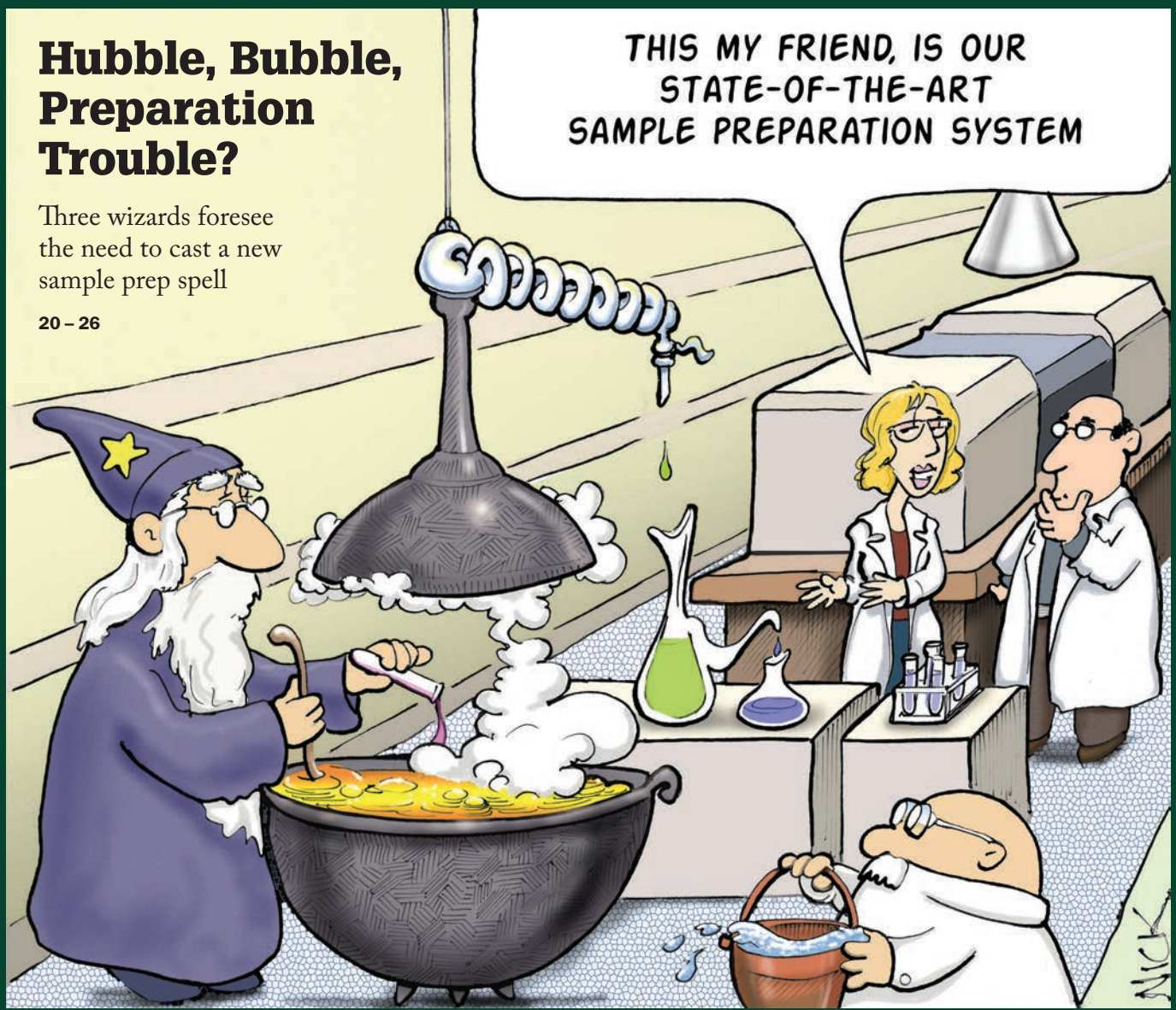
JULY 2014 # 18

the Analytical Scientist

Hubble, Bubble, Preparation Trouble?

Three wizards foresee the need to cast a new sample prep spell



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Online this Month

Please, Don't Go!
Building Mass Spec from the Inside
(tas.txp.to/0714/jardine)
After such a productive career in mass spectrometry, you surely can't just hang up the boots and do nothing! Don't disappear completely from the scientific world, there must be some low-stress activities out there? – *David Woollard, Senior Chemist/ Scientist New Zealand.*

Understand By Doing!
Learning Without Doing Equals Shortcoming
(tas.txp.to/0714/learnbydoing)
I agree! For every level of education, practical exercise is very important. In the Netherlands, this is a big part of standard education because we have a strong competance driven educational structure. I work with Avansplus and we give practical laboratory courses for employers in labs. We try to have practical sessions during all the short courses that we conduct. My experience is that this always helps the student to better understand the things we tell them in theory – *Helga Walters, The Netherlands.*

Sign up online for free to have your say: *theanalyticalscientist.com/subscribe*

Tea With Luigi

Have you seen our ongoing video series "Tea With Rich"? In our first interview, Luigi Mondello thanks Pat Sandra for the wonderful toy (the Riva conference) and introduces his latest grand research project. Oh - and there's camera drone footage over the lake…
tas.txp.to/0714/teawithluigi

Tea With Chiara

For our second episode, also set in the gardens of a beautiful old villa in Riva del Garda, Italy, Rich Whitworth invites Chiara Cordero from the University of Turin for tea. Chiara outlines her research into "sensomics", chats about the award she received at Riva 2014, and shares her deep passion for food – and tea.
tas.txp.to/0714/teawithchiara

Tea With James

For the third and final instalment from Riva del Garda, James Harynuk finds himself in great need of a cup of tea after 18 hours of travel, but dispatches jet lag to discuss GC×GC thermodynamics and the potential black box of chemometrics.
tas.txp.to/0614/teawithjames

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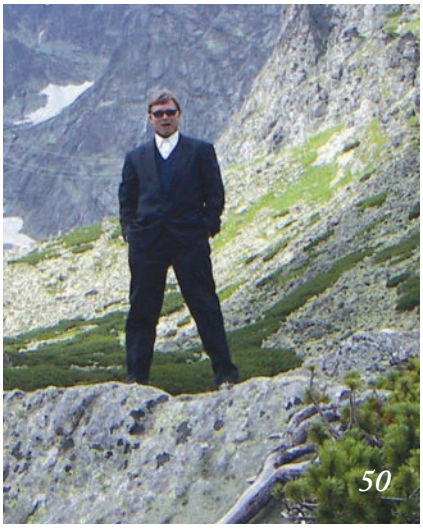

On The Cover

Nick Kim uses full artistic license to represent the archaic state of sample preparation. For more irreverence, see www.scienceandink.com

Upfront

In My View

the Analytical Scientist

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Fair Play at the World Cup

"Simulation" and biting aside, does the total lack of substance abuse mean that the beautiful game is clean?

Somewhat amazingly, every single player at the World Cup in Brazil was clean (1). And that's not because the authorities didn't bother testing anyone after the World Anti-Doping Agency (WADA) revoked the accreditation of Rio de Janeiro's Ladetec laboratory months ahead of the tournament. No. WADA and FIFA came to the agreement that an accredited laboratory in Switzerland would be used to analyze samples for the tournament – surely, a logistical and chain-of-custody nightmare.

It seems staggering to think that not one of the nearly 750 players involved hadn't at least eaten contaminated meat. (Mexico's coach banned his players from eating beef in Brazil for fear of being tested positive for clenbuterol after infringements in the 2011 CONCACAF Gold Cup). But for FIFA's World Cup at least, that's two decades of clean sheets; you have to go back to 1994 to find a positive test (ephedrine) and a disgraced Diego Maradona. So, either world class football players hail from an extremely clean culture where performance-enhancing drugs are frowned upon or... I would be interested to hear your views. Perhaps Juliet Macur of The New York Times has got the right idea: "Don't let it ruin this moment. No one ever tests positive at the World Cup. Just do what FIFA has done in the past: close your eyes and pretend that doping in soccer does not exist." (2).

The Analytical Scientist has covered sports doping several times, most recently when doping expert Douwe de Boer went head-to-head with the Dutch Doping Authority's Herman Ram (3). But, with two polar opposite views, the discussion left more questions than it answered. For example, how much effort is appropriate and what priority should doping receive?

Other big tournaments this summer, such as Wimbledon and the French Open Tennis Championships, also seem to be clean, which goes against the grain given that WADA recently announced a 20 percent rise in abnormal test findings recorded by anti-doping authorities worldwide in 2013 (4). So, have all top athletes cleaned up their act or has WADA dropped the ball?

Still, with nearly 6,000 adverse or atypical test results in 2013, there have to be casualties. One this year is runner Adrienne Herzog, who tested positive for elevated testosterone in an out-of-competition sample. She concluded in a very personal blog post (5): "It is so unjust - this one-sided balance of power. WADA does the initial test, the confirmatory tests, reaches a verdict, and then administers the penalty. Where are the checks and balances here to ensure I get a fair shake at things?" Discuss.

Rich Whitworth
Editor

References

1. *www.fifa.com/worldcup/news/y=2014/m=6/news=all-pre-fifa-world-cup-doping-controls-test-negative-2374232.html*
2. *www.nytimes.com/2014/06/21/sports/worldcup/no-doping-at-the-world-cup-thats-what-fifa-says.html*
3. *tas.txp.to/0714/doping*
4. *www.bbc.co.uk/sport/0/28194582*
5. *www.prorun.nl/columns/navigating-through-murky-waters*

Contributors:

Elizabeth Thomas

Liz Thomas qualified as an analytical chemist while working at Glasgow University and has been in the CRO and pharmaceutical industries for over 25 years. In 2011, she joined ICON as vice president and general manager of the European bioanalytical business with a major role in the harmonization and development of the bioanalytical business globally. "Before ICON, I worked at AstraZeneca, where I was responsible for providing scientific, regulatory and strategic leadership globally and locally." Liz has represented both ICON and AstraZeneca on the European Bioanalysis Forum (EBF) and is a member of a Global Bioanalytical Consortium (GBC) harmonization team.

Liz enlightens us with her entrepreneurial flair on page 40.

Jody Dunstan

While studying marine and environmental science at university, Jody Dustan was offered the chance to work on a masters research project looking at PAHs in marine sediments at Plymouth Marine Laboratory. "That project involved developing a GC-MS method; I enjoyed it so much that, 14 years later, I am still involved in GC-MS." Jody has worked in various technical positions within Waters before becoming a product manager in 2013. "I am still a geek at heart and like to get involved in the technical side of things when I get the chance."

Jody tells the story behind instrument development on page 44.

Jack Cochran

Jack Cochran is a recognized expert in GC and GC×GC for the analysis of pesticides, PCBs, explosives, PAHs, PCDDs, PCDFs, BFRs, and similar priority pollutants in food, soils, sediments, air, water, and other matrices. "I use my real-world experience in method development, sample preparation and analysis to help chromatographers worldwide. I'm still a 'hands on' scientist and like to spend as much time in the laboratory as possible". Jack is also an adjunct professor in the Forensic Science Program at The Pennsylvania State University.

Jack offers tips for easy GC×GC on page 17.

Aydogan Ozcan

Aydogan Ozcan, the chancellor's professor at the Unversity of California, Los Angeles (UCLA), and an HHMI Professor with the Howard Hughes Medical Institute, leads the Bio- and Nano-Photonics Laboratory at the UCLA School of Engineering. He is also the associate director of the California NanoSystems Institute. Aydogan has received many major awards for his seminal contributions to near-field and on-chip imaging, and telemedicine based diagnostics. In 2011, Aydogan co-founded Holomic with the aim of improving patient health care using smartphones and biophotonics.

Aydogan divulges the secrets of our smartphone-based analytical future on page 30.

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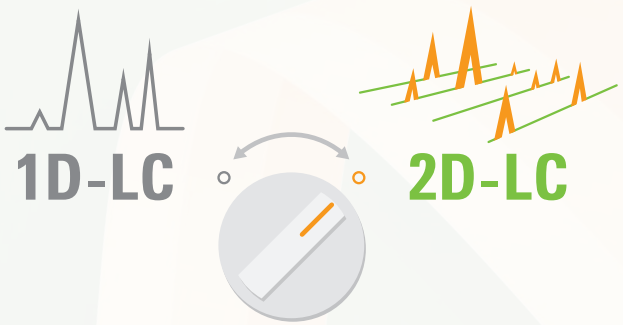

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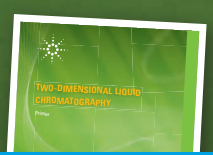

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Oil Spill Forensics

Measuring the impact of the Deepwater Horizon disaster four years on

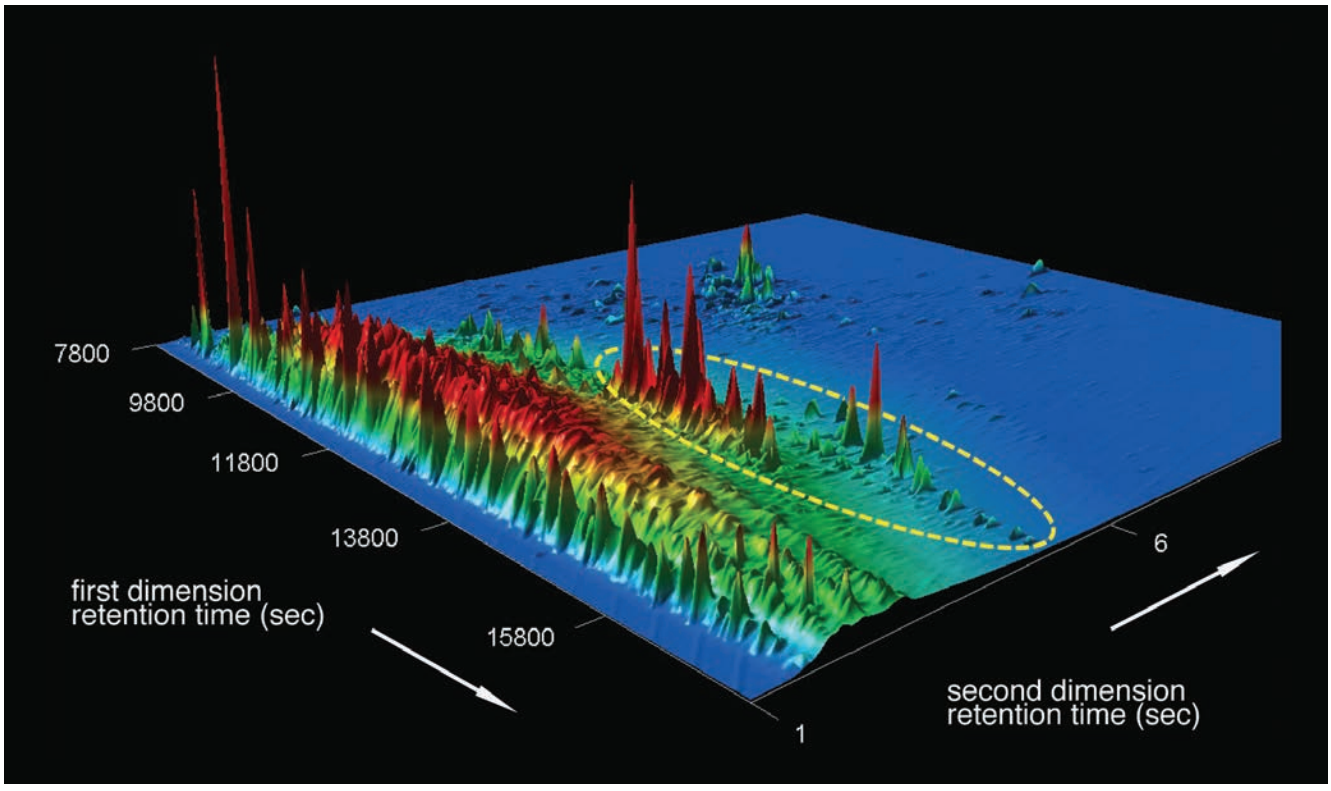
In 2010, the Deepwater Horizon spill discharged an estimated 4.9 million barrels of oil into the Gulf of Mexico. Even today, residual oil is being washed ashore along the northern Gulf of Mexico coast, particularly the Alabama and Louisiana sections. Researchers from Woods Hole Oceanographic Institution, Bigelow Laboratory for Ocean Sciences, and the University of California, Santa Barbara have been using oil biomarkers to track the oil back to Deepwater Horizon, and recently published their findings (1). All oil has a unique history of formation, which leads to a specific relative composition of its various compounds. Some of those compounds – also known as molecular fossils – are used as biomarkers to identify oil and its origin.

"Oil biomarkers are commonly used in exploration or after spills for identification," explains Christoph Aeppli, lead author of the study. "But there was some uncertainty about the recalcitrant nature of these biomarkers in the environment after years of exposure to sunlight, oxygen, and microbes." To that end, the researchers collected oil samples over a 28-month period from along the Gulf shore and used comprehensive two-dimensional gas chromatography (GC×GC) to study levels of oil biomarkers (see Figure 1). "For such complex mixtures, traditional one-dimensional GC reaches its limits to chromatographically separate compounds," says Aeppli, "GC×GC is quite a novel technique in oil spill analytics, and allows us to study the compositional change over time."

But that's not to say it was easy (for "Eight Tips for Easy GC×GC" see page 17). "Producing nice, reproducible

Catherine Carmichael, Woods Hole Oceanographic Institution

Four years after the Deepwater Horizon spill, oil continues to wash ashore as oil-soaked "sand patties."



Christoph Aeppli, Bigelow Laboratory for Ocean Science

Figure 1. Comprehensive two-dimensional gas chromatography (GC×GC) 3D plot of oil sample. Biomarkers are shown inside the dotted line.

Joseph Suffita, University of Oklahoma

Christoph Aeppli led a team that identified Deepwater Horizon Oil on shore

GC×GC chromatograms is not trivial," says Aeppli, "We used a setup that has been optimized over the years, and I was lucky to collaborate with co-authors Chris Reddy, who pioneered GC×GC for oil spill research, and Robert Nelson, who has been working with GC×GC for more than 10 years."

Long-term observation studies conducted over decades in arctic environments have shown that oil compounds eventually degrade, but Aeppli and his colleagues described themselves as "very surprised" when their analysis of the Deepwater Horizon oil spill revealed substantial degradation of compounds after just 18 months. One of the compound classes investigated, triaromatic steroids, have not been closely investigated before, but it is generally assumed that they are very stable. In fact, says Aeppli, "there are some laboratory studies showing that triaromatic steroids are more stable than hopanes. In contrast, we found that triaromatic steroids degraded quickly and were much less stable than hopanes, which are universally used in the oil spill community as one of the reference biomarkers."

"Our research shows that while biomarker analysis is very powerful in identifying oil (even four years after a spill in a place like the Gulf of Mexico where there are thousands of oil platforms), one has to keep in mind that chemistry and biology are still active, and biodegradation and photooxidation can alter biomarker composition," says Aeppli. The take home message is that the timescale and environment being investigated need to be carefully considered when selecting certain biomarker ratios for fingerprinting oil.

Pushing oil spill forensics even further, Aeppli is using the same GC×GC methods to investigate oil from the Exxon Valdez, which ran aground on a reef in 1989, dumping an estimated 260,000 barrels into Prince William Sound. "It will be very interesting to compare and contrast these two iconic oil spills," Aeppli concludes. *SS/RW*

Reference

1. *Christoph Aeppli et al., "Recalcitrance and Degradation of Petroleum Biomarkers upon Abiotic and Biotic Natural Weathering of Deepwater Horizon Oil", Environ. Sci. Technol., 48 (12), 6726–6734 (2014).*

Bombs Away

A nanowire-based prototype "supersensor" can detect the fingerprint of explosives down to parts per quadrillion

Most current methods for detecting explosives require physical collection of explosive particles, which isn't ideal for real-world applications. As a result, research into devices that can 'sniff out' a wide range of explosives by sampling the air is a hot area. One team based in Israel, headed up by Fernando Patolsky, has developed a detector based on chemically-modified nanodevices that exhibits sensitivity up to four orders of magnitude higher than commonly-used ion mobility spectroscopy (1). The portable platform can apparently detect explosives down to the parts per quadrillion level, including triacetone triperoxide (TATP), which is often used by suicide bombers.

The team created a multiarray of 144 silicon nanowire field effect transistors (FETs) split into eight subarrays. Each subarray comprises 18 FET nanodevices modified with a surface-binding agent. The subarrays are fed by a common integrated microfluidic channel that enables the flow and interaction of all analytes. "Our nanoarray chip allows for the differential yet simultaneous chemical modification of all eight subarrays with multiple surface binding agents," Patolsky explains. "Each explosive species is expected to exhibit a distinctive pattern of interaction, both kinetically and thermodynamically, with the chemically-modified FETs."

"Several improvised explosives display a relatively high volatility and can be found in the air at high concentrations," says Patolsky, "but other explosives such as RDX (C4) and pentaerythritol tetranitrate (PETN) exhibit low volatility and cannot be directly detected by current methods, or even by trained dogs, in air samples. This forces the physical collection of particulates – clearly a huge limitation." By identifying parameters to mathematically differentiate the responses of different explosives and other non-explosive materials – a process Patolsky describes as a 'fingerprinting' – the new platform is apparently sensitive enough to pick out explosives even in heavily contaminated conditions.

There are other potential security applications for the device too, including the detection of bio-threats and toxins. It could also be used to sniff out narcotics or to monitor the environment. But for now, the team will be focusing on field testing the prototype and expanding the detectable explosives library. "We believe the platform will be readily able to detect the most miniscule traces of explosives present in the air around the source without requiring physical contact. It will be relevant in many real-life scenarios," says Patolsky. *SS*

Reference

1. *Lichtenstein et al., "Supersensitive fingerprinting of explosives by chemically modified nanosensors arrays," Nature Communications DOI: 10.1038/ncomms5195 (2014).*

Hair Today, Gone Tomorrow?

Carcinogen levels in hairdressers' blood appear to be linked to number of coloring treatments

In the 1970s, the majority of aromatic amines that were found to cause cancer in animals were phased out of hair dyes by manufacturers. However, the debate over whether hair dyes still contain mutagenic and carcinogenic chemicals continues. Now, researchers from Lund University in Sweden provide evidence that concentrations of ortho-toluidine in the blood of hairdressers increases with the number of treatments given per week using both light-color permanent hair dyes and hair waving treatments (1). The US Environmental Protection Agency has classified ortho-toluidine as a "probable human carcinogen" (2).

The Lund study analyzed blood samples from 295 hairdressers, 32 consumers and 60 control subjects who had not dyed their hair in the past year for eight potentially carcinogenic aromatic amines (ortho-, meta- and para-toluidine; 2-, 3- and 4-ethylaniline; and 2,3- and 3,4-dimethylaniline). To assess long-term exposure, the researchers looked at those aromatic amines that had bound to hemoglobin to form 'adducts,' which have a life span of four months. "We used liquid phase extraction to extract the aromatic amines from the blood to hexane," explains Gabriella Johansson, a doctoral student at Lund University and first author of the study. "The samples were then analyzed using gas chromatography-tandem mass spectrometry. The results obtained in picograms were recalculated into picogram/gram hemoglobin."

A comparison of the adduct concentrations found in hairdressers, consumers and controls samples showed no statistically significant difference for six of the aromatic amines. However, the levels of ortho- and meta-toluidine significantly increased with the weekly number of treatments performed using light hair dye and waving products. According to Johansson, meta-toluidine has been assessed as "not classifiable as carcinogenic to humans," owing to lack of data.

The study included practical advice for hairdressers to help reduce their exposure to ortho-toluidine, and noted that while low levels should not produce a significant risk, any exposure should be considered undesirable. The researchers will follow up on the study by measuring selected aromatic amines in different hair dyes and hair waving products in Sweden so that possible measures can be discussed. *SS*

Reference

1. *G. Johansson et al., "Exposure of Hairdressers to Ortho-and Meta-toluidine in Hair Dyes", Occup. Environ. Med. doi:10.1136/oemed-2013-101960 (2014).*
2. *www.epa.gov/ttn/atw/hlthef/methylan.html#ref6*

September Science in Salzburg

Analytical scientists will descend on Mozart's hometown for two future-facing conferences – ISC 2014 and MSACL 2014 EU.

MSACL 2014 EU

First up, from September 2–5, is the inaugural European Mass Spectrometry: Applications to the Clinical Laboratory (MSACL) conference, which follows on from the success of the US version, which has been held in San Diego since 2008. Aiming to offer a forum to discuss the hot topic of MS implementation in the clinical laboratory, MSACL has drawn an excellent selection of speakers. Here are our highlights:

Direct mass spectrometric characterization of fluids, cells and tissues - the benefits and the price of real-time analysis *(Zoltan Takats, Thursday, 8:15am).*

Metabolic profiling as a tool for investigating diseases of pregnancy *(Elizabeth Want, Thursday, 10:45am).*

Rapid bedside diagnosis tools by coupling of bio-compatible solid phase microextraction (SPME) devices to mass spectrometry *(Janusz Pawliszyn, Thursday, 11:35am).*

MALDI molecular imaging of proteins, metabolites and drugs for preclinical and clinical research *(Axel Walch, Thursday, 3:00pm).*

The impact and potential consequences of machine intelligence on healthcare *(Randall Julian, Friday, 8:15am).*

Towards an LC-MS/MS based clinical-chemical analyzer for small molecules in body fluids *(Karl-Siegfried Boos, Friday, 11:10am).*

Touch spray mass spectrometry (TS-MS) used for rapid diagnosis of kidney and prostate cancer using tissue specimen obtained from surgery *(Kevin Kerian, Friday, 3:50pm).*

For more information, see www.msacl.org.

ISC 2014

Not satisfied with just one analytical conference per month, Salzburg Congress Center welcomes the 30th International Symposium on Chromatography from September 14–18. "The overarching goal of ISC is to promote research and knowledge in separation science in all its broad glory. We will cover the fundamentals and applications, from nano to preparative scale," says co-chair of ISC 2014, Wolfgang Lindner.

It would be a shame to miss the plenary lectures on Sunday evening; Pat Sandra, recipient of the Pregl Medal Award and excellent orator, takes the stage at 5:25pm to highlight advances in biopharmaceutical analysis. Sandra is followed by Jeremy Nicholson, director of the UK's National Phenome Centre, who will discuss how we can meet the analytical challenges of systems medicine and molecular phenotyping.

Here are our other top picks from the scientific program:

Non-traditional format of monoliths for separations of small molecules, peptides, and proteins *(Frantisek Svec, Monday, 10:50am).*

An ion chromatograph for extraterrestrial explorations *(Purnendu Dasgupta, Monday, 11:20am).*

Trends and applications of LC-MS-based methods to assess the exposure to the "mycotoxin cocktail" present in food and feed stuffs *(Rudolf Krska, Tuesday, 9:00am).*

A novel approach to low volume sample preparation *(Tony Edge, Tuesday, 10:10am).*

Plasmid DNA biopharmaceuticals analysis: new media and methods for iosoform and topoisomer separations *(Michael Lämmerhofer, Tuesday, 11:00am).*

Exploring selectivity and enantio resolution of sub 2 µm silica particles modified with vancomycin by nano-LC *(Salvatore Fanali, Wednesday, 9:30am).*

Ionic liquids in separations and MS *(Daniel Armstrong, Wednesday, 3:35pm – see Sitting Down With on page 50).*

Synergy between material science and analytical chemistry - potential of new materials in integrated air sampling and sample pretreatment *(Marja-Liisa Riekkola, Thursday, 10:30am).*

Ion mobility MS with direct surface analysis or LC workflows applied to drug metabolism and metabolomics *(Gérard Hopfgartner, Thursday, 1:05pm).*

For more information, see www.isc2014.at.

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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

Opening Up Environmental Analysis

How microfluidic paper analytical devices will make testing for the presence of toxic chemicals in air or water as simple, ubiquitous and inexpensive as measuring the temperature.

By David Cate, John Volckens, and Charles Henry, Colorado State University, USA.

Airborne particulate matter (PM) is dangerous to humans, and represents a significant source of exposure because of its ubiquity and chemical complexity. Tens of thousands of compounds ranging from relatively harmless (for example, Cl^- and Na^+) to toxic (for example, polyaromatic hydrocarbons) have been identified in PM studies. Airborne metals (for example, copper, chromium, and nickel) are also common in PM and are consistently identified as contributing factors to daily morbidity and mortality. Yet, despite the relatively high rates of such diseases, our inefficient paradigm for assessing exposure has remained relatively unchanged for the past 25 years. We still do not fully understand, mechanistically, how PM (and its chemical constituents) adversely affects the body, nor do we understand which of the thousands of sources of PM should be targeted for reduction to improve health.

A range of problems need to be confronted. Currently, exposure monitoring relies too much on single stationary sites, analytical instrumentation that is restricted to the benchtop, and analytical techniques that are time-intensive and prohibitively expensive for evaluating individual exposures. Moreover, collected samples are rarely analyzed in the field, but instead are shipped to a central laboratory for analysis. The consequence is that the time from sample collection to reporting – or hazard communication – is typically several weeks. On top of that, processing can cost over $100 per sample. There is an urgent need for alternative monitoring solutions that are more affordable, timely, and user-friendly.

Microfluidic paper analytical devices (or µPADs), an old technology repurposed in 2007 by the Whitesides group at Harvard, is making a comeback as a new, low-cost strategy for analytical measurements (see "Using Simplicity": tas.txp.to/0714/simplicity). This new class of sensors is designed for chemical analysis at the point-of-need. These sensors overcome restrictions posed by more expensive and complicated analytical techniques, namely user-friendliness, portability, and expense.

> *"There is an urgent need for alternative monitoring solutions that are more affordable, timely, and user-friendly."*

Instead of relying on active pumping, samples in µPADs are processed passively through capillary action in common filter paper. Channel patterns printed onto paper generate circuits that restrict flow to defined regions, allow chemical reactions to take place in a well-defined manner, and cost pennies per sample. Because of their advantages, µPADs are making waves as an inexpensive alternative to traditional exposure assessment techniques.

Paper-based analytics may challenge the longstanding belief that scientific research must be left to the scientific community, particularly in the field of environmental science. To this end, collaborative efforts are already being aided by volunteers who donate their time and resources to make distributed measurements for large-scale environmental projects. The advent of this 'citizen-science' represents a promising resource for public and environmental health research. Community-based monitoring initiatives are becoming increasingly significant and impactful in the scientific community. For example, the "Click to Cure" program (www.clicktocure.net) uses crowdsourcing to sort through enormous banks of culture slides (viewable online) for cancer cell phenotyping. Another initiative, the Foldit program (http://fold.it/portal/), lets computer gamers (most with no biochemistry background) help design protein markers for viruses. To date, gamers have assisted with the design of thousands of proteins, a feat that has saved researchers precious time and expense.

However, examples of citizen-science projects in the field of public and environmental health are less prevalent, largely due to a lack of sensor technologies that are inexpensive and easy for public use. Thus, the development of new and inexpensive sensors for environmental pollutants has great potential for citizen-science to improve public health through distributed measurements that can lead to further outreach, advocacy, and education.

Eight Tips for Easy GC×GC

Comprehensive two-dimensional gas chromatography is often presented as extremely complicated. It isn't. Here is some simple advice to help attract more users to the benefits

By Jack Cochran, Restek Corporation, Bellefonte, and The Pennsylvania State University, University Park, Pennsylvania, USA.

I love to attend the Multidimensional Chromatography Workshops organized annually in Toronto by Eric Reiner, an early adopter of comprehensive two-dimensional gas chromatography (GC×GC), who works for the Ontario Ministry of the Environment (MOE). The workshop has over 150 attendees, an impressive number given that Eric arranges it by himself and that it takes place in Canada – in January. The meeting features an excellent range of GC×GC presentations, from theory to instrumentation to applications to data processing, in an informal atmosphere that promotes discussion and stimulates collaboration. The audience includes people who have never used GC×GC, but may be considering it to solve their own separation problems, for example, in environmental research where the matrices are complex and the residues are often present at trace levels.

Just over two years ago, while attending Eric's workshop, I was sitting in the back row of the MOE auditorium with a person who pioneered the use of GC×GC with time-of-flight mass spectrometry (ToF MS) for the practical purpose of determining (and discovering) environmental chemicals for human biomonitoring. We listened to one of our colleagues describe the complexity of GC×GC method development in a lecture: two columns, two stationary phases, two lengths, two inner diameters, two film thicknesses, two flow rates, two oven temperatures, variable modulation times and modulator temperatures, two sample loading capacities... Oh, and all of those parameters may be independent of each other… I heard my colleague mutter, "No, no, no", and when I looked over he was shaking his head. I asked him what the problem was and he replied that we – the community – should not present GC×GC as being that complicated to

"We – the community – should not present GC×GC as being that complicated"

those considering whether to employ it (or not) for the first time. I emphatically agree!

In my view, GC×GC needs to be presented as simply as possible to increase the number of practitioners, or more appropriately put, to entice potential users to take advantage of its power for separation problem solving. I'm not talking about a disingenuous representation of GC×GC as a push-button technique. My suggestion is to recommend columns and operating conditions based on sound one-dimensional GC principles. Those principles include efficient carrier gas flow and optimal heating rate as presented by Leon Blumberg and Matthew Klee in several publications (1–3). So, here are eight simple tips on how to start method development for GC×GC-ToF MS, especially when doing broad semi-volatile compound screening work (for example, metabolomics, petroleum-omics, emerging environmental compound work):

1) Use 5% phenyl-type × 50% diphenyl-type or 50% diphenyl-type × 5% phenyl-type stationary phases. Or pick the set that offers the largest spread of compounds in first and second dimensional space.
2) For the dimensions of the first column, use 30m x 0.25mm x 0.25µm (a commonly used GC column in a wide variety of stationary phases). 60m length may be even better since it has higher peak capacity.
3) For the dimensions of the second column, use 0.60m x 0.25mm x 0.25µm. Use a non-restrictor, so that the first dimension separation is two to three times faster than if using 0.10mm. A column of 0.25mm also offers better sample loading capacity versus 0.10mm. If you selected a 60m first dimension column, use 1.3m x 0.25mm x 0.25µm in the second dimension.
4) Use helium as the carrier at a constant flow of 1–2 mL/min for efficient first dimension separation.
5) Use an oven program rate of 10°C / holdup time (minutes) – optimal heating rate maximizes first dimension peak capacity.
6) Use a modulation time of 1-3 sec (maximum). Slice the first dimension peak approximately three times to preserve the first dimension separation.
7) Use a modulator temperature offset of 15°C higher than the second dimension oven temperature offset (think of the modulator as the inlet for second dimension column – the inlet should be hotter).
8) The second dimension oven temperature offset should be 5-10°C or more (control wraparound, as necessary).

Another trick is to use highly selective columns in the first dimension that are known to separate isobaric congeners. Generally, we need selectivity and efficiency to separate structurally similar compounds. The second dimension should be chosen to move interfering matrix compounds out of the way.

I like to call the approach "true peak capacity increase GC×GC". And I often conclude my presentations on the subject with: "If somebody from Oklahoma can do GC×GC, anybody can…" Let's keep GC×GC data acquisition simple and attract more people to the club.

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Diagnosing Malaria Sooner

Our third effort at developing a simple analytical diagnostic for malaria looks promising. It could help prevent the spread of the most devastating disease on the planet.

By Bayden Wood, associate professor, Chemistry Department, Monash University, Victoria, Australia.

Over three billion people are at risk of malaria. In 2012, according to World Health Organization estimates, 207 million people were diagnosed with malaria and 600,000 people died from the disease; the main casualties were children under five and pregnant women.

There is an urgent need for diagnostics to detect the early stages of the parasite; they must be highly

sensitive, cost effective, simple to use, and rugged enough to be transported to remote areas in tropical jungle communities. Current diagnostic tools include optical microscopy, which has a sensitivity of around 40 parasites/μl but which requires an experienced microscopist; monoclonal antibody-based rapid diagnostic tests (RDTs), which are easy to perform but do not quantify parasitemia (parasite load) and take about 20 minutes per test; and polymerase chain reaction (PCR) assays – the current gold standard – which have excellent sensitivity (one parasite/μl) but require expensive technology and reagents – and results take up to two hours to generate.

For a diagnostic technique to be effective it must be able to detect both the immature asexual (ring) stage of the parasite (the only stage that is present in the peripheral circulation in new infections) and the mature sexual stage, which appears later and is the only stage capable of transmission to mosquitoes.

We initially investigated the potential of Raman microspectroscopy in combination with multivariate data analysis (1). The technology showed potential for detecting hemozoin, a by-product of the catabolization of hemoglobin and also known as malaria pigment, but it took several hours to produce results, which is not acceptable.

To accelerate the analysis, we next detected hemozoin in a whole drop of blood. Using an ultrasonic acoustic levitation device we could probe the droplet with a Raman microscope with a right angle lens. An acoustic levitation device consists of a piezo electric transducer and a reflective sound plate, which together generate a standing wave with very stable ultrasonic nodes. A droplet of blood can be placed in one of the central nodes and levitated in air, which has the advantage of concentrating the droplet though evaporation and reducing the attenuation of Raman laser light, as there is no container. This enabled us to record high quality spectra and detect later stage ring-form parasites. However, it was not conducive to routine analysis as the droplets can become unstable after time and explode, and it did not detect the early stage rings found in peripheral blood. But it did demonstrate the ability to investigate a large population of cells with a spectroscopic modality.

"For a diagnostic technique to be effective it must be able to detect both the immature asexual (ring) stage of the parasite and the mature sexual stage"

Building on this, we identified a unique fatty acid signature for each stage of the parasite's life-cycle at the single-cell level using the FTIR microscope on the infrared beamline at the Australian Synchrotron (2). Since a synchrotron clearly cannot be used as a routine clinical tool, we turned to total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. This detected the earliest ring forms of the parasite and the gametocytes by analyzing specific fatty acids associated with the parasite membranes rather than relying on hemozoin. ATR-FTIR in combination with partial least squares (PLS) regression analysis enables parastemia detection down to 0.00001 percent in laboratory-spiked red blood cell samples – an improvement upon the PCR assay. Moreover, the technique is portable and rugged, so it can be placed in a bag and transported to remote jungle communities. It quantifies parastemia and does not require highly trained technicians. Sample preparation involves blood centrifugation, removal of the plasma and white blood cells, and the addition of methanol. A 20 μl aliquot of packed red blood cells is placed onto the ATR-FTIR window and the spectrum recorded in about 20 seconds. The spectrum is run through the PLS algorithm and the diagnosis, including degree of parastemia is determined in seconds (3).

The ability to detect very low levels of parastemia is crucial. People with low levels of malaria parasites often show none of the classic fever symptoms but infect more vulnerable members of their communities via mosquito bites. We will soon conduct a pilot study in Thailand to test the efficacy of the ATR-FTIR approach with clinical patients in remote communities.

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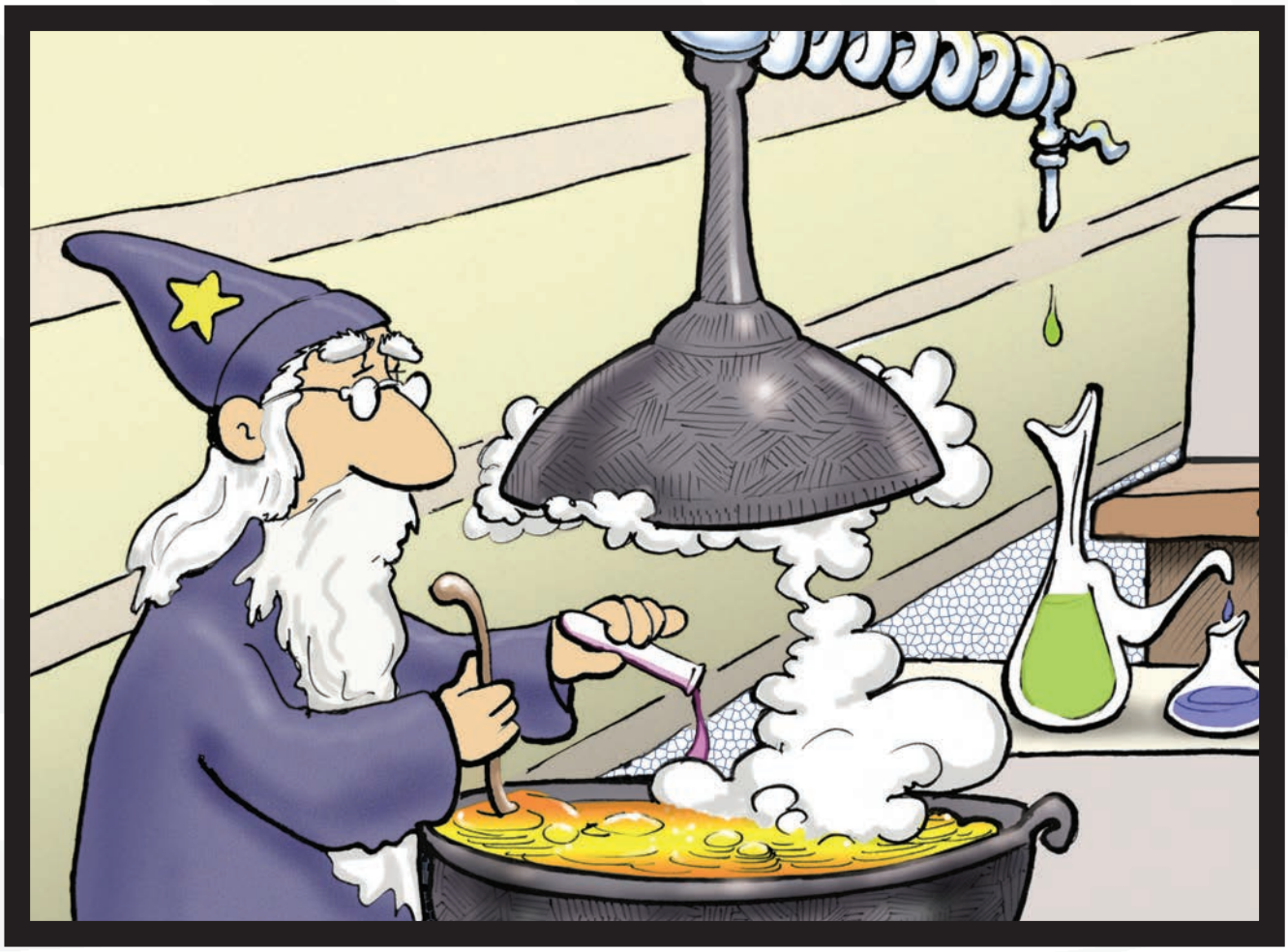

Courtesy of Nick Kim – www.scienceandink.com

Three Wizards* of Sample Preparation

Many sample preparation techniques originated a long, long time ago. Should we be satisfied with old separation magic or does our whole approach need to be re-cast? Here, three wizards of sample prep, Hans-Gerd Janssen, David Benanou, and Frank David, offer their recipe for transforming one of the ugly frogs of analytical science into a handsome prince.

**The spell of artistic license has transformed gurus to wizards, for one issue only.*

The Wizards

Hans-Gerd Janssen is the science leader for compositional analysis at Unilever Research and Development in Vlaardingen, The Netherlands. Janssen's team develops and applies methods for compositional analysis of food samples as well as home and personal care products. To do so, they apply a range of techniques from simple wet-chemical methods to complex instrumental approaches.

David Benanou started working for Veolia nearly 25 years ago, predominantly in research and innovation dedicated to analytical chemistry. Benanou is a specialist in separation techniques and mass spectrometry, as well as sample preparation, where he focuses on micropollutants and organic matter characterization in environmental matrices.

Frank David works at the Research Institute of Chromatography (RIC), the private research lab founded by Pat Sandra, where he is responsible for research and development projects in chemical analysis, including (petro)chemicals, polymers, food, environmental, consumer products and pharmaceuticals.

At Riva 2014 (the 38th ISCC and 11th GCxGC Symposium), Pat Sandra stood on the stage periodically shaking an imaginary separation funnel – much to the amusement of the audience. "I find it just incredible to see what people are doing in application notes," Sandra remarked. "They have the very best instrumentation, for example to determine the composition of drinking water at the sub-parts per trillion level with triple quadrupole mass specs and gas chromatography. But do you know what their sample preparation is? They take one liter of water, add 100 ml of dichloromethane, shake it for half and hour. Then they put the dichloromethane in a vial, evaporate it down to 0.5 ml – don't worry, it's just going into the air – and then they inject. We need to do something about that…"

While the delivery was humorous, it had a serious underlying message: the analytical community needs to rethink sample preparation. Using Sandra's provocative lecture as a springboard, we invited three experts to share their views and discuss the current state of the art.

What role does sample prep play in your work?

David Benanou: Analytical chemistry is a multistep endeavor: measurement is the final link at the end of a chain of operations that begins with sample prep. Sampling and sample preparation are therefore essential processes that underlie all subsequent work and impart relevance to what would otherwise be a meaningless exercise.

In all of the diverse forms of analysis, sample preparation is essential. This is especially true of our activities, whatever the environmental matrices considered and even if our goal is miniaturization or the use of green techniques without solvents.

Hans-Gerd Janssen: In food analysis, sample preparation is crucial. All foods contain high levels of lipids and proteins, and these two compound groups interfere in analyses. Moreover, food products are extremely complex and the spectrum of analytical questions is very diverse: for example, today, we might have to analyze the hydrogen content of an acid product packaged using aluminum foil; tomorrow, the target compounds might be protein aggregates.

Frank David: To give you a sense of its importance, our company offers analytical services in many different application areas – from petrochemicals to pharmaceuticals – and in many of the challenges we face, the development or optimization of the sample prep is an essential part of the solution.

How has sample prep changed over the past 20 years?

HGJ: Well, I can say what certainly has not changed: its importance. Modern chromatography and MS instruments are slightly more selective, and so able to analyze slightly more complex samples, but they have also become more vulnerable to 'dirt,' by which I mean compounds that are not of interest and contaminate the system. I would say that sample preparation is now more universal – think about QuEChERS or normal phase liquid chromatography as sample prep methods. There are also now better options for automation.

FD: For me, there have been three major trends: miniaturization, automation, and higher throughput. Miniaturization can also help make sample prep "greener" through solvent reduction or even elimination.

It is important to recognize that sample prep is part of the whole analytical workflow. The (r)evolution in mass spectrometry and the availability of more sensitive detection (for example, triple quadrupole MS in MRM mode) has allowed us to optimize and reduce complex sample prep procedures. This resulted in smaller sample sizes, and/or elimination of selective fractionation or clean-up, while maintaining or even improving sensitivity.

Moreover, in several application fields, such as environmental and food analysis, customers are seeking "generic" or universal methods that allow coverage of a wider range of compounds, as Hans-Gerd also mentioned. This trend encourages the development of sample prep methods that should be less selective.

DB: I offer two answers. One is that in many cases sample prep unfortunately remains the same old, time-consuming, expensive and boring technique that it has always been. The other, from an innovation point of view, is that we've moved from liquid-liquid extraction (LLE) and solid phase extraction (SPE) to solid phase micro extraction (SPME), micro LLE with large volume injection (LVI), PDMS-based enrichment, and so on. Following the increase in sensitivity of analytical systems, sample preparation has the same importance but can be miniaturized or adapted to be greener. Such approaches are typically well accepted by 'clever' analysts.

From a standardization point of view, the techniques do not seem to have changed at all. Take any European country or any standard (AFNOR, CEN, DIN), and you'll see that instead of pushing for innovation, such consortiums are happy to remain where they were 20 years ago, imposing ridiculous – and non-exhaustive – techniques on routine labs.

How important is sample preparation in your lab?

FD: As our activities include the development of methods for different industries, sample prep is of the utmost importance in our lab and receives appropriate attention. Our efforts focus on the three trends mentioned earlier but one should always keep the final goal of the analytical method in mind. Whether that is quantification of major solutes, impurity detection, or trace analysis, different goals require different solutions with different sample preparation approaches.

DB: Sample prep is not just important for my company, it is essential! In my field – micropollutant characterization and quantification at the sub-nanogram level – good sample prep is the only way to obtain the best and most precise results possible.

For the past 14 years, I have used (and promoted) a green and sensitive enrichment technique called stir bar sorptive extraction (SBSE) also known as 'Twister.' We have decreased our solvent consumption by around 800 percent using Twister.

HGJ: Without sample preparation, trace analysis is not possible because fats and proteins immediately contaminate your system. Moreover, many compounds are present in a food product. For a detailed understanding of the quality and safety of the food, analysis of all these compounds is relevant.

"It is clear that errors made in sampling, sample preparation or sample introduction (injection) cannot be corrected by using even the most advanced MS systems."

Pat Sandra ironically stated, "I don't know what's going on, but apparently, we are the only group who still have a problem with sample preparation and injection." Is sample preparation underestimated and, if so, why?

HGJ: Sample prep is not really sexy. It does not use expensive, shiny instruments, and there is no theory to it. And, in fairness, it is not always important, if you have just a few samples to analyze. Modern LC, GC and MS instruments can cope with a bit of dirt… I suppose that academic research into sample prep methods is not very rewarding and does not receive the attention it deserves as there is no perceived need. However, 'real' users know its importance – and have problems with it!

DB: Sample prep simply must remain on everyone's radar. I would suggest that if sample prep is underestimated, then it is the manufacturers that could be blamed or thanked, depending on your point of view. Vendors push the limit of sensitivity in order to avoid sample prep and allow direct injection of the sample or extract. Indeed, that is often the way new systems are promoted.

FD: Pat's statement was indeed made ironically. Lots of attention is paid to high-end mass spectrometry and comprehensive techniques, and it could be concluded that sample preparation is simply not important anymore. However, we are convinced that "high-end GC-MS systems" (that is to say, multi-dimensional GC coupled to high resolution QToF MS) should be paired with high-end sample prep systems. It is clear that errors made in sampling, sample preparation or sample introduction (injection) cannot be corrected by using even the most advanced MS systems.

Sample prep issues appear not to be recognized or communicated, what's the problem?

DB: It may be a controversial view but I believe that minimizing the need for sample preparation is seen as a positive by manufacturers as it enables them to sell the latest innovations in other areas with "no sample prep needed"! Unfortunately, the vendors are often more interested in selling systems than promoting real analytical chemistry.

FD: There are several issues. The fundamental one is the growing gaps between the academic world, the instrument manufacturers, and the end-users. Academic research is predominantly driven by the need for research results to be publishable, instrument companies are focused on best-in-class equipment, and industry is seeking productivity, which I define as robust solutions that give the correct answer to an analytical question in an appropriate time. These three drivers are not always in sync and are sometimes totally at odds. It is possible to attend a single session at an international symposium that begins with an academic presentation describing a new concept in comprehensive GC, continues with a company presentation of a new mass spectrometer with femtomole sensitivity, and concludes with an industry presentation that describes real issues with a simple GC-FID analysis…

Often, problems in the field relate to sample preparation, or incompatibility between some aspect of the sample – such as the matrix, solutes or concentration of solutes in the matrix – and the applied injection, separation or detection method. This can simply be down to a lack of user expertise: an example would be an effort in pesticide analysis to look for all pesticides in all foods using a single extraction method. The extracts will contain lots of matrix compounds and this will inevitably impact on productivity because even the most

expensive instrument can be contaminated and need cleaning offline. Is this of concern to the instrument vendor who is mainly interested in demonstrating the perfect performance of a brand new instrument using a standard solution or to the academic researcher looking for publications and not long-term testing of method performance? No!

This issue is not new. It was raised more than 10 years ago by Konrad Grob (Kantonales Laboratory, Zurich) during one of his provocative lectures.

HGJ: I agree somewhat with Frank – I would wager that only those who must analyze large series of samples know how relevant and difficult sample preparation is. Academic technique developers who analyze five samples to demonstrate their new method are unlikely to see the need. And, as I indicated earlier, obtaining funding for research into sample preparation is probably very difficult.

Sandra said that lab managers underestimate the importance of good sample prep: "Want to buy a €350,000 GCxGC QToF system? You have it, no problem. Want to spend €20,000 for sample preparation? No. That's too much." What are your thoughts?

HGJ: I don't think this remark is true – at least not in the food and pharma industry. Industry assesses cost of analysis and divides that into instrument costs and operator time. It is therefore easy to calculate the need for investment and the expected cost reductions of such an investment. In the end, it is all about the return on investment. If that number is okay, there is probably money.

DB: I partially agree; often lab managers believe that by spending a huge amount of money he or she will be the owner of a magic box that will avoid sample prep altogether. However, I can say that at Veolia we all push for sample preparation, whether in research or routine labs.

FD: Pat's statement is in-line with the observation that sample prep has not received much attention in the last 20 years. And the growing distance between academia, instrument suppliers and the end-user doesn't help. However, in the end, an appropriate analytical solution should solve the analytical problem at hand, and should include automation where possible.

What is hindering uptake of more advanced or automated sample prep methods?

FD: Over the past few decades, several sample preparation methods have been developed, some of which can be automated. David has already given the examples of SPME and SBSE, to which I would add dynamic headspace (DHS). In fact, great solutions are available from several vendors. I think uptake by routine labs is often hindered by a combination of internal conservatism (both from management and in the lab), external conservatism in the form of accreditation bodies sticking to their comfort zone, a lack of expertise in the lab, and some 'lay-back-and-relax' mentality.

HGJ: Method development in sample preparation is difficult. And during the development, the performance of a certain parameter set is difficult to quantify. Moreover, even after proper method development and validation, sample preparation methods are unfortunately not often rugged. Nominally identical samples behave differently due to variable water levels, particle sizes, and so on. Finally, I repeat: sample preparation is not sexy. There are fewer shiny expensive instruments, just cheap plastic tubes and messy laboratory benches…

DB: Old-fashioned techniques, such as LLE, are standard. As Hans-Gerd indicates, a new technique needs to be validated and it takes time and money to prove that it gives the same or better results than the 'traditional' method. I also believe that a strong and widespread effort to lobby for and promote clean, easy and green techniques is lacking.

Are there clear environmental or 'green' gains to be made?

HGJ: Absolutely. There is no longer any need to shake large sample sizes with huge volumes of solvent. Sample size and solvent volumes can be minimized. Large volume injection is a good tool here as well. And, of course, instruments have become

"The lack of education and expertise is not only at laboratory level, but also at management level. There is a need for training and the availability of correct, unbiased information."

more sensitive so that less evaporative preconcentration steps are needed. However, there is a limit to downscaling – after all, at some point it will no longer be possible to obtain a representative sample.

DB: I totally agree and am currently applying and spreading this philosophy throughout Veolia labs all over the world. Just consider the situation where we are trying to quantify a few nanograms of toxic compounds in water with hundreds of milliliters of toxic solvent. It is total nonsense! Many systems can benefit from LVI or high-speed chromatography – do customers not know that? By applying these two techniques we can make the dramatic switch from old-fashioned LE to micro LLE.

FD: Modern sample preparation should indeed include miniaturization, but with respect to the minimum sample size – as Hans-Gerd noted – to ensure representative sampling. In fact, miniaturization is one of the key drivers towards "greener" technologies.

Lastly, where do you see sample prep going in the next few years?

DB: I think progress can be made through lobbying and the presence of sample prep ambassadors in the various standardization consortiums. But it will take time and money.

Many teams are developing new concepts for sample prep, but it will take a decade or more before they are accepted as convention. The importance of sample prep should and must become more prominent in universities. That way, the next generation of research scientists and lab managers will have better instincts for green sample prep.

HGJ: In industry and quality control laboratories, sample preparation will get the attention it deserves. Quite simply, it is needed – there can be no analysis without sample preparation. Will it get the attention it deserves in academia? Perhaps in the applied analytical groups – again because they need it! However, I don't think we will see revolutionary new methods, just evolution of existing technologies. Will it get lots of attention in academic groups that focus on technique development? I doubt it. Despite more research being needed, this is not an area where research grants are easily obtained. There are no expensive instruments, no theories, just hard work.

I guess the short answer is that we need to raise awareness that, in real life, sample preparation definitely is an issue. Academia often presents new methods that work once or a few times, but are useless for routine use.

FD: There is no universal solution nor a small set of "tools" that can handle all sample types for all problems. In my opinion, the bottleneck is not the availability of methods and equipment, but rather education. The lack of education and expertise is not only at laboratory level, but also at management level. There is a need for training and the availability of correct, unbiased information. International meetings should play an important role; unfortunately, most have "fully-packed" programs with parallel sessions and without ample time for discussion or critical evaluation of presented results…

To conclude somewhat ironically, like Pat Sandra, I find it a little strange, indeed unacceptable, that papers are still being published that show amazing data concerning sensitivity but rely on sample preparation methods based on liquid-liquid extraction of a 1L water sample with 100 mL dichloromethane, followed by evaporative concentration to 100 µL and injection of 1 µL. Maybe Denis Desty's 50-year-old "hammer injection method" should be applied here…

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The New 2D-LC Kids on the Block

Concluding our series on two-dimensional liquid chromatography, we talk with a pair of relative newcomers who relate their experiences to date and discuss how they plan to take advantage of the technique in the future.

The metabolomics master

Bernd Kammerer heads the metabolomics facility at the Center for Biosystems Analysis (ZBSA), University of Freiburg, Germany, the purpose of which is to identify and quantify the full range of metabolites in biological systems. Bernd has broad scientific experience in metabolite and metabolome analysis, particularly with MS and NMR methods, and is familiar with bioinformatic methods for cluster analysis and metabolomic data mining.

What are the specific analytical needs of metabolomics?

Well, the complexity and high dynamic range of metabolite concentrations pose tough challenges for qualitative and quantitative analysis. Typically, the target compounds are small molecules with masses ranging between 100 and 1000 Da. These are studies in a wide variety of biological matrices, such as cell culture samples, urine and blood. On the one hand, you need precise MS analysis; on the other hand, a highly effective chromatographic separation is indispensible.

How has your view of 2D-LC changed over the last decade?

Ten years ago, 2D-LC was accomplished mainly by coupling the first and second dimensions offline. This provided fantastic separation power but it was very time-consuming, something that has been solved by modern 2D-LC systems. Today, it is possible to perform comprehensive 2D-LC without loss of time and with fewer potential sources of error.

What problem were you addressing when you first considered 2D-LC?

A major research project for us is the identification of a metabolic signature for early detection of human breast cancer. To do this, we are analyzing different biological samples with a high degree of complexity. Our target substances – modified nucleosides and ribosyl derivatives – differ only slightly in terms of their chemical structure and, therefore, retention in LC. Consequently, we decided to lift the chromatography to the next level, that is, to two-dimensional separation.

Metabolomics generally deals with complex matrices containing several hundred compounds, so separation power is essential. Insufficient separation can lead to the formation of highly reactive radical cations that interact with each other in the ion source (ESI or APCI) before entering the MS, causing ion suppression and artefacts. Since we are often looking to distinguish between two different biological states, it is important to improve the (semi-)quantitative analysis of target compounds, something that has particular relevance to ion suppression. An additional advantage of increased separation power is the possibility of determining previously hidden metabolites.

What were your expectations of 2D-LC?

Two things really. The structural class we deal with is differentially modified nucleosides, and we expected to achieve a better differentiation between isomers and nucleosides that could hardly be separated at all using a single column. And we were hoping for purification of peaks in the second dimension separation to help limit background noise.

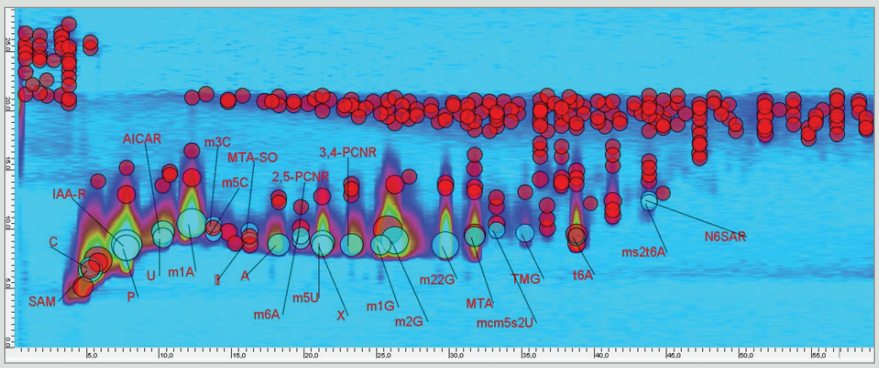

Figure 1. Detection of modified nucleoside in SPE-purified urinary samples by 2D-LC-QToF MS

What has been your experience so far?

We have just started with the new technique and are in the process of method optimization. Choosing the right separation mechanism is the first step of method development. Column dimensions, solvents and elution gradients all offer many possibilities to improve your analysis and different combinations of column materials lead to striking differences in orthogonality, retention and peak capacity. Our 2D-LC can be used as a standalone solution with a diode array detector, but it can also be coupled to different mass spectrometers. This high degree of functionality and flexibility is important for studies of complex biochemical pathways.

Currently, we are looking forward to the first comprehensive measurements from a large batch of real life samples. The first results look very promising (see Figure 1).

Do you anticipate 2D-LC being adopted more fully in metabolomics?

Yes. The importance of comprehensive 2D-LC will grow rapidly because of its universal applicability. I expect that 2D-LC in combination with different ion sources and MS solutions will become essential in applications of analytical chemistry, especially in research that must cope with complex matrices and/or complex analyte spectra.

How will you use 2D-LC in the near future?

We are planning to combine our 2D-LC solution with different mass spectrometers to address a range of sample and chromatographic challenges. Optimization of the chromatographic methods for the particular challenge at hand is clearly important here. In particular, for the separation of structural isomers, which occur frequently in modified nucleosides, I can see 2D-LC opening up new vistas.

The impurity analyst

Ole Gron has been in the pharmaceutical industry for over 10 years after finding his way into separation science via spectroscopy. Ole now works out of Vertex's San Diego R&D site in the analytical development department, which offers support functions from the lead generation stage of drug discovery right through to clinical trials.

How long have you been using 2D-LC?

We've been evaluating the technique for two years to see if it's something that Vertex wants to adopt at a larger scale.

What specific challenge prompted you to look at 2D-LC?

To be honest, when I first heard about 2D-LC it sounded like an interesting novelty, but I didn't see a real need in my environment – after all, we don't suffer from the overcrowded chromatograms seen in other fields. However, commercialization made us consider it more seriously, as it meant that we could test the technique without wasting time building a system that would be robust enough. Our major need is in impurity analysis; although our chromatograms are not overcrowded, we can have structurally related impurities that co-elute. We often run two separate but orthogonal LC methods to give us increased confidence; basically, we want to see as much as we can, as early as we can. We wondered if we could couple those two 1D runs into a single 2D-LC method.

How easy or hard have you found 2D-LC?

Of course, there is a learning curve – and there are a number of parameters that need to be considered carefully. However, after getting used to the setup, running the system is relatively easy. More importantly, I feel that I can trust a commercial system to provide the same result time after time. Robustness is important.

How are you using your 2D-LC system now?

I've been using multiple heartcuts to assess each impurity peak in my first dimension separation. Right now, I've got my system set up to store each impurity using 'peak parking', which gives me longer run time in the second dimension.

How fast do you think 2D-LC will be adopted by the pharmaceutical industry?

I don't think we'll see an explosive uptake; the pharmaceutical industry is pretty conservative when it comes to adopting new techniques, so it will take time. However, it's hard to go back once you've tried something superior. I've been assessing 2D-LC in a number of different applications and I can imagine wider adoption within the next year or two at Vertex.

You can access the whole "Demystifying 2D-LC" series online:
Embracing the Second Dimension: tas.txp.to/0714/2dlc01
Exploring Chinese Medicine with 2D-LC: tas.txp.to/0714/2dlc02
Two-dimensional Bioanalysis: tas.txp.to/0714/2dlc03
Harnessing 2D-LC for Big Pharma: tas.txp.to/0714/2dlc04

A Vision of Our Mobile Future

How smartphone power, coupled with the scale of its adoption globally, offers a compelling platform for analytics and diagnostics – and a chance to level the playing field for researchers in resource-poor countries.

By Aydogan Ozcan

Smartphones represent an enormous opportunity for the creation of field-portable, compact and cost effective analytical instrumentation of the type that you would normally find only in laboratories or hospitals. Such applications have the potential to tackle the lack of analytical and diagnostic capability in certain parts of the world or in field settings. The absence of such services is not just because advanced instruments are very expensive; beyond the initial expense, there is also the requirement for solid infrastructure, which, in developing countries, is often lacking.

Big numbers and big challenges

No-one could have predicted the current status of mobile telecommunications 10 or 15 years ago. The numbers are simply staggering: fifteen billion cell phones have been sold and there are currently seven billion cell phone subscribers worldwide, more than 75 percent of whom are in developing countries, despite a lack of basic infrastructure – or even roads in some cases. In such countries, cell phones are the most advanced technology that you will find; phone towers, communication networks, and mobile power stations for charging cell phones appear to have found their way into every corner of the globe.

Cell phones are extremely cost effective. The sheer economy of scale and fight for market share have driven unprecedented strides in technological advancement and capability at amazingly low cost. Let me illustrate exactly how cheap this technology has become: if you were to somehow magically remove three zeros from either the number of cell phones sold or the number of subscribers (that is to say, replace billion with million), the cell phone in your pocket would cost you the same amount as a high end car.

The megapixel count of cell phones has been doubling every two years for the last 10 years (from 0.2 to 40 megapixels). So, if, like me, you're a researcher who's interested in developing portable high-end microscopes, the constant improvement in cell phone performance offers regular opportunities to push for more and more functionality. We can now routinely see single viruses and sub-100 nanometer florescent particles using cell phones. Admittedly, these cell phones are very high end, but they have enabled us to expand the boundaries of mobile imaging, sensing and diagnostics. Virus imaging is no simple task so it is a real milestone that proves the worth of our approach and the potential that the technology has in other areas, such as environmental monitoring and materials science.

One of the next steps is commercialization and deployment of existing instruments and designs, and it's already happening to a degree. There are commercially available applications and hardware to convert cell phones into laboratory instruments. For example, I co-founded a company called Holomic LLC (www.holomic.com, see sidebar: Introducing Holomic), which develops devices to image and quantify lateral flow immunochromatographic assays. Such cell phone-based systems can quantify analytes at concentrations in the parts-per-million or even parts-per-billion range, depending on the test of interest.

Once this and other devices gain regulatory approval, it's not hard to imagine the rapid rise of "off-the-shelf" consumer products for a number of different applications from health monitoring to food analysis.

Ironically, one of the biggest barriers to the development of cell phone-based technologies is the very fast rate at which cell phones are evolving in terms of the hardware and software that they use. This is, of course, at the heart of the business model for providers and carriers. In diagnostic

Introducing Holomic

Holomic is a spin-out from UCLA that has licensed more than 15 intellectual property (IP) applications created by my lab. It has funding from the US government in the form of small business initiatives from the National Institutes of Health, NASA and the Department of Defense (Army), along with some private funding. Holomic's first product was introduced in 2011, and it will hopefully gain FDA approval by the end of 2014.

The company's main mission is mobile microanalysis. We aim to provide the complete readout solution for all diagnostic tests available, whether colorimetric or fluorometric. We have created an imaging platform that universally accepts all diagnostic tests, automatically recognizing and reading them. This functionality enables us to work with many other companies that are developing diagnostic tests. At the same time, we also provide the server end, so that when the user creates an image and diagnostic report, we offer extra analytics and mapping of the data. Essentially, we are positioning Holomic as a digital provider of field-portable, high quality data analytics for all available clinical tests.

Holomic also has an interest in microscopy and imaging. We have created a unique field-portable microscope, which may be useful in direct discovery or in imaging microarray plates among various other specimens. We are targeting mobile health, telemedicine and the research field as a whole with these high-end computational imagers.

applications, however, stability is a major requirement. If we wanted to develop an application for, say, the Samsung Galaxy S4, we need to know that it would still be available in its current guise for at least the next five years. This time is required to develop, test, gain regulatory approval and market our application while users still have access to the relevant phone model. However, the Galaxy S range is likely to evolve significantly over just the next two years – the S5 is already on shelves – and there is no real end in sight to this marketing strategy.

There is an old saying that "every challenge is an opportunity." New ventures could take advantage by taking control of the billions of used handsets and smart devices, communicating with the industry, discovering its needs, and offering a regulated supply chain to ensure that biomedical device manufacturers have access to the smartphones they need. Forget recycling – how about diagnostic upcycling? In this way, used phones become the hearts and brains of new portable analytical systems rather than add-on devices being made available to consumers.

Another solution to the problem could present itself, if whispers in certain circles about phone modularization come to fruition: imagine that rather than constantly changing phones, we could simply upgrade or change modules within an endoskeleton. Google has already staked out this potentially fertile field with Project Ara (www.projectara.com) – a forum that aims to bring together module developers with that exact aim. Clearly, from a diagnostic device point of view, this would be a very positive development.

Big data and big players

One of the reasons that mobile health tools are better than laboratory-based instruments that perform the same tasks is in the collection and use of data: mobile tools are inherently connected. The wireless connectivity of cell phones coupled with smart and secure servers means that rather than working with a single disconnected instrument or sensor, an entire network of instruments from all over the world can be accessed. Reference libraries would virtually self-assemble and databases would get richer and richer, enabling increasingly sophisticated analysis, such as the self-classification of images or signals, and automatic flagging of risk signatures.

There is a nonlinear threshold beyond which machine learning becomes very powerful – something that Google has taught the entire world. To breach that threshold requires progress on both the technology side and in terms of deployment. By bringing analysis to the masses at a fraction of the cost and by stabilizing the technology, the output of big data (and the analytics and machine learning that will result) will benefit not only the users, but also those who collate information for large-scale studies to discover wider patterns and trends. The new opportunities presented by such large amounts of networked analytical data and the potential size of the overall impact is hard to exactly predict right now. But perhaps a simplistic musical analogy is in the difference between only being able to access your own CD collection in the 1990s to having the ability to listen to almost any song ever recorded today…

"There is a nonlinear threshold beyond which machine learning becomes very powerful – something that Google has taught the entire world. To breach that threshold requires progress on both the technology side and in terms of deployment."

Google, Apple, Samsung (and others) are all building collaborations in the medical diagnostics area and working on products and business models behind closed doors. They have the cash and the muscle to make waves, and the outcome may look like the phone market: who's winning or losing at any given time will depend on the user interface, the relevance of the data captured, the level of integration into the consumer's life, and the "coolness" factor. If you remember when the iPhone was introduced, it was a different kind of phone and a different way of interfacing with a computing device – and that lit the fuse for an explosion of innovation.

I anticipate a fragmented market, which means that we will see leapfrog advances from these giant companies driven by the desire to be the first to present the next 'big thing'. The next couple of decades will be a frantic struggle to be increasingly involved in the consumer's daily life and routine

"The idea that we could replace people with gadgets and algorithms is a dangerous and misleading one that goes against the fundamental and centuries-old philosophy of medicine"

– to the point of monitoring the bodily fluids as well as biochemical and physical signals that we leak over the course of the day – and making insightful and actionable 'sense' out of the resulting data.

Where is health care in all this?

Our technological future should not strive to replace health care professionals. Rather, it should improve their performance by providing better, faster diagnostics and more in-depth patient data. Mobile diagnostics will simply bring in new complementary tools for the medicine of the future, driving us closer towards preventative health care.

The idea that we could replace people with gadgets and algorithms is a dangerous and misleading one that goes against the fundamental and centuries-old philosophy of medicine, which is all about "feeling empathy for the patient". We have to be very aware of our continued need for the human touch. I certainly don't want to live in a world where we replace doctors and other healthcare providers "entirely" with robots, no matter how advanced artificial intelligence and machine learning becomes. But surely such a view is not in conflict with the fact that health care delivery can be significantly improved with technology and new instruments that assist professionals with their medical practice.

Certainly, regulatory agencies will be strict with new diagnostic devices. Where there have been attempts to skirt around the rules, the US Food and Drug Administration (FDA) has been quick to point out the requirements. I don't think the FDA is going to fight against change, rather they will continue to set and monitor appropriate safety and performance standards. In the case of Holomic's platform, which is actually a diagnostic reader for many kinds of tests, the approval process for the cell phone-based system as a whole will be much shorter because similar (non-cell phone-based) systems already exist, which allows us to go down the 510(k) route of proving equivalent performance to a validated bench-top instrument.

Getting on board

The automation of signal reading is a no-brainer; it makes tests more robust, improving accuracy, sensitivity and repeatability. The cell phone provides everything needed for automated reading of a signal or image: an advanced camera for imaging, powerful processing capabilities for computational tasks, and a high-resolution screen to display data, all within a compact package. Even though not every application will make full use of all these abilities of the cell phone, anyone interested in developing field-portable devices who fails to utilize these advantages will quickly fall behind. Or at the very least they will find it extremely costly to improve specifications at the same rate as cell phone technology, which is simply not sustainable in the long run: how can a small biotech company compete with Samsung or Apple on those terms? Instead of competing with emerging consumer devices, we must accept them and leverage their power for our own applications.

Using the power of consumer electronics to bring the advanced functions normally found in a hospital or laboratory into field settings empowers applications in a whole range of areas, from environmental monitoring to material science to health care in developing countries. It also helps build research capacity in developing countries. Insufficient infrastructure and/or funding can make it impossible to buy and/or maintain expensive laboratory instrumentation or perform some research; however the innovation landscape generated by the coupling of consumer electronics with diagnostic tools changes the dynamic. Through democratization of measurement toolsets using mobile phones and other ubiquitous and cost-effective devices and interfaces, researchers in developing countries will be capable of generating high-quality scientific output, matching that of their colleagues in developed countries. Not only that; mobile analytics will also have a big impact on the democratization of science in general. Right now, the research world is highly polarized in terms of output: there is a close correlation between a country's GDP and the number of papers published.

In education, the same holds true. But the recycling of cell phones or their components to make innovative, high-

end analytical devices will boost science and engineering education. Hands-on education experience is very important, especially for science, technology and engineering fields; it enables skills in solving problems, the testing of hypotheses, and prompts students to ask the right questions. In developing countries, where even basic instrumentation is lacking, education suffers. And, in fact, it's unlikely, even in developed countries, that we would happily use a $50,000–$100,000 microscope to show a kid what a HIV virus looks like; however, now we can use a phone that costs less than $500 to do the same thing. That's a game changer.

The term "citizen science" is a little fuzzy – but it certainly hints at another facet of the current direction of innovation. Acquisition of high-quality data from large numbers of cell phones or other consumer electronics devices all over the world will enable us to discover patterns and trends that would be impossible to find otherwise.

To conclude, various benefits of mobile phone-based diagnostics, for example, improved implementation of health care and more widespread environmental monitoring, are immediately obvious. The slow-burning transformation in the behavior of researchers and educators in resource poor countries is less obvious – but it too is almost inevitable.

Aydogan Ozcan is the Chancellor's Professor at the Departments of Electrical Engineering and Bioengineering, University of California, Los Angeles, USA and founder of Holomic LLC.

Keeping Ahead in Life Science

Fresh from the excitement of ASMS 2014 in Baltimore, Ken Miller, VP of Marketing for Life Sciences Mass Spectrometry, provides a snapshot of Thermo Fisher Scientific's ambitions.

ASMS is clearly a big show for you…

Definitely. It's hard to directly measure the impact of ASMS but, beyond the product launches, it is very much about educating our customers and giving them an exemplary and highly memorable experience. This year, we had a live speed painter and turned our hospitality suite into something we called "Club Quan" – complete with robot DJ – in the evenings; it was pretty amazing. We hope it allowed us to truly show our appreciation to our customers.

What were the main messages at ASMS?

Firstly, having launched a number of new products to "transform science" last year, we wanted to prove that we'd delivered on the promises we made back then and share progress in terms of new features and applications.

Secondly, software was a big point of emphasis for us this year. We introduced some really powerful new workflows for small molecule and protein discovery. In particular, we were incredibly excited to announce the new PepFinder software. The biopharma market has really taken off. It's a huge opportunity for us as we can leverage our leadership in protein analysis and characterization. PepFinder allows detailed, quantitative characterization of protein drugs and appears to be the right software at the right time. We've been staggered by what it can do as well as the reception it has received. It created a big buzz at ASMS.

Finally, we were very proud to launch the Q Exactive HF, which is really all about productivity, particularly in proteomics.

How does the Q Exactive HF contribute to the advancement of proteomics?

This is the latest chapter in the Orbitrap story. It builds on our Q Exactive Plus platform by combining it with an ultra-high field Orbitrap mass analyzer. Essentially, the Q Exactive HF doubles the spectra acquisition rate, which means comparable results (to the Q Exactive) in about half the time – or twice the resolution in the same run time.

As Ian Jardine noted in his feature last month (see tas.txp.to/0714/jardine), in the early days, the ion trap coupled with John Yate's SEQUEST was a great starting point for proteomics. He described the move away from the linear ion trap/Fourier transform (FT) MS instrument as a gamble. And it's true; we knew the Orbitrap would harm our FT business, but the technology was so compelling that many of us had no doubts about moving forward. Sure enough, within two years, there had been an almost complete shift from what had been a very robust FT business to Orbitrap. But I guess it's better to eat your own lunch than have someone eat it for you…

What do you see as the near future of proteomics?

Proteomics can revolutionize patient health care. Marker discovery and clinical research requires the analysis of samples from many patients – and that's been very difficult to do from a proteomics perspective. As a comparison, next-gen systems allow whole-genome sequencing in about a day and costs have been falling dramatically. Up until recently, there has been no good way to address large studies at the proteome level; protein analysis typically required fractionation and very long LC-MS runs which could take days or weeks per sample. Methods have improved dramatically with faster instruments and multiplexing technology to a point where, for the first time, it's practical to think about proteomics playing an important role in large-scale biological or medical research studies, something that has simply been too labour-intensive and way too expensive to contemplate before. With workload and costs starting to come down, it's not hard to imagine personal proteomics beginning to play a role in routine health monitoring.

You seem very focused on the clinic…

That should come as no surprise as our corporate mission statement is to enable our customers to make the world healthier, cleaner and safer. We on the staff derive a great deal of pleasure from seeing evidence of that on a daily basis.

What few people realize is that Thermo Fisher Scientific is the fifth largest clinical company on the planet, making all kinds of reagents, kits, diagnostics and so on. For me, it's exciting to be working for a

company that can now add proteomic screening and genome-based clinical tests to this portfolio. The Life Technologies acquisition is clearly part of that ambition.

I see the life sciences as a vast continuum, starting with research, for example, in proteomics, metabolomics and lipodomics, to understand biology and identify potential markers of disease, health, toxicity or drug efficacy. That research must then be translated into specific assays or platforms that clinics and pharma companies can use to analyze patient samples and deliver improved health care. We aim to facilitate that entire translational journey from discovery to reliable diagnostics – a total analytical ecosystem.

Such a strong influence by a single company could be considered dangerous, does that concern you?

I see your point, but you have to consider that there is plenty of competition. Competitive research technology, such as QToF MS, is out there and constantly improving. It forces us to keep pushing forward. And while our competitors complicate our lives, we know that ultimately it's a good thing. It keeps us on our toes. We all have to fight to stay competitive. We have a lot of internal capability, but we also work closely with a handful of core collaborators, giving them full access to our technologies, so that they become true partners in the development process.

One great example of successful collaboration is our work with Amgen and one of their scientists, Zhongqi Zhang, who spent over 15 years developing the software that became the basis for PepFinder 1.0. At first, we wondered why Amgen would want to license its own software to us, but it became clear that, from a regulatory perspective, there are distinct advantages to moving everyone towards an industry standard. Driving science forward is a team effort.

What are your thoughts on data-independent acquisition (DIA)?

There's a real divide in MS applications. In targeted analysis, you know what you're looking for and the goal is to quantify as accurately, robustly and inexpensively as possible. In discovery, you need comprehensive analysis to reveal as much about your sample as possible by using fastest, highest resolution MS systems. Then there's a big grey area in the middle. In an ideal world, it would be great in all situations to look at a sample in an untargeted way, and identify and quantify as many components as possible. That's essentially the promise of data-independent acquisition.

From R&D to VP

I started along my career path with an undergraduate degree in chemistry and the intention to become a doctor but, after completing the first year, I realized it wasn't for me. However, the experience was not wasted; I gained both a solid grounding in biochemistry and a real insight into the huge impact of clinical and translational science. With that prominent in my mind, I joined Genentech as a research associate. I was fortunate to start at the infancy of the biotech industry, and got a great education in biologic drug development, from cloning, expression, purification, characterization, the approval process, and sales and marketing of protein-based drugs. The guys down the hall from our R&D lab were just figuring out how use mass spectrometry to analyze proteins, which really fascinated me.

As time went on, I realized that I was a people person and the lab started to feel a little restrictive. I went back to school and graduated with an MBA from the University of California, Berkeley, in 1991. Since then I've worked for a succession of analytical instrument companies, initially in sales and then marketing. It has been extremely valuable to once have been a customer – it helps me understand the challenges that our customers face and to develop products and programs to help them succeed.

I joined Thermo Fisher Scientific in 2000, which was really the first big proteomics wave with ion trap instruments and SEQUEST™ software. Supporting and sustaining collaborations continues to be a source of pleasure and inspiration. Now, I'm VP of marketing for our life sciences mass spectrometry business, and it's great to be working for Thermo Fisher at a time when we are deeply involved in so many aspects of a field that has always meant a lot to me.

We've been doing data-dependent acquisition (DDA) for a long time. It's incredibly fast and sensitive, but it's a stochastic process; the 10 precursor ions (for example) selected by a survey scan for further mass selection in one sample may not be the same in another sample. DIA has captured the popular imagination because it seems that we can have our cake and eat it too. Indeed, it has the potential to create a high-resolution digital archive of all components in a complex mixture in an unbiased way. In brief, you step up the mass range in increments (for example, 25 m/z windows in SWATH™) and fragment everything in each incremental window to create a very complex, multiplexed MS/MS spectrum. Of course, those complex spectra are both a blessing and a curse. Yes, you have spectral representation of everything present in a given window but, on the other hand, deconvoluting that highly complex data post-acquisition is an extremely complicated process.

SWATH™ and other DIA software attempt to get over that hurdle by matching components in the acquired spectra to a mass spectral library. Our DIA methods offer a considerable advantage in that Orbitraps acquire very high resolution, very accurate data. We can typically be accurate to within 5 ppm whereas QToF data is extracted in a 50 ppm window. If the search window is opened to 50 ppm, you will have contribution from a lot of different species, making it difficult to distinguish real signal from noise. The tighter the window, the easier it becomes to separate signal from noise, with the added benefits of improved sensitivity and analytical precision. It's all about selectivity. In other methods we've developed, we shrink the m/z mass range windows (for example, from 25 m/z down to 5 m/z) thus reducing the complexity of each spectrum, which has the same result. Where we must be careful (and I think the proteomics community has learnt this the hard way) is understanding the absolute need for analytical rigor in terms of how we assess data quality and mitigate the risk of false positive identifications. It caused problems in the early days of proteomics and I think it has the potential to cause further problems in DIA if robust statistical tools are not used. Ultimately, for simple samples, DIA works well; for more complex samples, it can be more challenging. Having said that, with Orbitrap we can dig deeper and derive more accurate quantitative data even in more complex samples, so I think there's plenty of potential to explore.

How far are we from Alexander Makarov's dream of an Orbitrap in every lab?

It's a nice idea! But I still think it's early days for Orbitrap technology. It's used extensively in the high end research environment right now, though it is beginning to be used in some interesting quantitative and routine applications. A good analogy is automobile vendors – most of them invest heavily in Formula One or rally race teams; and that's where the discoveries and inventions are made. But inevitably, the most useful innovations trickle down to the mainstream market. Thermo Fisher Scientific has much the same philosophy. We experiment at the high end and, through a process of refinement, make that technology more accessible to a broader cross-section of our customer base, both in terms of price and in terms of application. We must continue to push innovation into the mainstream.

Webinars

Virtual Events

Detecting Human Exposure to Toxins (ADME): 4 Cases

Event Overview:

It's easy to see when someone is ill, but sometimes it is a little more difficult to determine what is causing the problem. Join Elizabeth Hamelin from the Centers for Disease Control and Prevention for a discussion on how analytical chemistry is being used to identify and measure human exposure to harmful toxins.

Learning Objectives of Webinar

1. Human exposures can be identified using analytical chemistry
2. Selection of biomarker and biological matrix is paramount
3. Method evaluation is essential to determine potential biomarker sources, detect matrix biases, and minimize false positives and false negatives

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Speaker
Beth Hamelin
Chemist, Centers for Disease Control and Prevention (CDC)

Elizabeth (Beth) Hamelin works at the Centers for Disease Control and Prevention in Atlanta, GA, developing analytical methods to detect human exposure to toxins and poisons. Using analytical skills developed from years of method optimization and technical support, Beth continues to publish methods using GC, LC, and MS for the analysis of clinical samples.

Beth began her scientific career by obtaining a bachelor's degree in chemistry and Spanish from Coe College in Cedar Rapids, IA, and continued her education by obtaining a master's degree from Texas A&M University. She continued to gain analytical experience in product development, method development, technical service and support from a variety of industries, including motor oil, automotive supplies, carbon black applications and drilling fluids production.

Business

Economic drivers
Emerging trends
Business strategies

The Bumpy Road from Chemist to Entrepreneur

Starting my own bioanalytical research company after many years working for Big Pharma has been daunting, but also satisfying, and I wouldn't have missed it for the world. Here, I describe my journey from naïve beginnings to fully functional lab and the steep learning curve that led the way.

By Elizabeth Thomas

"Why don't we set up our own bioanalytical contract research organization?" That was the simple question that I posed to a group of close colleagues in 2004, back when I was an associate director of bioanalysis at AstraZeneca. It's fair to say that the response lacked enthusiasm. After all, why would my coworkers want to give up well-paid, secure jobs to do something much riskier and more demanding?

Fast-forward to July 2013 and I was asked the same question, and this time things were different, for at least three reasons. One, an established service provider, ICON Bioanalytical labs in Manchester, UK, was closing, leaving a real gap in the market. Two, skilled people would soon be made redundant both at ICON and at AstraZeneca who announced that it was moving research and development from Cheshire to Cambridge. And three, the BioHub was to open at AstraZeneca's Alderley Park facility, adding a tempting location to the mix. Yes indeed, times really had changed since that first conversation in 2004. Entrepreneurial urges had been fueled.

Setting the wheels in motion

After a few phone calls to exchange ideas and concerns, I got together with three recent former colleagues from ICON and AstraZeneca to seriously consider the possibility of launching our own bioanalytical contract research organization (CRO). For the early confabs we met up every Wednesday evening in the "Didsbury office", otherwise known as a pub called The Slug and Lettuce. After a couple of discussions we added a second weekly get-together, on Sunday afternoons in my kitchen. Little by little, our business plan began to take shape and all four of us agreed that we should go for it: we were going to set up our own bespoke bioanalytical CRO.

Reality soon started to set in. We began on several parallel activities, including a search for appropriate lab and office space (as we did not wish to limit options to the BioHub), investigating the possibility of start-up grants, and getting access to training, advice and business support. Some

government-run training courses were useful, although they seemed to be aimed at single individuals setting up small businesses: ours was definitely at the more ambitious end of the spectrum. It is strange what sticks in my mind from these courses; one was when the advisor on the bookkeeping course recommended keeping invoices in a shoe box(!) and the second was the absolute horror on people's faces when, after a show of hands in the social media course, I was identified as the only person in the room not on Facebook. "Is that really a problem?" I asked.

Training was also available via the BioHub in the form of a business Bootcamp event. This was very useful and really made me challenge whether we had a viable business idea. It also drummed into me the importance of managing the business – as opposed to being 'in' the business – and the need to focus on 'sales, sales, sales'.

All four of us had spent the majority of our careers in large pharmaceutical companies and CROs, so we were used to the corporate world and everything that it entails. Suddenly, we were way out of our comfort zone. The difference between working in – or even managing – a business unit and running your own company is immense, and I found my time taken up by a multitude of issues that I had never needed to consider before: "How do I do a VAT return? How do I run a payroll? What exactly is Corporation Tax?" Unfortunately, 3am seemed to be the time when I worried about these things the most. And yet, we kept working and progressing.

Let the science begin?

By January 2014, we had registered the company, becoming the proud owners of Alderley Analytical. We had a logo, our website was up and running, and we had reserved the lab and office space we needed to get started at the BioHub. This felt like a good start, but there was still equipment, rent, accounting, legal issues, insurance, finance and, of course, 'sales, sales, sales' (and marketing) to consider. There was still a very long way to go.

While conducting market research with potential customers and ex-colleagues, a number of them told me I was "brave" to set out on my journey. If I'm being honest, I could have substituted the word brave with many others (some of them good and others simply not publishable) depending on the challenges and hurdles I was trying to negotiate at the time. In those more difficult moments, I was very pleased to have colleagues who I knew well. Without others to share worries and concerns, things would have certainly been much more difficult – even impossible.

By the end of Febraury, we had signed the lease for the lab and office, purchased general lab equipment from the closing ICON lab and acquired a liquid chromatography-tandem mass spectrometry system. Although it was perched on the bench awaiting direction, when I surveyed my new

Five Top Tips to Get You Started

1. Be thorough with your market research. What problem are you solving? Is your business idea viable?
2. Get your website up and running as soon as possible.
3. Don't be scared to ask for advice (business, legal, accounting). You will be surprised how much free advice is available.
4. Look at Government websites – they can often guide you to small business advisors and free training.
5. Get out of the building. It is essential to get your business known and to grow your network.

Five Pitfalls to Avoid

1. Try not to spend too much time 'in' the business instead of managing it.
2. Don't get stuck in your comfort zone. You will need to take on a diverse range of tasks and challenges.
3. Don't pay full price for goods or services. Negotiate everything and try to get as much free stuff as possible!
4. Don't forget about Business Development and sales, sales, sales. No customers means no business.
5. Don't get disheartened. Find a colleague or mentor to help you meet the challenges.

environment, I felt satisfied that we had arrived – we had a fully functional lab. Let the science begin, I thought.

Alas, such thoughts were a little premature. We still had to prepare for Good Laboratory Practice (GLP) accreditation and there were many standard operating procedures (SOPs) to write and forms to design; we had to validate the LC-MS/MS system and the temperature-monitoring system for our fridges and freezers; we had to write company and health and safety policies and procedures. And still the list grew... We had to set up our accounting software, our customer relation management system and our IT infrastructure; we had to get our business cards printed, finalize the website and develop the marketing brochure. And grew… We had to sort out our business banking accounts and banking software, legalize the company, the decision-making, and the shareholdings.

There were the slightly disturbing 'what if' scenarios to consider, those things that 'could in theory' occur in the future and affect the stability of the company. What if one of us died? What if we all died? What if we couldn't work together anymore? What if one person wasn't pulling their weight? Oh – and, of course, we couldn't forget about

'sales, sales, sales.' The next topic to raise its head was 'investment'. Did we need it now, in the future, or at all? On several occasions, we were asked "What is your our exit strategy?" That question left us a little bamboozled. Exit strategy? Let us get started first.

Current Status

As I sit writing this on a beautiful evening in July 2014, Alderley Analytical is now doing some real bioanalytical science for a real customer. We have also developed and validated a very sensitive testosterone assay and analyzed some human serum samples. We will soon be moving on to our next project, which will include some exciting science: if successful, we will publish the results. We also have a positive emerging pipeline – and genuine smiles on our faces!

The science aside, we are looking around to see what (if any) grant funding might be available to us. The word 'investment' is still in the air. Indeed, interest has been expressed by a couple of potential investors. And, of course, we're still focused on 'sales, sales, sales'. It's too early to say what 2015 will bring but we are quietly optimistic. At the very least, we shall 'survive' – but our fingers are firmly crossed for successful execution of our five-year plan. Jump forward to the end of 2018, and we hope to have approximately 50 staff, six LC-MS/MS systems, and several large molecule analyzers, with a matching increase in lab/office space from 1,500 square feet to 7,000 square feet.

But whatever happens, I will never regret starting on my journey. I have learned so much and met so many great people along the way. I wouldn't have been able to get this far without a great deal of support, help and advice.

We are currently a small molecule bioanalytical CRO but I still can't get used to being called an entrepreneur, despite the fact that people have already used it to describe me. When I think of entrepreneurs I imagine Richard Branson and Alan Sugar but perhaps that's a generational (and British) perception. If I was younger (and American), I guess my first thoughts might turn to Mark Zuckerberg or Larry Page. Either way, I can honestly say that I still don't consider myself to be an entrepreneur. Perhaps that is because I am still a scientist at heart and always will be. However, life has definitely changed. I was looking at LinkedIn recently and realized that many of my new connections weren't scientists; they were accountants, solicitors, corporate finance people and – you've guessed it – entrepreneurs.

Last week, I had coffee with an acquaintance who is thinking about setting up their own scientific business. The very first question asked was "where do I start?" The answer to that is neither short nor easy – but I gave that my best shot, too.

Elizabeth Thomas is CEO and founder of Alderley Analytical, Cheshire, UK.

Solutions

Real analytical problems
Collaborative expertise
Novel applications

Who Says You Can't Do GC-MS on an LC-MS System?

Atmospheric pressure gas chromatography (APGC) showed great promise when it was originally unveiled, but significant development and input from key collaborators would be required to harness the technique for ultra-trace POP analysis. Here's that story.

By Jody Dunstan

The Problem

Existing atmospheric pressure ionization-mass spectrometry (API-MS) instruments are unable to analyze low-polarity compounds. How can the advances made in ionization sources for liquid chromatography (LC) be extended to gas chromatography (GC)?

Background

Mass spectrometry has increasingly become a key part of the modern analytical laboratory. But MS systems are a large investment for many organizations, so they must be used as efficiently as possible. One way to ensure optimal use is to maximize the number of compounds that can be analyzed per instrument by interfacing both LC and GC to a single MS platform.

Let's go back to 1973, when the technique of atmospheric pressure chemical ionization (APCI) was initially developed by Horning et al (1). APCI showed excellent promise for the ionization of relatively non-polar analytes, but lacked commercial instrumentation. Over time, ionization modes were developed for API-MS instruments, often combining multiple ionization techniques into one source design. Although this enabled a much wider range of analyte polarities, the technique was limited to compounds amenable to liquid chromatography. So, what about gas chromatography?

With that question in mind, many began seeking the elusive "universal detector". Though there has been a general trend towards LC, many analyses simply work better with GC. Therefore, the option to use either separation technique with one MS product is a real bonus for a lot of laboratories.

Fast forward to 2007, and Charles McEwen (then at DuPont) developed two new technologies: the atmospheric solids analysis probe (ASAP) and atmospheric pressure gas chromatography (APGC, see Figure 1). The ASAP probe allowed direct analysis of solids and liquids in an API source and APGC offered GC capability on an LC instrument (2). Charles (known as Chuck to his old friends) approached Waters to discuss these two new technologies, initially without much success.

Many new technologies are evaluated by Waters and we typically need to answer two fundamental questions: does it work in the real world (outside of R&D) and is there a commercial need? New technology always has a number of barriers to overcome and several innovations have come along, promised miracles, and (sometimes unsurprisingly) failed to deliver on those promises. However, good data tend to win people over, and so, about a year later, Michael Balogh (CoSMoS president and consulting principle scientist at Waters) heard about Chuck's work via the CoSMoS (Conference on Small Molecule Science) committee. Michael convinced Andy Jarrell (a scientific fellow) to investigate APGC further. After some communication with the MS team in Manchester, UK, it was decided that the technology could be a good fit with Waters' current and planned portfolio. Waters and DuPont agreed an exclusive license agreement and work commenced on both sides of the Atlantic in an attempt to turn the rough concept into a commercial reality.

The Solution

The process of taking an idea from the

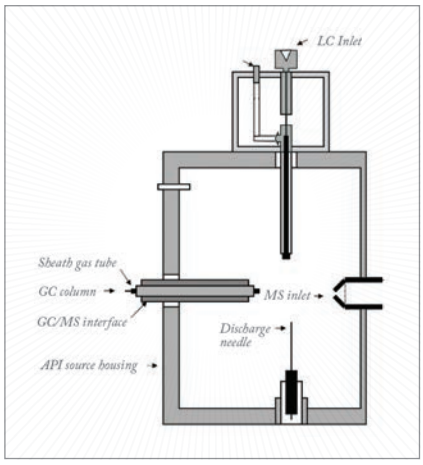
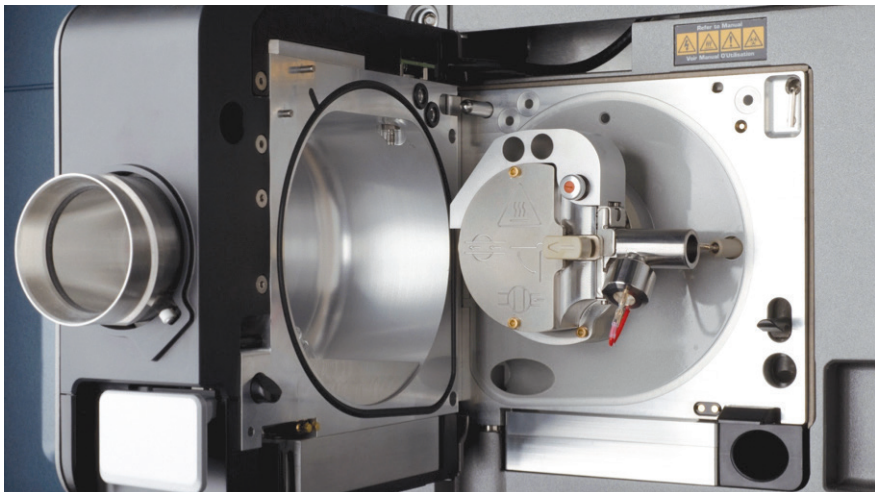


Figure 1. The original APGC design, taken from Reference 3.



The modern APGC source fitted to a Xevo TQ-S MS.

prototype stage to a commercial product can be long and fraught with challenges.

Our design philosophy – engineered simplicity – is to create products that are fit for the purpose intended but also easy for both customers and engineers to use and maintain. At the feasibility stage, various source geometries were trialed. The original source was open to atmosphere, but controlled ionization requires a closed source, which allows for a wider range of compound ionization possibilities (dry source for charge transfer and 'wet' source for proton transfer). The Universal (API) source was an existing closed source and deemed suitable for modification. After much trial (and some error) the geometry was settled, and in 2010 Waters filed for a patent of the modified design. The design uses a mini ionization chamber to provide a 30-fold sensitivity improvement on Chuck's already impressive design.

An important aspect of the design was that it could be integrated into Waters' portfolio of instruments. That is to say, the swap from LC to GC (and back again) needed to be as simple as possible. Past generations of instruments that could run with both LC and GC have required serious compromises (often in both modes). It was important to us that this system would be different. Our mechanical designers were set the task of creating a product that would allow tool-free changeover. The design also needed to be able to integrate with both the floor-standing MS instruments as well as the bench-top QToF and tandem quadrupole MS systems. The engineers were proud to deliver a final design in 2008 that worked well with all three instruments and, crucially, allowed both UPLC and APGC to be installed together.

The next and probably most important phase of development was our efforts to maximize instrument performance. Over the years, several groups in the analytical community have pushed to convert GC methods into LC, often with some sacrifice in performance. However, several analyses still require GC because of compound or matrix chemistry – this was a potentially ripe market, and we imagined APGC as an add-on to LC-MS and LC-MS/MS instruments. Initial data showed that a wide range of compounds could be ionized. We presented our work at various conferences and interest came mainly from the more technical mass spectrometry community.

On the quantitative LC-MS/MS instrument of the time, the performance looked good, but for ultra trace analysis of persistent organic pollutants (POPs) the overall system sensitivity was not good enough for the most challenging analyses. Soon after the introduction of APGC, development of the next generation of quantitative mass spectrometer began (later named Xevo TQ-S). One of the key components of this system was the implementation of a new type of T-Wave device – the StepWave. The original T-Wave concept was developed by Kevin Giles (another scientific fellow at Waters) as a fast, high-efficiency collision cell. Giles and MS development scientist David Gordon further developed the technology into an ion sampling/transmission device. In a nutshell, the step-wave is an off-axis ion-funnel that actively separates charged and neutral species, increasing signal and reducing background noise, which enables StepWave technology to sample the gas cloud entering from the sample cone orifice with high efficiency.

Initial work with the Xevo TQ-S with APGC was very promising, with

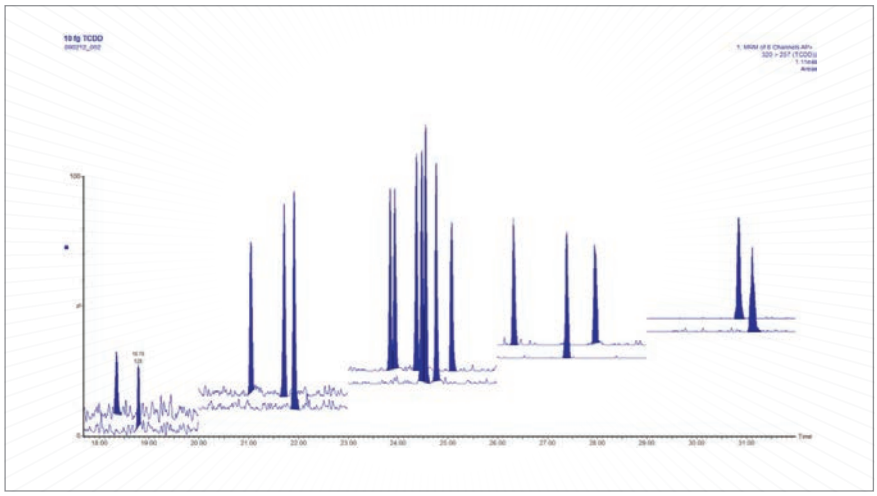


Figure 2. Results from Xevo TQ-S with APGC for low-level (10x dilution) dioxin/furan standard.

the sensitivity and reproducibility of the system exceeding our expectations. The initial results included much checking of calculations and dilutions to check the sensitivity was real rather than an error! Indeed, the Xevo TQ-S was released in 2010 and the improved results gave APGC further potential to perform applications we had not previously thought possible without using a magnetic sector GC-MS instrument.

Several demonstrations of the Xevo TQ-S with APGC took place in our global application laboratories, and there was some hesitation due to the lack of sensitivity for POPs using earlier generations of MS systems. Figure 2 shows an example chromatogram of a 1 in 10 dilution of the lowest dioxin/furan calibration standard (CSL). These data show excellent signal to noise for this standard, similar or better than data that can be achieved by magnetic sector MS ('gold standard' for dioxin/furan analysis). Good quality data were starting to interest the experts in this still developing technology.

Based on these experiences, we decided we needed to work with key opinion leaders, and a critical meeting occurred in late 2011 with Bert van Bavel, laboratory director at the Man-Technology-Environment Research Centre (MTM), Orebro University, Sweden. Bert has a long history of dioxin and POPs analysis, and saw great promise in the Xevo TQ-S with APGC as a possible replacement for magnetic sector MS, so a collaboration between Waters and MTM was cemented. In 2012, Rainer Malisch and Alexander Kotz from the EU Reference Lab for Dioxins in Food & Feed, Germany, and Wim Traag from RIKILT, The Netherlands, visited Waters in Manchester to run some dioxin and PCB samples. Once again, initial results showed great promise and soon plans were made to perform an extended side-by-side comparison with a magnetic sector MS.

Collaboration with key opinion leaders is always an important part of the Waters development process. It not only allows access to skills that are not available in-house but also gives a truly honest appraisal of the performance of the technology. It can sometimes be hard to take an unbiased view of a product that has taken up years of your life! The feedback about the data quality from the Xevo TQ-S with APGC was positive from both groups. The technology started to look very promising as a potential replacement for magnetic sector MS. There are several advantages to this solution. Firstly, the system is easier to use than a magnetic sector, and secondly, the system is flexible, allowing various different analyses to be run on a single platform rather than having a single, dedicated system just for dioxin/furan analysis. After much discussion both internally and externally, Waters launched the technology as a distinct product at the Dioxin 2013 meeting in Daegu, South Korea.

Beyond the Solution

Research and development of APGC technology continues within Waters, both to understand the fundamental principles behind the technology and to fully optimize the technique under different conditions. Acceptance of the technology has been increasing over time and our customers have moved from researchers and opinion leaders working at the leading edge of science to high-throughput commercial laboratories. The pace of development never seems to slow in mass spectrometry, so the next challenge is never far away.

Jody Dunstan is MS Product Manager, Waters Corporation, Wilmslow, UK.

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Absorbance of Anti-Oxidants in a Fruit Juice Blend

The acai berry has been reported to have the highest levels of antioxidant anthocyanins, measured as oxygen radical absorption capacity (ORAC), of any fruit or vegetable. Anthocyanins are natural pigments that not only give the acai berry its unique color, but also its remarkable anti-oxidant powers. In this application note, we measured the absorbance of a nutrient-dense juice blend comprising acai berries and other fruits.

By Ocean Optics staff

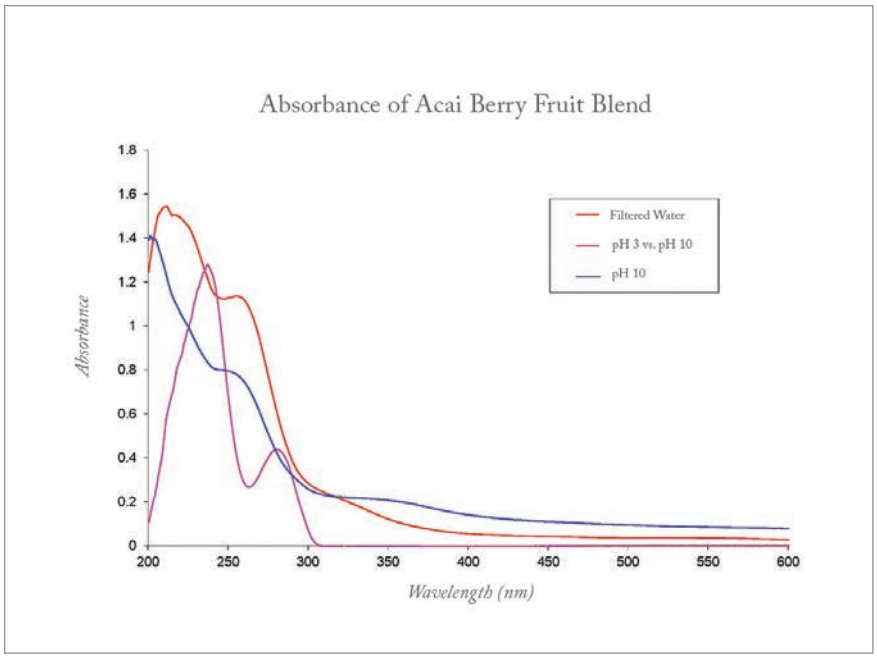

Figure 1: Absorbance spectra of the acai berry fruit juice blend diluted in water and different pH buffers show the likely effects of amino acids in the buffers.

Introduction

Absorbance measurements are a simple and non-destructive way to measure concentration. The amount of absorbance is proportional to the concentration of the sample. Often, components of a mixture, such as a fruit juice blend containing acai berries, can be identified as individual peaks. In the case of the acai berry, the main color pigment has a strong absorption band at 530 nm.

Component-based spectrometer systems are ideal for absorbance measurements. A good general-purpose spectrometer option such as the Ocean Optics USB2000+ or USB4000 spectrometers can be optimized for the wavelengths of interest and configured with optical bench accessories, such as optical slits and order-sorting filters. Indeed, if your absorbance application requires very high resolution to resolve closely aligned peaks, or significant light throughput is required to measure solutions, the grating and entrance aperture can be optimized to the samples being measured.

Measurement Conditions

Dilutions of a nutrient-dense fruit juice blend (composed more than 50% by weight of acai berries) were prepared in water, in a pH 3 buffer and in a pH 10 buffer. We used a USB4000-UV-VIS (200-850 nm) spectrometer to measure absorbance of the solutions in a 1 cm quartz cuvette in a cuvette holder, with a deuterium source for illumination and premium-grade, solarization-resistant patch cords both to carry light to the sample chamber and from the sample to the spectrometer. Solarization-resistant fibers are recommended for UV applications, where radiation <300 nm can degrade transmission in silica fibers. Although not part of our experiment setup, a NIST-traceable photometric absorbance standard is useful for quantitative, traceable absorbance measurements.

Results

Anthocyanins – the natural pigments giving the juice blend its dark color – absorb at 530 nm. These anthocyanins change color with pH. The spectra of the juice samples in all solvents showed a strong peak at 260 nm, most likely the result of high concentrations of amino acids. In pH 10 buffer, a secondary peak appears in the 350 nm region. The absorbance difference spectra of the juice blend in the pH 3 buffer versus the pH 10 buffer shows clearly a species with an absorbance peak at 280 nm (Figure 1).

In the case of fruits and vegetables, color is a good thing! The pigments that color our fruits and vegetables reduce damage from free radicals and may lessen damage from inflammatory processes, reduce the risk of cancers and improve the efficacy of our immune systems.

Living on the Endless Frontier

Sitting Down With Daniel Armstrong, Robert A. Welch Chair in Chemistry, University of Texas at Arlington, TX, USA.

Among your firsts is the original example of measuring proteins using mass spec. What is the background to that?

I was trying to synthesize peptides and proteins spontaneously using micellar catalysis. Other than size-exclusion chromatography we didn't have a good way of figuring out what we'd made. A professor of mine, Ron Macfarlane, had invented californium-252 plasma desorption mass spectrometry – a real breakthrough – that looked like it might work for proteins. Lo and behold, we were able to nail down high molecular weight materials for the first time. It wasn't recognized as an achievement at the time, partly because we emphasized the micellar catalysis in the paper, but I was enthralled by the method and it made me realize that the real ingenuity in science often comes from the people that invent methods and devices. Once you have those, anybody can and does do the rest.

That was an exciting start to your career. How did you maintain that level of engagement?

The most exciting thing of all is that I have been able to continually do new things. At any point in my career, what I was doing was exciting to me; five years earlier or later I'd be doing something quite different and be equally excited. That's what keeps me fresh. Being an expert in the same area for 20 to 30 years would be tremendously boring to me. My PhD mentor once told me that any person who has made a contribution to an area will become a detriment to it if they stay in it long enough!

Do you go into new areas and end up thinking, "what have I got into here?"

Absolutely. I am constantly coming up with things to pursue, but they are not always feasible. In fact, the majority of projects don't work out. The important thing is knowing when to go in a different direction or to bail completely. You get an innate sense for that although if I am tackling an extremely important problem or if the work has really piqued my curiosity, I focus on it a little longer. The freedom to pursue anything you want often takes you in more interesting directions than the original planned research.

"The freedom to pursue anything you want often takes you in more interesting directions"

Is the development of cyclodextrins for enantiomer separations an example of that?

It is. In the catalysis work, micelles contaminating the catalysis reaction mixture were affecting the chromatography. That's what got me thinking about using micelles as a chromatographic mobile phase in place of organic solvents. We then looked for things other than micelles that would do this and found cyclodextrins, which in the 1970s were expensive and esoteric materials. They worked quite well so we developed them as a stationary phase. Our interest was in molecular recognition and selectivity, rather than in developing a commercial opportunity, which was an afterthought. Of course, everybody jumped on that possibility and ignored the theory and the mechanism – they just wanted to know whether it could separate molecules. Right up until it happened, it hadn't dawned on me that that's what would catch the most attention.

That's surprising, as you are known for commercialization of your research.

Originally we never considered it, but when our work had a huge practical impact it was hard to ignore. And I've come to see it as important. If you publish something that nobody uses or even cites, it doesn't matter that you ever did it – and that's the case for most research, which consists of jumping on the bandwagon or making incremental changes. If I see another nano-something, I can almost turn my mind off right there. Let's face it, nano is just another word for colloid science, which has been around for over 100 years. Going in a completely new direction – that's what counts. And if it has a practical application, so much the better.

What is the biggest challenge you have faced in work in the past 12 months?

Without a doubt it's that there's not enough time. I occasionally do experiments but opportunities are more and more rare, and it is the thing I miss the most. If I am here and not traveling, I do the next best thing: I go to the lab to check progress, discuss problems and watch experiments being performed. That way I get to examine and discuss the most recent results. The paperwork, committee meetings, etc., are not fun. Going into the lab, getting interesting new results is fun, I hope I'll keep it going until I die.

How do you feel that analytical chemistry is faring?

It doesn't get the credit it deserves, definitely not, and it doesn't get the funding commensurate with its contribution to science and society. Despite this, there is no doubt that analytical chemistry is healthy and competitive. It is in good shape. And it provides great job opportunities, particularly separations and mass spectrometry. People in all branches of science need these.

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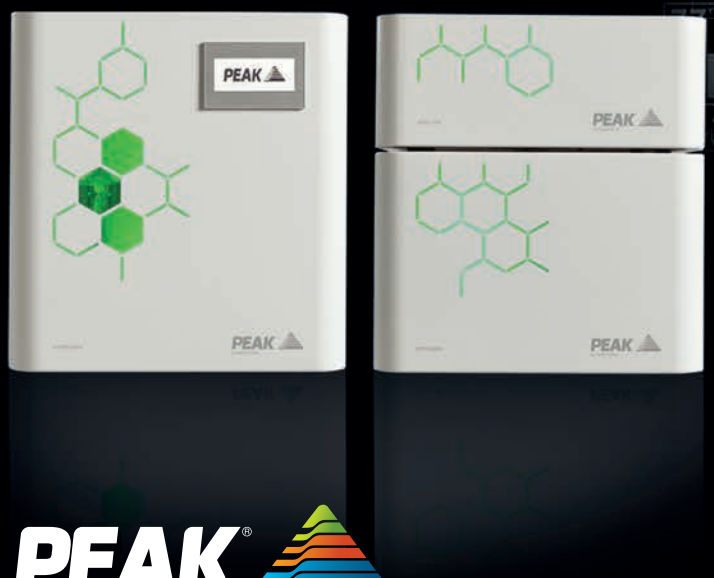

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