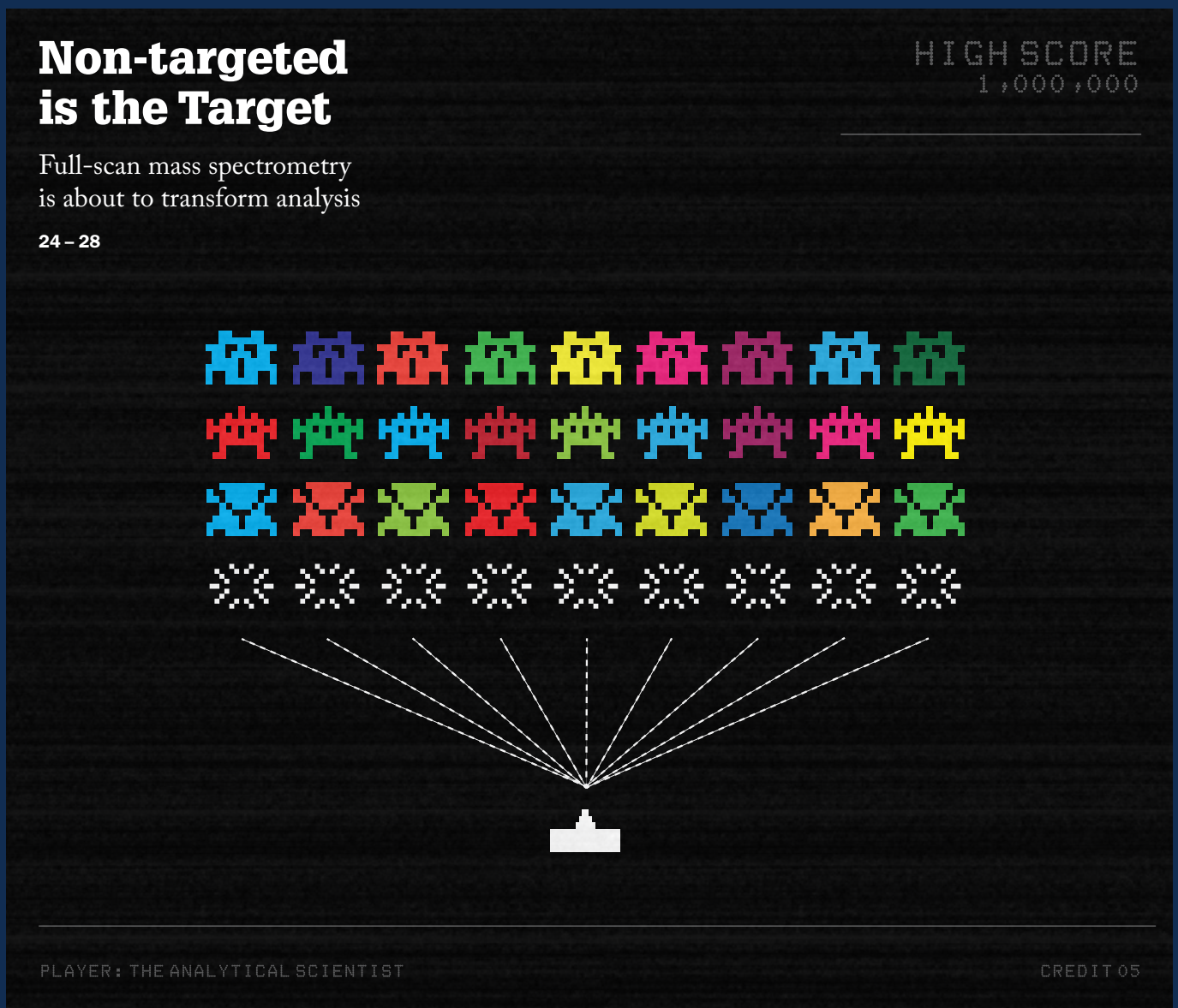
MAY 2013 # 05

the Analytical Scientist

Non-targeted is the Target

Full-scan mass spectrometry is about to transform analysis



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Online this Month

The Alert Reader

To facilitate discussion threads (see "Heating Up Part II"), we have introduced an important new function online. Now, when someone writes a comment on an article after you, you'll receive an email alert automatically. Put simply, you'll know if someone is trying to talk/agree/fight with you without even visiting the website.
Try it out today – we'll be sending souvenirs to the users who have left the best comments over the last 6 months.

Heating Up Part II

The online version of Ewa Szymańska's article, "Why Isn't Chemometrics Center Stage?", has stirred up considerable interest from our audience in the USA.

"Nearly 40 years after being the first Ph.D. out Bruce Kowalski's group, chemometrics has changed very little, people are still treating the technology as "black magic" and forget that chemometrics is a useful tool only when you have lots of data […] Chemometrics is not for a lazy analyst."– Doug Dierdorf, USA.
"Chemometrics has very powerful tools, but often the discussions are very theoretical. The more practical applications are published the better."– Astor Green, USA..

And by no means least:
"Any method that requires statistical treatment (eg., SAS) for interpretation is an inadequate method. Forget LC-MS or LC-MS-MS. We can't afford it with our frozen budgets. The only scientists I know who use chemometrics are biologists and silviculturists trying to quantify multiple ecosystem influences on tree growth. Their data is so poor the only way they can parse anything out is to use chemometrics techniques."– Joseph Fischer, USA.

The final point is surely one that is up for debate. Add your perspective by commenting at: theanalyticalscientist.com/issues/0313/302
If you feel particularly passionate, the editors are always open to In My View articles. Send your story to edit@texerepublishing.com with "In My View" in the subject line.

The Dotted Line

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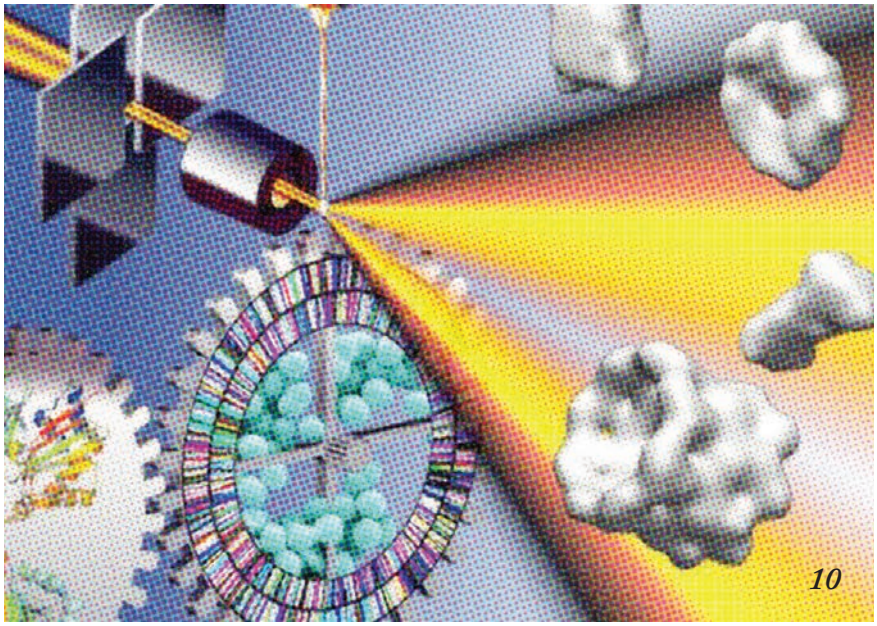
On The Cover

Concept: Space Invaders (mass spec) with a difference: instead of targeting individual objects (analytes), the laser zaps (measures) everything.

Upfront

In My View



Features

Departments

Sitting Down With

Going Dutch

To celebrate HPLC 2013 in Amsterdam, this issue features several contributions from the Netherlands, a hotbed of analytical science.

the **Analytical Scientist**

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Analytical Attrition

Despite its intrinsic importance, analytical science has a very low profile and is in danger of being dismantled completely. One organization, COAST, offers an alternative future.

A recurring concern in our field, shared across all techniques and application areas, is the attrition of the profile of the analytical sciences.

It was brought up in our first ever In My View article, when Samuel Kounaves of Tufts University wrote: "I find it disquieting that many universities have been dropping courses in analytical science over the past few decades; all too often, the material is integrated into lower level courses." And it crops up again in this month's Business article from Oscar van den Brink, Managing Director of COAST (see page 42). The profile of analytics is already low and, without adequate measures, will continue to drop.

True, this analytical attrition is partly a consequence of the evolution of science and its terminology. Fashions come and go, and today it is far more opportune to describe yourself as a proteomics researcher than as mass spectrometrist. While you may think that's not cause for concern, as the work is being done regardless of the label pinned to it, titles do matter. They help define what's hot and how the subjects are viewed from the outside, and the slide from somewhat unfashionable to obsolete is a quick and painful one. At that point, where shall we find the Csaba Horváths and Sir J. J. Thomsons of the future?

Van den Brink's COAST organization is one of only a handful that are trying to refocus on analytical science as a field rather than on the applications of analytical technology. The clear aim is to encourage basic innovation that will generate solutions to cut across several – or all – application areas. It's a sound idea and one that I believe more public-private bodies (and funding agencies) should embrace.

Analytical science is an enabling technology – essential for innovations in most other areas of research. Yet it takes a back seat when it comes to investment. Perhaps "invisibility" has a part to play; perhaps governments are quicker to act on areas that stir an emotional response from voters. Certainly, funding for the environment, healthcare, biotech, and energy all take precedence.

COAST provides us with a blueprint. The sense of urgency and direction shared by their industrial and academic leaders has attracted support, including government funding, for ambitious R&D projects. Equally important, they maintain a strong focus on education and human resources to help tackle the heart of the problem.

Do you share this concern about our field's profile? If so, what can we, the analytical community, do about it? Let us know by commenting: theanalyticalscientist.com/0513/104

Rich Whitworth
Editor

Monika Dittmann

"During my graduate studies in the early 80's I had the chance to work with GC and HPLC instruments from Hewlett-Packard. When we received our first 1090 HPLC, I was so impressed that I wanted to work with the people that had developed this system," says Monika Dittmann. She got that opportunity in 1988 and has worked for the company (now Agilent Technologies) ever since, developing instruments and technologies in HPLC, capillary electrophoresis, capillary electro-chromatography and microfluidics. "I like to work in a multi-disciplinary environment," Dittmann says, "only by combining expertise from different areas can we able to meet the evolving needs of our customers." See page 46.

John Miller

John Miller has wide-ranging experience in education and training. "I have taught high school biology and chemistry, college courses in management, and corporate courses in applied electronics, management, and sales and marketing," he explains. Now Director of Professional Education for the American Chemical Society, Miller's department provides training and education programs for professional scientists worldwide. Read his recommendations to companies that have cut back on professional training on page 19.

Lourdes Ramos and Serge Rudaz

Coming from different application areas, Lourdes and Serge join forces to describe best practice and prospects for sample preparation. Lourdes, Senior Scientific Researcher at the Institute of Organic Chemistry, IQOG-CSIC, Madrid, Spain is currently developing miniaturized sample-prep methods for the fast determination of organic pollutants in environmental and food samples. Serge, Associate Professor in the School of Pharmaceutical Sciences at the University of Geneva is an expert in pharmaceutical analysis and natural product science. His research interests include chiral substances, biological matrices, and clinical and preclinical studies. See page 30.

Hans Mol

Hans Mol has been engaged in food safety analysis for almost 20 years. "And since my PhD at the Technical University in Eindhoven in the early 1990s I've been in favor of getting the whole analytical picture of a sample, rather than a snapshot of specific analysis at a certain moment in time," he says. Hans points out that there is more information in samples than questions you can think of. "Rapid developments in chromatography combined with full scan mass spectrometry means we have access to more and more information. We can answer new questions, even when the analysis was done along time ago." His review of non-targeted analysis is on page 24.

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Upfront

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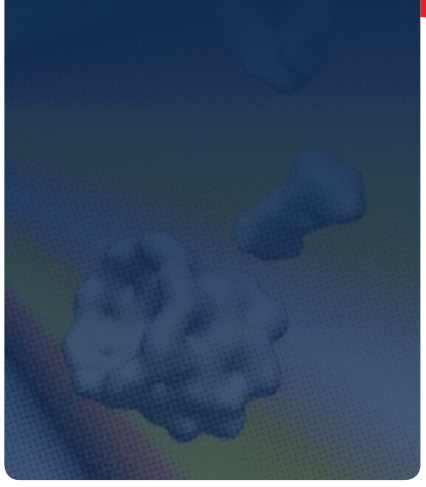


Reinvigorating SAXS

New metrics promise "game-changing" improvements in the structural analysis of flexible macromolecules by small-angle X-ray scattering (SAXS).

New structural information data has boosted SAS experiments with X-rays (SAXS) and neutrons (SANS). Developed by Robert Rambo, a scientist at the Lawrence Berkeley National Laboratory (Berkeley Lab) Physical Biosciences Division, and John Tainer of Berkeley Lab's Life Sciences Division and the Scripps Research Institute, the approach offers a 20-fold reduction in data collection time and provides quantitative measurements of flexible macromolecules. This means, Tainer says, that SAS will deliver "accurate and quantitative shape and assembly information, which provide a basis to annotate genome sequence function for biology".

The inspiration behind the research was the desire to connect the huge potential of genome sequence data and its meaning for biology with the relatively unrealized capabilities of SAXS. "There is a rapidly expanding gap between sequence data and the structural data needed to provide mechanistic and predictive biology, despite key investments in structural biology and many advances including powerful synchrotron facilities. We realized that SAXS had the potential to be a true high-throughput structural technique that gives complete information on the ensemble of structures in solution under physiologically relevant conditions, even for highly flexible complexes," Tainer explains.

Despite the ability of SAXS experiments to provide a complete set of electron pair distances – which provide structural information – the flexible nature of some macromolecules mean that traditional SAS analytics are unable to accurately use the full data set. Rambo estimates that 90% of the information is lost (1) and the risk of over-interpreting SAS data, and the subsequent generation of unreliable models, veiled the true value of the approach. Tainer explains further: "Our paper (2) deals with two

1960 – mid 1970's

Bio Sample Snapshot

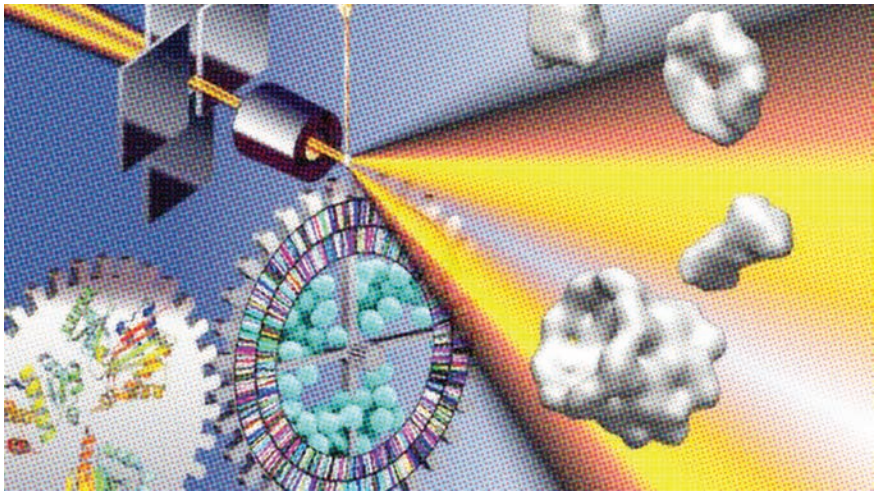
How clinical analysis tracks developments in analytical technology.

By Karl-Siegfried Boos and Rosa Morello

Clean-up of biological specimens, such as whole blood, serum, plasma and urine, for clinical chemical analysis does not change significantly between 1960 and late 1970's.

Pretreatment is centrifugation and liquid-liquid extraction (LLE) prior to GC-MS analysis.

Clinical chemical analysis is highly selective, with enzyme catalyzed and/or antibody triggered/boosted chemical reactions for quantitation of small molecules, such as metabolites and endogenous compounds, and large molecules like functional proteins.



major needs that limited SAXS for structural biology. First, it gets directly at information from the SAXS experiments on flexible samples without modeling. And second, it defines the accuracy and resolution of SAXS data and models. The combined results provide much needed objective and quantitative assessments and avoid over-fitting the data."

Rambo's earlier discovery of an SAS invariant – the "volume-of-correlation" or Vc – was key in developing the new metrics. The value of an invariant is independent of how or where the measurement was performed. "Vc uses the full range of the SAXS data to tell us about shape and assembly in solution, even for highly flexible samples – and shape is information in biology. Molecular forces are strongly distant-dependent so macromolecular shapes must fit closely together to interact strongly enough to achieve their functions," says Tainer.

The newly reinvigorated and reliable SAXS technique is likely to have wide-ranging impacts. According to Tainer: "Now that SAXS can reliably define protein, DNA and RNA shapes, interactions and assemblies, it can help define biological functions and annotate gene sequences. This has major implications for medicine, nano-materials, bio-manufacturing, responses to climate change, and development of biofuels".

When asked, "What's next?", Tainer's answer is clear: "Building upon these metrics to improve measuring flexibility and folding states in solution, and combining SAXS with other methods such as NMR. The SAXS data will helps to create models rather than simply acting as a filter to remove unsuitable predetermined possible models." *RW*

Do you currently use SAXS (or SANS) analysis? We'd love to hear your views on this development and what impact you think it will have. Sign up online and leave a comment: theanalyticalscientist.com/0513/201.

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2. *Robert P. Rambo and John A. Tainer, "Accurate assessment of mass, models and resolution by small-angle scattering", Nature 496, 477–481 (2013). doi:10.1038/nature12070*

Late 1970s - 1999

HPLC and a broad spectrum of stationary phases focus attention on sample pretreatment.

In subsequent years, solid phase extraction (SPE) becomes the dominant clean-up principle for pretreatment of body fluids, especially for HPLC-UV/VIS, field desorption (FD) or electron capture dissociation (ECD) analysis of small molecules in therapeutic drug monitoring (TDM), and for forensic specimens.

Routine SPE is mainly performed manually or semi-automatically by dedicated liquid/SPE handling systems.

~2000

HPLC instruments and software integrate SPE-based sample clean-up: SPE-LC is born.

On-line SPE-LC relies on tailor-made packings using restricted-access materials in small SPE-columns (20 x 2 mm inside diameter).

SPE-LC becomes increasingly attractive, saving costs on consumables and salaries, and allows complete automation. Sample throughput is a rate-limiting step.

Mid-2000s

Tandem mass spectrometry (MS/MS) enters routine clinical-chemistry laboratories with the message "dilute and shoot".

It becomes apparent that when operating MS/MS in electrospray ionization (ESI)-mode, ionization might be decreased or enhanced – so-called "matrix effects".

SPE packings to remove phospholipids and multidimensional SPE are introduced to maximize sample clean-up and minimize matrix effects.

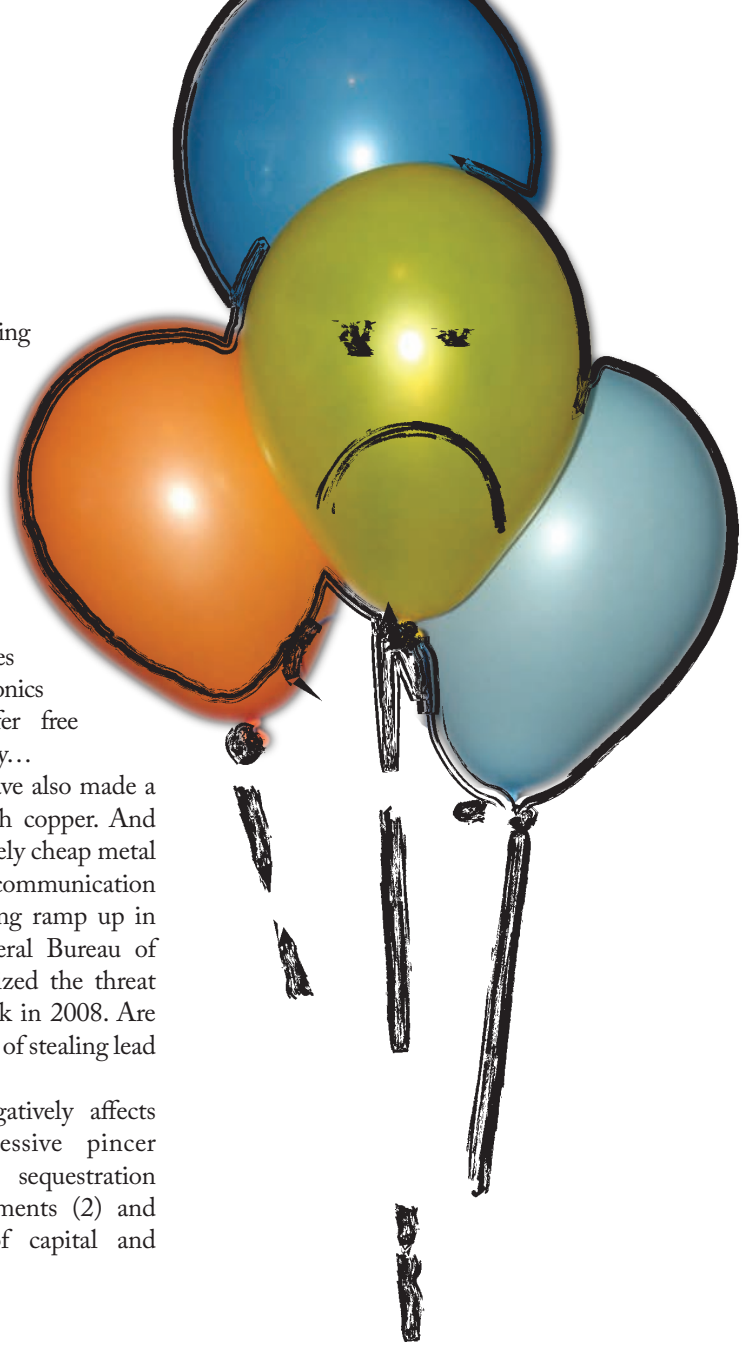
99 Dead Balloons

As we burn through our natural resources with gay abandon, all humans (analytical scientists included) must consider the impact of non-sustainable activities and prepare to make changes. Helium is just the latest in a long line of elemental casualties.

Shortage of natural resources is sadly not a new phenomenon. As we continue down our path of development (a word disputed by sustainability-minded individuals), increasing demand fast outstrips supply. Continually rising oil prices are an obvious testament to that fact. Likewise, the precious metals used in the technology sector have followed a similar trend, and recycling – of computers, mobile phones, and other gadgets – has found new favor given the small but valuable amounts of gold, iridium, and silver that can be "mined", often by enterprising Asian companies. According to a 2008 study by Japanese recycling firm Yokohama Metal Co Ltd., a tonne of gold mine ore yields 5 grams of gold on average – small fry compared with the 150 gram yield from a tonne of discarded mobile phones (1). No wonder electronics firms are keen to offer free recycling services so readily…

Scrap metal dealers have also made a killing, in particular with copper. And thefts of this once relatively cheap metal from electric and telecommunication hubs has seen a matching ramp up in security – the US Federal Bureau of Investigation (FBI) realized the threat to US infrastructure back in 2008. Are we really back to the days of stealing lead from church roofs?

Resource scarcity negatively affects research in an aggressive pincer movement: mandatory sequestration by once robust governments (2) and the increasing costs of capital and consumable equipment.



2008~

Dried blood spot (DBS) and dried spots of other biofluids, such as urine and plasma, become popular, especially for Phase I studies in drug development (See "Pharma's DBS Dilemma", page 36).

The combination of (micro) sampling, ease of shipment and storage using a single filter card boost its popularity.

2013 and beyond...

SPE

Intra-laboratory turn-around times in laboratory medicine are 3 min to 3 hours for common analytes. To compete, SPE is being miniaturized, multiplexed and/or hyphenated with UPLC under normal or high linear flow (TurboFlow).

MS

Besides well-established GC-MS systems, routine application of (UP) LC-MS/MS, TOF-MS, DART-MS and other MS platforms in the clinical setting is slowly but steadily catching on. To match classical clinical-chemical analyzers, improvements in ease of operation, robustness, downtime, and 24/7 runtime are required.

DBS

DBS has potential use in outpatient thereapeutic drug monitoring (see "Spot On", theanalyticalscientist.com/issues/0413/401).

Cell-Disintegrated Blood (CDB)

Offers the potential to simplify and fully automate the analysis of whole blood

Yet, despite the world as we know it crumbling around us , it seems somewhat surprising that helium, the second most abundant element in the observable universe and a gas found in the very air we breathe (albeit in very small amounts), should face the same fate. And while children's birthday parties will certainly be less buoyant in the future and hilarious squeaky voices may be a thing of the past, those still reliant on the inert carrier gas in GC applications face more serious consequences as helium supplies become increasingly erratic (read: more expensive). "Space mining" suddenly starts looking less silly (3).

At Pittcon 2013, Bruker announced two new helium-free GC platforms (Scion 436 and 456) that safely replace helium with hydrogen. Earlier this year, Agilent sponsored a webinar: "Converting helium carrier gas GC methods to nitrogen and hydrogen" – both surely signs of things to come.

In other areas of science research, especially where superconductors are essential, the problem has a more profound impact. According to Mark Stokes, a cognitive neuroscientist at Oxford's Centre for Human Brain Activity, helium is irreplaceable for his work: "Magnetoencephalogram (MEG) systems are based on superconducting sensors at near-absolute zero – liquid helium is the only element that can be used to maintain this critical operating temperature." Current MEG systems require weekly refills of liquid helium and, given the lack of an alternative, are very vulnerable to disruptions in supply. Stokes continues: "MRI, which is also essential for our research (and of course medical diagnostics), also depends on an annual supply of helium. If there was a prolonged disruption in helium supplies, it would have serious consequences for both research and medical practice."

It would appear that the time for change is now. *RW*

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prior to SPE-LC or point-of-care-testing (POCT). POCT uses sensor technologies that involve little to no sample preparation.

Improvements in microchip and nanoscale technology will yield automated, algorithm-dependent in-situ diagnostics that obviate the need for sample preparation.

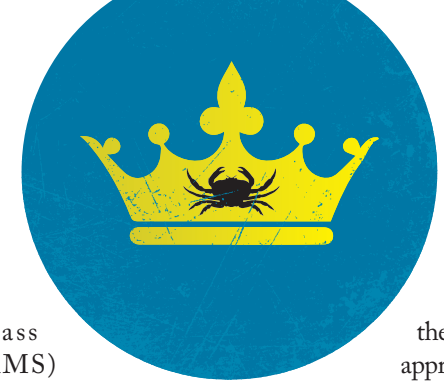
NMR

Routine application of high resolution NMR in the classification of subclasses of low-density-lipoproteins has been a door opener in laboratory medicine.

NMR, in principle, does not require sample clean-up, yet allows quantitative analysis of highly complex bodily fluids, such as urine.

NMR-based analysis protocols will become attractive in systems biology-based profiling and, eventually, in personalized (laboratory) medicine.

Karl-Siegfried Boos and Rosa Morello are at the Laboratory of BioSeparation, Institute of Clinical Chemistry, Medical Center of the University of Munich, Germany.



A Meal Fit For a King

Radiocarbon dating not only supported the identification of King Richard III's remains, it also shed light on his diet.

In March, we reported on the role of mitochondrial DNA in confirming the identity of a skeleton found in a Leicestershire car park as King Richard III. There's another analytical component to the story, involving the use of radiocarbon dating.

The Oxford Radiocarbon Accelerator Unit and the Scottish Universities Environmental Research Centre (SUERC) in East Kilbride both used accelerator mass spectrometry (AMS) radiocarbon dating, each analyzing two small rib bone samples.

The findings dated the remains to AD1475-1530, consistent with them being Richard III's. Analysis of stable carbon and nitrogen isotopes also gives information about the source of the protein that was eaten by the living individual. Derek Hamilton from SUERC explains: "δ13C gives us information about how much marine protein was in the diet. Essentially, if you only ate beef, the δ13C value of your bones would be around -21.0 per mille, but if all you ate was seal meat that value would be about -12.5 per mille. The average of the four measurements was -18.5 per mille. If we extrapolate between our two endpoints it suggests that the diet consisted of approximately 25 percent marine-derived protein."

So, King Richard was a fan of seafood. But what kind? "δ15N, provides extra information about where in the food chain the food was derived. As you move up the food chain its number increases. Marine environments have much longer chains than on the land, so this number can also cast some light on where in the marine food chain the individual was sourcing their protein. Our results suggest that he did not only eat species that were lower in the food chain, such as oysters, crabs, and mussels, but rather that his marine protein was from a variety of sources." *RW*

Read more at theanalyticalscientist.com/issues/0313/201.

7 DBS Solutions

Dried blood spot analysis is a useful, but not yet perfect, technique. Here are solutions to some currently vexing issues.

By Bert Ooms,
Principal Scientist at Spark Holland

1. Heat stabilization

Problem: Degradation of analytes during drying due to enzymatic activity.
Solution: Heat the samples for 30 secs.
Caveat: Degradation still occurred with three of six analytes tested.
Reference: http://1.usa.gov/YrWqPb

2. On-card derivatization

Problem: Complex handling procedures are needed to derivitize thiols.
Solution: Pre-treat cards with 2-bromo-3'-methoxyacetophenone, dramatically simplifying the workflow.
Reference: http://1.usa.gov/17Jyppy

3. On-line desorption

Problem: Direct desorption techniques suffer from ion suppression, interference and low sensitivity.
Solution: Online desorption to an SPE cartridge followed by online elution to LC–MS/MS results in excellent precision and linearity
Next: Online full-spot analysis to circumvent spot-size variability caused by hematocrit variations.
Reference: http://1.usa.gov/140R4uY

4. Therapeutic protein analysis

Problem: Biopharmaceutical industry wants to reduce pre-clinical animal use.
Solution: Direct enzymatic digestion of DBS followed by LC–MS/MS to identify signature peptides.
Reference: http://1.usa.gov/12dvFxi

5. Paperspray MS

Problem: Therapeutic drug monitoring with DBS tedious and difficult to automate.
Solution: Spraying directly from triangle-shaped DBS paper into MS/MS provides adequate performance with simplicity.
Reference: http://1.usa.gov/YrWw9H

6. Direct liquid junction DBS

Problem: No single assay for both screening and diagnosis of hemoglobin variants.
Solution: Liquid junction-based extraction and direct infusion into high resolution MS.
Reference: http://1.usa.gov/OmADpM

7. Colorless samples

Problem: Colorless samples (e.g., liver microsome incubation) are difficult to visually inspect on DBS card.
Solution: Spotting colored dye on card first indicates presence of samples as colorless patch.
Reference: http://bit.ly/Yrsq69

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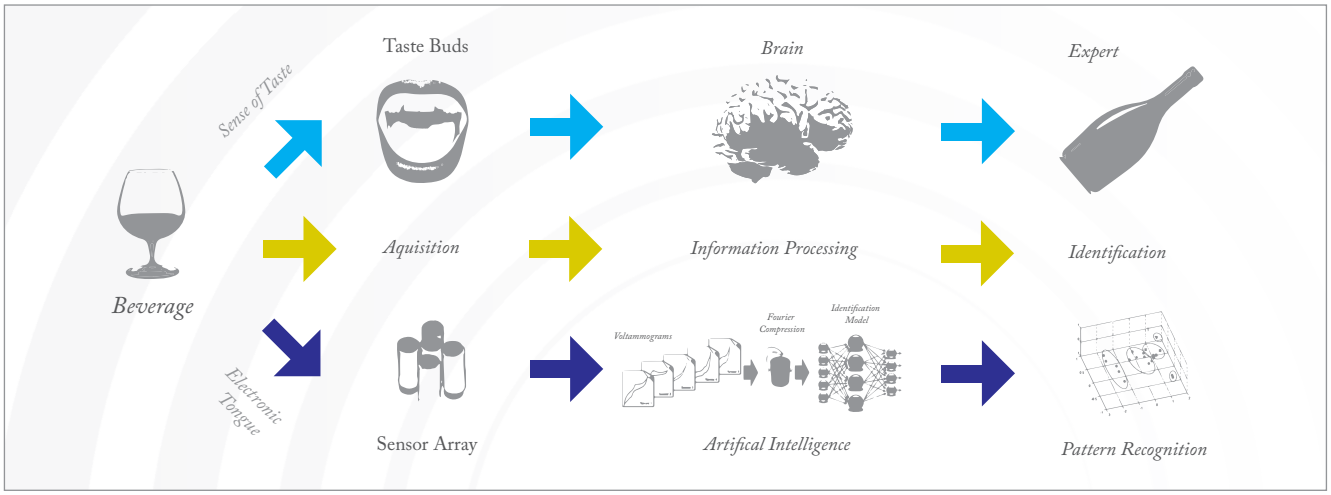

Alcoholic Intelligence (AI)

For centuries, taste experts have had the enviable – and indispensable – job of managing brandy quality control. Are they about to be replaced by E-Tongue technology?

"Claret is the liquor for boys; port for men; but he who aspires to be a hero must drink brandy." – *Samuel Johnson*

The Analytical Scientist spoke with Manel del Valle from the Sensors and Biosensors Group at the Universitat Autònoma de Barcelona. His system combines a voltametric electronic tongue with an artificial neural network model trained for the semi-quantitative identification of several undesired compounds that indicate brandy defects.

How is the voltametric array constructed?

Carbon electrodes are combined with chemical compounds, such as nanoparticles, conducting polymers, and certain redox catalysts, at around 5% proportion to the initial mixture to form "modified epoxy graphite composite electrodes".

Similar sensor arrays have already been successfully applied to wine characterization and beer evaluation. In the case of brandy, undesired compound markers include: butan-2-ol, ethyl acetate, acetaldehyde and butan-1-ol. We also quantify the polyphenol index.

And how is the signal from the array processed?

The data generated per sample are highly complex, featuring three components: current, polarization potential and sensor type. They undergo Fourier transform, much like the compression of a CD audio file into an mp3 file, in which a reduced number of Fourier coefficients per voltammogram are stored. High frequency components are discarded as noise. The Fourier coefficients are then analyzed using pattern recognition methods, including Fisher's Discriminant analysis for sample classification and identification, and an artificial neural network.

How does this relate back to the quality of the liquor?

A large number of samples previously processed by a human sensory panel have been translated into the artificial intelligence (AI) model. Cross-validation of the model with a second set of samples not used in the training of the system confirmed that we could correctly classify brandy samples.

Are there limitations to this type of system?

Not all quality factors in the food/beverage industry are easily tackled with E-noses or E-tongues, for example, the spoilage of wine corks by trichloroanisole (TCA), a metabolite of residual fungi in the cork. Human senses are extremely sensitive to TCA, an anomaly that makes for an extremely demanding application. The problem is caused by the lack of sufficiently sensitive sensors for this compound.

Drift is also an issue, in which sensors can alter in behavior, invalidating the generated model.

What are other possible for uses the E-tongue?

One obvious area is in environmental applications where continuous unattended monitoring of complex processes is required. In any use of E-Senses, shifting the complexity of the analysis from the measurement to the data treatment, a feature made cheaper every year, will be a key driver. *RW*

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In My View

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

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

Contact the editors at edit@texerepublishing.com

Closing the anti-doping circus

Are the right questions being asked about substance abuse in sport? I say no, and suggest that the entire process might be abandoned.

By Henk van 't Klooster, former director of the Analytical Chemical Laboratories Division of the National Institute of Public Health and the Environment in The Netherlands.

Alberto Contador, winner of the 2010 Tour de France, had to return his yellow jersey because clenbuterol was present in his urine. The amount found was 50 picograms per milliliter (1 pg = one millionth of a millionth of a gram). Clenbuterol was – and in certain parts of the world continues to be – used in the meat industry to promote the conversion of fat into muscle. Cyclists, swimmers, football players and other athletes use it for that purpose too, although it's on the list of banned substances. Contador, however, has always denied using clenbuterol; his explanation is that he ate meat that contained the substance. So far, his defense has been in vain but the results of a recent study (1) might change his position.

During the Under 17 world soccer championship in Mexico in 2011, researchers from the Dutch Wageningen Research Institute (RIKILT) were commissioned by soccer's governing body, FIFA, to analyse meat samples from the kitchens of the hotels where the players were staying. In 14 of 47 samples, clenbuterol was detected, at amounts between 0.06 and 11 micrograms per kg. "That's not strange in Mexico," says Saskia Sterk of RIKILT. At the same time, researchers from the Doping Lab of the German Sports University at Cologne analysed 109 of the 208 urine samples collected from the participating soccer players and found clenbuterol, ranging from 1 to 1556 picograms per milliliter.

Add to this previous work that established that nutritional supplements, such as the vitamins, minerals and proteins taken by many athletes, can be contaminated with anabolic steroids, which are also on the list of banned substances (2).

A huge amount of attention, energy and money has been focused on methods and procedures to demonstrate whether or not athletes have taken prohibited substances. It's an anti-doping circus. And many questions persist, such as:

- Which substances should be banned?
- What are the criteria that support banning?
- On whose authority should decisions to ban substances be made?
- How should interactions between body chemicals and doping substances be taken into account?
- How should foods and food supplements contaminated with doping agents be handled?
- How can masking agents and methods be revealed?
- What are the criteria for unequivocally demonstrating abuse?

- How should research methods and procedures be validated?

Even given all these questions, there is one more that, in my opinion, deserves more attention still. It is this: how sharp are the boundaries between the effects of nutrients as constituents of foods and nutritional supplements – which athletes can use – and the effects of banned substances that are considered to be performance-enhancing? If these boundaries are as vague as I suspect, the whole tedious and costly anti-doping circus can be closed.

To what extent do banned doping agents enhance performance anyway? The former professional athlete and physician Harm Kuipers, who was Holland's 1975 world champion in all-round skating and is now professor of kinesiology at Maastricht University, said in an interview about Lance Armstrong (3): "I do believe that he used EPO, but even then. Both EPO and a blood transfusion may provide a few percent more power, just that little bit extra to enable a slightly faster ride uphill in a final race. But doping cannot transform a Citroën 2CV into a Ferrari".

"How sharp are the boundaries between the effects of nutrients as constituents of foods and nutritional supplements – which athletes can use – and the effects of banned substances that are considered to be performance-enhancing?"

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Skills Gap = Foresight Gap

The abandonment of employee training and development is a false economy. Those companies that do invest will have a huge competitive advantage.

By John Miller, Director, Office of Professional Education, American Chemical Society.

A recent survey by the American Society of Training and Development (ASTD) asked organizations to identify and rank the reasons why there is a skills gap in their organization (1). Almost all the respondents cited reasons that are actually within their control, including:

- Training investment cuts and/or lack of commitment by senior leaders to employee learning and development.
- Lack of skilled talent in one or more of the company's lines of business.
- Skills of the current workforce do not match changes in company strategy, goals, markets, or business models.

Why are companies sitting on their backsides waiting for these issues to magically resolve themselves when solutions are right at their fingertips? And what will they lose while they wait? In my role in professional education in the chemical sector I've had a ringside seat.

In the past, an overwhelming majority of our customers would say that if they felt the need for training, the money would be found. That's the language they used. Training didn't require an elaborate, months-long approval process. Companies trusted their managers to develop the workforce responsibly. Especially in science, where innovation is key, where exposure to a wide variety of ideas creates advances, where team-based projects are the norm, and where technology and conceptual advancements are happening at a rapid pace, ongoing training was seen as essential.

Economic pressures have changed all of that. Training and travel was drastically cut in 2008 in response to the great recession; the long-term delay in restoring that culture of lifelong learning and internally supported training has

completely changed the atmosphere in most of the chemical sector. Many of our former customers have disappeared entirely from our radar screen because external training is no longer even discussed. Managers who were previously rarely questioned about the value or return on investment of their training decisions, and therefore have little skills on how to do so, aren't even willing to ask for training. The scrutiny and hassle just isn't worth it.

What is the prospect for the future of the chemical industry when learning is seen as too much of a hassle to bother with? What happens to the company that maintains only its current skill level and doesn't advance?

I'll acknowledge that I'm biased about the value and importance of training. I've worked in this field my whole life and I've seen the impact it can have. My contention is that this significant cultural shift has created an awesome opportunity for smart companies to grab hold of and ride to success.

Any company making a significant and strategic investment in employee development while its competitors restrain and constrict their employees is well-positioned to attract and retain better talent, which sets up just the right environment for innovation and high productivity. That investment is a powerful lever for a company to use to support its employees desires

Constant Quality Improvement

Scientific innovation combined with manufacturing excellence continues to drive improvements in GC. For trace analysis, the development of ultra-inert columns has been crucial. Here's how it was done.

By Allen K. Vickers, Product Development Chemist at Agilent Technologies, Folsom, California, USA.

Among analytical instruments and techniques, one stands above all others in terms popularity: gas chromatography (GC). This so-called 'mature' technique is ubiquitous in analytical labs and most often turned to for a first evaluation of sample composition, even though it is appropriate for only 10 percent of potential sample types. There are good reasons for GC having such clout. It is fast (meaning cost-effective), boasts high resolution (meaning unambiguous results), and is highly evolved (meaning relatively easy to use).

While the theory has been understood for years and the field is in many respects 'mature', GC is still an evolving analytical practice from one perspective: the instrument itself. As I shall illustrate here, each component of a chromatograph has a crucial role to play in constantly improving the generation of accurate, cost-effective data.

Take the heart of GC analysis: the separation column. Do you know how the columns that you rely upon were tested prior to packaging and delivery to your laboratory? I find it incredible that some people happily say that a column made by manufacturer A is "just like" that of manufacturer B without serious thought. Any difference is critical because during the course of a GC analysis, solutes have over 50 times the surface exposure in the column compared to the liner and injection port seal.

Dozens of process steps are involved in the manufacture of a capillary GC column, each with a list of parameters that must be optimized to produce a uniform, stable, inert stationary phase. At my company, Agilent Technologies, in-process evaluations are performed throughout the process and, once produced, every individual column is tested. Testing each column rather than batch testing is daunting, especially give that we produce thousands of columns in hundreds of different configurations on a weekly basis. However, we have ample evidence that in batches of columns that meet our specifications, there can be one column that does not meet the highest standards.

The quality of GC results emerge from a combination of multiple chromatographic parameters, including internal diameter (ID), retention factor, phase ratio, theoretical plates, bleed, and tailing factor. Most of these parameters will be very familiar to seasoned chromatographers but the importance of certain performance attributes may be underestimated. Inertness is a good example. In chromatography, inertness is the absence of reactive sites in the flow path towards the sample solutes. Stated simply, what goes in the GC, comes out. It is important to know not only what you are 'seeing' in the data, but also what you are not seeing. This is particularly important in the analysis of untargeted analytes, but also in routine analysis for the most reactive target solutes.

Our focus on improving column inertness goes back more than a decade. It was in this regard that in 2002, the co-

to succeed, to come up with the next big thing or mundane-but-valuable process improvement.

Simultaneously, technology advancements have made training and educational opportunities for professionals more diverse, more flexible, and more applicable than ever before. Professionals can gain access to experts from any part of the world and in multiple industries at once.

These conditions mean that the risk for this investment is lower, and the reward can be infinitely greater for the company that has the foresight and courage to realize it.

The bottom line is this: training done right provides a return that far exceeds the original investment. The ROI is clearly documented. Why some companies don't see that and act on it is a mystery.

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"I find it incredible that some people happily say that a column made by manufacturer A is 'just like' that of manufacturer B without serious thought."

founder of J&W Scientific, my mentor the late Prof. Walt Jennings came into work one day and proclaimed to the scientific staff that we were no longer trying to make better products at J&W; just the same old good products we had become good at making. To him, quality was always about improvement. He told us, "When the majority of the columns you make are passing your QC test, it is because your test isn't stringent enough." He was so ardent about this that in 2004, Walt challenged the chromatography community to become concerned about what was being missed in analyses due to solute losses by inadequate GC flow path inertness (1). We set out to test how "bad" the columns of the time were (2) and found that, while they were passing our already stringent QC tests, there was room for improvement.

Generating that performance improvement has taken time and expertise. While the measurement of theoretical plates and retention factors are straightforward ways to evaluate and compare individual columns, the chromatographic measurement of inertness is complicated by the solutes – and how much of each is being analyzed – and the conditions of the analysis. Furthermore, it was essential to ensure that any performance gains were not at the expense of performance loss in another parameter. For, example, some column-makers thermally condition their products at high temperatures for extended periods. This improves column bleed background, but column inertness is compromised.

By adhering to good scientific observation, focusing on quality in the manufacturing environment, and applying sound chemistry solutions, real improvements have been made, as evidenced by our Ultra Inert columns. This process continues on a daily basis: the only way a manufacturing facility can be competitive is to constantly apply scientific innovation on the factory floor and build products to demand. Quality must be an ongoing mission and it must be built-in, not achieved by chance.

MASStastic Voyage

A state-of-art mass spec system hits the road, offering a new way for analytical science providers to meet their customers.

By Steve Taylor, Market Development Manager, Pharma at AB SCIEX in Warrington, UK.

The pharma market has seen dramatic changes over the last few years and these changes are continuing in 2013. Gone are the days of cash-rich spendthrift departments attending meetings around the globe and purchasing every latest technology to hit the market. Nowadays, justification for any spend is the norm, with all budgets trimmed to save costs.

Instrument suppliers and meeting organisers need to recognise this paradigm shift and position value-added solutions. I witnessed one significant step last year, when AB SCIEX launched its MASStastic Tour across the USA prior to the annual American Society for Mass Spectrometry (ASMS) conference. Instead of asking customers to come to the vendor, this new approach involved taking instruments and scientific expertise out on the road in a mobile laboratory, directly to the customers. The result was a resounding success, with nearly 1,400 visitors from 30 cities across the USA. The obvious follow up was to export the idea to Europe.

It has been my pleasure to help drive (pun intended) this vision here in Europe. At the time of writing, the European MASStastic Voyage Tour has made 30 stops across the Netherlands, Belgium, Denmark, France, Italy, Hungary, Czech Republic, Poland and Germany, including to individual customer sites, science parks, and big and small scientific meetings. More than 1,000 scientists have visited the mobile lab, which features as its centrepiece an operational AB SCIEX QTRAP® 4500 System with an Eksigent micro LC system (I have lost count of the number of astounded visitors who have asked me, "Is it really running?"). Actually, the tour is quite a testament to the robustness of the 4500 System. It has been trucked over thousands of kilometers and endured temperatures so low that all of the LC eluents freeze solid, and yet it has started up every morning without fail. It is hard to imagine the MS systems of just a few years ago coping with even a weekly power-off and -on.

"Instead of asking customers to come to the vendor, this new approach involved taking instruments and scientific expertise out on the road in a mobile laboratory, directly to the customers. The result was a resounding success."

The mobile lab is also equipped with shell instruments, including the AB SCIEX TripleTOF® 5600+ and QTRAP® 6500 System, as well as instruments from our partners Phenomenex and Peak Scientific. Visitors have been able to bring and run their own samples, to ask technical questions from our experts, and to attend seminars on clinical, food, environmental and proteomics applications. The partnership of Phenomenex, Peak Scientific and AB SCIEX has provided an interactive experience for an end-to-end LC/MS/MS solution.

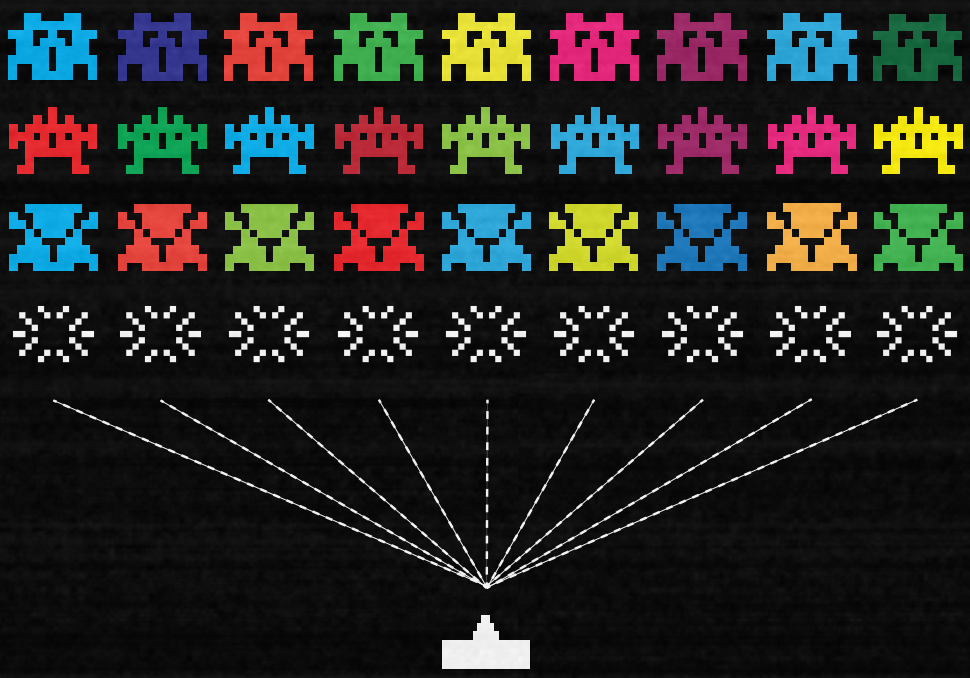
Our goal – meeting our customer base and bringing them up to date with the latest techniques and technologies without the need for them to spend time away from their base – has been achieved. Of course, there were some bumps along the way. At one site, a new building construction prevented access, and the arctic conditions experienced at certain locations undoubtedly kept some people from venturing outside. But what will live in my memory are the scientists I met who were appreciative of the opportunity to attend a seminar and to see the latest technologies without the need to travel great distances or spend a significant time away from their lab. The organizers of scientific meetings provided fantastic support, seeing the value of bringing something new and exciting to conference-goers.

So, is taking the message to the customer base the way forward? I certainly believe that it is. Budgets are unlikely to return to the heady days of the 1980s, so I predict that innovative ways of bringing events, information or new technologies to customers will become more common. I hope to be telling you about more of them in the years to come.

Non-Targeted is Our Target

Full-scan mass spectrometry is about to transform the way that we do analyses. Here, I describe the possibilities for non-targeted analysis that are opened up by recent improvements in instrumentation, compare the new approach to today's best (targeted) practice, and look for solutions to the bottlenecks that are hindering widespread adoption of non-targeted analysis.

By Hans Mol



Diverse domains of analysis, including food safety, environmental, clinical, forensic toxicology and doping control, face a similar, and substantial, challenge.

Take food analysis, for example. A foodstuff may contain one, two or multiple residues of pesticides, veterinary drugs, natural toxins (mycotoxins, plant toxins) environmental contaminants, packaging contaminants and processing contaminants. Within each of these categories, hundreds of substances are known. For many of these chemicals, legal limits in food commodities have been established; for others, limits have not been set, either because authorities are unaware that they occur in food or because the toxicant is not yet recognized as such.

In environmental analysis, the number of chemicals detected in surface water is increasing. These range from well-known persistent pollutants and pesticides to pharmaceuticals and metabolites that elude the sewage treatment system. Many of them pose a risk to aquatic life and to our drinking water

resources. Attempts are being made to generate a list of key chemicals to be monitored but this is hampered by a lack of data on their occurrence. (Of course, in an ideal world, everything should be assessed.)

Analysts from toxicology and doping control laboratories face a similar situation when analysing urine, oral fluids or blood samples for toxic substances and illicit and designer drugs.

What is shared across all of these domains is a requirement to detect and determine very high numbers of substances, known and unknown, in a wide variety of sample matrices, at levels ranging from sub µg/kg to low mg/kg level. It's a huge analytical challenge. At present, organic trace analyses are conducted as targeted methods (see "Past and Present: Targeted Measurement" on page 27). But the future looks rather different.

Non-targeted measurement

Using the classical, targeted approach, you must decide what you want to know, set up the method and perform your analysis. In contrast, a non-targeted approach analyses everything, then you decide what you want to know and extract the information from the raw data. Table 1 summarizes the targeted and non-targeted approaches.

Non-targeted measurement uses generic sample preparation and chromatography, combined with full-scan mass spectrometric detection. All ions are detected during the entire chromatographic run time and, in contrast to MS/MS, there are (in principle) no limitations to the number of substances that can be detected (see Figure 1).

Using non-targeted measurement, you may search the raw data of previously measured samples for analytes that were not of interest or not known at the time of analysis, without re-sampling or re-analysis of stored samples. Another feature is that a fingerprint, known as a total ion chromatogram (TIC), is obtained for each sample. This can be used for comparison with existing sample profiles to reveal deviations and/or to aid in the identification of unknowns.

The potential of non-targeted measurement was recognized long ago; the approach as such is not new. Applications were reported in the 1980s for the toxicological screening of drugs, and soon afterwards for detection of pesticides residues in food and of priority environmental pollutants in surface water. These early applications were based on GC-EI-MS (single quadrupole full scan) or GC-EI-ITD (ion trap detection). However, many substances not amenable to GC were not covered, and for those that were, the sensitivity and selectivity were limited. To improve selectivity, two approaches have been pursued. One is to increase chromatographic resolving power using comprehensive two-dimensional GC (GCxGC). This reduces peak overlap, resulting in cleaner mass spectra or less interference of the diagnostic ions. Since GCxGC refocuses the chromatographic peak, sensitivity is also improved although, since these peaks are very narrow (~0.1 s), fast-scanning (>100 Hz) time-of-flight (TOF) mass analysers are required. The second approach is to increase the resolving power of the mass spectrometer. Selectivity is improved because ions that have the same nominal mass but a different exact mass can be separated, again resulting in reduced interference of diagnostic ions. However, the GC-EI-hrTOF-MS instruments of the mid 2000s offered only intermediate resolution (5,000-7,000 FWHM) which meant that they suffered from limited dynamic range and unreliable mass accuracy. Only very recently have new instruments with better performance characteristics become available (13,000-25,000 FWHM).

Initially, high resolution TOF systems for LC presented these same shortcomings. However, dynamic range, sensitivity and

Table 1. Trends in developments in trace analysis of small molecules by chromatography.

Time	*Sample preparation*	*Chromatographic measurement*	*Data*
1970s-2000s	Target extraction/LLE IAC, columns, SPE derivatisation	Target GC-NPD, GC-ECD, GC-FPD, GC-MS HPLC-UV, HPLC-fluorescence	1-20 peaks
2000s-2010s	Non-Target Extraction/LLE extraction/dilution	Target (U)HPLC-MS/MS GC-MS/MS	1-200 peaks
2010-		Non Target (U)HPLC-full scan hr MS CG(xGC)-full scan (hr)MS	comprehensive 3-4 dimensional data

selectivity (resolving power/mass accuracy) have all improved dramatically over the past eight years. Resolving powers of 25,000-50,000 are the norm, while the benchtop Orbitrap MS systems go up to 140,000. Direct comparisons between high resolution MS and triple quadrupole MS/MS are not easily made. Roughly speaking, they are comparable in terms of selectivity when the resolving power is ~50,000 or higher (2) and, while high resolution MS falls short of the newest triple quadrupoles in terms of sensitivity, it is fit-for-purpose for most applications. Fragment ions to aid identification can be generated in the source or in a collision cell using alternating scan events and, when done without precursor ion selection, there are no compromises with respect to non-targeted measurement (see Figure 2). Given the quantitative performance of current instruments, even for targeted applications, full-scan measurement will be the method of choice in the near future because the measurement is more straightforward compared with MS/MS.

Developments in hrMS detectors for LC have resulted in them being coupled to GC using alternative ion sources (APCI, atmospheric pressure chemical ionization). This soft ionization technique is somewhat less generic than EI, but the abundance of molecular or protonated ions combined with sensitivity and resolving power make it an attractive possibility (4).

The potential of non-targeted measurement by chromatography with full scan MS has been demonstrated in numerous papers in food safety (2), environmental analysis (5) and clinical, forensic toxicology and doping control (6,7).

New approach, new challenges

Challenge 1: automated analyte detection

Untargeted measurements provide an overwhelming amount of data. For each substance, including analytes of interest but also all matrix compounds, information is obtained on retention time(s), accurate mass of ions (m/z 50-1000), isotope patterns and/or spectra, and intensity (from noise to saturation). The question is, how do you find what you're looking for in all this raw data?

In most applications, the analyst has a long target list of substances in mind, and interrogates elemental composition (provided by the exact mass and isotope signature) or an EI-spectrum. Although the measurement is untargeted, this approach, in the end, is equivalent to a targeted method but for a very high number of targets.

A consequence of this is that analyte detection has to be automated; manual evaluation of spectra or extracted ion chromatograms would be too time-consuming in routine practice. In the case of GC-EI-MS, spectra are more-or-less instrument-independent and large libraries are available. The instrument software performs searches of sample spectra against library spectra, although few packages are specifically designed for automated detection and reporting on larger numbers of samples and various in-house solutions have been described in the literature. With high-resolution MS data, the detection of analytes is based on a signal for the exact mass of one or more diagnostic ions at the expected retention time. Here, at least part of the relevant information for analyte detection is dependent on the instrument and experimental conditions, which complicates the establishment of comprehensive reference databases. At the moment, databases are still a work in progress, for both vendors and users.

The initial detection of a spectrum, signal or peak is performed by a software algorithm either directly on the raw data or after a pre-processing step. Often, a number of parameters can be set to influence peak detection. Once a peak is detected, tolerances are set on spectral matches, isotope fits, mass deviations, retention time, intensity thresholds, and so on, to ensure that the number of false positives and false negatives is acceptable. In practice, optimization of software parameters and thresholds is the most time-consuming part of the method development, especially when sensitivity is critical. New, instrument-specific software packages and updates are regularly released by vendors, which requires re-optimization of parameters. This has triggered some researchers to develop their own software solutions (8). Whereas the hardware is fit-for-purpose, the lack of comprehensive databases and inadequate software for automated detection are major bottlenecks hindering the widespread application of untargeted measurement for wide-scope screening at the moment.

Challenge 2: validation

Another challenge faced by non-targeted measurement aimed at the detection of very high numbers of analytes is method validation. To illustrate, consider veterinary drugs in food of animal origin and pesticides in food and feed. EU-guidance documents were established in 2010 (3), under which a method is considered fit-for-purpose when a substance can be detected in 95% of the samples (with N≥20 in an initial validation exercise that needs to be complimented by on-going analytical quality control). Because even similar substances can behave very differently when analysed by chromatography-MS, the guidelines require validation to be performed at the individual analyte level. We have carried out two validation studies for a total of 350 pesticides measured by GCxGC-TOF-MS and HPLC-Orbitrap-MS. The criterion was met for the majority of the tested pesticide/matrix combinations at levels down to 0.01-0.05 mg/kg. The constraint here is not so much sample analysis and validation as having all of the standards available and maintaining the collection: 350 pesticides is not comprehensive

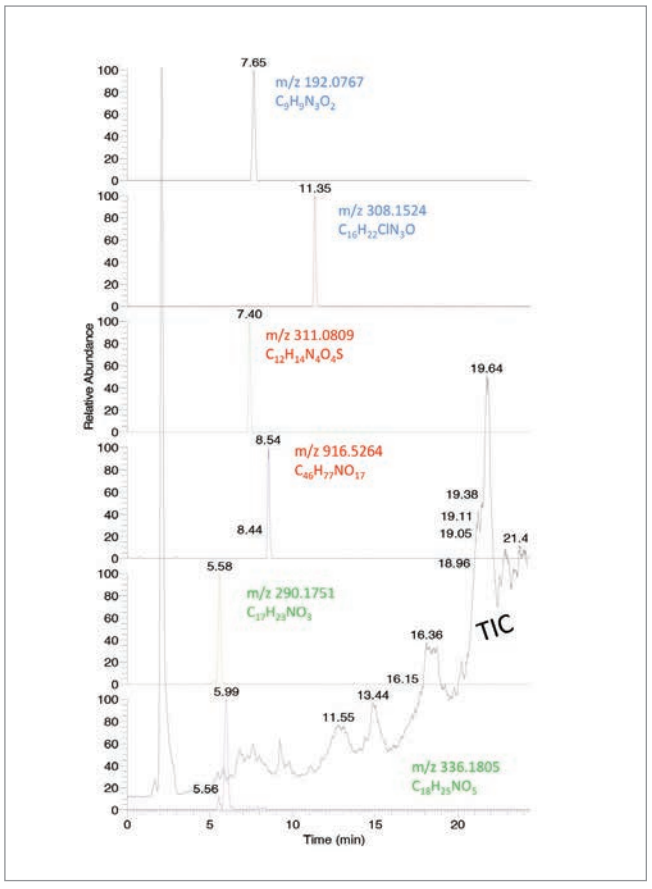


Fig 1. Honey (spiked with various contaminants at 10 µg/kg) analysed by HPLC with high resolution MS. Total ion current (TIC) (in black). The individual traces are extracted ion chromatograms (exact mass of [M+H]+ ±5 ppm) of various types of contaminants, from top to bottom: carbendazim, tebuconazole (pesticides), sulfadimethoxine, tylosine (veterinary drugs), atropine, senecionine (plant toxins).

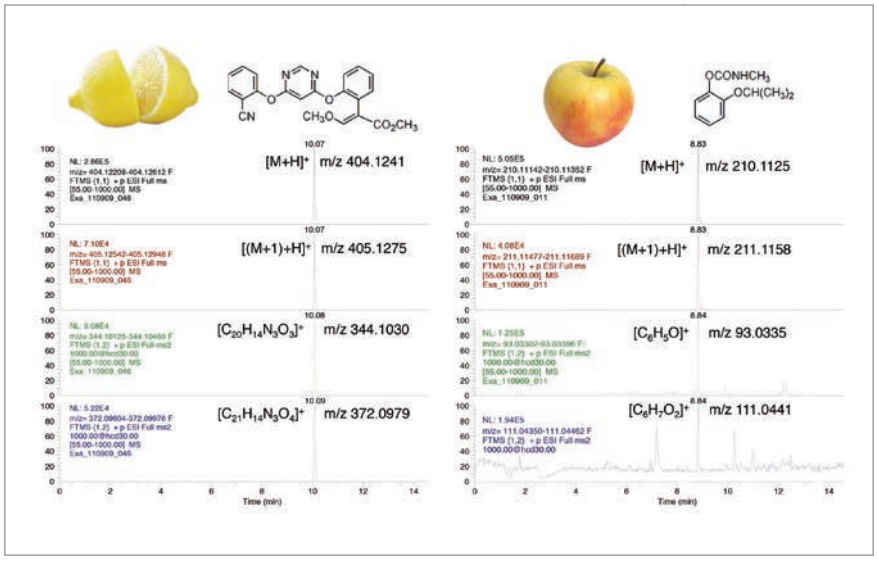


Fig 2. Pesticides in fruits (azoxystrobin in lemon, propoxur in apple, both 0.01 mg/kg). Diagnostic ions (protonated molecule, (M+1) isotope and fragments ions) retrieved from the raw data obtained after HPLC-full scan hrMS analysis. Alternating scan events were performed with and without fragmentation (3).

Past and Present: Targeted Measurement

In targeted measurement, the goal is set before analysis: "we wish to determine substance X in matrix Y". Next, a method is selected or developed to provide a quantitative measurement. The procedure has three steps: (1) sample preparation, which consists of extraction, clean up and optional derivatization; (2) chromatographic separation, and (3) detection. All three contribute to selectivity and sensitivity.

In the early days (1970s–2000), the selectivity of detectors included element-selective detectors for GC, and UV and fluorescence detectors for LC. The limitations of these systems were compensated for by extensive clean-up procedures that selectively isolated certain analytes or analyte classes. This inherently-restricted analysis changed with the introduction of MS detectors. In GC, single quadrupole and ion trap MS enabled generic and reasonably selective detection: generic because electron ionisation (EI) works for virtually any substance that is GC-amenable, and selective because ions are obtained that are often highly diagnostic for the analyte of interest. The improved selectivity in instrumental analysis made it possible to reduce clean-up efforts during sample preparation, retaining more classes of analytes in the final extract. This in turn offered a substantial increase in the number of analytes that could be determined by one method.

GC-MS was the dominant technique for multi-analyte determination for a decade (1990s). Then came the coupling of LC to triple quadrupole MS (MS/MS). This dramatically improved selectivity and sensitivity, allowing further simplification of sample preparation and eliminating the need for derivatization. Today, sample preparation is essentially non-targeted, being reduced to extraction and removal of the bulk matrix, and dilute-and-shoot approaches (1). LC-MS/MS predominates, complemented by GC-MS/MS where required. The overall measurement is still targeted, because with MS/MS acquisition, analyte-specific transitions are measured. Furthermore, there are limitations in the number of transitions, that is, analytes, that can be targeted simultaneously in one method (currently several hundreds). Consequently, a range of (very similar) methods are used, often focusing on certain types of analytes.

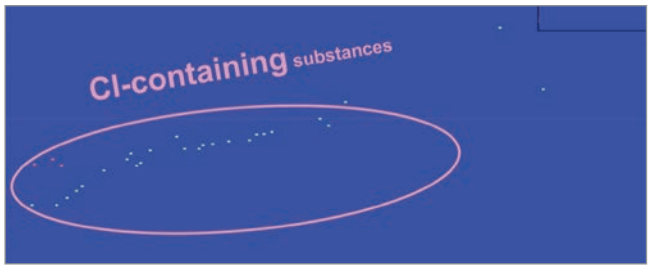


Figure 3. Two dimensional GCxGC-EI-TOF-MS chromatogram. Scripting software detects isotopic features of chlorinated analytes which are filtered from the raw data and depicted as dots. Spectra retrieved from a dots can then be searched against the halogenated compounds from a generic library (e.g. NIST) to aid in the identification.

and represents only a small part of the potential of the method.

These new analytical possibilities throw up new legislative and ethical issues. For example, if a sample of wheat taken to verify compliance with maximum limits for mycotoxins detects an illegal pesticide, can enforcement actions be taken for the latter? When urine samples are taken for biomarker analysis to investigate exposure to mycotoxins, but at the same time metabolites of drugs-of-abuse are found, what should be done with this information?

Beyond targeted detection

Currently, one way of identifying unknown substances is by analysing features; for example, the sample under investigation may share a fragment typical for a certain class of substances, or a characteristic chlorination or bromination isotope pattern (see Figure 3). High resolution/accurate mass data are ideally suited for this work, but with proper scripting software it can also be done using full-scan GC-EI-MS data.

The next step in data evaluation is non-targeted detection. Using GC-EI-MS, the spectrum for each peak can be run against a generic library of more than 200,000 spectra. Since the number of resolved peaks in a sample may be in the thousands and one spectrum may match reasonably well with multiple reference spectra, there may be various candidates that require further investigation. Using soft ionization and high resolution MS, an elemental composition derived from the accurate mass can be run against compound databases such as ChemSpider or PubChem Compound. Here, the number of candidates may be higher still, since one elemental composition may correspond to a lot of chemical structures. Fragmentation data are then needed to aid identification. A special case of non-targeted detection is provided by comparative studies in which fingerprints from non-deviating (non-treated) products are compared with fingerprints from deviating products. Multivariate analysis of the fingerprints may reveal certain peaks that are different in the two categories and which may then be focused upon for identification. A good example of this is the metabolomics approach to detect cattle treated with synthetic hormones (9). Similar types of approaches are being explored to assess the chemical safety of food (10).

Conclusions

Non-targeted measurement using full-scan high resolution MS is rapidly becoming fit-for-purpose for the determination of small molecules at low levels in a wide variety of matrices. It allows us to do the things that would otherwise require triple quadrupole MS, namely quantitative targeted detection, in a more straightforward way. At the same time, it offers many more new possibilities for targeted and untargeted screening of other analytes of interest, which can be done directly after the analysis or at any later point in time. Improvements in software to digest the enormous amount of data generated have been made in the past few years, but more efforts are needed to take full advantage of the potential. I foresee that for many applications, non-targeted measurement (full scan MS) will gradually replace targeted measurements (triple quads).

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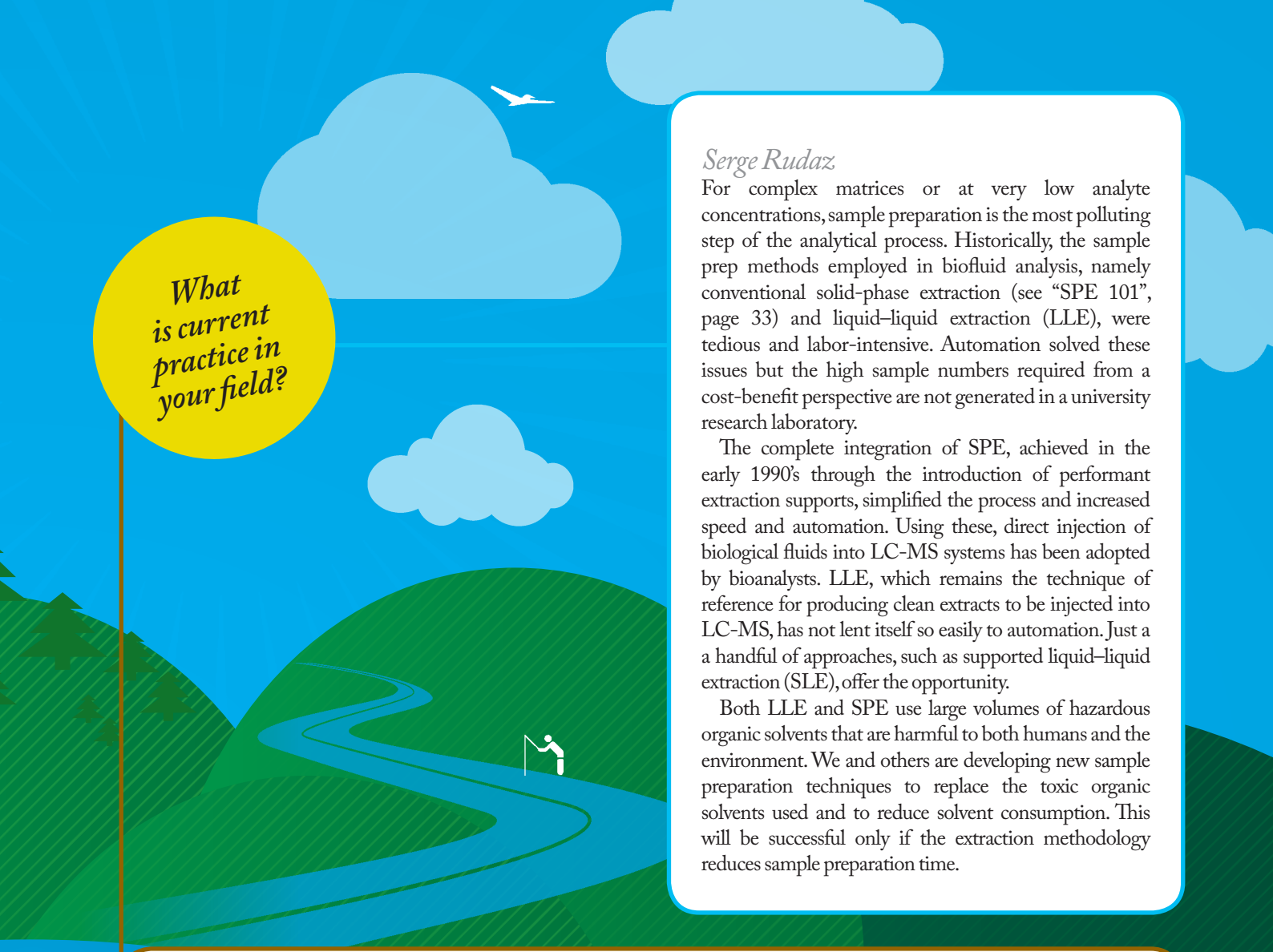
Fail to Prepare, Prepare to Fail

Where does sample preparation stand today, and where is it going? Two experts – one from environmental chemistry, the other from pharmaceutical science – share views on the current, short-term and long – term outlook for sample prep.

By Serge Rudaz and Lourdes Ramos

Over the last two or three decades, sample preparation has advanced from being a required step ahead of instrumental analysis to become an integral part of the analytical process with a profound influence on both the total time required to complete the analysis and the quality of the results obtained.

Is this the high point for sample preparation? Will ongoing improvements in liquid chromatography–mass spectrometry (LC-MS) and other techniques make sample preparation redundant or spur further innovation?



What is current practice in your field?

Serge Rudaz

For complex matrices or at very low analyte concentrations, sample preparation is the most polluting step of the analytical process. Historically, the sample prep methods employed in biofluid analysis, namely conventional solid-phase extraction (see "SPE 101", page 33) and liquid–liquid extraction (LLE), were tedious and labor-intensive. Automation solved these issues but the high sample numbers required from a cost-benefit perspective are not generated in a university research laboratory.

The complete integration of SPE, achieved in the early 1990's through the introduction of performant extraction supports, simplified the process and increased speed and automation. Using these, direct injection of biological fluids into LC-MS systems has been adopted by bioanalysts. LLE, which remains the technique of reference for producing clean extracts to be injected into LC-MS, has not lent itself so easily to automation. Just a a handful of approaches, such as supported liquid–liquid extraction (SLE), offer the opportunity.

Both LLE and SPE use large volumes of hazardous organic solvents that are harmful to both humans and the environment. We and others are developing new sample preparation techniques to replace the toxic organic solvents used and to reduce solvent consumption. This will be successful only if the extraction methodology reduces sample preparation time.

Lourdes Ramos

Although large-scale solvent-based techniques are still routinely used in many laboratories for certain analyses, SPE is widely used in routine and academic labs to preconcentrate and purify analytes from fluids and aqueous samples.

For (semi-)solid matrices, classical approaches, such as solvent-shaking and Soxhlet extraction, have been replaced by faster and more cost-effective versions, such as Soxtec. Enhanced solvent extraction techniques have become markedly more popular in the food and environmental fields, particularly pressurised liquid extraction (PLE).

In PLE, the sample, typically dispersed in a drying or inert sorbent, is packed in a stainless-steel cell and extracted in a closed flow-through system, using solvents at high temperatures and pressures (up to 200 ºC and 20 MPa). A range of extraction solvents and experimental conditions can be used, and the technique is straight-forward, with a limited number of variables to be optimized. Current trends include the packing of a co-sorbent on top of the sample column, providing in-cell (or on-line) purification of extracts. This approach, known as selective-PLE (s-PLE), yields analysis-ready extracts.

Conventional solvent-based techniques, such as LLE, SLE and Soxhlet extraction, have remained essentially unmodified for more than a century. They require large samples, consume much organic solvent, involve manual manipulation of the sample and extracts, and are difficult to automate. The extracts are often too diluted for direct instrumental analysis and, when used with complex samples, their non-selective nature make additional clean-up of the collected extracts mandatory. SPE and PLE permit faster, less manipulative, greener, more cost-effective, and automated sample treatment that in many instances result in ready-to-analyse extracts. They can be used in hyphenated procedures when the analytical approach is conveniently miniaturized.

What are the major challenges?

Rudaz

Gaining sensitivity is probably the key challenge. This can often be achieved through regular updating of our analytical tools, since improvement is continuous.

Our laboratory is accredited (ISO 17025), so new technologies must be carefully evaluated. We need to see a significant improvement to justify the amount of laboratory documentation required to introduce a revised methodology.

Ramos

The increasing demand for food and environmental controls means that the diversity of compounds and matrices that must be accurately determined is constantly expanding. In addition, public and legal pressure is driving more frequent analyses, for example, for trace pollutants in food and environmental samples. This means that massive numbers of sample-analyte combinations need to be analysed quickly and with ever-more sensitivity. The main current challenge is for higher throughput, cheaper, and increasingly sensitive analytical methodologies.

Many techniques developed during the last two decades have tried to fulfil these demands. Their success, particularly their final acceptance by commercial laboratories involved in food and environmental control, have varied widely. On-line SPE, commercialized more than twenty years ago, is well-established and accepted. However, complete hyphenated systems involving PLE await miniaturization of the system and the development of selective-PLE-based processes.

Other miniaturized techniques have been incorporated into automatic systems, allowing unattended preparation-plus-instrumental analysis of samples. These include solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE), which share advantages and shortcomings. On the plus side is their miniaturized nature, virtually solvent-free operation, simplicity, limited – if any – sample manipulation requirement, adaptability to different liquid samples and extracts, and capacity for automation and hyphenation with conventional separation-plus-detection techniques, namely LC and GC. On the minus side, the limited number of sorbent coatings commercially available restricts potential and legislation sets stringent performance criteria that are difficult to meet. Similar considerations have limited the impact of solvent-microextraction (SME) techniques in routine labs.

Where are things going in the near-to-medium term?

Rudaz

Sample pre-treatment, including dilution and protein precipitation, will be improved and faster, even where the quality of the sample is low. But I do not forsee a revolution as the basic principles of extraction remain the same.

New sample storage formats, such as dried blood spots (DBS), will gain in importance.

Miniaturization, which reduces the sample volume, analytical time, operating costs and loss of compounds will continue to make inroads as detection sensitivity improves. Both LLE and SPE can be miniaturized, the former by solid-phase microextraction (SPME) or disposable pipette extraction (DPX), the latter using liquid-phase microextraction (LPME) techniques, such as single drop microextraction (SDME), or dispersive liquid-liquid microextraction (DLLME).

From a personal point-of-view, I would like to see more electro-assisted sample preparation.

Ramos

I expect no dramatic changes in the short-term (that is, 1-5 years). Both SPE and PLE will benefit from progress in the synthesis of new materials, including very pure small-size sorbent particles, highly sortive sorbents, coatings based on nanomaterials, new molecularly imprinted polymers (MIPs) and solvents with improved solubilization properties, such as ionic liquids (ILs).

SPME and SBSE have probably reached their maximum development from a technical point of view, so advances will be associated with new coating materials. These will expand the application field for certain promising SME techniques.

Dispersive-solid-phase extraction (d-SPE), for which QuEChERS is the most popular application method, will enjoy increasing acceptance and use in routine laboratories. A constantly increasing number of applications, the simplicity of the procedures, and the commercialization of ready-to-use tubes will push this trend, while difficulties in automation may represent a real limitation.

SPE 101

An appropriate sorbent is packed in a syringe-like cartridge (glass or plastic, depending on the application). The liquid (or dissolved) sample is percolated through the SPE column followed by one of two analytical strategies. In the most common approach, target analytes are selectively retained in the sorbent while other interfering and matrix components are eluted out of the column. The preconcentrated analytes are then eluted with a relatively small volume of an appropriate solvent as a purified and relatively concentrated extract. In the alternative strategy, matrix components are selectively retained on the sorbent while the test analytes are eluted as a clean, but diluted, extract for concentration before instrumental determination. The wide variety of sorbents and formats commercially available allows determination of analytes with divergent chemical structures and polarities for a wide variety of application studies.

And in the long term?

Rudaz

I see greater focus on the use of chemical processes in an environmentally- and human-friendly way to suit green approaches. The latter goal consists of designing chemical processes to either reduce or eliminate hazardous substances. New analytical procedures have been developed to protect people's health and to eliminate, or at least reduce, the negative impact of chemical products (e.g., organic solvents) on the environment. This was the case for separation techniques, with the advent of ultra-high performance liquid chromatography (UHPLC), capillary electrophoresis (CE) and supercritical fluid chromatography (SFC), which use significantly reduced quantities of organic solvents. Sustainable and solventless approaches are likely part of the future for sample preparation.

As briefly discussed, miniaturized sample preparation that drastically reduces solvent consumption is the most obvious route. Toxic solvents should then be replaced with alternative, nontoxic extraction agents. This goal will also be a direction requested in regulations like REACH (the European Community Regulation on chemicals and their safe use).

Ramos

Miniaturisation is a clear (and necessary) trend in food and environmental analysis. Advances in instrumentation made over the past two or three decades has allowed the development and commercialization of a number of separation-plus-detection techniques. These have improved selectivity and sensitivity and are used in many laboratories for routine analysis. In most cases, these techniques involve mass spectrometric detection for final unambiguous determination, allowing a significant reduction in initial sample size without affecting performance. Large volume injections without affecting chromatographic performance is an additional tool that can achieve similar detectability levels. These are key components of the development of miniaturized methods. Another is the coupling of analytical treatments for the preparation of complex matrices for automated and/or unattended sample analysis. Here, selective mass spectrometry-based techniques will reduce or eliminate tedious multistep purification protocols, simplifying and speeding up the analytical process. This is already being done for liquid samples and fluids while the development of equivalent methods for (semi)solid samples awaits the commercialization of appropriate miniaturized systems.

Although I believe that miniaturization is the way ahead, the representativeness of the subsample used for the analytical determination can become an issue when analysing complex heterogeneous matrices. Careful homogenization of the sample before analysis is essential.

Do you see a bright future for automation?

Rudaz

That is my strong expectation. I remember the development of selective extraction supports, allowing the direct and multiple injections of biological matrices, as an attractive means to reduce sample preparation time. Now, with ten years experience behind us, my conclusion is that the full automation of sample preparation is possible for a limited number of applications, which need to be very well characterized.

Ramos

Full automation of food and environmental samples is absolutely required to meet the increasing demand, as described earlier.

Full automation has already been achieved for certain techniques and applications, particularly for those related to the analysis of volatiles and liquid samples. For many others (including semi-solid and solid matrices), today's sample preparation and instrumental techniques could yield novel hyphenated instruments that allow equivalent approaches for many other application studies. To achieve this, more work is required of academia and, especially, of manufacturers in developing new analytical instrumentation.

Rudaz

Yes and no. Resolution has always depended on three steps: sample preparation, separation and detection. The tremendous increase in the power of detection in recent years has modified how we view resolution, arguing against the need for separation.

However, new problems have arisen, most notably the matrix effect, which is the suppression or enhancement of the MS signal. These effects are generally not reproducible and the desired deuterated internal standard is not always available. This compromises quantitative analysis. I therefore believe that sample preparation, even simple, should always be considered in most cases. (Where qualitative estimation or low-level quantitation is needed, direct MS injection may be considered.)

This sample preparation may be rather generic since the subsequent steps (separation and detection) can compensate for some loss of selectivity. Rapid, simple, generic, and automated sample preparation methods are on the way. For example, simple protein precipitation or dilution could easily be automated, which would be particularly useful for samples containing a high concentration of analytes.

Note also that good sample preparation maintains the analytical platform. The dirtier your sample, the more often you have to clean your device. This is an important aspect of productivity.

Ramos

The selectivity provided by modern MS-based (and multidimensional chromatographic) techniques has already reduced the requirement for sample purification. Strategies like in-line purification can be incorporated into the instrumental procedure to preserve column integrity.

I have assisted in the development of a number of analytical procedures that allow direct determination of selected target compounds without (or with minimum) sample preparation. The feasibility of the approach, for example, for lipids or fibre in foodstuffs, or for impurities in drugs, has already been demonstrated. However, for the determination of trace components in complex matrices, such as food and environmental samples, a sample-preparation-free approach is far from being a real analytical alternative, despite being an attractive concept.

Serge Rudaz is associate professor in the School of Pharmaceutical Sciences at the University of Geneva, Switzerland. Lourdes Ramos works in the Department of Instrumental Analysis and Environmental Chemistry at the Institute of General Organic Chemistry (IQOG-CSIC), Madrid, Spain.

Pharma's DBS Dilemma

Advantages of dried blood spot (DBS) analysis for the pharmaceutical industry abound yet analytical and regulatory challenges are hindering widespread uptake. Here, two leading proponents of the approach discuss what can be done to overcome the barriers to its implementation.

By Paul Abu-Rabie and Neil Spooner

There has been much recent interest in the use of dried blood spot (DBS) sampling for the determination of circulating drug concentrations, particularly in support of clinical and non-clinical studies performed as part of regulated pharmaceutical development (1,2).

DBS offers an alternative to conventional wet plasma sampling, which is widely employed in pharmaceutical development due to ease of handling, shipping and storage compared to whole blood, and its historical acceptance by practitioners and regulators.

DBS's primary advantage over conventional plasma sampling is that it is readily employed as a microsampling technique. In practice, DBS sampling – typically involving the collection of 3 x 15µl spots – requires 50 – 100 µl of blood, including unavoidable wastage, compared to ~500 µl for conventional plasma sampling (2). This volume reduction provides an ethical advantage, particularly for toxicology studies with rodents. The physiological effects of conventional blood sampling approaches mean that extra study animals are added (known as toxicokinetic (TK) satellites) to obtain good quality drug exposure data, without compromising other endpoints for the main toxicology study animals. With DBS, TK samples can be taken from central study animals, reducing the overall number of animals needed. Using DBS as a microsampling approach is an important step forward in demonstrating a progressive approach to animal use. Furthermore, warming of rodents is required to obtain sufficient blood volumes for conventional sampling, which can be stressful to the animals. Overall, DBS is a significant contribution to the implementation of the 3Rs (replacement, reduction and refinement) in animal use for drug development.

A further benefit of microsampling is that the reduced sample volumes enable clinical pediatric studies to be performed, particularly in very small children, and facilitate the juvenile toxicology studies that are required to support pediatric registration. These studies, which would otherwise be difficult or impossible to conduct, support the provision of medicines for children and potential patent life extensions.

Other DBS benefits include reduced shipping and storage costs, simplified procedures, and a reduction in the amount of test substance required. Samples for many analytes can be shipped and stored at ambient temperature, eliminating the need for refrigeration or freezing. This generates savings that are particularly notable for multi-site clinical studies; shipping at ambient, rather than frozen temperatures, can reduce costs by tens or hundreds of thousands of Euros (3).

Chemically treated DBS paper substrates lyse cells and denature proteins on contact, meaning that sample shipping can sometimes be carried out without the extra costs incurred when handling potentially harmful biological samples [web reference: a]. DBS also offers the potential of less invasive sampling – via a finger or heel prick, rather than venous cannula in human studies – especially when the blood sample is only for pharmacokinetic assessment. This may aid recruitment of subjects for clinical studies, which is a perpetual problem for drug development projects.

The fact that DBS sampling eliminates the need for centrifugation of blood and freezing of the derived sample makes it ideal for Phase II/III drug development studies in locations where specialist equipment and consumables for sample processing are not readily available. Similarly, it is ideally suited to drug screening where the collection of wet samples is not feasible. Another potential application is therapeutic drug monitoring to enable sampling in a patient's home or local doctors surgery. Finally, there are also advantages for monitoring critically ill patients, where it may be important to minimize the volume of blood being taken.

So, with all the advantages DBS has on offer, why hasn't it already replaced conventional wet plasma analysis as the primary sampling technique to support pharmaceutical

development? The remainder of this article explains the barriers to the widespread adoption of DBS sampling for regulated drug development, and the prospects of the technique.

Bioanalytical barriers and direct analysis

Current interest in DBS for regulated quantitative bioanalysis stems from work initiated by bioanalytical groups. Somewhat ironically, one of the major barriers to the broad acceptance of DBS sampling also comes from bioanalytical scientists.

Compared to conventional wet plasma analysis, the DBS extraction procedure is more complicated, assay sensitivity is lower (microsampling means less sample, and thus, less drug is available to be analysed), and ion suppression is higher. There is understandable reluctance to switch from a tried and tested technique that is accepted by regulatory authorities to one that is more complex and that is under greater regulatory scrutiny. Given the overall benefits to patients and drug development organisations, these challenges should perhaps be a minor inconvenience but any barrier could dilute the ethical and financial advantages.

"With all the advantages DBS has on offer, why hasn't it already replaced conventional wet plasma analysis as the primary sampling technique to support pharmaceutical development?"

You could be forgiven for thinking that the need to punch out a disc from a DBS sample is a very minor increase in extraction complexity and, in isolation, this is certainly true. Indeed, it barely involves any more effort than the equivalent step in conventional wet plasma analysis, which is aliquotting an accurate volume into a sample tube using a pipette. However, considered from a high-sample-throughput perspective, where a staff member will analyse hundreds of samples per day, this can become a rate-limiting step, severely impacting efficiency. In conventional (high throughput) wet plasma analysis, this problem has been eliminated by introducing the use of automated liquid handling robots, which reduced this part of the process from hours to minutes and allowed the analyst to undertake other activities whilst their samples were being processed. This partly explains the consternation of some bioanalysts faced with going back to a manual process of individually punching DBS samples. To curtail this issue, the manual handling limiting factor also needs to be addressed for DBS sample extraction. For many bioanalysts to even consider DBS to be a suitable alternative, the effort involved with sample extraction needs to be on a par with conventional wet plasma analysis.

Automated DBS analysis

Semi- or fully-automated 'card-punching' instruments [b, c, d] can be used in tandem with a liquid handling robot [e] to relieve some of the manual burden of DBS analysis. Such instruments are available that use the workflow bioanalysts are accustomed to, which is a clear advantage, but they do not deliver the seamless workflow provided by direct elution and direct desorption. The numerous automated DBS analysis instruments used in our laboratories have not been overwhelmingly popular with our analysts.

Direct analysis

Direct analysis encompasses direct elution and direct desorption techniques, and describes any technique that eliminates the manual steps in sample extraction, including punching the DBS. The ideal workflow is simple: DBS samples are loaded onto the direct analysis instrument and extraction, separation and detection of the analyte is completely automated (see Figure 1).

In direct elution (also known as direct extraction), the analyte of interest is extracted with a liquid solvent, without sub-punching the spot. Specific techniques include on-line DBS; liquid microjunction-surface sampling probe (LMJ-SSP) and liquid extraction surface analysis (LESA) [f]; the sealing surface sampling probe (SSSP); and digital microfluidics (DMF) (4–8). The mechanism of extraction is conventional liquid extraction but it uses a sealed sampling area or confined liquid stream, low extraction solvent volumes, and direct introduction of the extract into the detector. These techniques are very well suited to DBS direct analysis. Crucially, unlike most direct desorption techniques, direct elution produces a liquid extract that is compatible with the currently used HPLC-triple quadrupole MS/MS bioanalytical workflow, which is an important regulatory and financial consideration. In addition, DBS direct elution offers an order of magnitude increase in assay sensitivity compared to manual extraction, as the entire area of the spot eluted is directed onto the HPLC column for analysis, rather than a subaliquot being injected for sub-punching and elution workflows (9). This is an important factor since low sensitivity limits the application of DBS in the pg/ml region, for example in studies of low dose or inhaled drugs.

A major concern was that the elimination of sample clean-up in direct elution techniques would results in a 'dirtier' extract

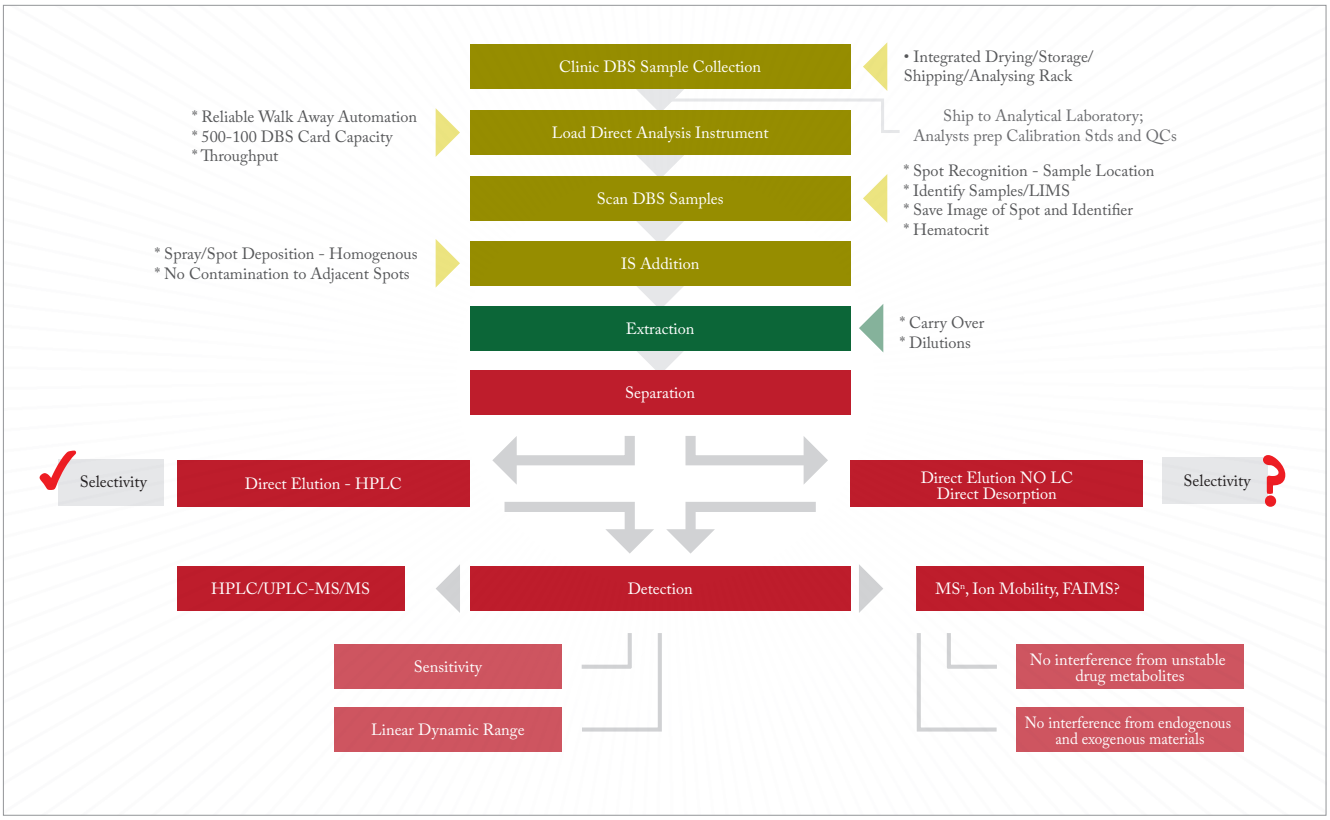


Figure 1: DBS direct analysis theoretical workflow

being introduced into the MS, potentially compromising the robustness of the analytical procedure. However, to date, no studies have found a significant difference in robustness between DBS direct elution and manual extraction (10).

Development of the functionality of direct elution instruments has made them compatible with the workflow detailed in Figure 1 [g, h, i], including:

- Sample capacity of up to 500 DBS cards
- Fully automated card handling
- Intelligent visual recognition systems to recognise the quality of samples and where to elute
- Integral wash stations to reduce cross contamination
- Integrated internal standard (IS) modules that allow the analyte and IS to be co-extracted (11).

The emergence of commercially available, fully automated instruments make the use of DBS in regulated quantitative bioanalysis a realistic prospect in the near future.

Direct desorption

Here, we use the term 'direct desorption' to describe direct analysis techniques that use non-liquid elution for analyte extraction and do not produce a liquid extract that is compatible with HPLC. This encompasses techniques that have also been referred to as atmospheric pressure surface sampling/ionisation MS; ambient ionization MS; ambient MS, and ambient desorption ionization MS techniques.

These techniques include thermal desorption, for example, direct analysis in real time (DART); laser desorption, for example, laser ablation electrospray ionization (LAESI); and gas jet desorption, for example, desorption electrospray ionization (DESI).

One of the potential advantages of direct desorption, namely the elimination of liquid chromatographic (LC) separation, is also one of the challenges for successful introduction into a regulated bioanalytical environment. While eliminating LC would simplify the bioanalytical workflow, offering greater sample throughput, its removal may result in poor sensitivity due to ion suppression, reduce selectivity, and risks assay interference via metabolite decomposition into parent compounds during MS ionization.

It is possible that different types of separation techniques

than those commonly found in the bioanalytical laboratory (ion mobility, FAIMS, or some novel approach) will overcome these drawbacks (see Figure 1). Significant resources, both financial and time, are required to develop and underpin these techniques to ensure that they are acceptable for regulatory approval.

This is not the only barrier to the practical application of direct desorption. A direct desorption technique that offers adequate sensitivity across a range of representative pharmaceutical small molecule compounds has yet to be described. DBS analysis using DART on the widely used cellulose paper substrate format is considerably less sensitive than manual DBS extraction. DESI (12), and in particular Paper Spray (13), has sensitivity close to, and in some cases exceeding that of manual DBS extraction for some compounds, but is disappointing for others.

In the regulated quantitative environment, direct desorption is a long-term goal. In the meantime, it has found a useful role in applications such as drug screening and therapeutic drug monitoring.

The hematocrit issue

Hematocrit (HCT) values that deviate from the 'normal' expected range can significantly affect assay bias when using a sub-punch approach to obtain the sample for the quantitative bioanalysis of drugs (14). The issue can be broken down into three components: area bias, recovery bias, and ion suppression bias (15).

The area bias is the influence of the viscosity of the whole blood sample, which is directly related to HCT. For a fixed volume of blood on cellulose substrate, high HCT produces a relatively small spot area; low HCT a relatively large area. Consequently, the analyte density of each spot is different because the area that a fixed volume of blood will spread over depends on the HCT value and a sub-punch taken within the sample will contain differing amounts of matrix. The recovery of analyte can also be dependent on the degree of hematocrit. The effect can be the opposite to that of the blood spot area, causing an apparent reduced overall effect on analytical data. Clearly, if DBS sampling is to be used for regulated quantitative bioanalysis to support pharmaceutical development, the relationship between DBS sample HCT and assay bias is a key issue.

The ideal solution for controlling the area bias would a novel substrate that behaves independently of HCT, which would enable the current workflow to remain unchanged. It could be a novel substrate material, substrate configuration or the addition of a modifying agent that, for example, eliminated differences in drying rates. Such a modifier could be added to the sample prior to spotting although this would compromise the simple workflow. Unfortunately, such a substrate or modifier has yet to be reported. Currently, the effect of HCT on assay bias is assessed during method validation to ensure that bias is within acceptable limits, which are typically <±15% (16). However, HCT values for study samples may be unknown and certain disease states, or exposure to certain drugs, could take a patient's hematocrit level beyond normal ranges.

The most practical way to avoid HCT based DBS area variability at the moment is to extract the whole spot rather than take a sub punch. However, to ensure confidence in the analytical result, an accurate volume must be dispensed at the clinic – a general inability to do so is the reason for taking a sub punch in the first place! Reliably dispensing accurate volumes in a clinical setting is a challenge. Blood collection devices must be developed that are accurate, cheap, disposable, and faster and easier to use (by non-analysts) than conventional positive displacement pipettes.

Existing automated DBS analysis and direct elution techniques can be made compatible with whole spot elution through relatively minor modifications, such as larger punches or sealed sampling areas. Another advantage of whole spot extraction/elution is that sample volumes could be further reduced without affecting assay sensitivity, as the same amount of blood could be sampled in total as is currently sampled in the sub punch, eliminating the wastage currently associated with manual extraction. Alternatively, current DBS volumes could be retained, eliminating many of DBS's sensitivity issues. It is not currently clear how hematocrit level effects direct desorption techniques, but it can be assumed that the area bias will be relevant in any analysis technique that samples a localised area rather than the whole sample. Further investigation is required into the mechanisms of direct desorption sampling and how hematocrit variation will bias analytical results.

High HCT levels often result in lower analyte recovery, an issue that becomes increasingly significant as overall assay analyte recovery decreases (17). Maximising the recovery of analytes will reduce the range of the recovery bias as HCT changes. An improved method of IS addition, that allows the analyte and IS to be co-extracted, would greatly assist in this regard as would additional sample clean-up to remove interfering species and/or modifying chromatography to move the analyte away from areas of suppression. The hematocrit issue certainly complicates the ultra-simple workflow on offer from DBS sampling, but the introduction

of an elegant sampling device in the clinic, coupled to a fully automated direct elution instrument, should enable the benefits of DBS to be reaped without major compromise.

Regulatory issues

Whole blood is a suitable matrix for pharmaceutical drug development studies (18). However, as with any new technique in this highly regulated field, DBS faces intense scrutiny from regulatory authorities, whose role it is to ensure maximum confidence in the data produced in support of new drugs, and ultimately that the drugs are safe for public consumption. A considerable body of research is required to prove any new technique. Currently, guidance from the US Food and Drug Administration (FDA) requires the collection of DBS and wet matrix data in parallel for a subset of samples for any particular drug development program, to prove concordance between the two sample methods (19). If this is clearly demonstrated, then it is likely that the regulator will allow DBS sampling to be used on its own for subsequent studies. Presumably, once enough positive data has been collected for a number of molecules, regulators will deem DBS to be reliable and relax the requirements. In the short-term, however, the introduction of DBS faces a financial hurdle. Supporting parallel sampling and analysis greatly increases costs, which project managers and drug development organisations are reluctant or unable to bear. Given that so many are unwilling to make the required financial commitment, progress in DBS has slowed after the initial surge of interest.

We hope that the clear long-term benefits will drive the establishment of DBS. A number of organisations are already using DBS sampling for studies where this approach can demonstrably provide benefits over conventional wet sample – good examples include clinical trials in remote areas, studies in small children and pre-clinical rodent microsampling. Publication of these data will enhance understanding of the technique. In addition, consumable manufacturers and vendors see the potential benefits of DBS and are working to overcome, or minimise, the issues associated with HCT and homogeneity. When these projects come to fruition, DBS sampling will rival wet plasma sampling in regulated drug development. To help accelerate the process, a number of industry consortiums, such as the European Bioanalysis Forum (EBF) and the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ), are running projects and sharing the data with the wider community. These multi-company initiatives should generate broader acceptance of the technique for use in regulatory quantitative bioanalysis as part of drug development.

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Business

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Coasting Ahead

How a public-private community is transforming the way analytical chemistry research is approached in The Netherlands.

By Oscar van den Brink

In early 2008, a group of Dutch analytical lab managers and academics were sitting together bemoaning the lack of human capital in analytical science. We all felt that if something wasn't done, the situation would get even worse but that if we simply shouted, "Help!" no one would respond.

"Which of us is willing to take action?" we asked each other. Eight people – myself included – raised their hands, and went on to form the interim board of what is now COAST: Comprehensive Analytical Science and Technology. We board members committed to getting things off the ground and the group as a whole pledged support. The 30 or so people in the room, representing academic groups and companies, all agreed to donate a few thousand Euros.

Three root concerns were identified. First, the difficulties that were being experienced in recruiting high quality researchers and technicians. Second, a trend towards application-driven and sector-specific analytical R&D at Dutch universities, with a concomitant decline in innovative fundamental research. And third, the limited sharing of and access to rare, high-end analytical instrumentation. In all of these respects our comprehensive partnership aims at having the widest possible influence by covering the full width of the discipline, from sampling to interpretation and from bulk chemical characterization to single molecule mapping, while serving multiple application areas.

Today, COAST still comprises these three main pillars: human capital, research and development, and infrastructure. The roof over the pillars is valorization – the conversion of knowledge into capital – which is hugely important for real progress. Here are examples of the multiple projects that we have in each category:

Developing human capital

Research labs, particularly in industry, employ a large percentage of people with an applied science background. Our Analytical Sciences Talent Programme (ASTP) stimulates the development of talent amongst students in vocational education at universities of applied science. We link students up with participating companies to give them industry experience alongside their regular education. All candidates undergo a thorough selection process; they need good first year grades, a letter of support from the director of their university, and they must show enthusiasm

and a willingness to work 400 hours per year at the company. Once on the program, students must also attend an extra 150 hours of ASTP lectures given by professors from academic universities and application scientists in industry. In exchange, we provide them with a grant – they certainly don't have time for a part-time job pulling beers. Participating companies have found it to be a successful recruitment mechanism.

ASTP is something we are very proud of; Peter Schoenmakers, our education director, has worked very hard to realize it (see page 50).

Promoting R&D

TA COAST (TA stands for technology area) brings academia and industry together to dig deeper and make step changes in analytical science. The key is linking together analytical science experts from different application areas. For example, participants from chemistry, food, and life science areas may collaborate together on a project to develop an affordable lightweight mass spectrometer or NMR techniques to study polymers under processing. To date, TA COAST has provided grants totalling 9 million Euros to support nine projects, that range from four to fourteen partners. Twenty-four private partners, including 10 SMEs, and 17 academic groups are involved. COAST, as a foundation, takes no intellectual property (IP) rights from the projects supported. Project participants negotiate on IP, sharing the success.

When several application areas join forces and gather interests, a more fundamental description of their analytical needs is generated, which allows research to dig deeper. In reality, that means working on a solution that is closer to basic innovation. Collaborative approaches help spread the risks and efforts required; it's a more business-based approach to research and development. Indeed, our research programs attempt to facilitate a better balance between the chance of success (or risk) and the magnitude of investment. Without such collaborative efforts in the current economic climate, conducting comprehensive analytical research would be much more difficult.

Improving the Infrastructure

There are many unique and/or rare analytical facilities in The Netherlands. COAST facilitates access to both techniques and instrumentation, from the latest high-end LCxLC set-up to something very specialized, such as Willem van Raalte's interface for the analysis of oily residues (theanalyticalscientist.com/issues/0113/503). If you want to perform oily residue analysis only once or twice, why not do it in Willem's lab? That way, immediate capital investment is unnecessary. Of course, Willem may charge for the service, but he also has an interest in your success. At the more expensive end of the spectrum, COAST provides access to the 850 MHz solid-state NMR facility in Nijmegen and the 900 MHz liquid-state NMR in Utrecht –facilities that you wouldn't dare suggest to management that you should buy! COAST provides fast access to 49 instruments on our database, each of which provides specific, unique or rare capabilities to the community. A by-product of the database is the creativity it can engender – you may discover something that you didn't even realize existed.

Generating Economic Value

If technical innovation is important for economic growth, the well-being of the population, or the sustainability of the ecosystem of a country or region, then

Beyond Borders

Could the COAST model be exported to other countries? Certainly it could, and we would be willing to join forces and support similar initiatives to form a global network.

However, I believe there are some cultural issues at play; public-private partnerships of this type are perhaps easier in Dutch culture (or Belgian or German) than in some other cultures. As far as I can tell, bilateral agreements are preferred over multilaterals when it comes private partnerships in the US. You could form a community made up of bilaterals, but it would be an inefficient and lengthy process. By the time the initiative reached critical mass, the benefit of multilateral collaborations may become obvious, but that remains to be seen. The reason for COAST's success is the very fact that two or more private partners get on board with each project.

In future, COAST may well look to expand beyond the borders of The Netherlands by being open to companies or institutes who have a strong motivation to link up with us. We interact a lot more internationally nowadays, and this has encouraged a wider perspective and increased the trust necessary for the kinds of partnerships we promote. As communication technology progresses, things can only improve further.

governments must pay attention. On the basis that analysis meets all three of these criteria, we approached the Dutch government for matching funds. In fact, it was this move that prompted the formal establishment of TI COAST (our official title – TI stands for top institute) as a professional organization in December 2008.

The government had already defined specific economic areas to be developed using public-private partnerships – the so-called "Topsectoren" (www.top-sectoren.nl). Among them are chemistry, life sciences and health, agro-food, and water. All of these areas are linked to tangible products and, in most cases, analytical science is a crucial success factor in continued technical innovation. And the better you measure, the faster the innovation. We see a parallel to information and communications technology (ICT): without it, you can achieve very little – it is an enabling cross-sector discipline; the same is true of analytical science, though admittedly more modestly so. The essential nature of analytical science is something that COAST highlights to the funding agencies within our government. Until recently, the message did not resonate, but people are starting to wake up as we continue to show that companies within many of the Topsectoren are willing to link up in analytical science research to share the risk, share the investment, and, most importantly, multiply the success.

Maintaining the Structure

The government contribution to COAST is all put into research programs; nothing is used for the day-to-day running of the organisation, which is funded by participant fees. That means that if government decides to reduce its commitment, the organization remains sustainable and won't collapse in on itself.

Legally, COAST is a foundation but it is structured like a society or club. It is run by a participant council made up of some 70 companies, universities and knowledge institutes; the strength of their vote depends on what they contribute, with voting values from zero (no contribution) to three – he who pays the piper, calls the tune!

The main reasons for participants joining COAST are concerns about the availability of well-trained analytical professionals and interest in innovation within analytical science. The industries and public institutions that use analytical science are of course primarily interested in applications but see the need for investment in basic innovation as well; the academic partners are of course interested in performing research; and the instrument companies may be primarily interested in selling instruments but certainly see the benefits of developing new techniques, methods and applications in collaborations with their customers. These needs and interests are complementary and the reason COAST has been so successful in connecting those disparate stakeholders.

I am often asked how "the project" is doing. It is doing very well but COAST is not a project – it is a community. We want people to work together not just in the short term but for many, many years to come. We want to create a community that not only collaborates on research but also recognizes that to do good research, we need good people. And investing in them now will enable even better research in the future.

Oscar van den Brink is Managing Director of COAST, Amsterdam, The Netherlands.

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Solutions

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Volume-Based HPLC

Moving away from constant flow to multiple modes of operation heralds a revolution in high performance liquid chromatography.

By Monika Dittmann

The Problem

Utilizing the full power range of high-performance liquid chromatography (HPLC) instruments is constrained by constant flow operation. How can we improve separation speed and robustness of current ultra high-performance liquid chromatography (UHPLC) separation methods by removing these shackles?

Background

When we consider the basic thermodynamic principles of liquid chromatography (LC), the retention factor of an analyte is governed by the volume ratio of stationary and mobile phases and a peak elutes after a certain volume of mobile phase has passed the column, independent on flow rate (1). The original definition of the retention factor k' in LC is given by:

$$k' = K \cdot V_s / V_m$$

where:
K = distribution coefficient of the solute
V_m = volume of mobile phase
V_s = volume of stationary phase

The elution (retention) volume is given by

$$V_r = V_m \cdot (k'+1)$$

In the early days of LC, the driving force separating ions was gravity and the identification of compounds was achieved by elution volume and spectral properties.

With the advent of modern HPLC it became common practice to record an elution time rather than elution volume. This was mostly for a good, practical reason: it is easier to measure time than to measure elution volume.

There is, however, a prerequisite for interchangeability of run volume and run time, which is operation at a constant flow rate. Only when this is preserved can the run volume be determined directly from the run time.

$$V_{run}(t) = \int_0 F(t)dt = F \cdot t$$

What price is being paid to keep the flow rate constant during a

chromatographic separation? We give up the flexibility to operate our system at the highest possible separation speed, since flow rate has to be adjusted to solvent properties such as maximum viscosity. In constant flow operation, flow rate is limited by the pressure drop at the composition with maximum viscosity and it is not possible to speed up the separation when the viscosity decreases.

To illustrate this, imagine running a gradient separation using water and an organic solvent like acetonitrile or methanol. The solvent properties, such as viscosity, heat capacity, and density vary with the composition of the eluent. As a result, the pressure drop and temperature gradient due to frictional heating vary across the gradient. For a gradient of acetonitrile and water the viscosity reaches a maximum (1.2 cp at 20°C) at 20% acetonitrile and decreases to a minimum (0.45 cp @ 20°C) for pure acetonitrile. This means that large parts of the gradient could be run at higher flow rates if we were free to vary the flow over the course of a run (see Figure 1).

In UHPLC separations, the power generated by the pump leads to a temperature increase along the length and the radius of the column. The magnitude of this increase depends on pressure drop, heat capacity, density and adiabatic expansion factor of the eluent. As all these values vary during a solvent gradient, the column excess temperature varies significantly over a run. When the temperature equilibration times are long (10 – 30 min) compared the gradient time, this can lead to differences in temperature profile in a series of gradients. With variable flow operation, the flow rate could be adjusted to keep the excess column temperature constant, leading to better retention time reproducibility.

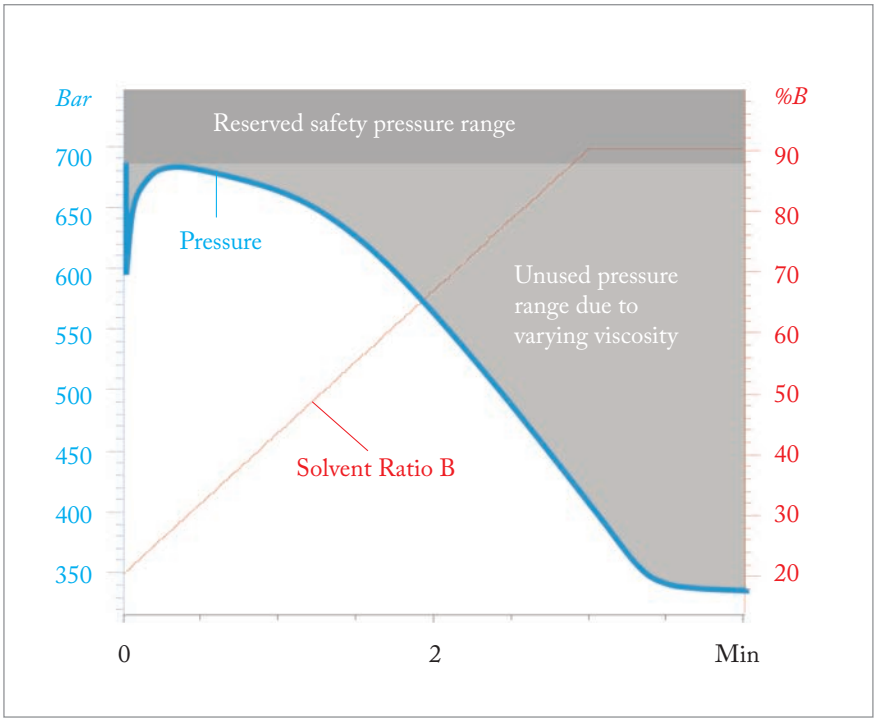

Fig 1: Typical Pressure trace for a gradient from 20 – 90 % acetonitrile

The Solution

If the constraint of constant flow rate is removed from HPLC operation, different operation modes become possible. This opens up new opportunities for control and optimization, such as operation at constant pressure, operation at constant frictional heat, and real time flow adjustment for special applications. These applications could include stopped-flow operation for HPLC-nuclear magnetic resonance (NMR) coupling or fractionation, selectively increasing or decreasing flow at specific time points in a separation.

In pressure-controlled operation, the flow rate would be adjusted to keep the pressure drop constant, being low in the high viscosity region and increasing with decreasing viscosity. This has a number of potential benefits: the overall separation time would be decreased, there would be no shutdowns due to overpressure and the pressure stress on columns would be reduced (2-5).

In frictional heating-controlled operation, the variation in temperature increases over a series of gradient runs could be reduced, leading to improved reproducibility of retention times and resolution (6, 7).

What is needed to enable volume-based HPLC? The following are prerequisites:

- The gradient shape, that is, composition versus run volume, must be maintained independently of flow rate variations. To provide clearly defined and reproducible gradients, programs must be based on run volume rather than on elapsed time.
- Accurate, real-time data on run volume at varying flow rate must be available for evaluation of detector signals versus run volume.

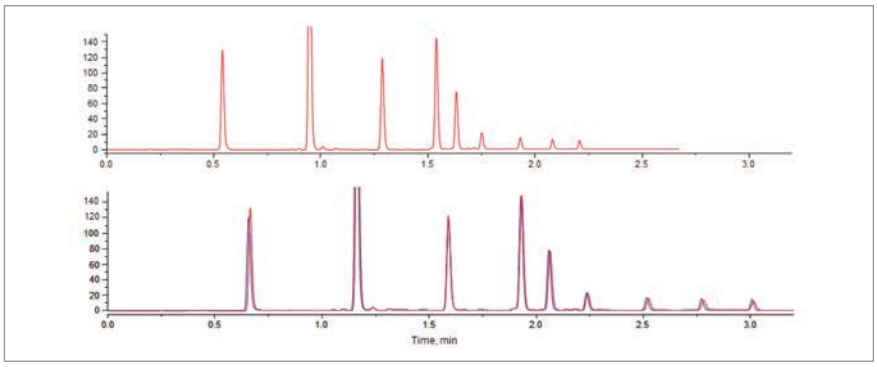

Fig 2: Separation of alkylphenones in constant pressure mode (1000 bar) (upper trace) and constant flow mode (0.6 ml/min) (lower trace, red = constant pressure vs virtual time, blue = constant flow vs real time).

- Software tools must be available that use the run volume data to rescale the signal versus real time into signal versus run volume data as these are independent of the speed with which the peak passes the detector.

To achieve these goals, the pump must be capable of measuring the run volume in real time and adjusting the solvent composition such that the delivered gradient in volume units remains as programmed, independent of flow rate variations. Such a pump requires a controller with increased computing power and very precise control of piston position to make the necessary changes to the volume displaced in real time. However, the value of primary importance for volume-based operation is the decompressed solvent volume after the column outlet. The Agilent 1290 series pumps can provide this information reliably given the very low system elasticity and the availability of accurate compressibility tables for the solvents used, which allow exact calculation of the decompression ratio.

Precise knowledge of the run volume is needed to (a) control the gradient formation in volumetric units in real time and (b) to rescale the detector signal with respect to run volume or virtual time (that is, the time that would have elapsed if the separation had been run at a selected constant flow rate).

It is important to note that the gradient is programmed by the operator in time units for a certain flow rate, just as in conventional HPLC operation. The gradient is then executed by the pump in volumetric units exactly as programmed, independently of the actual flow rate used.

The chromatogram and analysis of data can be performed with respect to run volume or in virtual time, depending on the preference of the user. Figure 2 shows chromatograms obtained at constant pressure (1000 bar, upper) and at constant flow (0.6 ml/min, lower). In the lower trace, the constant pressure chromatogram is converted to virtual time and overlaid.

The time savings that can be obtained in constant pressure operation will depend on the type of organic modifier and the start and end composition of the gradient. In practice, time gains of up to 25 percent can be anticipated, taking into account that the pressure safety margin can be reduced.

Implementing the Solution

Volume-based LC could be used in virtually all areas of modern liquid chromatography. The increased separation speeds would have great impact on high-throughput applications or in speeding up comprehensive 2D-LC. Another potential application area is routine HPLC, where the volume-based operation mode could be used to maximize robustness by avoiding shut-downs due to overpressure. The operation of columns at constant pressure might also provide greatly enhanced column lifetime, although this needs further evaluation.

Monika Dittmann is R&D Senior Scientist at Agilent Technologies GmbH, Waldbronn, Germany.

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TOSOH BIOSCIENCE

The High-Impact Chromatographer

Sitting Down With **Peter Schoenmakers**, professor at the Faculty of Science, van't Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands, and co-chair of HPLC2013.

You've run many conferences – what's the appeal?
Starting close to home, one of the great things about organizing a conference is the exposure it can give to your own group. We learnt to focus on short courses for our own students – making it accessible and interesting to them – and then built the conference around it. The step towards a larger conference is a big one!

You're talking about HPLC2013?
Yes. If you organize a conference for 250 people, your own group may make up 10 percent of the meeting – that's the motivation. At a larger conference, you have to bring together the community and create exposure for your entire country! As of today, about 10 percent of HPLC2013 attendees are Dutch. But when you live around the corner, it makes it easier to register late. I expect more.

What's so special about HPLC2013?
It's special for me, personally, because I've been part of that community for such a long time. But it is also special in its extreme diversity. There are hardcore chromatographers, as I call them, so the program must cover fundamentals and technology. But in other communities, HPLC is vital as a tool. That's what High Impact LC is all about. The big challenge is trying to find the right balance.

What would be a satisfying outcome for HPLC2013?
Ahead of the meeting, we set many targets – the number of oral presentation abstracts, poster abstracts, short course participants – and, in all cases, the lights are green. But that's numbers. Now it comes down to making it happen and ensuring a good atmosphere for science.

How many people do you expect?
Predictions are dangerous. Last week, we didn't know how many poster abstracts we would have by the deadline. We had around 500-600. When I checked this morning, there were 920. I couldn't have predicted that. The biggest HPLC in recent history was in Budapest in 2011 with 1285. That's a nice target. It also proves that the meeting is still very much alive.

"To be invaluable to a company and fully respected as a great scientist by the community – that's a difficult balance to strike."

You spent twenty years in industry before switching to academia – was it simply time for a change?
I worked in industry for a long time, but I always felt like a scientist; I was happiest in the lab. At some point, it became difficult to concentrate on science, and that's part of the reason. There should always be an opportunity in industry to do new things, to publish, and to keep it exciting.

Do you think there are enough great scientists in industry?
They do exist, but they're a rare breed – and getting rarer. To be invaluable to a company and fully respected as a great scientist by the community – that's a difficult balance to strike. To raise the profile of industry researchers, HPLC2013 is introducing a new award to commemorate Uwe Neue – a brilliant chemist and an amazing contributor to the community. The first recipient is Jack Kirkland. Jack is the epitome of an industrial scientist and his 55-year-long career is legendary.

You organize conferences, lecture, edit, and take on many other responsibilities… Why?
I am an analytical scientist. I believe that if we don't take ourselves seriously, no one else will. You will recognise the feeling: "It's only analytical chemistry". But the change must come from within. It's up to us to explain what we do and make it very clear how important that is. The Netherlands is a relatively small country and yet COAST (see page 42) has over 60 partners standing as a community and taking analytical science very seriously indeed. My feeling is that if you invest in a community, you also invest in yourself.

You act as education director for COAST, how do you tackle that responsibility?
When building a community, it's not just about good research; it's about training the right people. Industry says correctly "there aren't enough good people" and I've found the same problem when hiring post docs. Our university programmes try to address that imbalance.

What do you find most rewarding?
When things run well and work out. The fact that we have such a strong public-partnership like COAST, which took us many years to get off the ground, with all partners involved because they consider the field so important – that's rewarding. We've come a long way.

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