APRIL 2014 # 15

the Analytical Scientist

The Art of Simplicity

George Whitesides' 50 years of research experience has lead to a single, basic philosophy: keep it simple.

HiQ®

The only word you need to remember in the world of specialty gases.

High purity gases, ISO accredited calibration gas mixtures and precision gas control equipment for laboratories, research institutes and universities.

http://hiq.linde-gas.com

HiQ® Precision matters in everything we do.

Linde AG
Linde Gases Division, Seitnerstrasse 70, 82049 Pullach, Germany
Phone +49.89.74 46-1661, hiq@linde-gas.com, http://hiq.linde-gas.com

Online this Month

Webinars

Boost Pharmaceutical, Food, Clinical and Toxicology Method Development with Kinetex Biphenyl Core-Shell Technology.

Enjoy a brief overview of the benefits of core-shell technology for HPLC, UHPLC, and LC/MS/MS, as well as practical advice and case studies.

April 30, 2014 – 11am EST

Sign up now:
tas.txp.to/0314/pheno/web-reg

GPC Solutions – Optimizing your Polymer Separations.

Gain an understanding of the important variables, learn how to optimize those variables, and then discover how to put the knowledge into action to achieve reliable, robust, high-resolution polymer separations.

May 13, 2014 – 3pm GMT

Sign up now:
tas.txp.to/0414/agilent/web-reg

Hot Topic

Diving into the Black Box (tas.txp.to/0414/blackbox)

"Can the science be saved...?

I agree completely with your comments and share your concerns. I have spent my entire career fighting for a role on the core project team. Too often, analytical is called in at the last minute when the project is in crisis mode. Analyses are needed immediately and the appropriate methods have not been developed. If analytical is part of the core team, these situations can be avoided. I have been fortunate in that a number of project team leaders have recognized the value of having analytical involved early on. These projects have always gone much smoother. Analytical chemists must, as you suggest, educate themselves in the areas that they are supporting. You do not need to become an expert synthetic organic chemist, but you must have a basic understanding of the chemistry involved. I have maintained for some time that the 'chemistry' has gone out of analytical chemistry. We have become so infatuated with what the computer can do that we forget to consider what is going on inside the 'Black Box'. I have found it very frustrating over the years when I am trying to evaluate a new instrument and all that is discussed is the computer software and all the manipulation that one can do with it. We seem to have forgotten that the computer is merely a collection of electronic components and we are the ones that can determine if we are getting the expected results and if those results make sense based on our knowledge of the project or study.

It is person, not the box, who understands what the box can and cannot do, and what the results mean.
– *Frank, USA.*

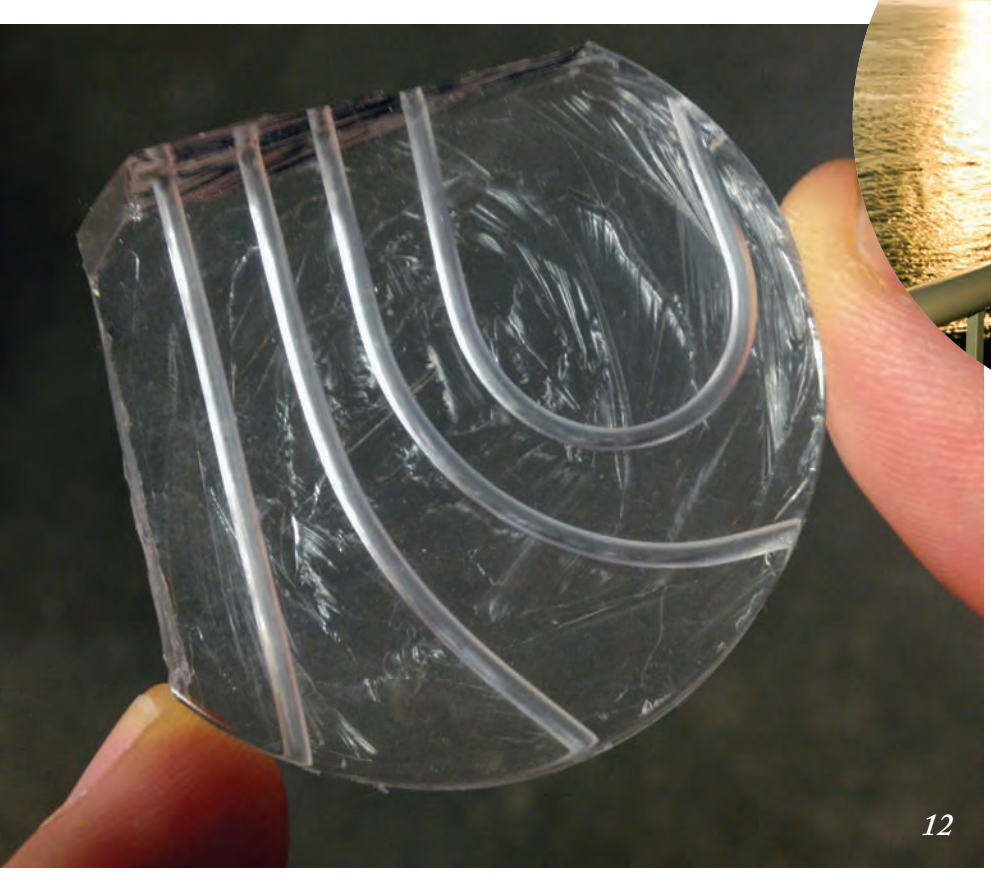
Reformation of Education Programs for Analytcal Chemists...

I totally agree with the points raised by the [Wolfgang Lindner]. The analytical data we obtain is just the window that helps us to look into the 'Black Box'. The inside meaning of the data is very much linked with the samples we analyze and the background and/or matrix of the samples we collected. An analytical chemist should not only know how to obtain the data and/or results correctly but also how to interpret the data and/or results meaningfully. In this sense, an analytical chemist should be equipped with the knowledge that relates with samples and the environment from where the sample comes from."– *James Xiong, China.*

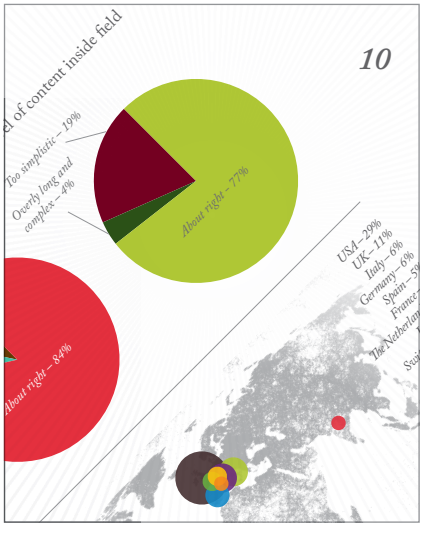
Sign up online for free to have your say:
theanalyticalscientist.com/subscribe

Contents

50



12



On The Cover

The ultimate in simplicity: a paper-based diagnostic chip from Diagnostics For All (www.dfa.org).

Upfront

In My View

22

Features

Reports

Departments

Sitting Down With

the **Analytical Scientist**

ISSUE 15 - APRIL 2014

Published by
Texere Publishing Limited, Booths Hall, Booths Park, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

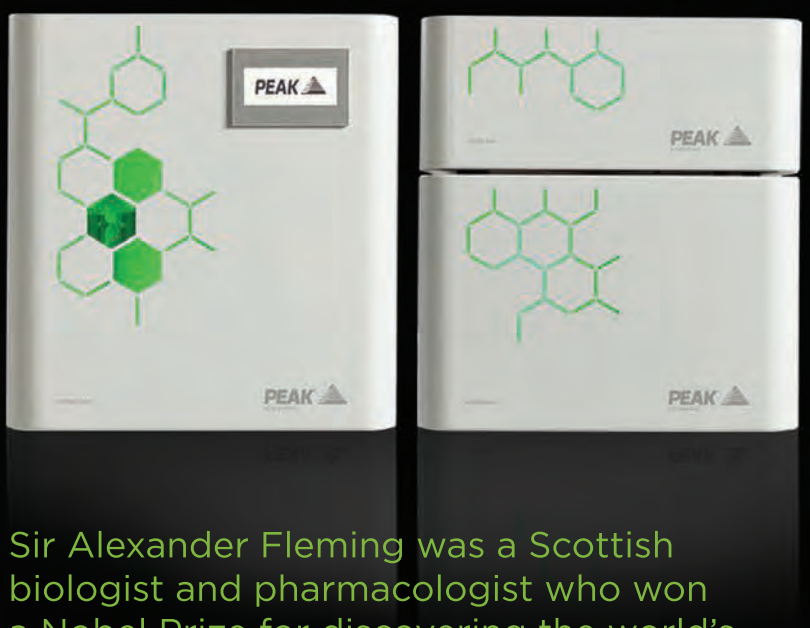
General enquiries:
www.texerepublishing.com
info@texerepublishing.com
+44 (0) 1565 752883
sales@texerepublishing.com

Distribution:
The Analytical Scientist is distributed worldwide through 21,000 printed copies to a targeted European mailing list of industry professionals and 58,750 electronic copies, including 27,583 to North/South America, 26,509 to Europe, and 4,658 to the Rest of the World.
ISSN 2051-4077

texere publishing ltd

It was a curious Scot that discovered life-saving penicillin.

We also design the world's best Gas Generators.



Sir Alexander Fleming was a Scottish biologist and pharmacologist who won a Nobel Prize for discovering the world's first antibiotic. But Scottish inventiveness didn't stop there...

Today, Peak Scientific gas generators also proudly represent Scottish innovation. Manufactured to ever higher standards and with new features appearing regularly, Peak's gas generator range offers innovation, reliability, and the assurance of peak performance.

Add to this, world class after sales care and you can see why Peak Generators are used in some of the most sophisticated labs in the world.

PEAK® SCIENTIFIC

EXPECT INNOVATION

FIND OUT MORE

Call: +44 141 812 8100
Email: marketing@peakscientific.com

www.peakscientific.com

What You Think

More than 500 of you provided us with your opinion on what we cover and how we cover it.

There is something both unsettling and exciting about having your work rated, especially if you promise to publish the results. So it was with some trepidation that we asked readers to complete an online questionnaire about the content of The Analytical Scientist. You kept your part of the bargain: in all, we received 523 completed surveys, a laudable total, which I estimate to be around 1 percent of the hard-core readership. Without getting into the statistical minefield of sample sizes, it is more than sufficient to provide meaningful information.

Now, we are completing our side of the bargain by presenting the results of the survey in graphical form on pages 10 and 11 of this issue. Here, I shall offer the editorial team's thoughts on the trends and specific issues that were raised in the free-form section of the survey.

The overall rating of the publication – the combined figure for 'excellent' and 'very good' of 86 percent – is inspiring to us, and sets a great benchmark for the future.

Readers use The Analytical Scientist to provide a broad, integrative overview of the field (see "Why I read TAS") so I was especially interested in how the poll ratings for content from inside and outside readers' areas of expertise would compare. In the latter case, 84 percent feel that the tone and pitch is about right, while the 9 percent who think that it is too complex and 7 percent too simplistic balance each other out. Seventy-seven percent think that coverage within their area of expertise is about right; 19 percent feel that it is too simplistic. Since more in-depth (but less accessible) information is readily available on all aspects of analytical science, we believe that we are striking a good balance between complexity and accessibility. However, we do intend to add more graphical information that will offer agreeable visual summaries for the non-expert, and allow us to delve more deeply into select subjects.

Ratings on individual sections also provided us with food for thought. Features, Solutions and Application Notes were given relatively high ratings and Business and Profession relatively low ratings. This charges us to reimagine the Business section in particular, something that we had already started. You may have read the perceptive article from Marcus Lippold, Head of [iito] Business Intelligence, in last month's issue; we will have more from Marcus and additional articles of this type in future issues.

To all those of you who participated in the survey, thank you. We welcome further suggestions and feedback; our goal is to provide you with the most useful and engaging magazine possible.

Richard Gallagher
Editorial Director

Contributors:

Lourdes Ramos and Juan Muñoz-Arnanz

"'It must be possible to make this faster' I remember thinking many times during my PhD when I monitored the slow development of large columns used for sample preparation in the determination of PCDD/Fs and PCBs in (semi-)solid foodstuffs," says Lourdes Ramos. With this idea in mind, Lourdes moved to The Netherlands after receiving her PhD in 1998 where she learned about systems for on-line treatment of aqueous samples and started to develop equivalent approaches for the analysis of microcontaminants. "Since then, this type of research has become an essential part of my work at the Department of Instrumental Analysis and Environmental Chemistry (IQOG-CSIC)," says Lourdes.

Concerned about the widespread presence of persistent organic pollutants (POPs) and their impact on people's lives, Juan Muñoz-Arnanz strives to better understand the occurrence, behavior and fate of POPs and related compounds in the environment. "As an researcher at the Spanish National Research Council (CSIC) in Madrid, a key aspect of my studies and never-ending learning experience centers around the analyses of contaminants that are often present at ultralow-trace concentrations," says Juan.

Lourdes and Juan tackle the future of POP analysis on page 16.

Jean-François Focant

Jean-François (Jef) Focant leads the organic and biological analytical chemistry group of the mass spectrometry laboratory at the University of Liège in Belgium, where his research interests include the development of new strategies in separation science and the implementation of emerging strategies for human biomonitoring and food control. "I've been active in the field of dioxin analyses for the last 15 years and chaired the international Dioxin 2011 symposium in Brussels," says Jef. Well known as a dioxin expert, he is also active in characterization of complex mixtures of volatile organic compounds (VOCs) for medical and forensic applications. "Working on the hyphenation of state-of-the-art analytical techniques to solve practical analytical issues is what I really enjoy doing," he says.

Jef proposes a new strategy for dioxin monitoring on page 34.

Ashley Sage

After gaining a PhD in analytical chemistry (separation, mass spectrometry and spectroscopic science), Ashley Sage joined Micromass as an applications scientist, where he developed MS solutions for food, pharmaceutical and life science applications. After 7 years, Ashley moved into the business side of the industry and became the Global Product Manager for the time-of-flight MS platform and continued to develop the use of high-resolution MS for both life science and applied applications. Ashley is currently Senior Manager of the European Market Development team at AB Sciex for the food and environmental business unit.

Ashley talks us through a novel MS-based solution for meat speciation on page 44.

MERCK MILLIPORE

Do you need trouble-free speed?
... then speed-up to Chromolith® HR

The Chromolith® HR introduces a brand new approach to HPLC.
Its monolithic, solid silica rod technology offers:

- A smooth, fast track past the pressure and congestion of packed columns
- Greater flow rate and solvent choice than packed columns, even on standard HPLCs
- Higher consistency of results throughout much longer lifetimes

Discover the New Chromolith® HighResolution
www.merckmillipore.com/analytical-hplc

Merck Millipore is a division of MERCK

Upfront

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

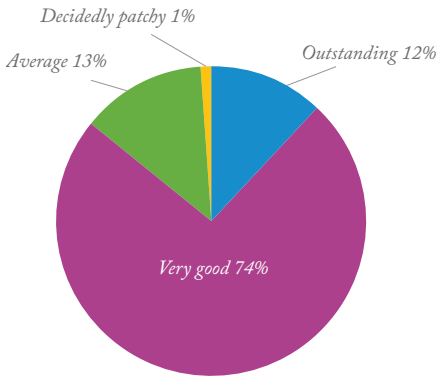
We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email:

rich.whitworth@texerepublishing.com

Under Scrutiny

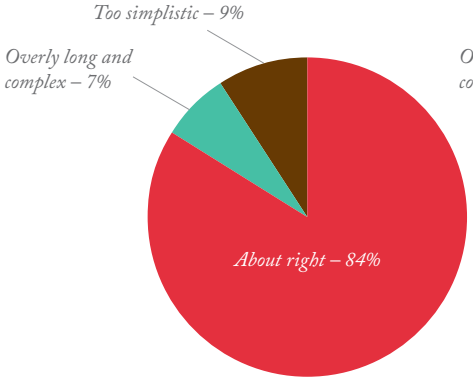
523 of you completed our reader survey, providing us with a great deal of invaluable feedback – thank you! Here, we share what you really think about The Analytical Scientist.

How do you rate us overall?

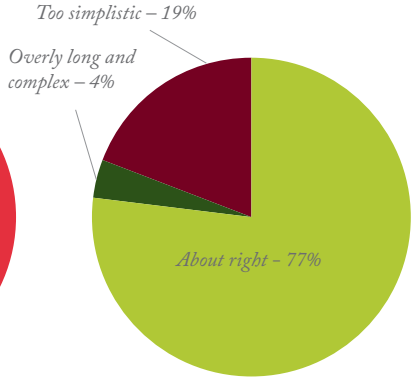


Bewildered, bored or beaming?

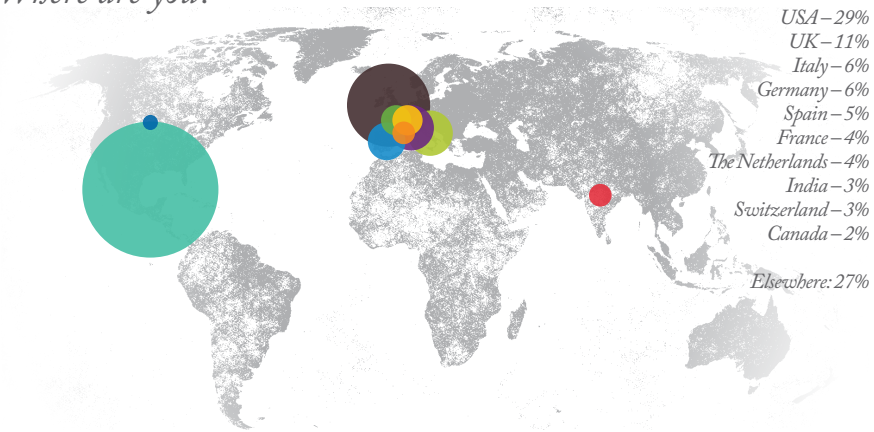
Level of content outside field



Level of content inside field



Where are you?



Why and how you read The Analytical Scientist

Why?

"…for its broad coverage of the life of analytical scientists" 16%

"…to keep up to date with new and exciting research in my area" 15%

"… to stay abreast of trends in analytical science" 19%

"…for the expert-written feature articles" 13%

"… to gain insight on professional development topics" 10%

"…for the interviews with key opinion leaders" 8%

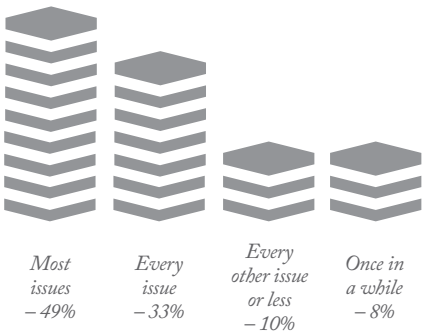
"…for diverse international opinions" 7%

"… to gain insight on business topics" 4%

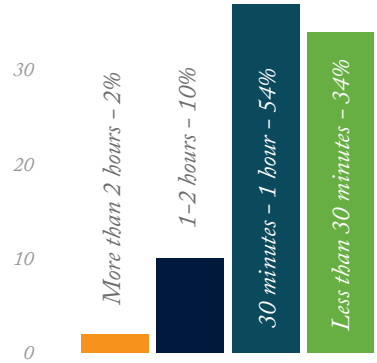
"…for something to do on my coffee break" 4%

How?

How often?

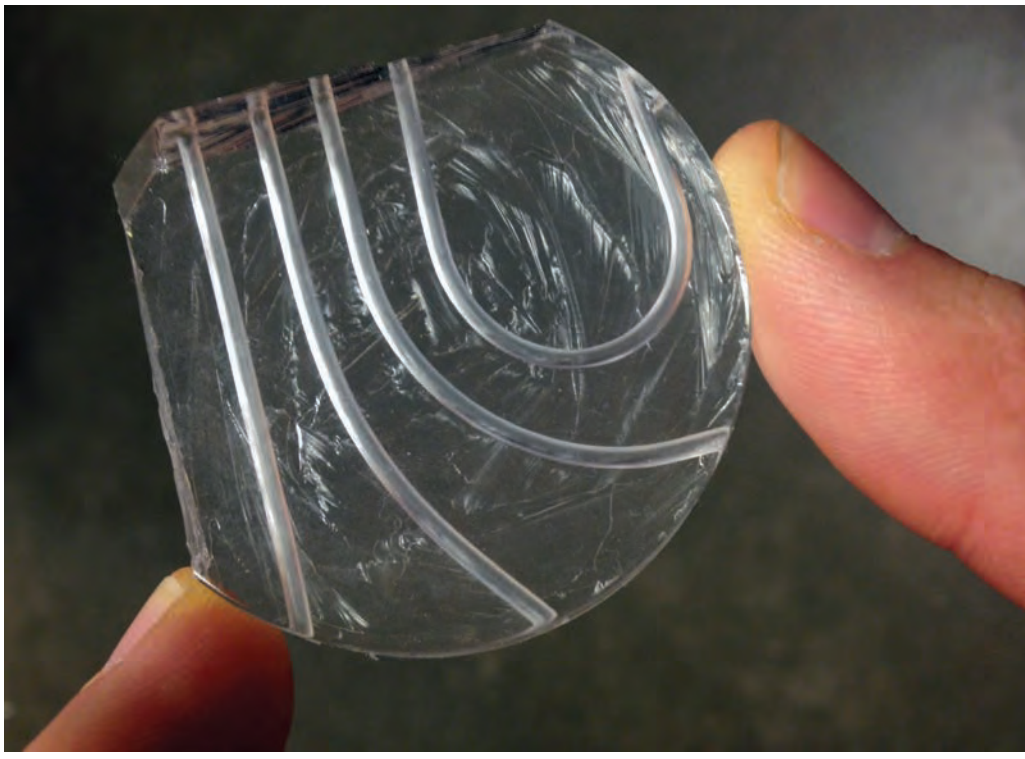


How long for?



Macrofluidics Meets Pathology

Biopsy tissue sample preparation in a tube promises to be cheap, fast, reproducible and automated



The simple fluidic transport system designed to help automate and streamline biopsy tissue sample preparation and handling. (Photo courtesy of the University of Washington.)

University of Washington (UW) scientists and engineers are working on a low-cost device that will help pathologists diagnose pancreatic cancer faster. The first-generation device is extremely simple (see photograph). It uses a fluidic transport system to expose a needle biopsy tissue sample to the sequential steps involved in fixing and staining samples for diagnosis.

The team presented its initial results at SPIE Photonics West 2014. We spoke with Ronnie Das, a UW postdoctoral researcher in bioengineering and lead author on the related paper (1).

The manufacturing process seems very simple – like making jello in a mold...

Yes. It is a very simple process that lends itself to quick fabrication and modification of channel designs. The curved, circular channels are, to our knowledge, the first of their kind, especially as we are transporting large pieces of tissue.

Is this strictly speaking microfluidics as noted in the paper?

The total volumes of the solid samples we're dealing with are 0.5-3.0 mm^3. To hardcore microfluidics experts this could be considered "macrofluidics" but I'd say it still fits the definition of microfluidics.

What steps are you trying to replicate?

For now, we are trying to replicate in a precise, reproducible fashion the very basic steps of pathology, which are chemical fixation of the tissue, staining of the tissue with a histological dye, and optically clearing the biospecimen.

How will the device be coupled to imaging?

Currently, we are attempting to flow tissue from the device to the 3D imaging platform. Future designs under consideration will incorporate onboard optics or even include an interface for smart phone cameras to collect imaging data.

How far away is a fully working prototype?

This current design is rudimentary and proof-of-concept. Humbly, I believe with sufficient resources that a fully working prototype could be developed in a year or two. But perhaps my lab director may have a more accurate time line!

What challenges do you foresee along the way?

The actual cancer diagnosis. Sure, the technology is great, but ultimately we are servicing medical doctors, pathologists and clinical professionals, who make the the hard call. The challenge is simple: our device must be able to reproduce exactly what pathologists are used to seeing on a daily basis, by matching or emulating the traditional processes that have been established for nearly half a century.

Have you identified other potential applications?

On a strictly brainstorming level, large tissue sample processing could be employed for a variety of cancers, and in other areas of pathology. For now, we are focusing on pancreatic cancer because it is such a terrible disease; helping these patients in any way is a win.

Reference

1. *Ronnie Das et al., "Pathology in a Tube: Step 1. Fixing, Staining, and Transporting Pancreatic |Core Biopsies in a Microfluidic Device for 3D Imaging", Proc. SPIE 8976, Microfluidics, BioMEMS, and Medical Microsystems XII, 89760R (2014).*

Setting the Scene, with Odors

Research using lobsters shows how discontinuous odor information is integrated at the neuronal level to produce an 'olfactory scene'. It could have practical applications in identifying the location of explosives or drugs.

Lobsters are known to use odors to detect food, track fellow lobsters and avoid predators but the mechanisms have been obscure. Now a study by Il Memming Park and colleagues at the University of Florida has advanced understanding at the neuronal level of how lobsters perform these tasks.

The team sought to understand how neurons process and represent information by studying the cells' "spiking activity", a measure of how they communicate with other neurons and collectively compute. "The olfactory world, unlike the sense of sight or hearing, is perceived through a filament of odor plume riding on top of complex and chaotic turbulence," Park explains. "This means that you are not going to be in constant contact with an odor." To illustrate this, consider how you locate a barbeque. While searching for the source of the odor, you are not always in direct contact with the smell but instead follow 'waves' of increasingly intense odor.

"Lobsters heavily depend on their ability to constantly analyze olfactory sensory information to form an olfactory scene," Park continues. "One critical component for olfactory scene analysis is the temporal structure of the odor pattern. We wanted to find out how neurons encode and process this information."

The neurons under investigation were a group of rhythmically active primary olfactory receptor neurons (ORNs) called bursting ORNs (bORNs), which detect odor signals to be processed by downstream neurons. "It is very surprising that those neurons seemed to be spontaneously generating signals even after the odor stimuli disappears," says Park, "We wanted to understand why a sensory system would generate its own signal – especially as the downstream neurons would not know if the signal was genuine or not". The group came to realize that the neurons acted like tiny clocks; when the neuron is stimulated by external odor molecules, it repeats the signal in a time-dependent manner. "Each neuron is too noisy to be a precise clock, but there is a whole population of these neurons such that together, they can measure those temporal aspects critical for olfactory scene analysis," Park explains. In some ways, the system has parallels to the echolocation (bio sonar) used by bats and dolphins.

Can research on lobster neurons be applied to electronic nose (E-nose) technology? Typically, E-noses focus on discriminating 'what' the odor is. However, in the case of dangerous chemicals, such as explosives or potent drugs, the location of the source could prove invaluable. "We show how animals might use the 'when' information to reconstruct the 'where' information (the olfactory scene)," says Park, "Such knowledge could inspire neuromorphic chips full of artificial neurons using the same principle to encode temporal intervals into instantaneously accessible information."

Reference

1. *I. M. Park et al., "Intermittency coding in the primary olfactory system: A neural substrate for olfactory scene analysis", J. Neurosci, 34 (3) 941-952 (2014).*

Sense a Revolution?

DNA aptamer-based sensors could take the world of personalized medicine by storm, but only if aptamer selection doesn't prove to be a roadblock

Towards the end of 2013, Tom Soh and his team at the University of California, Santa Barbara, US, developed a microfluidic-based sensor device to measure real-time concentrations of drugs in vivo. It uses DNA aptamers, which are nucleic acid species engineered through repeated rounds of selection to bind to a molecular targets, to trigger an electrical signal (see Figure 1). The sensor is known as the microfluidic electrochemical detector for in vivo continuous monitoring (MEDIC).

The group demonstrated the system's capability by measuring in vivo concentrations of a chemotherapeutic in live rats and of an antibiotic in human whole blood; in both cases, MEDIC provided high sensitivity and specificity with good temporal resolution (1). A video (tas.txp.to/0414/medic) shows a cartoon of the device in action, which highlights how the liquid buffer filter allows the system to be used with whole blood.

Looking at the potential widespread applicability of the technology, the big question is how quickly or easily DNA aptamers can be generated against target molecules. Ryan White, who was involved in MEDIC before moving to the University of Maryland, Baltimore, is co-author of a recent paper on the current and future role of aptamers in electroanalysis (2). The paper indicates that the limited number of proven aptamers is hindering progress, slowing uptake beyond academic laboratories and proof-of-concept studies.

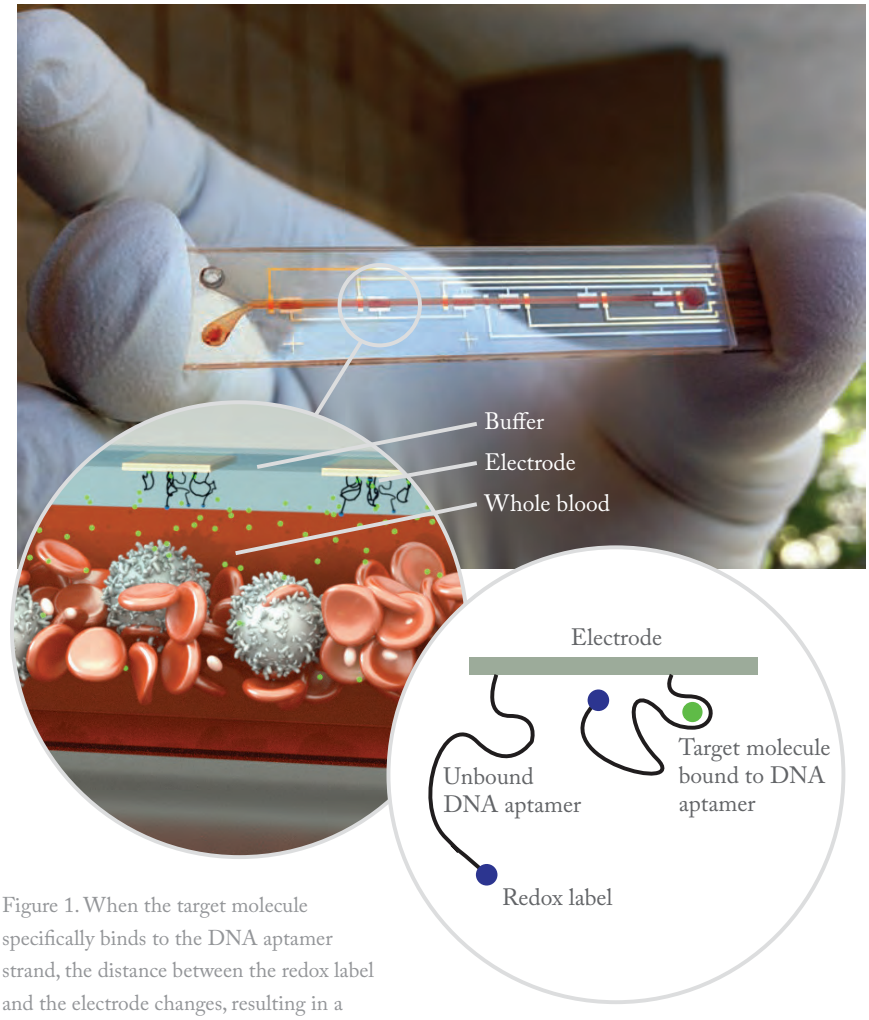


Photo and 3D render courtesy of UC Santa Barbara

Figure 1. When the target molecule specifically binds to the DNA aptamer strand, the distance between the redox label and the electrode changes, resulting in a measurable signal change.

"In our review, we highlight MEDIC as one of the advances in the field. I think that the MEDIC example shows a synergistic marriage between sensing chemistries and engineering for a device that is truly capable of taking on the challenge of something like personalized medicine," says White. He believes that the sensing technology has matured to a level where the analytical field must now identify what is needed in terms of detection. "We need to talk with clinicians and physicians to find out where this technology can have a real impact?"

Feel free to jump in with your ideas by commenting online: tas.txp.tp/0414/aptamers

References

1. *B. S. Ferguson et al., "Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals", Sci Transl Med 5:213ra165 (2013).*
2. *Juan Liu et al., "The Current and Future Role of Aptamers in Electroanalysis", J. Electrochem. 161 (5), H301-H313 (2014).*

SHIMADZU
Excellence in Science

Breakthrough

Tracera merges the GC-2010 Plus GC with the brand-new BID-2010 Plus detector

The brand-new universal BID-2010 Plus detector applies the patented, breakthrough Barrier Discharge Ionization (BID) technology. It targets organic and inorganic compounds at the ppb level and enables their high-sensitivity analysis in a single detector only. Coupled with the GC-2010 Plus capillary gas chromatograph, Tracera reveals trace components which are difficult to see for other GC detectors.

- **Exponential increase in sensitivity**
  over 100 times that of TCD, and over twice that of FID detectors
- **Single detector approach**
  utilizing the universal detection system also for complex analyses
- **Improved efficiency**
  based on the system's long-term stability and operation temperatures up to 350 °C

The Barrier Discharge Ionization Detector (BID) generates low-temperature helium plasma at close to room temperature which re-invents the future of GC detection.

www.shimadzu.eu

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 Endless Frontier of POP Analysis

Accurate determination of persistent organic pollutants has come a long way in twenty years, but we should not rest on our laurels: increased speed, improved economics and a reduced environmental footprint are important targets.

By Juan Muñoz-Arnanz and Lourdes Ramos, Department of Instrumental Analysis and Environmental Chemistry, IQOG-CSIC, Madrid, Spain.

The analysis of dioxin-like persistent organic pollutants (POPs) is commonly perceived as a difficult process that involves several sequential treatments of a sample and a sophisticated final instrumental determination. The perception is correct: analysis of POPs can be complicated.

The main issues are (1) the need to accurately determine specific compounds at very low concentrations in the presence of other species that are present at much higher levels, and (2) the presence of potentially interfering compounds in the extracts. Interlaboratory exercises have proven that most labs provide satisfactory results for standard solution analyses. This might be expected, given the highly sensitive and selective instrumentation, the well-established analytical conditions to ensure proper analyte determination, and the strict application of quality assurance/quality control (QA/QC) criteria, including regular running of blank samples to keep potential interference under control.

Also, as might be expected, the situation changes when complex matrices are being analyzed, such as sediments, foodstuffs and biological fluids. While satisfactory results are consistently provided by well-equipped and experienced labs, discrepancies are often observed with less well-trained labs. These inconsistencies should galvanize lower-performing labs to implement appropriate QA/QC programs and validate their sample treatment methodologies as, with appropriate training, an adequate level of accuracy can be achieved.

Does this mean that the methodologies used in POP monitoring programs have reached maturity and that no further improvement is required? We would say no, they have not, and that further improvement is essential.

Today's selectivity and sensitivity of instrumental analysis of POPs could have been barely imagined twenty years ago; this is not where improvements are needed. Instead, emphasis should be focused on speed – aiming to improve throughput and reduce analysis response times; economics – aiming to reduce the price per analysis both in terms of time and reagent consumption; and sustainability – aiming to dramatically reduce waste generation and the long-term environmental impact of analyses.

While some modifications might be suggested regarding the methodologies and techniques used for instrumental determination, the biggest gains could be made by improving protocols for sample extraction and purification.

The initial sample volume needed for

many types of POP analyses could be significantly reduced without affecting the detectability of the investigated analytes. In the last two decades, researchers have proposed alternative extraction and enrichment techniques, and many conventional, that is, large-scale, methodologies have been replaced by more efficient, faster and greener analytical approaches that retain quality and efficiency. Multi-residual methods are valid approaches that enhance sample throughput and reduce reagent consumption without compromising data quality. New sample preparation techniques and approaches that enable miniaturization and solventless operation are often more productive and cost-effective than conventional sample treatment methodologies in use for routine POP analysis, while reducing occupational health risks and wastes. Apart from benefits derived from the fact that some processes exhibit slightly different behaviour at smaller scales, miniaturization is crucial when setting up (semi-)automated analytical protocols, speeding up sample preparation, decreasing reagent consumption and increasing sample throughput.

Changing a validated method to a multi-class, miniaturized or greener one is a challenge and requires effort and investment from laboratories, and from industry. In the case of POP analysis, where large quantities of reagents and toxic solvents are used in multi-step protocols, these analytical alternatives make sense not only from an economic standpoint, but also from an environmental point of view.

Dioxin Madrid 2014, the 34th International Symposium on Halogenated Persistent Organic Pollutants, runs Aug 31–Sep 5 (www.dioxin2014.org).

Toxic Cocktails

What are the effects of mixtures of toxic substances, all present at low concentrations in our foodstuffs, and how should we go about measuring them?

By Alberto Mantovani, Istituto Superiore di Sanità, Roma, Italy

Consider these five real-life scenarios:
(i) The monitoring of pesticide residues in Europe has generated little concern about single substances but the simultaneous occurrence of multiple toxic residues could be a very different matter.

(ii) Lipophilic pollutants, such as PCBs, dioxins and brominated flame retardants often occur together in fatty foods, such as milk and dairy products, and in certain fish.

(iii) Mixtures of polychlorinated aromatic hydrocarbons are present in grilled or smoked meats.

(iv) A range of molds, which may produce different mycotoxins, are commonly found on pistachios, peanuts, corn and sorghum.

(v) Multiple essential minerals and vitamins, as well as enzymes, preserving and flavoring agents, can be found in feeds for intensive farming.

As for pesticides, 15-20 percent of samples contain residues of

"The combinations of different substances may be additive in effect or may reciprocally modify the action or metabolism of other substances."

multiple monitored substances, albeit each within the maximum permitted level. The combinations of different substances may be additive in effect or may reciprocally modify the action or metabolism of other substances. How should such threats be assessed?

The most straightforward approach is the one adopted for dioxins, namely assessing the overall impact on a given mechanism, in this case interaction with the aryl hydrocarbon receptor. Since potency of each compound is known, the overall threat can be judged. But do we know the toxicity mechanisms for all relevant chemicals? Can we wait to gain knowledge of these mechanisms and in the meantime avoid making a decision? Do substances with dissimilar mechanisms always act independently and in isolation? The answers are no, no and no.

In 2013, the European Food Safety Authority (EFSA) took a bold approach when dealing with the toxicological assessment of combined exposures to pesticide residues. Now, all substances that induce the same effect in a given target tissue or system are considered to act together in an additive way; for example, two pesticides that induce hypothyroidism are grouped together, irrespective of their different chemical structure. Beyond the mechanism at the molecular or biochemical level, it is the adverse outcome that matters, the EFSA states - provided that the outcome is clearly defined. Thus, a toxic cocktail in food may now be characterized by the components that produce the same effect. Available knowledge determines the criteria for grouping substances: the "effect" may be an alteration in laboratory animals or a pattern of metabolomic changes in a robust in vitro system.

One tricky issue for the approach is how to identify the effector components of any given cocktail. Although a cocktail may include a large number of, for example, lipophilic contaminants, only a few chemicals may drive the overall effect of the mixture. In straightforward cases, such as dioxins, a few potent and/or highly concentrated/persistent congeners are responsible for the lion's share of the toxicity, and assessing just these substances provides a reasonable measure of the biological impact of dioxins as a whole for practical risk assessment. Clearly, the main drivers of toxicity are exposure and potency.

Potency has to be measured using assays and parameters that are comparable. Exposure is a topic that becomes exponentially more complex as the focus shifts from residues within a given commodity through to exposure across the whole diet; and since diet is impacted by factors as diverse as ethnicity, region, income and age, it is clear that exposure to relevant substances is also highly diverse. This is demonstrated in biomonitoring studies of the total burden of endocrine (for example, estrogenic) activity in human serum, which have shown that natural substances and xenobiotics from diet (and also from the environment) contribute to a given endocrine activity.

The downside of the approach is that the chances of drowning in complexity are just around the corner. The in-depth assessment of cumulative risks should, therefore, be better focused to answer risk management questions: for example, what pesticides should be regulated more strictly or what pollutants drive risk at a contaminated site? Finally, toxicologists should be most interested in producing new experimental data on whether shared adverse effects can be defined at the molecular level.

Free Up Funding

Following trends and buzzwords is delaying progress. We need more support for diverse, curiosity-driven research projects.

By Gary Hieftje, Distinguished Professor and Robert & Marjorie Mann Chair, Department of Chemistry, School of Public and Environmental Affairs, and School of Informatics, Indiana University Bloomington, USA.

Those who manage to get to the top of their game do so by keeping up with advances across many fields, because principles and concepts developed in one area can be applied elsewhere.

In my field, for example, advances in radio and microwave research, such as double resonance methods and multi-pulse approaches, are finding their way into optical spectroscopy. In short, it often pays to widen one's focus. Yet, in academia at least, the system almost seems organized to frustrate this. We divide into mass spectrometry, nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) or infrared spectroscopy researchers (I could go on): it's quite plain to see that the majority of us concentrate on one area, and it is mainly because of the instrumentation that we have access to in our labs. That's a serious practical limitation: if you're doing Raman spectroscopy, it's not easy to get into NMR even if you see a need. Cost is an issue.

This current instrument-limited approach to research is far from ideal but it isn't going to be easy to alter. At the heart of the reasons why not is funding, which acts as a double brake on change. The first brake is the shortfall in funding resources, which limits the investments that can be made; that's nothing new. But the second brake stems from the fact that funding tends to follow the latest fashions.

In actual fact (and slightly contrary to what I've just stated) my group moved somewhat away from optical spectroscopy into mass spectrometry (MS), partly out of the need to follow funding patterns. It means that we have more instrumentation at our disposal, which allows greater freedom in research projects, but it wasn't an easy transition. And clearly, while learning and applying new knowledge is fun, it does require money. To summarize, we lucky to be able to expand our lab by riding a great wave of interest in MS back in 1983.

Right now, the wave of interest, or fashion, requires that you include the prefixes "bio" or "nano" and add "materials," if you want to maximize your chances of receiving funding. And many of us feel the pressure to do so. To me, it's a really negative feature of academic research today. If we are all going in the same direction, none of us will dare to take a big step forward – it's too risky because while you're trying to take your big step, all the smaller steps are being filled behind you! With everybody marching in lock step, big breakthroughs become unlikely.

"The current instrument-limited approach to research is far from ideal but it isn't going to be easy to alter."

We need a rethink. To paraphrase Richard Feynman, if a person publishes in a less crowded field, he would not only seem better and bigger, he would *be* better and bigger. So, in my mind at least, there are clear benefits of being involved in new and different areas, but everything from funding patterns to impact factors argues against going for it.

So, is there a way forward? Here, in the United States at least, funding is largely driven by governmental agencies, meaning that it is driven to a certain extent by political considerations. I offer a potential seed of a solution: a shift from hypothesis-driven science to curiosity-driven science.

Bioscience and nanoscience, while valuable, are not the only important areas of research. Many other areas are not even being considered because there's no obvious hypothesis-driven

funding mechanism: nanoscopic imaging and multispectral imaging methods, for example, should get more attention, as should two-dimensional infrared spectroscopy. The problem is that, currently, funding isn't provided on the basis of simple curiosity alone. A possible solution is to provide some funding solely on the basis of track record; this would offer flexibility but is contrary to most current thinking.

But it is not all doom and gloom. Instrument limitations and funding patterns point towards collaboration and that's a good thing. Collaborative research is not only popular right now, it's also important. It offers us a chance to break away from the limitations of our own labs and explore and profit from a wider world. Physicist recognized this truth a long time ago – chemists and biologists are finally following suit.

"To paraphrase Richard Feynman, if a person publishes in a less crowded field, he would not only seem better and bigger, he would be better and bigger."

WoTS in a Name?

How reimaging the HET Instrument exhibition will meet the demands of today's integrated business chain model.

By Paul Petersen, Director at Federatie van Technologiebranches, based in Leusden, The Netherlands.

Even if you never visited the HET Instrument exhibition in The Netherlands, you may have read about it in magazines or heard about it from colleagues – it's renowned in the industry. Just how memorable it had become was illustrated to me while vacationing on the tiny island of Alderney; when the cab driver discovered my connection to HET, he waxed lyrical about his experience at the exhibition – though that was many years before he changed profession and location.

Despite its renown, from 2014 HET Instrument will change its title to World of Technology & Science (WoTS). The new event is a collaboration between two Dutch associations, FHI and FEDA, and presents four themes or "worlds": industrial automation, industrial electronics, laboratory technology, and "motion and drives". The event will provide an opportunity for visitors from industry and science to get a sense of the full breadth and complexity of today's technologies (and technology companies) by uniting exhibitions that were related but previously separate in time and location. The World of Laboratory Technology is closest in spirit to HET Instrument, which, since 1956, has served as a showcase for instruments for scientific and industrial customers.

WoTS will present exciting technology for all kinds of practical and abstract applications. The practical component takes the form of high-tech equipment while the abstract is in the coverage of interactions in business chain development. Many industries function in this way: strong partnerships across several global players underpin operations that have new levels of speed and quality. Of course, the scientific world has always been a 'global village'.

Integration is the order of the day and the real power in WoTS is the collective members who create added value for their customers and provide a strong connection to specific areas of technology. Previously, around thirty members of the Laboratory Technology sector built a "Live Lab" at each HET Instrument event. The theme of the lab varied, ranging from water analysis to crime scene investigation to sports laboratory. For WoTS, the concept has evolved into X-peditions, which develop storylines that delve into the knowledge of several companies. X-peditions will guide visitors through six to eight booths, illustrating technology, content and cooperation that builds into a solution around different broad themes, for example, safety.

We believe that visitors are looking for knowledge in the form of solutions to problems. The Live Labs and X-peditions will be entertaining projects that do this in part by encouraging interactions. Visitors are no longer satisfied by learning about the innovations of individual exhibitors, they are looking for dynamic combinations that provide solutions to match the complexity of the business chain. Our goal at WoTS is to help make this possible.

Rediscover Your Curiosity

"I wonder what happens if…"

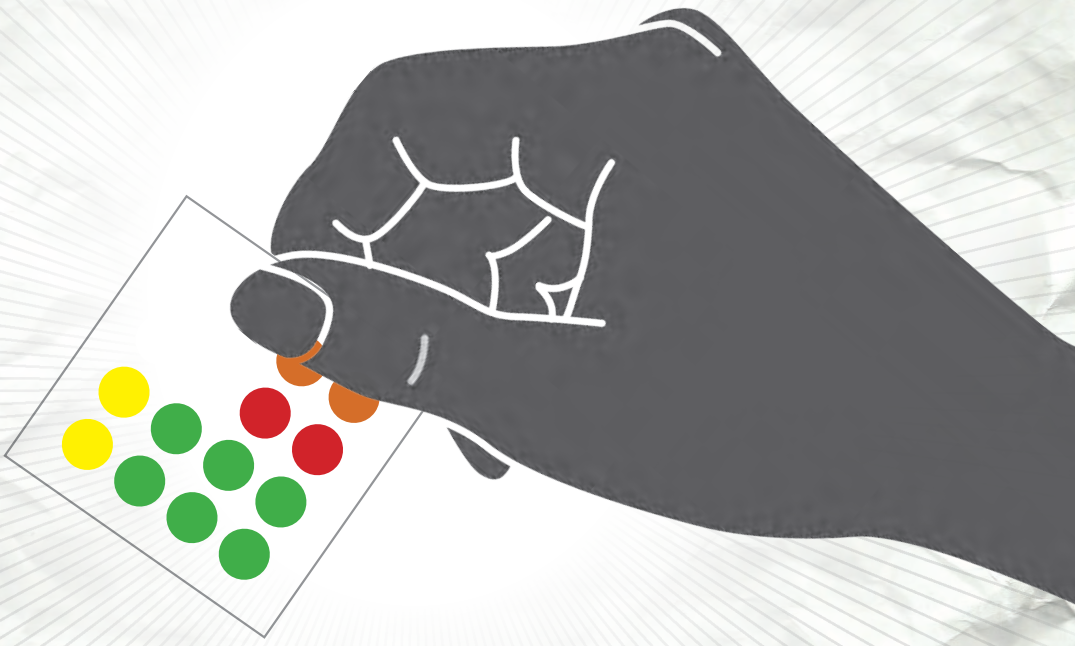
Remember your first discovery? That moment when you first realized you had found something new? Curiosity drives science, and real breakthroughs depend on analytical tools that provide accurate, reliable data. We develop innovative, high-quality chromatography products and provide expert technical support, so you can trust your results and focus on asking the questions that lead to new advances. With Restek, you can ***rediscover your curiosity and push the frontiers of science.***

RESTEK
Rediscover Restek

Want to learn more about how Restek products can help you advance your science?

Sign up to receive free literature in your area at **www.restek.com/rediscover**

Using Simplicity



For more than five decades, I have worked in academic research. The questions I and my colleagues – graduate students, postdocs, and collaborators – addressed in the beginning were "academic", meaning that they focused purely on curiosity. They were usually great fun, but often seemed a little other-worldly. More recently, I have become interested in how best to make university research both intellectually interesting (that is, science for the sake of understanding) and practically useful (that is, technology that works). When the two occur together – or at least in the same project – the result is research centered in what is called "Pasteur's quadrant": these are problems of major societal importance for which the science base to generate a solution does not yet exist. The problems in Pasteur's quadrant raise philosophical and practical issues about the value of sophisticated, complex research, relative to research guided by the idea of a simple (functional) solution. My current belief is that while complexity can be beautiful, simplicity works better.

By George M. Whitesides

As a scientist, I have become increasingly radicalized with age: science has purpose beyond simply amusing scientists. For many years my research was almost entirely curiosity-driven, but at a particular point – starting roughly with the initial development of self-assembled monolayers (SAMs) – I began to suspect that this style was neither the best possible use of my time, nor the best way to provide a broad education for students. It was no coincidence that the field of surface science that developed around SAMs had a low barrier to entry, and could address both scientific and practical problems efficiently. By combining simplicity with broad experience in multi-functional teams (and, occasionally, start-up companies), our approach to research has gradually become more interventionist – both in terms of science and applications. The range of problems on which we now focus includes the lack of appropriate healthcare technology in the developing world (and the exorbitant cost and ineffective performance of the healthcare system in the United States is a problem that isn't far behind), the origins of life, the behavior of dissipative systems and the mechanisms of quantum tunneling; a research program that combines "curiosity" and "use."

As scientists who get our money from the public purse, we have an obligation to spend some time producing science that helps to solve problems. (There are, of course, differences in opinion on what strategies for research best serve the interest of society.) In some cases, rather than developing more and more elaborate extensions of the very sophisticated science that's already out there, I (and especially my colleagues in the research group) prefer to start with a clean page and ask, "what can we do that solves a problem intellectually or practically, and that's *very* simple?"

That's where I am right now. How I got here is a long and winding story…

Scientific origins

When I was young, there was no such thing as a helicopter parent. Children were almost thrown out of the door after they'd been fed breakfast and were expected to come home for dinner when they got hungry. In between, they amused themselves, got into trouble, and explored. Not only was it mostly wonderful fun, it also instilled the idea that the world is full of things that you can't always understand but that you can tinker with.

My father was a chemical engineer and ran a small chemical engineering company in my hometown of Louisville in Kentucky. Despite this connection to chemistry, my route into academic chemistry was based on luck, and, to some extent, coincidence. I hadn't considered fancy schools on the East Coast as an option (in fact, the idea of "options" was not part of my vocabulary: as most children, I simply went to school), even though I was raised in a middle class family and attended private school. One day, seemingly out of the blue, a teacher from my school called my parents and said, "Can I come out and talk to you about your child?" (a question that usually makes a parent's heart sink). This teacher – a stranger to me – insisted that my parents send me east to prep school, and had arranged for me to go to Phillips Academy in Andover, Mass – a move that he had organized purely out of the goodness of his heart. It didn't strike me as being as remarkable back then as it does now. The step up from Kentucky to Andover was a big one; but from there, Harvard, CalTech, and MIT followed quite naturally.

I went to Harvard University in 1957, intending to study English or mathematics, but found that I lacked the talent to be in mathematics. I also couldn't imagine how I might make a living from English. As a teenager, I had worked at my father's company over a couple of summer holidays as a technician, so I was reasonably experienced at routine laboratory work. I liked

working with my hands, washing dirty dishes, doing distillations and so on, so chemistry seemed like an effortless choice. It was not a deeply analytical and thoughtful decision on my part. Still, to be a good fit for a profession you need to enjoy doing the day-by-day stuff, rather than being focused on some ultimate (and usually not well-imagined) view of success. Since I was at least an experienced technician, I did well enough, and chose to stay in Chemistry. Chemistry at graduate school was a logical progression, and from there I went to work in a university, because it seemed more appealing to work on whatever I wanted to work on, rather than doing what somebody else wanted me to do.

Jumping off the springboard

My PhD work, in the early 1960s, was done with a distinguished physical organic chemist named Jack Roberts at the California Institute of Technology ("CalTech"). A big component of Jack's work was applying the tools of physical chemistry to problems in organic chemistry, and one of the hot instrumental techniques at the time was nuclear magnetic resonance (NMR) spectroscopy. By today's standards, it was unbelievably primitive: you didn't need to scan the NMR spectrometer in any direction because the drift in the field was so rapid it would just scan itself randomly up-and-down and back-and-forth – you'd get half the spectrum of ethanol and, later, the other half of it backwards, all of which was pretty amusing. Thankfully, we were not analytical chemists; our interest was in structure and mechanism.

By the mid-1960s, I had settled into the Massachusetts Institute of Technology and was exploring an interest in polymers, which I knew very little about. MIT was, and still is, a wonderful place to be because of the diversity of interests and experience to be found there. Around that time, the first steps towards biotechnology were being taken (it was called bioengineering then) and Ray Baddour, the chairman of Chemical Engineering, was putting together a team for a bioengineering project. In such a team, you need one of everything: a fermentation engineer, a geneticist, a fluid mechanic, a microbiologist, and so on. The team was all set and ready to go when the chemist, who was an expert on enzymes, decided to move to Israel, leaving a gap. Ray started with the letter A in the list of chemistry faculty at MIT and called everybody in alphabetical order, asking, "Would you be interested in helping us to figure out what bioengineering is?" And everybody, very sensibly, said, "no". Until he got to W for Whitesides, at which point I said, also very sensibly, "yes". In general, I think it's a better answer than "no," and knowing nothing about polymers was intellectually very similar to knowing nothing about enzymes. I was, however, doing a lot of work in organoplatinum chemistry and part of the reason to be interested in biology was because platinum is such a good catalyst – and, of course, the same is true of enzymes.

The subsequent four years were fantastic. I worked on biology, applied biology and bioengineering – fields that had previously been completely unknown to me. One lesson from this experience that has really stuck with me is that it's okay, if you don't know anything. Just jump off the end of the springboard and see if there's water in the pool! You'll win more often than you lose, and if there's no water, well... I discovered that there are things you can do with enzymes that you can't do with ordinary catalysts. Interestingly, when I suggested to the superb synthetic chemists around me that they might try an enzyme in place of platinum or sulfuric acid catalysts, the idea was met with horror. Regardless, and insensitive to this most unenthusiastic reception, I set off down that path, with chiral compounds and sugars being the two main areas of focus. I never thought of it as applied research, more as a program in exploratory catalysis.

Catalysis – at least the large-scale heterogeneous catalysis practiced in the chemical industry – is, of course, all about surface chemistry. At that time, everybody in surface chemistry was working with beautifully clean single crystals of, for example, nickel, prepared in ultra-high vacuum, and studying reactions on these beautifully prepared surfaces, using sophisticated instruments to do different kinds of spectroscopy. To me, it all seemed just too complicated, slow and expensive, and I wondered if we couldn't come up with a form of surface chemistry that was a lot easier to do and also more relevant to biology and organic chemistry. We started with polyethylene, which is the prototypical organic polymer, and found that it could be oxidized to introduce carboxylic acids onto the surface; organic chemists like carboxylic acids because you can do a lot of different kinds of chemistry with them. This was in the eighties and, now back at Harvard, I worked on the topic for a decade, a progression that took me from catalysis to heterogeneous catalysis, to surfaces, to organic surfaces, to polyethylene.

My interest in self-assembly once again came from the notion of doing something that no one else was doing. Organic chemistry was dominated by extraordinarily complicated, interesting, sophisticated synthesis, of mostly natural products. The reactions were really beautiful constructs of science and, because they involved making covalent bonds, were based on making non-equilibrium structures. By this time I was a functional biochemist, and realized that most of the important structures in biology – lipid bilayers, folded proteins, paired strands of DNA – are not made entirely out of covalent bonds. Amino acids are linked together covalently, but that string is completely inactive until it is folded; a bilayer lipid membrane isn't stitched together, it just forms itself like a soap bubble. Everything in biology basically puts itself together. I became fascinated by the idea of using systems that put themselves

Diagnostic device images © Diagnostics For All
George Whiteside photo by Stephanie Mitchell, staff photographer, Harvard University News Office

"The big change in analytics is the migration from generating analytical data to generating actionable, affordable information."

Diagnostics For All

Over the course of my career, I have been involved in a dozen start-ups in different areas. The process from invention to product (and by a product I mean something that solves a specific problem that's sufficiently valuable that somebody will pay you for it) is not a simple one. It sometimes (but not always) starts with a new idea; then you have to show that the idea actually works and that it might be good for something; then you have to make a crude prototype of the product and somebody has to understand that it could be adapted to solve a real problem; and then they have to make a prototype of the real thing that someone might want to manufacture. Manufacturing is another art form altogether, requiring lots and lots of money, regulatory clearance and so on. You have to think about distribution and training, advertising and sales, all kinds of things. The process requires cooperation among a series of entities, ranging from the university to a pharmacy chain like CVS. A really interesting intellectual problem is how you put together these entities.

One of the things that I have concluded is that it is very difficult to run a single research organization in which part is tasked with creating new things and part is tasked with doing engineering development. Engineers are clever at taking something that exists and making it better, cheaper and faster, but they need some starting point; scientists are good at imagining and inventing things but not at development. It's not that engineering is more or less creative than science, it's just that engineering and science are creative in different ways, and each requires people who enjoy the particular set of activities required for their part of the problem.

Given all this, Diagnostics for All (DFA) was launched as a not-for-profit company explicitly to do product development based on the inventions from my lab at Harvard in the paper diagnostics area. Today, about five years, DFA's first product has undergone successful field trials in Africa and Vietnam, and several other technologies – for immunodiagnosis and nucleic acid analysis – are at advanced stages. Meanwhile, in the research lab we are developing hand-held electrochemistry and two-phase polymer systems. It is a very successful collaboration in which we, Harvard (and any other relevant entity – technology should be agnostic on the subject of parenthood), invent, and they, DFA, look at problems and engineer solutions.

When involved in start-up companies as a university scientist, you have to work with them in those periods when you can contribute (there are times that they don't actually need – in fact, don't like – outsiders very much). At the moment, DFA is something that I'm spending a lot of time on, because I believe that I can make a contribution. With for-profit start-ups there is a clear goal, which is to make money and to pay back the investors. With not-for-profits the story is a little bit more complicated because you have been given money with the expectation of contributing to societal good. I can't say which I prefer, that's like being asked which of your children you prefer. But doing something that's very interesting, possibly useful, and new is always satisfying.

together rather than relying on bond-after-bond synthesis by chemists. Self-assembly is the answer (or one answer) because it creates molecular aggregates that are very much bigger than those that can be made with conventional synthesis: crystals, protein-like structures, films on surfaces, all kinds of stuff.

Our focus was on developing new methods of making large molecular ensembles or ways of mimicking biology – but these were exploratory studies that were not directed towards solving a specific problem. Nevertheless, the invention SAMs was really a big deal for us (and for surface science more broadly). Our work started in a joint activity with Ralph Nuzzo, a distinguished surface and material scientist, now at the University of Illinois. Ralph had been a student with me, working on organoplatinum chemistry, and had gone off to work in Bell Laboratories. He came by the lab one day, and very generously told us about a process he and Dave Allara were using to create very well-ordered SAMs on evaporated gold films. We started to work together, with Ralph providing the crucial spectroscopy and with us doing the physical organic chemistry to show how you went from molecules to ensembles of molecules with material properties.

Stepping into another unknown

The idea of simplicity was a very powerful concept that I took, in part, from our work on self-assembly. It was, for example, straight-forward to print SAMs, which got us into something that looked like lithography. Then we realized that by sealing the elastomeric stamps we used for printing to the surface (instead of using them to transfer the "ink" that became the patterned SAM), they became microfluidic systems. This led to a ten-year run in which we – along with Andreas Manz at the Institute for Advanced Studies in Freiburg, Germany, and Steve Quake at Caltech and Stanford – invented much of the foundation of what's known as microfluidics.

Paper diagnostics – the ultimate in simplicity – was the next step in the chain. While we had a versatile fluid-handling device that offered a relatively inexpensive way of doing analysis, it was by no means cheap. One of my post-docs, Sam Sia, was very interested in using microfluidic systems to create analytical devices that would be inexpensive enough for the developing world. He introduced us to the subject and his devices worked well, but I became interested in making simpler and even less

expensive solutions. Certainly, the diagnostics for the developing world was another driver of this interest in simplicity, but so was the idea of more broadly-applied health surveillance in the USA. A further motivation for paper diagnostics was our desire to undertake another kind of experiment: to determine if it is it possible for a university research group to create a new technology to be used in the field by real people.

This was a step into the unknown of a different kind – one no longer restricted to research questions, but encompassing societal, political, regulatory, and financial issues. One wonderful thing about being a scientist is that, within reason, you get to do anything that you want, and very smart people will often help you learn. I had been involved in projects with the Defense Advanced Research Projects Agency (DARPA) and with a number of other advisory groups in Washington, DC. This work was very instructive, teaching me something about technology and about public policy. I learned all kinds of things that then became part of how I think about science.

One of the consequences of this broadened education has been the development of Diagnostics For All (DFA, see sidebar), a not-for-profit company that makes analytical devices for biomedical use in the developing world. Our laboratory at Harvard now works cooperatively with DFA to invent, prototype, engineer and validate low cost biomedical devices.

To me, an analytical instrument is simply something that generates information. The big change in analytics is the migration from generating analytical data to generating actionable, affordable information. I attended Pittcon this year and the exhibition contained endless booths full of fascinating devices, and most interesting solutions to problems in analysis; some of them – but only some – also considered their product as information.

More focus on information in biomedicine could perhaps help us to think beyond individual health, and beyond those diseases where all that can be done is to put off the inevitable temporarily. Instead, we could think about public health, about managing cities, about understanding the environment, about food security and water safety. To measure these complex systems requires lots of data, so data acquisition has to be cheap. And, although there are some things where critical measurements require laser spectroscopy or some other highly sophisticated instrument, anything to do with food, water, your internal plumbing, or the safety of animals in South Africa also requires taking samples of fluid, be it blood, serum, tears or sweat. That's where wet (bio) chemistry is always going to come into it. Our focus has been on using technology to make things fundamentally cheap, and ideally convertible into smaller pieces that can go into the analytical web.

Challenging the medical system

The American medical system works in a for-profit mode; for example, if I get a prostate-specific antigen (PSA) measurement it might cost fifty bucks. Every other high-technology industry has made the transition from "analysis for profit" to "information for free" that medicine has not made. For most high-technology areas, measurement has ceased to be a profit opportunity. Instead, information is free, and the value is added by those who manipulate it, store it and distribute it. To give an example, the number of bits required to fly an airplane is simply staggering, from how you set the ailerons, to how the aircraft is tracked by air-traffic control. Yet the pilot doesn't come out of the cockpit and get you to drop five dollars in quarters into a slot beside the wing every time he or she wants to reset the ailerons. But that's basically the way the medical system works – every time data is needed, somebody gets paid for it. I believe that's simply the wrong approach, so part of our interest in paper diagnostics is to create a situation where information in medicine is as close to free as possible. The value should then be added by doing other things with that data. You see this concept in spades in genomics. The cost of sequencing has fallen at an astonishing rate – faster than electronics. It is now possible to sequence an entire human genome for $1,000, and the cost will continue to fall. Soon, every baby will start life with a full sequence on a USB drive. That economy of scale – presently made viable by the functional simplicity of Illumina sequencers – makes it possible to think about medicine in fundamentally new ways.

Looking at the American business model in healthcare, it is hard to say how much of what gets done is for patient benefit and how much is for profit. Instead, we should think about information in the biomedical system as being a public good at lowest possible cost. Don't misunderstand, I'm a hardcore capitalist, but I think that the reason that society tolerates the entity called a corporation is both because it generates a return on investment and because it provides solutions to societal problems. One has to be a little careful that the tail does not wag the dog.

A wonderful thing about universities is that if we get interested in bringing high technology to public health – as opposed to bringing it to end-of-life medicine – and if we can find somebody who will pay for it, we can do it. That, in my view, is exactly what a university should be doing. We should be doing things that are not accepted as a proven paradigm in society. Ideally, we should be doing them simply so that other people who are interested can get involved and take those that turn out to be really good ideas, and expand them to the benefit of society. Do I think that it is important for scientists to do things that have societal benefit in mind? Yes.

38th International Symposium on Capillary Chromatography

Palazzo dei Congressi
Riva del Garda
Italy

38th International Symposium on Capillary Chromatography

and

11th GCxGC Symposium

May 18 - 23, 2014

Chairman
Prof. Dr. Luigi Mondello

THE PROGRAM

- Review papers by leading scientists in the field covering the latest developments
- Key note lectures by young scientists.
- Contributed papers presented in poster sessions.
- Discussion sessions to stimulate intense scientific exchange.
- Workshop seminars presenting the latest developments in commercial instrumentation.
- 11th GCxGC Symposium
- Course on GCxGC - Sunday May 18th

SUBMISSION OF PAPERS

Authors intending to submit papers for the symposium will be required to adhere to the following deadlines:

- A 300 word abstract must be received no later than **February 1, 2014**. For abstract submission see the website.
- Notification of acceptance will be mailed to the authors by **March 1, 2014**.

REGISTRATION FEE

Advanced registration, prior to April 1, 2014

Registration 38th ISCC	450,00 €
Registration 11th GCxGC	200,00 €
Combined Registration (38th ISCC/11th GCxGC)	600,00 €
* Student Registration 38th ISCC	225,00 €
* Student registration 11th GCxGC	100,00 €
* Combined Student Registration (38th ISCC/11th GCxGC)	300,00 €
Course on GCxGC	75,00 €

* (verification of student status)

Registration fees include entrance to all technical sessions and the exhibition, a copy of the final program, a book of abstracts and participation in social events.

CENTRAL ORGANIZATION

Prof. Luigi Mondello
Chromaleont a start-up of the University of Messina
Phone (+39)-334-3612788 - Fax (+39)-090-9080155
e-mail: iscc@chromaleont.it
website: www.chromaleont.it/iscc

LOCAL ORGANIZATION

Mrs. Sara Gagliardi
Thermo Fisher Scientific
Strada Rivoltana - I-20090 Rodano (Milan) Italy
Phone (+39)-02-95059238
Fax (+39)-02-95059256
e-mail: sara.gagliardi@thermofisher.com

TECHNIQUES

- Capillary Gas Chromatography
- Micro Liquid Chromatography
- Electromigration Methods
- Microfabricated Analytical Systems - Lab-on-a-chip
- Column Technology
- Coupled and Multidimensional Techniques
- Comprehensive Techniques
- Hyphenated Techniques
- Sampling Systems
- Sample Preparation
- Trace Analysis
- Automation

APPLICATIONS

- *Environmental Applications*
- *Energy, Petrochemistry and Industrial Applications*
- *Biomedical and Pharmaceutical Applications*
- *Analysis of Natural Products, Food, Flavours and Fragrances*

Scientific Committee
C. Bicchi (Italy)
F. Dondi (Italy)
S. Fanali (Italy)
C. Huber (Germany)
H.-G. Janssen (The Netherlands)
K. Jinno (Japan)
J. Jorgenson (USA)
R.T. Kennedy (USA)
M. Lee (USA)
L. Mondello (Italy)
M. Novotny (USA)
P. Sandra (Belgium)
M. Ramsey (USA)
F. Svec (USA)
G. Xu (China)

Organizing Committee
F. Bedini (Italy)
A. Cappiello (Italy)
F. David (Belgium)
P. Dugo (Italy)
P. Myers (UK)
A. Sironi (Italy)

The Forum on Microcolumn Separations

Web page: www.chromaleont.it/iscc

The Solution Evolution

Agilent Technology's Markets and Applications Programs – MAPs for short – connect you to a growing network of expertise to provide working solutions for your analytical business.

Welcome to a service that redefines the term 'solution'; a service that doesn't just take you one step ahead of current offerings, it takes you several leaps beyond.

The Markets and Applications Programs (MAPs) integrate application experts (both internal and external), Agilent's leading instrumentation, and a total commitment to support your laboratory's needs. The goal is to maximize your efficiency: instead of wasting time adapting your new instrument to its intended task, Agilent's effective and efficient team will assist you. They will help develop an application that meets your analytical goal or solves your measurement problem: MAPs is the very definition of 'solution.'

MAPs is a new initiative from Agilent's Chemical Analysis Group for the EMEAI (Europe, Middle East, Africa, India) region, and covers five applied markets: food, environmental, forensics and toxicology, chemical and energy, and materials.

Agilent's willingness to take full responsibility for seeing your evolved solution delivered (and that means working), is a testament to its belief in MAPs and the benefit that it offers to those of you on the frontline, who face practical problems but have an analytical business to manage.

To provide this remarkable service, Agilent has recruited the best independent experts in each of the five market sectors and, by integrating with in-house know-how, forged it into a network with a single purpose: application development. This MAPs team is ready and willing to solve your analytical needs.

MAPs defined

To present the full scope of MAPs, Armando Miliazza (MAPs Manager) and Marco Zanotti (Partner Labs Manager and Food Market Manager) describe the network, its goal, and their passion.

The expert network

How did it all begin?

Marco Zanotti: The obvious truth is, we are an instrument manufacturer. And while we are very good at what we do, our customers are interested in buying applications – not boxes. Customers don't buy a new gas chromatography system because they want a shiny new instrument in the lab; they buy a new GC because of its analytical abilities. A general trend of all markets is the increasing volume of analytical load, which has shifted the experience level of users. Today, supplying a box is not enough; most customers want and need a full method. Packaging consultancy expertise with our systems was a logical next step.

Though we have a wealth of experience, we don't have in-house application expertise in all market areas – that would be impossible. Instead, we recognized that external consultants, whose knowledge extends from sample preparation to data analysis and includes any overarching regulations or practical issues, would be of huge benefit to our customers. With this in mind, we started building a network of partner laboratories in 2010. Since then it's grown from six to 23 well-respected groups today, meaning we can offer expertise in any application. Pat Sandra's Research Institute for Chromatography is just one of those partners and is a great example of the level of capability to hand (see "High Profile Partners", on page 32).

Who else is involved in the network?
Armando Miliazza: There are four components: the customer, Agilent, the partner labs described by Marco, and our channel partners. The channel partners offer customization options – both hardware and software – for Agilent systems, for instance, automated sample preparation or complex integration. Acknowledging the need for this extra dimension of support, we developed a much more defined working relationship with our channel partners, placing them under the MAPs umbrella.

With everyone working together in this way, we can avoid overlap and share knowledge across the whole network to offer the customer multifaceted support that meets all of their specific application needs.

We have been developing the MAPs initiative since November 2010. Now, we are excited to be able to share it with our customers. We are ready for any request or challenge!

The passion

Who makes up the MAPs team?
Armando: The core comprises the five market managers (one each for food, environmental, forensics and toxicology, chemical and energy, and materials). Our managers were recruited on the basis that they are highly motivated and very experienced, which means they are able to "speak the right language" – that is to say, they have deep insight into their own specific market area and already understand much of the complexity and many of the challenges that customers face on a daily basis. This level of experience allows them to find real solutions and support customers very comprehensively.

Until recently (before I took on responsibility for the whole program), I was the chemical and energy market manager; before that, I worked for one of Agilent Technology's primary channel partners for over 10 years. That's why I am so passionate about the channel partnerships and what they can bring to MAPs – I've seen the potential from both sides.

Beyond the market managers, we have local 'MAPs champions' in each country, who connect the network together. They know the partner labs and channel partners in their own country, of course, but they are also aware of expertise in other regions, and can direct requests to the right person.

Where does MAPs fit within Agilent?
Marco: It is part of a transparent structure. When a customer requests specific application help or has a demanding project to complete, the local MAPs champion introduces it into the network. Such direct access to our well-structured network means we can address challenging requests much more easily. MAPs allows us to help customers who may not have application expertise in a certain area, but who need to get a particular analysis set up and running in no time at all. MAPs

CSI: Italian Pioneer

Customer profile

CSI, despite its shared named with the popular forensics-focused American TV show, is actually an Italian Certification and Behavioral Analysis Center that was founded in the early 1960s and has since become a leader in product certification and qualification. CSI is an early adopter of Agilent's MAPs, and here Daniele Rigoldi from the food packaging materials division explains why CSI chose MAPs and how it benefits the company.

What was your analytical problem?
We were interested in the potential of multi-pesticide residue solution for fruits and vegetables; however, we recognized that the big challenge was not necessarily addressing the long list of target compounds but rather doing so without compromising the sensitivity of the analytical method.

Typically, how long would it take CSI to develop such a solution in house?
That's hard to say, but we would have probably needed at least three months. Thanks to Agilent's MAPs approach we were able to implement everything in just two weeks. That's a huge time saving and, quite clearly, the biggest benefit of all. By shifting focus from the instrument to the solution in hand and applying outside expertise, Agilent were able to fast track method development and get the solution up and running in our lab faster that we had hoped.

Did anything surprise you about the MAPs approach?
I think Agilent's flexibility throughout the process was impressive. It was a very pleasant surprise for the team here.

What was most impressive?
I think this ties in with the flexibility – Agilent's team offered almost constant availability, and that enabled us to interact with them on a profound level.

Describe the impact of MAPs...
Thanks to the speed of implementation of the new solution, we've been able to expand our analytical portfolio faster but crucially, whilst also maintaining high quality. We're confident this will result in higher visibility in the market and, subsequently, direct revenues for our company.

High Profile Partners

Pat Sandra founded the Research Institute for Chromatography (RIC) in 1986. RIC has extensive experience in developing and applying solutions to a broad range of samples and analytes and has been collaborating with Agilent Technologies (or antecedents) for over 30 years. RIC is now very much part of MAPs, so we reached out to Pat, Koen and Tom Sandra to ask how they fit into the expert network.

How and why did RIC become involved in MAPs?

One of our core business imperatives is method development and validation; over the years, we have developed many analytical methods for food analysis, environmental monitoring, the chemical and energy industries, and so on. Our colleagues at Agilent Technologies have, over time, become aware of our unique application expertise, so I think our involvement in MAPs is a logical continuation of our long standing collaboration.

What expertise does RIC bring to MAPs?

The focus at RIC is the development of total analytical solutions. One of our strong points lies in the initial steps (often the decisive steps in terms of the quality of data produced) in the analytical organogram: sampling and sample preparation. This is very complementary to the instrumental portfolio of Agilent and allows us to develop turn-key solutions, including the preparation of standard operating procedures (SOPs) and completion of validation studies. Very often, this is accompanied with an in-depth demonstration at RIC laboratories and the training of technicians both on sample preparation and the instrumentation used.

How does MAPs resonate with RIC's philosophy of "Creating new techniques, finding new applications…Together"?

RIC is primarily a research center; but private research only makes sense (and becomes financially viable) when those new techniques are used for new applications. "Together" means that we must first learn about the new analytical challenges customers are facing – either directly from them or through partners, such as Agilent Technologies and Gerstel. The result is a final solution that is based on our research and the instrumentation of our partners.

In what way does MAPs benefit RIC?

RIC is both a partner lab and a channel partner; depending on our role, we benefit in different ways. As partner lab in MAPs we gain:

- excellent visibility within the Agilent organization
- access to a network of Partner Labs that allows us to collaborate in fields in which we are less specialized, for example ICP-MS
- increased appreciation of our research activities
- new contacts
- fresh awareness of analytical challenges.

As a channel partner, we gain an understanding for:

- new region-specific problems
- instrumental needs in our region.

Pat Sandra is founder and president; Tom Sandra is managing director; and Koen Sandra is R&D director of Life Sciences and Metablys, all at the Research Institute for Chromatography, Belgium. www.richrom.com

is also perfect for complex projects, where a suite of instruments for a new lab is needed – we offer a full guarantee that that the customer will quickly become productive.

The promise

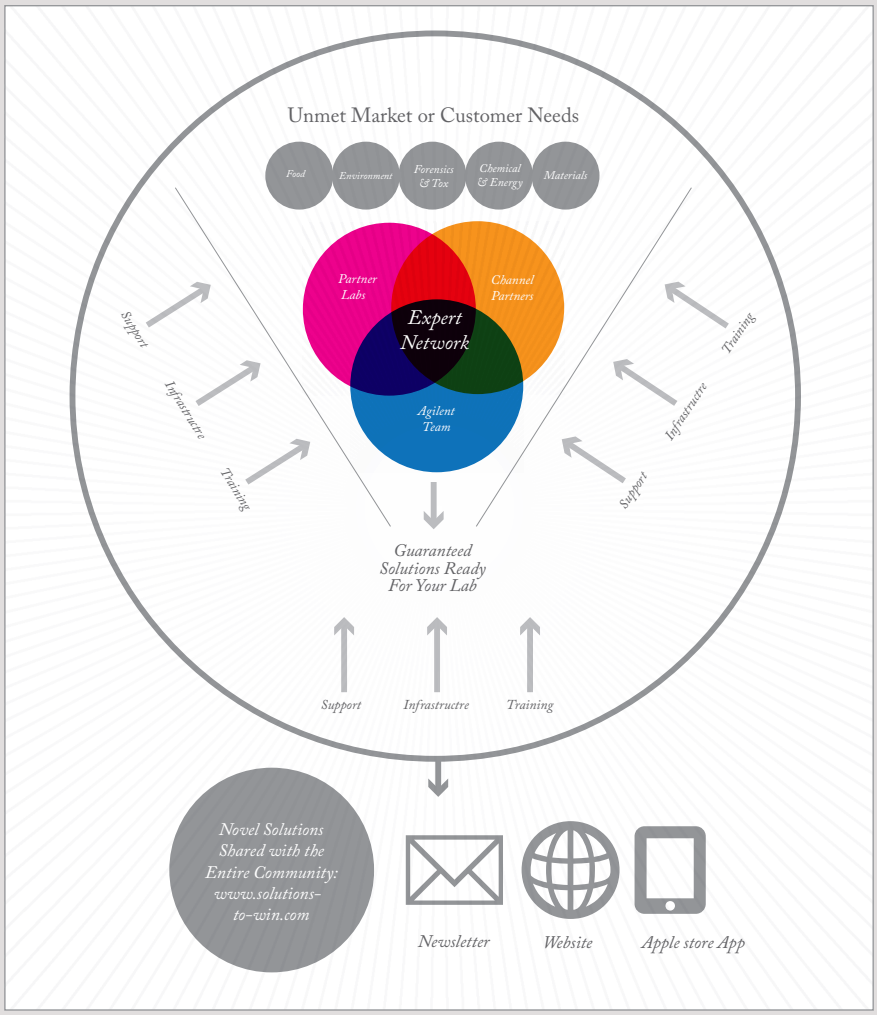
Could you tell us more about support?

Armando: Providing expertise is just one half of MAPs; the other half is our support function. Historically, the level of support delivered to customers could vary – the same is true for most instrument manufacturers. Now, we've added clarity, in the form of five different levels of solution guarantee. The support team gets involved from the very beginning, and that enables them to identify the necessary resources for each individual solution installation. For example, in our highest-level installations, our partner labs perform robustness tests, validate methods, provide standard operating procedures, and even train the customer and Agilent support team ahead of installation. Such advanced support means that our promises – or our solutions – are always fully delivered.

How is MAPs changing Agilent?

Marco: The culture shift within Agilent has been significant. Application support is very much the Number One goal. With our expert network, we offer top support to our customers, and we also use it to grow the knowledge base of our own support team by learning from each and every installation – and that allows us to take full responsibility.

We have set entirely new expectations; our customers are not buying an instrument they are buying a complete solution, and they not only expect us to install the hardware, but to see it running their application in their lab. We are shouldering a lot of responsibility by offering this level of support, but it is very much in line with the needs of current and future customers.



A finger on the pulse

How do you keep ahead of the game?

Armando: We classify each information request, including market area, the most common matrix, and the analytes of interest to discover how people plan to use our instruments, which gives us insight into market – or application – trends. The granularity of our approach has helped distill real market drivers and allows us to focus on developing applications and solutions that our customers really need, instead of relying on a gut feeling. Being so aware of market trends also enables us to have a much closer relationship with our customers and a much deeper understanding of their future requirements.

Marco: A welcome byproduct of working with such a tight-knit network is that any knowledge gained becomes valuable feedback for our R&D department. Sooner or later, customers can expect to see next-generation hardware and software that is very much aligned with market trends and needs. After all, continuing to drive the entire field forward is in everyone's interests.

For more information on MAPs or to sign up for the MAPs newsletter: www.solutions-to-win.com.

Savvy Channeling

The Channel Partners are the final piece of MAPs. Here, we present a selection that covers an impressive analytical and geographical spread.

Gerstel

Specialty: automated sample preparation and sample introduction devices for GC-MS and LC-MS in food, environmental and forensics.
Location: Germany, with major country distribution.
www.gerstel.com

JSB

Specialty: complete solutions from sample prep to data handling. GCxGC thermal modulation technology.
Location: Austria, Benelux, Germany, Switzerland, UK.
www.go-jsb.com

AC Analytical Controls

Specialty: chromatographic solutions for energy and chemical market.
Location: The Netherlands, with global presence.
www.paclp.com

SRA Instruments

Speciality: purge and trap, thermosorbers, pyrolizers with chromatography, MS systems, LC-GC and GCxGC.
Location: Italy and France.
www.srainstruments.com

Ingeniería Analítica (IA)

Specialty: Turn-key solutions from a wide portfolio of techniques. Mobile solutions for environmental and forensic analysis.
Location: Spain
www.ingenieria-analitica.com

KEEPING TABS ON DIOXINS

Exposure to dioxins and polychlorinated biphenyls (PCBs) remains a significant threat to a substantial proportion of the European population. To maintain high levels of vigilance, analytical strategies need to evolve. Here, I propose substantial changes to the EU's monitoring strategy, changes that channel the power of modern analytical technology to accurately, rapidly and inexpensively monitor food and feed for dioxins, PCBs and as-yet-unidentified toxicants.

By Jean-François Focant

Dioxins first entered public consciousness indirectly. In the late 1950s, chick edema disease appeared: high death rates were reported in broiler chickens in the United States, associated with excessive fluid in the heart cavity, necrosis of the liver, and subcutaneous edema. It transpired that feed manufacturers had been using low-cost fat to increase the energy value of their products and the 'toxic factors' responsible for the high levels of chicken mortality were narrowed down to new fatty acid by-products of inedible tallow. It was another decade before 1,2,3,7,8,9-hexachlodibenzo-*p*-dioxin (which originates from the use of chlorophenols as a preservative in hide-stripping operations) was finally identified as the molecule responsible for the hydropericardium disease (chick edema disease) that killed more than a million chickens (1).

Dioxins and dioxin-like compounds (DLCs) are by-products of various industrial processes, and are commonly regarded as highly toxic compounds that are environmental pollutants and persistent organic pollutants (POPs). The heavy use of Agent Orange during the Vietnam War and Italy's 1976 Seveso incident raised awareness about the threat of dioxins to human beings. Meanwhile, the Yusho (Japan, 1968) and Yu-Cheng (Taiwan, 1979) food poisoning incidents highlighted the risk of polychlorinated biphenyls (PCBs) – which are precursors of dioxins – entering the food chain. Medical follow-up of the exposed populations in all incidents identified a broad range of severe health effects, some of which were passed onto offspring long after the original exposure (2).

In 1999, although concern over dioxins had faded among the general public (and at health protection agencies), there was another food contamination episode in Belgium (3). The accidental incorporation of contaminated oil (containing about 40-50 kg of PCBs and 1-2 g of dioxins) to a batch of slaughterhouse fat intended for feed production resulted in the distribution of 500 tons of contaminated feed to more than 2,500 poultry and pig farms in Belgium and neighboring countries. This was far from being the largest or most dramatic dioxin-related food incident; however, poor management of the resulting crisis had striking political and economical effects, including some 9,000,000 chickens and 60,000 pigs being destroyed, and an estimated economic cost of €2 billion (4). Fortunately, the general population was not exposed to significant levels of dioxins; even in the highest risk populations, exposure to dioxins barely reached levels that had been routinely observed 10 years before the crisis (5).

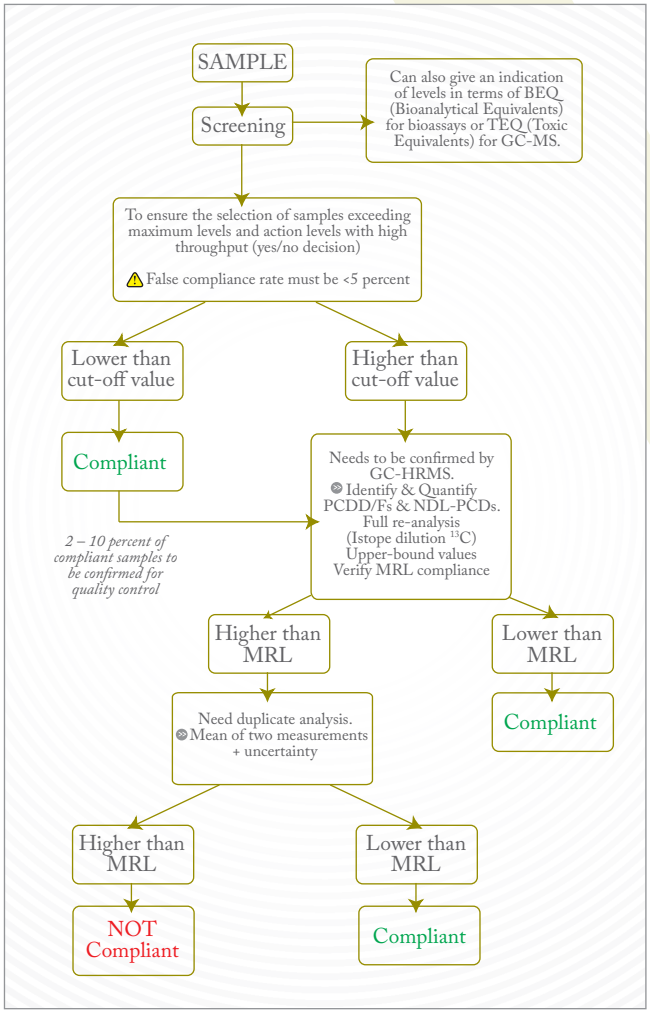


Figure 1. Scheme of the EU screening-confirmatory approach currently in place for the continuous monitoring of food and feed.

The 1999 crisis pushed Belgium to implement maximum residue levels (MRLs) for PCBs in a systematic national monitoring program for food of animal origin (the CONSUM program), and to create the Federal Agency for Food Safety (FAVV-AFSCA) in 2000. This prompted action at the European Union (EU) level, resulting in the

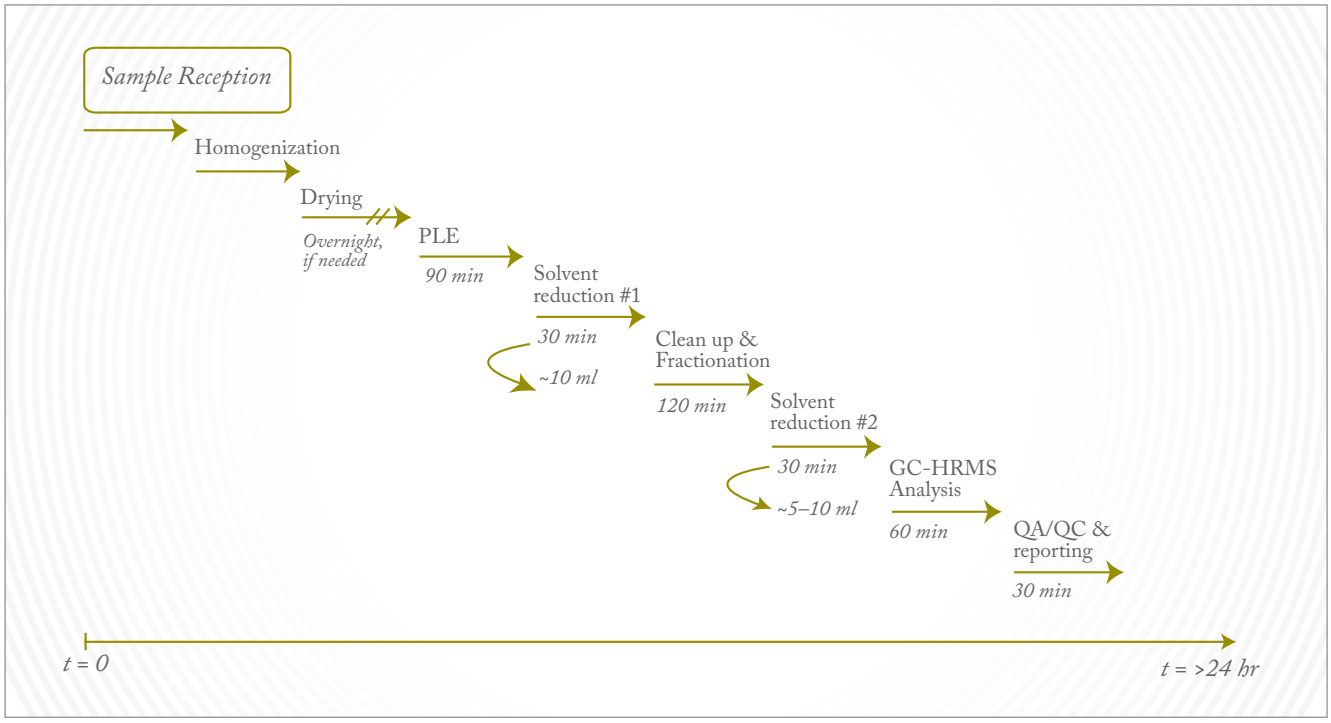


Figure 2.Time line of a typical sample processing procedure for food or feed.

implementation of matrix-dependent MRL EU-harmonized norms for dioxins and, later, for dioxin-like (DL)-PCBs. The legislation paved the way for the EU to set up a strategy of continuous monitoring of food and feed in all member states.

The demands of such an ambitious monitoring program required the means and capacity to perform measurements that were cost-effective, precise, accurate and robust. State-of-the-art ^{13}C-labelled isotope dilution gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) was too expensive to implement on a wide scale. Therefore, a "screening-confirmatory" approach was selected (see Figure 1). Screening uses bioanalytical and GC-MS methods, while GC-HRMS is mandatory for confirmation of suspected non-compliance. Expert working groups proposed strong and detailed analytical guidelines for both GC-(HR)MS and bioanalytical methods to ensure quality. Because it is among the most stable and sensitive assays, the screening method of choice is chemical-activated luciferase gene expression (CALUX) response binding assays (6).

A key aspect of the working groups' recommendations was the need for a performance-based measurement system (PBMS) that states 'what' needs to be accomplished, but not prescriptively 'how' it must be carried out. This permitted flexibility in method selection and also allowed for new developments to meet mandated monitoring requirements as improvements of existing procedures became available. Today, 15 years after the introduction of the first post-1999 dioxin crisis EU documents, and after the release of more than 20 major regulations, directives, or recommendations, the PBMS approach is still followed in European Standards (7). The strategy was enhanced by the development of a rapid alert system for food and feed (RASFF), which requires member states to immediately notify the European Commission (EC) about any information on serious health risks related to PCDD/F contamination derived from food or feed.

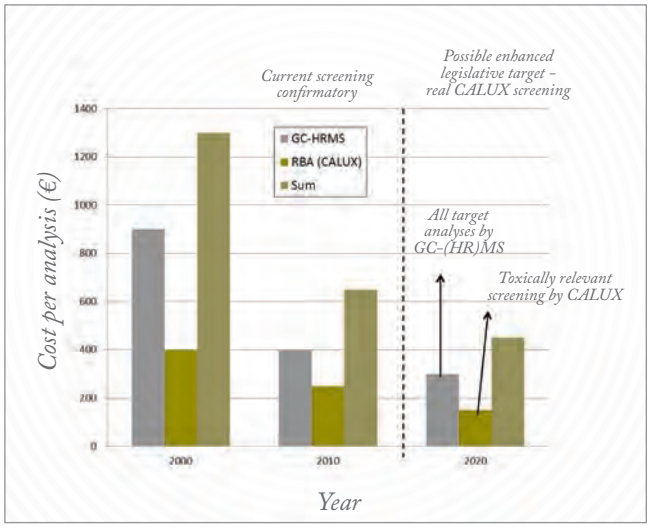


Figure 3. Evolution of the relative costs of screening and confirmatory analyses.

Improving sample preparation

Food-feed control is far easier to describe than to properly implement. This is because target levels are as low as pico- or femtogram per gram of matrix, with matrix-related interferences present in concentrations at orders of magnitude higher than the analytes of interest. As a result, a multi-step approach is required to (i) extract the analytes from the matrix core, (ii) separate out potentially interfering compounds, and (iii) isolate, separate and quantify analytes of interest.

The clean-up procedure for dioxins in biological samples has its roots in organochlorine pesticide residue analysis. It evolved from single-stage procedures to more advanced multi-steps approaches, such as acid washes and partitioning, silica gel, alumina, and carbon column chromatography, as well as size-exclusion chromatography. In the mid 1980s, due to increasing demands for sample throughput, an automated sample clean-up apparatus was developed for the isolation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from adipose tissues and human serum by the US Centers for Disease Control and Prevention (CDC) (8). Soon thereafter, automated sample clean-up was developed for other bio-accumulative polychlorinated dibenzo-*p*-dioxins (PCDDs, or dioxins), polychlorinated dibenzofurans (PCDFs, or furans), and ortho unsubstituted (planar, non-ortho) polychlorinated biphenyls (PCBs).

Prior to the clean-up step, several simple variants of preliminary extraction may be used, such as Soxhlet, liquid-liquid, solid-phase, and column extraction. Instrument approaches including microwave-assisted extraction (MAE), pressurized fluid extraction (PFE), and supercritical fluid extraction (SFE) are also popular for the extraction of the lipid portion containing the targeted dioxins.

To date, no "best combination" of extraction and clean-up techniques has been identified. There are several good options, but more than 30 years of investigation have not identified the definitive dioxin sample preparation approach. Thank goodness for PBMS! Essentially any working combination that fulfils regulation criteria can be accepted as a reference method.

Irrespective of the combination used, analysis times are long. This is problematic in the context of food-feed control because foodstuffs cannot stay under inspection for a long period without generating economic losses. More importantly, food safety agencies need fast response strategies to deal efficiently with potential dioxin contamination incidents. Remember, all this is in advance of the measurement itself which, for both biological screening and GC-HRMS confirmatory analysis, will require additional time.

Automation and integration

The proper management of dioxin incidents must be pursued with urgency and effectiveness; this means that reference accredited laboratories must have a certain level of automation. Once alert systems identify a potential issue, the precautionary principle is enforced: this can result in a few thousands farms being blockaded, again with tremendous economic effects. Proper traceability mechanisms and liberation of non-incriminated farms can only start once full analytical data sets become available.

In practice, this means that hundreds of complex sample matrices must be analyzed in matter of days, if not hours. The networking of national reference laboratories ensures widespread geographical analytical capability, while coupling and hyphenation of the various analytical steps ensures rapidity. For example, pressurized liquid extraction (PLE) coupled to automated solvent reduction-exchange devices can produce

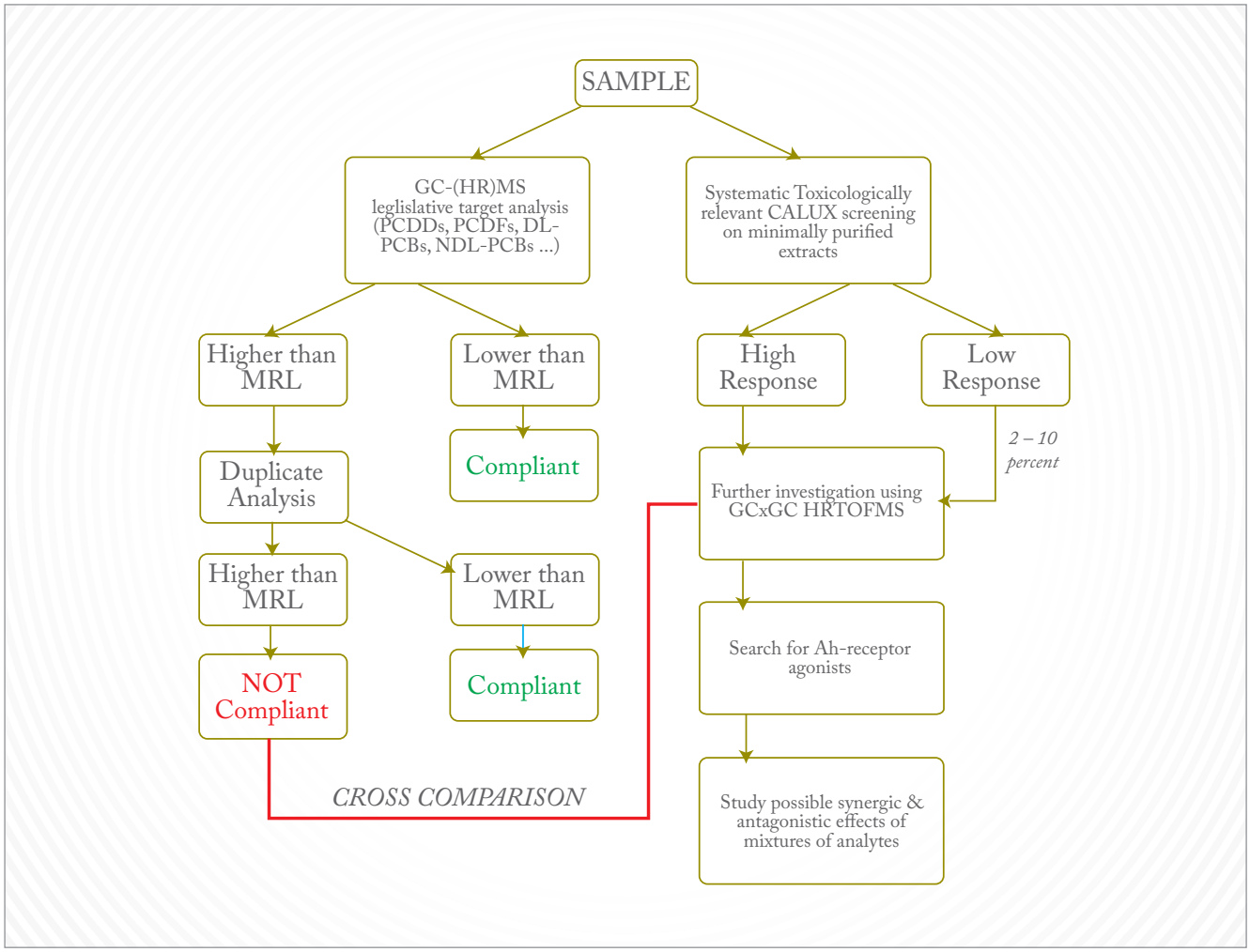


Figure 4. Scheme of a possible new approach for the continuous monitoring of food and feed, as well as screening for other untargeted toxicants.

sample extracts that are further cleaned-up in-line via multi-step liquid chromatography (LC) setups (9). The sorbents used in such LC setups allow the separation of the cleaned extract into subgroups of compounds (PCDDs, PCDFs, PCBs) depending on their polarity and geometry. The fractionated extracts can be further evaporated using a hyphenated solvent reduction-exchange device to reach the required concentration factor, at which point the extracts can be transferred to GC injection vials for separate GC-MS measurements.

Following such approaches, medium-sized laboratories can reach throughput levels of around 20 customer reports per day, including the congener-specific data (on 29 analytes) that are necessary for patterning and source identification. In fact, the current EU confirmatory task force, based in the 27 official or national reference laboratories (OFLs and NRLs), has a combined capacity of approximately 1000 samples per week, which is surprisingly much larger than the screening capacity of 300 samples per week provided by the seven OFLs responsible.

The death of screening?

The tremendous increase in sample throughput afforded by automation and general analytical capacity inside EU laboratories has been mirrored by a reduction in the cost to perform analyses. Today, most food and feed samples can be fully processed, with levels reported on a congener basis, in less than 24 hours (see Figure 2) and for less than €350.

Such confirmatory analysis speed forces us to reconsider the wisdom of following the screening-confirmatory approach. As shown in Figure 3, prices have drastically decreased since the 1999 dioxin crisis era and this trend is expected to continue for the next few years. Perhaps it is time to revise the strategy by using only confirmatory tools to monitor the food-feed web for PCDD/Fs and selected PCBs – this could be renamed the 'legislative target approach'.

In fact, such an approach was tested in Italy in 2008 (10), when high rates of MRL non-compliant samples were anticipated. In cases where the non-compliance rate exceeds 35 percent, performing the confirmatory analyses exclusively is less expensive than first screening all samples to estimate a global level of contamination and then performing analyses on suspected non-compliant samples. It also reduces the response time and allows congener-specific data to be used to trace the source of contamination and take rapid action. All this can be done at a lower cost than non-congener specific screening analysis in the year 2000.

Given the fact that congener-specific data are a crucial component of the EU's pro-active approach to reduce the presence of dioxins and DL-PCBs in food and feed, it seems illogical to follow any other path.

Looking five and 10 years ahead

The 'legislative target approach', using GC-(HR)MS instruments, would be a cost-effective approach to regulatory analysis of target analytes. Note that 'HR' is set in brackets because other types of MS analyzers of the latest generation could also be used. As an example, triple quadrupole systems performing in tandem mode are now also recognized for the official control (confirmatory) at the EU level (11). Such GC-MS analyses benefit from the latest advances in sensitivity enhancement, which is useful given that levels of dioxins are continuously decreasing in food and feed. Tools based on cryogenic zone compression (CZC) should be commercially available in the near future and offer the possibility of boosting chromatographic signals 'on demand' to attain low femtogram to high attogram performance levels (12, 13).

"Perhaps it is time to revise the current strategy by using only confirmatory tools to monitor the food-feed web for PCDD/Fs and selected PCBs."

This approach removes screening from the equation. However, this does not mean that the CALUX assay ceases to be of value. Instead, it is liberated for broader objectives than simply looking for dioxins. The CALUX response is based on the binding of dioxin-like compounds to the aryl hydrocarbon receptor (AhR); however, CALUX assays are not only sensitive to PCDD/Fs and DL-PCBs, but also to many other AhR agonists, including brominated dioxins and biphenyls, polyaromatic hydrocarbons, benzimidazole drugs, and natural occurring flavones (14). This lack of specificity is a major issue for the legislative use of CALUX as it requires extracts to be processed through several clean-up steps to isolate target analytes from other AhR agonists. Even with these clean-up steps, the use of matrix specific reference samples and cut-off strategies are often required to minimize the risk of false-positive/false-regulatory results.

With the CALUX no longer needing to be 'tuned' to satisfy the specific dioxin monitoring program implementation, it can be re-purposed as a real toxicity-screening tool. The CALUX response to extracts is of considerable toxicological relevance. The potential synergistic or antagonistic effects of mixtures of untargeted toxicants is a wide-open question but analyzing high-response CALUX extracts by sensitive GC-MS methods could identify emerging analytes that are capable of triggering an Ah receptor response – in other words, analytes capable of

exerting dioxin-like effects. A potential scheme of analysis that uses this is presented in Figure 4.

The selected ion monitoring (SIM) MS scanning mode used for sensitive target analysis cannot be used for the identification of unknown substances; full-scan mass acquisition is needed to screen chromatographic signals for new compounds, and for this the latest generation of time-of-flight (TOF) MS systems has a lot to offer. Current GC-TOF MS instruments offer the mass resolution of sector instruments whilst maintaining limits of detection (LODs) at the low picogram level. The complete deconvoluted mass spectral data set produced by high resolution TOF MS of high mass accuracy can be processed against reference spectra libraries to identify compounds based on fragmentation and exact mass. The high spectral acquisition rates of the last generation of HRTOFMS instruments also allows the chromatographic separation to be operated in comprehensive two-dimensional mode (GC×GC) to enhance both the separation power and compress chromatographic signals for better detectability (15).

The take-home message

Although the EU's continuous monitoring strategy has been in place for nearly 20 years, I do not consider the dioxin problem to be solved. Levels of dioxins and PCBs in our food web have been decreasing but are still not below the level that would ensure that the entire population is safe. Analytical procedures have evolved, making such measurements more rapid and cost-efficient, to the extent that the current screening-confirmatory approach should be revisited, in my opinion. A legislative target approach not only makes sense from cost and efficiency perspectives, it also frees up CALUX to screen for as-yet-unidentified toxicants responsible for false-regulatory results that could present dioxin-like adverse effects.

A comprehensive strategy that includes legislative target analyses using state-of-the art analytical weapons, coupled to a toxicologically-relevant CALUX screening backed by compound identification tools would more efficiently protect consumers' health. Taking full advantage of what modern separation science offers will deliver better standards of food safety. In short, we still have some work ahead of us.

Jean-François (Jef) Focant is a professor in the Department of Chemistry at the University of Liège in Belgium.

References

1. *D. Firestone, "Etiology of Chick Edema Disease", Environ. Health Perspect. 5, 59–66 (1973).*
2. *K. Tsukimori, "Long-term Effects of Polychlorinated Biphenyls and Dioxins on Pregnancy Outcomes in Women Affected by the Yusho Incident", Environ. Health Perspect. 116, 626–630 (2008).*
3. *A. Bernard et al., "Food Contamination by PCBs and Dioxins", Nature 401, 231–232 (1999).*
4. *A. Covaci et al., "The Belgian PCB/dioxin Crisis—8 Years Later: An Overview", Environ. Toxicol. Pharmacol 25, 164–170 (2008).*
5. *A. Bernard et al., "The Belgian PCB/Dioxin Incident: Analysis of the Food Chain Contamination and Health Risk Evaluation", Environ. Res. Section A 88, 1–18 (2002).*
6. *L. Hoogenboom et al., "The CALUX Bioassay: Current Status of its Application to Screening Food and Feed", TRAC 25, 410–420 (2006).*
7. *CSN EN 16215, Animal Feeding Stuffs – Determination of Dioxins and Dioxin-like PCBs by GC/HRMS and of Indicator PCBs by GC/HRMS, 2012.*
8. *C.R.Jr. Lapeza et al., Automated Apparatus for the Extraction and Enrichment of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Human Adipose Tissue", Anal.Chem. 58, 713–716 (1986).*
9. *J.-F. Focant et al., "Automated Sample Preparation-fractionation for the Measurement of Dioxins and Related Compounds in Biological Matrices: a Review", Talanta 63, 1101–1113 (2004).*
10. *M. Esposito et al., "Contamination Levels and Congener Distribution of PCDDs, PCDFs, and Dioxin-like PCBs in Buffalo's Milk from Caserta Province (Italy)", Chemosphere 79, 341–348 (2010).*
11. *SANCO/11950R2/2013 to become Commission regulation (EU) No …/.. of…. Amending Regulation (EC) No 152/2009 as regards the determination of levels of dioxins and polychlorinated biphenyls.*
12. *D.G.Jr. Patterson et al., "Cryogenic Zone Compression for the Measurement of Dioxins in Human Serum by Isotope Dilution at the Attogram Level Using Modulated Gas Chromatography Coupled to High Resolution Magnetic Sector Mass Spectrometry", J. Chromatogr. A 1218, 3274–3281 (2011).*
13. *D. Krumeweide et al., "Zone Compression (t-CZC): A Novel Gas Chromatographic Tool for Increasing Sensitivity", Chromatogr. Today Feb/March 2012, 19–23.*
14. *R. Hoogenboom, "The Combined Use of the CALUX Bioassay and the HRGC/HRMS Method for the Detection of Novel Dioxin Sources and New Dioxin-like Compounds", Environ. Sci. & Pollut. Res. 9, 304–306 (2002).*
15. *J.-M.D. Dimandja, "Comprehensive 2-D GC Provides High-performance Separations in Terms of Selectivity, Sensitivity, Speed, and Structure", Anal. Chem. 76, 167A–174A (2004).*

RESOLVE YOUR SEARCH FOR ACCURACY

The Agilent 7000C Triple Quadrupole GC/MS is the latest addition to the industry's broadest, most feature-rich portfolio of GC and GC/MS systems and software. It is designed to deliver highly accurate quantitative results and confident identification even with the most complex matrices thanks to new, high productivity enhancements.

- Enhanced Agilent MassHunter software with new MRM optimization tools
- Extended Pesticides and Environmental Pollutants Database
- New environmental PAH analyzer with automated Self-Cleaning Ion Source
- Programmable Helium Conservation Module
- Early Maintenance Feedback to assure maximum productivity search
- And more integrated intelligence tools for the GC/MS system

The Measure of Confidence

RESOLVE YOUR SEARCH FOR ACCURACY
Visit **www.agilent.com/chem/7000C**

Solutions for your analytical business
Markets & Applications Programs

Agilent Technologies

Exploring Chinese Medicine with 2D-LC

Two-dimensional liquid chromatography certainly sounds impressive and produces some pretty pictures, but is it essential in the analysis of complex samples? Here, we show that 2D-LC offers both style and substance.

By Oliver Schmitz and Duxin Li

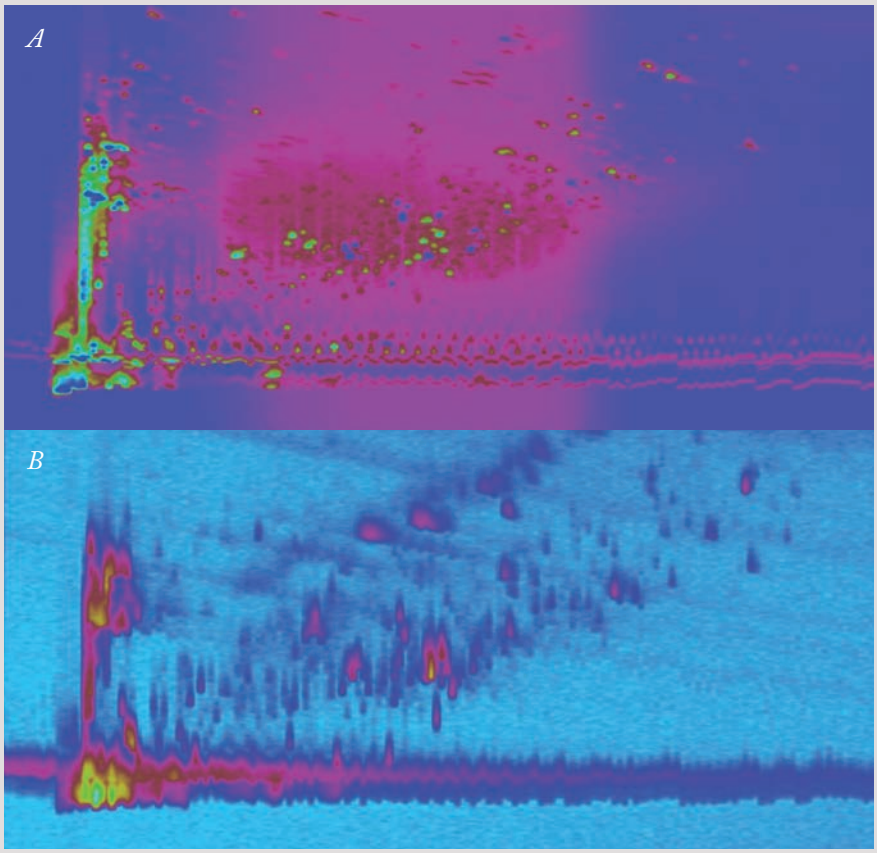


Figure 1. LCxLC analysis of an aqueous extract of two herbs, *Scutellaria barbata* and *Oldenlandia diffusa* (A: diode array detector, B: ESI-qTOF-MS)

Chinese medicine is an ancient art. The earliest existing Chinese text on medicine dates back to the second or first century BC – the Huang Di Nei Jing. Typically, in early Chinese medicine, several plants, each with hundreds or even thousands of compounds, are formed into a single drug formulation. Today, much has remained the same – the normal formulation of a Chinese herbal medicine (CHM) is extremely complex. Product control that is comparable to Western medicine is a huge challenge. CHM has, unsurprisingly, attracted a lot of attention throughout the world as our search for more effective medicines delves ever deeper. It follows that researchers are interested in the chemical constituents of Chinese herbs and the origins of their pharmacological and thus therapeutic activities.

Essential separations

We've probably all heard someone say that because the number of labs with high-resolution mass spectrometry (HRMS) is increasing, less and less chromatography will be required ahead of detection. For the non-chromatographers out there, that might even sound quite tempting. However, in reality, it's a statement that does not really make much sense: if all components of a sample are injected into the ion source at the same time and if a large percentage of those compounds are ionized, then – in a complex sample like Chinese herbs – several thousands of radical cations will be formed. And in an atmospheric-pressure ion source, such as ESI or APCI, all of these resulting radical cations can react or interact, each encountering approximately 20,000 collisions from the point of ionization to the entrance of the MS. The result is potential ion suppression and/or formation of artefacts. That's a problem. The solution? The addition of a high performance chromatographic platform in front of the MS! Such a combination is (and may always be) the gold standard, even with HRMS.

Comprehensive 2D-LC – or LCxLC – offers the high performance separation desirable for complex samples. Indeed, our group specialises in the use of comprehensive techniques to analyze various herbs in CHM. More non-polar species are analyzed using GCxGC-MS, whereas more polar compounds are analyzed using LCxLC-MS (see Figure 1).

Of course, as with any analytical technique, there are advantages and disadvantages of LCxLC, which are dependent on the application. For us, when compared with one-dimensional

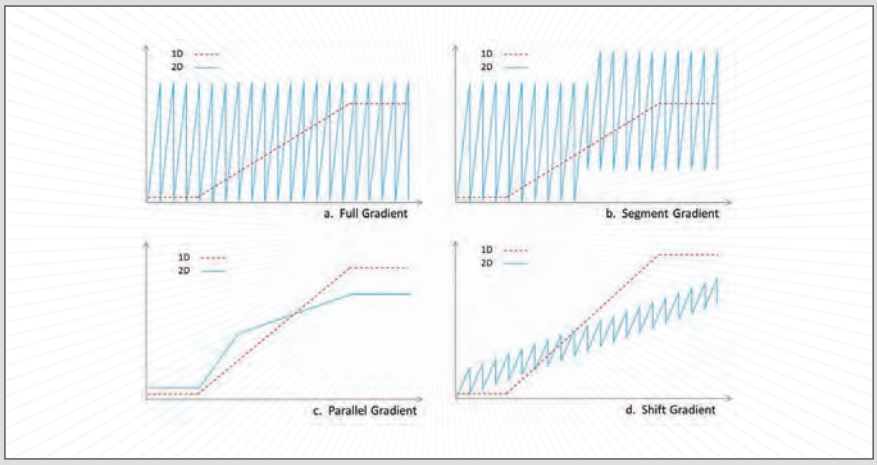


Figure 2. Graphical representation of different gradient programs used in 2D-LC.

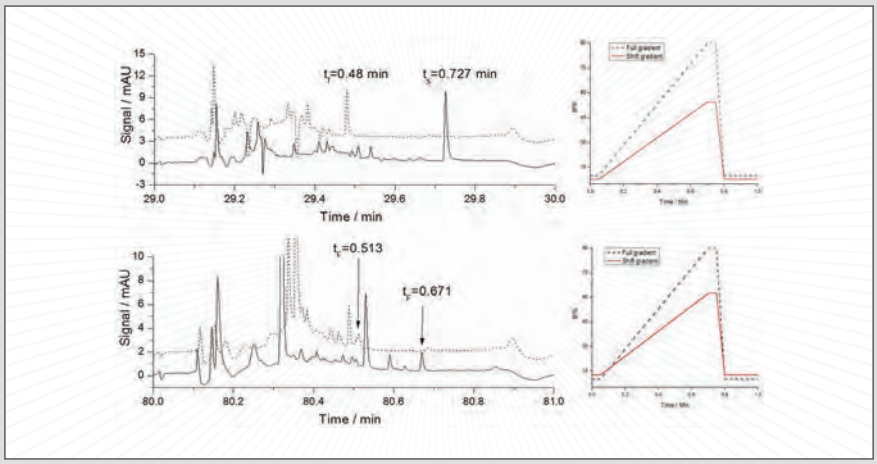


Figure 3. Comparison of full gradient (dotted line) and shift gradient (solid line) in fractions 29 (upper chromatogram) and 80 (lower chromatogram) of an LCxLC analysis from an aqueous extract of *Hedyotis diffusa* and *Oldenlandia diffusa*, with corresponding gradient programs on the right.

LC, the disadvantages of LCxLC could be summed up as potentially lower sensitivity with MS-detection and the more complex method development (1). However, the advantages are clear: much higher peak capacity (as noted in the first article of this series: tas.txp.to/0314/2DLC) and the ability to produce contour plots that display peak intensity as a function of the retention times in the first and second dimensions – these are excellent for fingerprint-style analysis.

A shift in gear

So, how can we further optimize LCxLC separations? The answer is in the gradient programs used (see Figure 2). Our system allows the use of a constantly shifted gradient in the second dimension, which uses a narrower range of mobile phase composition than the full gradient program but continuously shifts the concentration range according to retention. The shift gradient is really a combination of a parallel gradient and a full gradient; the lower concentration range enables the retention of weakly retained fractions, while the higher concentration range is sufficient to elute strongly retained fractions, as with a parallel gradient. The shift gradient offers bandwidth suppression but also reduces the probability of "wrap-around" behavior, just like a full gradient.

In a correlated RPLCxRPLC system, the early-eluted analytes in the first dimension will have a weak retention in the second dimension; the analytes eluted in the middle of the first separation will be eluted in the middle of second dimension; and the late-eluted analytes in the first

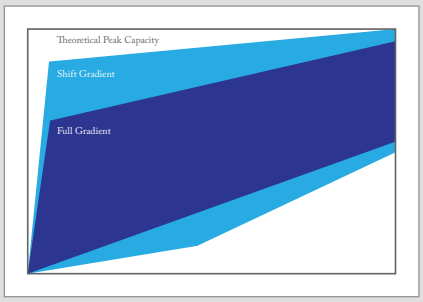


Figure 4. Comparison of peak distribution area of full and shift gradients (adapted from reference 3).

dimension will have a strong retention in the second dimension. And because the shift gradient runs in a continuous way, the cluster information in real world samples is preserved.

Undoubtedly, the shift gradient increases the separation power in the second dimension significantly as shown in Figures 3 and 4 (2).

2D-LC or not 2D-LC, that is the question

Should you be leveraging the power of 2D-LC? If you want increased separation of complex samples, then absolutely! Non-targeted analyses, such as identification of disease biomarkers, have become much more powerful using LCxLC-MS. And we are only at the beginning. The next step is LCxLC-IMS-qTOF-MS. Some of the first investigations of this sort are being done in my lab right now…

Oliver Schmitz is a professor of applied analytical chemistry and Duxin Li is a post-doc at University Duisberg-Essen, Germany.

Next month, the "Demystifying 2D-LC" series will tackle biopharmaceutical analysis.

References

1. *A. P. de la Mata and J. J. Harynuk, "Limits of Detection and Quantification in Comprehensive Multidimensional Separations", Anal. Chem. 84, 6646-6653 (2012).*
2. *D. Li and O. J. Schmitz, "Use of Shift Gradient in the Second Dimension to Improve the Separation Space in Comprehensive Two-dimensional Liquid Chromatography", Anal. Bioanal. Chem. 405, 6511-6517 (2013).*

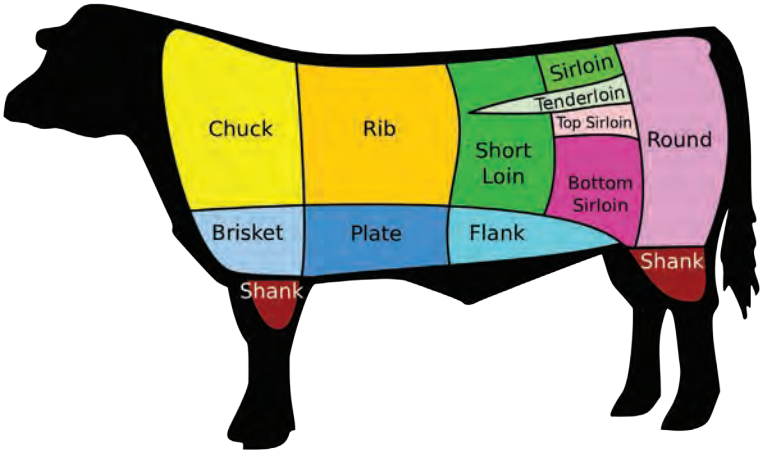
Solutions

Real analytical problems
Collaborative expertise
Novel applications

Keeping it Kosher

The University of Münster and AB Sciex join forces to enhance detection of fraudulent meat using LC-MS/MS

By Ashley Sage



The Problem

Food fraud is not going away – a fact that was cemented by the horsemeat scandal that first surfaced in early 2013. How can speciation testing be improved to quell consumer fears about the authenticity of meat?

Background

At the height of the horsemeat scandal, supermarket chains were forced to announce that some "beef" products actually contained up to 100% horsemeat. This led to concerns about the regulations that were in place for products entering the human food chain and cast serious doubt on the ideal of "farm to fork"; many people stopped trusting what they had been happily eating, resulting in calls from governments, industry and, of course, consumers for a better way to guarantee the authenticity of meat.

The substitution of beef products with horsemeat is likely to have been motivated by cost – what else? Pork, which also trades at a lower price than beef, has also been fraudulently substituted (possibly along with more unsavory species…). And while contamination of beef with meat from other species is unappealing to many people, it can be especially distressing for those with strict religious beliefs. Judaism and Islam both forbid eating food containing any type of pig meat. It follows that Jewish and Muslim communities need to know of any contamination of foods that are otherwise considered permissible to eat (kosher or halal, respectively). Beyond ethical or religious grounds, microbiological and chemical product safety is also at risk with such blatant disregard of food regulations. We need to authenticate our meat.

Common methods that are widely used for meat authentication testing include polymerase chain reaction (PCR) and protein assays, such as enzyme-linked immunosorbent assays (ELISA). These techniques are fairly quick and reproducible, but not always reliable.

PCR is one of the most commonly used methods for meat speciation testing. And though it generally allows for the detection of different species of meat simultaneously, the quality of the sample is important; any sample DNA degradation can be problematic. Unfortunately, such degradation can occur during certain meat processing and food manufacturing processes, giving plenty of potential for contaminants to remain undetected.

Protein assays are also commonly used because they are widely available and easy to use. However, it is difficult analyze for more than one protein marker at a time, making the approach time-consuming and somewhat limited. ELISAs require a greater sample volume because of the difficulties in multiplexing assays. And the generation of false positive or false negative results (caused by cross-reactivity of antibodies or by sample processing, respectively) is a final big issue.

PCR and ELISA sensitivity is considered "acceptable" – that is to say, it meets the the UK's Food Standards Agency (FSA) guidelines , for example, which recommends a one percent threshold for reporting cross-species contamination of meat. Some in the

Species	*Protein*	*UniProt accession*	*Biomarker peptide sequence*	*AA position*
Pig/horse	Troponin T/ unknown	Q75NG7/ F6X010	YDIINLR	239-245/185-191
Pig	Myosin-4	Q9TV62	TLAFLFAER	619-627
Horse	Myosin-2	Q8MJV1	EFEIGNLQSK	1086-1095

Table 1. Marker peptides identified for pig and horse

Jewish and Muslim communities would say that the requirement is to provide quantitative results at the lowest limit of detection possible – a view shared by others with a zero-tolerance policy towards meat contamination.

The Solution

Scientists at the University of Münster, Germany, and AB Sciex in the UK have developed an alternative speciation method for detecting pig, horse or beef proteins in meat. The method was published recently in the Journal of Agriculture and Food Chemistry (1).

A key objective of the work was to develop a solution that is easy to use. The new method, which uses liquid chromatography (LC) and tandem MS (LC-MS/MS), does exactly that while also offering a more accurate, reliable approach to meat speciation than other methods. Steve Lock, Business Development Manager at AB Sciex, suggested in 2012 that: "Mass spectrometry could potentially be used to look for over 10 different species in one go, as has previously been shown in the detection of 16 allergenic species in one analyses" (2). Indeed, the potential for detecting multiple, different animal species in a single run is a further advantage over currently accepted methods.

The first step in method development was to identify species-specific polymorphisms in proteins that would be detectable by MS. The research group at the University of Münster focused on myofibrillar and sarcoplasmic proteins because they are highly abundant and, therefore, allow higher sensitivity. The team extracted protein fractions from commercially available meat samples, including cow, pig, wild boar, horse, chicken and lamb, and used high-resolution MS (HRMS) to identify species-specific biomarker peptides.

The second step was to confirm the presence of targeted meat peptides in unknown samples. A multiple reaction monitoring (MRM) approach was taken, which is capable of providing sequence information, allowing peptides to be identified (see "The System"). The proteins found in horse and beef meat, for example, may differ by only two amino acids; mass spectrometry can detect these differences and indicate where they are in the sequence, avoiding the risk of false positives.

The most abundant biomarker peptides for horse, beef, and pork identified by the non-targeted proteomic approach were used to develop the MRM method. The MS parameters and conditions were optimized, and species specificity confirmed for all chosen peptides.

The three most intense marker peptides identified are shown in Table 1. These abundant biomarkers can be used for the detection of trace contaminations of pork or horse in beef.

Using MRM, the detection limit of horse spiked into beef was 0.55 percent. To achieve higher sensitivity and enhance the signal-noise ratio, the MRM3 mode of the QTRAP system was used. This allowed detection of pig and horse down to concentrations of less than 0.25 percent. Figure 1 shows the detection of two peptide sequences in beef using MRM3.

The sensitivity of the method was enhanced even further, by using the QTRAP 6500 system (equipped with micro-LC) to detect 0.13 percent pork in beef (see Figure 2).

In total, the scientists at the University

The system

Hardware: Tandem Mass Spectrometry (MS/MS) was carried out using the QTRAP 5500 LC-MS/MS system (AB SCIEX)

Software: The iterative workflow of the AB SCIEX MRMPilot software, version 2.1 was used to develop the MRM method.

The possible MRM transitions were identified and introduced into the initial method using predicted MS/MS spectra of target peptides. Purified peptide extracts from respective species or synthesized peptides were used to determine the retention time of target peptides and optimization of collision energy (CE). The most intense transitions were then identified and optimized for a second time.

To enhance sensitivity further, a micro-LC system was used. Positive control samples from respective species were used to assess the retention time stability and relative intensity of MRM transitions.

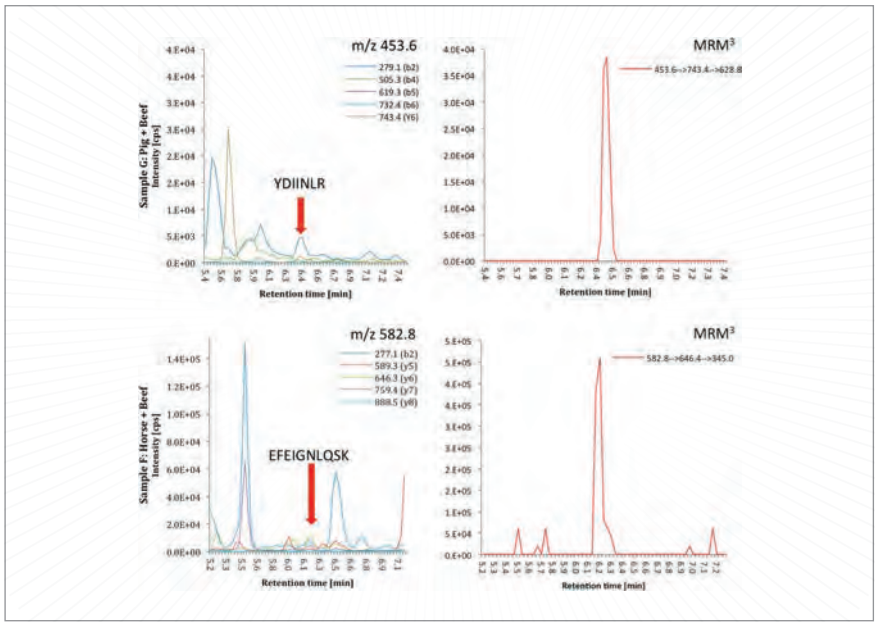


Figure 1. Detection of YDIINLR (upper panels) and EFEIGNLQSK (lower panels) using MRM3. Sample G contains 0.24 percent pork in beef (upper panels) and sample F contains 0.55 percent horse meat in beef (lower panels). The MRM trace is observable for each sample (left). The MRM3 experiment (right) gives an additional specific signal with a highly improved signal-to-noise ratio. Reproduced with permission from (1).

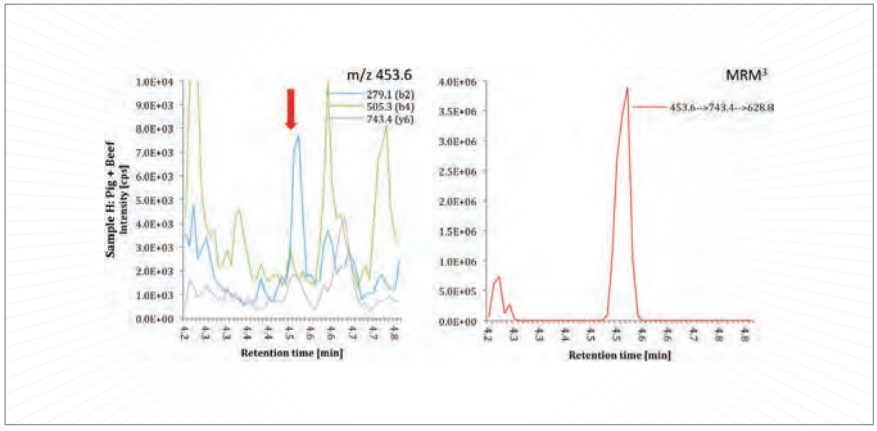


Figure 2. Detection of YDIINLR peptide at a concentration of 0.13 percent pork spiked in beef using the QTRAP 6500 MS by MRM and MRM3. Only the most sensitive transition is observable in MRM mode (left), while the MRM3 experiment gives an additional specific signal with a highly improved signal-to-noise ratio. Reproduced with permission from (1).

of Münster identified 12 tryptic biomarker peptides specific for pork and/or horse meat. To their knowledge, they were the first to use MRM or MRM3 as a sensitive and rapid MS-based technique. The sensitivity of the method is comparable with the most sensitive PCR and ELISA methods and uses only a small amount of sample. Another advantage of the approach is that it does not require extensive pre-fractionation for proteome characterization.

Beyond the Solution

The new method can be used to test meat products quickly and easily for the presence of pork, horse and beef.

Next steps include identifying peptide biomarkers for other species, and the subsequent development of a multi-species identification tool. The method will be optimized for foods that have been heavily processed (for example, pre-cooked); unprocessed (raw) meat was used in experiments outlined here and the sensitivity of the method is likely to be reduced in processed samples or low-quality meat.

At present, MS is often over looked. According to Hans-Ulrich Humpf of the University of Münster, "Many labs have the QTRAP system, but customers don't always realize the full power and capabilities of the QTRAP". But analysis is evolving and, given its many advantages, it is likely that MS-based techniques will be increasingly common in food analysis labs. In fact, MS techniques are already being used for allergen testing in food and wine.

Another future development will be the detection of gelatin from cows, pigs or horses in non-meat foods, which will be of particular interest to those with specific dietary preferences. Watch this space.

Ashley Sage is senior manager of the Food and Environmental Business (EMEA) at AB Sciex, Warrington, UK.

References

1. *C. von Bargen et al., "New Sensitive High-Performance Liquid Chromatography–Tandem Mass Spectrometry Method for the Detection of Horse and Pork in Halal Beef", J. Agric. Food Chem., 61 (49), 11986–11994 (2013).*
2. *S. Lock, "Allergen screening in Food by LC/MS/MS", conference proceedings 126th AOAC Annual meeting, Las Vegas 2012.*

Improving Absorption Measurements through Light Source Selection

Stray light limits the maximum absorbance level achievable in a spectral measurement. Once the stray light limit is reached, sample dilution or a shorter pathlength is required to measure more concentrated samples. In this Application Note, we show the impact of stray light on absorbance levels and describe how optimizing the choice of light source can minimize stray light and increase the maximum absorbance measured.

By Yvette Mattley, Ph.D.

Introduction

The maximum absorbance level achievable with a spectrometer is limited, in part, by stray light. Stray light is undesired light of any wavelength that reaches the detector, including light that reaches the detector from an unintended source (reflection and scatter from optical bench components). Since the detector cannot distinguish stray light from light coming from the intended light path, the system's dynamic range is lessened and the maximum achievable absorbance measurement level is reduced.

Experiment Details

Concentrations of salmon DNA (Sigma D-1626) were prepared in deionized water. DNA absorbance was measured in a 1 cm pathlength cuvette with an Ocean Optics STS-UV microspectrometer and a balanced deuterium-tungsten halogen light source. Measurements were made with both lamps and with the deuterium lamp only to show the impact of out-of-band light on the maximum absorbance level achievable.

Results

The impact of the light source used for the salmon DNA absorbance measurements is shown in Figure 1. Using both the deuterium and tungsten halogen lamps increases the total stray light in the bench due to the addition of visible light outside of the region where the DNA absorbs, resulting in a lower maximum absorbance of ~1.7. When the DNA is measured under the same conditions, but with only the deuterium light turned on, maximum absorbance increases to ~2.1. Also, the linear range of the system drops from 1.2 AU when both lamps are used versus 1.6 AU when only the deuterium lamp is used.

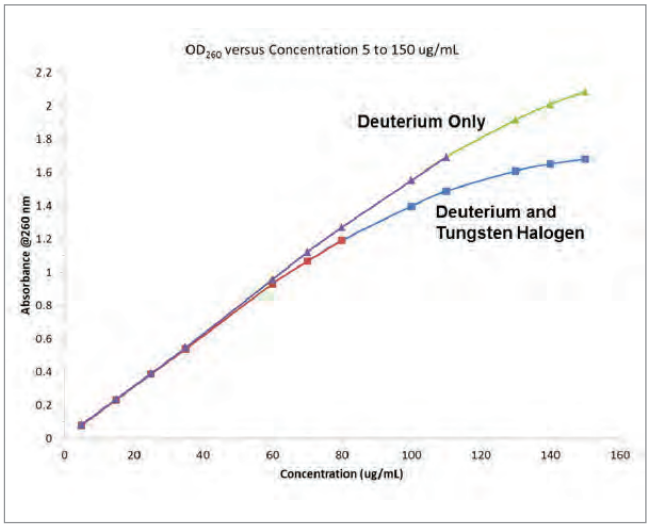


Figure 1. Impact of light source on salmon DNA absorbance measurements.

Conclusion

Stray light is always present in the total system used for absorbance measurements. It limits the maximum absorbance measurement that can be achieved, requiring sample dilution or shorter pathlength sampling cells for highly absorbing samples. As demonstrated by this data, simply avoiding the use of light outside the wavelength range of interest lowers stray light in the spectrometer and enables a wider linear measurement range for a higher maximum absorbance measurement. While many of the typical causes of stray light are out of the user's control, light source optimization is one option that has a significant impact on absorbance measurements.

Ocean Optics
830 Douglas Avenue
Dunedin, FL 34698
Tel: +1727-733-2447
Fax: +1727-733-3962
Info@OceanOptics.com

Profiling of Citrus Oils and Determination of Furocoumarins in Citrus Oils

Comprehensive 2D-LC Analysis with Combination of Normal-Phase and Reversed-Phase

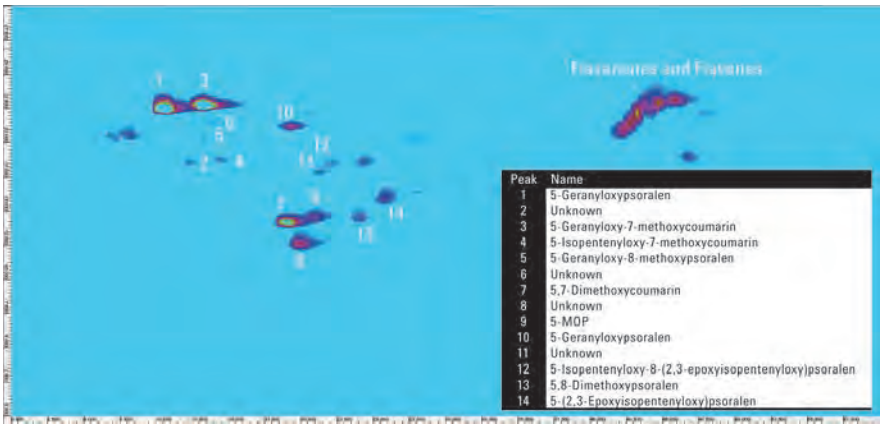


Figure 1

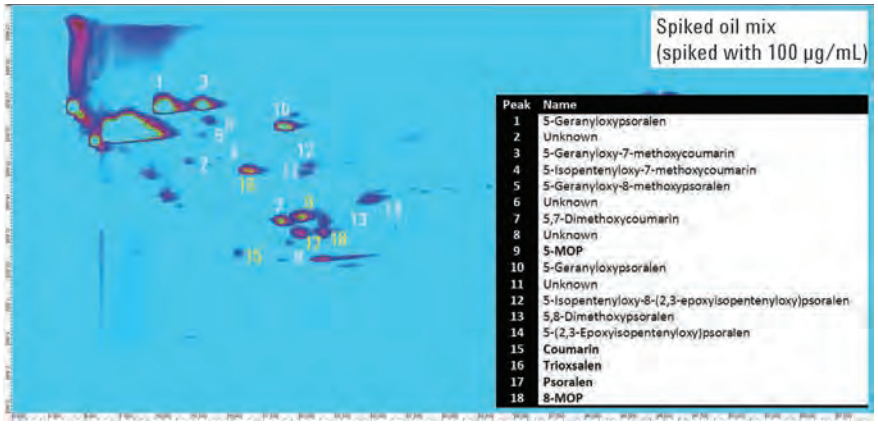


Figure 2

Introduction

Citrus oils are applied in numerous consumer products such as cosmetics and sun protection, food flavoring, and so forth. These oils contain significant amounts of psoralens, methoxylated flavonones, and flavones. A specific species of psoralens, namely furocoumarins, have been identified as photomutagenic and photocarcinogenic products. The analysis of such compounds in citrus oils can be done by (U)HPLC, however, chromatograms of such samples can be complex, and there is only limited certainty that a small peak for a target compound will be separated from other sample constituents. To increase the separation probability significantly, a radical increase in peak capacity is required. This can be accomplished by using comprehensive two-dimensional LC. A combination of normal-phase LC (NPLC) for the first dimension and reversed-phase LC (RPLC) for the second dimension resulted in good orthogonality.

Results and Discussion

Oil profiling

Various oil samples were analyzed and Figure 1 shows the plot for the analysis of a lemon/orange oil mix. The mixture contains various psoralens and analogs, and methoxylated flavonones and flavones. The latter are considerably more polar compared to the lemon oil compounds and need a stronger mobile phase to elute from the normal-phase column. Therefore, a fast gradient to 70 % ethyl acetate is applied from 35 to 36 minutes. As a consequence, the second dimension gradient needs to start with a 100 % aqueous mobile phase throughout the run. This focuses the loop content on the reversed-phase column.

Furocoumarins

Figure 2 shows the plot of a spiked mixed oil sample. It is clear from the contour plot that there are coelutions between the target compounds themselves, and between targets and matrix constituents in both of the single dimensions. The orthogonality with the second dimension results in separation for these compounds.

Conclusion

Different lemon oils were compared with the Agilent 1290 Infinity 2D-LC Solution and the method proved to be useful for characterization of the oils regarding their psoralen, methoxylated flavonone, and flavone content.

Agilent Technologies

Determination of anions with suppressed conductivity detection

Mareike Margraf, Dr. Silvia Marten, Wissenschaftliche Gerätebau Dr. Ing Herbert KNAUER GmbH, Germany

Determination of the common anions, such as bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate, is often needed in water analytics.

Conventional colorimetric, electrometric and titrimetric methods are available for the determination of individual anions, but ion chromatography provides a single instrumental technique that may be used for rapid, sequential measurement.

This Application Note presents the sensitive determination of anions in water samples using the isocratic AZURA Compact System with suppressed conductivity detection. Typically, anions can b rapidly and easily analyzed using conductivity detection with additional apparatus for the suppression of the eluent's conductivity.

Experimental

The stock standard solution was prepared by weighing in anion salt standards and dissolving them separately in deionized water. For the analysis of water, samples are often just filtered through a 0.45 µm syringe filter and injected to the IC system as also described in US EPA method 300.1 and the standard method 4110 (1, 2). More complex sample pretreatment is required if very low concentrations of anions have to be determined or if matrix constituents are interfering with the IC separation (3).

Method parameters

Column	*Anion Column, 250 x 4 mm*
Eluent A	*4.5 mM Na2CO3, 1.4 mM NaHCO3*
Gradient	*Isocratic 100 % A*
Flow rate	*1.2 ml/min*
Injection volume	*50 µl*
Column temperature	*25 °C*

Detection Conductivity Detector CDD-10A VP (5 Hz, 0.02 sec)
SeQuant® SAMS™ robust suppressor for anion chromatography
SeQuant® CARS™ Continuous Regeneration System for SAMS™

Results

The isocratic AZURA Compact system in combination with suppressed conductivity detection was found to be well suited for the analysis of anions in mixed standard solutions even in the low ppm region. Under the chosen conditions, the applied anion column separates all anions within 15 min as shown in Figure 2.

The column was designed specifically for compliance monitoring of inorganic anions in accordance with US EPA Method 300.0 (A) and 300.1 and low molecular weight organic acids. Common inorganic anions can easily be separated in a variety of sample matrices including drinking water, wastewater, process streams, and scrubber solutions with an optimized operating temperature of 30 °C to ensure reproducible retention times.

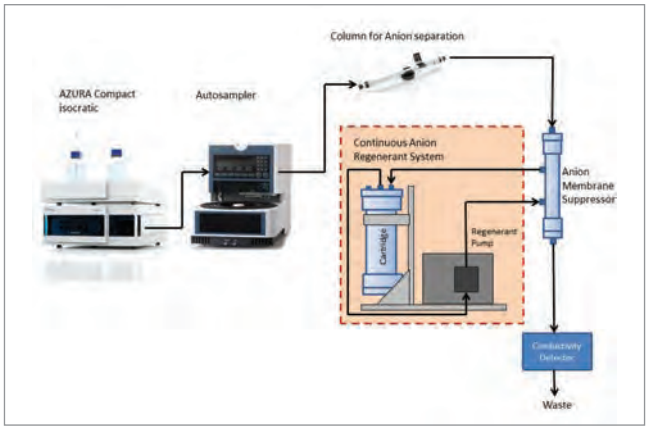


Figure 1: Flow chart of the IC system with anion Membrane Suppressor and Continuous Anion Regenerant System (4)

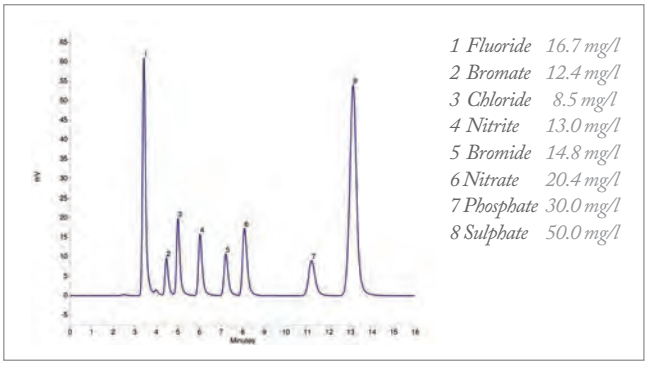


Figure 2: Chromatogram of the anion analysis.

References

1. *John D. Pfaff, Daniel P. Hautman: US EPA method 300.1: Determination of Inorganic anions in drinking water by ion chromatography (Revision 1.0), National Exposure Research Laboratory Office of research and development, U.S. Environmental Protection Agency, Cincinatti, Ohio 45268*
2. *American Public Health Association: Standard Methods for the Examination of Water and Wastewater, Standard Method 4110: Ion Chromatography with Chemical Suppression of Eluent Conductivity http://www.standardmethods.org/store/ProductView.cfm?ProductID=31*
3. *B. López-Ruiz, "Review: Advances in the determination of inorganic anions by ion chromatography", Journal of Chromatography A, 881, 607–627 (2000).*
4. *SeQuant: A practical guide to Ion Chromatography, ISBN 978-91-631-8056-9 (Sweden, 2007).*

A Leading Light

Sitting Down With… Gary Hieftje,
Distinguished Professor and Robert & Marjorie Mann Chair of Chemistry, Indiana University Bloomington.

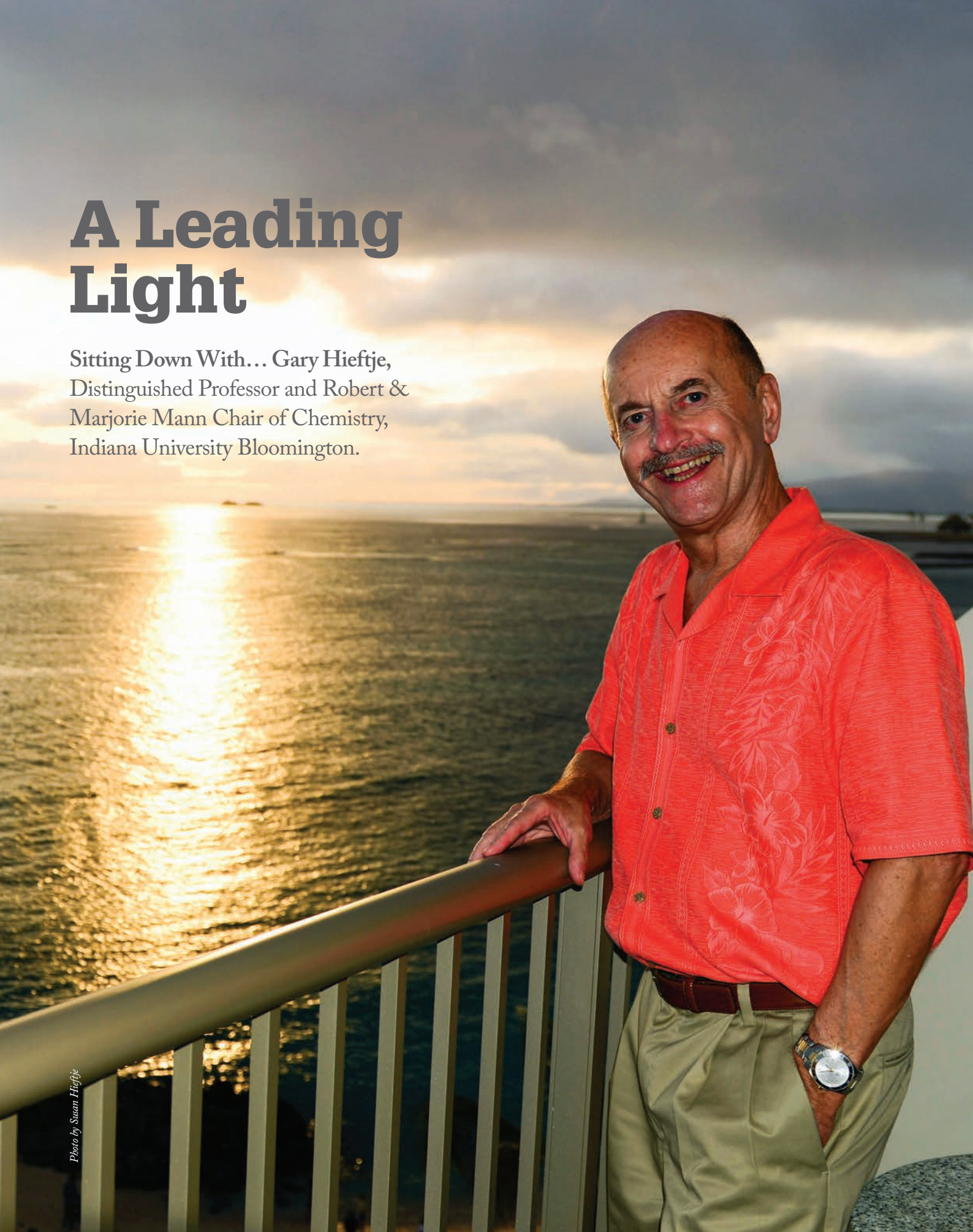


Photo by Susan Hieftje

What gives you the greatest pleasure professionally?

Working with students. Our research publications and patents are, to me, just a by-product; talented students are the real 'product'. Of course, I get a kick out of coming up with new ideas, testing them and publishing the results. But seeing students' eyes light up and watching them get involved in science is tremendously exciting. I had a pre-med student in my group a couple of years ago and the infectious enthusiasm for instrumental analysis corrupted him completely… He's just finished his PhD with Graham Cooks at Purdue. Apologies to the medical community, but that's very satisfying.

How do you instil enthusiasm?

Perhaps the way I run my research group naturally selects those who are enthusiastic, motivated and independent. Graduate students need freedom – and perhaps one of the most important freedoms is the chance to make a few mistakes… that's when we really learn.

How did you dive into spectroscopy?

Dive is perhaps not quite the right word. Going way back, I started out playing with chemistry as a kid, doing the kinds of things most parents disapprove of. I wound up pursuing synthetic organic chemistry at a tiny but well regarded placed called Hope College in Holland, Michigan. When I left, I was convinced to take a graduate course at the University of Illinois but the salary of a student wasn't enough to support my family, so I took a position for a year in physical chemistry at the Illinois State Geological Survey. The following year, I entered Illinois graduate school as an inorganic chemist, where I met Howard Malmstadt, a great analytical chemist who had a major influence on my life and my career. That's really when I got into spectroscopy. But it was somewhat by accident.

How so?

Before graduate school, while at the Geological Survey, I heard a lecture by David Hercules, one of the patriarchs of analytical chemistry. He was focused on fluorescence at that time, and it led me to become interested in time-resolved fluorescence. And so when Howard Malmstadt talked to me about doing time-resolved spectroscopy I was excited; however, it turns out he meant spark spectroscopy and not fluorescence. Not that I was discouraged, but it required quite a shift in my thinking.

You show the same kind of flexibility in collaborations...

Yes. We've had a number of collaborative relationships that have been able to access small business innovative research and small business technology transfer research grants. The idea is to foster small business development through collaboration with universities. Several of our research products have been commercially developed. It's very rewarding – and the students benefit from it too.

Can you provide examples?

We worked with LECO to develop an inductively-coupled plasma time-of-flight mass spectrometer (ICP-TOF-MS). We worked with Technicon to develop algorithms and instrumentation for near-infrared reflectance analysis. Our atomic absorption background correction system was commercialized. We've worked with several smaller companies to commercialize fiber optic sensors. There's a device for ambient MS that we developed a few years ago, called the flowing atmospheric pressure afterglow (FAPA). Right now, we're also working with a company in Australia on a new concept in TOF MS, called Zoom-TOF. And we've just patented something called distance-of-flight MS, for which we are seeking a partner.

You and others seem MS-focused right now. Is there a danger that optical spectroscopy is dying out?

Heavens, no, not at all. Mass spectrometry has become prominent, and for good reason: it provides very high sensitivity and a lot of information. But let's compare it with optical spectroscopy or nuclear magnetic resonance (NMR). As far as sensitivity goes, optical methods will usually win out over MS. That's because even though MS has a low background, it's ordinarily a destructive method; each ion is annihilated when it hits the detector. In contrast, if you have an isolated atom or molecule in solution, you can 'look' at it over and over again using optical spectroscopy. In fact, you can get about a million photons from each molecule before it photolyzes, so it's relatively easy to do single molecule or atom detection with optical spectroscopy. And photons are clean! In contrast, it seems crude to inject samples directly into your mass spec instrument and get it all gummed up. NMR offers many different parameters to gain exquisite detail and thus fills up the information space much more fully.

So, where is spectroscopy going?

I'll make a bold statement: spectroscopy allows us to push the limits of space and time. We're now using it to perform research on the attosecond (10^{-18} second) timescale and with sub-nanometer spatial resolution. Stand-off detection, for example, laser-induced breakdown spectroscopy, is an important trend right now. It's been around for a while but is really catching on now – you spoke with Rick Russo (one of my former PhD students) and covered the Mars Curiosity Rover a couple of issues ago, right?

A final bold statement: within the next few years, real-time imaging of individual molecules in motion will be possible; even now, researchers are approaching that goal.

RIVA 2014

COMPLETE YOUR AGILENT TOUR

INTERESTED IN CHALLENGING YOUR GC SKILLS?

COMPLETE THE GC SURVEY ON THE REVERSE, TAKE IT TO THE AGILENT BOOTH #1 AND COLLECT YOUR GIFT!!

The Measure of Confidence

Agilent Technologies

RIVA 2014 - COMPLETE YOUR AGILENT GC SURVEY

TAKE THIS CARD TO THE AGILENT BOOTH #1, CHECK YOUR GC SCORE, AND COLLECT YOUR GIFT!

1.- Keeping same chromatographic conditions, when changing from He to H_2 as GC carrier gas it is true that

- ☐ Efficiency will always improve
- ☐ Costs are lower
- ☐ The resolution will always be better
- ☐ Retention times will always be longer

2.- What are the benefits of decreasing the column internal diameter?

- ☐ Increased sample capacity
- ☐ Reduced risk of column overloading
- ☐ Increased resolution
- ☐ All the others

3.- Which of the following are not used as stationary phases in a GC column?

- ☐ Polysiloxanes
- ☐ Cyclodextrins
- ☐ Silica
- ☐ None are used as stationary phases

4.- Helium is generally preferred as carrier gas over nitrogen and hydrogen because

- ☐ It is inert
- ☐ It has a higher viscosity
- ☐ It doubles up as a party gas for balloons and funny voices
- ☐ All the others

5.- Which of the following gases is unsuitable for use as a GC carrier gas?

- ☐ Nitrogen
- ☐ Oxygen
- ☐ Helium
- ☐ All the others

I would like to receive information about

☐ GC ☐ GC/MS ☐ Triple Quadrupole GC/MS ☐ GC/Q-TOF ☐ Micro GC ☐ Sample Prep Workbench

Are you planning to purchase?

☐ GC ☐ GC/MS ☐ Triple Quadrupole GC/MS ☐ GC/Q-TOF ☐ Micro GC ☐ Sample Prep Workbench

When do you plan to purchase?

☐ Immediate ☐ <3 months ☐ 4-12 months ☐ >12 months ☐ Not now

Do you have budget available?

☐ Yes ☐ No ☐ In progress ☐ Other

First name: _____ Last name: _____

Company: _____

Department: _____

Address: _____

Postal Code: _____ City: _____ Country: _____

Phone: _____ Fax: _____

e-mail: _____

☐ Yes, I would like to receive product and service information from Agilent Technologies to the above e-mail address

1405_RIVA

The Measure of Confidence

Agilent Technologies

Improving Chemistry

New Reduced Price for 2014

Electrothermal OMNI Reaction Stations - Conduct parallel synthesis in multiple reactions simultaneously, maintaining precise heating, cooling and stirring at each position.

- Faster, more controlled reactions for increased productivity and throughput
- In block heating for better thermal conduct to the sample
- Cooling plug allows accurate, controlled cooling down to -30°C
- Powerful magnetic stirring up to 800rpm
- Excellent energy efficiency
- Available in 6 or 10 positions

Electrothermal
www.electrothermal.com

SYMPOSIUM CHAIR
Professor J. Michael Ramsey

SYMPOSIUM ORGANIZER
Professor Edward Yeung

SYMPOSIUM/EXHIBIT MANAGER
Ms. Janet Cunningham, Barr Enterprises

HPLC 2014

41st International Symposium on High Performance Liquid Phase Separations and Related Techniques

May 11-15, 2014 • Hilton New Orleans Riverside • New Orleans, Louisiana, USA



CONTACT
Ms. Janet Cunningham
Symposium/Exhibit Manager
Barr Enterprises
www.LinkedIn.com/in/BarrEnterprises
janetbarr@aol.com

www.HPLC2014.org

July 21-22, 2014 Gaithersburg Marriott Hotel, Gaithersburg, MD

CMC STRATEGY FORUM

ADVANCING BIOPHARMACEUTICAL DEVELOPMENT

Best Practices for Managing Emerging Trends and Challenges of CMO Oversight and Other Service Providers

Forum Co-chairs:

Siddharth Advant, *ImClone Systems Corporation*
Julia Edwards, *Genentech, a Member of the Roche Group*
Jeffrey Staecker, *Genzyme, A Sanofi Company*

SCAN to browse CASSS for program updates at casss.org

CASSS™
AN INTERNATIONAL SEPARATION SCIENCE SOCIETY
www.casss.org

Webinars

Virtual Events

Boost Method Development with Core-Shell

Transforming HPLC/UHPLC Method Development for Pharmaceutical, Food, Clinical and Toxicology Research with Kinetex Biphenyl Core-Shell Technology

Event Overview:
This webinar will present a brief overview of the benefits of Core-Shell Technology for HPLC, UHPLC, and LC/MS/MS separations and provide practical advice and case studies to chromatographers who are focused on developing new industry specific methods with core-shell columns or converting existing methods over to Core-Shell Technology.

Date: 30 April 2014, 11am EST
Register Free at:
http://tas.txp.to/0314/pheno/web-reg

Speaker
Dr. Jeff Layne,
Manager – Product Management and Technical

Sponsored By phenomenex

GPC Solutions – Optimizing your Polymer Separations

This webinar from The Polymer Analysis People will enable you to get the most from your GPC separations.

Event Overview:
You will gain an understanding of the important variables, learn how to optimize those variables, and then be able to put this into action to achieve reliable, robust, high-resolution polymer separations – each and every time. We will discuss solvent, sample, flow rate, temperature, columns, calibration, and detection.

Date: 13th May 2014, 3pm GMT
Register Free at:
http://tas.txp.to/0414/agilent/web-reg

Speaker
Stephen Luke, *GPC Product Manager*

Sponsored By
Agilent Technologies

Kromasil®
Eternity™

Kromasil EternityXT

UHPLC/HPLC columns with extended lifetime

EternityXT family of columns designed for improved efficiency and increased flexibility in your laboratory.

EternityXT columns for fast turnaround, easy method transfer and seamless scale-up from R&D to production.

For separations and purifications that can be run under a wider range of pH conditions.

With particle sizes between 1.8 and 10 µm.

www.kromasil.com

AkzoNobel