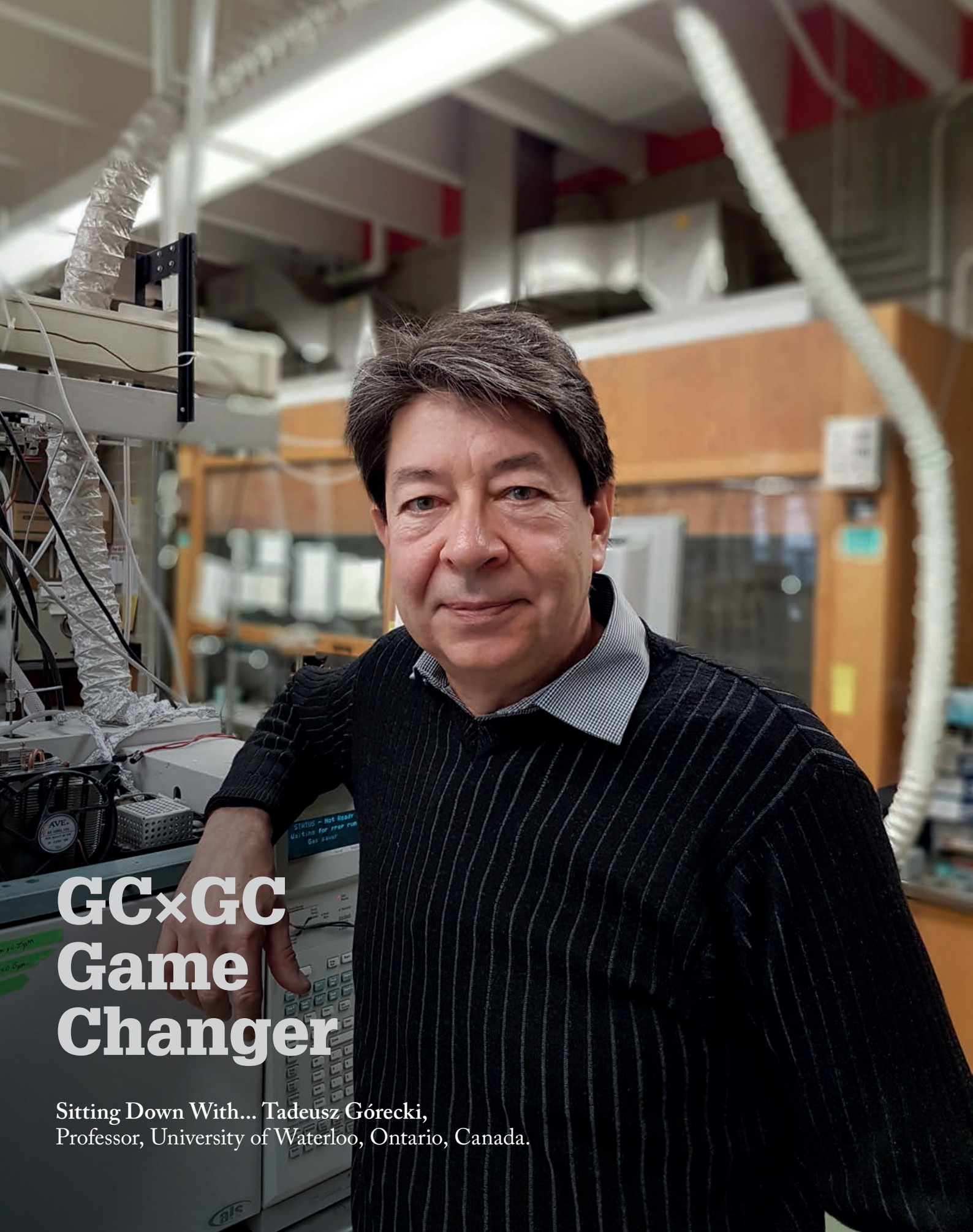
### Discussion

This result allows us to conclude that the fermentation process proceeded quickly and then was prematurely stopped in white wine. Consequently, a wine is produced with high residual sugar content, and higher fructose and lower glucose concentrations. The flavor of the wine is dependent on the tartaric acid and malic acid concentrations, as well as the total acid content comprised mainly of lactic acid, citric acid, and succinic acid.

### Materials & Method

An AZURA Analytical HPLC Plus system was used for this application. The system consisted of an AZURA P 6.1L pump, a manual injection valve, an AZURA CT 2.1 column thermostat, an AZURA RID 2.1L refractive index detector and an Eurokat H column. Eurokat H is a sulfonated cross-linked styrene-divinylbenzene copolymer. The 40 min isocratic method ran at a flow rate of 0.6 mL/min of 1.25 mM sulfuric acid. The column thermostat was set to 85 °C and the data rate of the detector to 10 Hz. 20 µL samples were injected.

*Contact:*
*Kate Monks*
*+0049 30 809727 320*



# GC×GC Game Changer

**Sitting Down With... Tadeusz Górecki,**
Professor, University of Waterloo, Ontario, Canada.

Congratulations on winning the 2017 GC×GC Lifetime Achievement Award – what achievement are you most proud of?

I hope that the biggest achievement is still to come! But I'm proud that I have been able to steer the discussion in new directions. For example, when my group published work on stop-flow GC×GC – de-coupling the flow between the first and second column to improve resolution – it was met with a lot of skepticism. Back then, it was considered almost a blasphemy to say that you could stop the flow in the first columns and not ruin the separation due to excessive band broadening. Today, intermittent stopping of the flow in the first dimension is part and parcel of flow modulators. A total transfer valve-based modulator based on this principle has also been described. I would like to believe that our research helped overcome the preconceptions that prevented fellow scientists from exploring this idea in the past.

We developed one of the first modulators with no moving parts, and we have now developed a consumable-free modulator, which I believe could be a real step forward. The field settled on cryogenic modulation for GC×GC almost 20 years ago, but I believe there are better solutions out there.

How did you get interested in GC×GC?

It started with a project on non-discriminating pyrolysis in the mid-1990s with Juergen Poerschmann at the Helmholtz Center for Environmental Research in Leipzig, Germany. He believed that the pyrolysis products we saw in the GC were only part of the story – many of the heavier analytes never reach the GC column. His idea was to create a pyrolysis device that would allow all analytes to enter the column. The device I built was essentially a piece of stainless steel tubing, which we zapped with a pulse from a capacitive discharge power supply to heat it rapidly. When the first papers on GC×GC came out, I could see a clear application to our work.

It was when the first cryogenic modulators were developed that it all clicked – we could couple cryogenic cooling with capacitive discharge heating, so that there are no moving parts. That was the inspiration for our first modulators, which we developed in the late 1990s and early 2000s. It was an uphill battle at times, but ultimately it allowed us to do really cool things, and it generated a great deal of excitement.

How has your research changed over time?

We still explore new approaches to modulation, but have been broadening our horizons. As GC×GC reaches a wider audience, we are seeing the re-emergence of LC×LC. It's older than GC×GC – but it reaching the mainstream has been an uphill battle. Recently, I have been applying my GC×GC expertise to LC×LC, and I will be presenting some interesting ideas at ISCC in Fort Worth, Texas this year... Watch this space!

What challenges face GC×GC in the next decade?

The next big step is to move the technology into routine use. I'd like to see the technique available to every lab, without having to hire a PhD who specializes in the field. You should be able use a modulator with any existing instrument – just hook up your columns and you're good to go.

How can we achieve that?

Cost is a huge obstacle; right now, you can buy two GC instruments for the price of one modulator – the sums simply don't add up for most labs. We also have to simplify the technology. And the need to haul liquid nitrogen to the lab every day is a deal breaker for many people.

We can only get there if the field has support from major instrument manufacturers. Unfortunately, GC×GC is essentially a side project for many of the bigger companies, and smaller companies lack the deep pockets needed to fund development. The field needs a champion on the vendor side.

You teach at both graduate and undergraduate level – what makes a good teacher?

A good teacher has to love not only the process of teaching but also the topic itself. I love teaching analytical chemistry and I think my students pick up on that. I try to develop their appreciation for what analytical chemistry can do. It is too often relegated in people's minds to a service role. But analytical chemistry in itself is a magnificent field; no chemical advance would be possible without the involvement of analytical chemistry. I tell them that being able to do the calculations is not enough – a good analytical chemist is open to new ideas and can synthesize knowledge from many areas to come up with a solution to the problem at hand.

It's wonderful to see their enthusiasm grow. Some of my best graduate students have been those I infected with the analytical chemistry bug during their undergraduate degree.

What motivates you?

I like the technology, it's as simple as that! GC×GC is very powerful – and it's fun to work with. People think it's complicated, and though it's clearly more complex than running a single GC, you get so much more information. I believe that the future is bright for the field, and that the day will come when GC×GC is in widespread use for routine analysis.

How do you define success?

For me, success is contributing to the community. You can spend years in the lab doing interesting work, but if there is no practical benefit, to me, that's not success. That's why I truly appreciate receiving the GC×GC Lifetime Achievement Award, because it acknowledges that I'm not just doing research for the sake of research, but helping to move the field forward.

# Lab Growth

Metabolomics, Flavor and Fragrance, Food Safety, Pharmaceutical, Environmental… the list is long. In every field, laboratories must deliver improvements in throughput and performance that are only possible with efficient automation.

The MultiPurpose Sampler MPS performs sample preparation, analyte concentration and introduction to GC/MS or LC/MS.

The MPS even generates your standards, adds them to samples, runs calibrations. All by mouse-click. Highly precise and efficient automation for accurate results.

**How can we support your growth?**



Agilent Technologies
Premier Solution Partner

GERSTEL

GERSTEL 50

www.gerstel.com