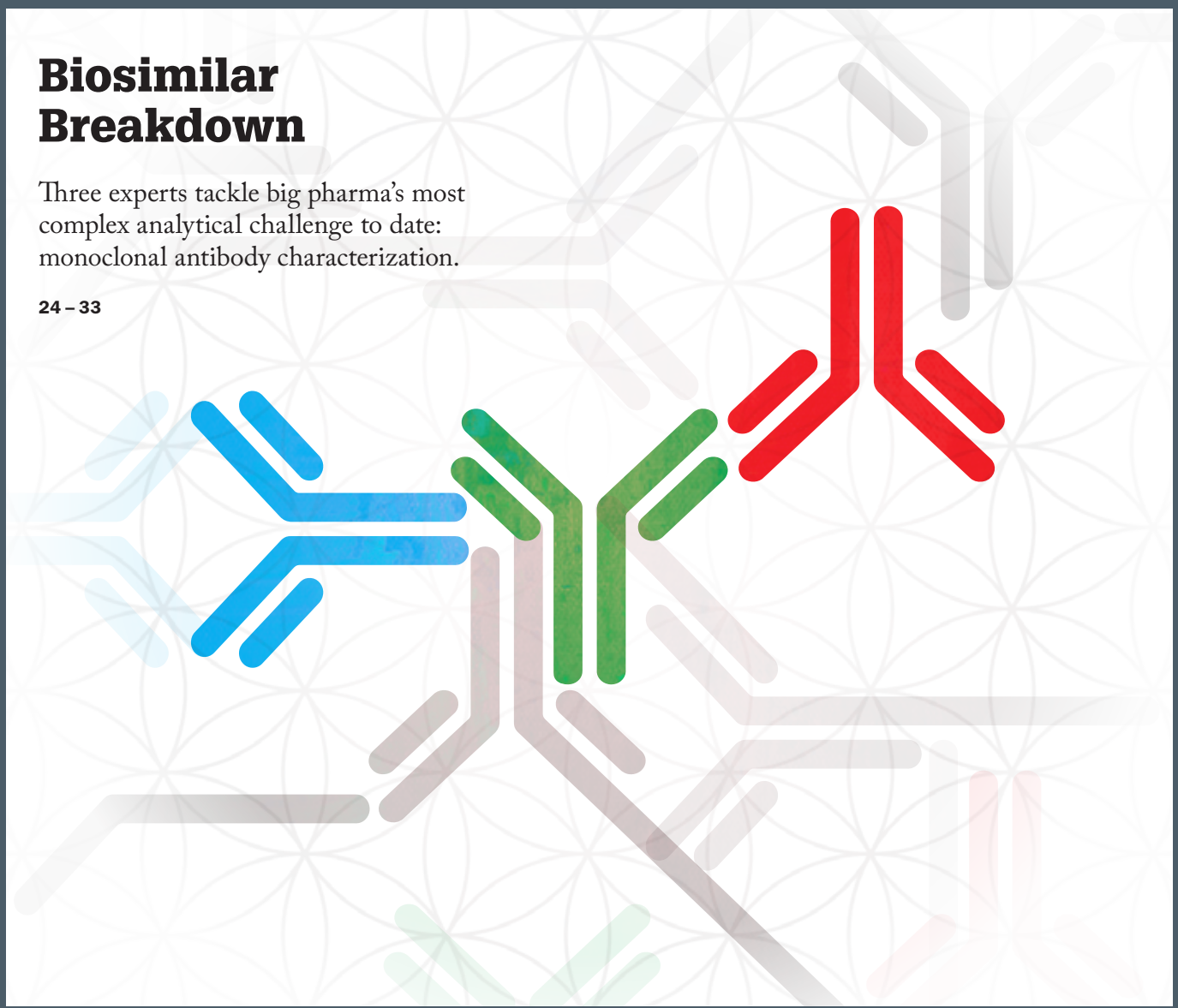
SEPTEMBER 2016 # 44

the Analytical Scientist

Biosimilar Breakdown

Three experts tackle big pharma's most complex analytical challenge to date: monoclonal antibody characterization.



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TOSOH BIOSCIENCE

Image of the Month

Giving Luciferase the Green Light

Luciferases catalyze bioluminescence reactions in insects such as glow-worms and fireflies – and have been adopted as useful tools by the bioanalytical community. Different species emit a range of colors from green to red – but "the structural determinants and mechanisms of bioluminescence colors and pH sensitivity remain enigmatic," say researchers from the Federal University of Sao Paolo (1). Using site-directed mutagenesis of luciferase in three families of bioluminescent beetle, the team was able to dig deeper into the role of two amino acids in the catalytic process. Credit: Robert Sisson/National Geographic Creative

1. V Viviani et al, "Glu311 and Arg337 Stabilize a Closed Active-site Conformation and Provide a Critical Catalytic Base and Countercation for Green Bioluminescence in Beetle Luciferases", Biochem, 55, 4764–4776 (2016).

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

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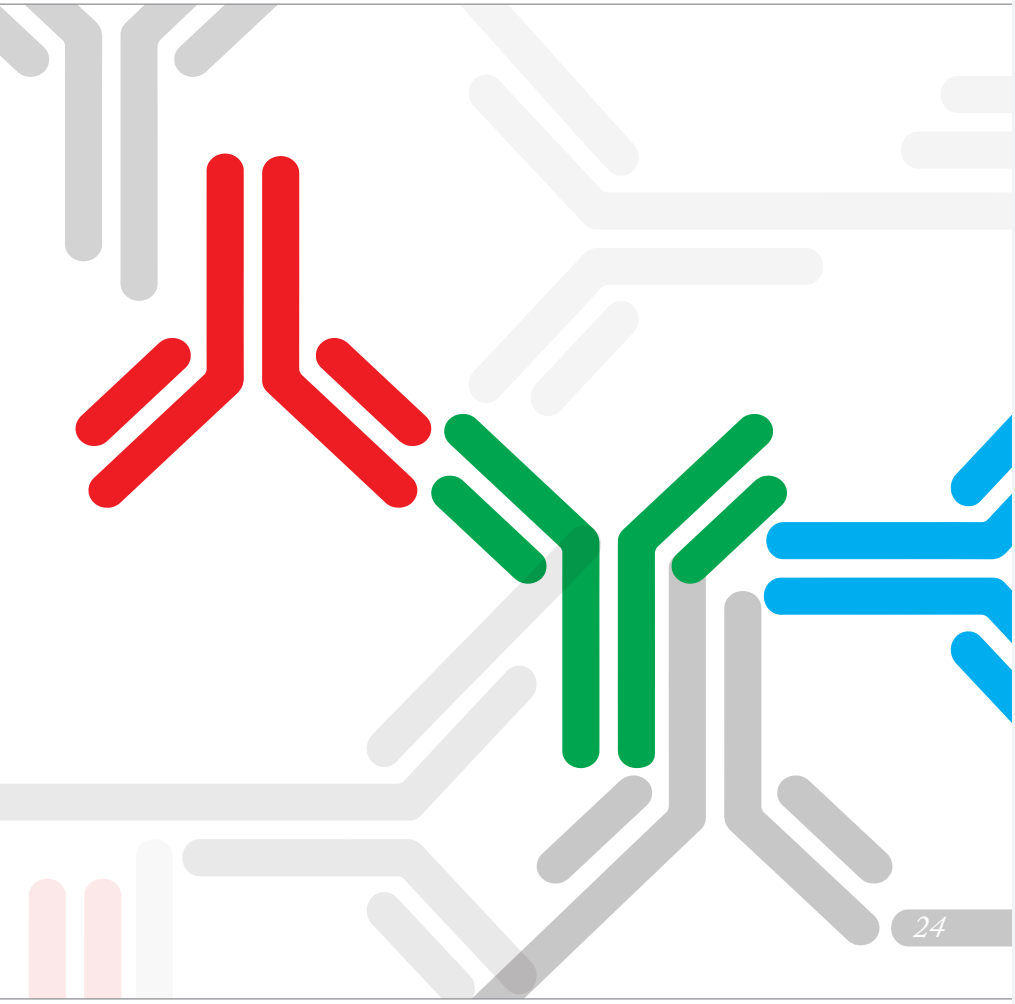

On The Cover

Simplified monoclonal antibodies belie the complexity of the analytical challenge.

Upfront

the Analytical Scientist

ISSUE 44 - SEPTEMBER 2016

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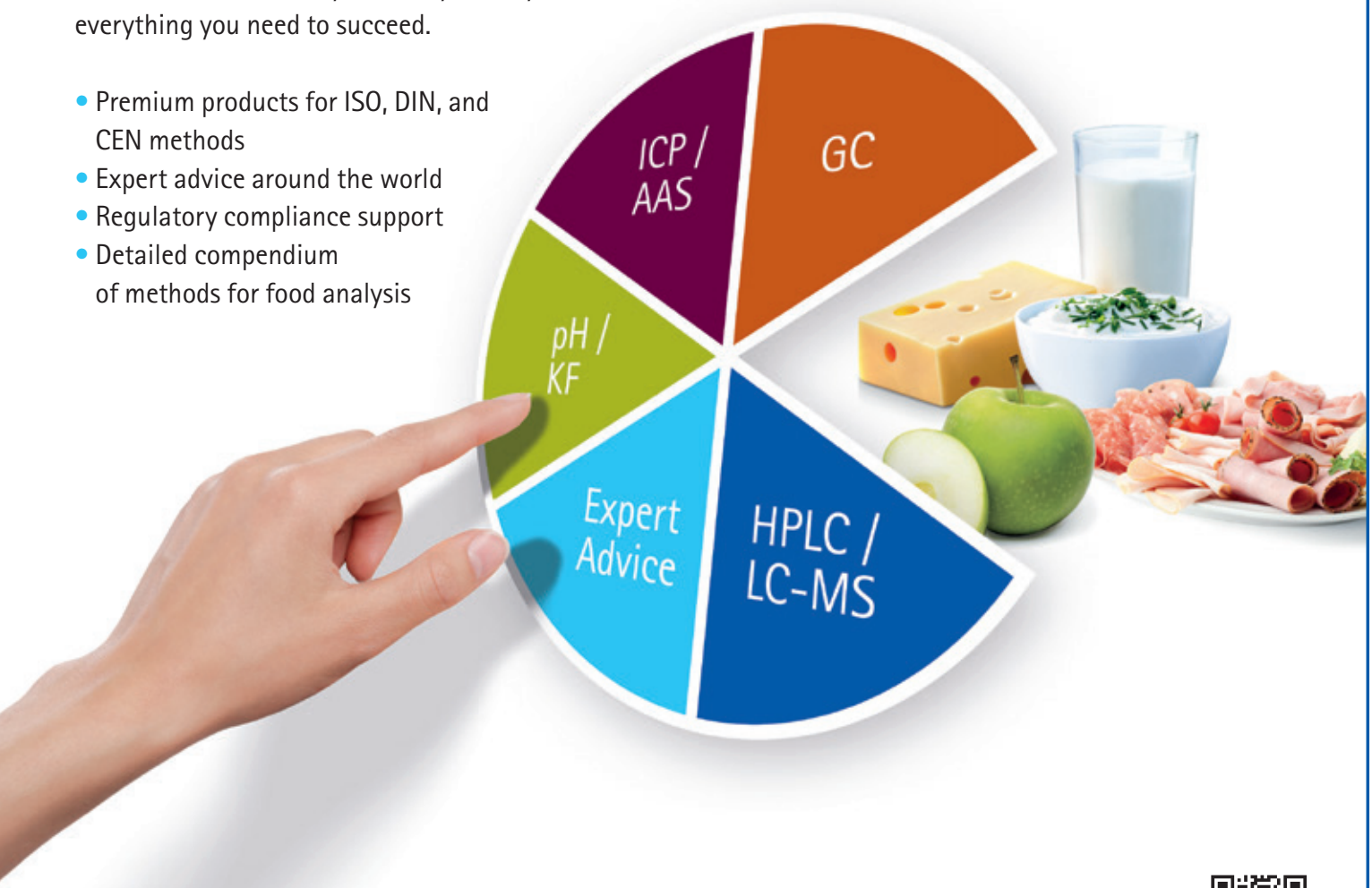

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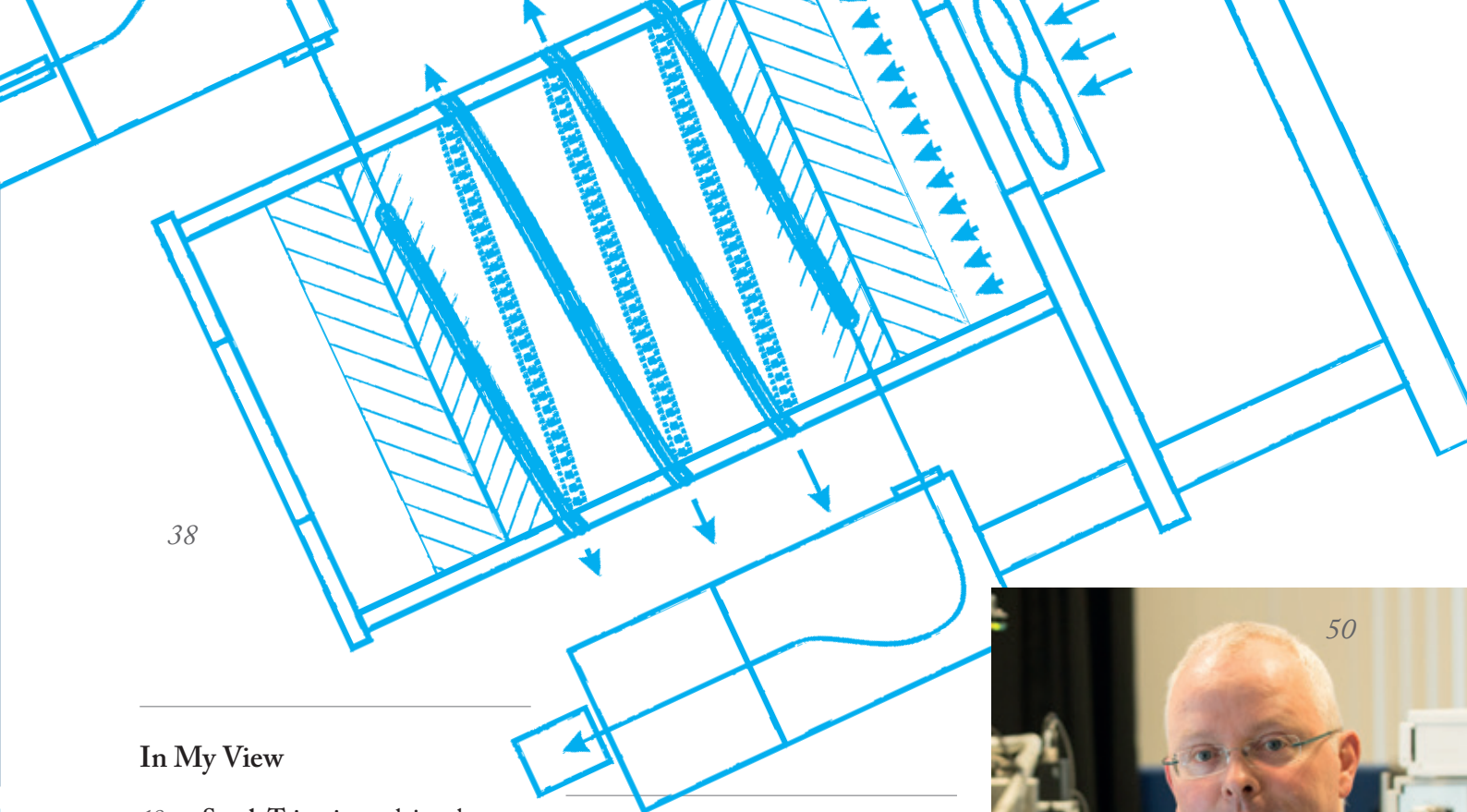

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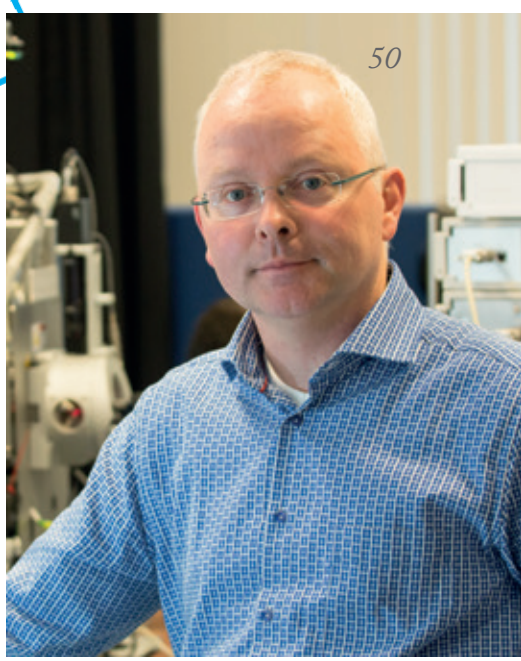

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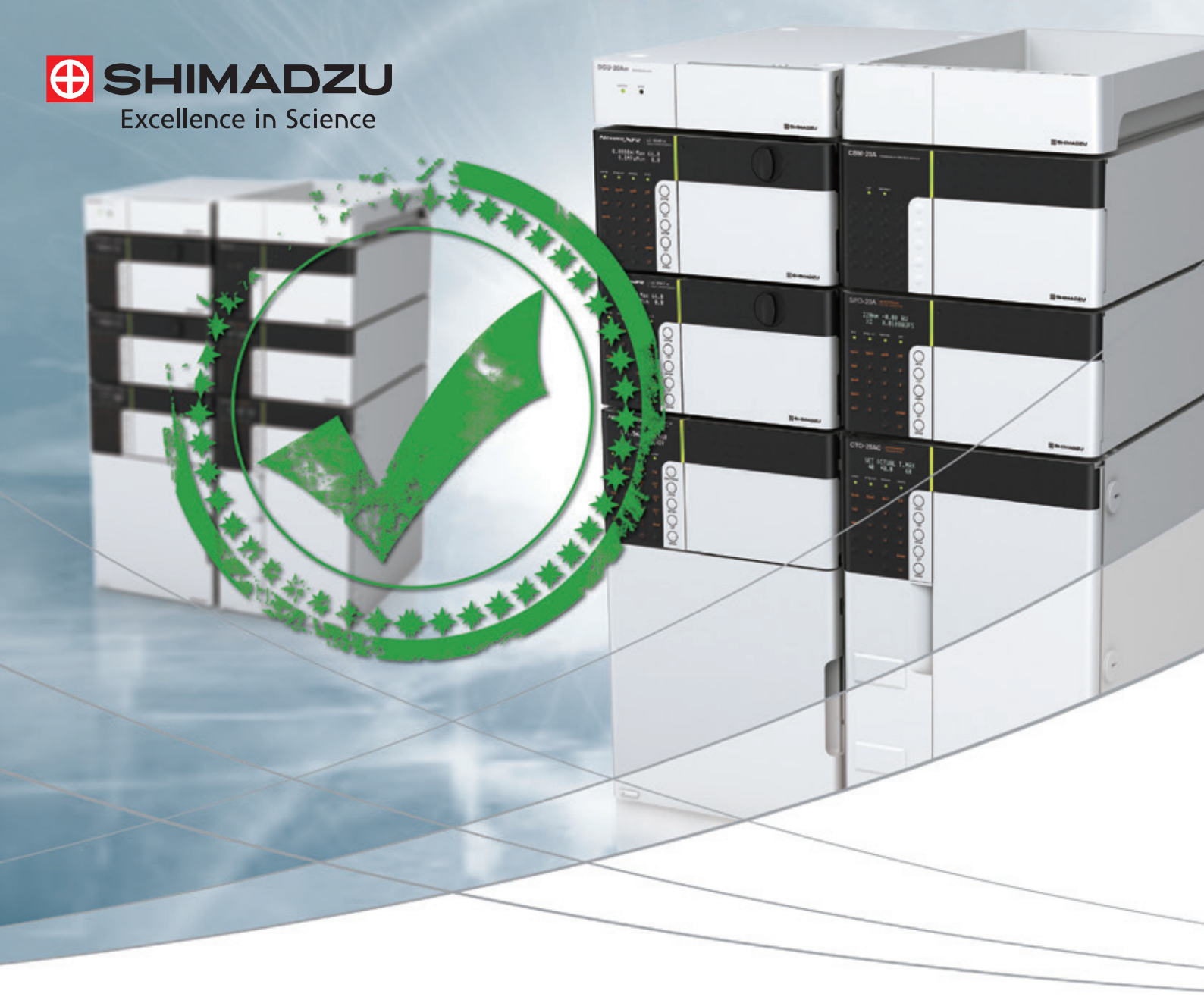
Excellence in Science



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A Gold Standard Community

A toast to friendship and mentorship!

We often see the term 'gold standard' in analytical science. In fact, having searched for a quick example online, I found this heavily plagiarized and slightly vague sentence: "GC-MS has been widely heralded as a 'gold standard' for forensic substance identification because it is used to perform a specific test." Borrowed–stolen from economics, where the term refers to a system that defines the value of a given currency in terms of gold, it is more commonly used today to describe the ultimate benchmark – a thing against which all others should be judged.

And 'gold standard' is the very term that sprouted in my mind when I was thinking about the analytical science community. Right from day one, I have been overwhelmed by the friendly and welcoming nature of people in this field; attending my first HPLC conference in Amsterdam (2013) was like being at a huge family party. Since those early days of The Analytical Scientist, I've never stopped being enchanted by the warm smiles and genuine handshakes that greet me at the various (numerous!) conferences that punctuate the normal routine.

One such smile and handshake this year came from Harold McNair at the ACS Annual Meeting in Philadelphia. Harold – for those of you who don't know – received the 2016 ACS Award in Chromatography; there was a dedicated symposium at the meeting to celebrate the fact. Before the presentations from his former students began, Harold made a point of making his way over to the sidelines to say hello – a small and yet great gesture – and entirely characteristic. Kevin Schug (one of the speakers) and I felt the sentiment at the symposium deserved to be shared with a wider audience; on page 34, several of Harold's friends share stories of his wonderful mentorship and friendship – and how he taught them not only to be good scientists, but also good people. It's a touching tribute to Harold – and I suspect that many of you have similar stories to tell about your own mentors.

Clearly, a great community can only be formed by many good people. Educators and mentors who successfully pass down skills that go beyond 'the science' should never be taken for granted. Without them – and the 'pay-it-forward' momentum they generate – the positive and welcoming community we all enjoy might cease to be. And conferences will be much less fun.

I would struggle to list here the many people who I have the great pleasure of knowing. But you know who you are. And for those of you I don't know – I'll never become tired of warm smiles or genuine handshakes.

Rich Whitworth
Editor

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: rich.whitworth @texerepublishing.com

Paleolithic Protein Analysis

250,000-year-old proteins show sophistication of hominin hunting habits

Paleoanthropologist April Nowell and team have discovered the oldest identifiable animal protein residues on Stone Age tools – suggesting our Paleolithic ancestors feasted on everything from camel to rhino. Nowell of the University of Victoria, tells us more about the findings and analytical method used to identify the proteins.

How did you come to work on this project?

I have been working in Jordan since 2000 on a variety of different projects. It's an interesting place to work because it is situated in what is known as the Levantine corridor (the Levant is modern day Jordan, Israel, Syria and Lebanon) between the African and Eurasian continents. For thousands of years, plants and animals – including hominins (ancient human ancestors) – passed through this corridor, referred to by one of my colleagues as a "Paleolithic bus station." It's a great place to ask questions about ancient human migration patterns and about how they survived in an often challenging environment. In the early 1990s, the water table in Azraq began to drop due to climate change, which has meant that sediments of the age we are interested in are now accessible to archaeologists.

What did you discover?

Seventeen stone tools tested positive for either horse, camel, rhinoceros, duck or wild cattle (bovine). And I can now tell you that a specific tool was used to hunt or scavenge or kill a specific animal, for example, "this hand axe was used to butcher a horse"!

Our findings also showed that a surprising range of animals were exploited, from duck to rhinoceros, suggesting that the cognitive abilities, social organization and technical know-how of these hominins were surprisingly sophisticated and human-like. Hunting is cognitively, socially and technologically demanding – hunting

Nowell on site (Azraq, 2014). Credit: James Dolkines

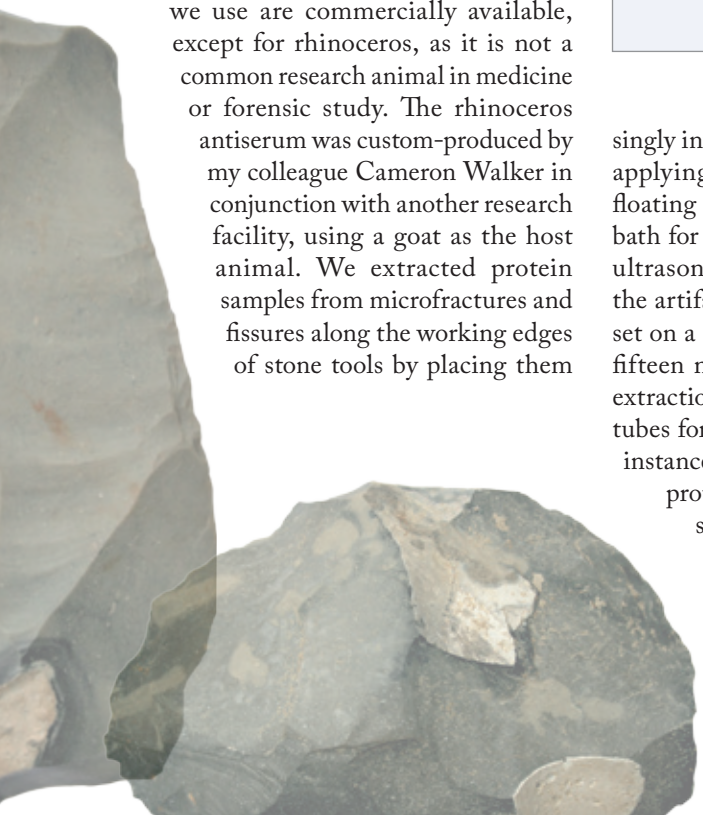
a duck is not the same as hunting or scavenging a rhino, but both require a great deal of skill. We think that they organized task groups to collect water and plants and to hunt or scavenge animals as the opportunity arose. The story of human evolution is the story of the generalist – and our findings give us the beginnings of that story.

Now we know that identifiable proteins survive this long, we hope that other scientists will use our technique on tools as old or older than ours to help build a comprehensive picture of what our ancestors were eating and how they survived in a variety of environments.

Tell us more about the protein analysis…

The method we used is called crossover immunoelectrophoresis (CIEP). It is highly sensitive – able to detect 10–8 g of protein in a 5 µl sample – and has been extensively tested in forensic contexts. Like less sensitive methods, such as ELISA, radioimmune assays or Western blots, it's based on antigen-antibody reactions and demands high-quality antisera. Many of the antisera we use are commercially available, except for rhinoceros, as it is not a common research animal in medicine or forensic study. The rhinoceros antiserum was custom-produced by my colleague Cameron Walker in conjunction with another research facility, using a goat as the host animal. We extracted protein samples from microfractures and fissures along the working edges of stone tools by placing them singly into sterile single-use containers, applying the extraction liquid, and floating the container in an ultrasonic bath for at least 15 minutes. After the ultrasonic bath, the containers with the artifacts and extraction liquid were set on a mechanical rotator for another fifteen minutes prior to pipetting the extraction liquid into micro-centrifuge tubes for refrigerated storage. In some instances, variations of this extraction protocol were followed due to the size of the artifacts being tested.

As for selecting the tools, we have excavated about 10,000 stone tools and looked at a large number of them under a microscope. My colleague Daniel Stueber and I chose 50 or so that we thought had clear evidence of being used in the past. Because these artifacts are so old and the procedure is expensive, I then selected six for testing. One came back positive for horse. I then sent a larger sample of 38 tools, which has provided us with 17 positives altogether. Our faunal remains now also include a possible lion...



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1. *A Nowell et al., "Middle Pleistocene subsistence in the Azraq Oasis, Jordan: Protein residue and other proxies," J Archaeol Sci, 73, 36–44 (2016)*

A Nest of Vapers

GC-MS indicates that chemicals from e-cigs are still a hot button issue

As debate over whether vaping is a healthier alternative to smoking rages on, a team from Lawrence Berkeley throw a little more fuel onto the fire, finding that the hotter an e-cigarette gets, the more likely it is to release irritants – and a couple of carcinogens to boot.

The indoor environment group at LBNL has been working on tobacco chemistry and exposure in indoor environments for about 30 years, but with the increasing popularity of e-cigarettes, they started looking at the chemical composition of both direct and passive (exhaled vapor) emissions. In particular, they focused on the effect of heating on the composition – as well as how the design of the device and nature of liquids can affect the levels of chemicals in the vapor. The overall objective? To assess the impact of the vapor on indoor air quality and estimate the health outcomes of exposure for both users and non-users.

The team identified 31 compounds in vapor, including glycidol – a probable carcinogen – and acrolein – a powerful irritant. Neither of the compounds had been identified in vapor previously. Emission rates ranged from tens to thousands of nanograms of toxicants per milligram of e-liquid vaporized, and they were significantly higher for a single-coil vs a double-coil vaporizer (by up to an order of magnitude for aldehydes).

GC-MS was selected as an appropriate technique to measure the partitioning of volatile compounds in the e-liquids to the gas phase. "Headspace analysis allowed us to quantify the fraction of each constituent that is emitted at different temperatures but also to mimic the heating of the e-cigarette – verifying if some chemicals undergo thermal decomposition or if new chemicals are formed during heating," says Mohamad Sleiman, Environmental Chemist and a Research Scientist at LBNL. "And by comparing direct injection with the headspace analyses for standard mixture and e-liquids, we were able to determine the percentage composition of the major constituents of the e-liquids."

Thermal desorption GC-MS (TD-GC-MS) was used to analyze the vapors emitted by e-cigarettes and exhaled by users. "The technique uses sorbent tubes containing, for example, a carbopack C bed, which traps and preconcentrates a wide range of volatile organic compounds (C3-C14)," Sleiman says. The trapped compounds were thermally desorbed at 250-300 C and separated and identified through GC-MS.

What else did the LBNL team 'smoke out'?

- Increasing voltage leads to higher emissions.
- By increasing the voltage applied to a single-coil device from 3.3 to 4.8 V, the mass of e-liquid consumed doubled from 3.7 to 7.5 mg per puff and the total aldehyde emission rates tripled from 53 to 165 µg per puff, with acrolein rates growing by a factor of 10.
- Harmful emissions increased with device age Aldehyde emissions increased by more than 60 percent after the device was reused several times, likely due to the buildup of polymerization byproducts that degraded upon heating. These findings suggest that thermal degradation byproducts are formed during vapor generation.
- Thermal dehydration of e-liquid solvents at high temperatures was the major source of toxicants.
- Glycidol and acrolein were primarily produced by glycerin degradation.

Acetol and 2-propen-1-ol were produced mostly from PG, while other compounds (for example, formaldehyde) originated from both. Because emissions originate from reaction of the most common e-liquid constituents (solvents), harmful emissions are expected to be ubiquitous when e-cigarette vapor is present.

But before vapers are forced to put that in their pipe and smoke it, it is worth noting that irritant levels were still lower than in conventional cigarettes. What's more, Sleiman suggests that since harmful emissions originate from heating the common constituents present in every e-liquid, protection of users may be gained by simply improving e-cigarette design to reduce coil and vapor temperatures. "Research into e-cig vapor's impact on health is needed to better assess the risks," he says. *JC*

References

1. *Sleiman et el, "Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals," Environ. Sci. Technol., 50, 9644–9651 (2016)*

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Hitler's Acid in Uranus?

'Exotic' chemical compounds could be present in planet cores

The core of Uranus could contain significant amounts of carbonic and orthocarbonic acid polymers, according to scientists at the Moscow Institute of Physics and Technology (MIPT) and the Skolkovo Institute of Science and Technology (Skoltech).

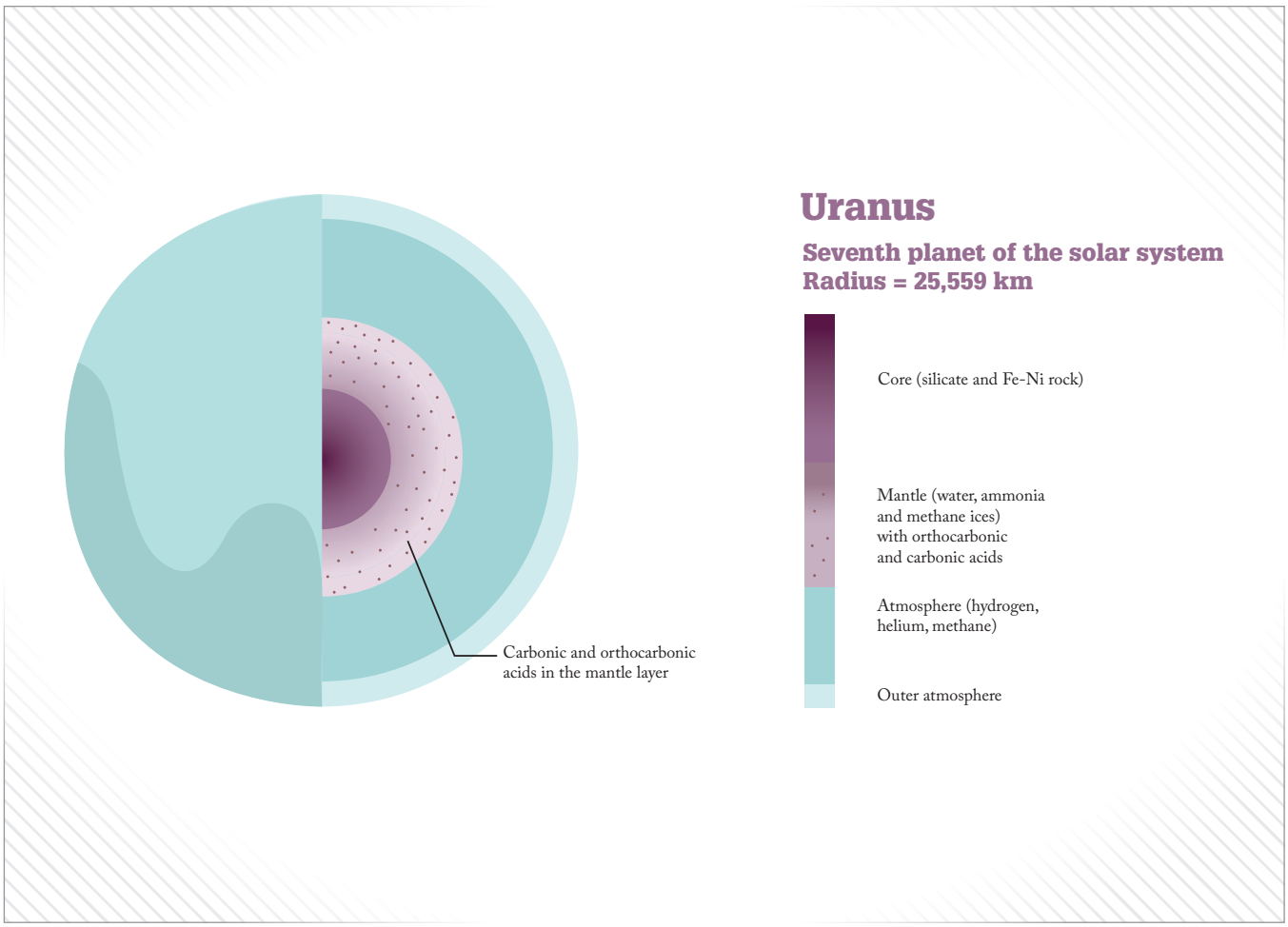
"The chemistry of the elements is heavily altered by high pressure, with stabilization of many new and often unexpected compounds, the emergence of which can profoundly change models of planetary interiors, where high pressure reigns," write the authors (1). "The C-H-O system is one of the most important planet-forming systems, but its high-pressure chemistry is not well known."

Using computer modeling, the team simulated the high-pressure conditions of planet interiors and discovered that when the pressure rises to 314 gigapascal, an exothermic reaction between water and carbonic acid results in the formation of orthocarbonic acid (H_4CO_4) – also known as "Hitler's Acid" because of the molecular structure's supposed resemblance to the infamous Nazi swastika.

USPEX (Universal Structure Predictor: Evolutionary Xtallography), the algorithm used by the team to carry out the analysis, was developed by Artem Oganov's team (MIPT's Computational Materials Discovery Lab) and has been used previously to analyze 'forbidden' substances that may be stable at high pressures. *JC*

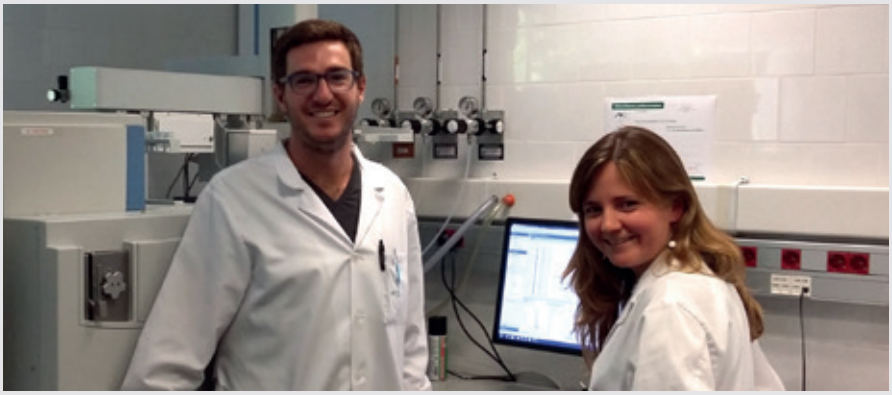
Reference

1. *G Saleh and A Oganov, "Novel Stable Compounds in the C-H-O Ternary System at High Pressure", Sci. Rep, 6, (2016), DOI: 10.1038/srep32486*



Hitting LOQs and Confirming Hits with GC-HRMS

Then & Now, with Nuria Cortés-Francisco, Emerging Contaminants and Mass Spectrometry Specialist at the Laboratori Agència de Salut Pública of Barcelona, Spain.



Then: only two years ago...

I started working with the LASPB (Public Health Agency of Barcelona Laboratory) even before I joined officially two years ago. I got the opportunity because of my experience in high-resolution mass spectrometry – specifically Orbitrap-based MS systems; it was the focus of my PhD at CSIC (the Spanish National Research Council) and I've been using Orbitrap technology ever since. We are an official control lab and we analyze more than 35,000 (mainly food) samples per year at LASPB – so we're pretty busy; in fact, we have an 'open accreditation scope', which means we're obliged to analyze any food commodity that a client sends to us – including requests for new analytes. The lab here introduced LC-Orbitrap systems five years ago, which are particularly useful for confirmation or to troubleshoot problematic analyses, but GC-Orbitrap was unfortunately unavailable.

We were facing two main problems in GC-MS analysis. First, we found it challenging to reach very low limits of quantitation (LOQ) for some emerging compounds – polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants. In 2014, the European Commission requested that such compounds be monitored, with LOQ recommendations. Limited to a triple-quadrupole MS system, we had to work hard on sample preparation to concentrate the analytes of interest – if your instrument can't catch the standard, you've got no hope in the matrix... We managed to hit the LOQs for all PBDEs except one – the notoriously tricky BDE-209. It's a big molecule, which causes column difficulties but also sensitivity problems; sensitivity of triple quad instruments drops off significantly at higher molecular masses.

The second challenge in our GC-MS applications was in pesticide analysis – not because that is particularly challenging, but because, unlike LC-MS where we had Orbitrap-based systems, we didn't have a confirmatory analytical method or an alternative technology for challenging matrices/interferences when it came to GC. Having confidence in our analytical results is extremely important, because they can have legal and financial implications; we must avoid false positives or false negatives!

Now: September 8, 2016

Now that we have the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system, we still use the triple quadrupole instruments we did before for pesticide analysis – but if we get any doubtful or strange results, we've got advanced technology to dig deeper; GC-MS has finally caught up with LC-MS in that regard. The strategy with high-resolution MS is different to triple-quad methods, so we had to work hard on it. Analysis may be done in full scan, and certain parameters must be set. We developed a database that includes all the retention times, exact masses, and confirmatory ions – and from the database you can quickly set up a method for a confirmatory analysis. In fact, the work led to a poster, which received an award at the 2016 European Pesticide Residue Workshop held in Cyprus – a proud moment! I can also say, with some satisfaction, that I recently applied our protocol with great success when addressing an alert for propargite in oranges. We were pretty confident that most of the samples analyzed with the triple-quad method were negative for propargite, but one sample was not so clear. Was it actually positive? I quickly set up the method for our Exactive GC using our new database, and sure enough – we had a positive.

As for the PBDEs, we're easily reaching all the LOQs now – including pesky BDE-209! The Exactive GC has good sensitivity and isn't deterred by higher masses, and the high resolution allows us to pick out all of the interferences, so we're obtaining beautifully clean chromatograms with very defined peaks. By creating a simple method with selected ion monitoring (SIM) windows, we can monitor not only the main peak but also the isotopic pattern – and we know for sure if we have a positive hit.

The upshot is that we now have the analytical confidence in the method to extend the analysis to other food commodities, beyond fish and seafood. On that note, I might add that we pushed the instrument really hard when it was installed – running 200 samples of salmon, tuna, and other fatty fish in the first couple of weeks. The instrument never missed a beat. We need such robust instruments.

Innovation, Generation and Automation

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. Some exciting new products this month, with potential impact on food analysis, sample throughput and forensics.

Products

- Peak Scientific introduces Solaris Nitrogen Gas Generator and FlexFlow gas subscription service.
- Agilent introduces the Intuvo 9000 GC system.
- Shimadzu has had a busy month launching the i-Series Food Safety Analyzers, the Quick-DB GC/MS/MS Forensic Toxicological Database, the GCMS-TQ8050 Triple Quad GC/MS system, and the Nexera MX system, which doubles sample throughput.
- SpectralWorks releases AnalyzerPro Version 5.3.
- Sciex presents forensic solutions with X500R QTOF System at TIAFT.
- Waters and NIBRT announce new RapiFluor-MS Glycan GU Scientific Library.
- Phenomenex introduces Yarra 1.8µm UHPLC SEC-X300 column.
- Leco unveils the Pegasus BT GC/MS ToF system.
- Bruker launches the solariX 2XR FTMS system at IMSC 2016, and the MetaboScape 2.0 solution for metabolomics.
- Waters introduces iKey HT device at IMSC 2016.

Collaborations

- Laboratory at Kyungpook National University named Waters Center of Innovation.
- Thermo Fisher to offer Hamilton Bonaduz Automation in EMEA & China.
- Illumina Partners with FlowJo for single cell analysis s/w.

Investments and acquisitions

- Danaher to acquire Cepheid for $53 per share in cash, or approximately $4b net of cash and debt acquired.
- Tecan acquires SPEware Corp for $50m in cash plus $10m potential earn-out.
- Microsaic announces £5.4m conditional placing.
- VUV Analytics closes $6.5m Series B financing.
- Metabolon closes $15 Million financing with Essex Woodlands.
- Vanderbilt University: $10.5m NIH grant for Richard Caprioli.
- Agilent completes asset acquisition of iLab Solutions.

Cheers to the Beermaster!

Liisa Otama describes how discrete analyzer technology has found its way from the clinic to the beer brewing industry – and beyond.

I joined Thermo Fisher Scientific somewhat unexpectedly; I needed a summer job during my university studies, and it seemed a great place to gain some experience – I was working with discrete analyzers and reagents in process engineering. I must have done something right, because I was asked to stay until the end of the year. I finished my Masters in analytical chemistry (at the University of Helsinki), and was invited back to work on another project by my previous manager at Thermo Fisher. I accepted – and I'm still here over six years later!

In those six years, discrete analyzers have changed somewhat. Notably, the new instruments have moved from the floor to the benchtop and are supported by more sophisticated software. Essentially, our discrete analyzers are based on photometric detection of analytes in disposable reaction cuvettes that are served by automated dispensing probes. The instrumentation is designed for heavy routine use in industrial applications and allows fast and accurate detection of a wide range of analytes. Indeed, our Thermo Scientific™ Gallery™ Plus Automated Photometric Analyzers are open systems, which means that customers can develop and use any reaction reagents; but our reagents come with the application itself (a fully preprogrammed automated method), which makes adoption of the system – or a new test – much easier.

From the clinic to the brewhouse

Discrete analyzers started out in clinical diagnostics. But their utility in other areas quickly became clear. Someone figured out that juices and wines could benefit from the speed and cost efficiency afforded by discrete analyzers – in addition to the increase in accuracy gained by full automation. Since that early introduction, the market has grown fast; dedicated analyzers are readily found in wineries around the world.

The beer brewing industry is a relatively new market, but once again the move to automated testing is driving gains in efficiency and accuracy. The Gallery Plus Beermaster Automated Photometric Analyzer is aimed squarely at that market and comes with methods for a range of tests, including bitterness – a key quality parameter, as all beer drinkers will know! The traditional manual test for bitterness is slow and laborious, and uses liquid-liquid extraction with isooctane, which must be disposed of properly. The Beermaster's bitterness module, on the other hand, uses 'green' solid-phase extraction ahead of a fully-automated testing protocol – and performs the whole analysis in 10 minutes. Moreover, it can be conducted alongside other photometric tests, using the same small amount of sample.

Beta-glucan concentration is another important parameter in breweries and malthouses; excessive amounts can clog process filters or impair the taste of the beer. Our unique method for rapid determination is more robust than the traditional method using fluorescence spectroscopy – and once again it's 'green'. The Beermaster can also determine SO2, free amino nitrogen, beer color, sugars, pH, acetaldehyde and acids quickly and accurately.

And let's not forget one of the most important raw materials in beer production – water! Discrete analyzers have been used in water quality analysis for many years, so the Beermaster really is a feature-rich system that can be used for the complete brewing process. It's built for breweries!

Brand consistency

I think today's consumers are more aware than ever. Consistency doesn't just mean batch-to-batch for breweries, but also between sites and countries – the beer brewed in Holland must taste the same as that brewed in Nigeria, for example. It's not just about following the 'recipe' – raw ingredients vary by location and by season. Taste testing is not enough and brewers are turning to chemical analyses; fast, accurate and robust measurements of key parameters – in the brewing process and the finished product – are essential.

Personally, I'm a fan of IPA beers, so anything that enables breweries to get the bitterness levels right has got to be a good thing. That said, I still think sensory testing is very important!

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

From MALDI and ESI to MAI

Simplifying ionization in mass spectrometry.

By Sarah Trimpin, CEO at MSTM, LLC, Newark, DE, and Professor at Wayne State University, Detroit, MI, USA.

The achievable sensitivity and specificity of mass spectrometers makes them the premier analytical technology for both targeted and non-targeted analyses of minute amounts of material. The high resolving power, along with the ability to obtain mass measurements that are frequently accurate enough to determine elemental composition, allows analysis of complex mixtures, especially when interfacing with chromatographic separations. And yet, despite huge successes, mass spectrometry (MS) still has ample room to grow.

Successful MS analysis begins with the ionization step, which converts molecules into gas-phase ions. In the early days of MS, compounds were vaporized and subjected to an energetic event, such as electron ionization of the gas-phase molecules, which made analysis of most biological compounds inaccessible. A great deal of research went into developing methods capable of converting nonvolatile compounds into (quasi) molecular gas-phase ions, culminating in the development of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) in the 1980s. These are now the most widely used ionization methods in MS, and both are capable of converting a wide range of (intact) compounds – regardless of volatility – efficiently into gas-phase ions.

Recently, these ionization methods have been supplemented by new ionization processes. One of these methods, termed matrix-assisted ionization (MAI), uses a matrix, similar to MALDI, but does not require a laser and produces multiply-charged ions nearly identical to ESI, without the use of high voltages. Astonishingly, MAI requires no external energy input to convert solid- or liquid-phase compounds to gas-phase ions; the energy that drives the ionization process is contained in the matrix and released when exposed to the vacuum of the mass spectrometer. Simply inserting a small quantity of the matrix:analyte sample into the atmospheric pressure inlet designed for ESI produces abundant protonated (and deprotonated in the negative mode) ions from low femtomoles (ca. 10-14 moles) of analyte. Alternatively, the sample can be introduced directly into the vacuum region to achieve ionization – similar to MALDI but without the laser. Using MAI, the expense of an ion source is eliminated while bewildering simplicity and competitive sensitivity is achieved – using the very same mass spectrometers optimized over the past 25 years for ESI or intermediate pressure MALDI.

Multiply-charged ions produced by MAI allow use of mass spectrometers that have a limited mass-to-charge (m/z) range, high mass resolution, and/or high performance fragmentation technology. For example, the singly-charged molecular ions of a small protein, such as insulin (5730 Da), produced by MALDI will have an m/z value that is outside the mass range of most of these mass spectrometers. For this reason, MALDI requires a laser and a specialized mass spectrometer for high-mass compounds, and is also poorly applicable to small compounds because of the high chemical background. In contrast, with MAI, even the

66,000 Da bovine serum albumin protein forms highly-charged molecular ions that fall within the range of mass spectrometers commonly used with ESI. Meanwhile, the same instruments are used with MAI to analyze and – if desired – quantify, for example, metabolites from undiluted smokers' urine, illicit drug-addicted infant urine, and a schizophrenia drug from tissue surfaces.

MAI also has advantages relative to ESI in terms of reducing energy requirements and consumables, because it eliminates the need for a hot inlet and nebulizing/desolvation gases, while notably increasing the speed of analyses and simplifying field portable mass measurements. Examples include the membrane protein bacteriorhodopsin, protein toxins, and Ebola protein, all measured within a few seconds. Selective use of MAI matrices has allowed successful analysis of even a monolayer of compound on a surface where both ESI and MALDI have failed. This new ionization discovery is successful with applications that are difficult or impossible using the traditional ionization methods, and the simplicity diminishes the requirement for highly trained personnel. Thus, MAI is especially promising for applications outside of analytical laboratories, such as field portable MS, homeland security applications, as well as in clinical and bedside diagnostics.

Easier Electroanalysis in Flowing Liquids

Carbon fibers could prove effective and versatile in the shifting world of liquid-flow electrochemical detection.

By Jan Vacek and Jan Hrbac, Associate Professors, Palacky University, Olomouc, Czech Republic.

Progress in the field of flow electrochemical detectors has experienced a technological shift in the last decade – high-surface-area flow cells are being replaced with microelectrode systems, which are enabling applications of chip technologies and microfluidics.

Currently, flow electrochemical detectors (EDs) are used for the speciation, biochemical, environmental or industrial monitoring of a wide variety of analytes. The majority of these are electrochemical analyses with the use of large electrode systems. The main limitation of current flow systems is the low reproducibility of analyses, related to passivation of the working electrode surfaces and also to the lack of standardization for electrode regeneration procedures. Hence the reason why recent research and development in the field of flow EDs is oriented towards applications of microelectrode systems.

We believe that carbon fiber is a highly effective and not yet fully appreciated microelectrode material. Indeed, carbon fiber cylindrical microelectrodes (CFMEs) are mechanically highly resistant working electrodes with advantageous electrochemical properties. Regeneration of the CFME surface in between analyses can be primarily achieved by repeated optimized potential ramps. Potential-controlled regeneration of the CFME surface can be performed in two ways:

"We see great application potential in the interconnection of CFMEs with microfluidics, chips and microseparation techniques."

i) mildly, so that only desorption of the undesirable oxidation products occurs, or ii) vigorously, which leads to mechanical restoration (ablation) of the surface (1).

CFMEs can be used in continuous flow systems, in which case the carbon fiber is installed directly into a PEEK capillary. CFMEs can be used without surface treatment but, if required, selectivity can be enhanced by applying appropriate layers of functional polymers that provide permeability for a target analyte that may be subsequently electrooxidized on the carbon fiber surface (2). Another way to ensure the selectivity of the determination is to modify the surface of the carbon fiber with metal layers, metal oxide layers, or semiconducting components, preferentially in the form of nanostructured deposits (3). Metal-modified CFMEs enable analysis not only in the positive but also in the negative potential region, which enables the analysis of substances undergoing reductive transformations.

From an application point of view, a microelectrode-based flow ED can be used in conjunction with flow injection analysis, capillary electrophoresis, liquid chromatography and microdialysis techniques. But we see great application potential in the interconnection of CFMEs with microfluidics, chips and microseparation techniques; for example, a microcolumn adapted for solid-phase extraction. Microelectrode systems need potentiostats working in the current range of <1 nA – a requirement that is met today by a wide range of devices, such as the experimental LabFlow sensing platform fully adopted for CFME applications. Certainly, analysis in flowing fluids – and the subsequent data evaluation – has specific software requirements, but these have already been met by a number of software applications. Indeed, we recently developed the freeware – eL-Chem Viewer – in our lab for this very purpose (4).

Electrochemical analysis in flowing liquids is moving beyond the concept of large-area electrodes, with research and development interest turning to microelectrodes that can be suitably modified and adapted to perform specific analytical tasks. In the future, one can also expect applications of microelectrode systems to grow as a field, appropriately modified with semiconducting polymers, microelectronic and optoelectronic components (5, 6). Given the potential applications and the versatility of advanced microelectrodes, we can see a bright future for the field of electrochemical detection.

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Foodomics: Work in Progress

How can analysis of food products be used to optimize their health and nutritional benefits?

By Peter Zahradka, Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Research Centre and Department of Physiology and Pathophysiology, University of Manitoba.

Most people are familiar with the adage "you are what you eat." For the general public, this concept would typically be associated with the various food groups that are used to help promote good eating habits. But none of the foods consumed consist of just a single item. Rather, everything we eat is a complex mixture of thousands of different compounds. Though the initial focus of food research was to understand how the nutrients contained in food contributed to health, over time it has been recognized that even the very minor constituents present in our foods may be capable of affecting our health. As a result, identifying which ones can be used for this purpose has become of great interest.

A century ago, food chemists spent years isolating and characterizing single molecules to determine their role in human nutrition. Considerable progress has been made since then in profiling the compounds present in our food, something that has become possible through the development of analytical methods and equipment with ever greater

sensitivity and speed. It is now possible in just a few days to prepare an extensive list of the compounds present in a single food sample and compare that list to others obtained from samples via other sources. These achievements have gone hand-in-hand with the increases in computing power that now make it possible to quickly identify many of the compounds detected with our analytical instruments. However, whether certain compounds produce health benefits cannot be determined by these approaches, and our ability to establish the biological significance of individual compounds has not expanded at anywhere close to the same rate as our analytical capabilities. We must now ask ourselves if this limitation needs to be addressed.

"A century ago, food chemists spent years isolating and characterizing single molecules to determine their role in human nutrition."

A PubMed search using the term "metabolomic" now produces almost 14,000 results. The earliest papers using this term were published in 2000, and 2015 had over 2,000 papers on this topic. The bulk of these papers provide lists of compounds detected in certain samples, and a large number looked at associations between the intervention or manipulation used on the system and the metabolite profile. In a number of instances, the profile was examined for potential use as a predictive marker or diagnostic in relation to the biological condition under investigation. In rare instances, papers were trying to determine whether certain compounds identified during profiling would serve as biomarkers. The latter approach has been assisted in large part through the ability to interrogate large biochemical pathway and disease databases. At the same time, determination of a causal relationship requires perturbation of the system with the compound of interest, an approach that requires a workable dose.

Analytical chemistry is certainly moving in the direction of detecting ever smaller amounts of these compounds, but determination of biological effect needs large amounts of pure compound. Unfortunately, extraction and purification methodology has not changed much over the past few decades, although computer automation of these systems has helped to improve consistency and speed. A similar scenario occurred in the period from 1970 to 2000, when the ability to rapidly clone individual genes became possible, but the ability to define their biological function was time consuming. However, it is worth noting that the most significant publications were those that established the contribution of the gene to a biological system. Unfortunately, the current situation is a much more difficult one, since unlike genes, not all of the compounds present in a food will have a biological effect. Given this state of affairs, it is quickly becoming imperative that closer ties must develop between analytical chemists and researchers in the biological sciences to bridge the huge gap that is forming. It is only through better connections between these disciplines that it will be possible to develop a comprehensive understanding of how the foods we eat or the compounds they contain can be used to benefit our health.

The Reformation of Analysis

On one level, today's analytical scientists may know more than the analytical chemists of the past – but at what cost?

By Apostolos Gerontas, Lecturer at Coburg University of Applied Sciences, Bavaria, Germany.

A decade ago, when I left the laboratory for the sake of the history of science, I had some ideas about the instrumental revolution in chemistry, and I believed I knew the basics concerning the changes in chemical practice brought about by the introduction of analytical instrumentation (1, 2). I was surprised, however, to find that these changes ran deeper than I thought, both socially and epistemologically. The analyst of today is no longer the analyst of the 1930s, and the analyst of the "instrument era" is less than aware of this fact.

Instrumentation shifted the focus of analysis silently from "separation" to the "identification" of compounds. As late as 1940, the analytical chemist's job – namely to "separate" and "quantitatively manufacture" compounds using their reaction properties – was downgraded and given to available research technicians. On the epistemological level, this means that although analytical chemistry remains the discipline that focuses on "signal production and interpretation," the very nature of the signal read and interpreted has changed; the focus of analytics has shifted from the chemical properties of substances to the properties of "molecular species" (3). On the sociological level, the analytical scientist of the post-war decades gradually became a professional "manager" of personnel, freed from the "dull" work of separation, and able to focus on the elemental properties of compounds – less a laboratory worker, and more a "scientist" of the type that a physicist is.

In this shift, the chromatographic instruments played a pivotal role – both by altering the separation procedures themselves, and by offering a paradigm for all the subsequent laboratory instrumentation. By the end of the 1960s, gas chromatography was the closest to being the dominant analytical method among all the available methods of instrumental analysis. As a method, GC was, from the beginning, characterized by its versatility: capable of analyzing samples over a broad qualitative range, easily adapted for preparative work, and able to operate at different scales of quantity and precision. Most importantly, the machine was complicated, involving too many different types of knowledge to exist. The production of the apparatuses was delegated outside, away from the chemical laboratories per se, and for the first time in the history of chemical analysis, an important part of the analytical process was effectively made invisible. While nowadays almost any chemist has an inbuilt psychological distance from the instruments that he is using, this distancing of the research apparatuses from their users was then a new and unexplored path.

The outsourcing turned the instruments into commercial objects like any other, creating a vibrant market and giving production companies the incentive to create meaningful R&D facilities. The companies would now compete not only through improved technology and products, but also through service structures, advertisements, "lobbying" and "special relations" with the "clients" – in this case, universities, hospitals, public institutions. For the first time and on a massive scale, there was a clear divide between the users of the instruments and the designers and builders.

The dissemination of the new techniques followed the chaotic paths familiar to us nowadays from the dissemination of computer and communication technologies (4): it took us away from academic curricula, to the modular transmission of knowledge packages – discouraging theoretical depth and instead encouraging "user-friendly" applications. In this way, the transformation of analytical science into a full science has come at a price: the loss of knowledge and control over basic laboratory processes. The new analytical scientist knows more on a higher level; yet the knowledge of acquisition processes has been irremediably black-boxed – and is now out of his or her control.

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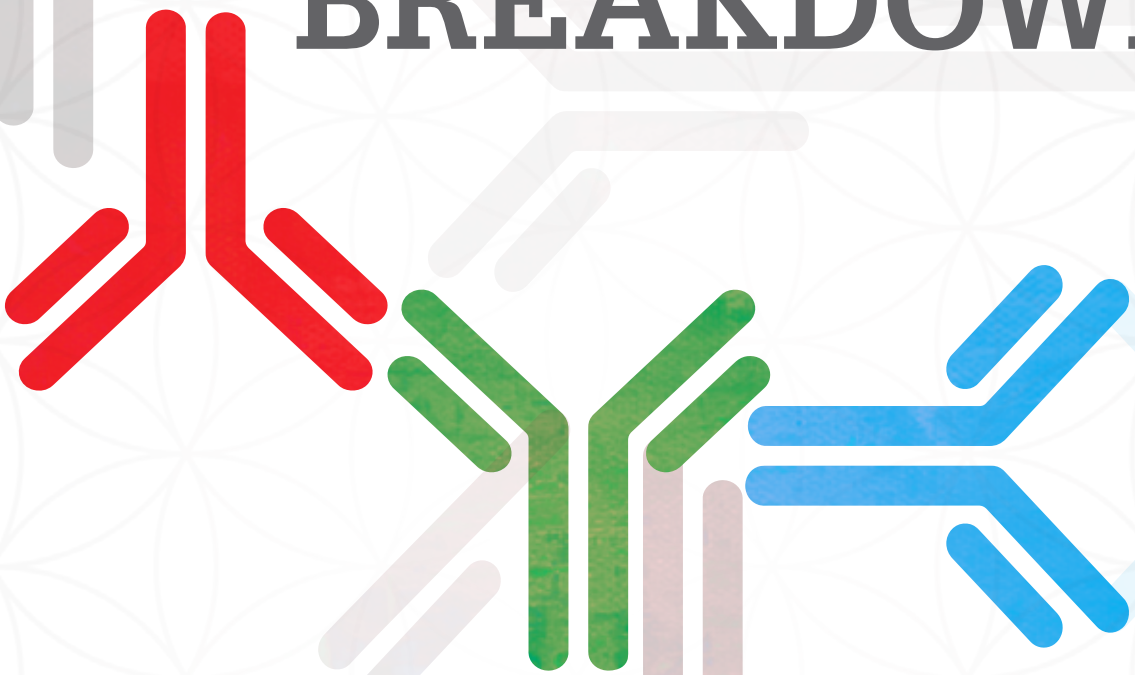
BIOSIMILAR BREAKDOWN



The biopharmaceutical industry's excitement for biosimilars has demanded the very best – and more – from analytical scientists and their tools. Here, Fiona Greer – long-time specialist in biopharmaceutical analysis – describes the need for instrumental orthogonality and the drive towards "fingerprint-like" comparative data. And on page 31, Pat and Koen Sandra discuss the power of a rising analytical star in the field: two-dimensional liquid chromatography.

Chasing the Biosimilar Fingerprint

After finally breaking into the US market, biosimilars have created a real buzz in the industry, but demonstrating similarity can be daunting to say the least. Here, I share the tools of the trade and discuss the role of analytical science.

By Fiona Greer

Things have come on apace since the first biosimilar was given authorization in the EU in 2006. That was Omnitrope, a somatropin, a simple biosimilar product because it was a small non-glycosylated protein. The EU issued its first guidelines in 2005, but SGS M-Scan, as a company, had been working with biosimilar manufacturers prior to that. We were working a little bit 'blind' and were treating these molecules as you would any other protein, using the same techniques to interrogate the structure. Now, we have far better and more clearly drafted guidelines, and in addition to overarching directives and guidelines on quality, clinical and non-clinical requirements, there are product- or class-specific guidelines for certain molecules (1). The EU now has over 20 biosimilar products, including monoclonal antibodies (mAbs). Seven or eight years ago, people didn't think we would ever have biosimilar mAbs – the analytical and clinical challenge appeared too great. Today, we have two products in the EU, etanercept and infliximab from two different manufacturers, and even have approval in the US.

Regulations: getting in on the acts

The European market is already being targeted by many non-European manufacturers, and numerous other countries worldwide have published and promoted their own pathways for biosimilars (many have chosen to adopt or adapt the European guidelines). The World Health Organization has also got in on the act by publishing guidelines on the evaluation of similar biotherapeutic products (SBPs) in 2009; supplementing these in March 2016 with a specific draft document on the evaluation of monoclonal antibody SBPs (2, 3).

The US was late to enter the biosimilars arena, with the introduction of the Biologics Price Competition and Innovation (BPCI) Act in 2010, which proposed the 351(k) pathway of the Public Health Services Act. It took another two years for the FDA to issue guidance for biosimilar manufacturers wanting to use this pathway, and further time for finalization; biosimilars have only started being licensed in the country within the past year (4, 5). As an accelerated pathway, 351(k) grants access to licensing based on a comparison with a reference product that has been approved via the standard 351(a) pathway. You can't proceed onwards with either the FDA or EU pathway until you've shown biosimilarity at the analytical level and that there are no clinical meaningful differences in terms of safety and efficacy. In fact, at the discretion of the FDA, a full suite of clinical trials may not be required for the biosimilar – as long as similarity to the originator is proven beyond "residual doubt" (see Figure 1). It has been postulated that within a few years, clinical efforts may be reduced down to one or two PK/PD safety, efficacy and immunogenicity trials, so most of the biosimilarity could be based on detailed analytics. The 351 k pathway of the BPCI Act defines two types of biosimilar: an ordinary biosimilar and an interchangeable biosimilar. An interchangeable product may be substituted for the reference by a pharmacist without the intervention of the prescriber. The wording is something along the lines of: "the interchangeable biosimilar has to be demonstrated to produce the same clinical effect in any given patient". Right now, as there are no specific guidelines, companies are trying to understand how to demonstrate this.

When the EMA authorize a biosimilar, they're saying the molecule has been demonstrated to be similar to the originator and that it is safe – they are not involved with the interchangeability issue . Each member country then has to decide whether they will allow this. Biosimilar uptake varies country to country, driven by their healthcare system – in some, depending what the biosimilars are, they are now overtaking the originator molecule in terms of market share. One of the countries within Europe which has seen high uptake is Norway. They are so keen on trying to ensure that biosimilars are introduced into the Norwegian hospitals, so the Norwegian Health Agency have decided to conduct a two-year clinical trial funded entirely themselves to look at interchangeability of an infliximab.

Establishing "fingerprint-like" biosimilarity

The first step in developing a biosimilar is to determine detailed structural information for the originator molecule, which can then be used as a structural template for the putative biosimilar. It is important that many different batches of the originator are studied, as variation is likely to have occurred over time. The source of the reference product can also be an issue, particularly when developing a biosimilar for a global market, as some countries' regulators will only permit proof of biosimilarity to a batch from another country if appropriate demonstration is made to show that it is indeed representative of the authorized product in the country of application.

The concept of the fingerprint was introduced by the FDA because they wanted a careful consideration of the techniques used to demonstrate comparability. There is little point simply saying you'll use ten techniques and show some data, if the ten techniques all looked at one parameter or if they didn't look at interrelated parameters. With fingerprinting, you need to think about the

Property To Be Determined	Available Methodologies
Amino acid sequence and modifications	Mass spectrometry, peptide mapping, chromatography
Glycosylation	Anion exchange, enzymatic digestion, peptide mapping, capillary electrophoresis, mass spectrometry
Folding	Mass spectrometry S-S bridge determination, calorimetry, hydrogen deuterium exchange and ion mobility mass spectrometry, nuclear magnetic resonance, circular dichroism, Fourier transform spectroscopy, fluorescence
PEGylation and isomerization	Chromatography, peptide mapping
Aggregation	Analytical ultracentrifugation, size-exclusion chromatography, asymmetric field flow fractionation, dynamic light scattering, microscopy, transmission electron microscopy
Proteolysis	Electrophoresis, chromatography, mass spectrometry
Impurities	Proteomics, immunoassays, metal and solvents analysis
Subunit interactions	Chromatography, ion mobility mass spectrometry
Heterogeneity of size, charge, hydrophobicity	Chromatography, gel and capillary electrophoresis, light scattering, ion mobility-mass spectrometry, capillary electrophoresis-mass spectrometry

Table 1. Potential analytical tools for demonstrating biosimilarity.

whole molecule; if the molecule is a glycoprotein, then how are these carbohydrates going to interact? Is the shape/conformation of that molecule similar to that of the originator? Developing a fingerprint for a biosimilar involves the use of multiple orthogonal analytical techniques, with appropriate quantitative ranges. By using completely different techniques, you can ensure that there is no bias, carefully building up the fingerprint to cover all quality attributes – those aspects of the molecule that could or would have an impact on its safety or efficacy.

Both clinical and non-clinical data are used to determine similarity. The basis of the biosimilar fingerprint is a statistical approach that demonstrates the two products are analytically similar, but some product attributes are more important than others. Data for the first tier, representing critical quality attributes, should include a statistical equivalence test to prove comparability – and the FDA recommends that these should include those attributes that pose the highest risk when different. A good example is a protein's glycosylation pattern – the presence of sugars (oligosaccharides) attached to certain amino acid residues – or protein content. Second tier attributes are still important, but less critical, and quality ranges based on standard deviations may be appropriate for these. Those quality attributes in the third tier are the least critical, so graphical or raw data are likely to be sufficient.

The analytical toolkit

The impact of biosimilars over the last ten years has had a hand in driving the analytical sciences forward. As noted, you've got to fully understand the originator, down to the last amino acid and the last variant, and that demands excellent analytical tools. I have no doubt that such analytical challenges have been responsible for continuing research in advanced techniques, such as mass spectrometry. And although we already have good mass spectrometry that tells us about the primary protein structure, we need techniques that bridge the information from the protein structure through to its functional activity. One technique that has emerged from an R&D backround in the last four or five years is hydrogen deuterium exchange (HDX) MS. It used to be a tricky technique, but manufacturers worked hard to make it more easily assimilated into the laboratories where it needs to be applied. We're also seeing techniques filtering through from proteomics, where we can use mass spectrometry, either labelled or non–labelled, to strive for full quantitation, for example, of the host cell proteins that are present in the final products.

ICH Topic Q6B is a guideline which lays down test procedures for setting quality specifications for biological drug products. It demands multiple physicochemical and structural analyses, and is an excellent starting point when determining a strategy for proving biosimilarity. Six specification requirements for structural characterization are mentioned:

- amino acid sequence
- amino acid composition
- terminal amino acid sequences
- peptide map
- sulfhydryl group(s) and disulfide bridges
- carbohydrate structure (if appropriate).

There are also six specifications for physicochemical properties:

- molecular weight or size
- isoform pattern
- extinction coefficient
- electrophoretic pattern
- liquid chromatographic pattern
- spectroscopic profiles.

Many different analytical techniques and tools can be used to obtain and collate this information, from classical chemical methods to newer, more advanced techniques, such as ion mobility MS and HDX mass spectrometry (see Table 1 for a more comprehensive list). If the molecule is an antibody, for instance, there are many types of interrogation that could and should be applied to structural comparison with the reference. Strategies analogous to those used for peptide mapping can be applied to determine overall sequence, disulphide bridging and glycosylation; for example, the glycoprotein can be analyzed intact or digested to form glycopeptides to detail the sites of glycosylation. Carbohydrates can also be released from the protein backbone. The resulting glycans can then be analyzed using LC-MS.

Not so sweet

Glycosylation is perhaps one of the most important post-translational modifications (PTM) that occurs during the translation and manufacture of a protein, because it can affect efficacy and, in some cases, result in immunogenicity. Glycosylation modifications cannot be predicted from the gene sequence and have to be determined experimentally. Furthermore, the unpredictable addition of sugars greatly adds to the heterogeneity of the biologic medicine. As an example, just one immunoglobulin G-type molecule has been estimated to have 3x108 potential variations (see Figure 2). One technique that can be applied here is electrospray ionization (ESI) MS, which can provide insight into the number and nature of carbohydrates that are attached on both the reference drug and the biosimilar.

Higher order structure

Biosimilars require study not just of the primary protein structure, but also the secondary, tertiary and quaternary structure – the way the protein folds and then interacts in the biological matrix. The conformation of the biologic also has a bearing on its activity and is another important area of investigation when developing a fingerprint for biosimilarity. The renewed focus on structure has led to the resurgence of many physicochemical techniques, for example, circular dichroism, field-flow fractionation, and techniques to look at dimerization and aggregation, such as analytical ultracentrifugation.

Indeed, many techniques – both qualitative and quantitative – can be applied to determine higher order structure. One of the most commonly applied quantitative techniques is circular dichroism, which is sensitive to helix content and provides information about both secondary and tertiary structure. On the down side, the presence of buffers in the formulation can interfere with the results. Fourier transform infrared (FTIR) spectroscopy is another quantitative method for secondary

The Analytical Challenge

Celltrion cited a number of analytical methods in its successful European application for approval for Remsima (a biosimilar to infliximab).

The primary structure was assessed using:

- liquid chromatography-mass spectrometry (LC-MS) peptide mapping
- LC-MS intact mass measurements
- amino-acid analysis/molar absorptivity studies
- N- and C-terminal sequencing.

The higher order structure was assessed using:

- FTIR
- differential scanning calorimetry
- circular dichroism
- free thiol and S–S studies
- antibody arrays
- X-ray crystallographic techniques.

The oligosaccharide profile, N-linked glycan, sialic acid and monosaccharide analyses were used to identify glycosylation patterns. [using what tools?] Purity and impurities were investigated using:

- size exclusion chromatography (SEC)
- SEC with multi-angle light scattering (MALS)
- analytical ultracentrifugation
- capillary electrophoresis-SDS studies.

The charged isoforms were assessed using isoelectric focusing (IEC) and IEC-HPLC.

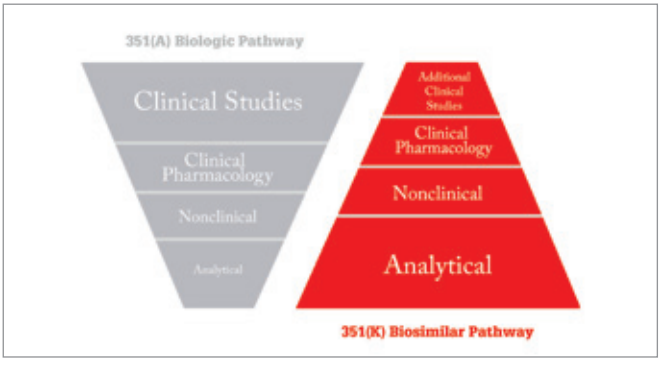


Figure 1. The newer 351(k) pathway places increasing importance on analytical methodology.

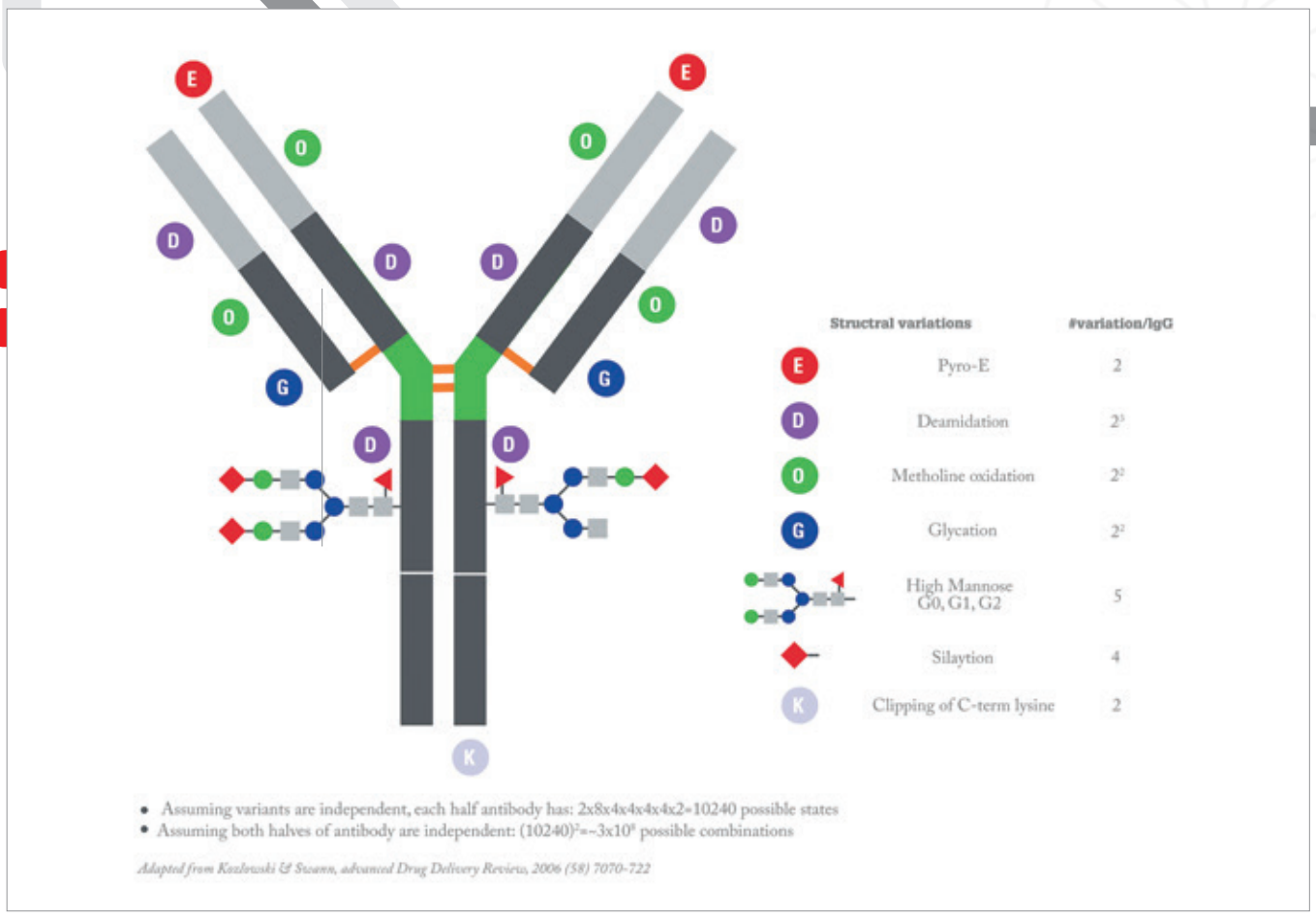


Figure 2. Example of complexity in the case of an antibody.

structure determination that is sensitive to sheet content and less likely to be affected by buffers, thus illustrating the need for orthogonal techniques.

Both intrinsic and extrinsic fluorescence techniques can be used – the former for local tertiary structure, and the latter for surface hydrophobicity – but only give qualitative results. Other qualitative methods include differential scanning calorimetry, which looks at thermal stability, and UV-vis spectroscopy for local tertiary structure. As previously noted, a technique that has emerged from research applications is HDX-MS, which highlights details of dynamics, conformation and interactions, but is expensive and has significant data processing requirements. Another technique more normally applied in a research setting is two-dimensional protein nuclear magnetic resonance.

The way that biologics oligomerize and aggregate must also be studied. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is an inexpensive, routine but low-throughput tool for assessing aggregates, and dynamic light scattering (DLS) can be used to look for high-molecular weight aggregates. Oligomers and aggregates can both be investigated using sedimentation velocity analytical ultracentrifugation (SV-AUC) and size-exclusion chromatography with multi-angle light scattering (SEC-MALS), both of which give quantitative results.

I hope I'm painting a picture here about the complexity of the task. The biosimilar boom has really driven analytical science to apply new techniques but also utilize techniques that have been around for a while but perhaps needed updating. In fact, we now have about 100 different orthogonal techniques that we can use to look at the structure of a biosimilar in a comparative way.

The science of safety: now and in the future

It is not sufficient merely to assess comparative structure: comparative functional assays also have to be performed. Suitable quantitative biological assays have to be developed and run to link product attributes with biological properties – and the results for the originator and biosimilar must correlate well if similarity is to be accepted by regulators. The assay must also be able to assess properties appropriate to the nature of the biosimilar. Again, a range of techniques can be applied, including biochemical assays, such as ligand binding, immunoassays, enzymatic assays and radioimmunoassay studies. Others techniques are cell-culture based, including cytotoxicity, cell uptake, proliferation, secondary messenger and PCR-based functional assays.

The chosen techniques, both structural and functional, will vary from one biosimilar to another. However, the resulting information should always cover a sufficiently wide range of

parameters to give regulators confidence that the biosimilar will behave in a similar fashion to its reference product in patients. For an example of how many different techniques may be needed for one product, see "The Analytical Challenge."

Big challenge, big data

A large amount of data is generated, so the crunching of data is a major problem and has to be addressed, either by the manufacturers of the instrumentation or the companies working with them. In either case, it needs to be tackled in a way that is satisfactory to the regulatory authorities. In particular, those authorities expect analysis to be performed in a GMP environment and will require any computerized data interpretation to be fully compliant with GMP. We need to ensure that we have good, robust methods that produce reliable quantitative data so that comparability can be conducted using head-to-head statistical analysis.

With the inevitable variability between biologic products manufactured in different cell lines, careful comparative studies are essential if regulators are to be convinced that a biosimilar is both safe and effective. By applying multiple orthogonal analytical techniques to both the reference originator product and the biosimilar, including functional studies, an all-important fingerprint of biosimilarity can give confidence that patients will not be adversely affected if they are prescribed a biosimilar instead of the originator product. The role of analytical science has never been more vital.

Fiona Greer is Life Sciences Global Director, Biopharma Services Development at SGS.

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Profiling a Protein Guru

Fiona Greer, Life Sciences Global Director, Biopharma Services Development at SGS, shares her analytical journey through the biotechnology boom.

How did you get into analytical science?

At school I was always into medicine. I initially wanted to be a surgeon but had second thoughts. I knew I wanted to be involved in science though, and was extremely interested in microbiology, so I went to university to do a degree in Food Science and Microbiology. That was back in the late 1970s – at the start of the biotechnology industry and the use of microbial fermentation. At that point, I got sidetracked into analytical chemistry by an Masters in forensic science, before becoming very interested in analytics and doing a PhD at Aberdeen University in Protein Chemistry. Many years ago, a Bulgarian diplomat was killed with ricin from the castor oil plant. I worked with a similar toxin, a lectin from the kidney bean plant (which is not as potent as ricin; it was very interesting to isolate this toxin and look at its capabilities. I think it was this investigative nature of analytical chemistry that piqued my interest.

What was your route into mass spectrometry?

My PhD was spent between Aberdeen University and the Rowett Institute for Nutrition and Health, where they had one of the first gas phase sequencing instruments – a piece of kit that was revolutionizing protein sequencing at the time. Around the same time, Professor Howard Morris, FRS – professor of biochemistry at Imperial College London – was setting up a company (M-Scan) to use mass spectrometry to sequence proteins – pioneering work. I joined Howard's company in 1984, where we initially used an ionization technique called fast atom bombardment (FAB) to sequence a variety of proteins and glycoproteins from the new biotechnology industry. That was my first foray into applying analytical instrumentation to biotech problems.

Sounds like an exciting field...

It was! But actually, protein science was not very trendy at that point – everybody wanted to be a geneticist or molecular biologist. Up until that time – and even during that time – a lot of the scientific focus had been on genetics, working on constructs that could express proteins. It wasn't until they'd succeeded in engineering and process development that they needed protein science to confirm that the product was the right one. To begin with, we were a small operation, about five or six people in the UK. But by 2010, we had four international sites operating with about 65 people. We had a reputation as the foremost protein and carbohydrate structural lab offering analytical services. At that stage, all four labs were acquired by SGS.

Is staying at the cutting edge important to you?

Very much so. In the beginning, we were a very small privately funded company; we had to keep driving forward so we could offer new techniques and capabilities to survive. And it wasn't just about running the instrumentation – determining the analytical strategies and interpreting the data were also crucial to solving problems. It's actually a considerable time since I wore a white coat in the lab, but with SGS I'm still focused on pushing forward our capabilities in the laboratories – ensuring that we keep introducing the most up-to-date, properly qualified and validated techniques.

What has kept you in the same company for so long?

The interest and excitement. The field has developed rapidly – driven by the challenges we were given by the biotechnology industry. When I first started, we were using a state-of-the-art high-field magnet mass spectrometer made by VG – now Waters – and the largest intact molecule it could look at was probably 6–7,000 Daltons. We had to drive forward both the instrumentation and the ionization techniques to be able to look at intact proteins at high sensitivity and perform MS/MS sequencing. We picked up electrospray very quickly along with MALDI-TOF and Q-TOF instrumentation. Biotechnology is a global industry and I have worked around the world, interacting with a lot of very bright scientists who were setting up companies, trying to exploit their research and bring it through into a commercial product.

Why do you think you've had such a successful journey?

Sheer bloody-mindedness! Everybody makes their own choices, and maybe I was lucky in that I chose something that I enjoy doing. I get intellectual stimulation from working with very bright people, and it's scientifically rewarding to look at the new techniques that are coming through and to try and introduce them to the labs that I work with.

Taming Biopharma with 2D Separations

Two-dimensional liquid chromatography represents a powerful addition to the analytical toolbox for protein biopharmaceuticals.

By Pat Sandra and Koen Sandra

In recent years at the Research Institute for Chromatography (RIC), we have noted a remarkable shift in analytical development projects from the pharmaceutical industry. Ten years ago, requests were mainly related to small molecule synthetic drugs, such as the blockbuster atorvastatin (trade name Lipitor from Pfizer) with a formula of $C_{33}H_{35}FN_2O_2$ and a molar mass of 559 g/mol. However, today's demands often involve large recombinantly expressed protein biopharmaceuticals, such as monoclonal antibodies (mAbs) – exemplified by trastuzumab (trade name Herceptin from Roche/Genentech), a highly heterogenous product with a formula of $C_{6560}H_{10132}N_{1728}O_{2090}S_{44}$ and molar mass 148,057 g/mol for one of its main variants! A big shift indeed.

This shift at RIC from small to large also has consequences for the titles and content of our contributions at International Symposia – and this short communication (requested by the editors of The Analytical Scientist) is based on two recent presentations, namely at ISCC 2016 in Riva del Garda, Italy (by Pat Sandra) and at HPLC 2016 in San Francisco, USA (by Koen Sandra).

Many of you will know that mAbs have emerged as important therapeutics for the treatment of life-threatening diseases, such as cancer and autoimmune diseases. Today, more than 40 mAbs are marketed in the United States and Europe, of which 18 display blockbuster status. Over 50 are in late stage clinical development. mAbs are currently considered the fastest growing class of therapeutics, with sales more than doubling since 2008. In other words, the analytical challenge is not going away.

From a structural point of view, mAbs are large tetrameric immunoglobulin G molecules of approximately 1,300 amino acids, forming Y-shape structures composed of four polypeptide chains that are two identical heavy chains (Hc) of ca. 50 kDa and two identical light chains (Lc) of ca. 25 kDa. The chains are connected through several disulfide bounds that can chemically be reduced to give the Hc and Lc fragments (see further). All mAbs have two N-glycosylation sites in the crystallizable fragment (Fc) that are occupied by specific glycan structures.

Substantial heterogeneity, subtle differences

During expression, purification and storage, a variety of modifications (chemical or enzymatic) can take place providing a macromolecule with a substantial heterogeneity. With several mAb blockbusters open to the market or soon evolving out of patent, the biosimilar market has exploded in recent years. Often subtle differences between originators and biosimilars must be revealed and addressed. The size, complexity and heterogeneity of mAbs requires a large toolbox of techniques for a detailed characterization and comparability assessment.

The portfolio of analytical techniques presently applied in these studies embraces all contemporary separation (liquid chromatography and capillary electrophoresis) and mass spectrometric techniques. Concerning liquid chromatography (LC), nearly all modes developed over the years are used; affinity chromatography (AC), ion exchange chromatography (IEX), size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC), hydrophilic interaction liquid chromatography (HILIC) and reversed phase liquid chromatography (RPLC). All of them provide specific information in helping to unravel the complexity of mAbs (1, 2).

Since the beginning of the 21st century, tremendous improvements have been made in LC column technology (for example, sub-2 µm porous and superficially porous particles) and instrumentation (pressure of 1000 bar and higher). These developments are commonly exploited and very successfully applied in one-dimensional (1D) LC format for mAb analysis in combination with mass spectrometers that offer accurate mass, high resolution, MS/MS and ion mobility capabilities at very high sensitivities. Notwithstanding this, the higher the chromatographic resolution or peak capacity at the front-end, the better the data in terms of content and sensitivity.

On-line two-dimensional (2D) LC represents an elegant way to increase peak capacity (3). In on-line 2D-LC, individual peaks, certain parts or the whole chromatogram are subjected to two different separation mechanisms. On-line 2D-LC can be divided into two main types. In comprehensive two-dimensional LC (LC×LC), the whole stream of effluent of the first (1D) column is transferred to the second (2D) column. In heart-cutting LC (LC-LC), one peak or one part of the chromatogram is transferred to the second column. Multiple peaks or multiple parts of the chromatogram can also be selected for transfer to the second column (mLC-LC). Multiple heart-cutting LC (mCEX-RPLC) combined with high resolution MS has recently been used to identify the main isoforms of the mAb rituximab (4) and to characterize the antibody drug conjugate (ADC) ado-trastuzumab emtansine (Kadcyla) (5).

In our recently presented lectures, the emphasis was mainly on LC×LC of the tryptic digests of mAbs and ADCs (5, 6). Indeed, analysis of the peptides provides detailed information in amino acid sequence and modifications and is an excellent way of elucidating where modifications (for example, deamidation,

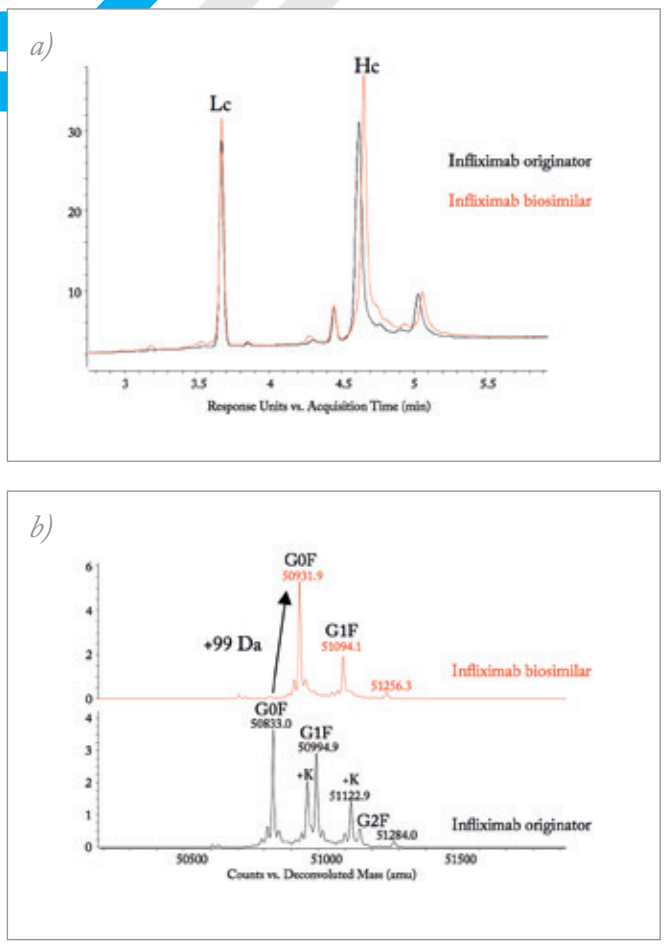

Figure 1. RPLC-QTOF-MS analyses of infliximab originator (black) and candidate biosimilar (red) on an Advance Bio RP-mAb column. (a) Chromatogram showing the Lc and Hc peaks and (b) the mass spectra recorded for the Hc showing the glycoforms and the +99 Da shift.

oxidation, isomerization, conjugation, mutation) are taking place.

In contemporary LC×LC, the target is to combine completely different separation modes, for example, IEX×RPLC, HILIC×RPLC. However, for tryptic digest characterization, we prefer the combination RPLC×RPLC. High orthogonality (optimum occupancy of the 2D-space) is obtained by running both dimensions at a different pH, resulting in a different charge on the peptides. Note that in the above combinations, RPLC is always used in the second dimension. Why? Because of the commercial availability of high quality "fast" RPLC second dimension columns and their compatibility with MS.

Mapping mAbs

To illustrate the attractiveness of 2D-LC for detailed characterization and comparability assessment of biopharmaceuticals, we describe the comparison of the tryptic digest of the originator infliximab (trade name Remicade from Johnson & Johnson) and a biosimilar candidate, for which, apparently, the recombinant expression in Chinese hamster ovary (CHO) cells was going wrong. Note that infliximab produced by Celltrion and commercialized as Remsina (Celltrion) or Inflectra (Hospira) is the first mAb biosimilar approved by the European Medicines Agency (EMA). The Food and Drug Administration (FDA) very recently approved Inflectra (Pfizer) for commercialization in the US.

Figure 1a compares the LC analysis of the infliximab originator and the candidate biosimilar after chemical reduction to cleave the disulfide bridges. The Lc fraction is identical but a retention shift is noted for the Hc fraction. The m/z difference in the Hc fractions is 99 Da (m/z 50,833.0 in the originator and 50,931.9 in the biosimilar) as illustrated in Figure 1b. RPLC×RPLC peptide mapping was subsequently performed to elucidate the origin of this shift and Figure 2 compares the relevant part of the 2D plot.

Both plots are very similar but some striking differences are noted when also taking the MS data into consideration. The spots SLSLSPG and SLSLSPGK clearly present in the originator are replaced by one spot – SLSLSPGI – in the biosimilar, which according to the mass spectral data corresponds to the addition of an isoleucine (I) to SLSLSPG or the replacement of lysine by isoleucine in SLSLSPGK at the C-termini. From a biochemical point of view, this makes sense and results in a positive move of 113 Da. The origin of the two spots SLSLSPG and SLSLSPGK in the originator mAb can be explained by the knowledge that heavy chains are historically cloned with a C-terminal lysine, but during cell culture production, host cell carboxypeptidases act on the antibody, resulting in the partial removal of these lysine residues.

A very small retention shift was also noted in another spot that has been identified by MS/MS as NYYGSTYDYWGQGTTLTVSSASTK in the originator and as NYYGSSYDYWGQGTTLTVSSASTK in the biosimilar. The change from T (threonine) to S (serine) is -14 Da. If we combine both modifications (+113 Da and - 14 Da), we find a difference of +99 Da! Therefore, two point mutations are at the origin of this wrong recombinant expression, namely ACC to AGC (T to S) and AAA to AUA (K to I). This was confirmed with reverse transcription polymerase chain reaction (RT-PCR) and DNA sequencing (illustrated in Figure 3) for a CHO clone that was producing a biosimilar candidate with the good sequence and another with the wrong sequence.

An important point is the present ruggedness of LC×LC and 2D-LC in general. The fact that major instrument manufacturers entered the 2D-LC market and made robust instrumentation

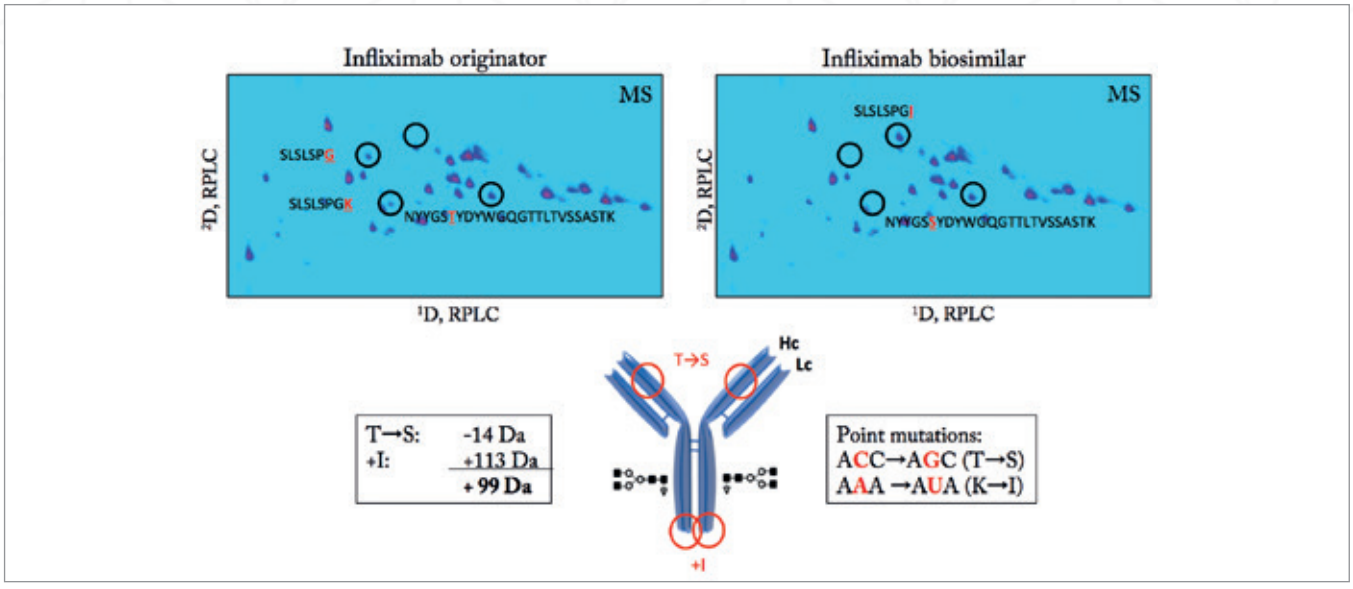

Figure 2. RPLC×RPLC-QTOF-MS analysis of infliximab originator and candidate biosimilar. Drawing of the mAb with annotation of the modifications.

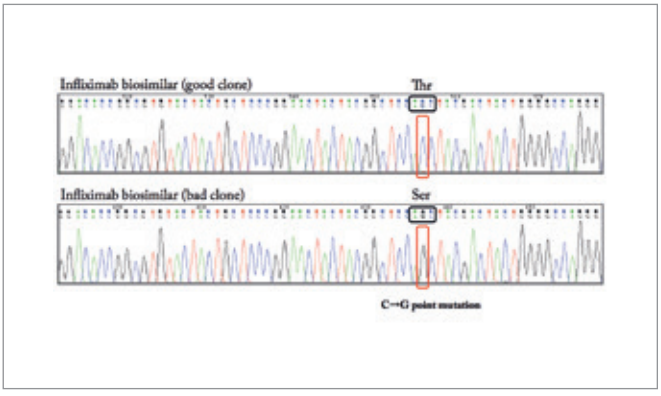

Figure 3. RT-PCR and DNA sequencing of CHO clones expressing the correct (top) and incorrect (bottom) infliximab sequence, illustrating the C to G point mutation and thus the threonine-serine substitution in the latter case.

available is an important asset for its breakthrough and applicability. Biologics are driving demand for reliable analytical techniques, not only in R&D but also in QA/QC. Our experience in LC×LC is such that we consider RPLC×RPLC ready to be implemented in routine environments.

Pat Sandra is President and Koen Sandra is Scientific Director at the Research Institute for Chromatography, Belgium. *They would like to acknowledge coworkers Gerd Vanhoenacker, Isabel Vandenheede and Mieke Steenbeke for their contributions to the 2D-LC work and biopharmaceutical characterization.*

For newcomers to the field of 2D-LC or for colleagues needing more information on the principles, practical implementation and applications, they refer readers to "Two-dimensional Liquid Chromatography" by Peter W. Carr and Dwight R. Stoll (7).

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Paying Tribute to "Doc"

Harold McNair is the 2016 recipient of the ACS Award in Chromatography. A few of his past students – Kevin Schug, Nick Snow, Chris Palmer, Vince Remcho, and Lee Polite – try to sum up (in too few words) why McNair is such an outstanding analytical chemist and special gentleman. They also share the lifelong lessons they have learned from him – and why the award is long overdue.

Nicholas Snow, Professor, Seton Hall University.

I cannot begin to enumerate the impact that Harold McNair has had on my own career and that of so many other scientists. In short, I owe everything to him. I started in his group with no knowledge of chromatography. He took me under his wing, even taking the time to personally teach me how to manually inject and how to pack, install and evaluate a column. He loves students and he loves the fundamentals.

My first read in chromatography was his book, Basic Gas Chromatography, first published in 1964 at Varian (four editions, two follow up editions with Wiley, 130,000 English copies sold and published in seven other languages) which represents the single most important contribution to gas chromatography (and chromatography in general) becoming a widely used technique. Basic GC advanced chromatography from the realm of the specialists and made it accessible to practitioners. Numerous authors later attempted and published similar texts for various chromatographic techniques, though none have been as successful, effective or as widely read. How many other instrumental techniques would have benefitted from a Basic GC-like book during their early development?

In his lab, several times per year, we had to take all of our research systems apart and set them up for the short courses. At the time it seemed a pain; each time it took several weeks away from our research, but looking back, we could not have asked for better professional training. McNair is the father of the modern chemistry short course. He taught the first ACS short course in GC in 1966 and was recognized by ACS in 1991 for 25 continuous years of short courses, training thousands of students and influencing numerous other instructors, including many of his own students that teach today. His short courses were the "gold standard" in continuing education for analytical scientists. McNair developed the first audio/visual program, a tape and booklet, based on Basic GC in 1974, and over the next 30 years, published several best-selling multimedia programs in GC and HPLC. McNair was doing multimedia and technology in teaching before most of us knew what it was. He has directly trained more practicing chromatographers than any other scholar in the history of chromatography.

McNair is still inspiring students and colleagues alike. With contributions in seven separate decades, from the 1950s to the 2010s, McNair has been teaching all of us how to be chromatographers and scientists. From his early work with pioneers including Martin, Keulemans, Dal Nogare and Golay, developing components and ideas still seen in today's GCs, through the authoring of Basic GC, pioneering short courses and training and creative contributions across the field of chromatography by himself and his students, McNair has been an 'icon' of chromatography and is directly responsible for developing many of the original methods and techniques we consider 'obvious' today.

Chris Palmer, Professor and Chair, Department of Chemistry and Biochemistry, University of Montana.

I spent eighteen months in Harold McNair's laboratory at Virginia Tech as a postdoctoral research associate; a period that was among the most productive and instructive of my career both professionally and personally. Harold is a consummate scientist with a well-deserved international reputation for his contributions to chromatographic science, and I benefited significantly from his scientific mentorship. I learned a great deal about the fundamentals and applications of chromatography, both in the laboratory and as an instructor in short courses, and we published some interesting work.

Just as important to me, though, is what I learned and how I developed personally while observing Harold and his interactions with others. Harold is a gentleman and a scholar who set a stunning example for me and others to try to emulate. His scientific talent is equaled by the quality of his character, and I could not help but be impressed and affected by this. Watching Harold, it became clear to me the extent to which a career in science could and should be a collaborative, international effort driven by interpersonal relationships built on mutual respect. I learned from Harold that, no matter how eminent or busy one might be, one can still make special efforts to compliment a young scientist who has just given a good presentation, make helpful scientific suggestions to any who are interested, and show genuine interest in the professional and personal lives of others.

Since leaving Blacksburg, I have had the pleasure of visiting and usually enjoying a meal and some wine with Harold (and often his late wife Marijke) in multiple countries on several continents, and

each time I was reminded of how much I still have to learn from his excellence of character and scientific intellect. I am fortunate to have had Harold as a mentor and friend for more than twenty years, and I congratulate him on this well-deserved honor.

Kevin Schug, Professor and Shimadzu Distinguished Professor of Analytical Chemistry, The University of Texas at Arlington.

No one has shaped my love for analytical chemistry or set my career path more than Harold M. McNair. In fact, McNair knew me even before I knew him. I grew up running around the halls of Davidson Hall at Virginia Tech, where my father was also a Professor of Chemistry. In college, I asked my father for some names of people to contact for internship opportunities. McNair was the first to respond, and he had one of the best offers. I spent my sophomore summer with McNair, learning about chromatography, and then the following summer, he arranged an industrial internship for me. Following graduation from college, I enrolled at Virginia Tech to pursue my PhD studies under McNair. He trained me not only to be a good analytical chemist, but to be a good citizen in the science community.

I have taken away so much from him in terms of how I manage my own group and interact with others, but there were two key events I would like to share. My college education at William and Mary was an eye-opener. I struggled early on to balance football, joining a fraternity, my social life, and school life – unfortunately, prioritized in that order. When I finished, I had a not so stellar GPA of 2.55, but McNair went to bat for me along with a couple of other key faculty members at Virginia Tech to get me admitted to the PhD program. In life, there are often a few such instances that set you on the right track – and that was a key one for me.

Even before I got to Virginia Tech, the industrial internship was another key event. I worked in the quality assurance lab at S.C. Johnson & Wax in Racine, Wisconsin. While it was an excellent experience, I later learned that McNair arranged this to show me what many BS chemistry graduates would be doing when they graduate – essentially the same thing day in and day out. That experience convinced me that I needed to pursue a graduate degree. Without someone with foresight like McNair looking out for my best interests, I do not know where I would be today.

Congratulations to Harold McNair on his well-deserved and overdue honor of receiving the ACS award in Chromatography. It has been amazing to be a part of such a wonderful person's life and to have been the recipient of so much good will and good friendships as a result. I am so humbled and thankful. Thank you, Doc!

Vincent Remcho, Professor of Chemistry & Materials Science, Patricia Valian Reser Faculty Scholar, UHC Eminent Professor, Oregon State University.

Every once in a while, if we are fortunate, we are afforded the opportunity to "apprentice" with a great mentor. For me, that opportunity came with Harold McNair. By the time our paths crossed in 1986, Harold was firmly established as both a great researcher in the field of chromatography and as a great educator in the broader field of analytical chemistry. He had built a reputation – to which most of us aspire – as a capable, accomplished, intelligent and innovative scientist. That was enough for me! I knew he was the kind of scientific mentor I wanted to work with. What I had yet to learn, but soon did, was that he has qualities that are far more important in a mentor than these. Harold is a fair-minded, warm, encouraging, accepting, giving, hard-working, selfless ally. He has had great success in attracting students from all walks of life and all educational levels to the field of analytical chemistry.

Harold built a formula for overcoming the activation energy barrier to engage students in the practice of chemistry by ensuring that the chemistry experience is contemporary: encompassing current public interest; accomplished with tools that are compelling, current and accessible; and capable of tackling measurement tasks that positively impact broad, diverse groups of people. He taught us, as members of the McNair group, how to appreciate one another more fully, how to support one another more effectively, how to celebrate one another more genuinely, and how to accomplish more together than we possibly could individually. That is a gift! He did this by taking us around the world to conferences at a time when that was far from the norm. He did it by including us in teaching short courses both at Virginia Tech and on-site at a variety of government and industry laboratories, giving us the distinct advantage of practical exposure to analytical chemists at work in the world. For most of us, this cemented our belief that this was indeed the profession for us. For some, it led to the conclusion that another path beckoned. All of us, though, came to know right away that we had a steadfast and lifelong supporter in Harold McNair, and a permanent home as a member of the McNair group.

I am so fortunate and thankful for all that Harold has done for me and meant to me. My goal as an educator and mentor is to be as much like him as I can.

Lee Polite, President and Laboratory Director, Axion Analytical Labs; Axion Training Institute.

Harold McNair is best known for his contributions to the world of chromatography. Literature searches will turn up that he was probably the first person in the US to do a PhD thesis on chromatography, that he built one of the first industrial gas chromatographs in the US,

"There is no better way to truly learn a topic, than to have to teach it."

that he was a Fulbright Scholar in Holland, and that he has trained some 50+ PhDs. But what this literature search might not reveal is his excellence as a communicator and his willingness to share his knowledge with the world around him.

I had the pleasure of spending 4.5 years working under McNair in graduate school. One of the traits that is common among all of his students is this willingness to share their knowledge. It is not just a side trait that we picked up in graduate school, but an integral part of our graduate career and training. McNair used to make us teach week-long HPLC and GC short courses for the American Chemical Society four times per year! At the time, that was seen as a major disruption to our research. When short course time rolled around, you dropped everything to become an instructor, repairman, chauffeur, tour guide and friend to the industrial scientists attending the courses. Most of us hated it as first, but it wasn't long before we realized that this was not just some type of indentured servitude, but an incredible learning experience for us! Imagine having to stand up in front of a group of paying customers, and answer questions on the spot regarding your craft. I remember studying harder for my first lecture (HPLC instrumentation), than any exam in my academic career. There is no better way to truly learn a topic, than to have to teach it. It didn't matter if the participants were MD/PhDs or high-school dropouts: they were there to learn, and we were there to teach them.

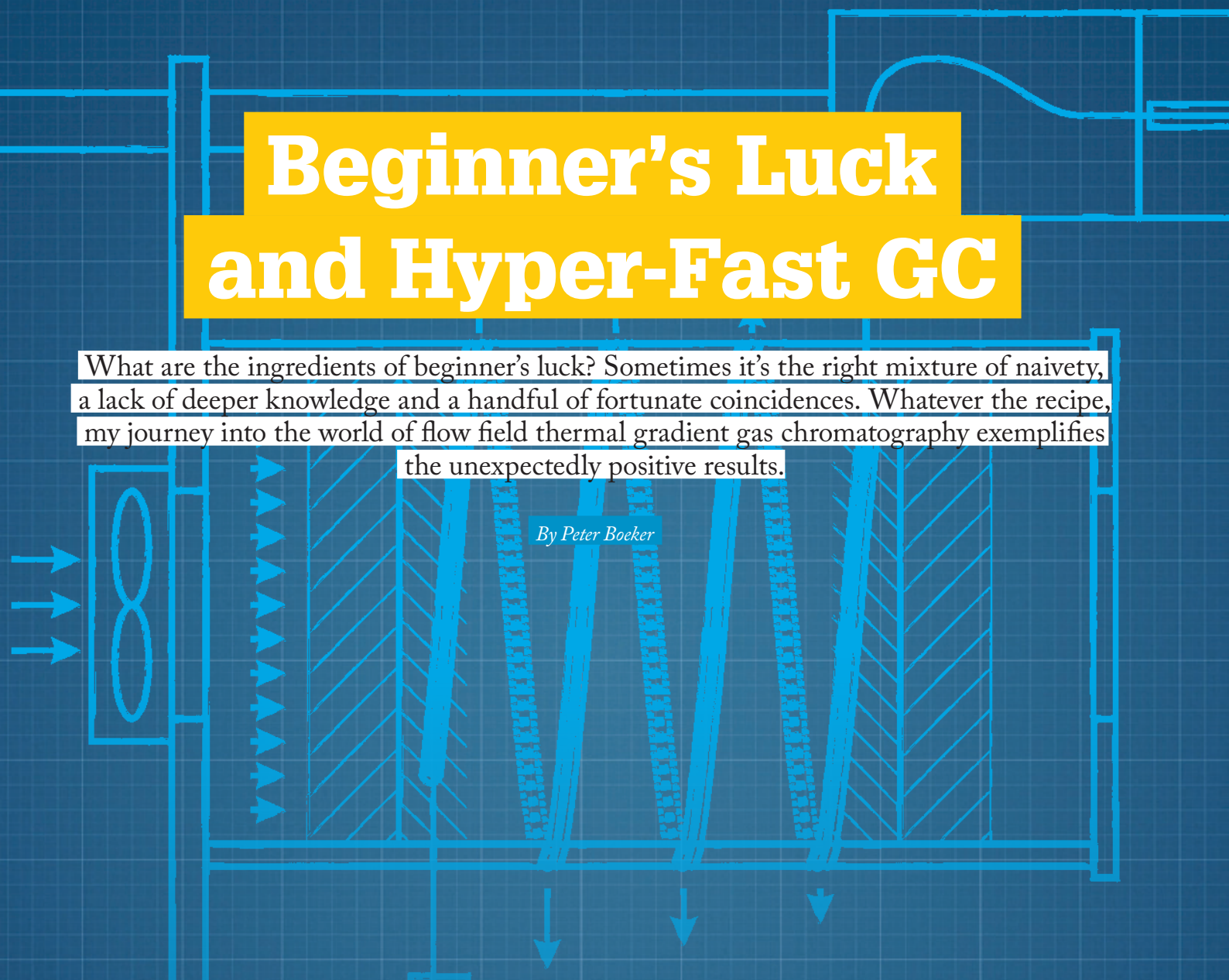
What we didn't realize was that this was Harold's way of solidifying our own knowledge of chromatography, along with problem solving, public speaking and interpersonal skills. McNair's goal was to teach the world what he had learned about chromatography, and we were his disciples. It seemed like a worthy cause at the time, but this training really paid off when it became time to find a job. The interview process usually includes giving a seminar to your perspective employers. To most job applicants, it was pretty intimidating to stare out at a crowd of adult, professional scientists as you present your research and answer their questions. But to us McNair students, we call that "day one of a short course"! By the time we were applying for jobs, we had each given hundreds of lectures to professionals in a variety of industries and settings. We had done it so many times that lecturing and giving seminars became second nature.

I remember giving a pre-application seminar at Amoco Corporation back in 1988. Unbeknownst to me, they had no intention of hiring someone right out of graduate school (like me!) – the job required five years' industrial experience beyond the PhD. They contacted Harold looking for recent graduates with industrial experience, and he suggested me. Amoco agreed to an interview and seminar, but only out of respect for Harold. I was two minutes into my seminar on high temperature ion chromatography, when a hand went up in the audience, followed by a GC peak shape question! I answered the question, and then went back to the seminar. A few slides later, another unrelated question came up on gel permeation chromatography, followed by a question on capillary electrophoresis and then one on supercritical fluid chromatography. Honestly, I didn't think much of it. I just thought they have a wide variety of questions (sounded like a fun place to work!). Years later, I was told that this was a test of my separation science knowledge and problem solving abilities. I spent nine fun years with Amoco as a research scientist and group leader. Thank you, Harold!

I have since gone on to teach chromatography to the world, as Harold instructed. I started Axion Analytical Labs about 20 years ago. In that time, we have trained professionals from every major pharmaceutical, chemical and petroleum company in the US, along with most of the major government labs. I have had the pleasure of lecturing on chromatography in 17 countries. Harold continues to be a great mentor and friend not only to me and my fellow McNair brethren, but also to my kids, employees, customers... and to just about anyone else he meets!

Congratulations to Professor Harold McNair on winning the coveted ACS McNair... I mean, ACS Chromatography award!

Beginner's Luck and Hyper-Fast GC



What are the ingredients of beginner's luck? Sometimes it's the right mixture of naivety, a lack of deeper knowledge and a handful of fortunate coincidences. Whatever the recipe, my journey into the world of flow field thermal gradient gas chromatography exemplifies the unexpectedly positive results.

By Peter Boeker

For a long time, my field of research has focused on electronic nose systems, gas sensors and odor measurements. And it was with this background that I jumped into more analytical approaches, including GC-TOF-MS, during the course of a security research project.

As always, I had written some pretty ambitious objectives into the proposal, with the aim of gaining the interest of the reviewers. In this particular case, I included the development of a fast GC system for near real-time explosives detection. Not too unrealistic, I thought – admittedly, without too much real experience in this particular field... Nevertheless, we were eager to please and full of ideas; we are always desperate to gain such funding because the research group consists of only two scientists, me and Jan Leppert – both essentially scientific freelancers without permanent positions. Indeed, we swing from one project to the next like Tarzan moving through the forest on unlikely lianas (to use an image from my childhood films). Unfortunately, unlike Tarzan, we are always in danger of missing the next one!

Luckily, we got access to the funding and started the tough task of thinking about how we would fulfill expectations. Jan had attended the 2010 Riva conference (ISCC and GCxGC symposia), where he heard a lecture by Aviv Amirav on a fast GC, based on resistive heating of a stainless steel capillary that was coaxial to the standard separation column.

Learning the ropes

Around the same time the funding came in, a physicist approached us for a diploma thesis and I thought that it would be a good opportunity to learn more about the thermal conditions of resistively heated capillaries. In this experimental work, we learned a great deal about heat loss due to natural convection and radiation heat loss, in addition to gaining a first-hand experience of the problems encountered when measuring the temperatures of tiny structures! We saw huge differences in the temperatures between vertical- and horizontal-oriented capillaries and between lower and upper coils, respectively. We surmised that attaining uniform temperatures using the concept of resistive heating may be an irresolvable problem. At one point, we briefly discussed the idea of using a fan inside a heated coil, but I stopped the discussion dead with the killer argument that we would create even worse conditions with forced convection and turbulence.

Also around the same time, we had trouble with a variable splitter in a GC-olfactometry set-up. The split ratio between the MS and sniffing port changed drastically during the temperature program. We worked hard on the theory of this splitter type and later on a Deans' switch to find a solution. Eventually, we published this work in two papers and during the review process one reviewer recommended additional references. I opened the journal archives to download the references and – by chance – at the bottom of the screen a 2013 paper caught my eye: "Dynamic thermal gradient gas chromatography" by Jesse Contreras, Milton Lee and colleagues (1). I immediately associated thermal gradients with cold spots and therefore thought the paper would cover such gradients – and how to avoid them – in great detail. But when reading this excellent paper, I became more and more excited. The idea of peak focusing on a thermal gradient was so obvious and convincing. Looking back, I was very naïve, but at the time I thought the effect should catapult GC resolution to unlimited heights!

There was only one problem: realization.

Paper trail

I started to collect all available papers on the subject. The first idea dates back to Russian scientists in the 1950s, with contributions from Zhukhovitsky and Turkel'taub (2). In those Cold War times, the concept had remained isolated in the East, with publications only in Russian and written in Cyrillic. Not until 1968 did Rudolf Kaiser (who had worked in East Germany before coming to the West) published a German paper on the subject (3). I also found some patents for short packed column arrangements that had been filed by Siemens in the late 1960s. Unfortunately, it seemed that the thermal gradient idea had not been applied to longer capillary columns. I also considered the later work of Rubey and Jain & Phillips from the 1990s, but they were 'proof-of-principle' set-ups – very promising but complicated to realize (4, 5).

The challenge sparked my engineering and physics ambitions. How could I design a simple TG-GC using standard capillaries and no cryogenic gases that was as easy to use as a conventional GC? At the beginning, the very idea seemed akin to squaring the circle. Imagine the challenge: apply a very smooth thermal gradient along a capillary of 2 meter or even more – without cold spots and in a controlled and programmable manner up to temperatures of 400 °C.

Inspiration strikes

Sitting on the backseat of a car on a long journey armed with only a pencil and a sheet of paper, I entered a state of creative nirvana and suddenly came up with an idea. Shame on me for rejecting forced convection! Dissipated heat in a long capillary cannot be controlled, but the corresponding heat loss through forced convection can. And that change of mindset shifted the construction problem from gradient heat generation to a fluid mechanics task of a controlled flow across the capillary.

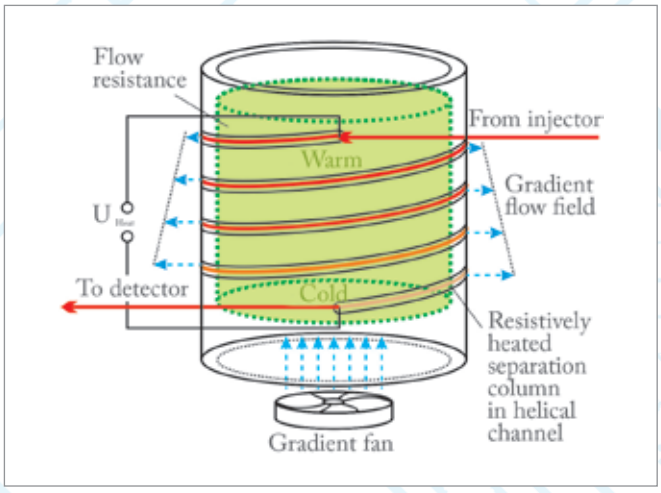
When I arrived home, I immediately studied engineering text books on heat transfer. The calculation of heat transfer from tubes is a very old area of engineering science. Even the designers of steam engines needed such knowledge. Therefore an excellent and proven theory existed, making it possible to calculate the energy balance of a heated capillary under forced convection conditions. When I looked at the results, it was clear that I had the key to building a thermal gradient GC! More than a hundred degree Celsius difference was easily achievable with moderate flows of ambient air.

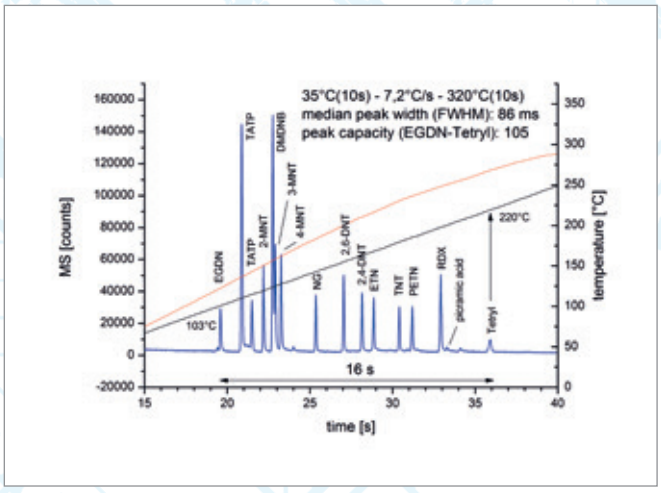
After a few more long car journeys and some experimental trials with other concepts, the final and very simple realization of the flow field thermal gradient GC was born. The heated column is mounted in a helical channel in the wall of a tube. Inside the tube, a porous material, which is used to form flow resistance, creates a very smooth flow field. As a result, the forced flow on the resistively heated column continuously decreases from one end of the column to the other. See Figure 1 for an illustration of the concept.

With the help of excellent staff at the workshop of our Institute of Agricultural Engineering – namely the electronic engineer Roland Lutz and the precision mechanic Wilfried Berchtold – we have now built several prototypes of the instrument. It took more than two years from the final idea to the measurement performance we have now acquired.

To come back to the idea of naivety and lack of knowledge, I suspect that we would have never started this project if we had known in advance how much detailed work was necessary and how much missing knowledge we had to acquire – a true drawback of being a newcomer! Indeed, the basic idea needed countless additional 'micro inventions' until it performed well.

Since those early days, we have performed many measurements with the system, especially in the main application field for which it was built: the near real-time detection of explosives.





Clockwise from top left: i) schematic of FF-TG-GC; ii) Explosives measurement with liquid injection of an explosives mix. SSL-injector temperature 170°C. Ramp time from 30 to 320°C within 40s. Column 0.1 mm/0.1µm DB1 of 2 m length. Detector Time-of-Flight MARKES BenchTOF. iii) Prototype of the FF-TG-GC system. iv) Jan Leppert, Leonid Blumberg and Peter Boeker at Riva 2016.

But the concept has a special advantage for basic research. We are capable to perform measurements with and without the thermal gradient – just by switching the gradient fan off. We have found that the gradient does indeed have advantages; it improves the peak sharpness but, perhaps more importantly, reduces elution temperatures. Our findings are very robust and can be explained using theoretical considerations.

Getting deeper

Now some words on 'deeper knowledge.' The outstanding Leonid Blumberg published papers in the 1990s on the topic of gradients in gas chromatography (and recently also in liquid chromatography). He very clearly deduces that thermal gradients, in theory, are of no benefit. We cannot be better than the 'ideal basic separation' (6). It is somewhat like the Carnot principle of GC and I refer to it as 'Blumberg's theorem.' Thankfully, I did not read (or understand) these papers at the beginning of our project. If I had, once again, we probably wouldn't have got started.

So the big question: is Blumbergs's theorem a contradiction to our experimental results? Maybe not. Theory – and theoretical modeling – usually require a reduction of the complexity of the system under evaluation. During our work on very fast GC, we have seen that many of the published theories are not valid for our system and sometimes have guided our efforts in the wrong direction. GC not only comprises the transport of the analytes but also the processes at the head of the column. The FF-TG-GC shows a very strong focusing effect because we can start the temperature program near ambient temperatures. I suspect that the remobilization effect plays an important role and is maybe not sufficiently accounted for in the theory and models of GC. We now have the very nice task of filling the gap between theory and experimental findings. For us, the interplay between experimental results and theoretical considerations is always the most fruitful way to gain a deeper understanding.

In 2015, we finally published our first paper – "Flow Field Thermal Gradient GC" – in Analytical Chemistry (7); I thank the reviewers for their benevolence. Unfortunately, after publication the paper seemed to disappear in the silence (or noise?) of the ever-expanding publication universe...

A new hope, a new community

One night, I received a newsletter from The Analytical Scientist magazine that showcased the content from the November 2015 issue. A diligent reader, I looked at the topics and "Landmark literature 2015" caught my eye. I was curious to see what was recommended by my peers and all of a sudden suffered an unexpected adrenaline rush! Pat Sandra's title – "Go with the flow (field)" – surely could only refer to our paper? I am still grateful to Pat Sandra for his very friendly and completely unexpected appreciation of our work.

My first attendance at the ISCC conference in Riva del Garda this year closed the circle after Jan's first attendance in 2010. From numerous papers, I knew many of the speakers by name but not in person. It was a great experience to come into contact with so many chromatographers whose work has been inspirational, setting the benchmark for our efforts. To name only a few directly connected to thermal gradient GC, I was able to talk with Rudolf Kaiser, Milton Lee and his co-workers, and had first discussions with Leonid Blumberg. And I was pleased that my own presentation received a friendly reception that led to other new contacts and further inspiration. I felt like I was finally arriving in a new and exciting community!

The next chapter

OK. So we have developed a new and powerful tool for hyper-fast GC – but who needs it? Is sample preparation not always the time limiting step? What about the sample capacity of micro bore capillaries? Is such a system as stable and reliable as a state-of-the-art GC?

These are just a few of the questions that people raise when it comes to their concerns about fast GC. My guess is that their experiences with previous systems on the market were not too encouraging. But either way, resistance against change is strong.

The reality is that we have only explored but one percent of the parameter space of TG-GC. Our two-person workgroup is unable to do much more than technical development and a few basic measurements, which is why many more questions are left open than answered – and also why so few applications have

Real Peer Review

Milton Lee, Brigham Young University
Peter Boeker brings up some good points, especially regarding the differences between ideal theory and reality. Some assumptions made when theory is developed are not valid under all conditions – this is the case with Leon Blumberg's 'ideal basic separation.' Although his theory is useful under most conditions, for extremes, such as short columns and fast analyses, as in Boeker's work, experimental results deviate from the ideal. For this reason, thermal gradient GC has potential. Theoretically, it will never match a temperature programmed separation using a 30 m column with respect to peak capacity; but for rapid analyses, trace detection, column-induced band broadening and tailing, and injection bands that are not infinitely narrow, it should be very useful. Eventual commercialization of this technique is dependent on the instrumentation becoming as simple and easy as a conventional GC convection oven.

Robert Synovec, University of Washington
I am fascinated by the recent development of Flow Field Thermal Gradient Gas Chromatography (FF-TG-GC) by Peter Boeker and Jan Leppert (7)! This is a very exciting and noteworthy advance for the field of capillary separations, with great potential to lead to significant improvements in peak capacity for fast one-dimensional GC, and to open up new opportunities in instrumentation design and performance for comprehensive two-dimensional gas chromatography (GC x GC), such as using FF-TG-GC as the second dimension. Though separations theory indicates traditional temperature-programmed GC and thermal gradient GC should both provide the same efficiency performance, in experimental practice the theoretical optimal performance has been challenging, if not essentially impossible, to achieve for fast separations. The key then becomes determining which of these two approaches to address the general elution problem can do so while minimizing additional sources of band broadening – the two main culprits being uneven heating (essentially manifested as a multi-path broadening term) and sample injection pulse width.
The design of the FF-TG-GC column module, in elegant fashion, provides very uniform heating – and the potential is there to make marked improvements. Furthermore, the inherent design and implementation of FF-TG-GC provides on-column focusing at the head of the column, which is effective in providing a narrow sample injection. I think it is these two aspects of FF-TG-GC that may set it apart from traditional temperature-programmed GC. While challenges remain in realizing the theoretical optimum for fast GC separations by further reducing the unwanted sources of band broadening, the introduction of FF-TG-GC should have an immense impact on this quest.

Jan Blomberg, Shell Global Solutions International
By controlling heat loss instead of heat input, Peter has come up with a stunningly simple solution to realize thermal-gradient GC using (long) capillary columns. On-line coupling of high-speed GC has always suffered from the unavailability of similar high-speed sample introduction devices. Since thermal-gradient GC automatically compensates for non-ideal chromatography, this implicates that (Ultra) High-Speed GC can be realized using robust, traditional sampling devices. With FF-TG-GC, Peter has finally brought what has long been an academic curiosity to the real world.

been developed thus far. Nevertheless, our expectations of the new basic technology are high. On our agenda: the application of a gradient column as a second dimension in GCxGC and the construction of a comprehensive heart-cutting GC to obtain very high peak capacity within a very short cycle time.

Maybe that's naïve again. But I hope that efficient low thermal mass fast GCs will have a promising future in gas chromatography!

Peter Boeker is a research associate at the Institute of Agricultural Engineering, University of Bonn, Germany.

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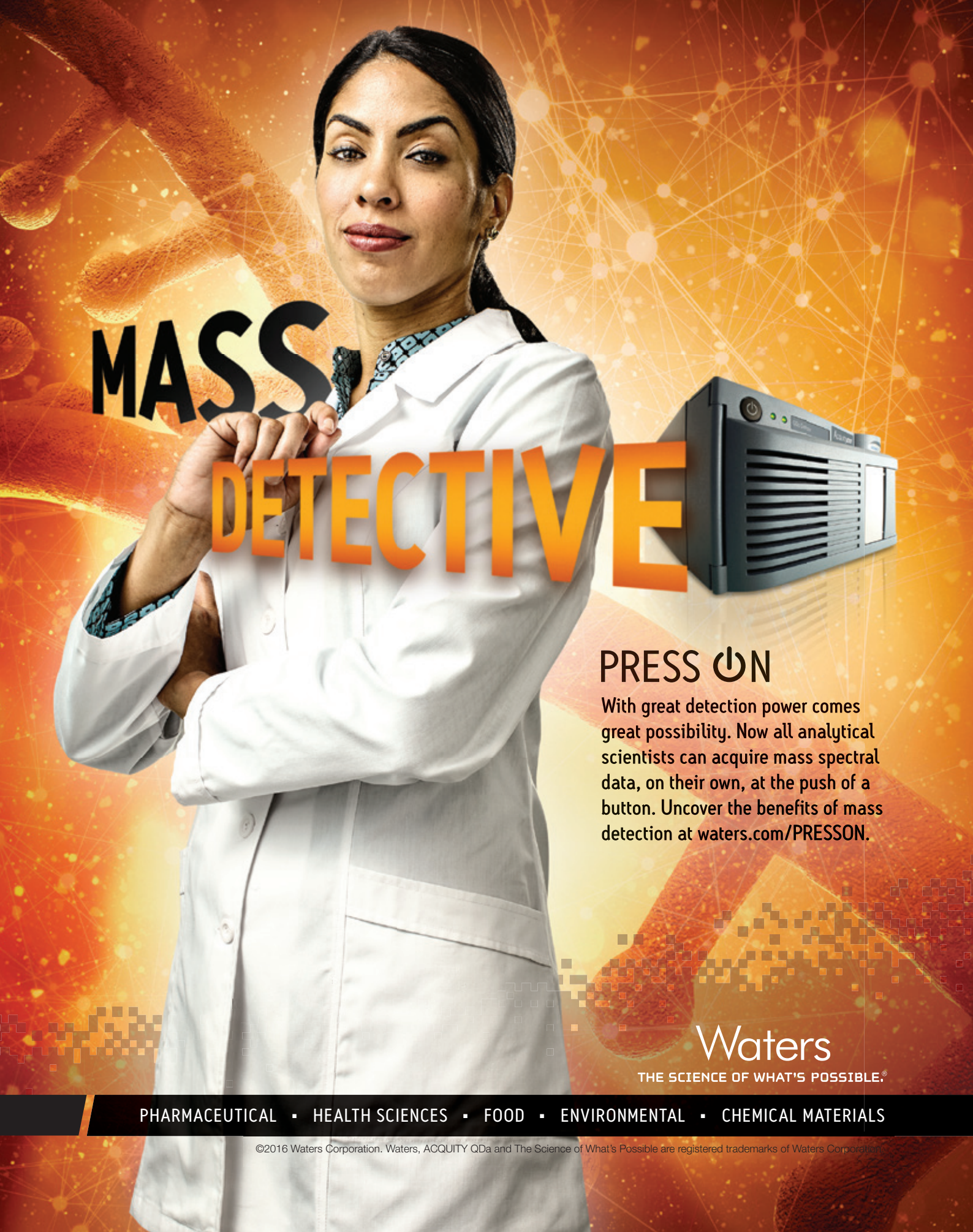
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Profession

Leadership
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An Education in ESI

Finally, an affordable way to conduct practical undergraduate courses on electrospray ionization mass spectrometry.

By Michel Nielen, Pepijn Geutjes, Barend van Lagen, and Teris van Beek

Every year, as part of our undergraduate course – Analytical Methods in Organic Chemistry – one or two hundred students from molecular life sciences and biotechnology are introduced to the wonders of mass spectrometry (MS). In the education space, it can be tricky to keep up with the fast pace of MS advances, and we're pleased when we are able to widen the experience to prepare students for the real world.

But before we share the details of our latest educational endeavors, a little history... Back in the 1990s, electron ionization (EI) was the most accessible technology in educational circles, and so the focus of the MS course was on both ion formation and interpretation of EI spectra. Over the years, gas chromatography (GC)-MS systems with EI became more affordable and entered into the practical course program; nowadays, we have two GC-(EI)MS systems permanently situated in our education building.

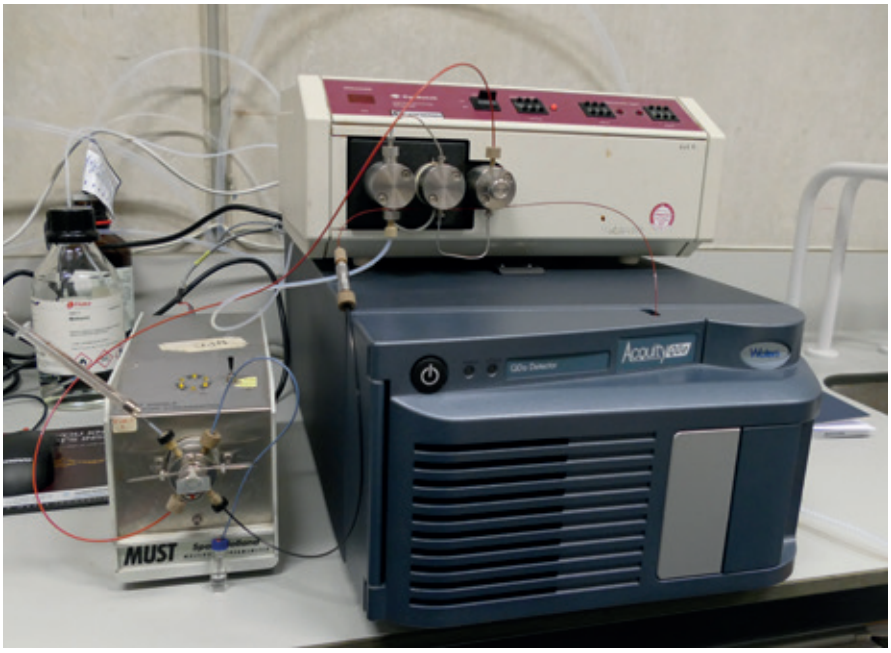

Figure 1. Experimental set-up featuring a transportable quadrupole ESI MS (Acquity QDa, Waters) operated in a straightforward flow injection set-up. The turbopump of the MS is supported by a simple membrane pump attached to the rear of the instrument.

Introducing ESI

Over the same period of time, the course had to be updated with the theory of soft ionization techniques, such as electrospray ionization (ESI; 1, 2) and matrix-assisted laser desorption ionization (MALDI). Unfortunately, hands-on practical experience was not feasible, so students were exposed to ESI through visits and demos at a research lab. Given the importance of ESI-MS in molecular life sciences and biotechnology in general, this was definitely not an ideal situation. Thanks to recent developments in affordable and transportable quadrupole-based ESI-MS systems (for example, the Acquity QDa detector from Waters, and the Microsaic 4000 MiD from Microsaic Systems), it is now feasible to purchase a system exclusively for educational purposes. We've done just that and it enabled us to develop a dedicated undergraduate practical course on ESI-MS.

Transportable ESI-MS systems are typically marketed as selective detectors

that can complement or replace photo diode array UV-Vis detectors in liquid chromatography (LC) systems. But for educational purposes, we have been using a quadrupole ESI-MS system as a standalone unit in a flow injection analysis set-up, which consists of an old isocratic LC pump and a manual injection valve with an inline filter between the injector and the MS (see Figure 1 and "Setup").

Our new system means that students are exposed to both GC-(EI)-MS and ESI-MS in practical hands-on courses. And our MS course objectives have been updated to ensure that students: i) learn and understand the differences between EI and ESI ionization in terms of the type of ions formed, the degree of fragmentation and the structural information obtained; and ii) understand the opportunities and limitations in the applicability of EI- and ESI-MS for the characterization of (bio)organic (macro) molecules and mixtures thereof.

Covering all bases

Thanks to the flow injection set-up and the very short start-up time and robustness of the quadrupole MS, we can typically get groups of 2-4 students to perform each of the following three experiments in under one hour of instrument time. Mass spectral interpretation and reporting requires extra time, of course.

Low molecular weight compounds

We prepared a number of mixtures based on their potential to highlight the spectral differences that will be typically obtained when analyzed by both EI and ESI-MS: primary amines (octylamine, decylamine, dodecylamine), secondary amines (dipropylamine, dibutylamine, dihexylamine), primary alcohols (decanol, dodecanol) and primary carboxylic acids (octanoic acid, decanoic acid, dodecanoic acid). As expected, the primary and secondary amines ionized very well in positive ESI, yielding intact $[M+H]^+$ ions (Figure 2a/b). On the other hand, in EI the mass spectra of the primary amines are dominated by the homolytic fragment ion at m/z 30, thereby losing the entire molecular weight information.

The primary alcohols are difficult to ionize in positive or negative ESI and no information is obtained at all; in EI, extensive fragmentation and water loss occurs and the molecular ion cannot be seen either.

Setup

We use a model Acquity QDa (Waters) single quadrupole MS with a mass range of m/z 50-1250, operating in alternating positive (capillary voltage 1.2 kV) and negative (–0.8 kV) ion mode at a source temperature of 120 °C, a probe temperature of 600 °C and a cone voltage of 15 V. The LC pump is an old Gynkotek model 300 operated at 0.4 ml/min. The injection valve has a volume of 5 µl. Following each injection, raw data are combined and background subtracted in Masslynx 4.1 (Waters) to obtain high-quality mass spectra for student interpretation. All chemicals are from Sigma-Aldrich, except for the aliphatic carboxylic acids and the amino acids, which are from Alfa Aesar. Standards are dissolved in HPLC-grade methanol (Rathburn) at 100-200 µg/ml, except for phenylalanine, lysine and PEG 400 (20, 20 and 50 µg /ml, respectively).

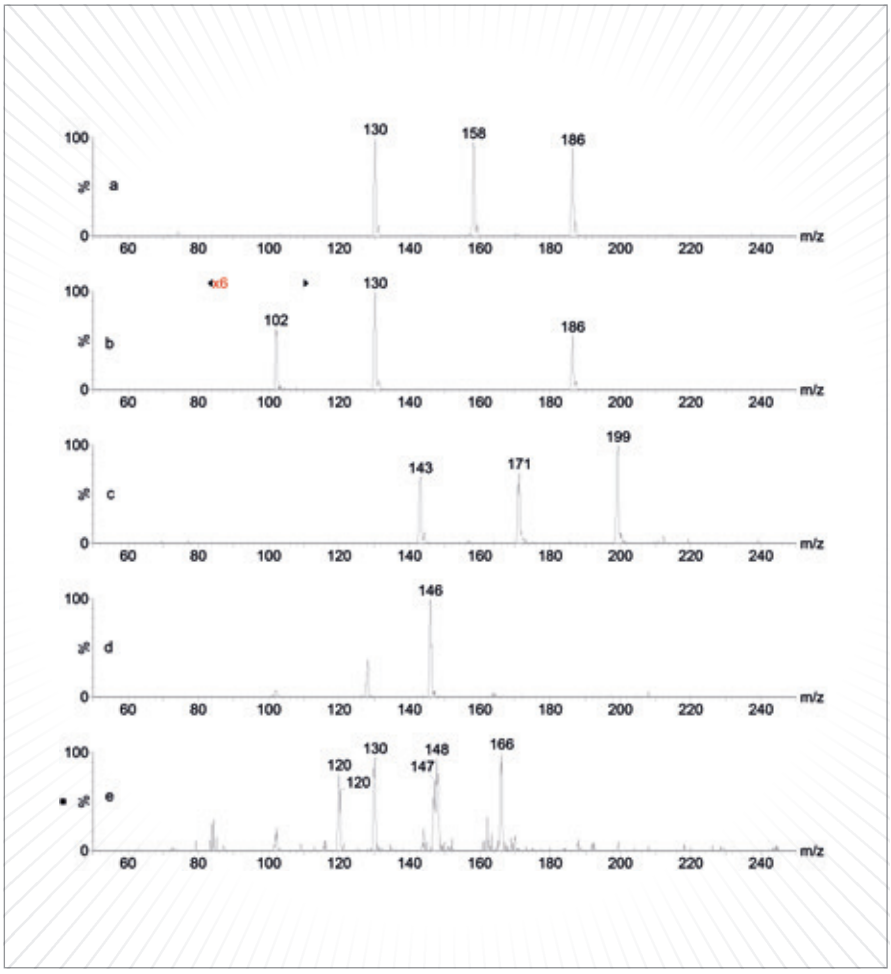

Figure 2. Background subtracted ESI mass spectra of low molecular weight compounds. Positive ESI: a) the primary amines octylamine, decylamine, dodecylamine; b) the secondary amines dipropylamine, dibutylamine, dihexylamine. Negative ESI: c) carboxylic acids octanoic acid, decanoic acid, dodecanoic acid. Negative (d) and positive (e) ESI: amino acids lysine, glutamic acid, phenylalanine.

The carboxylic acids hardly ionize in ESI unless the negative ion mode is selected and the pH is adjusted above the pKa with some ammonia: the intact $[M–H]^-$ ions obtained allow a straightforward assignment of the mixture (see Figure 2c).

When analyzing the mixture of amino acids (lysine, glutamic acid, phenylalanine) GC-MS obviously fails, while ESI in both positive and negative mode does a complementary job: negative ion ESI highlights the $[M–H]^-$ ion of glutamic acid at m/z 146 (see Figure 2d) while $[M+H]^+$ ions of phenylalanine, glutamic acid and lysine can all be found in the positive spectrum at m/z 166, 148 and 147, respectively – including a loss of water from protonated glutamic acid at m/z 130 (Figure 2e).

In all these cases, we can pose a number of questions to students, for example:

- How many compounds are present in the unknown mixture?
- Which ions are the (quasi) molecular ions?
- Are there any sodium or potassium or solvent adduct ions?
- Is there an odd or even (including zero) number of nitrogen atoms?
- What would be a possible elemental composition?
- Are there fragment ions and if yes, which cleavage reaction occurs?

Synthetic polymers

Poly(ethylene glycol) is easy to ionize in positive ESI and typically yields a series of intact $[M+Na]^+$ ions separated

by the monomer mass of 44 Da (Figure 3). High molecular weight PEGs may extend beyond the upper mass range of the quadrupole and, in addition, yield multiply-charged ion series causing too much confusion for the students, so typically PEG400 or 500 would be appropriate. Now the same questions as above will challenge the students a bit more since they are not really familiar with polydisperse polymer mixtures. Following some hints, they should be able to identify the polymer and discover that we are facing a mixture of single-charged polymer ions having the general formula $[A+(C_2H_4O)_n+B+C]^+$ in which A and B represent the end-groups of the polymer chain and C a species that provides the positive charge (proton, sodium, potassium). Finally, they may come to the conclusion that the spectrum represents polyethylene glycol having an average degree of polymerization of 10 and a value for A+B+C of 41 Da, which suggests a PEG400 having H and OH as end-groups and sodium as the cation.

Carbohydrates and proteins

Low molecular weight oligosaccharides such as maltose (MW 342), raffinose (MW 504) and maltotetraose (MW 666) show simple single-charged $[M+Na]^+$ ions in positive, and $[M-H]^-$ ions in negative ESI (see Figure 4a/b). Proteins, on the other hand, are rather confusing – especially for those students who have yet to study the theory of protein ESI-MS!

Clearly, the MS system is also pushed to (and beyond) its limits because of the fact that proteins show multiply-charged $[M+nH]n+$ ion series in the range of m/z 700-1500 – that's beyond the upper mass range and the nominal mass resolution of this small quadrupole MS.

The mass spectrum of ubiquitin (see Figure 4c) is rather noisy, as expected, but shows a sufficient number of multiple-charged ions for precise calculation of the protein mass based on the assumption that adjacent multiple charged ions differ by one charge state only; using the three most intense multiple charged ions, the deconvoluted protein mass would be 8566 Da, which is only 1.5 Da higher than the real molecular weight of ubiquitin.

Challenging questions for students:

- How many substances are present in this sample?
- Which ion(s) is/are the (quasi) molecular ion(s)?
- What is the molecular weight of the substance(s)?

We found that the relatively high accuracy of the calculated protein mass is very often a pleasant eye-opener for students who still recall their previous hands-on gel electrophoresis experience!

"We found that the relatively high accuracy of the calculated protein mass is very often a pleasant eye-opener for students"

Give it a try?

If you were wondering, the flow injection ESI-MS set-up is simply switched off when not in use and powers up in approximately five minutes – including auto-tuning and mass calibration – so the system is really education-ready. And of course, the same set-up can be applied as a detector to complement an LC-UV set-up in chromatography courses. We hope we've shown here that it's possible to develop a highly affordable and straightforward solution for undergraduate practical courses in mass spectrometry. Why not drag your MS courses into the 21st century? We'd love to hear how you get on.

Michel WF Nielen, Pepijn Geutjes, Barend van Lagen, and Teris A van Beek are all based in the Laboratory of Organic Chemistry at Wageningen University, The Netherlands.

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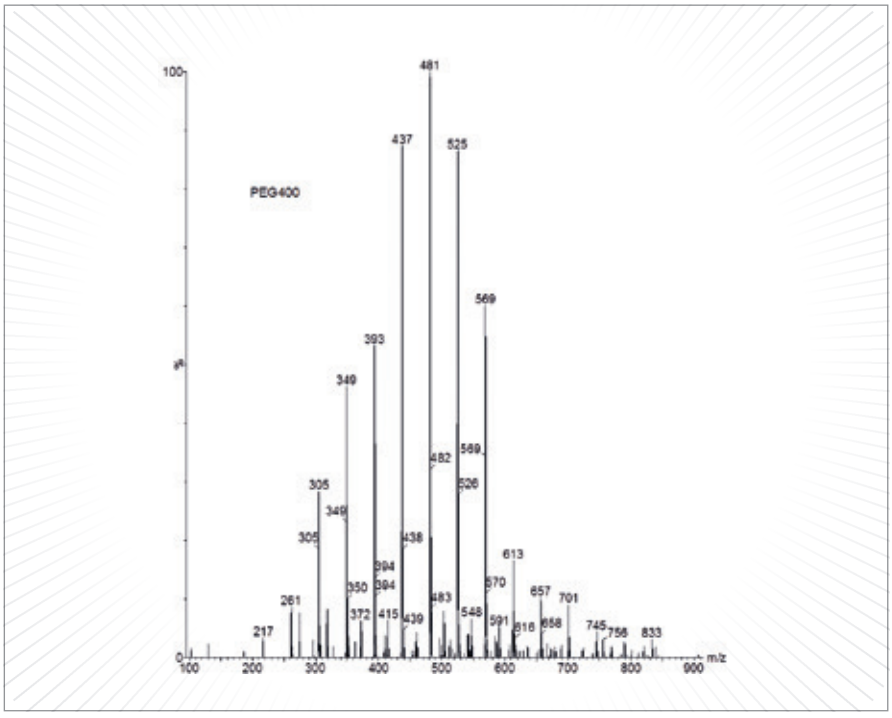

Figure 3. Background subtracted positive ion ESI mass spectrum of the synthetic polymer PEG400.

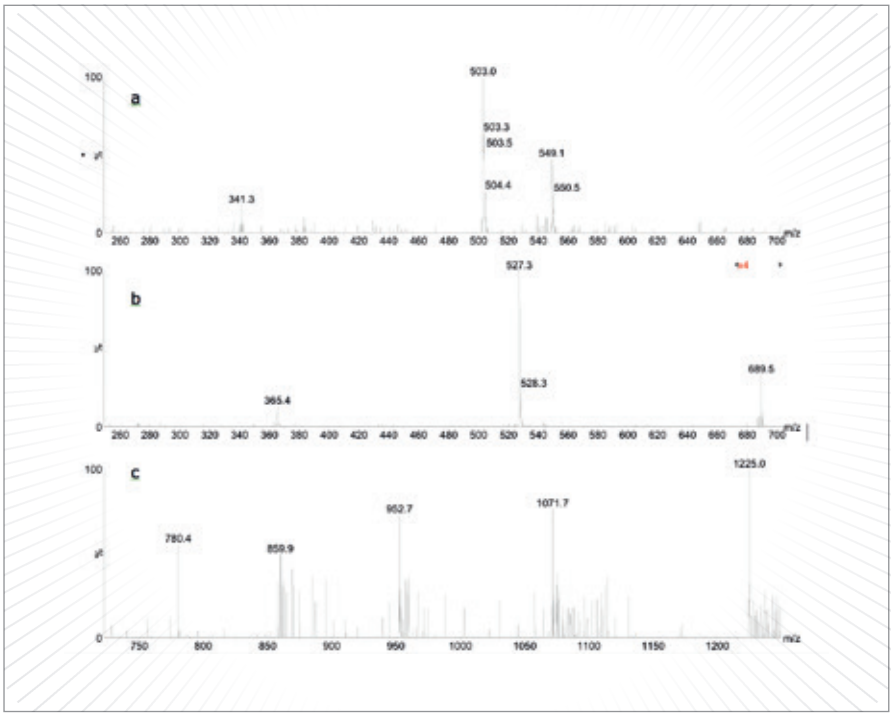

Figure 4. a) Negative $[M-H]^-$ and b) positive $[M+Na]^+$ ion background subtracted ESI mass spectrum of maltose, raffinose and maltotetraose; c) positive ion background subtracted ESI mass spectrum of ubiquitin in methanol/water (40:60) acidified with 0.1% formic acid.

Sensitive Online SPE Determination of Bisphenol A in Water Samples

An HPLC system with additional switching valves can be used in continuous online SPE operation without time-consuming sample preparation steps.

By Juliane Böttcher and Mareike Margraf

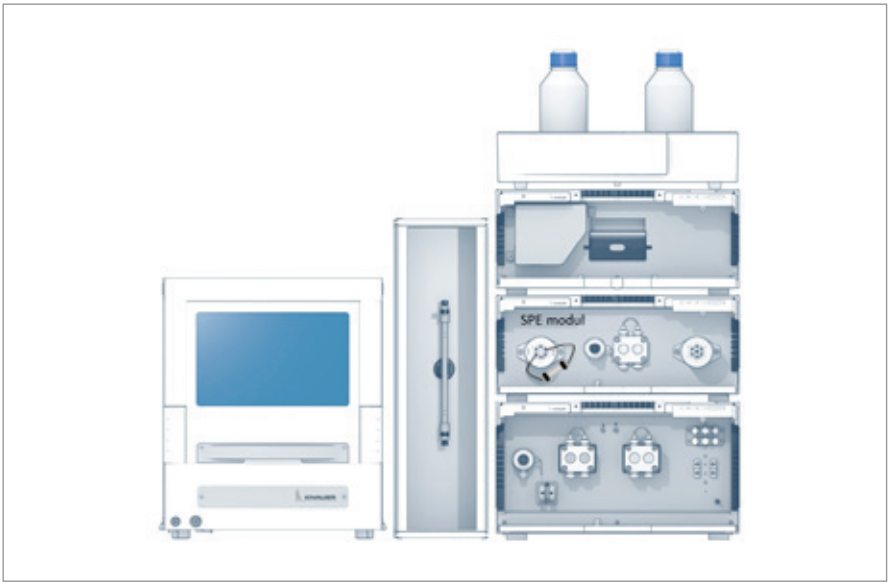

Figure 1. Schematic arrangement of the involved system components for the HPLC system coupled to online SPE extraction.

Abstract:
In this application note a method for the sensitive determination of bisphenol A (BPA) from water samples is presented. The use of online SPE coupling avoids time consuming and manual sample preparation steps, making the method well-suited for routine analyses of BPA in low concentration samples like drinking water.

Introduction

Solid-phase extraction (SPE) is an effective preparation method for concentrating analytes prior to HPLC analysis. Classically, this method is done offline via time consuming steps. The advantages of online coupling result in a reduction of analysis time, sample contamination, and analyte loss. This automated method is perfectly suited for pre-concentration of Bisphenol A (BPA) in drinking water. This substance is known for its endocrine effects similar to the hormone estrogen even at very low dosage and is associated with environmental and health problems. Derived from various studies, a maximum entry of <1 µg/ml is expected in cold drinking water. In warm water (70°C) a concentration of up to 30 µg/ml is possible. All of these facts necessitate a fast HPLC method that reaches very low detection limits for BPA.

Experimental preparation of standard solution

All standards were prepared and diluted with LC-MS grade water to eliminate matrix effects during calibration and to ensure high quality of the standard.

Results

After calibration by direct injection, the recovery rate is determined with the online SPE column in the flow path. Differing concentrations down to 0.07 ng/ml have been extracted from prepared water samples with constant extraction time. Afterwards the extraction time was varied using a solution with a constant concentration of 0.1 ng/ml. A recovery rate of 98% for BPA was found.

Conclusion

The presented automated method is well-suited for the sensitive analysis of BPA in water samples like drinking water. For a lower detection limit, the online SPE extraction time can simply be increased. The new method makes time consuming manual sample preparation steps obsolete.

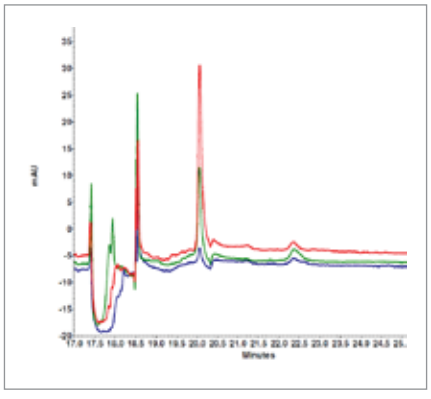

Figure 2. Chromatogram of three different concentrations with the same online SPE extraction time.

KNAUER

Protein Protagonist

Sitting Down With… Albert Heck, Scientific Director at the Utrecht Institute for Pharmaceutical Sciences, Netherlands and Scientific Director of the Netherlands Proteomics Centre.

What was your route into proteomics?

I originally trained as a physical chemist but gained experience in mass spectrometry (MS) during my PhD. But my route into proteomics started when I joined the University of Warwick as a senior research fellow in around 1995. I was working on biomolecular MS – looking at intact peptides and proteins – and even though I was a newcomer to molecular biology, I soon realized that it had enormous potential and decided to stay in the field. I'm not sure it was even called proteomics back then...

What are you working on at the moment?

For ten years or so, my group has had two focal points: i) developing new fragmentation techniques and new separation techniques for shotgun or peptide-centric proteomics, and ii) developing tools for structural biology – mostly by native MS and hydrogen deuterium exchange (HDX) MS. The aim has always been to bring these two areas closer together and now feels like the right time.

What are the biggest challenges in the field right now?

I think one of the big questions or challenges is: can we really understand how all these molecular players work together to regulate the whole system? Every simple question has a complicated answer. All the protein, DNA, and metabolites are all entwined with each other. We are getting very good at generating high-quality data, but we still don't know what to make of it from a biological standpoint – and that's very frustrating. I hope that by measuring more or by answering more hypothesis-driven questions, we can get a better understanding. Everyone knows that systems biology is essential, but no one really knows how to do it!

What do you think the answer is?

A simple answer that is not a simple answer! Though I certainly think more and better bioinformatics is important, we need to ask the right questions and to do the right experiments. I am humble; human biology is very fine tuned and I think the questions we ask and the experiments we do are still way too simple compared to the complexity of how a cell in a human being solves a particular problem.

Do you agree that proteomics hasn't delivered on its early promises?

Yes and no. Ten or fifteen years ago, there was this hype about proteins being simple biomarkers for diseases, and in clinical proteomics, the hype has never been fulfilled. However, the hype was not introduced by core proteomics people, but rather by those who saw an opportunity. It's the same today, I think. We are likely to hear bold claims about CRISPR from some quarters, for example, while core researchers will remain excited but modest about what can be achieved. What has proteomics delivered? Well, it has fundamentally changed how we look at molecular and cellular biology.

You're a strong believer in collaboration...

It's the way science should be. In the past (and maybe still), science has been too ego-centric, when it should be problem-centric. There are many scientists who want to do everything and take all the credit. But you can't do everything by yourself anymore, and certainly not to the same degree as when you work with experts in other fields. Moreover, collaboration essentially means free learning!

What drives you?

I'll try not to get too philosophical, but I would say curiosity drives me. I am really happy if we see something that no one else has ever seen. The feeling that you reached a new level of understanding – that excites me very much. It's a little bit like being the first man on the moon – exploring new territories. In that sense, I'm very technology driven. Maybe you'd like to hear that I want to cure cancer or something – and of course I would love to do that – but I believe we understand so little about how life is organized that my biggest contribution comes from improving technologies so that we can better look at life and how it works.

"Science has been too ego-centric, when it should be problem-centric."

What advice do you give young people coming into the field?

I tell them that it is one of the most exciting areas to work in! And I always tell them to go off the beaten track and try to be creative. Sometimes you can start with a naive idea and go with it because you don't know all the details, and find something new. When people read all the literature, it can actually stop them from being creative... But it's a fine line.

What would you like to achieve in the next few years?

Ultimately, I'd like to sit on top of a protein in the cell and see what it's communicating, how it's behaving, how it's influenced by its environment. I'd like to see how that protein operates, and if it's similar to how a human being operates in society. I'm pretty sure proteomics is going to be part of getting there, but we also need other technologies. If we could gain that insight – even for one protein – then I'll be able to say, "That's the dream I've had for 20 years – and now I'm pretty sure we're there."

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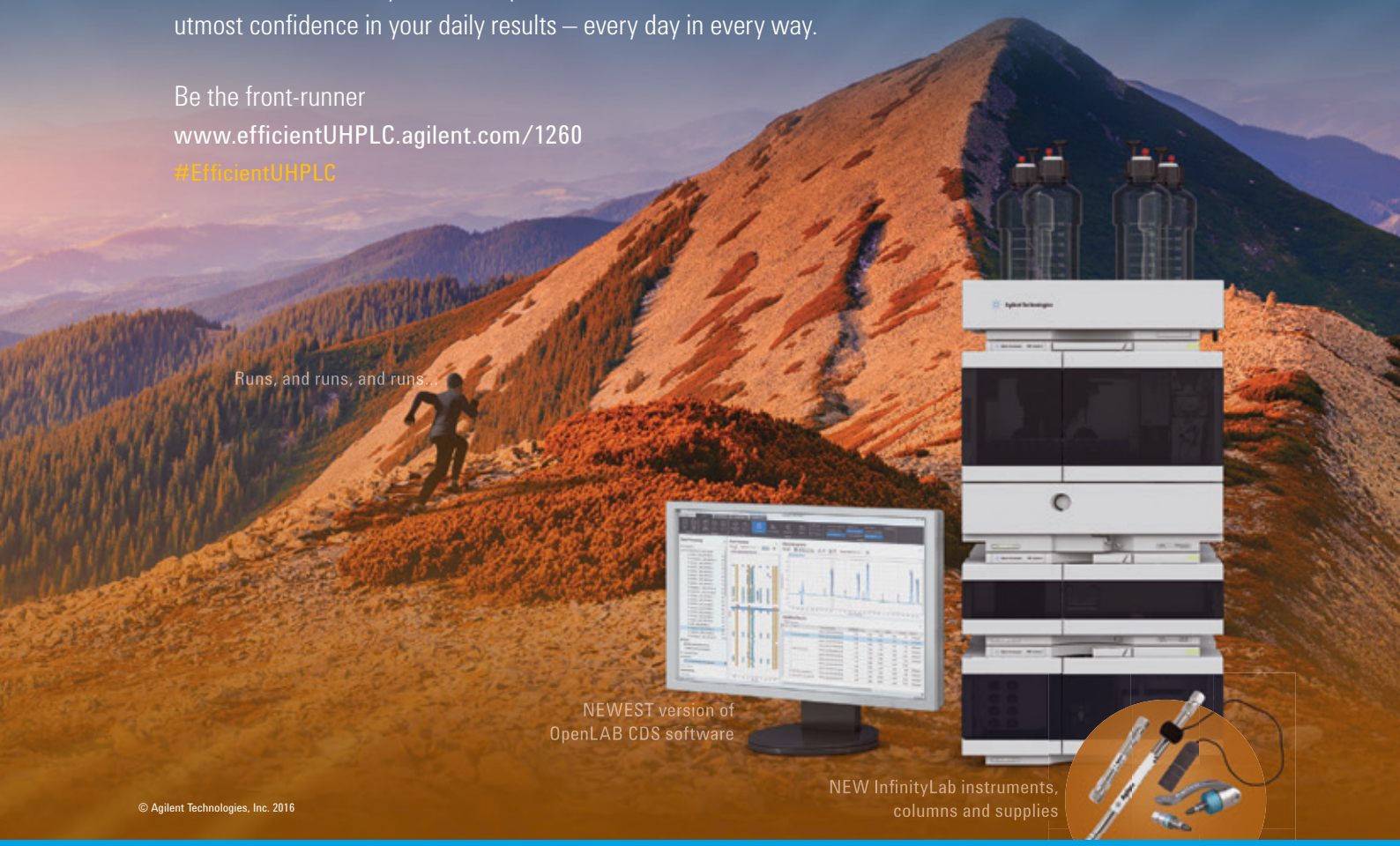

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