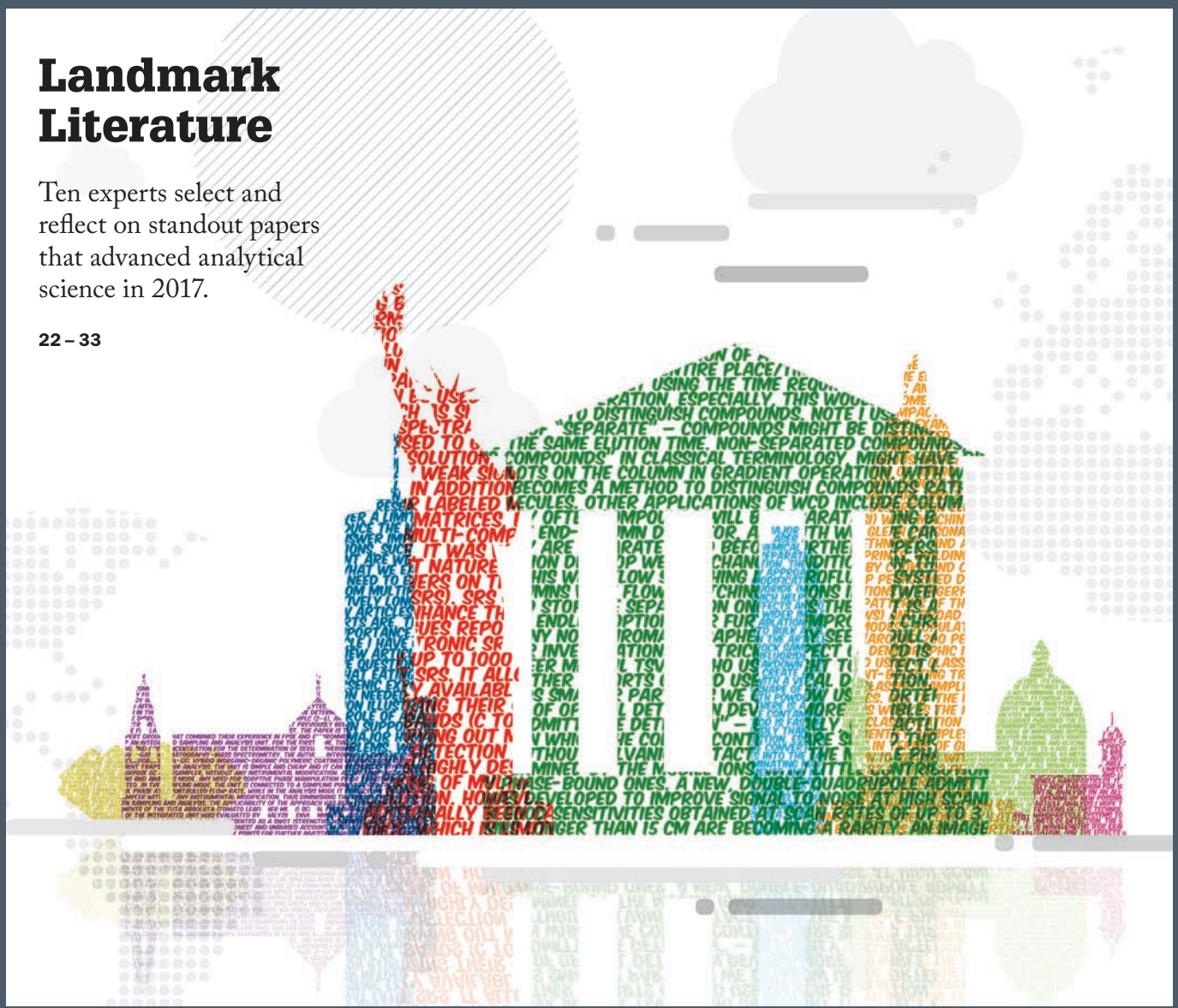
JANUARY 2018 # 60

the Analytical Scientist™

Landmark Literature

Ten experts select and reflect on standout papers that advanced analytical science in 2017.



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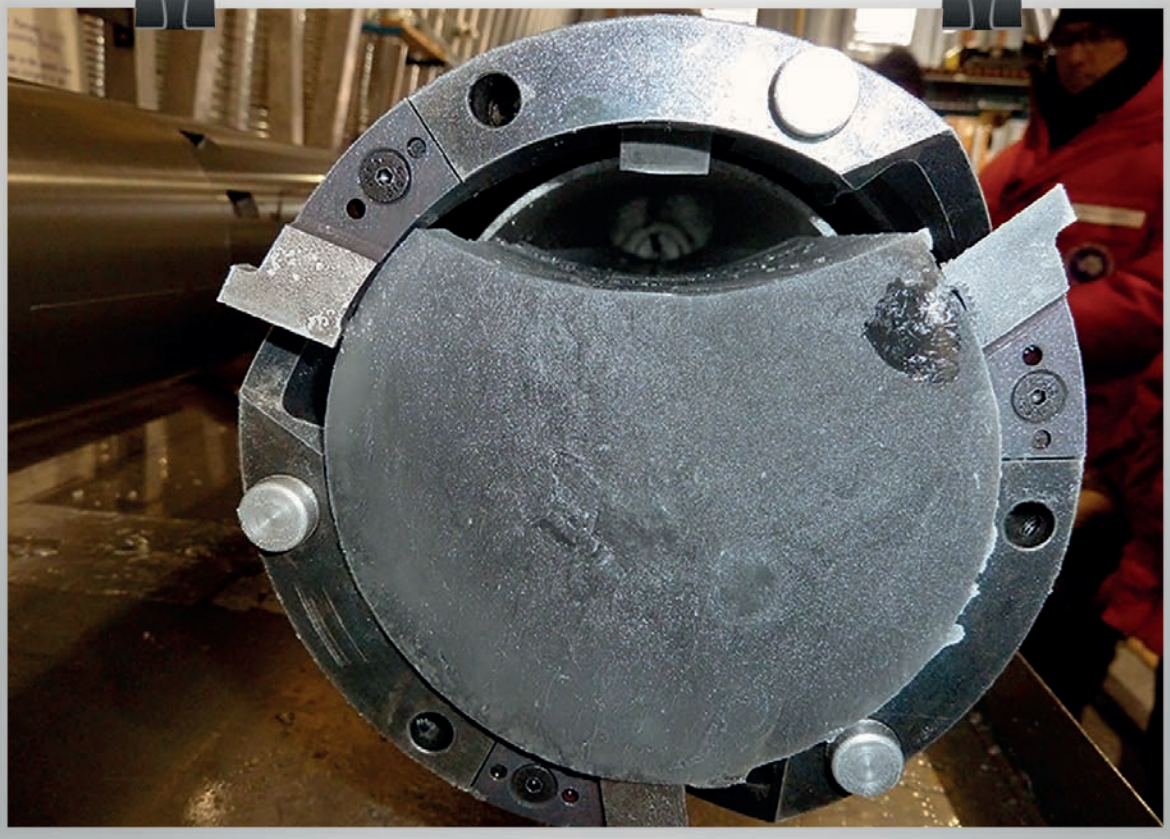


Core Values

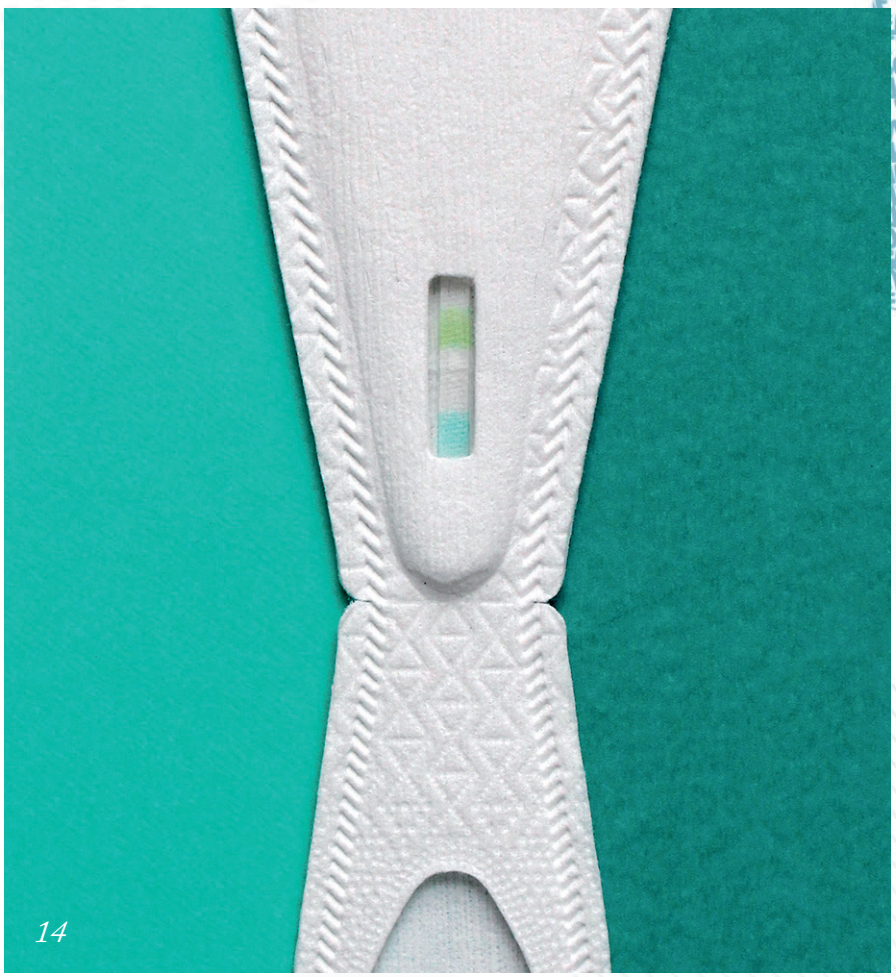
Researchers at Scripps Institution of Oceanography have developed a new way to measure the average temperature of the world's oceans over geological time. The scientists used a dual-inlet isotope ratio mass spectrometer to measure noble gases trapped in Antarctic ice caps, and showed that mean global ocean temperature increased by 2.57 ± 0.24 degrees Celsius over the last glacial transition (20,000 to 10,000 years ago). Seen here is an ice core from West Antarctica, drilled in 2012.

Credit: Jay Johnson/IDDO.

Reference: B Bereiter et al., "Mean global ocean temperatures during the last glacial transition", Nature, 553, 39–44 (2018).

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

Contents



On The Cover

Landmarks from New York to Wuhan feature in our round-up of the top papers of 2017.

Upfront

the Analytical Scientist

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Reflections and Resolutions

With one year at the helm of The Analytical Scientist, I consider my own "lessons learned."

The close of the old year (and the start of the new one) is traditionally a time for reflection. Inspired by the latest article in our "Lessons I've Learned" series on page 44, I find myself asking: what lessons have I learned from my first year as Editor of The Analytical Scientist?

First, my fascination for analytical science has intensified beyond my bioanalytical background. Ever since the first article I edited, I've been constantly reminded that the wider field is at once surprisingly simple and fathomlessly complex. Though most analytical techniques are based on straightforward concepts and a deep well of fundamental knowledge, there remains a constant stream of iterative innovation that is always pushing the field forward. (Don't miss the fantastic array of advances in our 2017 Innovation Awards: tas.txp.to/1217/Innovation).

Second, I've learned that, although techniques and technology are the lifeblood of the field, people are its beating heart. From the patient being conned by counterfeit drugs (page 34) to the planet's 7.6 billion humans (and 20,000 lions – see page 13) who need a clean and safe environment, analytical science is having a huge impact – everywhere.

Finally, I've come to realize that analytical scientists are a truly self-effacing bunch – a comment often made by Content Director Rich Whitworth in these pages. Our authors and readers often make little of the important role they play in diverse and groundbreaking research; Perdita Barran provides a perfect example on page 10, saying, "It is very humbling as a mere measurement scientist to help find some signature molecules to diagnose Parkinson's." Interestingly, although individually modest, as a community you are more than happy to give credit to colleagues and mentors, as witnessed by the overwhelming number of Power List nominations.

Another New Year's tradition is to make resolutions. Here are three resolutions for 2018 that (unlike most such commitments) we will keep as we head into our sixth year:

1. We aim to meet as many readers and contributors as possible. This spring, look out for Jo, Frank, Rich and me at HTC in Cardiff, Pittcon in Orlando, and Spring SciX in Glasgow.
2. We won't shy away from unusual or controversial topics – e-cigarettes, life on Mars and the unsung heroes of industry are all coming soon.
3. We will continue to celebrate analytical scientists and their achievements.

I wish you all a wonderful 2018.

Charlotte Barker
Editor

Upfront

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

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

Sniffing Out a Test for Parkinson's

A British woman who can identify people with Parkinson's disease by smell alone is helping scientists pinpoint the molecules behind the giveaway scent

Joy Milne has an unusual gift. Years before her husband was diagnosed with Parkinson's disease, Milne started to notice a strange "musky" smell around him. When they later attended a support group for the disease, she realized that the odor was unique to people with Parkinson's – and the more advanced the disease, the more pronounced the scent.

Milne, a former nurse, attracted widespread press coverage when she proved her "superpower" in a series of controlled lab tests, where she correctly identified clothing worn by Parkinson's patients versus controls. Her only misstep was to identify one "control" as having Parkinson's – initially recorded as a false positive, the subject went on to be diagnosed with the disease soon afterwards. Now, Milne is working with scientists who hope to develop a much-needed early test for Parkinson's disease.

Manchester University chemist Perdita Barran, who appeared in our 2016 Power List, is attempting to isolate the molecules that make up the distinctive odor, which appears to originate in sebum. Changes to sebum production in Parkinson's sufferers are well known, with many patients reporting "waxy" skin as a symptom.

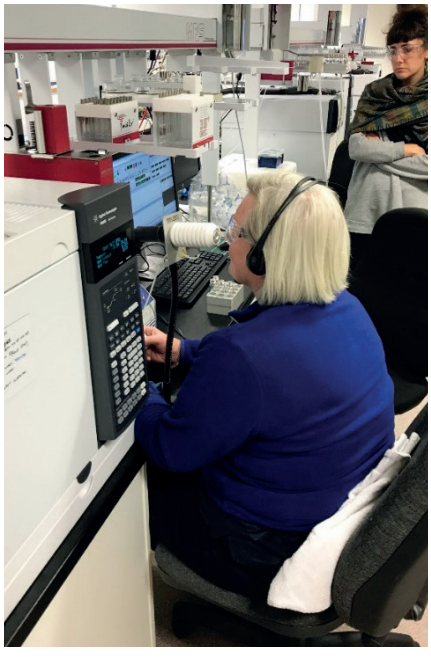
The team used dynamic headspace sampling to collect volatile compounds from clothing worn by people with and without Parkinson's. An olfactory detection port (ODP; Gerstel), allowed the samples to be simultaneously analyzed by the human nose (in this case, Milne) and GC-MS. By tallying Milne's reactions with the read-out from the GC-MS, the researchers have so far identified 10 potential biomarkers that appear to be distinctive to Parkinson's patients.

Milne is delighted with the initial findings, and hopes that by helping to develop a test she can spare others the pain of watching a loved one struggle with Parkinson's – her husband died in 2015, 20 years after being diagnosed with the disease. She told the BBC, "It's horrible watching your partner change like that. If we can get the test right, it may never get to that stage" (1).

Barran added, "It is very humbling as a mere measurement scientist to help find some signature molecules to diagnose Parkinson's."

Reference

1. *E Quigley, "Scientists sniff out Parkinson's disease smell", Available at: ttp://www.bbc.co.uk/news/uk-scotland-42252411. Accessed January 8, 2018.*



Resisting Arrest

Could resonance Raman spectroscopy be the key to your heart?

For healthcare practitioners, being able to measure the oxygen levels in tissue is crucial – especially during surgery. Currently, venous blood is required to check the delivery of oxygen to mitochondria, but monitoring levels over time necessitates repeated blood draws, which can pose a problem in critically ill patients who may already be losing blood. Moreover, the method cannot give local information on specific tissues, and can be misleading in some patients.

Scientists from the Boston Children's Heart Center have developed a device based on Raman spectroscopy that can not only provide reliable organ-specific information on the delivery of oxygen to the mitochondria, but also predict cardiac arrest during and after surgery (1). Predicting when a patient's heart will stop is not possible with current technologies, according to John Kheir of Boston Children's Heart Center (2), because the heart is able to compensate for low oxygen conditions – until breaking point. "Once cardiac arrest occurs, its consequences can be life-long, even when patients recover," said Kheir.

The device is made up of a laser pump source and a fiber optics probe, which includes a jacketed glass fiber bundle that delivers the Raman signal back to a custom-built high-resolution/high-throughput spectrometer. In rat and pig models, the system detected changes in mitochondrial redox state, which correlated with tissue oxyhemoglobin saturation, and also predicted weakening contractions in the heart muscle (1). However, the device has not been tested for its ability to provide a measurement of overall oxygen levels in the body – and its current depth of penetration in vivo means it requires direct access to the tissue being monitored.

Despite the limitations, a device that is able to directly measure oxygen levels within tissue does have utility; according to the authors: "[It] may be used to monitor tissue viability during surgery, for the protection of organs explanted for transplantation, or the identification of critical ischemia" (1).

Reference

1. *DA Perry et al., "Responsive monitoring of mitochondrial redox states in heart muscle predicts impending cardiac arrest", Sci Transl Med, 9 (2017). PMID: 28931652.*
2. *SpectroscopyNOW.com, "Heart stopping science: Raman sensor", (2017). Accessed January 8, 2018. Available at: http://bit.ly/2EjWI3d*

Good Vibrations

Microelectromechanical resonators could help detect biomarkers in small sample volumes

What?

A "microelectromechanical resonator" – a small, vibrating sensor that may allow the sensitive, specific and affordable detection of biomarkers in even small volumes of blood (1). Senior author Jeffrey Rhoads explains…

Why?

"What made us consider looking into biomarker detection? To be honest, George Chiu, Eric Nauman and I decided upon this research path over a water cooler conversation! George and I have worked together for nearly a decade on various sensing systems, primarily focusing on industrial and national security applications. Eric had been working on research problems related to traumatic brain injury, and he felt that our sensors might be able to have an impact in that space.

The advantage of our method over other existing ones appears to stem from a combination of the high sensitivity of our devices, our statistics-based detection approach, and the fact that we require very small test volumes."

How?

"The system is based on resonant mass sensing. The basic idea is that you have a small-scale vibrating element – in this case, a small plate. The element has a series of frequencies at which it 'likes' to vibrate – natural frequencies, which depend on a number of design parameters, including the mass of the device. If you have a clever way to bind specific masses, like proteins, to your device – for instance, through a functional polymer layer – then you have a sensitive way to detect that biomarker. The challenge is to avoid false positives without using a large sample volume of test fluid. We circumvent this through the use of large sensor arrays, which leverage the power of statistics, and a sensor functionalization and material deposition technique based on small-scale bioprinting.

Generally speaking, the sensing system is agnostic to what it detects. Functionality and selectivity are set solely by the functional layers that we deposit (and, if necessary, develop). For this work we deposited specific antibodies, embedded in a polymer, that have an affinity for the protein of interest. In our earlier work, we used similar techniques to detect analytes ranging from explosive vapors to volatile organic compounds."

Who?

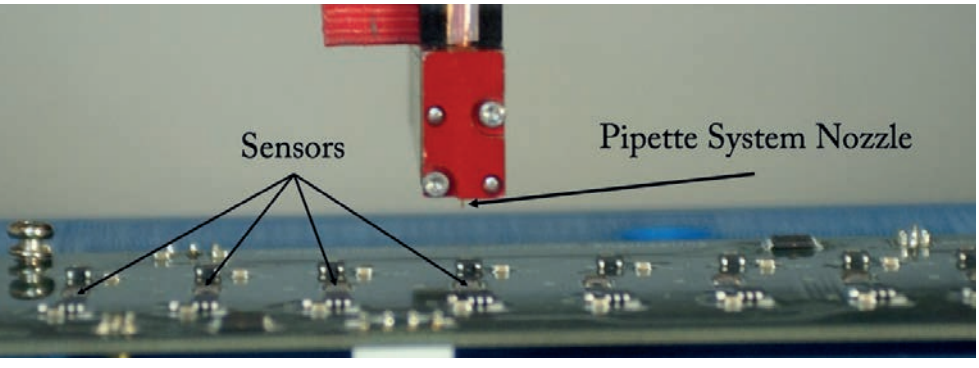
"Given the low-cost nature of our sensor, I think it might be well-suited for lab-based and perhaps even point-of-care diagnostics in the field. Our hope is to provide laboratory medicine professionals with an affordable sensing solution that takes less time to process than current gold-standard methods. Given that the required sample volumes are also low, we may also be able to help reduce the need for large volume fluid withdrawals – for example, enabling the use of finger sticks instead of full blood draws for some applications."

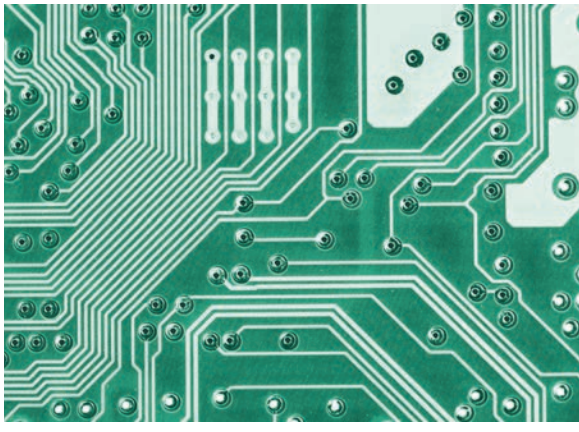
When?

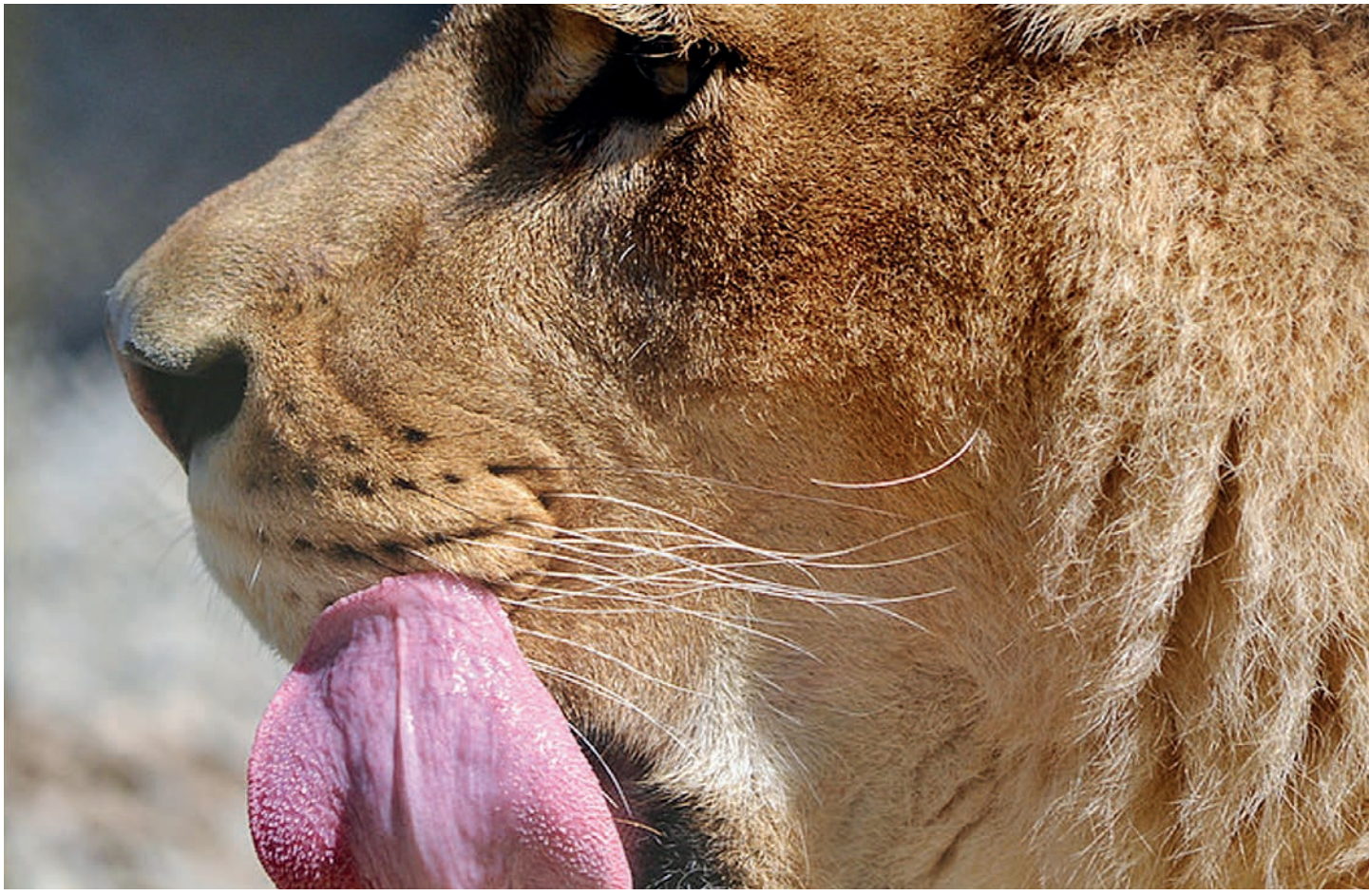
"Before we can bring our biomarker detection sensor to the clinic, I suspect the next logical step is to conduct a small-scale clinical trial. We are currently looking for external partners who work in the clinical medicine space to aid with further testing. At the same time, we are working with the Purdue Research Foundation to identify potential technology transition partners. It's our hope to be able to provide laboratories and field clinics with a better, cheaper biomarker sensor as soon as possible."

Reference

1. *MJ Wadas et al., "Detection of traumatic brain injury protein biomarkers with resonant microsystems", IEEE Sensors Letters, 1, 2501304 (2017).*







Feline Forensics

When analyzing the diet of animals, IRMS is the cat's whiskers

When it comes to the conservation of endangered species, such as the big cats (or felids), understanding more about the food being eaten in the wild can be useful. Though it is possible to analyze the dietary intake of animals through analysis of their whiskers, insufficient data on whisker growth rates for different species has thus far got in the way of accurate assessment. Thankfully, five wild cats from the National Zoological Gardens in Pretoria, South Africa have stepped up for the good of their species.

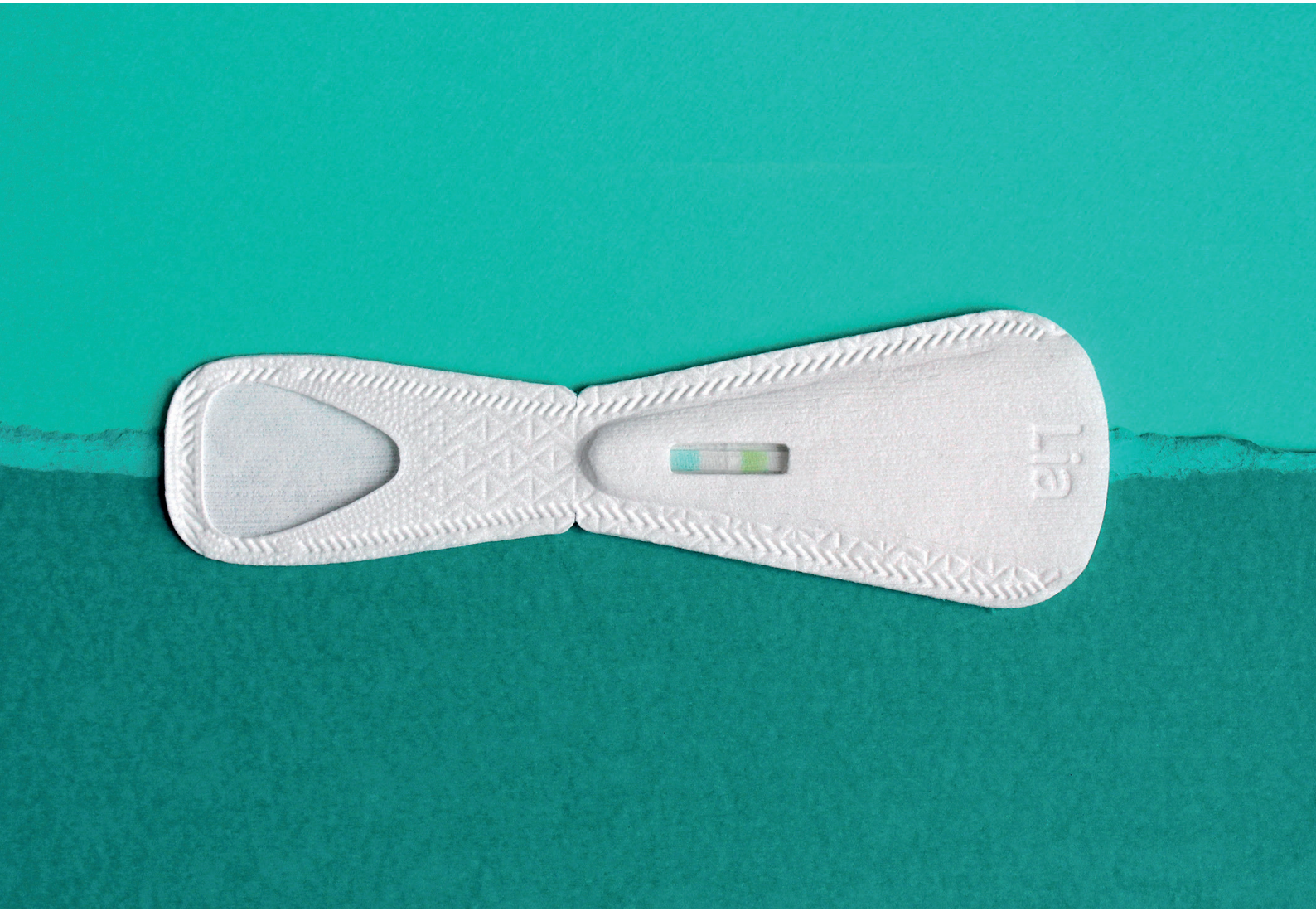
Four lions – Emma, Bianca, Tess and Boesman – and Diesel the leopard valiantly (if not willingly) donated their whiskers for isotopic analysis. The human contingent included researchers from Cape Peninsula University of Technology and the University of the Free State, Bloemfontein. Over a period of 185 days, giraffe meat was added to the wild cats' staple diet of chicken and beef at four stages, while the animals' whisker growth was monitored. The whiskers were then removed and segmented, before being analyzed by isotope ratio mass spectrometry (IRMS) to determine nitrogen and carbon ratios.

The ^{13}C-depleted, C_3-based giraffe meat presented as clear peaks in the ratio plots, correlating in three cases with the feeding bouts (1). Trophic discrimination factors (TDFs) – the isotopic difference between the whisker tissue and the diet – were also taken into account.

The conclusion? Big cat whiskers can clearly provide important dietary information – though the authors of the resulting paper (1) noted that TDFs did differ between felid species. They acknowledge that to provide more insight into isotope discrimination, further studies are needed on the full trajectories of whisker growth and shedding, as well as possible life history influences. As for Emma, Bianca, Tess, Boesman and Diesel, despite the undignified plucking of their whiskers, we hear they're still feline fine…

Reference

1. *R Mutirwara et al., "Growth rate and stable carbon and nitrogen isotope trophic discrimination factors of lion and leopard whiskers", Rapid Commun Mass Spectrom, 32, 33– 47 (2018).*



Mum's the Word

A radical re-design of the home pregnancy test aims to protect women's privacy – and the planet

The humble pregnancy test has altered little since the 1980s. Home tests are extremely accurate, but there are drawbacks – for one thing, disposing of the test in the trash means the result might not be as private as you'd like. Then there's the environmental impact – it is estimated that two million pounds of plastic and digital pregnancy test waste is produced each year in the USA alone (1).

Lia Diagnostics think they have the answer: the world's first flushable (and compostable) pregnancy test. Small, discreet and made of plant-based materials, the pad boasts the same 99 percent accuracy as regular pregnancy tests, but is fully water-dispersible and decomposes by 98.2 percent after three months in soil.

Women urinate on the test as usual, and wait for it to do its job – detecting the presence of human chorionic gonadotropin (hCG, produced when a fertilized egg attaches to the uterus). After getting the result, women can simply flush the test away – or bury it in the garden (which seems a little strange, but to each her own).

Discreet, easily disposable and eco-friendly? Seems that from now on, pregnancy testing could be as easy as 1, 2, pee...

Reference

1. *Lia Diagnostics, "Why Lia", Available at: https://meetlia.com/why-lia/. Accessed January 10, 2018.*

Cerebral Spectroscopy and Centers of Distinction

Business in brief: what's going on in analytical science?

Products and launches

- The TRACER 1000 MS-ETD, a mass spec explosives detector developed by Astrotech Corporation, had its first demo for shareholders in early December. The company believe TRACER can improve on the "shortcomings" of current ion mobility spectrometry systems, with near-zero false positive rates and an expanded list of compounds of interest.
- Medical device company Cerebrotech has received CE approval for its Intracranial Fluids Monitor, which uses volumetric integral phase-shift spectroscopy to detect changes in distribution of brain fluids caused by ischemic stroke.

Collaborations and acquisitions

- The fight against food fraud continues, with PerkinElmer and TeakOrigin collaborating on a new food authenticity platform. The research will combine Raman, mid-IR and NIR and molecular spectroscopy techniques with GC/MS, HPLC and wet chemistry to develop fast, portable food screening technology.
- Phenom-World, a manufacturer of desktop scanning electron microscopy solutions, has been acquired by Thermo Fisher Scientific, who are integrating the company into their Analytical Instruments segment.
- Last month, Eurofins Scientific acquired Brazilian company Pasteur Group – representing a new foray into the Latin American clinical diagnostics market.

Company updates

- SCIEX has opened its Center of Distinction in collaboration with the National University of Singapore. Paul Matsudaira, who heads the Department of Biological Sciences at the university, said at the opening ceremony: "To be SCIEX's first regional Center of Distinction is a particular honor for us […] With SCIEX, we want to be the place that trains the next generation."
- Bruker has consolidated its subsidiary companies in an attempt to streamline its internal structure. On January 1st, it was announced that Bruker Daltonics K.K., Bruker Optics K.K., and Bruker AXS K.K. have merged with Bruker BioSpin K.K., with the resulting company renamed Bruker Japan K.K.

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

The Road to HPLC2018 Part II: Sampling the Future

The sampling and sample preparation symposium at HPLC2018 will feature a raft of exciting new developments, from streamlined workflows to nanoscale sampling that reveals the detailed chemistry within the brain.

By Janusz Pawliszyn, Professor, Department of Chemistry, University of Waterloo, Ontario, Canada.

At HPLC2018, I will be chairing a symposium focused on new developments in sampling and sample preparation technologies – an area that has seen real progress in recent years. It may not be the most "glamorous" aspect of analytical science, but it is of fundamental importance – all analytical data depends on the quality of the sample. In particular, when investigating living systems, in-vivo sampling is important, as the removed sample (biopsy) is not stable and changes over time. In addition, in-vivo sampling makes the whole analytical process simpler, particularly if the sampling is well integrated with sample preparation and introduction to the analytical instrument. During the HPLC sampling symposium, two approaches will be discussed:

1. microdialysis, a more established technique suitable for very polar to mid polarity small analytes
2. solid-phase microextraction with matrix compatible coatings, which performs well for more hydrophobic analytes.

Both approaches extract the free form of the analyte, which is typically biologically active.

Applications will include pharmaceutical, medical, food, environmental and forensic, including both targeted and untargeted determinations. R. Graham Cooks and Zoltan Takats, the pioneers of ambient mass spectrometry, will discuss the advantages and limitations of eliminating sample preparation, chromatography and other separation steps when analyzing complex samples. Robert Kennedy will cover nanoscale sampling coupled to LC-

"Particular emphasis will be placed on integration of sampling and sample preparation with instruments providing quantitation."

"Will the technological advances we discuss at HPLC2018 eventually render sample preparation redundant? "

MS/MS for high-resolution exploration of brain chemistry. My own presentation will cover in-vivo solid-phase microextraction in medical practice, including evaluation of quality of transplantation organs, brain cancer surgery and deep brain stimulation.

Particular emphasis will be placed on integration of sampling and sample preparation with instruments providing quantitation. Efficient integration facilitates high-throughput, on-site and in-vivo determinations. Design of such approaches requires a reduction in the use of organic solvents. A good example includes our recently developed matrix compatible coating morphology in solid-phase microextraction applied in different optimized geometries for specific applications. Such developments will eventually allow us to achieve a major goal of analytical chemists – to perform analysis at the place where the sample is taken rather than moving the sample to a laboratory, and so reducing errors and the time associated with sample transport and storage. Ultimately, on-site analysis leads to more accurate, precise, fast analytical data and quicker decisions. Hyphenation of sampling/sample preparation with HPLC-MS as well as direct coupling to mass spectrometry are emerging technologies that could have a big impact in the near future.

Will the technological advances we discuss at HPLC2018 eventually render sample preparation redundant? Is this the death of chromatography? As mass spectrometry technology improves, in particular ion mobility mass spectrometry coupled with modern mass analyzers (which you will hear about in Richard Smith's plenary talk), rapid screening measurements may no longer require LC separation – or even sample preparation – provided the sample matrix is simple. However, for investigative analysis of problem samples, chromatography will have a role to play for a long time to come. In addition, complex sample matrices will require sample preparation to keep the instrument "clean" and create on-site or high-throughput analytical apparatus. Integration and simplification of sampling, sample preparation, and chromatography processes hyphenated to evolving mass spectrometry tools will likely drive development of modern instrumentation for the foreseeable future.

HPLC 2018 takes place on 29 July to 2 August in Washington, DC. HPLC2018.org

The Road to HPLC2018 Part III: Catching the Next Wave in IMS

Technological advances are putting IMS at the forefront of separation science – so it's a guaranteed hot topic for HPLC2018.

By Richard D. Smith, Battelle Fellow and Chief Scientist, Biological Sciences Division, Pacific Northwest National Laboratory (PNNL),Richland, Washington, USA.

Ion mobility spectrometry (IMS) originated more than a century ago and, to date, has been mainly used for chemical weapon agent detection and airport passenger screening. Though the field has been developing steadily over the years, IMS is only now transitioning to the top tier of analytical separation methods.

In IMS, ions collide with a buffer gas and separate due to their different shape-dependent velocities in an electric field. In high vacuum, without collisions with gas molecules, the result would be a separation based upon the ion's mass and charge – in other words, mass spectrometry (MS). On the other hand, if the IMS separation were to occur in a liquid (and in the presence of oppositely charged ions) it would be electrophoresis. Since ions attain much higher velocities in gases than in liquids, IMS separations occur much faster than liquid-based separations, with some forms achieving useful separations in milliseconds for compounds that might take minutes to an hour to effectively separate by HPLC.

IMS has become an increasingly popular front-end adjunct to MS. In fact, nearly every major MS vendor now offers an IMS-MS system in its portfolio. As well as faster analysis speeds, the two-dimensional platform provides significantly increased overall peak capacities over MS alone – and even greater increases when combined with more conventional separations; for example, LC-IMS-MS.

Currently, limited IMS path lengths constrain its resolving power (Rp) or peak capacity when compared to MS, or even LC. Achievable Rp increases with path length for both IMS and MS, with maximum path lengths limited by the practical maximum electric field and voltage (for example, in drift-tube IMS), cost of building longer path devices, reasonable platform size, and the increased ion losses incurred with longer ion drift paths. At very low gas pressures, the effects of ion–gas collisions are a minor perturbation to MS, generally causing a broadening and subtle shifting of peaks, which results in some loss of both Rp and mass measurement accuracy. The Rp feasible with IMS is constrained by diffusion in the gas to much smaller values (no more than a few hundred at present and often much less depending on the form of IMS).

But new opportunities are opening up due to the combination of two key advances in technology. One of these developments occurred more than a decade ago – the use of traveling waves (TW) in the Waters Synapt IMS-MS platform. TW IMS combines time and space variation of electric fields to induce the movement of ions along the path of the waves (a 25 cm path in the Synapt 2), with separation based upon the mobility-dependent ability of ions to "keep up" with the TW.

The second, more recent, development was Structures for Lossless Ion Manipulations (SLIM), from my group at PNNL. SLIM are literally constructed from electric fields, using arrays of electrodes patterned on two closely spaced planar surfaces to generate the fields. SLIM represents a dramatic departure in how ions in gases are focused and manipulated, allowing ions to be efficiently transmitted through turns, switched between paths, and stored losslessly over long time periods. The use of TW in SLIM enables the design of long compact serpentine IMS paths. A 13 m SLIM IMS provided over five-times greater Rp than the best drift tube or TW IMS commercially available, allowing baseline separation of structurally similar and previously challenging isomers, for example.

Though these early results are exciting, they are only the start of IMS separation enhancements to come. SLIM multi-level 3D implementations will allow much longer path separations (perhaps kilometers!) and provide the basis for compact IMS designs. SLIM ion switches open the door for multi-pass separations over potentially unlimited path lengths. SLIM also allows the temporal and spatial compression of peaks and even the "squeezing" of whole IMS separations without loss of resolution, as well as the injection and effective use of much larger ion populations for increased S/N.

SLIM IMS is riding a wave to exciting new separation capabilities, and I look forward to sharing more about the possibilities in my plenary talk at HPLC2018!

HPLC 2018 takes place on 29 July to 2 August in Washington, DC. HPLC2018.org

The World (of IHC) Is Not Enough

As a qualitative assay, immunohistochemistry does the job. But when it comes to accurate quantitation, don't we need something more?

By Dean Troyer, Professor, Departments of Microbiology, Molecular Cell Biology and Pathology, Eastern Virginia Medical School, Norfolk, USA.

Immunohistochemistry (IHC) detects PD-L1 as a companion diagnostic for pembrolizumab, a humanized monoclonal antibody used in cancer immunotherapy. The assay provides a semiquantitative score pathologists can use to determine the likelihood of treatment success using PD-1/PD-L1 inhibitors.

"Histabolomics overcomes several hurdles in the application of metabolomics to human tissues."

Recall that the original test for estrogen receptors in breast cancer was a quantitative radioimmunoassay (RIA). It required relatively large amounts of fresh or cryopreserved tissue. IHC replaced the RIA method largely because it fits into the existing histology workflow for formalin-fixed, paraffin-embedded tissue, making its convenience obvious. Histopathology became the go-to approach for personalizing breast cancer treatment, and soon, the HER2 assay also became part of the tissue pathology toolkit.

IHC offers a powerful way to determine whether a protein is present in cells or tissue – and where that protein of interest is located. But how much is present? We're able to say to some extent, but accurate quantitation remains challenging. Morphometric algorithms and technical automation don't overcome the intrinsic variables that affect IHC, such as fixation, tissue processing, antigen retrieval, antibody avidity, antibody titer, and chromagen development. So is it reasonable to assume that IHC can deliver everything we need?

Smaller molecules (<2000 kD), such as metabolites, are largely undetectable by IHC. Metabolites are part of the wider

"It is possible to imagine [a future] where quantitative, normalized metabolomic data is combined with histopathology, DNA sequencing, RNA expression and IHC."

"omics" landscape, and metabolic changes have been associated with cancer since the initial description of the Warburg effect in 1956. Metabolomic studies have really evolved since those early days, and my colleagues and I have recently developed a way of incorporating metabolomics into the histopathology workflow by using alcohol as a primary tissue fixative, followed by secondary fixation in formalin and embedding in paraffin. The alcohol extracts metabolites and lipids, but preserves tissue architecture and allows proteins, RNA and DNA to remain in the tissue. Then, we use liquid chromatography-mass spectrometry (LC-MS) to determine the metabolites in the alcohol. Thus, we can perform repeated microscopic analyses on the same tissue – just as we have always done.

Our new method, for which we have coined the term "histabolomics," overcomes several hurdles in the application of metabolomics to human tissues. One such hurdle is the small size of human tissue biopsies; an assay that competes for tissue will not be welcomed onto the playing field. Another is the need for normalization – the expression of the quantity of an analyte per unit of sample. In clinical chemistry, the analyte is expressed per volume of serum or plasma; tissue RNA is often expressed in relation to a housekeeping gene. But all of these approaches require extraction and disruption of the tissue. Normalization of metabolomics data is usually performed "post-acquisition," when the LC-MS data are analyzed, processed, and normalized in relation to total ion counts or similar values – or by tissue weight. Even if the tissue is weighed, the amounts of disease or tumor relative to non-diseased tissue and stroma remain variable. Histabolomics downscales the method to accommodate as little as 5 mg of tissue, the typical yield of an 18-gauge core needle biopsy. This bypasses the need for tissue cryopreservation, commonly used for metabolomics. When combined with a chemical labeling technique, the method is quantitative and normalized.

Histabolomics is complementary to existing methods of RNA and DNA analysis, and to in situ methods such as FISH or CISH. Both tissue metabolomics analysis and routine histopathology and IHC can be performed on exactly the same tissue. Although it's inconceivable to picture a future without IHC, it is entirely possible to imagine one where quantitative, normalized metabolomic data from human tissues is combined with histopathology, DNA sequencing, RNA expression and IHC to enhance clinical decision-making.

Such an approach is useful in distinguishing aggressive from indolent cancers, and my colleagues and I suggest that it could also be applied to other medical conditions where we operate with sparse or imperfect data – inflammatory bowel diseases, liver diseases, identification of drug targets, toxicology and more. In my opinion, pathologists should pursue diagnostic methods that yield as much information as possible, with as little impact as possible on the patient – and our histabolomics approach fits nicely into that picture.

HUMANITY IN SCIENCE AWARD

the Analytical Scientist

In partnership with

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Richard Jähnke

Meet the Winner

Richard Jähnke

Richard Jähnke from the Global Pharma Health Fund (GPHF) has received the 2017 Humanity in Science Award for "development and continuous improvement of GPHF Minilab™ (www.gphf.org), which represents a breakthrough for the rapid and inexpensive identification of substandard and falsified medicines in low- and middle income countries in Africa, Asia and Latin America".

Richard received his award at a special jubilee reception in Berlin, Germany on October 2, 2017 hosted by KNAUER to celebrate the company's 55th birthday this year. Read more about Richard's work on page 34.

Could it be you in 2018?

Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

Has your own work had a positive impact on people's health and wellbeing? Details of the 2018 Humanity in Science Award will be announced soon.

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Landmark Literature

As a new year begins, we turn to 2017 for inspiration, asking experts from across analytical science to select one paper that stood out from the crowd.

Good Vibrations

By Karen Faulds, Professor of Chemistry, University of Strathclyde, UK.

Enhanced-Raman spectroscopy is an excellent tool for probing biochemical processes – the molecular information that can be gained from the vibrational fingerprint spectra produced is invaluable. Raman has led to great advances in non-destructive analysis in recent years, particularly in the biomedical field, where it can be used to non-invasively probe systems, such as single cells, to gather information-rich spectra. However, though the technique can be used to gain molecular information at sub-cellular resolution, sensitivity is limited by the intrinsically weak signals obtained from Raman scattering. In addition, it can be challenging to track specific or labeled molecules within complex biological matrices, because of the complexity of the multi-component spectra obtained.

Therefore, it was with great interest that I read a recent Nature paper from Columbia University researchers on the use of stimulated Raman scattering (SRS). SRS uses non-linear Raman effects to enhance the Raman signal, and Wei and colleagues reported using pre-resonance electronic SRS (epr-SRS) to enhance signals by up to 1,000 times compared with non-resonant SRS. It allowed the authors to use commercially available fluorescent dyes, as well as synthesizing their own suite of dyes containing alkyne bands (C to C triple bonds), with a view to carrying out multiplex imaging. Multiplexing – the detection of multiple analytes simultaneously – is highly desirable in imaging, as it allows tracking of multiple species with subcellular resolution. However, multiplex imaging has traditionally been carried out using fluorescence, which is limited in the number of species that can be detected using one excitation wavelength. In addition, the broad nature of fluorescence bands can make deconvolution of the data challenging. Therefore, using vibrational techniques with narrower peak widths for multiplexing could bring major benefits, and this work demonstrates the potential for 24 different resolvable codes.

> *"Multiplexing – the detection of multiple analytes simultaneously – is highly desirable in imaging."*

The use of alkyne labels – first reported by Sodeoka and colleagues (1) for imaging of alkyne-tagged EdU in cells using normal Raman – is particularly attractive, as the alkyne band falls in the so-called cellular silent region (1800–2800 cm^{-1}) of the spectrum – a region where there are no vibrational bands from the cell components. Wei's group used the imaging approach to observe metabolic activity in the form of DNA replication and protein synthesis in hippocampal neuronal cultures and cerebellar tissue. The increased sensitivity and rapid imaging achieved by epr-SRS in combination with multiplexed dyes make this a very powerful technique for cellular imaging and provides a compelling argument for using SRS over fluorescence in future studies.

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Karen's Landmark Paper

L Wei et al., "Super-multiplex vibrational imaging", Nature, 544, 465 (2017).

Closing the Gap

By Martin Giera, Head, Metabolomics Group, Leiden University Medical Center (LUMC), Leiden, the Netherlands.

Asked to put forward my landmark paper of 2017, I didn't hesitate; for me, it has to be an article from Huan et al. in the Siuzdak group. The paper fills an important gap – for me personally, but also for the metabolomics community.

The story starts in the 2000s, when I applied for a postdoc at the Scripps metabolomics center… and totally blew the interview. Looking back, untargeted metabolomics involved too much programming for a wet-lab scientist like me – I wasn't ready for it, or perhaps it wasn't ready for me!

Ten years later, and I recently joined the group as a visiting professor. I had a wonderful time, with many intriguing scientific discussions; even when we could not agree on certain concepts or ideas, the general idea of combining systems biology and (untargeted) metabolomics always prevailed. There is no doubt about the value of integrating multi-omics datasets to obtain a better systems-wide understanding of biology – and eventually manufacture tools shaping biological phenomena to our needs. However, there are several gaps in our current capabilities. One is that statistically relevant information needs to be extracted from different types of data and integrated to give a detailed mechanistic understanding of molecular biology – and that's not just about the numbers, it's also about people; scientists from different fields need to understand each other and work together hand in hand.

Though some software tools, such as MetaboAnalyst, allow for integrated pathway analysis using different datasets, in its 2017 paper the Siuzdak group for the first time described a completely integrated approach, using raw LC-MS data in the XCMS workflow. The work is a first step and many bottlenecks remain, but I am convinced that such approaches are the future when it comes to the generation of meaningful biological information from complex datasets. Ultimately, they will boost our success in the search for new diagnostics and therapeutics. Imagine an integrated platform like XCMS, with tens of thousands of users, bringing together people from different fields and stimulating their interaction. This landmark paper closes the gap between untargeted metabolomics and systems biology, and demonstrates that untargeted metabolomics is ready for deeper biological meaning.

"The work is a first step and many bottlenecks remain, but I am convinced that such approaches are the future when it comes to the generation of meaningful biological information from complex datasets."

Martin's Landmark Paper

T Huan et al., "Systems biology guided by XCMS Online metabolomics", Nat Methods, 14, 461–462 (2017).

Manipulating the Matrix

By Jhanis J Gonzalez, Lawrence Berkeley National Laboratory, Environmental Energy Technologies Division, Laser Technologies Group, Berkeley, California, USA.

The paper I have chosen addresses a major roadblock in advancing laser ablation for routine chemical analysis – sample preparation and matrix manipulation. The authors present a new approach to solid matrix modification – ammonium bifluoride (NH_4HF_2) digestion – which helps eliminate matrix effects and opens the door for wider implementation of laser ablation-based techniques into bulk analysis (1,2).

As described in the article, digesting silicate rock samples with ammonium bifluoride causes complete breakdown of silicate minerals, to create an ultrafine powder with a very consistent size and shape of grains. Compared with powders achieved by milling, there is a reduced risk of sample contamination, and the method is faster and more eco-friendly than traditional acid digestion. Essentially, the technique converts varied solid matrices into the same matrix, thus eliminating matrix match requirements and matrix effects between samples during chemical analysis by laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS). The work is a game changer – not only can the approach be used to modify solid sample matrices, but it also serves as a new way to prepare solid standards, and so on. In the next few years, I hope to see a surge of applications based on this approach across a number of fields.

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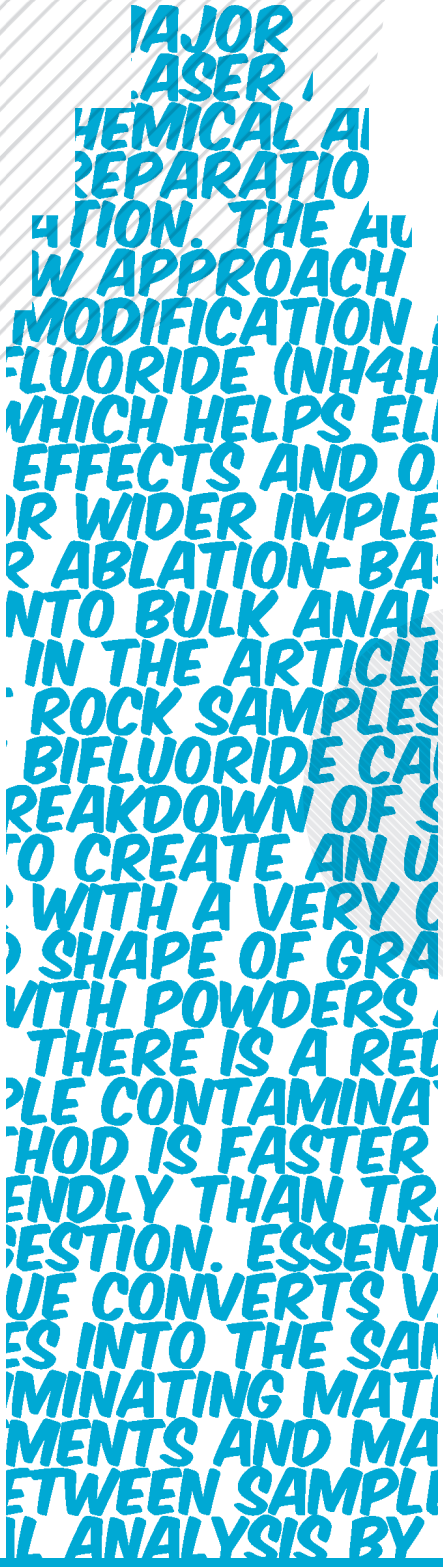
2. *MJ O'Hara et al., "Decomposition of diverse solid inorganic matrices with molten ammonium bifluoride salt for constituent elemental analysis", Chem Geol, 466, 341–351 (2017).*

"Not only can the approach be used to modify solid sample matrices, but it also serves as a new way to prepare solid standards."



Jhanis's Landmark Paper

W Zhang et al., "Quantitative analysis of major and trace elements in NH_4HF_2-modified silicate rock powders by laser ablation – inductively coupled plasma mass spectrometry", Anal Chim Acta, 983, 149–159 (2017).

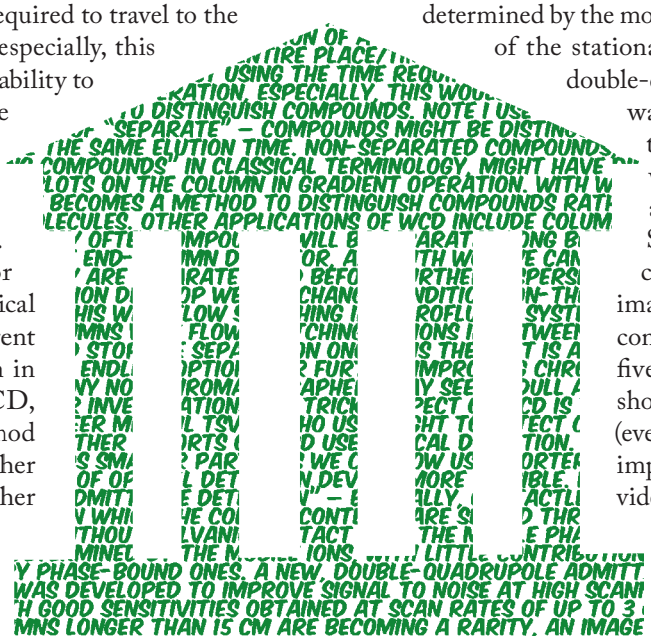
The New Old-Fashioned Way

By Hans-Gerd Janssen, Science Leader Analytical Sciences, University of Amsterdam and Unilever Research, Amsterdam, the Netherlands.

"The mature technique of chromatography still has tricks up its sleeve!"

"Whole column detection (WCD) is as old as chromatography itself."
"In Tsvet's first experiment on chromatography of plant pigments, he necessarily carried out visual whole column detection (WCD)."

These are just two quotes from an excellent article that showed us once again that the mature technique of chromatography still has tricks up its sleeve! In regular chromatography, the criterion for peak identification is solely the time of appearance of the analytes at the column exit. Unfortunately, many compounds have the same retention time. If we could track the location of a compound as it travels along the column, we could use the entire place/time plot for compound assignment, rather than just using the time required to travel to the detector. In gradient operation, especially, this would significantly increase our ability to distinguish compounds. Note I use the word "distinguish" instead of "separate" – compounds might be distinguishable even if they have the same elution time. Non-separated compounds, or "co-eluting compounds" in classical terminology, might have different place/time plots on the column in gradient operation. With WCD, chromatography becomes a method to distinguish compounds rather than to separate molecules. Other applications of WCD include column length reduction. Very often compounds will be separated long before they are seen by the end-column detector, and with WCD we can detect them as soon as they are separated and before further dispersion. Finally, if we see separation develop we can change conditions on-the-go. Imagine if we combine this with flow switching in microfluidic systems – using segmented columns with flow-switching options in between two segments, we could stop the separation once it is there. It is another proof that there are endless options for further improving chromatography, a technique many non-chromatographers may see as dull and not requiring further investigations.



The tricky aspect of WCD is the detection. Like early pioneer Mikhail Tsvet, who used light to detect compounds, virtually all other reports on WCD use optical detection. With recent trends towards smaller particles we can now use shorter columns, making arrays of optical detection devices more feasible. My landmark article uses "admittance detection" – basically, contactless conductivity detection, in which the column contents are sensed through the column wall without galvanic contact with the mobile phase. Read-out is mainly determined by the mobile ions, with little contribution of the stationary phase-bound ones. A new, double-quadrupole admittance detector was developed to improve signal to noise at high scanning rates, with good sensitivities obtained at scan rates of up to 3 cm/s. Since columns longer than 15 cm are becoming a rarity, an image reflecting the position of all compounds can be obtained every five seconds, or even faster for shorter columns. The drawings and (even better!) videos in the article are impressive. In one gradient elution video, a trailing analyte band can be seen "overtaking" one that was initially ahead – something that would never have seen by an end-column detector.

Hans-Gerd's Landmark Paper

BN Stamos, PK Dasgupta, S-I Ohira, "Admittance scanning for whole column detection", Anal Chem, 89, 7203–7209 (2017).

Love Is in the Air

By Victoria F. Samanidou, Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece.

No matter what matrix is analyzed, what analyte is determined, or which analytical technique is applied, sample preparation is a crucial step. My choice for the landmark publication of 2017 brings the benefits of fabric phase sorptive extraction (FPSE) to gaseous samples.

FPSE is a sample preparation approach that was introduced in 2014 by Kabir and Furton (1). When it first came to my attention, I was impressed by its simplicity and efficiency – it requires no matrix modification or clean-up and still achieves great performance, effectively assimilating most of the benefits of solid-phase microextraction while avoiding most of the shortcomings of conventional sample preparation techniques. FPSE's versatility makes it a suitable technology for a wide range of applications and allows it to resolve diverse analytical problems.

There are already hundreds of sol-gel coatings that can be readily used as sorbents for FPSE, with unique selectivity and affinity towards an extensive range of target analytes. In our laboratory we have used the approach in the analysis of milk and biological fluids for the determination of several compounds, such as endocrine disrupters, antibiotics and antidepressants by HPLC (2–6), and metals by flame atomic absorption spectrometry (7).

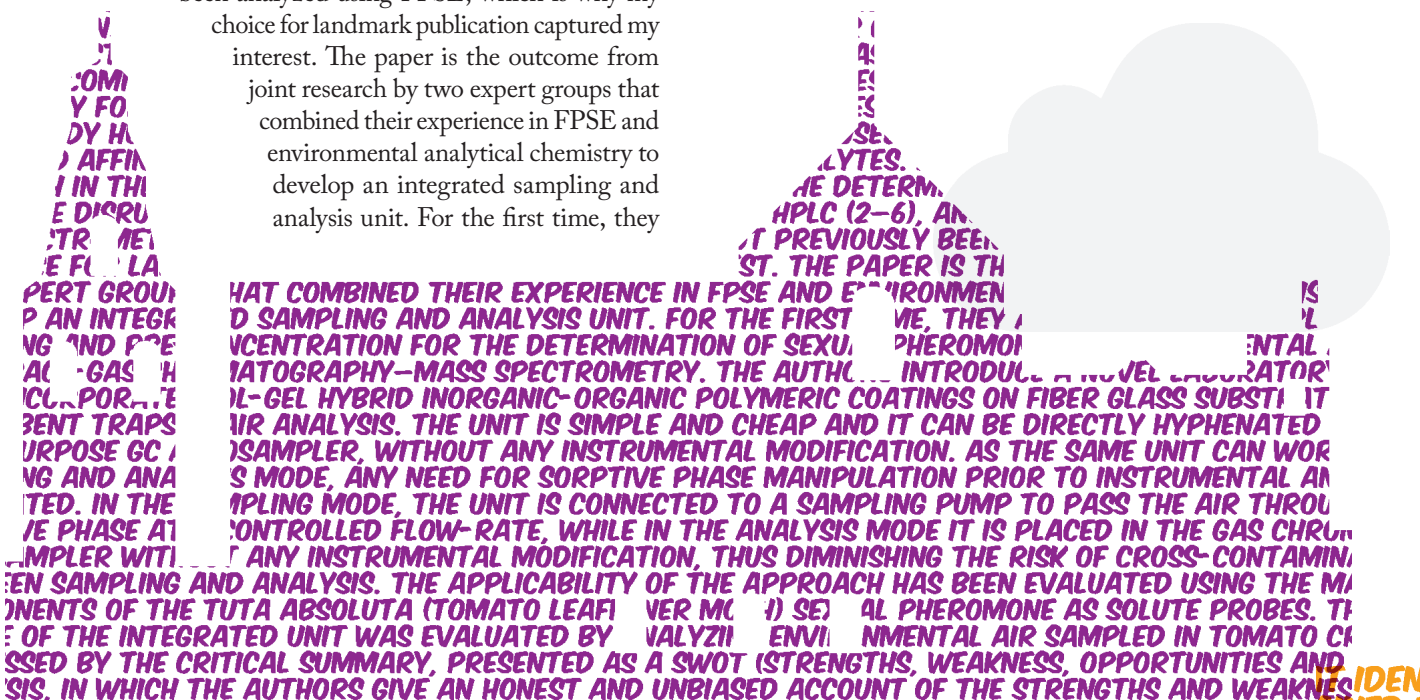
However, gaseous samples have not previously been analyzed using FPSE, which is why my choice for landmark publication captured my interest. The paper is the outcome from joint research by two expert groups that combined their experience in FPSE and environmental analytical chemistry to develop an integrated sampling and analysis unit. For the first time, they applied FPSE principles to air sampling and pre-concentration for the determination of sexual pheromones in environmental air by headspace-gas chromatography–mass spectrometry.

The authors introduce a novel laboratory-built unit that incorporates sol-gel hybrid inorganic-organic polymeric coatings on fiber glass substrate surface as sorbent traps in air analysis. The unit is simple and cheap and it can be directly hyphenated with a multipurpose GC autosampler, without any instrumental modification. As the same unit can work under sampling and analysis mode, any need for sorptive phase manipulation prior to instrumental analysis is eliminated. In the sampling mode, the unit is connected to a sampling pump to pass the air through the sorptive phase at a controlled flow-rate, while in the analysis mode it is placed in the gas chromatograph autosampler without any instrumental modification, thus diminishing the risk of cross-contamination between sampling and analysis.

The applicability of the approach has been evaluated using the major components of the *Tuta absoluta* (tomato leafminer moth) sexual pheromone as solute probes. The performance of the integrated unit was evaluated by analyzing environmental air sampled in tomato crops.

I was impressed by the critical summary, presented as a SWOT (strengths, weakness, opportunities and threats) analysis, in which the authors give an honest and unbiased account of the strengths and weaknesses of their proposed method, and identify key points for further investigation.

The results presented in my landmark paper open a door to further FPSE applications, enabling the determination of volatile and semi-volatile compounds in air – and once again prove that imagination in sample preparation and sample handling has no limits.



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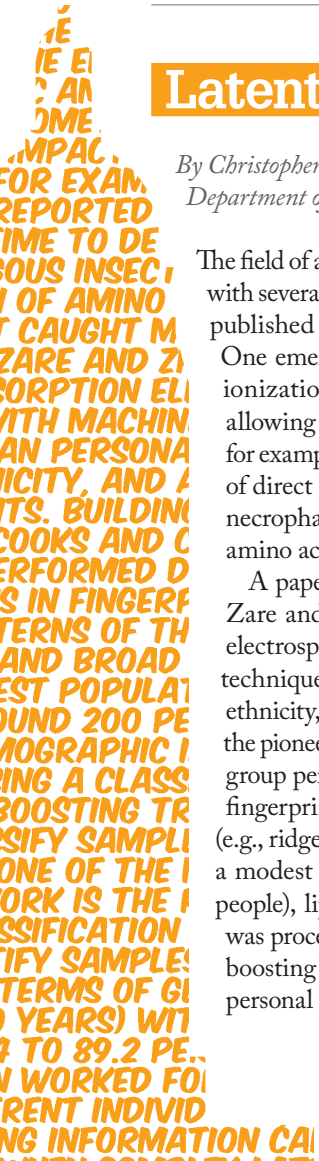
Victoria's Landmark Paper

MC Alcudia-León et al., "Integrated sampling and analysis unit for the determination of sexual pheromones in environmental air using fabric phase sorptive extraction and headspace-gas chromatography–mass spectrometry", J Chromatogr A, 1488, 17–25 (2017).

Latent Talents

By Christopher C. Mulligan, Professor of Analytical Chemistry, Department of Chemistry, Illinois State University, USA.



The field of ambient mass spectrometry continues to flourish, with several reports of novel methodologies and applications published in the top analytical journals during 2017. One emerging trend was combining simplistic ambient ionization methods with chemometric processing, allowing rapid, yet impactful, profiling of target samples; for example, Rabi Musah and co-workers reported the use of direct analysis in real time to determine the species of necrophagous insect eggs by statistical examination of amino acid profiles (1).

A paper that caught my eye was the work of Richard Zare and Zhenpeng Zhou, who combined desorption electrospray ionization (DESI) with machine learning techniques to glean personal information (e.g., gender, ethnicity, and age) from latent fingerprints. Building upon the pioneering work by Cooks and co-workers (2), the Zare group performed DESI imaging of lipid distributions in fingerprints, yielding both spatial patterns of the print (e.g., ridges, furrows) and broad chemical mapping. Using a modest population of study participants (around 200 people), lipid distribution and demographic information was processed using a classification algorithm (gradient-boosting tree ensemble) in an effort to classify samples by personal characteristics.

One of the most intriguing aspects of this work is the reported accuracy of their classification model, which was able to identify samples from a diverse test set in terms of gender, ethnicity and age (within 10 years) with accuracies ranging from 82.4 to 89.2 percent. The methodology even worked for overlapping prints from different individuals, suggesting that identifying information can still be obtained even when complex latent fingerprint evidence is present. Furthermore, smeared fingerprints that have lost the spatial patterns needed for dactyloscopy can still yield information of probative value in criminal investigations. In terms of forensic processing of fingerprint evidence, this method could take Locard's exchange principle that "every contact leaves a trace" to a whole new level.

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Christopher's Landmark Paper

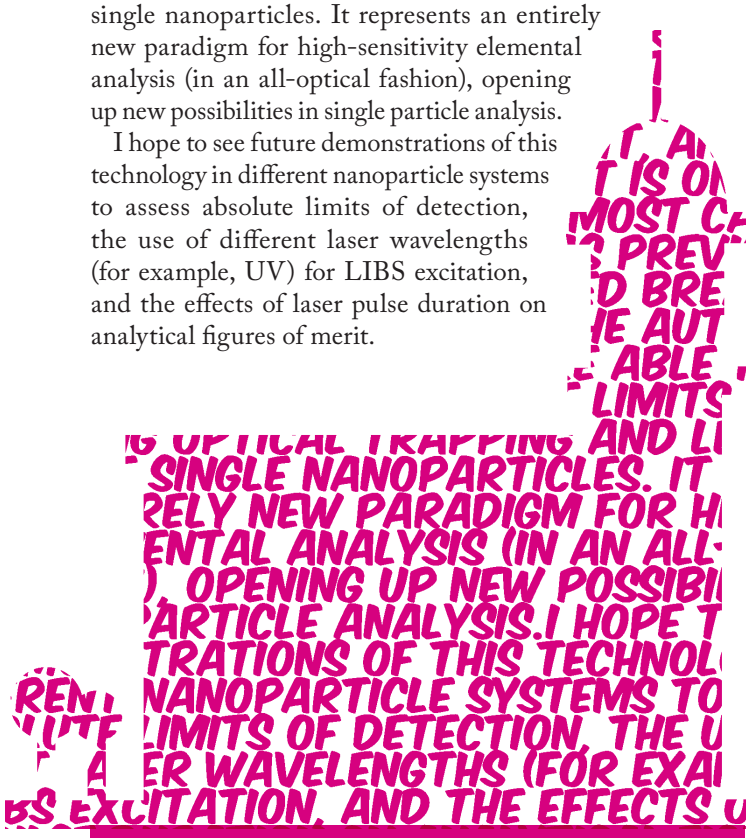
Z Zhou, RN Zare, "Personal information from latent fingerprints using desorption electrospray ionization mass spectrometry and machine learning. Anal Chem, 89, 1369–1372 (2017).

Attogram-Scale LIBS

By Vassilia Zorba, Group Leader, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

Nanoparticle analysis is extremely useful in many disciplines and applications. Optical excitation techniques are limited in resolution by the diffraction limit, and imaging below the diffraction limit is only possible with near-field optics in most cases. Single nanoparticle analysis was previously unattainable with laser induced breakdown spectroscopy (LIBS), but the authors of my 2017 landmark paper were able to achieve attogram-scale absolute limits of detection by using optical trapping and levitation to isolate single nanoparticles. It represents an entirely new paradigm for high-sensitivity elemental analysis (in an all-optical fashion), opening up new possibilities in single particle analysis.

I hope to see future demonstrations of this technology in different nanoparticle systems to assess absolute limits of detection, the use of different laser wavelengths (for example, UV) for LIBS excitation, and the effects of laser pulse duration on analytical figures of merit.



Vassilia's Landmark Paper

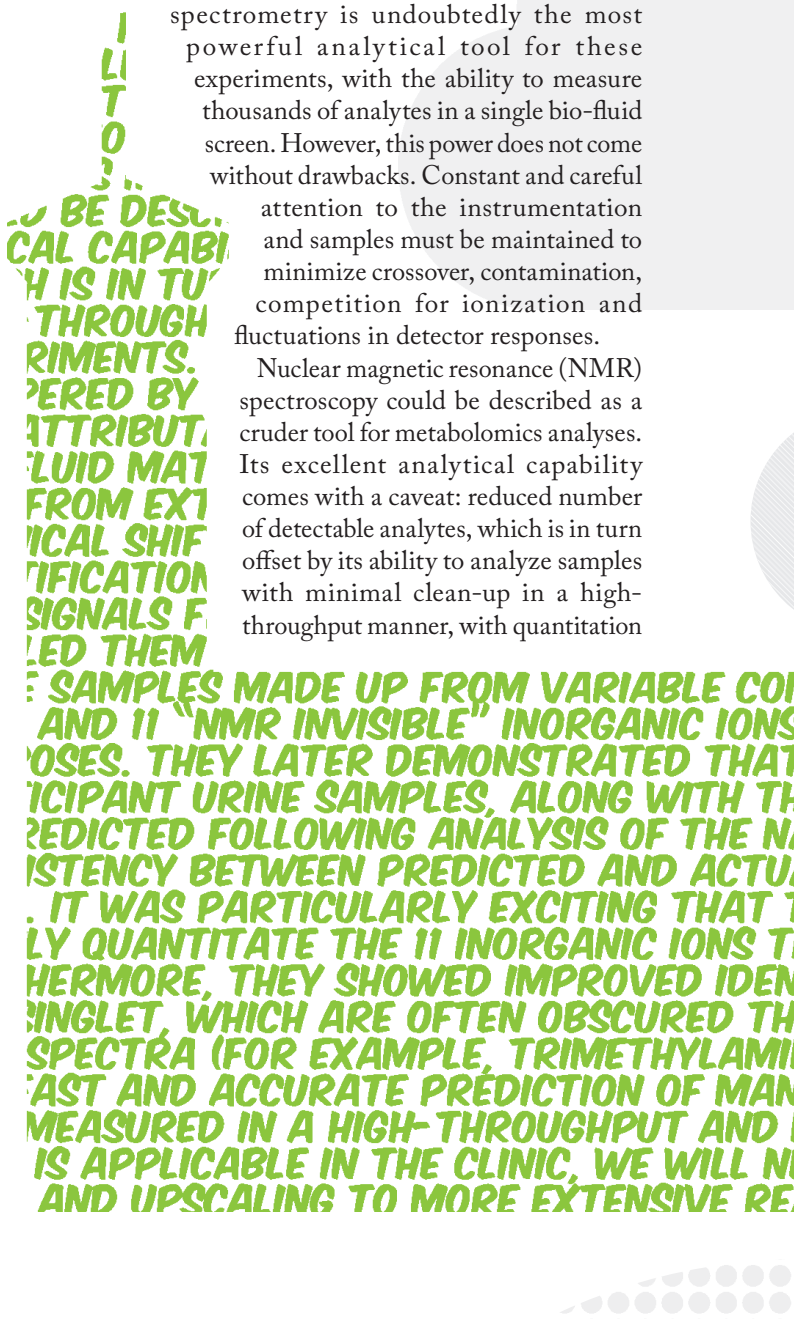
P Purohit, FJ Fortes, JJ Laserna, "Spectral identification in the attogram regime through laser-induced emission of single optically trapped nanoparticles in air", Angew Chem Int Ed, 56, 14178 –14182 (2017).

Shifting Gears

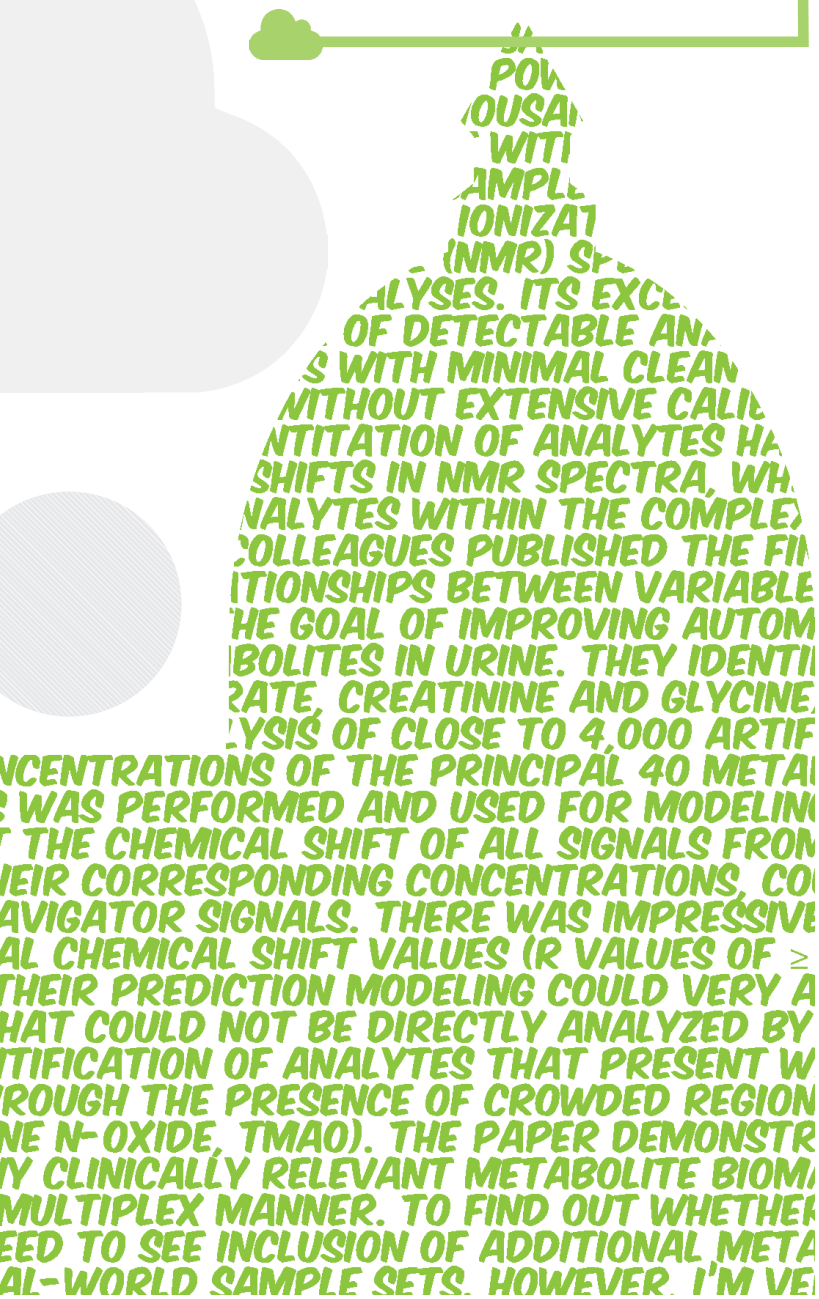
By Liam Heaney, Research Associate, Department of Cardiovascular Sciences, University of Leicester, Leicester, UK.

As a researcher with an interest in both targeted and non-targeted metabolomics, it is encouraging to see the constant evolution of technical and computational advances for the analysis of metabolites in complex biological samples such as urine and plasma. Mass spectrometry is undoubtedly the most powerful analytical tool for these experiments, with the ability to measure thousands of analytes in a single bio-fluid screen. However, this power does not come without drawbacks. Constant and careful attention to the instrumentation and samples must be maintained to minimize crossover, contamination, competition for ionization and fluctuations in detector responses.

Nuclear magnetic resonance (NMR) spectroscopy could be described as a cruder tool for metabolomics analyses. Its excellent analytical capability comes with a caveat: reduced number of detectable analytes, which is in turn offset by its ability to analyze samples with minimal clean-up in a high-throughput manner, with quantitation



"There was impressive consistency between predicted and actual chemical shift values."



possible without extensive calibration experiments. That said, automatic detection and quantitation of analytes has been hampered by the variability of metabolite chemical shifts in NMR spectra, which are attributable to multifaceted interactions of analytes within the complex bio-fluid matrices.

In 2017, Claudio Luchinat and his colleagues published the findings from extensive experiments to clarify the relationships between variable chemical shifts and analyte concentrations, with the goal of improving automatic identification and subsequent quantitation of metabolites in urine. They identified five signals from three main urine metabolites (citrate, creatinine and glycine) and labeled them as navigator signals. Subsequent analysis of close to 4,000 artificial urine samples made up from variable concentrations of the principal 40 metabolites and 11 "NMR invisible" inorganic ions was performed and used for modeling purposes. They later demonstrated that the chemical shift of all signals from participant urine samples, along with their corresponding concentrations, could be predicted following analysis of the navigator signals.

There was impressive consistency between predicted and actual chemical shift values (R values of ≥ 0.98). It was particularly exciting that their prediction modeling could very accurately quantitate the 11 inorganic ions that could not be directly analyzed by NMR. Furthermore, they showed improved identification of analytes that present with one singlet, which are often obscured through the presence of crowded regions of NMR spectra (for example, trimethylamine N-oxide, TMAO).

The paper demonstrates the fast and accurate prediction of many clinically relevant metabolite biomarkers, measured in a high-throughput and multiplex manner. To find out whether this tool is applicable in the clinic, we will need to see inclusion of additional metabolites and upscaling to more extensive real-world sample sets. However, I'm very hopeful that these initial results will stand the test of translation to more demanding scenarios.

A beta version of the team's NMR modeling tool can be found at http://150.217.146.252:8080.

Liam's Landmark Paper

PG Takis et al., "Deconvoluting interrelationships between concentrations and chemical shifts in urine provides a powerful analysis tool", Nat Commun, 8, 1662 (2017).

Arsenic: an Old Trace

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

For several years, my research group has been developing methods to measure potentially hazardous arsenic compounds in our diet. However, establishing an unambiguous link between the consumption of particular foodstuffs and ill health is an extremely challenging proposition. No single research group working over a limited timescale could produce the necessary data to answer important societal questions, such as: "To what extent are we being poisoned by what we eat?" Researchers need to examine the results from multiple studies over relatively long time periods. Review articles by recognized experts are, therefore, of critical importance.

The landmark article I have chosen is one such a review article. It addresses the question: "Is there evidence that eating rice increases arsenic exposure?" The information needed to answer this question illustrates very nicely the role of analytical chemistry in supporting ongoing studies of major humanitarian health problems.

We now know that the foodstuff that contains the highest concentrations (triple digit µg per kg) of inorganic arsenic (a non-threshold human carcinogen) is rice. We also know that rice and tuna contain similar concentrations of arsenic in the form of dimethylarsinate (less toxic to humans, but probably not innocuous). Although scientists make such statements on a regular basis, it is important to remember that inorganic arsenic and dimethylarsinate are the species that appear in solution as a result of the sample preparation procedure. So we know that rice-eaters are probably eating more inorganic arsenic than non-rice eaters. It is necessary to specify the chemical species, because fish-eaters are consuming much higher amounts of arsenic per serving, as fish contains high concentrations (up to 5 mg per kg) of arsenobetaine, a tetramethylarsonium derivative that is thought to be harmless.

To assess exposure, it is necessary to measure the internal dose. As the internal dose cannot be measured directly, biomarkers are used; the concentrations of arsenic species in urine is the most widely studied biomarker, but nails, hair, and blood have also been analyzed. The reviewers identified 16 studies whose designs ensured that the arsenic species in urine could be correlated with the consumption of rice, specifically. Most of the studies involved the determination, by HPLC-ICP-MS, of inorganic arsenic and dimethylarsinate (whose concentration is derived from the metabolism of inorganic arsenic – via monomethylarsonate – and from the ingestion of dietary dimethylarsinate) and compared the values obtained with those obtained from the urine of non-rice eaters.

The reviewers scrutinized the study design and statistical tests applied, to ascertain whether the differences observed were significant. They concluded that, despite the variations in study design, and ethnicity and age of participants, the results showed a consistent positive association between rice intake and arsenic exposure.

> *"We know that rice-eaters are probably eating more inorganic arsenic than non-rice eaters."*

Julian's Landmark Paper

MA Davis et al., "Assessment of human dietary exposure to arsenic through rice", Sci Tot Environ, 586, 1237–1244 (2017).

The Shape of Things to Come

By Koen Sandra, Scientific Director, Research Institute for Chromatography, Kortrijk, Belgium; co-founder and R&D Director anaRIC biologics, Ghent, Belgium.

It will come as no surprise that my selected paper is related to biopharmaceutical analysis. Biopharmaceuticals are becoming a core aspect of the pharmaceutical industry and, in recent years, we have observed many advances in the analysis of these huge and complex structures. 2017 saw a number of exciting papers and lectures in the field – a few that spring to mind are:

- Four-dimensional liquid chromatography (4D-LC) in hyphenation to mass spectrometry (MS) for the characterization of monoclonal antibodies (mAbs) (1);
- Detailed characterization of the glycosylation profile of the fusion protein etanercept by high-resolution native MS (2);
- Hydrophilic interaction chromatography (HILIC) for the separation of mAbs at middle-up level (3);
- Direct coupling of cation exchange chromatography (CEX) to MS to directly assign a molecular weight (MW) value to acidic and basic variants of mAbs (4).

And though all of these developments are landmarks in their own right, the paper I ultimately selected is related to higher-order structural analysis of proteins using mass spectrometry. The higher-order structure (secondary, tertiary, quaternary) defines the function of a protein and as such contributes to the quality, safety and efficacy of biopharmaceuticals. At protein level, higher-order structures can be assessed using native MS and ion mobility MS (IM-MS). Higher-order structures can also be assessed at peptide level, despite the fact that all structural information is lost upon generating peptides. Limited proteolysis can provide insight into higher-order structure but nowadays we typically rely on more advanced tools, such as cross linking, footprinting and hydrogen deuterium exchange (HDX). HDX is especially promising and is quickly being implemented in biopharmaceutical analysis. The principle is simple but clever. Upon placing a protein in a D_2O solution, amide hydrogens can exchange with deuterium based on their solvent accessibility. Exposed and dynamic regions exchange rapidly, while rigid and protected regions exchange more slowly. Peptide measurements following labeling and digestion allow discrimination of these regions (due to the mass shift induced by deuterium) and so provide an indirect read-out of the higher-order structure.

In my chosen paper, the authors demonstrate the applicability of HDX-MS to monitor structural changes caused by chemical modifications. Though earlier HDX-MS studies in a biopharmaceutical context have typically focused on major structural differences, what makes this paper stand out is the sensitivity of the workflow with its ability to detect tiny local structural differences. Indeed, an increase in oxidation of just one percent in the conserved region of a mAb (methionine at position 254) resulted in statistically relevant deuterium uptake differences. Furthermore, the article demonstrates the importance of studying biopharmaceuticals using different complimentary techniques. Indeed, chromatographic and spectroscopic techniques – size exclusion chromatography (SEC), CEX, circular dichroism (CD) and Fourier-transform infrared (FT-IR) – were used to monitor global structural changes, indicating preserved structural integrity.

HDX-MS is extremely sensitive to back-exchange (D → H), which results in the loss of structural information and so demands substantial user expertise. All post-labeling handling involves compromises; digestions are typically performed at low pH using pepsin (as opposed to neutral pH using trypsin in traditional peptide mapping experiments) and separations are performed at very low temperatures (as opposed to elevated temperatures). The authors perfectly understand the weaknesses of the technique and took sensible precautions to limit back-exchange, dictating the success of their work. The set-up involves a nice interplay of valves and columns, similar to that in a 2D-LC set-up. Indeed, the deuterated mAb is injected on a pepsin column for digestion (low pH, 15°C), before the resulting peptides are directed to a trapping column and subsequently eluted from the trap onto the analytical column operated at 0°C for high-resolution MS and MS/MS measurements.

In the coming years, we expect this methodology to be more widely applied during the development of biopharmaceuticals. In the development of biosimilars, a comprehensive comparability exercise to the innovator product is required to demonstrate similarity in terms of physicochemical characteristics, efficacy and safety. In that respect, an enormous weight is placed on analytics, and the analytical package for a biosimilar submission is considerably larger than that of an innovator product. In my opinion, the value of HDX-MS data in such dossiers is indispensable. Further developments are needed to take this tool out of the hands of the experts and into routine use, but with instrument vendors now offering HDX-MS workflows, this seems only a matter of time.

To my great satisfaction, my landmark paper demonstrates that cutting-edge research is not the sole preserve of academic laboratories, but is also being performed within industry.

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Koen's Landmark Paper

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TAKING DOWN A GOLIATH

Big pharma, vulnerable supply chains, an international religious network… The story of the Global Pharma Health Fund reads like the plot of a conspiracy novel. But for its developer Richard Jähnke – winner of the 2017 Humanity in Science Award – the reality of fighting counterfeit medicine is far more prosaic. Equipped with his sling – a case full of chemicals, a basic TLC test, and a training manual – his aim is simple: to help spot fakes before they reach consumers.

By Joanna Cummings

Counterfeit medicines are a problem of epidemic proportions, particularly in resource-poor countries. In 2000, the World Health Organization (WHO) reported (1) that of all poor quality and substandard counterfeit falsified products, 80 percent do not contain any active ingredients, do not contain enough active ingredient, or even contain the wrong ingredients – leaving patients with drugs that are at best ineffective, and at worst potentially fatal. Complex supply chains provide too many opportunities for adulteration, while understocked labs and expensive analysis equipment only compound the problem of detection, particularly in countries with limited financial resources and patchy power supplies. What is needed is a simple, low-cost and transportable analytical toolkit to protect the supply chain – and, ultimately, consumers.

A tough challenge, perhaps. But Richard Jähnke, as part of the Global Pharma Health Fund (GPHF, based in Frankfurt - a charitable intiative led by Merck, Germany) has spent the last 20 years working with a small team of chemists and pharmacists from universities and the pharmaceutical industry in Germany to develop and deliver a life-saving solution. Its name? The "GPHF-Minilab."

A bicycle built for… chemical analysis

How did the Minilab come into being? Jähnke, a former Principal Scientist at Beecham Pharmaceuticals, UK, and Business Development Manager at the German branch of PCI Pharmaceutical Services, Philadelphia, recalls his bold (and wonderfully naïve) declaration on finishing his pharmacy degree in Bonn: "I was talking to friends about what we were going to do with our lives. I said I wanted to go into international health development work – and that I would try to make sure the pharmaceutical industry was paying for it. It was a big statement at that time!" In search of a worthy venture after being awarded a Master of Business Administration (and brushing up on his English language skills), a fortuitous meeting with the GPHF – and a subsequent discussion with the WHO – led to the birth of the Minilab project.

The WHO provided a clear but also challenging specification for the project: a test kit for rapid medicine verification and quality monitoring in the field, for low- and middle-income countries. The kit needed to be transportable, reliable, affordable, and unsophisticated, allowing people on the ground to monitor medicine quality with minimal training. It needed to be used by health or medicine-supplying facilities, as well as drug supply organizations in the private and public sectors, and in places with little access to fully fledged operational laboratories. But Jähnke is quick to point out that the Minilab was never intended as a laboratory replacement. "We wanted a 'complement' to the lab – when it's not in full working order, or one simply doesn't exist." He spent the next two years developing the Minilab.

Jähnke in no way takes full credit for the idea behind the kit, acknowledging the importance of good timing – and the foresight of his colleague Tom Layloff (Senior Environmental Health Advisor at the Partnership for Supply Chain Management), who he dubs the "grandfather" of the Minilab. "In 1985, there was a big WHO conference in Nairobi, about the quality of medicine in sub-Saharan Africa. People were aware of the circulation of fake medicine, and wanted to discuss ideas for improving pharmaceutical supply," he says. "Lots of observers from the industry were getting involved – but we needed to stop talking and take action. Tom was working for the FDA at that time, and started to develop some simple thin-layer chromatography test methods – but didn't have an appropriate toolbox and couldn't get funding for it. It wasn't the right time. Ten years later, it started to gain momentum."

Jähnke realized that to be a success, the Minilab must improve upon the accuracy of previous dye-testing methods, yet be cheaper than the HPLC methods used in the lab. "HPLC is the Mercedes Benz of instrumental analysis, but we only needed a bicycle," Jähnke explains. "In this context, we did not need fully fledged, sophisticated testing – detecting the absence of a drug is relatively easy." The resulting Minilab uses thin layer chromatography to test for the presence of 90 drugs, and also includes physical tests for degradation or solidification, which can prevent adequate release of the drug and thus render them useless.

As well as working with input provided by the WHO, the team consulted churches and faith-based organizations who are involved in health initiatives in low- and middle-income countries. "Such organizations gave input as to the cultural

> "We did not need fully fledged, **sophisticated testing** – detecting the absence of a drug **is relatively easy.**"


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"When you order or use a Minilab, **what is written in the text can be instantly performed.** There's a starter kit of chemicals, **and everything you need to do the job is right there.**"

background, and what would and wouldn't work," Jähnke says. "For example, when I came to develop the manual, they told me I needed far more extensive operational procedures than in the British or US pharmacopoeia. On the other hand, I was told the list of materials in the pharmacopoeia is too long; when people have to do a test on, say, amoxicillin, they would normally consult the pharmacopoeia, then run around the lab, identifying the equipment and chemicals needed – of which 50 percent were likely to be missing." To prevent the problem, the team had to include all the chemicals, reference standards and solvents needed, so that testing could be performed on the spot. "When you order or use a Minilab, what is written in the text can be instantly performed. There's a starter kit of chemicals, and everything you need to do the job is right there," says Jähnke.

Have lab, will travel

From a logistical standpoint, the Minilab comes in a heavy-duty flight case, which contains all the appropriate labware and consumables. The 'hub' weighs approximately 25kg, but a starter kit of about 20 boxes of chemicals and solvents is also included in the shipment. Sending scientific equipment to remote regions is a challenge, but Jähnke is proud to note a solid track record in his own supply chain. "We have sent Minilabs to every corner of the world – to the Philippines, to Tanzania, to Ghana – and although they have arrived late in some cases, we've never lost one in transit completely," he says.

The bulk of the Minilabs go to national medicine control labs or public health facilities run by the state. "At the beginning, we focused on the quality of antibacterial and antimalarial medicine. These are public health concerns, so it's the responsibility of the state to make sure the medicine is accessible and of good quality for people in that country," says Jähnke. If there is no manufacturing capability in the country itself, the state buys the medicine by tender process, probably cheaply from China or India. Medicine is delivered to central medical stores, then distributed to regional medical stores – and from there goes to general and referral hospitals. Faith-based drug supply can be even more complex.

For African countries in particular, churches have proved to be an invaluable partner in interactions with local communities. "UNICEF and other global tender organizations might order 10 Minilabs for Congo, but they will not tell me precisely where they are going. We procure and send the kits, but are disconnected from their use," he says. "I find with church groups, there is more of a rapport – I talk to them, I know them, and it's more transparent." And though the state might have to answer to its people, faith-based groups answer to a higher power. "They don't have much money and are quick to spot when they are being cheated. They track fake medicine down even more effectively than the police – because in their eyes, delivering counterfeit medicine with nothing inside is 'like cheating God'."

The human factor

Jähnke believes that cooperation with partners has played a crucial part in getting the Minilab in front of the right people and into the hands of those who benefit the most – describing the Minilab as "a success not only of science, but also of public relations." Recognizing the value of the Minilab, the US pharmacopoeia has helped to market the kit as part of its global health impact program.

"They are very well connected, with access to many governmental labs… but most importantly, they care about the technology," Jähnke says. The result of such support is few limitations in geographical reach, or in funding. "They were very good at negotiating with the US Agency of International Development to get the funding and, through the Center of Disease Control (CDC), they had access to every embassy. In terms of marketing, when they became involved in the project it was like a hot knife going through butter."

Working closely with the WHO has also been a real boost

What's Inside the Minilab?

- Glassware for sample extraction, preparation, pipetting and spotting
- High performance chromatographic plates
- Developing and detection chambers
- Electronic pocket balance
- UV lamps with different wavelengths
- A hot plate
- Calliper rules
- A full collection of secondary reference standards for 90 active ingredients
- A set of manuals providing simple operation procedures.

A three-point plan

Testing with the Minilab involves three steps:

1. A physical inspection scheme of dosage forms and associated packaging material for an early rejection of the more crudely presented counterfeits
2. A simple tablet and capsule disintegration test in order to verify label claims on enteric-coating and other modified-release systems
3. Easy-to-use thin layer chromatographic tests for a quick check on drug content, thus verifying label claims on potency.

Supplies include sufficient quantities in order to perform about 1,000 assays while ensuring that the total material costs for one test run do not exceed two Euros.

https://www.gphf.org/en/minilab/index.htm

> "Wherever I go, I travel there as a human being and, **ultimately, that's how you make connections.**"

to the Minilab team, affording a type of protection that might otherwise have been difficult to attain for a micro enterprise; Jähnke describes the relationship as "a gentleman's agreement" rather than contractual protection. "They told me that to survive as a small operation, I should follow them as long as I can."

Jähnke's own visibility has helped build public trust in the product. "I gave a presentation in Africa, and an audience member said, 'This is the first time I've seen a professor and not a politician!' They trust me because they can see I have no hidden agenda – I'm not telling them what they want to hear. The most interesting parts of this job go beyond the professional – wherever I go, I travel there as a human being and, ultimately, that's how you make connections."

But that's not to say that he has never caused a stir. The authorities in certain countries have been known to keep a watchful eye on his activities. "One Minister of Health admitted that the state was unable to carry out the testing themselves, but went on to say that it doesn't mean anyone else is allowed to do it. They reminded me that they were observing me – and that they could have thrown me out of the country at a moment's notice! But we always find a way around..."

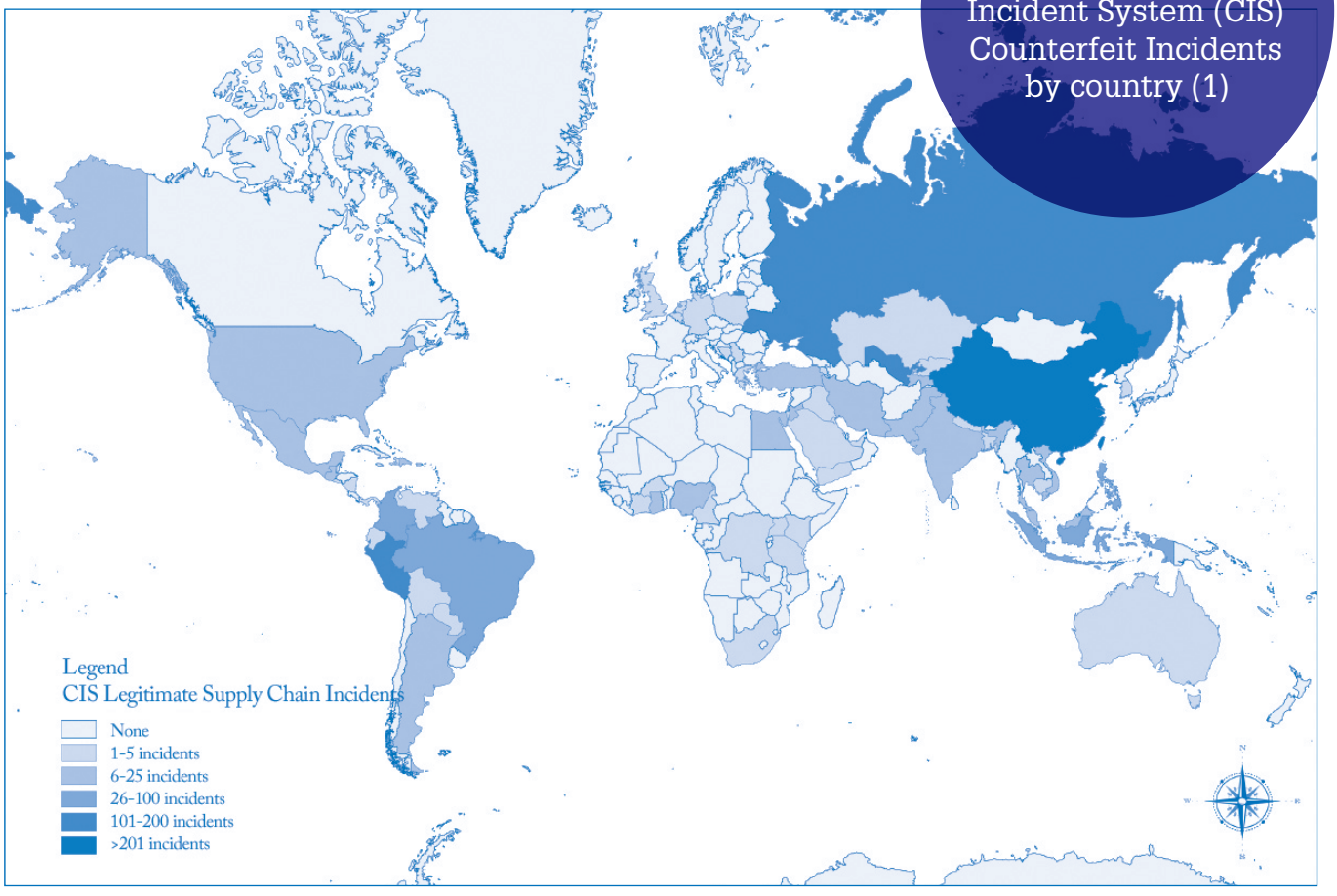
Put to the test – and then further optimized

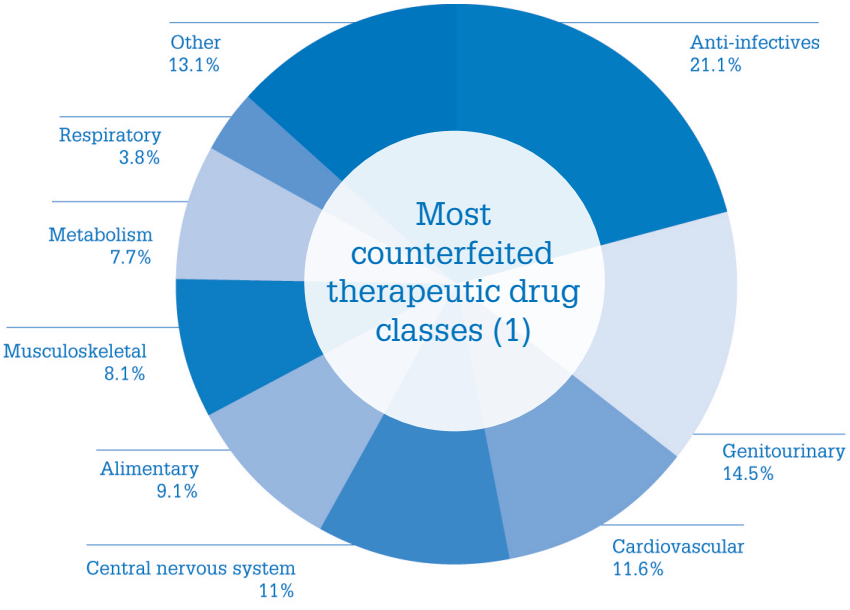
Jähnke was delighted to have the impact of the Minilab retrospectively confirmed by a WHO report. In a study to identify the scale of the problem of falsified medicine, the WHO checked 100 publications in 88 countries from the last ten years (2), comparing the Minilab with HPLC and other technologies. They checked the reports on 48,000 samples, of which 20,000 were tested by the Minilab – and of these, 1,000 were found to be fake or of extremely poor quality.

"We now know the impact of the Minilab statistically.

Counting the Cost
of Counterfeit Medicines

Global map of Counterfeit Incident System (CIS) Counterfeit Incidents by country (1)





Most counterfeited therapeutic drug classes (1)

An estimated **1 in 10** medical products in low- and middle-income countries is substandard or falsified (2)

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"Twenty years ago, when we were talking about counterfeit medicine, not many people wanted to listen. But now it's discussed everywhere."

Case by case, we knew we were doing well, but our claim that the Minilab saves lives has now been backed up by the authority of the WHO. The GPHF is a micro enterprise, so it can feel like David versus Goliath!" The constant observation that goes hand in hand with a higher profile has increased the pressure on the team, however. "We've essentially been developing test methods in the public arena, so there is nowhere to hide!"

The Minilab covers a broad spectrum of drugs within the anti-infective arena – the next step is to expand into testing of drugs for non-communicable diseases; for example, cardiovascular, anti-diabetes, and gastrointestinal medicines. Jähnke believes there is room for technical improvement for the Minilab too, such as using a smartphone camera to improve the assay reading, which is currently done by eye. "We want to combine the TLC plate with a final assay reading and interpretation connected to smartphones."

There is also no shortage of countries still in dire need of the Minilab's capabilities. "We would like to focus on regions where there are not enough Minilabs available. I'd like to supply more Minilabs for Congo, Cameroon, Chad, Benin, Togo, and the Ivory Coast. If there's a counterfeit medicine hot spot in the world, it's Francophone West Africa – whenever we go there, we find something. I would also like to supply countries like Libya, Sudan, Djibuti, Syria and Yemen - but currently, it is just too dangerous."

Finally, Jähnke would like local workers to take over training on the device. "I have done about 50 training sessions in the past 18 years, and I would love for them to be run independently. I want to empower local people to do the job."

Hold the line

Fighting fake pharma could be regarded as an overwhelming task, but in Jähnke's case, persistence (and by his own admission, a little luck) has paid off. "When I finished my degree, I knew what I wanted to do with my life, but I couldn't gain access to the public health arena. Ten years after my final examination as a pharmacist, I got my chance – and since then, I have followed the Minilab from development to production, to advertisement, to delivery, to training. Twenty years ago, when we were starting the project and talking about counterfeit medicine, not many people wanted to listen. But now it's discussed everywhere."

Considering the innumerable challenges, does he ever feel disheartened? "On the contrary – I am filled with gratitude that I got the opportunity to carry out this task. I've been to big conferences, with legal factions, public affairs, the consumer power groups, and you wonder how anything is moving – they make it so complicated. But I don't get ground down by the scale of the task. If I am blocked in one area – I just pop up somewhere else!"

In 2017, Jähnke was "extremely flattered" to win the Humanity in Science Award for the Minilab. "You work all your life in a lab, hoping that maybe you've made a difference... But an award like this helps you realize you have had some influence. It's another part of the story that has drawn the Minilab from the lab and onto the world stage."

A recent post on Facebook about the GPHF's detection of counterfeit medicine attracted many memorable and heart-warming comments. For Jähnke, one comment in particular struck a chord, when a fellow pharmacist stated, "It's the first time I have been proud to be a pharmacist!"

"That's one of the reasons I do this job," Jähnke explains. "It's not just about counterfeit medicine; it's also about promoting the pharmaceutical profession. It gives us a voice."

Although the battle against counterfeit medicines is far from over, Jähnke feels content. "It's overwhelming to still be working on the Minilab 20 years later, when projects these days can be so short-lived. We have survived the test of time. I've no plans to retire yet, but when I do, I will feel I have made my mark on Earth."

For further information, see www.gphf.org

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Training Workshops on Antimalarial Medicines Quality Monitoring and Performance Analysis of Their Dissolution Property
PQM in Collaboration with DFDA, VSDC, DMR-LM, WHOind GPHF
Nay Pyi Taw, Myanmar ◆ Dec. 2-6 and 9-13, 2013


GPHF-Minilab

Business

Economic drivers
Emerging trends
Business strategies

Lessons I've Learned, with Andrew Alpert

PolyLC founder and President Andrew Alpert received the Uwe Neue Award at HPLC 2017 in Prague for his contributions to the field of separation science, which include the introduction of hydrophilic interaction chromatography (HILIC) as a general-purpose mode of chromatography. In 1985, he founded PolyLC, with a mission of providing life scientists with the separation tools they need – even if it means developing completely new methods. Here, he shares some of the lessons learned from his years at the interface of business and science.

Don't automatically discount outliers

My development of the HILIC concept stemmed from observations involving i) analysis of Agent BZ, a chemical warfare agent; ii) the effect of organic solvents on retention of peptides on PolySULFOETHYL A; and iii) a paper by Colin Mant, Robert Parker and Robert Hodges on size exclusion chromatography (SEC) of peptides in various solvents. What these three unrelated cases had in common was a dramatic increase in retention of polar solutes on polar stationary phases at levels of organic solvent greater than 70 percent. It was an anomaly. Most scientists ignore anomalies. I've always viewed them as the doors to new fields in science.

You don't have to be in academia to build a fulfilling research career

During my second postdoc I applied for assistant professor positions at 31 different colleges and universities – I didn't even get an interview. In the mid-1980s, academic departments only wanted applicants who were working in recombinant DNA, and the nascent biotechnology industry didn't have many proteins to analyze yet. The prospect of a third postdoc was unappealing. The fact that I was able to go on and construct a career in science is profoundly gratifying.

A good mentor can have a profound impact

I was fortunate to join Fred Regnier's group as a graduate student when he was developing the first materials for HPLC of proteins. Being able to perform a protein separation in 20 minutes that normally took 24 hours in the cold room made a powerful impression. In 1978, a member of the Regnier group – Karen Gooding – started a company called SynChrom with her chemist husband Dave to manufacture the materials being invented by our group. Their efforts served as an inspiration when my own time came.

I was also encouraged by Robert (Bob) Brownlee, who started an innovative HPLC column manufacturing company in Silicon Valley in 1978. As a graduate student, I developed an anion-exchange material for proteins that was subsequently manufactured under license by SynChrom. Bob bought the bulk material from them and liked it! He expressed a desire to add a material for cation-exchange of proteins to his product line – and, during my first postdoc in 1982, I developed one: PolyCAT A. Bob immediately ordered

"Most scientists ignore anomalies. I've always viewed them as the doors to new fields in science."

Clockwise from top left: Receiving the Uwe Neue Award at HPLC2017. PolyLC at Pittcon 1987 in Atlantic City. In Regnier lab during grad school in 1978. With Fred Regnier in 2007. At Scripps in 2006 with Ken Kessler and John Yates.

"Your customers and collaborators will tell you what new fields your products should be in. You should listen to them."

200 grams, but this time directly from me. And that was the start of a sideline that I named PolyLC and which became my day job three years later. It's an open question whether or not that would have been possible without Bob's faith that a postdoc with no company behind him would keep supplying the material that Bob had committed to. I wish I could express my appreciation to him today but, sadly, he died prematurely a few years later.

You cannot overstate the importance of family support

When I started the company, I was married with two small children and very little capital. My wife was encouraging and supported the family for years while all profits were ploughed back into capitalizing the company as it grew. Without that support, the company could not have grown. Help also came from my father, Robert Alpert, who was an executive in manufacturing, and provided considerable assistance in helping me to organize my new company.

Many labs are using my innovations

HILIC is the most widely used method I've developed. The concept is so general that many other companies have been able to sell their own stationary phases for it. Our most widely cited material is PolySULFOETHYL A. In the mid-1990s, I worked with John Yates to develop the SCX-RPC (strong cation exchange-reversed-phase chromatography) sequence for fractionating complex peptide digests in bottom-up proteomics. When he introduced MudPIT in 1999, the proteomics community followed his lead in using PolySULFOETHYL A for the SCX step. An Internet search under its various spellings returns close to 10,000 publications...

However, our best-selling material is PolyCAT A. After I'd developed it, I first used it for the clinical analysis of hemoglobin variants – my first postdoc was in a pediatric genetics group, and I knew that mutations of hemoglobin were the basis of humanity's most widespread lethal genetic diseases. Today, PolyCAT A is used for the majority of clinical hemoglobin variant analyses in the USA and for a large percentage in other countries. It's probably the most widely used material for analysis of intact proteins by HPLC.

There are many factors behind the success of a new technology

I have been on three patents. Two resulting products are still being manufactured: an anion-exchange material I helped develop as a grad student in the Regnier group and materials with coatings based on polysuccinimide, including PolyCAT A. The third was an application patent involving the use of HILIC in an immunoassay for pathogenic prion protein. The assay was developed at the height of the scare over Mad Cow disease (bovine spongiform encephalopathy) and remains the only assay that's sensitive enough to detect the pathogenic prion protein in the blood of a diseased animal several years before symptoms develop. However, as panic about Mad Cow disease waned, so did interest in this particular patent.

If you have a great product, then advertise!

During the first 12 years after I introduced HILIC as a general-purpose mode of chromatography, some 80 papers were published by us and our customers on its use for a wide variety of compounds and application types. Both Waters and SeQuant took note and introduced their own materials for HILIC in 2003. Unlike PolyLC, they promoted their HILIC products vigorously. Guess who owns that market now? Even for a scientific method, it pays to advertise. I shouldn't complain, though; the promotional campaign has played a major role in the burgeoning growth in the use of HILIC.

Engage with the field

Your customers and collaborators will tell you what new fields your products should be in. You should listen to them. A case in point is the work I did with Phil Andrews. We were graduate students in the same department at Purdue University. He ended up at the University of Michigan, identifying polypeptides from natural sources. When I launched PolyLC, I initially focused only on proteins, but Phil worked with me to develop applications for peptides with several of the new materials that I'd produced, including PolySULFOETHYL A. It went on to become very successful.

I enjoy working at the interface of academia and industry

My background as a biochemist in academia has helped me in a number of ways, most importantly in recognizing impactful analytical problems in need of solutions. It's also equipped me with the skills to perform some of the necessary method development in-house. In larger companies, the people who work on

Left: In the PolyLC lab in 1996. Right: PolyLC at Pittcon 2006 in Orlando.

materials development are generally not the people who develop new methods, leaving one side without guidance and the other without optimal tools.

Our company has a small footprint but a big shadow

PolyLC currently has five employees. Notwithstanding our small size, we are the dominant company in several of the applications that we focus on, such as proteomics fractionations and clinical hemoglobin variant analysis. We have also introduced a version of hydrophobic interaction chromatography (HIC) that can be used directly with mass spectral analysis, and which we believe is going to play an outsized role in quality control analysis of commercial monoclonal antibodies and antibody–drug conjugates.

Running a small business is always precarious though; PolyLC could be described as a research group that gets its funding in increments of one column sale at a time. We have to get it right every time, too. My customers have to be able to reproduce my research immediately or I don't get paid – unlike in an academic lab.

In research, just because you can doesn't mean you should

There's no point in performing an experiment if an outcome either way isn't going to make you do anything differently.

My main reward in my working life is being able to influence how analytical biochemistry is done.

Getting paid for it isn't bad, but a less tangible reward is influencing the biochemistry community's approach to analytical problems. My 1990 paper introducing HILIC as a general mode of chromatography has been cited over 1,600 times. Thousands of labs have used materials and methods that I developed. In effect, they found my research compelling enough to implement it themselves, which is hugely rewarding.

"When I started the company, I was married with two small children and very little capital."

Real-Time Speciation of Ethylbenzene from the Xylenes Using Direct MS

Direct mass spectrometry (DMS) techniques struggle to speciate ethylbenzene from the xylene isomers, yet increasingly regulators are imposing different emission and exposure limits for these compounds. This application note describes a significant breakthrough for DMS, because selected ion flow tube mass spectrometry (SIFT-MS) readily achieves direct, real-time speciation of the xylenes from ethylbenzene.

Mark J. Perkins,[1] Vaughan S. Langford[2]
[1]Anatune Limited, Cambridge, United Kingdom
[2]Syft Technologies Limited, New Zealand

Resolution of ethylbenzene from the xylenes is important when regulators impose different emission or exposure limits, as is the case for occupational exposure limits in the European Union (time-weighted averages of 100 and 50 ppm, respectively). Direct mass spectrometry techniques traditionally struggle to distinguish these isomers, so measurement has been reported as a total concentration. In this application note we apply the SIFT-MS technique to achieve direct, real-time speciation of the xylenes from ethylbenzene.

A Syft Technologies Voice200*ultra* SIFT-MS instrument – which applies multiple, rapidly switchable reagent ions with different ionization properties (see syft.com/SIFT-MS) – was used in this study. Air was sampled continuously through the instrument's high-performance inlet at approximately 25 sccm. The total ethylbenzene + xylenes concentration was measured using the NO^+ reagent ion. The O_2^+ reagent ion reacts with ethylbenzene and the xylenes to form the same product ions (m/z 91 and 106), but in almost inverse ratios. Separation of the isomers is achieved through calibration of the 91 to 106 ratios using several ethylbenzene/xylene mixtures at two nominal concentrations (150 ppbv and 10 ppmv). Calibration results are summarized in Figure 1.

The success of this approach is illustrated from experiments conducted in the relatively complex air matrix of the Anatune Limited laboratory (typical concentrations in the 10s to 100s of ppbv). Data were acquired with a temporal resolution of just over three seconds.

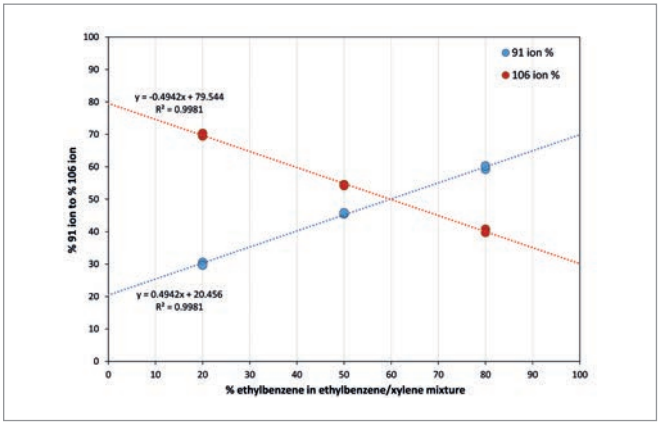


Figure 1. Calibration of relative abundances for the m/z 91 and 106 product ions formed when O_2^+ reacts with different mixtures of ethylbenzene and the xylenes.

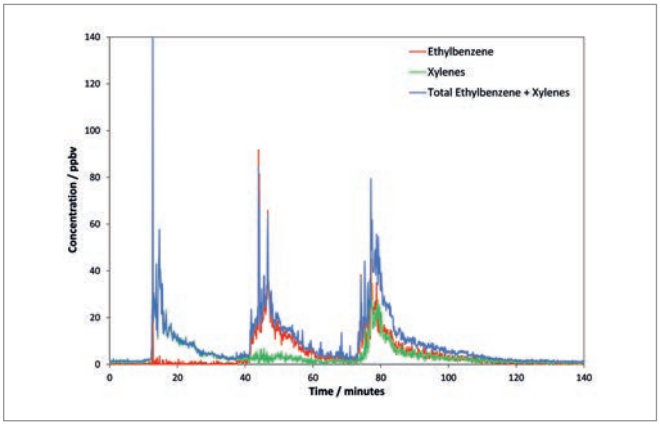


Figure 2. Separation of ethylbenzene and xylenes using O_2^+ with the total concentration obtained using NO^+ superimposed.

A small volume of each of m-xylene, ethylbenzene, and a mixture of the two was introduced into lab air from 20 mL headspace vials, which were shaken and uncapped for a few minutes. Laboratory windows were opened to ventilate the room between samples. Figure 2 shows the total concentration measured using the NO^+ reagent ion, plus speciated ethylbenzene and xylenes concentrations obtained using O_2^+. It is clear that the first exposure was xylenes, followed by ethylbenzene and then a 50/50 mixture of the two.

Excellent speciation of ethylbenzene from the xylenes in real time can be achieved at very low ppb levels by using the SIFT-MS O_2^+ reagent ion. This approach can be applied to both real-time and high-throughput applications, providing benefits in testing laboratories and continuous monitoring applications.

For the full application note, visit syft.com/ethylbenzene-and-xylenes.

Good in a Food Fight

Sitting Down With… Chris Elliott, Faculty Pro-Vice Chancellor and Founder of the Institute for Global Food Security, Queen's University Belfast, Northern Ireland.

How did you get started in food analysis?

In the 1980s, I worked on methods to detect residues of growth-promoting compounds in animal production, such as anabolic steroids, beta agonists, beta blockers and tranquilizers. At that time, legislation had just been introduced in Europe to ban these substances, so I was not only developing methods, but putting them into regulatory programs.

My goal has always been to do good science that ultimately helps to keep people safe.

What have been your biggest successes?

It's gratifying to see methods developed by my team being used all over the world, by regulators and by industry – it shows that our research is having a real and meaningful impact. For example, I carried out a review for the UK government after the "horsemeat scandal," and the recommendations I produced were not only accepted by the UK government, but are also being used by agencies in many other parts of the world to improve the integrity of their food supply systems.

What have been the toughest aspects of the journey?

Often, the hardest part is getting the funding in the first place. Like most academics, I spend a lot of time writing grant applications, many of which get turned down. Though the research itself is often very complex, I have put an excellent multidisciplinary team in place over the years, including analytical chemists, immunologists and food psychologists, to make sure that we can deliver.

Finding the right methodologies to do high-quality analysis can be really difficult. We examine innovations in technology platforms across multiple sectors – life science, pharmaceutical science, and so on – to see how those methods can be applied to food safety analysis. We're lucky to have two excellent technology centers at the university: one focused on rapid screening tests using sensor technologies, the other on using targeted and untargeted spectroscopy and mass spectrometry to produce high-quality quantitative data.

How do you ensure your research is applicable to routine use?

A big part of what we do is translating complex analytical techniques into easy-to-use methods. We would like government inspectors and others involved in quality control of food to be able to operate these systems and interpret the data. In addition, we work with many different food companies, small and large, all of whom are very keen to have these types of tools. So we work very closely with industry in trying to get methods that are fit for purpose.

Could you summarize your major projects?

Our most important project aims to improve food safety and food fraud detection between the EU and China. Food safety failures have become a growing problem in terms of ensuring the integrity of our food, but also for trade. We've embarked on an 11 million Euro, four-year project to look at best practice in both territories and drive forward standards. It's jointly funded by the EU and China, and there are 33 partners in total (16 from Europe and 17 from China), with academia, industry and government all working together. Another project we're very enthused about deals with fraud in the global supply chain of rice. Here, we are developing techniques that will identify adulteration and counterfeiting of rice, and also flag a number of potential food safety issues.

Looking at your work, "transparency" appears to be a key theme...

I have a strong belief that people have lost touch with how their food is produced and where. We want to rebuild the trust in our food supply systems – and the only way we can do that is with food transparency.

First of all, consumers need to understand that, although food supply chains are highly complex, information is increasingly available to them. Exciting innovations, such as block chain technology, are making supply chains more transparent. In November 2017, we launched the world's first block chain beer in Northern Ireland – by scanning the QR code you can see where all the ingredients came from, when it was manufactured, and lots of other details. The beer also tastes good!

You are supporting the Food Fraud conference in London. What is the main goal of the event?

We had a very successful conference on food fraud in 2017, bringing UK food industry representatives together to determine what the big issues are. At Food Fraud 2018, we will be expanding the discussion to an international level, and finding out how companies can collectively deal with some of the issues in food fraud prevention.

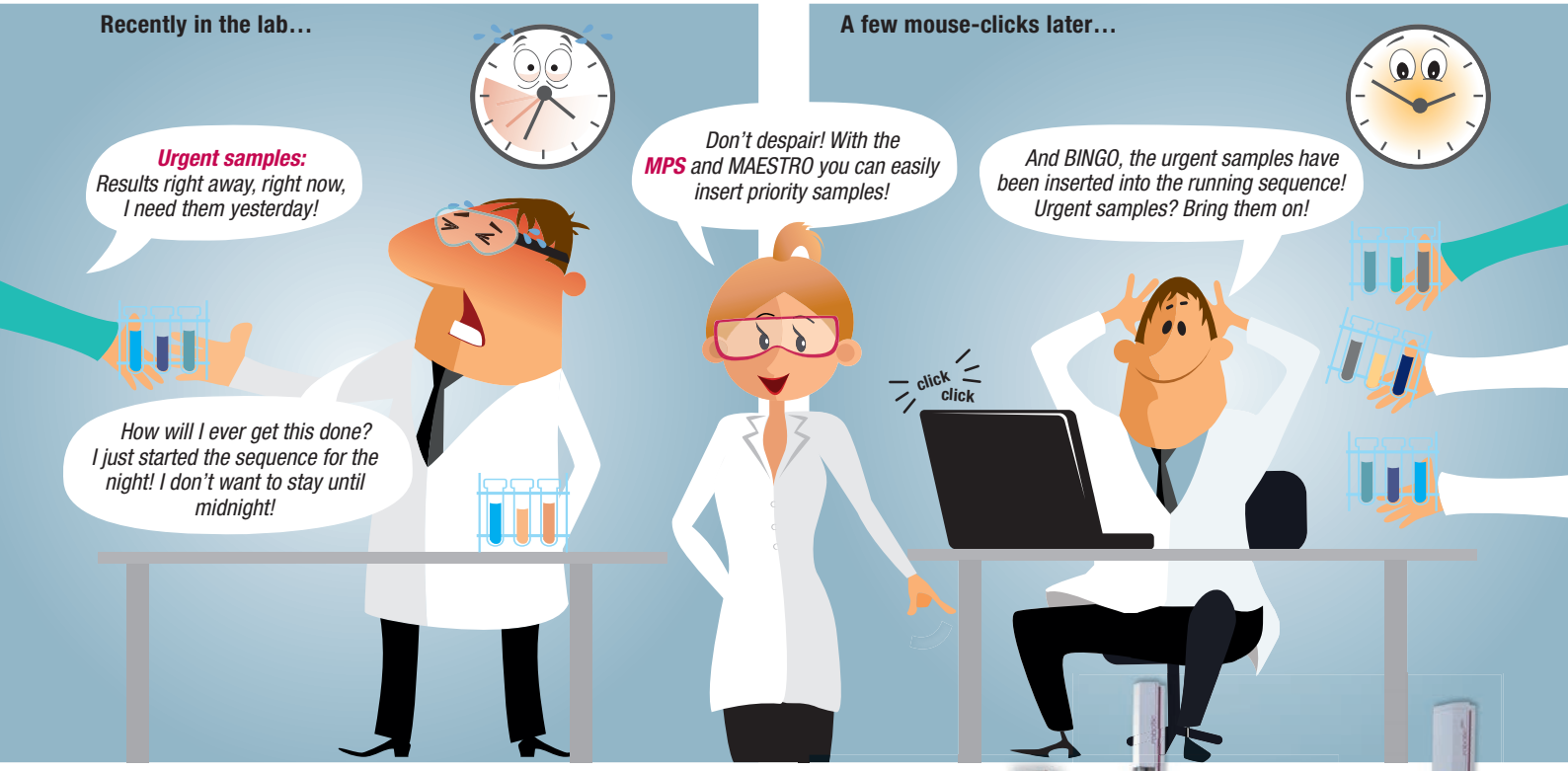
What type of food is more vulnerable to fraud?

The simple answer is that all types of food are vulnerable – and most already suffer some form of fraud. But more complex food supply chains are strongly linked to an increased likelihood of fraud. The food industry operates on very small profit margins, so there is always pressure to cut costs – one way to do that is to cheat. If the fraud is successful (it goes undetected), vast sums of money can be made, so the motivation for criminals is huge.

Where would you like to see the field in five years?

I'd like to see a stronger network of collaboration at a national and international level. Here at Queen's University, we want to develop a global hub for food fraud detection, working with other laboratories across the world to standardize our methodology. Harmonization has been the trend for analytical science in many other areas, such as veterinary drugs and pesticides, and I'm optimistic we can do the same for food safety.

Priority 1A+!



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