NOVEMBER 2018 # 70

the Analytical Scientist™

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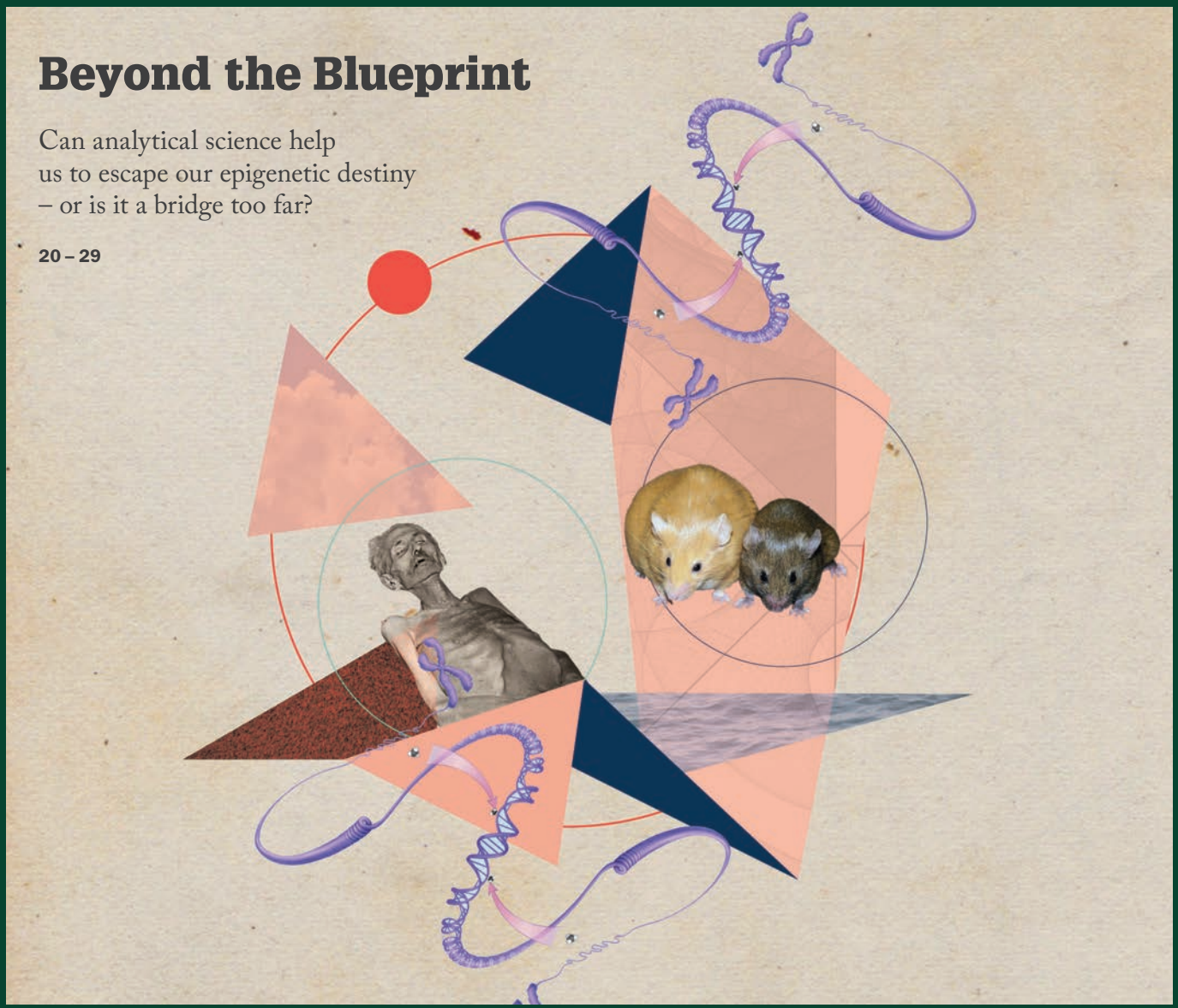

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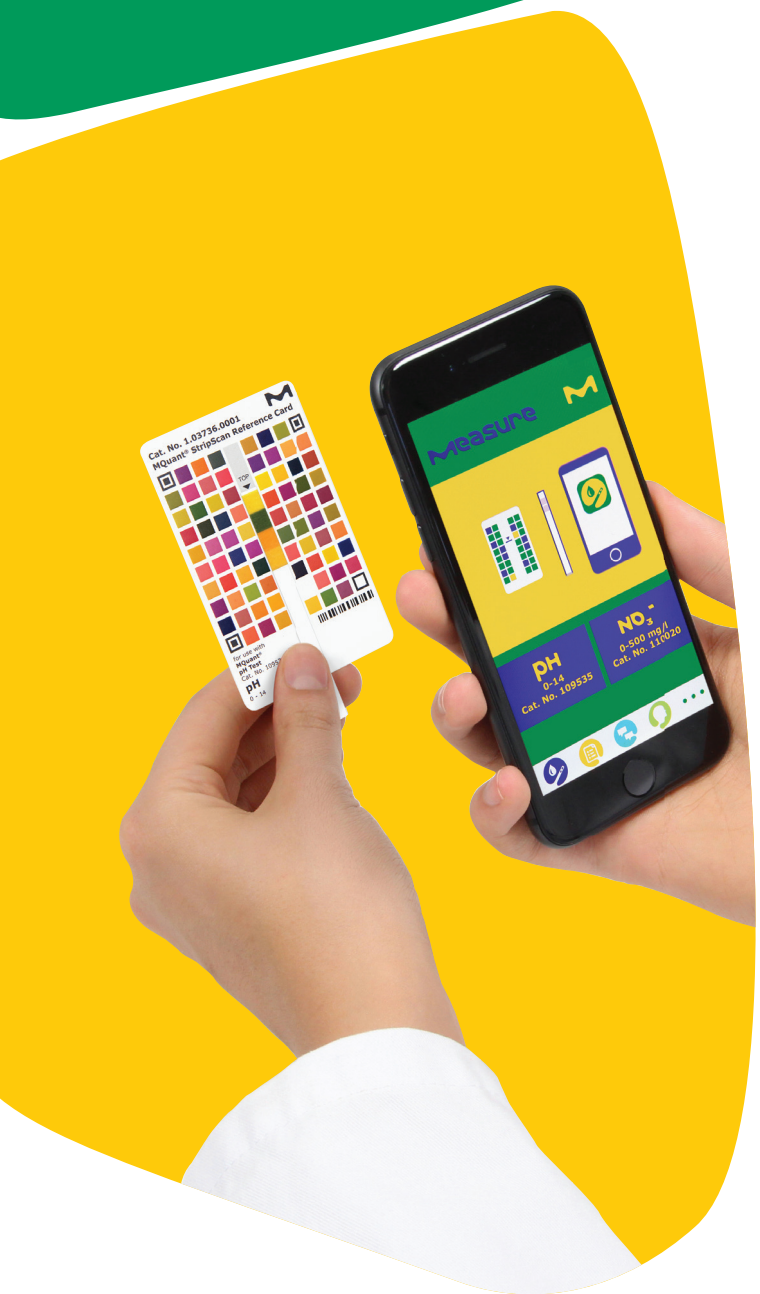

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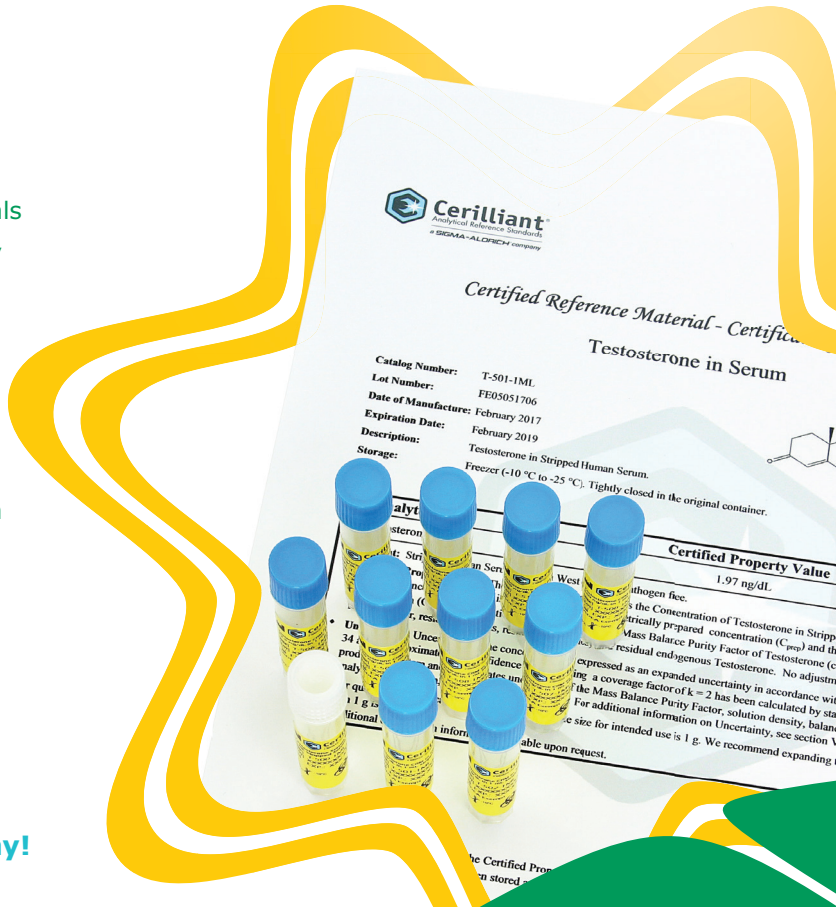

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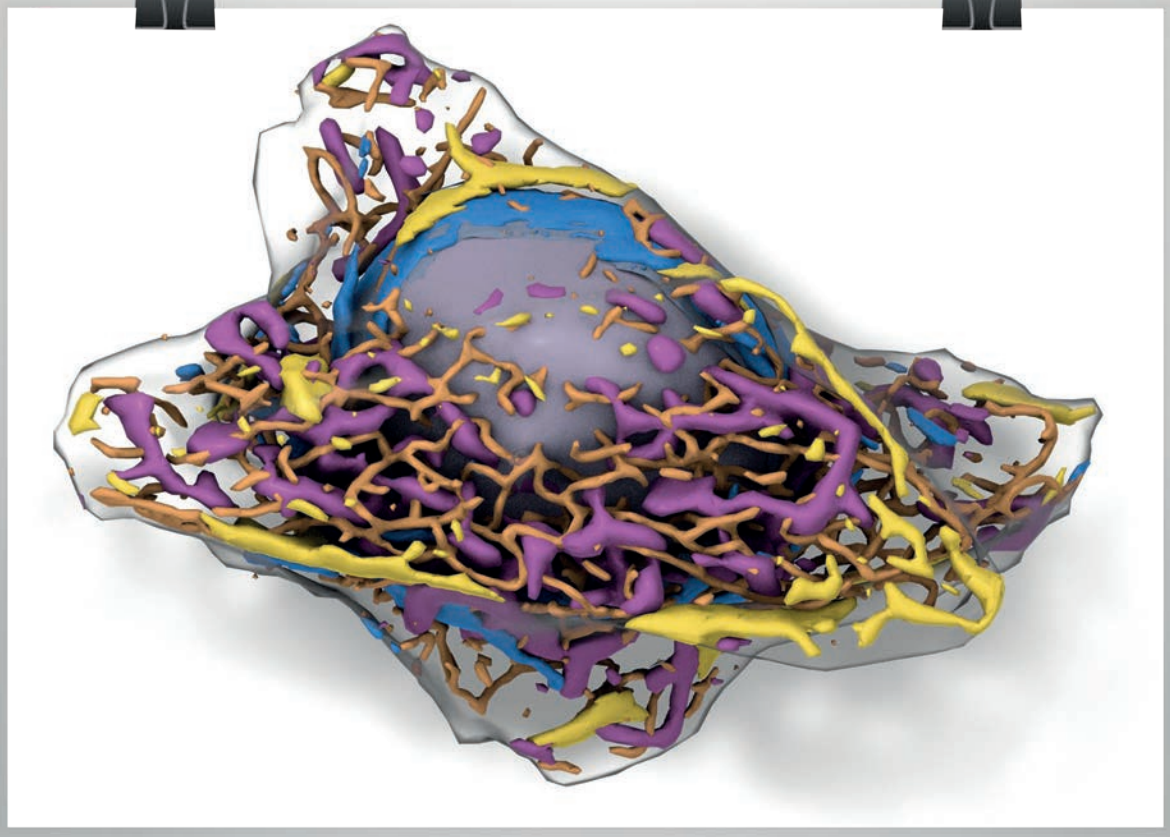

Journey to the Center of the Cell

A new 3D modeling tool allows scientists to view live human cells in an unprecedented way, using a computer algorithm to determine the shape and location of its structures without the need for fluorescence microscopy.

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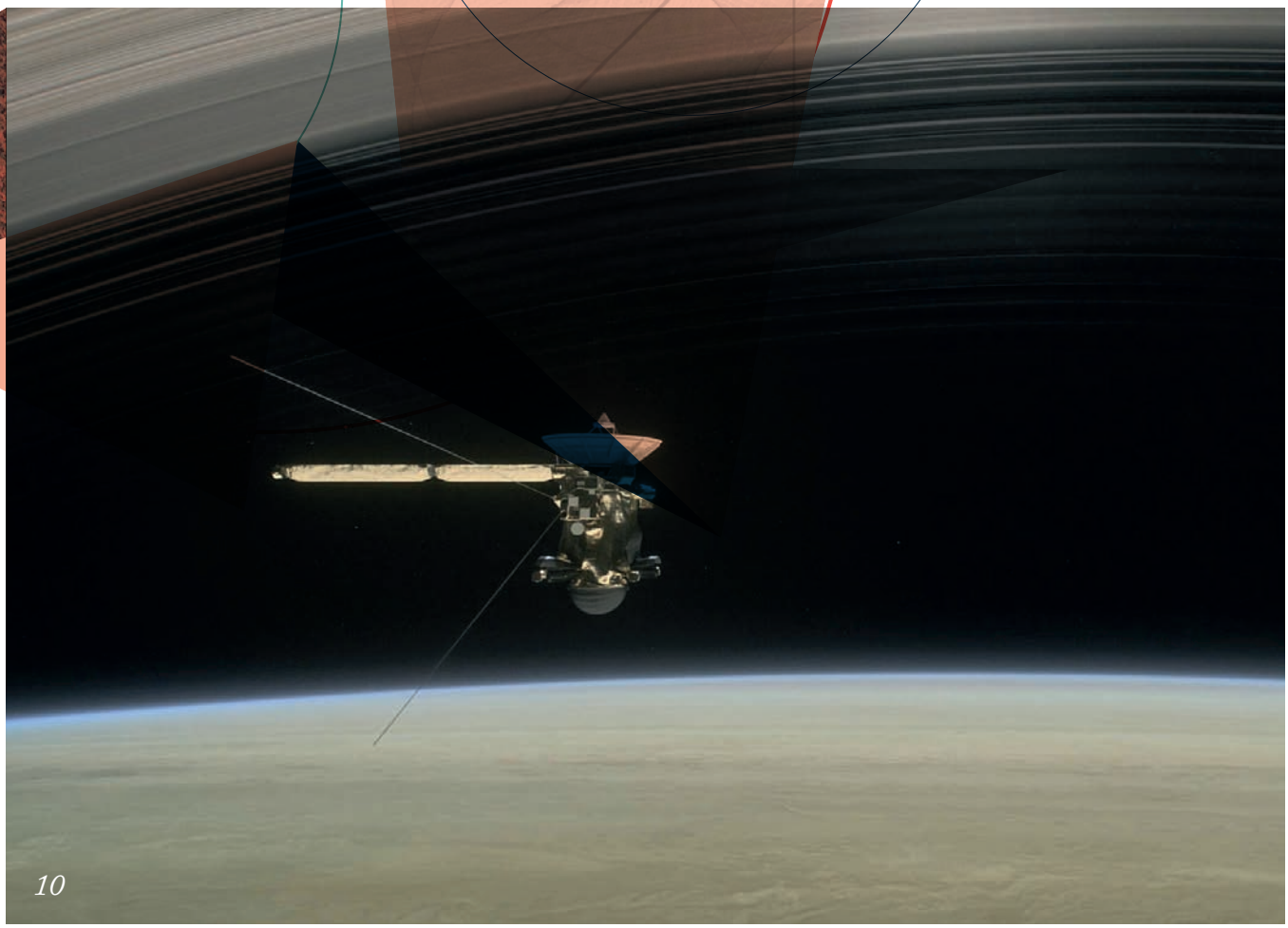

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Sitting Down With

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Just What the Doctor Ordered

We need to increase diversity – and talking is not enough.

One of my favorite moments of SciX 2018 was the unveiling of Karen Esmonde-White's costume for the Gala celebration (you'll already know this, if you follow me on Twitter: @Editor_Jo). There was something immensely satisfying about being at a conference chaired by a woman – and a woman who chose to represent the first female iteration of a much-beloved sci-fi character (Doctor Who, if you're lost!).

SciX, for me, is a highlight of the analytical conference calendar, because – among other reasons – it has an overt history of addressing issues of diversity through its workshops. Its 2015 workshop on "Women in Spectroscopy" inspired my first ever cover feature for The Analytical Scientist, "I Love the Sound of Breaking Glass (1)," which tackled the topic of sexism in analytical science. The workshop was more recently expanded to cover diversity and underrepresentation more broadly.

But this year, there was no diversity session at SciX. Some people felt that it had become an echo chamber, with successful women preaching to the converted. Surely, as valuable as it is to share experiences, it's time for more tangible efforts.

Any publication, any workforce, any institution needs to periodically ask itself if actions to ensure (more) equal representation are i) adequate, ii) appropriate, and iii) effective. However far we've come, thrusting those who face discrimination of any sort into the public eye still appears to be an uphill battle. You only have to read the intense media reaction to this year's Nobel Prize winners to see that a woman's name on the list is still newsworthy. And let's not forget the female Doctor Who…

There are many possible reasons for this – lack of opportunity or recognition, entrenched prejudice, unconscious bias – but it's time for us all to do our bit, and that goes beyond raising awareness. Could you, as a senior academic, champion underrepresented students? What can you, as a lab manager or department head, do to make the working culture more supportive and flexible? How could you affect change as a conference committee member?

Achieving equality could be the result of a revolution or – more likely – gradual evolution. But one thing is certain – when it comes to boosting diversity, we need less talk and more action.

Joanna Cummings
Deputy Editor

Reference

1. *tas.txp.to/0516/breaking-glass*

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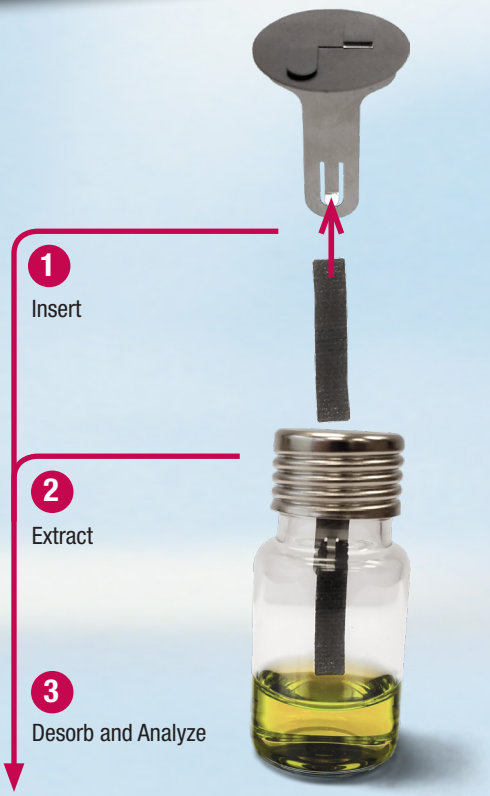

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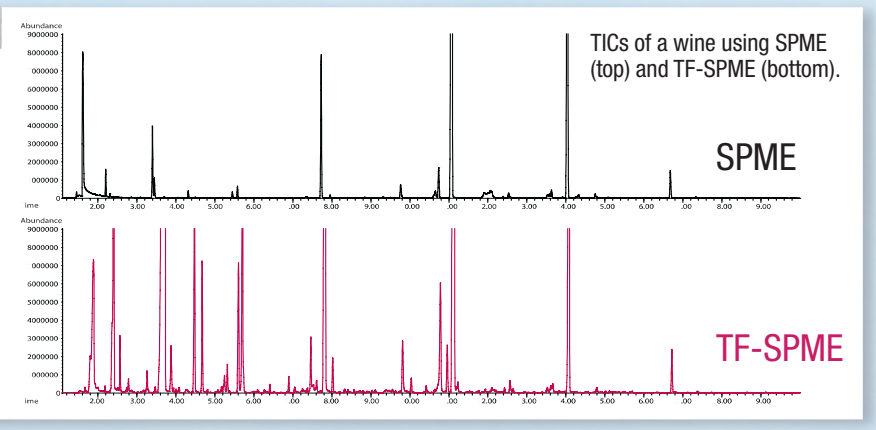

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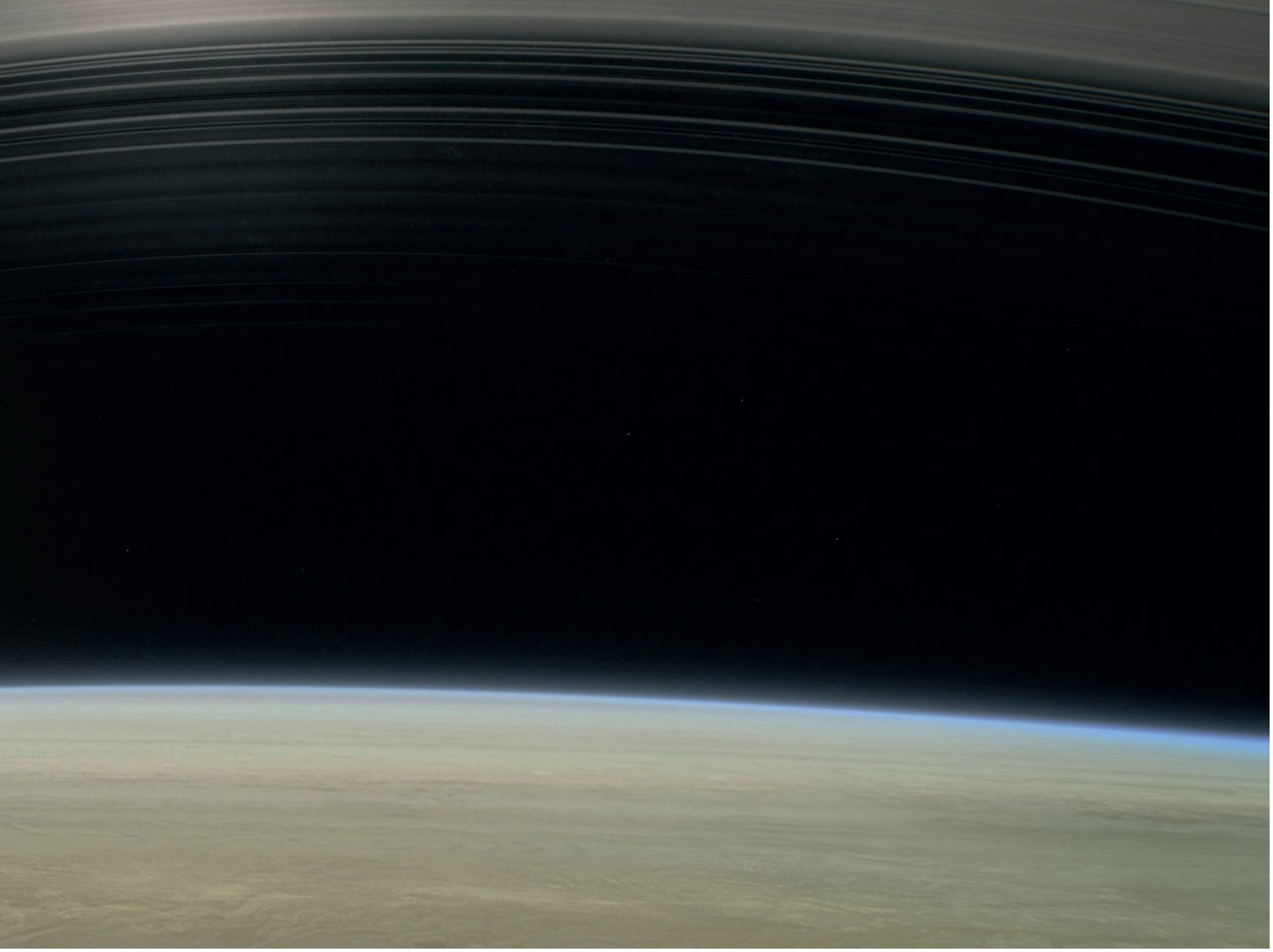
Ring the Changes

NASA's pioneering Cassini spacecraft captures Saturn's atmospheric composition in its "Grand Finale"

What?
NASA's Cassini spacecraft has managed to analyze the chemical complexity of Saturn's atmosphere, overturning existing theories on the molecular composition of the planet's distinctive rings. Previously thought to be chiefly comprised of water, the rings were found to be composed of water, methane, ammonia, carbon monoxide, molecular nitrogen and carbon dioxide – which are being expelled into the planet's atmosphere at a rate ten times faster than previously believed.

How?
The Ion and Neutral Mass Spectrometer (INMS) on Cassini recorded the data as part of its "Grand Finale," a series of five dramatic plunges through Saturn's rings, culminating in a final dive into the planet's atmosphere in September 2018. The spacecraft has been recording data for around 13 years.

Who?
Cassini's INMS team. Thomas Cravens, co-author of a paper describing the findings (1) said, "My interest was in the ionosphere, the charged-particle environment […] Per atom, it's pretty energetic stuff because of the speed



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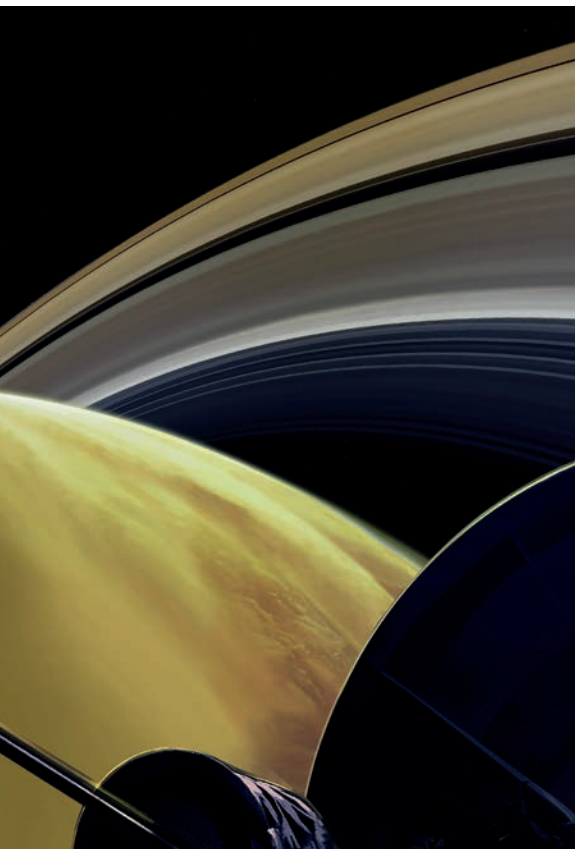


differentiation between the rings and the atmosphere. We think it may be heating the upper atmosphere, changing its composition."

What next?

Though there is no future for Cassini – which vaporized soon after entering the planet's upper atmosphere – the authors believe we are a step closer to understanding the formation of planetary rings. Cravens said, "This is a new element of how our solar system works."

Reference

1. *JH Waite Jr et al., "Chemical interactions between Saturn's atmosphere and its rings", Science, (Epub) 362 (2018).*

Yummy Mummy?

GC-MS reveals ancient Egyptian embalming "recipe"

Analysis of textile wrappings on a 6,000-year-old intact mummy has unearthed the embalming techniques of the ancient Egyptians, giving further insight into their early funeral rituals and what one researcher described as the "first ever unequivocal scientific evidence for the use of embalming."

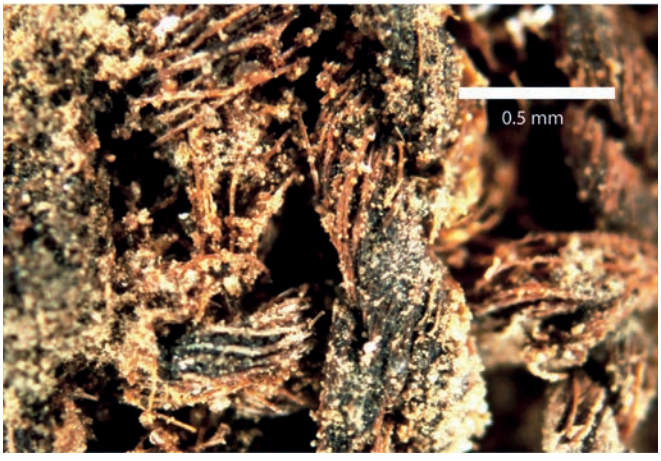
The British–Italian research team analyzed the textiles using a variety of techniques, including gas chromatography-mass spectrometry (GC-MS), thermal desorption/pyrolysis (TD/Py)-GC-MS, radiocarbon dating and shotgun metagenomics.

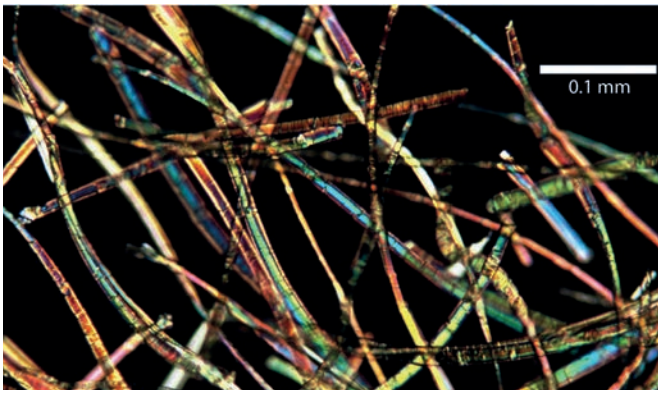
The recipe was found to be a composition of plant (possibly sesame) oil, a balsam-type root extract, and a plant-based gum. The addition of a conifer tree resin to the concoction is thought to have been a crucial step in protecting the body from decomposition, and demonstrates – the authors believe – the sophistication of the Egyptians' embalming techniques.

Co-author Tom Higham (Deputy Director of the Oxford Radiocarbon Accelerator Unit, Oxford, UK), said, "Our radiocarbon dating shows it dates to the early Naqada phase of Egyptian prehistory, substantially earlier than the classic Pharaonic period, [offering] us an unparalleled glimpse into funerary treatment before the rise of the state." He added, "The results change significantly our understanding of the development of mummification […] and demonstrate the power of interdisciplinary science in understanding the past."

Reference

1. *J Jones et al., "A prehistoric Egyptian mummy: Evidence for an 'embalming recipe' and the evolution of early formative funerary treatments", J Archaeol Sci, [Epub before print] (2018). DOI: 10.1016/j.jas.2018.07.011*





Clockwise from top left: The Turin mummy, microscopic view of linen from body, ultimate fibers; microscopic view of linen from body.

Suppressed Excitement

New microfluidic membrane suppressor technology wins the prestigious Solvay Award for the best PhD thesis

Suppressor technology was first introduced in 1975. By lowering the conductivity of the mobile phase prior to conductivity detection, it made ion-exchange chromatography more broadly accessible. Now, a team from the Vrije Universiteit Brussel is aiming to take the technology a step further, and in the process talented PhD student Sam Wouters won the Solvay Award for Young Chemists. Wouters and supervisor Sebastiaan Eeltink tell us more.

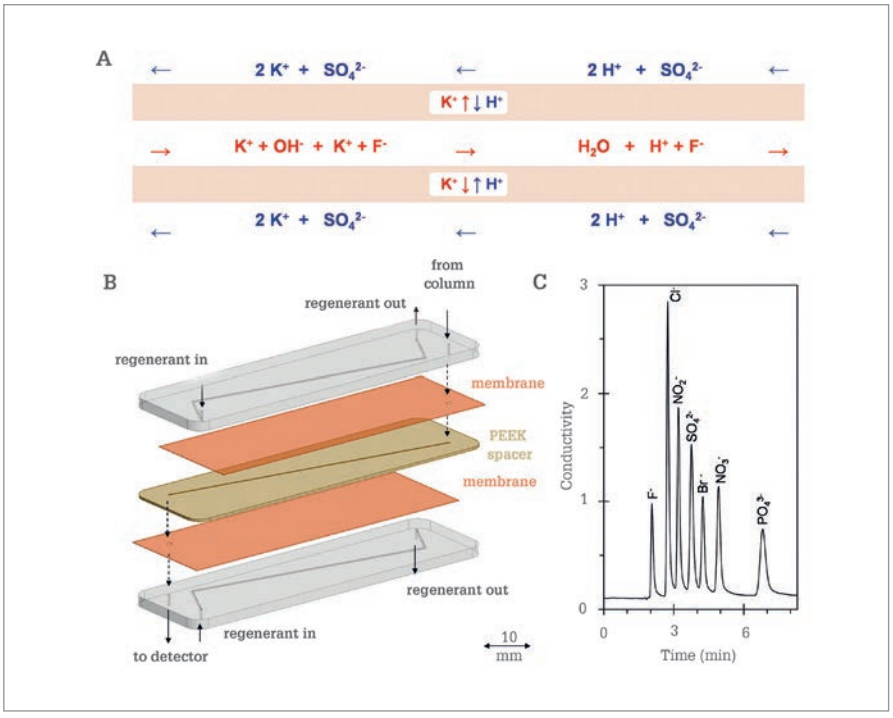


Figure 1. (A) The principle of chemical suppression in anion-exchange chromatography using a chemically-regenerated membrane suppressor. (B) Illustration of the stack and channel configuration in the double-sided chip suppressor, featuring a PEEK spacer with eluent channel, placed between two ion-exchange membranes, and two COC plates allowing us to establish a regenerant counter flow. (C) Isocratic separation of 7 anions at 10 µL/min (solid line) applying 30 mM KOH on a capillary 150 mm AS18-4 µm column (30°C) hyphenated with the double-sided chip suppressor and a capillary conductivity detector (baseline-subtracted chromatogram).

What prompted you to work on this technology?

As a team, we actually worked not only on the suppressor module but the complete miniaturization of an ion chromatography system. Our aim was to make a portable device allowing for on-line/at-line process monitoring and field analysis – a work in progress!

How is membrane suppressor technology used?

The suppressor is included after the separation column and prior to the detector. In ion chromatography, we try to detect ions using an ionic solution such as NaOH as the mobile phase. As conventional UV detection cannot be used (many ions do not have chromophores) conductivity detection is most frequently applied. However, with so many ions already in the mobile phase it is very difficult to detect "target ions" as the linear range of the detector is very low (most of the range is "used" by the ions present in the mobile phase). We therefore need to use a suppressor to remove these ions.

What were the limitations of previous technology?

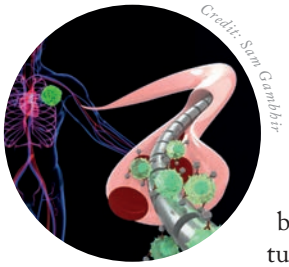
Early suppressors or "strippers" consisted of large i.d. columns packed with ion-exchange resins, and had limited capacity. There were also problems with mechanical stability of the fiber and with establishing an interface with other system components.

How is your suppressor different?

As shown in Figure 1, we have developed a continuously chemically-regenerated microfluidic membrane suppressor chip for anion-exchange chromatography. When the separation in the column is completed, the analytes and mobile phase (composed of potassium and hydroxide ions) are introduced to the suppressor chip via the eluent microchannel located in a PEEK spacer, that is sandwiched between selective cation-exchange membranes and two thermoplastic chip substrates containing microfluidic channels, which provide the regenerant flow (in this case a diluted sulfuric acid solution). The sulfate counter ions are relatively large and the negatively charged membranes repel these ions. Protons will diffuse through the membrane, replacing K+ ions to pair-up with the hydroxide ions and form water, hence reducing the background conductivity and enhancing the signal. Figure 1C shows the potential of the microfluidic suppressor chip for the high-throughput separation of a mixture of seven anions by coupling a microfluidic membrane suppressor chip to a capillary anion-exchange column.

How will this technology help analytical scientists reach their goals?

Ultimately, we hope this chip will allow you to analyze very small volume samples (important for clinical applications) but also establish modules so you can measure at-line and improve process control. There is still work to be done – it needs to be a very robust "plug and play" chip device – but we believe that microchip suppressors could prove useful in a number of fields.


Credit: Sam Gambhir

Gone Fishing

A magnetic wire inserted into a vein captures circulating tumor cells and cell-free tumor DNA

Blood biomarkers for cancer are often present only in low concentrations. But how can you interrogate the entire contents of an adult's circulatory system? Sanjiv "Sam" Gambhir and his colleagues at the Canary Center at Stanford for Cancer Early Detection have developed a unique approach: an injection of magnetic nanoparticles designed to bind circulating tumor cells, combined with a thin, magnetic wire that captures them directly from the vein.

What prompted you to investigate in vivo tumor cell retrieval?

We were trying to develop a strategy that goes after rare biomarkers (such as circulating tumor cells or cell-free tumor DNA) in blood. When shed by small tumors, these markers are rare – and that poses a diagnostic and monitoring problem. To capture them, we needed a strategy to sample the entire blood volume. If you remove a few vials at about 7 mL of blood each, you may get lucky and spot rare biomarkers – but you won't see much of them. We needed a way to sample the entire five-liter blood volume of an adult. That's why we came up with inserting a magnetic wire into the patient and leaving it in for about 20 minutes to collect rare biomarkers.

What inspired this magnetic "cancer cell catcher?"

I knew that we needed a totally new approach to find biomarkers that are present at very low concentrations (if at all). I originally thought we might need an external magnet to make the idea work, but we ended up being able to do it with tiny 1 mm magnets strung together to form a 60 mm magnetic wire.

How would the new technique fit into the clinic?

Initially, I expect it to be useful in patients at high risk of cancer, who are tested every six to 12 months for rare biomarkers so that disease can be spotted early. It could also be used to remove circulating tumor cells from blood to reduce the likelihood of metastasis, or in non-cancer applications such as capturing bacteria in the blood.

The technique won't be much more expensive than a standard blood draw, but the injection of magnetic nanoparticles will increase the cost. It will also require the wire to be left in for 20 minutes and then withdrawn, which may be stressful for the patient – although hopefully less so than the risk of cancer.

What's next for your lab?

We continue to work on novel strategies for the early detection of cancer. Right now, we are working on a "smart toilet" to routinely sample biomarkers in stool and urine. We are also working on the molecular imaging of cancer so that, after a biomarker test reveals that a patient may have early disease, we can detect its location in the body.

Reference

1. *O Vermesh et al., "An intravascular magnetic wire for the high-throughput retrieval of circulating tumour cells in vivo", Nat Biomed Eng 2, 696–705 (2018).*

Safe and Sound

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

Products and launches

- B&W Tek have launched their latest handheld Raman analyzer, TacticID-1064, which they claim is capable of measuring colored, impure and darker substances.
- A new 'flash purification system' has been released by Swedish company Biotage.
- The new RenataDX screening system from Waters is an in vitro diagnostic device designed for dried blood spot analysis.

Collaborations

- AstraZeneca is collaborating with the UK's Medicines Discovery Catapult to speed up the adoption of Acoustic Mist Ionization Mass Spectrometry (AMI-MS) in the pharma industry.

Company and people updates

- AkzoNobel's chemicals business has been relaunched as an independent company, Nouryon, by new owners the Carlyle Group.
- University of Illinois chemistry professor Renske van der Veen has been awarded the Packard Fellowship to develop ultrafast microcopy techniques.
- Agilent Technologies has announced the appointment of a new senior vice president of strategy and corporate development, Eric Gerber.
- Milton Lee, co-founder and chief science officer of Axcend Corporation, received the 2018 Csaba Horvath Memorial Award.

For links to original press releases, visit the online version of this article at: tas.txp.to/1118/BUSINESS.

Bearfaced Research

A new way of discovering antibiotics makes use of an unusual source of complex microbial communities

A team of scientists has developed a screening technology for antibiotics using microbes from a curious source: saliva taken from an East Siberian brown bear.

Why would anyone want to collect bear drool? Co-author Konstantin Severinov explains that diversity is the key: "Bears have a diverse diet, so we assumed that their microbiome will also be diverse. They also have lots of drool!" The challenge, of course, was to catch one. They used a trained hunting husky to lure a bear into a cage; once inside it was offered a stick covered with absorbent canvas, which it duly bit, says Severinov. "There was plenty of saliva to pack into test tubes once it let go!" After this rather undignified treatment, the bear was given a consolation prize of honey and released.

Current procedures for screening microbes for antibiotic production are tedious and require testing individual isolates one by one. Instead, Severinov and the team cultivated microbes from various communities (in this case, the oral cavity of a wild bear) in oil drops filled with nutritious medium, where they are isolated from each other and cannot affect each other's growth. "In the presence of a target microbe – in our case, Staphylococcus aureus, which had been made fluorescent with a green protein and identified using MALDI and mass spectrometry – we can detect the effect of these droplet-incarcerated microbes on growth."

They were able to sort the droplets at tens of thousands per minute using fluorescence activated sorting to isolate droplets (and the microbes contained in them) with lower fluorescence, where S. aureus growth is inhibited – presumably due to a noxious compound produced by the microbe, However, the technique is not without limitations. "Our method still depends on cultivation, which is a major limitation, as most microbes are not easily cultivated in the lab," says Severinov.

So what does this mean for the discovery of new antibiotics? The high throughput of this procedure should allow scientists to screen orders of magnitude more microbiota cells to find those producing antibiotics. "Once identified with our procedure, there will be the "normal" workflow of identifying the compound, and determining its structure, genes responsible for its synthesis, spectrum of antibacterial action, and so on," says Severinov.

Next, the plan is to apply this method to microbiota from other microbial communities – both "exotic", such as a Komodo dragon, and "standard" (human), to find new antibiotic leads.

Reference

1. *SS Terekhov et al., "Ultrahigh-throughput functional profiling of microbiota communities", Proc Natl Acad Sci USA, 115, 9551–9556 (2018). DOI: 10.1073/pnas.1811250115.*

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 charlotte.barker @texerepublishing.com

Biotherapeutic QC: Time to Meet MAM

How high-resolution mass spectrometry and the multiple attribute method could give a boost to biopharma analytics, particularly with continuous processing on the horizon.

By Simon Cubbon, Senior Global Marketing Manager for the Connected Laboratory, Thermo Fisher Scientific, UK.

The well-known clinical benefits of biologics come at a price: large molecule drugs are highly complex, leading to analytical challenges throughout the development pipeline – from discovery, through to bioprocessing, quality control and release. Monoclonal antibodies (mAbs) and other complex drug products, such as antibody-drug conjugates (ADCs), must be exhaustively characterized to ensure the safety and efficacy of a batch before release. Even the smallest change or post-translational modification, such as oxidation, deamidation, or glycosylation, has the potential to render the drug batch ineffective, or worse, create negative off-target effects for the patient.

Process development also faces challenges that are unique to the bioproduction environment, which, when combined with technological limitations of selectivity, fluidics and sterility can create an arduous sample analysis process, if the correct methodologies are not carefully chosen.

Characterization of biotherapeutics typically requires multiple labor intensive or time-consuming analytical techniques in offline QC labs; for example, cation-exchange chromatography, imaging capillary isoelectric focusing, and capillary electrophoresis sodium dodecyl sulphate (3). Typically, each technique provides information on only one or possibly a handful of critical quality attributes (CQAs) – and only after significant analysis.

In my opinion, high-resolution accurate mass (HRAM) mass spectrometry (MS) coupled with high performance separation represents the cutting-edge of biotherapeutic characterization, not only because it offers high-resolution data and impressive levels of sensitivity, but also because it increases confidence in results. Although MS is integral to biopharma R&D processes, its use is still evolving in bioproduction and QC (4). Why? Historically, HRAM MS has required skilled users to operate the instruments, sample processing has been slow and complex, and software hasn't always been up to scratch. And although HRAM MS certainly results in high resolution data, the fact that it still focuses on single or a handful of attributes makes it difficult to scale up to fit commercial bioprocessing and QC needs (5). Ultimately, these barriers to adoption have been too high to implement in QC and lot release. However, the situation is starting to look very different thanks to new and improved analytical systems and software – and a market place hungry for new solutions.

A relatively new analytical approach that is particularly well suited to biotherapeutics QC is the multiple attribute method (MAM) – and a

number of research papers back its advantages (1). MAM is based upon traditional peptide mapping; the biotherapeutic must first be digested into peptides – a critical step that requires 100 percent sequence coverage, high levels of reproducibility, and minimal process-induced modifications (for example, deamidation). The resulting peptides are separated using liquid chromatography, and detected with HRAM MS, before being processed using software tools. High resolution MS with MAM provides a comprehensive view of the CQAs present in biotherapeutics, down to the individual amino acid sequence of each molecule. Detailed information can then be obtained on post-translational modifications (PTMs), glycoprotein structures, the presence of any sequence variants at extremely low levels, and minute amounts of potential process impurities (2, 4). In short, MAM has the potential to consolidate multiple analyses from QC to batch release, enabling us to work towards consistent biotherapeutic structure from batch to batch, and across the entirety of the process.

For some time, there has been discussion about the need to improve biopharma manufacturing processes, and a common thread is the need for continuous manufacturing and real-time lot release, as alluded to within ICH guidelines (5). To get there, we need effective – and online – analytical methods for process monitoring and data generation. Here, MAM's ability to simultaneously characterize multiple attributes could provide comprehensive and timely support for the consistent flow of products from continuous processes, assessing quality and ensuring proper control.

Regulators encourage the use of new and improved technologies, but until there is regulatory acceptance of MAM, the technique must run in parallel with existing methods to prove its equivalency and demonstrate its key benefits. There is certainly work ahead, but I do believe it's time for HRAM MS and MAM to start moving into new areas – in particular, QC. Expanding the use of MS beyond R&D will not only reduce the number of experiments required per sample, but will also save time and resources – and provide increased confidence throughout the development and testing lifecycle.

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Digital Sample Prep

Advanced 3D printing is already boosting analytical efficiency – but shouldn't the wider community be embracing its transformative potential?

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

As many of you will already know, three-dimensional (3D) printing is a broad term that describes a production process that combines computer aided design (CAD) with innovative techniques and diverse starting materials to creates objects of various shapes and geometries. Most 3D printing uses layer-by-layer deposition of suitable materials – including organic polymers, ceramics, and metals – to create the final product, which is why the term "additive manufacturing" is also used to describe the same process.

First introduced in the 1980s, 3D printing is relatively new technology, but it has already been successfully adopted in various scientific fields. Medicine, dentistry, veterinary science, biology and chemistry have all taken advantage of its versatile manufacturing capability. And with 35 years of further advances and applications under its belt, it has reached a stage where it is mature enough to become a mainstream manufacturing process in many more areas.

Stereolithography (SLA) is one popular 3D printing technology, but there are others: fused deposition modeling (FDM, based on the consecutive layering of softened/melted thermoplastic materials), selective laser sintering (SLS), as well as inkjet and PolyJet. The selection of the technology used largely depends on the requirements of the final printed object in terms of material biocompatibility, strength, composition, surface roughness, and dimensions, for example. Whichever technology is used, all 3D printing processes begin with the modeling of a 3D object using CAD software. The CAD model is saved as a file (for example, an .STL file for stereolithography), and can be interpreted by any suitable 3D printer.

Analytical chemistry – a demanding field in terms of reproducibility – has recently started to benefit from the increasingly accurate ability of 3D printing to rapidly construct a variety of scaffolds and platforms. Electrophoresis devices, flow-cells for chemiluminescence detectors, or injection valves for flow systems that can be coupled with sophisticated analytical instruments as for example with ICP-MS are among the various tools reported so far in literature (1,2).

The ability to manufacture reproducible and complex scaffolds (at low cost, in many cases) is specifically suited to the preparation of sample preparation materials. 3D-printed electrodes are one widely available platform, but printed membranes with biorecognition elements are expected to gain more attention in the future. With regards to solid phase extraction (SPE) – one of the most broadly used approaches for sample preparation – 3D printing can be used to integrate flow-through devices and SPE capability into a single unit. And because such devices are produced from a computer template and recreated accurately by 3D printing, files encoding the best performers (or even those devices with promise) can easily be shared between researchers and labs across the world, allowing for further iterative improvements – and giving everyone access to sample preparation advances (3,4).

Current examples of SPE applications of 3D printing include the production of devices for "multisyringe flow injection analysis" (MSFIA), which automates SPE across various sorbents, including packed beds, magnetic particles, stir bars and disks (5). Three-dimensional printed devices have also been used to automate pre-SPE and post-SPE integrated sample preparation for the preconcentration and further determination of metals, such as lead and iron, in water samples (6,7).

SPE columns or cartridges constructed using 3D printing techniques can integrate multiple components such as, for example, various hormones, and provide supplementary functionality and improved performance over conventional SPE formats. The potential benefits? i) pre-concentration can take place with high extraction efficiency, accuracy and precision, ii) matrix interferences can be reduced because of high tolerance to salt matrices and iii) high sample loading flow rates can reduce interference, facilitating highly sensitive determination of trace elements in environmental samples (1,4,7).

Recently, molecularly imprinted polymer (MIP)-functionalized scaffolds were proposed as novel SPE sorbents for the extraction of a psychoactive drug (8). Such tailor-made scaffolds can be 3D printed in a relatively

straightforward way to meet the exact shape and geometry of a defined SPE column. Would it be possible to apply the same approach to a multi-analyte application; for example, by integrating several such MIP-functionalized scaffolds (each targeted to a specific analyte) into a single SPE column? If so, such novel SPE sorbents could find their way into food and clinical analysis, too.

I consider 3D printed SPE sorbents to be a milestone in the field of chemical analysis, but the application of additive manufacturing is not limited to SPE. It can be applied in other novel ways; one good example being "on-line carbon nanofiber reinforced hollow fiber-mediated liquid phase microextraction" – a 3D-printed extraction platform that serves as a "front end" to the liquid chromatograph, allowing automatic sample preparation (9).

In short, 3D printing is an exciting trend that could potentially lead to increased efficiency and miniaturization in analytical applications. Devices tailored to specific analytical needs allow us to experiment with an entirely new – and continually expanding and changing – toolkit. How fast can we move forward? Well, that very much depends on how quickly the wider analytical science community embraces both the technology – and the potential – of 3D printing.

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A LASTING LEGACY

In 1944, famine struck the Netherlands. Babies born soon after the end of the famine initially appeared to suffer few ill effects, but as they reached middle age they began to exhibit an unusual prevalence of heart disease and other metabolic disorders. Studies of the "Dutch Hongerwinter cohort" ultimately discovered that environmental factors can not only affect gene expression in an individual, but leave a lasting epigenetic imprint on their children. How can events that happened decades earlier affect later generations – and could we one day manipulate our epigenome to live longer, healthier lives? To find the answers, we need better, faster analytical tools.

By Mary J. Wirth

Over the past half-century, advances in the life sciences have been profound, and epigenetics is one of the most exciting new frontiers. The "epi" prefix is from the Greek for "above" - or in this case "in addition to." It was once thought that the genetic code we were born with was a static blueprint for all that would happen in our cells throughout our lives. Epigenetics describes the revelation that, in fact, that blueprint can be affected to a large degree by the environment. Changes in gene expression dictated by the environment can even be passed down to the next generation and beyond. Many of us would have got a failing grade in high school biology, if we said that information about someone's life experiences could be passed down to their children. Now, epigenetics is recognized as a basic biological phenomenon.

One of the first documented manifestations of epigenetics in humans arose from dire circumstances: war and famine. During World War II, France was liberated after D-day, but the Netherlands remained occupied by the Germans. To liberate the Dutch and hasten the end of the war, British Field Marshall Montgomery developed a plan that became known as Operation Market Garden. The Allies would drop 20,000 paratroopers into the Netherlands to seize a series of bridges along a highway leading to Germany, allowing subsequent Allied troops to move swiftly into Germany.

If you have seen the movie "A Bridge Too Far," you will already know that the plan was a failure. The Allies encountered heavy resistance from German forces at Arnhem, the bridge over the Rhine River just before the German border. The Allied paratroopers were surrounded by German defenses and sustained heavy casualties, with about 15,000 killed and many more wounded. Even before Operation Market Garden, food was in short supply and, with no reinforcements coming, the surviving Allied paratroopers were soon starving along with the Dutch.

One soldier's story

One of the paratroopers of the US 101st Airborne, a sharpshooter, flew out of England on September 17, 1944, and his division was dropped over Veghel under enemy fire. His pack was so loaded with supplies that his combat helmet popped off when his parachute opened. But it was easily replaced; so many of his fellow paratroopers were shot as they descended that the ground below was littered with their helmets. His division captured the bridge at Veghel and then marched to Nijmegen, where they helped take control of the bridge over the Waal River. Then came the order to march onward to Arnhem. With German panzer divisions defending Arnhem, and no supplies coming, the Allies ran out of food. They had been told that the Dutch would feed them, and some did, but they had very little food to share, and those who were caught helping the Allies were executed by German soldiers.

One day, the hungry sharpshooter and his best buddy, Bob Sherwood, were out scouting for enemy soldiers when they saw an apple tree laden with fruit. Delighted, they raced to

Figure 1. Paratroopers commemorate Operation Market Garden in the Netherlands.

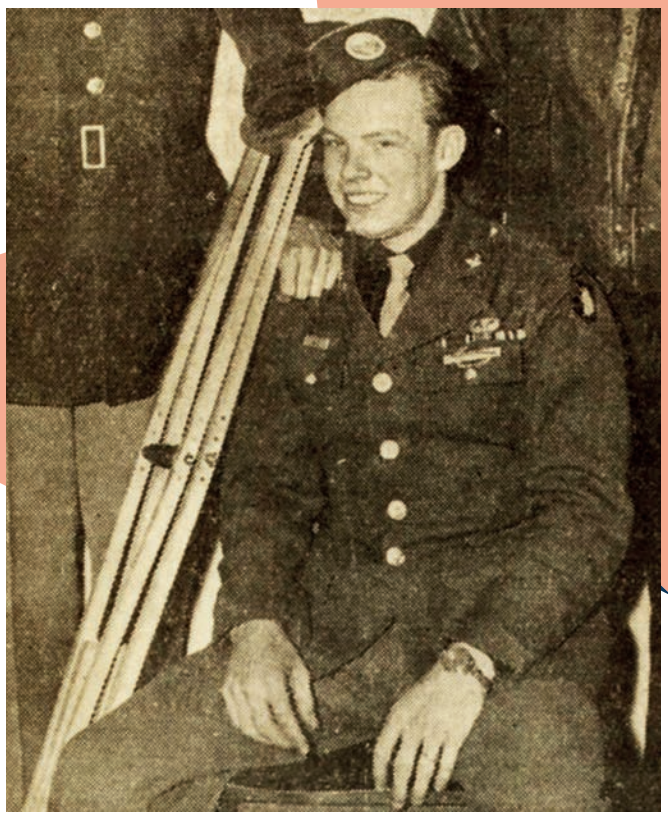

Figure 2. My father, Robert "Red" Wirth – a sharpshooter from the US 101st Airborne Division – who survived the battle of Arnhem.

"One of the first documented manifestations of epigenetics in humans arose from dire circumstances: war and famine."

the tree. Bob climbed and started shaking the branches to drop apples down to his friend below.

A single shot rang out.

Bob fell from the tree, killed instantly by a German sniper. Afterwards, the sharpshooter lay on the ground motionless for hours, until he was able to escape under the cover of darkness.

The sharpshooter was not so lucky on October 5, 1944, when he ran in to replace a gunner who was killed by heavy German fire. He knew he would be the next high-value target, and quickly wiped out five gunners with five shots. But a sixth, hidden in the woods, fired a mortar shell that gravely wounded the sharpshooter with shrapnel. He lay in a wine cellar, bleeding, for days before being loaded into a truck

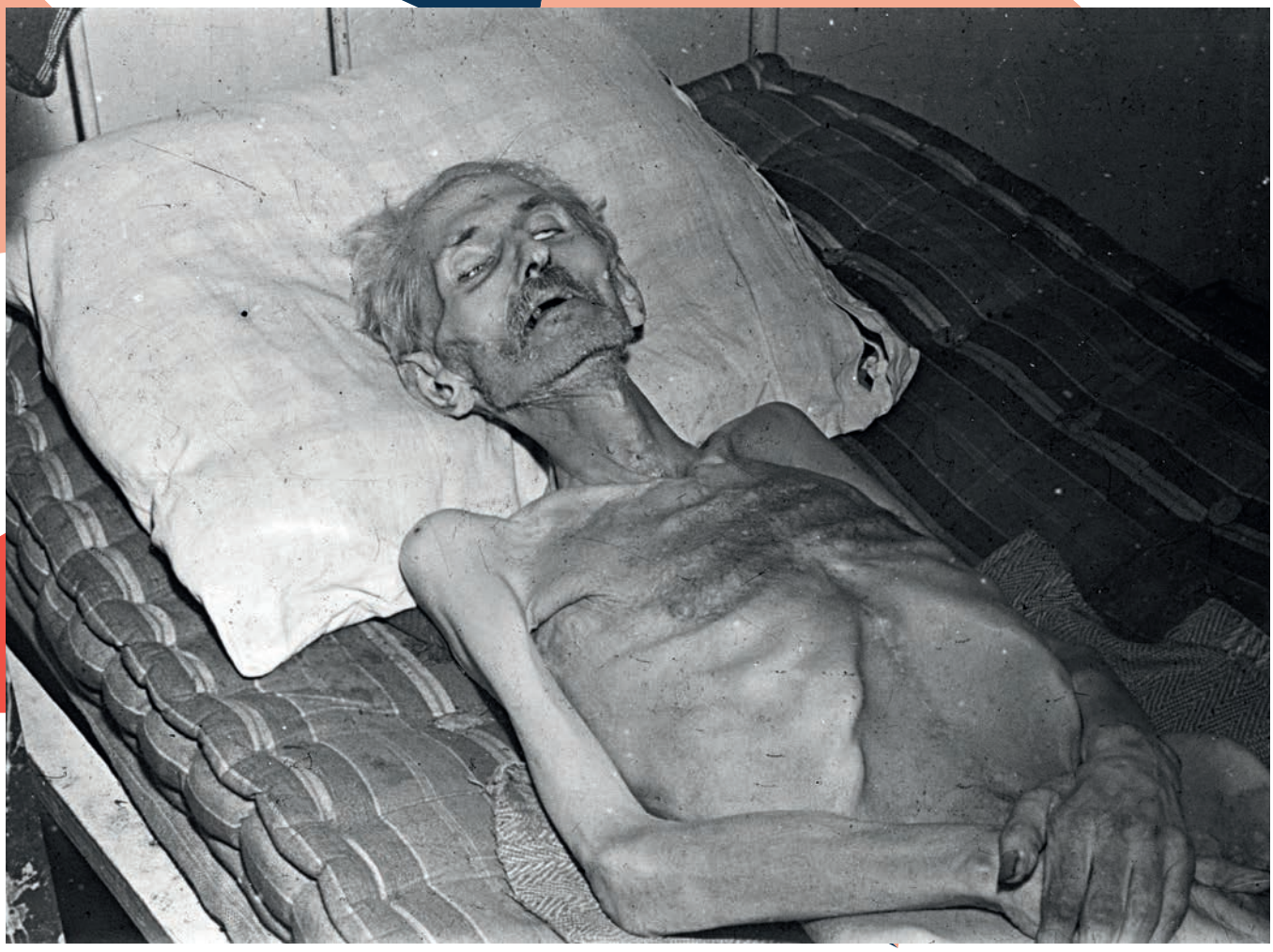

Figure 3. A victim of starvation in the Dutch Hongerwinter. Credit: Menno Huizinga.

"A half-century after World War II, Dutch people born just after the famine found that their bodies behaved as though they were still living under conditions of starvation."

overflowing with casualties. A week later, the sharpshooter was transported to the US by hospital ship – and was finally released 10 months later. For his efforts, he earned a Bronze Star for bravery. I am particularly thankful that he survived as he is my father: Robert "Red" Wirth. Now 93 years old, he still tells stories of his harrowing wartime service.

The thrifty gene

During and after the battle at Arnhem, the Germans destroyed the transportation infrastructure in a bid to secure their hold on the Netherlands, cutting off the food supply just as an unusually hard winter approached. The result was widespread famine. The winter of 1944-45 is known as the

Re-writing the Code

The key mechanisms through which genes can be silenced or amplified by the epigenome.

1. DNA methylation

Genes are described by a sequence of the DNA bases: thymine, adenine, cytosine and guanine (T, A, C, G). A gene is expressed when a transcription factor binds to the promoter region of the gene sequence and commences the construction of mRNA for translation into protein. A gene can be silenced by blocking the binding of the transcription factor through methylation of a cytosine in the promoter region – where the C is followed by a G in the 5' to 3' direction (a CG pair or CpG). Gene silencing is nothing new, of course; the cells in your heart contain the entire genetic code, so many non-heart-specific genes must be silenced to allow for specialization. However, it is only relatively recently that we have recognized that environmental factors can silence or de-silence genes through methylation of a cytosine.

2. Histone modification

The other main epigenetic mechanism involves chemical modification of histones in the tail regions, affecting electrostatic interaction with DNA. There are many types of modifications – for example, lysine acetylation removes the charge on lysine to loosen the connection between histones and DNA, for easier gene expression. The various histone modifications are dynamic and reversible, and can work in concert with DNA methylation.

3. Non-coding RNA

A third mechanism occurs later in the process, after the gene is expressed. At this stage non-coding RNA can bind to the mRNA to block its translation into protein.

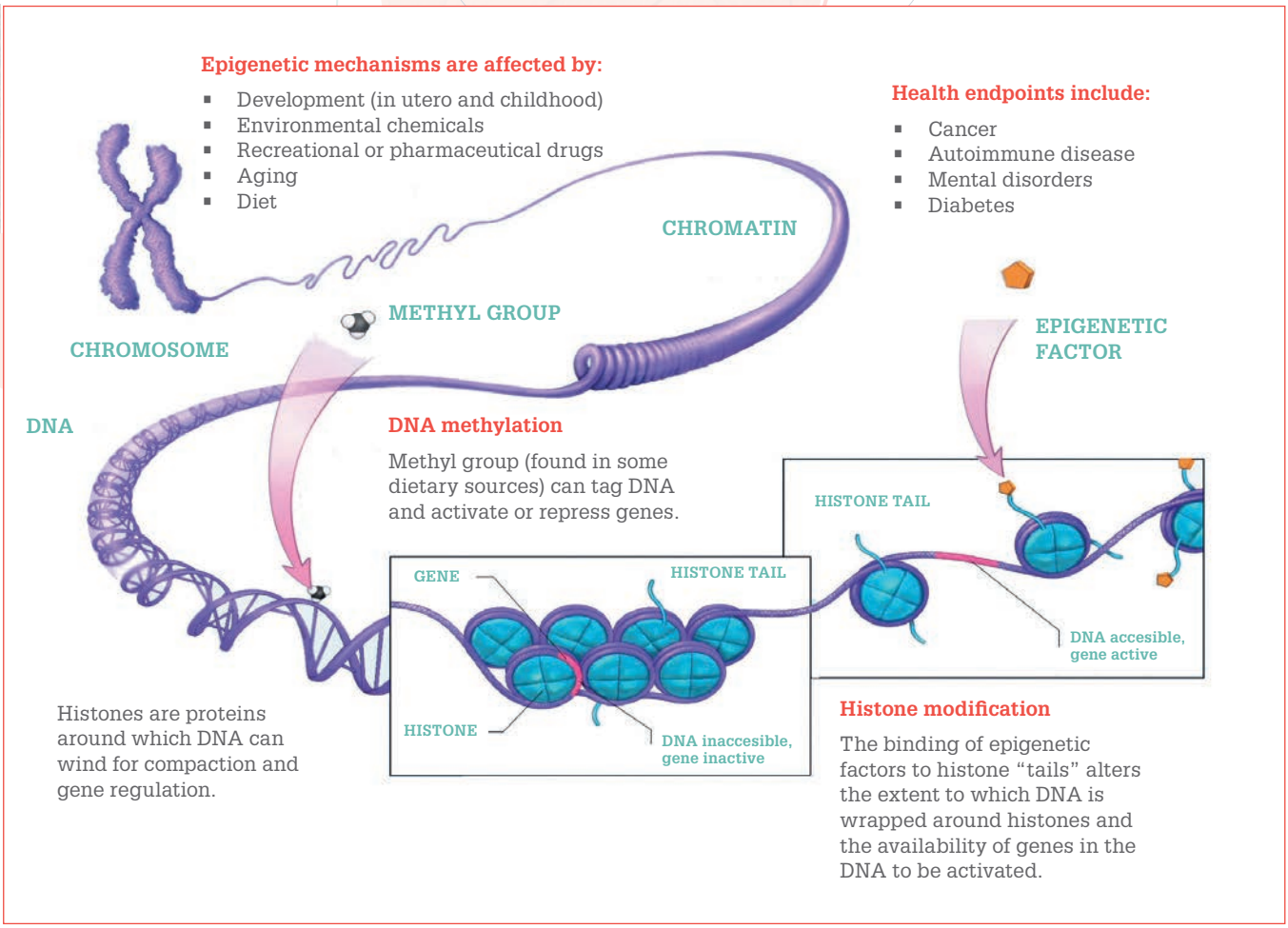

Figure 4. Epigenetic mechanisms.

Hongerwinter – "the hunger winter". It was an inadvertent and tragic experiment on the long-term effects of famine. From December 1944 until Germany surrendered in May 1945, food was rationed to under 1,000 dietary calories per day, and dropped as low as 580 calories per day in the height of winter. More than 20,000 people died of starvation.

Dutch babies born early during the famine were born underweight, but after the war, many grew to normal weight. Babies spending only the first trimester in utero during the famine, and born after the war, were born with normal weight. Initially it appeared that this latter group had escaped the worst impact of the famine. However, in the long-term, this group were in fact some of the hardest hit. Far from being cushioned from the effects of the famine, they suffered increased rates of obesity and a host of metabolic diseases affecting cardiac health once they reached middle age. They had somehow acquired what is popularly called the "thrifty gene." A half-century after World War II, Dutch people born just after the famine found that their bodies behaved as though they were still living under conditions of starvation. We now know that the malnutrition suffered by parents caused epigenetic changes in offspring conceived during the famine.

The conventional understanding of molecular biology was once simple: genes are transcribed to make mRNA, which is translated to proteins:

genes -> mRNA -> proteins.

In reality, things are not so simple – transcription can be blocked by environmental effects, and genes that are supposed to be blocked can be unblocked.

"Can we do anything about our aging epigenomes or do we have to make do with what we inherited and put up with whatever changes occur over our lifespan?"

Two main types of gene silencing occurs in cells – one is by modification of the DNA and the other is modification of histones. Figure 4 shows how histones are related to DNA. In the upper left is an X chromosome, and if you started to unravel it, you would find that the DNA is wrapped around histones like pearls on a necklace. The histones are opposite in charge to the DNA to give a strong electrostatic attraction, and modifications can affect how tightly the DNA is held by histones.

Not quite a clean slate

In reproduction, the egg and sperm typically lose their DNA methylation, erasing any memory of the parental environment. The DNA is then re-methylated during development. Folic acid is a source of methyl groups for this critical process, hence the mother's nutrition in the first trimester is critical. For the Dutch Hongerwinter Cohort, as they are called by researchers, maternal malnutrition meant that methylation was not completely erased, and the epigenetic changes caused by the famine were passed down to their children, and in some cases even their grandchildren – imprinting them for life with the traits needed to survive conditions of starvation.

Scientists in the Netherlands and the USA recently pinpointed the mechanism behind the higher body mass index and elevated serum triglycerides seen in the Dutch Hongerwinter Cohort: DNA methylation at CpG sites in genes mediating energy metabolism (1).

Experiments on animals shed light on the epigenetics of starvation. The two mice pictured in Figure 5 are genetically identical - the only difference is in the diets of the mothers during pregnancy. Both mice were engineered to be pre-disposed for obesity, but the mother of the smaller mouse was fed a much more nutritious diet. Though the genetic blueprint of each mouse is identical, the expression of genes relating to metabolism is radically different, reflected in their disparate appearance. In other words, they have the same genotype but a different phenotype. Hypomethylation of DNA even affects the coat color.

In this case, it was the mother's diet responsible for passing on unfortunate epigenetic traits, but a study on extreme exercise (mimicking starvation) in male mice showed that the father can pass down the so-called "thrifty" gene or genes through epigenetic changes in sperm, leading to obese offspring (2). However, for women thinking that they would be better off choosing a man who lies on the couch watching TV all day to father their children, too little exercise in male mice causes other epigenetic problems (3).

DNA methylation, once presumed to be a persistent gene silencer, is now appreciated to be a reversible, dynamic process, with an individual's genome undergoing significant change over their lifespan (4). The average centenarian has significantly less methylation of cytosines than a baby – with de-silencing of certain genes responsible for some age-related conditions (5). Fraga et al showed that identical twins have virtually identical epigenomes at age 3, but by age 50 their epigenomes significantly differ (6), which may help to explain why identical twins usually die of different diseases. For example, the famous twin-sister advice columnists in the US, Abigail Van Buren and Ann Landers, were amazingly identical when they were young, and even pursued the same career path. But they died of completely unrelated diseases, Ann at age 83 from multiple myeloma and Abby at age 94 from complications from Alzheimer's Disease. As well as this genetic drift, epigenetic changes also lead to predictable effects. The epigenetic clock, for example, is based on a steady rate of DNA demethylation for a specific set of genes as people age – and it is even being used in forensics to estimate the age of crime suspects (7).

Figure 5. Obese and normal mice. Credit: Randy Jirtle and Dana Dolinoy

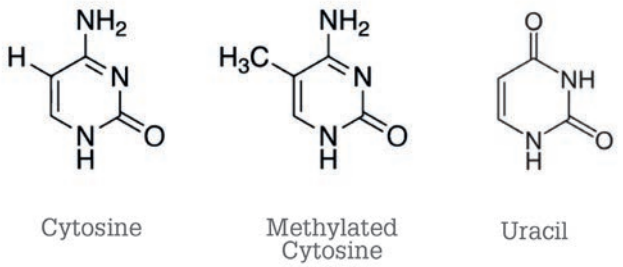

Figure 6. Chemical structures.

Overcoming destiny?

Can we do anything about our aging epigenomes or do we have to make do with what we inherited and put up with whatever changes occur over our lifespan? It appears that we can in fact manage epigenetic changes to some extent. A quick online search for "DNA methylation and exercise", for example, brings up a wealth of studies. Heart disease is the most common cause of death in industrialized countries, and we know that exercise helps prevent heart problems. Now we know why: our epigenetics change with exercise to help lower our risk (8). And if your genetics pre-dispose you to heart disease, your epigenetics can offset some of the risk.

The breadth of studies on epigenetics is vast. Though we have focused on metabolism thus far, DNA methylation plays a role in a huge assortment of diseases (9). The epigenome of a cancerous cell is very different from that of a healthy cell, and this fact is being exploited for new therapies (10). In other diseases of old age – everything from Parkinson's disease to chronic obstructive pulmonary disease – you will again find epigenetic factors at work. The diseases that the famous advice column twins succumbed to are both known to be associated with epigenetic changes.

How can analytical science help us to understand - and maybe even control – our epigenome? The key ingredients for a major (or minor) scientific advance are, first, asking the right questions and, second, having the ability to answer them. Analytical chemistry allows us to measure DNA methylation and histone modification to answer the interesting questions raised by biologists. The method presently used for detecting methylation of cytosine was invented in 1992 by Frommer et al, who demonstrated that bisulfite deaminates cytosine, converting it to uracil (11). The structures are shown in Figure 6. Methylated cytosine, also shown, is much slower to react with bisulfite; therefore, methylated cytosines are still visible as C's in DNA sequencing after treatment with bisulfite.

Analyses in epigenetics began with single-mode measurements; for example, detecting average methylation

across all DNA or average modifications across all histones. Once scientists began to understand that DNA modifications and histone modifications work together, new methods were needed (12). The overlapping nature of epigenetic changes poses a measurement challenge, because there is just one copy of each nucleosome per cell. PCR does not amplify methyl modifications, and, of course, protein concentrations cannot be amplified.

As a result of the high sensitivity needed, single-molecule techniques are now widely used for these analyses. In a technique called ChIP-seq, a highly specific antibody for a histone modification (for example, lysine acetylation) selects the nucleosomes with this modification by chromatin immunoprecipitation (ChIP) (13), and single-molecule DNA sequencing using nanopore technology (14) identifies which cytosines in the chromatin are methylated. The nanopore technology sequences DNA and detects modifications without the need for PCR and does not require bisulfite treatment. Future measurement technology will need to address an even larger challenge: there are multiple modifications of histones and other modifications of DNA besides methylation of cytosine at CpG sites. The same histone can have multiple modifications, all of which work in concert with one another and with the DNA modifications. These comprise a complex epigenetic code that describes how our cells operate, how they respond to the environment, and how diseases arise. The limitations of current technology are clear when considering the vast complexity of analytical measurements required to unravel this code (15).

Epigenetics has captured the interest of social scientists, who are concerned about the epigenetics of social status. It is striking that an environment that we have no control over can cause deleterious biological changes, and that this effect can be passed down to the next generation and beyond. The Hongerwinter demonstrated that there can be a critical window, such as the first trimester of gestation, that impacts one's entire life and sometimes the lives of one's children. Beyond heart disease, folic acid deficiency during gestation in the Hongerwinter has been associated with a higher level of schizophrenia (16). But malnutrition is just one factor affecting the epigenome - in mice, maternal care in the first months of life has been demonstrated to epigenetically effect stress response in later life (17). Plus, pollution, drug addiction, family dysfunction and stress may all play a part in our individual epigenetic code. These issues and more are discussed in a recent review in the sociology literature (15).

Epigenetics is a rapidly growing and expanding science, encompassing nutrition, exercise, disease, substance abuse, family life and socioeconomic status. There is much we still don't know, but one thing is certain: progress in all areas would be accelerated with better, faster analytical tools and techniques.

"How can analytical science help us to understand – and maybe even control – our epigenome?"

Mary J. Wirth is the W. Brooks Fortune Distinguished Professor – Analytical Chemistry at Purdue University, West Lafayette, Indiana, USA.

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Remembering Lloyd Snyder

In September 2018, Lloyd Snyder – best known for uncovering fundamental principles in liquid chromatography – passed away at the age of 87. These recollections by four colleagues and friends paint a picture of Lloyd as a generous and gifted scientist – and a wonderful human being.

Giant of Chromatography

Barry L. Karger, Director Emeritus, Barnett Institute, James L. Waters Chair and Distinguished Professor Emeritus, Northeastern University, Boston, USA.

I was very saddened to learn of the passing of Lloyd Snyder, a true giant in the field of chromatography – and one whose contributions have been truly seminal. I knew Lloyd for 50 years; in the late 1960s and early 1970s we collaborated on multiple projects, including co-authoring the textbook "An Introduction to Separation Science" with Csaba Horváth. In the early 1980s, when Lloyd was at Technicon, we developed a high-throughput LC clinical assay technology – boxcar chromatography. John Dolan was a postdoc in my lab at that time, and he went to work for Lloyd at Technicon. The rest is history.

The field of HPLC would not be where it is today without Lloyd's contributions. With Jack Kirkland, he is well known to several generations of separation scientists as the author of the essential textbook "Introduction to Modern Liquid Chromatography" and organizer of ACS courses. Lloyd was co-author of a number of other books, all with a focus on translating chromatographic fundamentals to practice. From his earliest days at Union Oil to LC Resources in the 2000s, Lloyd was a builder of models, taking fundamental concepts and generating practical approaches to aid analysts in optimizing separations. His contributions included, among many:

1. Retention in liquid-solid chromatography
2. Classification of mobile phases and solvent selectivity
3. Linear solvent strength gradient elution theory
4. Column selectivity via hydrophobic subtraction theory.

On a personal level, Lloyd was someone with unbelievable energy and focus. His productivity was impressive, his impact great. He collaborated widely in the field, even to the end of his days. Everyone enjoyed interacting with him, as he was invariably kind and made sure his collaborators shared in his success. He was a unique scientist who will be sorely missed. In the "Hall of Fame of Chromatography," Lloyd Snyder will be considered one of the major figures.

A Life in Science

By Imre Molnár, President, Molnár-Institute for Applied Chromatography, Berlin, Germany.

Lloyd came to chromatography early in his professional life, and his achievements in the field are many and varied. He made major contributions in normal phase liquid chromatography (NPLC), the compositional analysis of petroleum, the theory of retention in adsorption chromatography, and the development of high-performance liquid chromatography (HPLC).

In 1970, Lloyd was invited to join the editorial board of the Journal of Chromatography and in 1987 became the Editor of the journal – a position he held up until 2000. Lloyd was known as an editor with strong opinions, and if he agreed to accept a paper, it was a great honor for the author(s). Lloyd will also be remembered by the chromatography community for his contributions to several major textbooks and hundreds of scientific papers.

I first met Lloyd in the summer of 1976 through my then-supervisor, Csaba Horváth, at Yale. I returned to Berlin, Germany in 1977, and founded my private research institute a few years later. It was Csaba who saw the potential in a collaboration between me and Lloyd. Based on Csaba's suggestion, I invited Lloyd to join me to teach reversed phase HPLC courses in Berlin. After teaching several such courses together, we eventually started to develop software to support our classes. Lloyd suggested the name for the software – DryLab – based on his early experience with chemistry kits. As a child, he once caused a (small) explosion in his parents' cellar while trying to achieve a chemical reaction. He said that this always reminded him of the importance of working out experiments on paper beforehand – so DryLab struck him as an apt name.

Although in those days computers were not taken very seriously, we thought that it could be a business opportunity for the future. In 1983, Lloyd formed a new business, called "LC Resources Inc," with John Dolan – a former postdoc from Barry Karger's lab in Boston – and Tom Jupille as partners. I was the European partner, and at first had a hard time explaining the concept. Nobody believed that something like this could be successful. However, Novartis in Basel saw the potential of DryLab early on, other companies like Pfizer, Sanofi and others followed, and we started to get more and more interest.

> "LLOYD SNYDER WAS A TRUE FRIEND, AND A WONDERFUL HUMAN BEING."

We felt we were generating something innovative and unique – something revolutionary. We fixed thousands of bugs and were proud of the precision of our predictions. In 2006, DryLab was transferred to the Molnár Institute and has continued to develop.

The work of Lloyd Snyder will long outlive him. His excellence in teaching HPLC and his contributions to the theory of chromatography, especially in gradient elution, will influence young chromatographers for years to come. In addition, DryLab is now used in almost every pharmaceutical company, plus at the FDA and other regulatory agencies, so Lloyd's work will continue to support the vital work of drug discovery.

Lloyd Snyder was a true friend, and a wonderful human being. He always sought out relevant scientific questions, answered quickly, and drove science forward with great enthusiasm. We in the scientific community will all miss him very much. Bless you, Lloyd, for all you did for us.

Badges of Honor

The list of professional honors for Lloyd Snyder shows the scientific community's appreciation for his extraordinary work:

- American Chemical Society Award in Petroleum Chemistry
- Steven Del Nogare Memorial Award in Chromatography, Delaware Valley Chromatography Forum (1976)
- Biography in "75 Years of Chromatography – A Historical Dialogue," Elsevier (1979)
- Chromatography Memorial Medal, Scientific Council of the Academy of Sciences of the USSR (1980)
- American Chemical Society Award in Chromatography (1984) Pittsburgh Society Award in Analytical Chemistry (1984)
- LS Palmer Award in Chromatography, Minnesota Chromatography Forum (1985)
- IP Martin Award, the Chromatography Society (Europe) (1989)
- Northeast Regional Chromatography Discussion Group National Chromatography Award (1991)
- Journal of Chromatography, 60th Birthday Honor Volume; Vol. 550 (1991)
- American Chemical Society Orange County Section "Service Through Chemistry" Award (1993)
- Eastern Analytical Symposium Award in Separation Science (1994)
- Recognition of five "HPLC pioneers"; HPLC 1995 in Innsbruck, as reported in J Chromatogr A, 703 (1995).
- James L Waters Sixth Annual Symposium recognizing four Pioneers In the Development of Analytical Instrumentation (High Performance Liquid Chromatography); organized by the Society for Analytical Chemists of Pittsburgh (1995)
- California Separation Science Society Award for Distinguished Contributions in Separation Science (1996)
- Anachem Award (Association of Analytical Chemists) (1998)
- Memorial Medal of the Medical University of Gdansk (2001)
- Biography in Chromatography – A Century of Discovery, 1900-2000, Elsevier (2002)
- Csaba Horváth Medal, Connecticut Separation Science Council (2005)
- Chicago Chromatography Discussion Group Merit Award (2005)
- Waksmundzki Medal of the Polish Analytical Society (2005)
- Member, Honorary Editorial Board of the Journal of Chromatography A (2009)
- Lifetime Achievement in Chromatography Award, LCGC North America (2012)

Lloyd's (typically modest) response to this list?

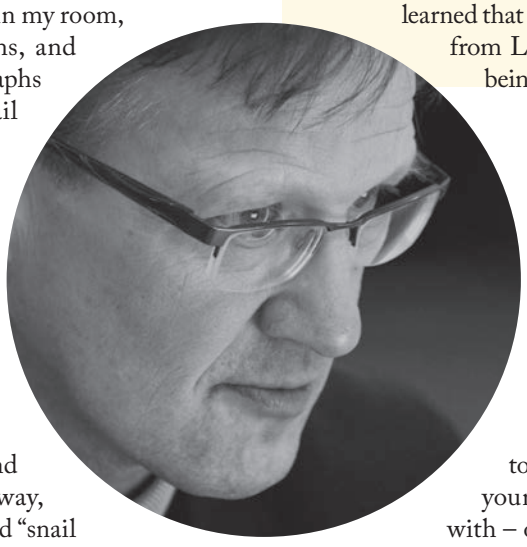
"I very much appreciated each of the above 'prizes.' But sometimes I wonder, were these acknowledgements for good work, merely the appearance of good work, or mainly a popularity contest? In any case, the number of awards seems out of all proportion to what was actually accomplished."

Mentor and Inspiration

Peter Schoenmakers, Professor, Faculty of Science, Mathematics and Computer Science, Van 't Hoff Institute for Molecular Sciences, Universiteit van Amsterdam, Amsterdam, The Netherlands.

I met Lloyd Snyder for the first time in September 1977 at the HPLC meeting in Salzburg, Austria. It was my first month as a PhD student and my first international conference. It was also the first time that posters were featured at the conference.

My poster focused on gradient elution and Lloyd Snyder spent almost the entire poster session in my room, asking questions (and more questions, and then more questions) about all my graphs and equations. He was into every detail and he made the young student in front of him think and explain. At that moment – and from that moment on – he helped me tremendously. My impression of Lloyd from that first day proved to be very true to who he was: absorbed by his scientific studies and able to concentrate on every detail, without losing track of its place in the bigger picture.



In the years that followed, Lloyd and I communicated the old-fashioned way, through long letters – what is now called "snail mail." Our first instance of electronic communication came more than 10 years later, when it turned out that Lloyd and I had the same calculators that could read and write little magnetic strips. I sent Lloyd a strip containing a primitive gradient-scanning program, which constituted great 20th century progress.

Lloyd and I kept communicating about our research, but we also developed another relationship. Lloyd was an editor of the Journal of Chromatography for many years (there were no A and B versions in those days) and I was one of his reviewers for long and difficult papers. Lloyd identified me as good but slow (not much has changed over the years). I tried to be faster, but I learned that I would always receive a new manuscript from Lloyd by return mail, and decided that being good and slow had its advantages.

Lloyd himself barely changed over the years; he remained absorbed by science, keen on every detail. He was a true gentleman, and very helpful to younger people, which eventually was almost everybody else. I will remember Lloyd for the great contributions he made to science in general and to chromatography in particular, and I will remember him for the great contributions he made to the professional growth of the many younger scientists he came into contact with – of which I was just one.

A group photo of Lloyd on his 70th birthday with his friends and colleagues (2001). The event was organized by Elsevier in Ellecom in the Netherlands. Top row: Erich Heftman, Peter Schoenmakers, John Dolan, Peter Carr, Jack Kirkland, Imre Molnár, Klaas Bij, John Dorsey, Roger Giese, Rob Marx, Shigeru Terabe, Nobuo Tanaka; middle row: David McCalley, Gyula Vigh, Roman Kaliszan, Csaba Horváth, Lloyd Snyder, Sandra Poole, Sarah Rutan, Edward Soczewinski; bottom row: Pavel Jandera, Colin Poole, Hans Poppe, Vadim Davankov. (Georges Guiochon is missing).

As we were gathering memories of Lloyd Snyder, we learned of the passing of another chromatography pioneer – John Knox. Perhaps best known for the Knox Equation by analytical scientists (but for the Knox Anchor by fellow yachtsman), we'd love to honor him by sharing your memories in an upcoming issue.

Please email: charlotte.barker@texerepublishing.com

A Generous Spirit

John W Dolan, LC Resources, Oregon, USA.

Over the 41 years that I knew Lloyd – 34 years of which we were business partners – he was my mentor, colleague, and friend. In addition to his numerous contributions to the field of liquid chromatography, Lloyd was more open and generous than any scientist I have ever met. He freely shared data and suggestions for future research with the entire community, so that our understanding of chromatographic processes would advance, instead of hoarding information for his own benefit. He always gave more credit to his collaborators than any of us deserved. Lloyd had a larger impact on my career than anyone else – and he leaves a big hole in my life.

Solutions

Real analytical problems
Collaborative expertise
Novel applications

Think Twice

We're (re)developing twin column "recycling" chromatography as a semi-preparative technology that separates and identifies trace impurities.

By Fabrice Gritti

The problem

The problem originates from the pharmaceutical industry: the need to unambiguously identify trace impurities that nearly co-elute with the main active pharmaceutical ingredient (API). The identification can be easily achieved by nuclear magnetic resonance (NMR) spectroscopy, but it requires that about 1 mg of the impurity be prepared at a purity level of at least 90 percent. Given the current state of instrumentation, the pharma industry could not find acceptable technologies capable of producing a (nearly) co-eluting trace impurity at this level within a reasonable timeframe – either because of a lack of resolution power (semi-prep instruments, see Figure 1) or a low production rate (analytical instruments). An alternative semi-prep technology is therefore desperately needed to solve this problem.

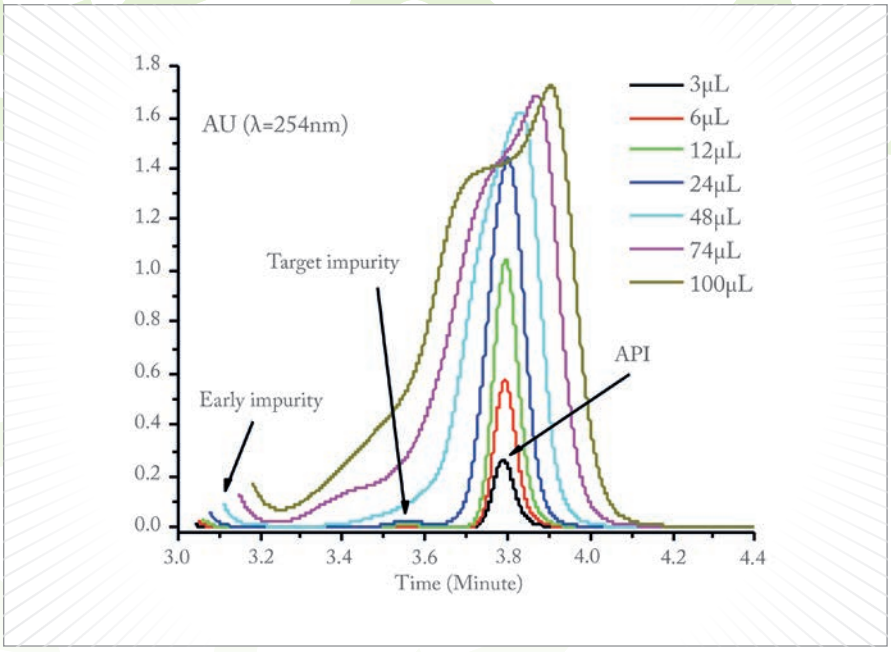


Figure 1. Illustration of the failure of conventional batch preparative systems to isolate a trace impurity (left peak) from the main API compound (right peak) when injecting a large volume (up to 100 µL) of a highly concentrated (10 g/L) API solution.

Background

First of all, by "recycling" liquid chromatography, we do not mean some sort of "green" separation process based on a clean or regenerated mobile phase. The main challenge that we attempted to tackle with this technique is the need for unprecedented high-resolution performance – resolution that cannot be realized even with today's state of the art ultra-high pressure LC systems (up to 1.5 kbar) and columns packed with very fine particles (< 2 µm).

We envisage our technique primarily being applied when compounds have very similar structures (take isomers or isotopes for example) and cannot be baseline separated, even after intense screening to optimize mobile and stationary phases or with the use of 2D-LC systems.

The core concept behind recycling chromatography is quite straightforward, and was first proposed in preparative LC in the 1960s, when particle sizes were too large (> 30 µm) and the resolution power was too low. The use of a two-position valve and a pair of twin columns, results in a virtual infinitely long column that can still be operated at low pressures with standard pumps. The key part of the recycling technique consists in re-injecting the targeted mixed sample zone from the outlet of one column into the inlet of the second twin column (see principle in Figure 2). Twin column recycling chromatography was then born (1) and there are many successful applications of this technique under analytical conditions ([2]; see one example

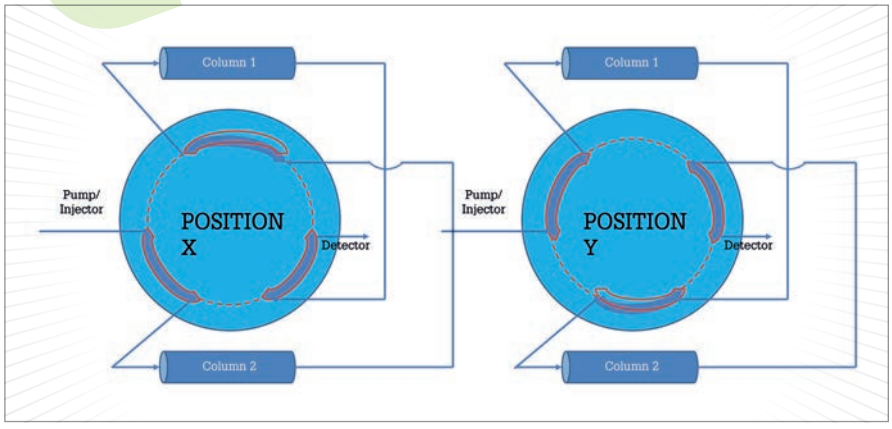


Figure 2. Standard recycling liquid chromatography: the critical pair of compounds to be separated is alternately sent to columns 1 and 2 using a two-position (X and Y) six-port valve.

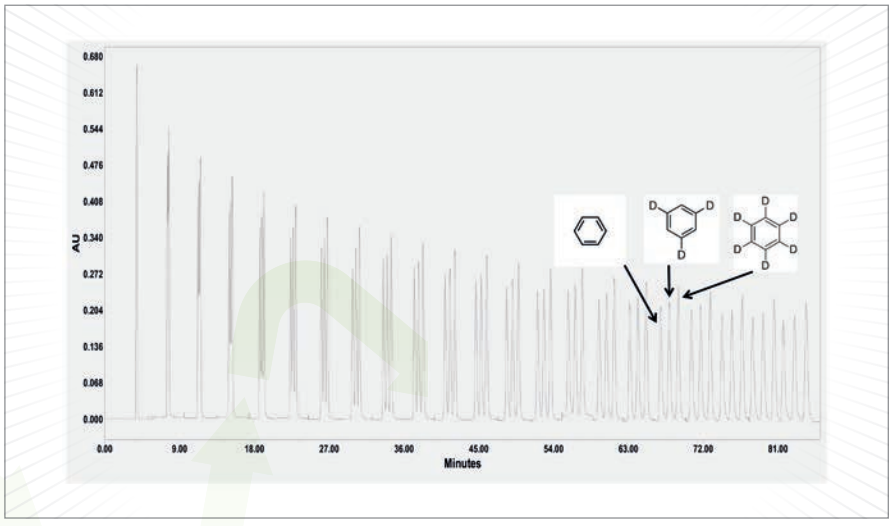


Figure 3. Direct application of recycling chromatography under linear conditions (small injection volume, diluted sample concentrations, analytical columns): baseline isotope separation (benzene, d-benzene, and 1, 3, 5-d3-benzene) in less than 90 min. See experimental details in (3).

in Figure 3). We then designed a new semi-preparative purification technique (high-resolution semi-preparative liquid chromatography) including the above-mentioned recycling LC unit.

The problem of detecting trace impurities in pharmaceutical manufacturing requires an unusual combination of system capabilities: not only ultra-high performance levels (to isolate the impurity from the API) but also a separation system with semi-preparative capabilities (to produce 1 mg of material). High-resolution chromatography can be achieved today with classical HPLC, UHPLC, or 2D-LC heart-cutting systems. Yet, these systems operate under linear chromatography at very small injection volumes and low impurity/API concentrations. It would take years before 1 mg of isolated impurity could be prepared – not an acceptable solution. On the other hand, currently available preparative instruments such as batch Prep-LC, simulated moving bed (SMB), or steady state recycling (SSR) systems can easily prepare 1 mg of material in a very short time. However, they cannot cope simultaneously with a very low selectivity factor between impurity and API, an extremely low relative abundance of the impurity relative to the API (~ 1/1000), and a severe mismatch between the properties (elution strength, viscosity) of the sample diluent and those of the eluent, causing severe band distortion (see Figure 1). We were therefore asked to design a new separation system capable of combining incompatible analytical (small sample volumes and concentrations) and preparative (large sample volumes and concentrations) features. In brief, a real conundrum stood in front of us.

The solution

To solve the pharma industry's problem, we had to build an integrated system capable of injecting large volumes of very concentrated API samples (~ 10 g/L), separating the two distorted bands (impurity and API) by performing recycling chromatography, and collecting the isolated impurity using a fraction manager – the high-resolution semi-preparative LC (HR-SP-LC) system (Figure 4). The two most critical features of the system are i) the temperature control of the pair of twin columns for the sake of cycle-to-cycle and injection-to-injection repeatability and ii) the minimization of sample dispersion upon sample transfer from the first to the second column. Figure 5 demonstrates the success of the HR-SP-LC system in the case of the isolation of an unknown impurity present on the left side of the main API (10 g/L estradiol sample). It required a total of six cycles to achieve a complete baseline separation with an enrichment factor of the impurity relative to the API in the order of 5000 (Figure 6).

Of course, this principle was tried nearly 50 years ago in preparative recycling chromatography, but for much

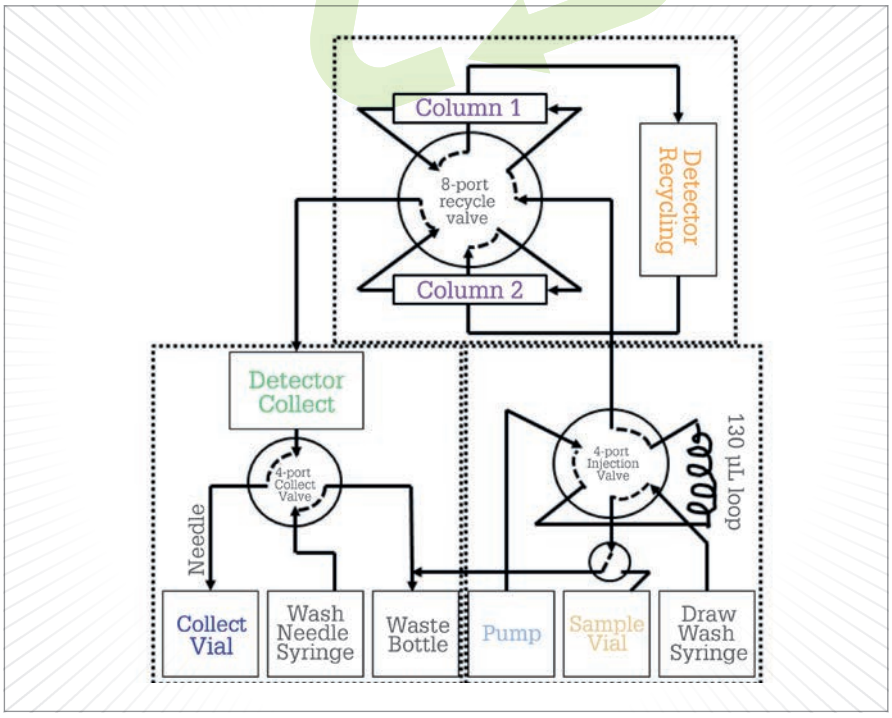


Figure 4. Principle of the proposed high-resolution semi-preparative LC system based on three operational sub-units: 1) pump-injector unit, 2) recycling and separation unit, and 3) fraction collection unit.

less challenging separation problems: the goal was essentially to prepare pure fractions of the most abundant compound present in the sample mixtures by GC (no problem of diluent-to-eluent mismatch) or by LC (relatively easy purification at moderate or low yield). In the present case, the targeted impurity is present in trace amounts at concentrations as low as a few mg/L while the concentration of the nearly co-eluting API compound is as large as tens of g/L. Therefore, the yield must remain close to 100 percent, as we cannot afford to lose any of the targeted impurity; meanwhile, we have to maintain a high purity level (> 90 percent for NMR characterization).

Additionally, the performance of today's pumps (high pressure) and valves (low dispersion valve), as well as the quality of connections (face seal connections) between the different parts of the HR-SP-LC system have improved so much that it is now possible to use modern UHPLC columns packed with very fine particles without losing too much resolution upon transferring the analyte from the first to the second column. This was not possible decades ago. Finally, the stability of the column temperature set by today's active eluent preheaters ensures an unprecedented reproducibility of the chromatograms shown in Figure 5 and a complete automation of the system including sample injection, recycling separation, and fraction collection. The RSD of the retention times remains within 0.3 percent over an entire week (5). The process can be kept running for weeks without user intervention and still provide the targeted purity level for each of the collected fractions.

In principle, the experimental set-up (Figure 4) is not more complicated than that of a standard 2D-LC process, incorporating: a sample manager that can accommodate large sample volumes (50 mL vials), a two-position six-port valve, a two-column oven with active preheater, a fraction manager connected to the recycling unit, one low-dispersion detection cell, and one standard chromatography software to control the quality of the purification process immediately before collection of the trace impurity. Once these different units are connected to each other and commanded by the same chromatographic software, the HR-SP-LC system can be entirely automated by tabulating the times at which the recycling and collection valve should be switched. Therefore, a non-specialist analytical chemist can easily run the process. The only experimental information requested from the user is the measurement of the retention time of the targeted impurity after elution through one column length. Once this retention data is known, the user defines a unique isocratic chromatographic method (sample injection volume, flow rate, temperature, eluent composition, impurity band shaving times, recycling and collection times), the method is validated (or not) once by the signal recorded immediately before the fraction collector, and a sequence of as many runs as necessary to collect the desired amount (1 mg) of pure (>90 percent) impurity is programmed.

Beyond the solution

As yet, the system is still a prototype. We are still experiencing challenges in terms of production rate; so far, the optimum production rate is around a few micrograms per hour using standard 4.6 mm i.d. x 150 mm long analytical columns and injection volumes up to 250 µL. If the initial impurity concentration is only 1 mg/L, one can only produce about 0.1 mg of impurity per week for a half an hour run and six cycles. This is clearly insufficient (1 mg is needed within a day). Therefore, we are planning on scaling-up the HR-SP-LC process by using 10 mm i.d. × 250 mm long columns packed with 5 µm

particles. Each injection will consist of a 1–2 mL sample injection volume. This will bring the production rate up to the desired level, but will add three new challenges: i) how to accurately control the temperature of these large i.d. columns with a wide air-convection oven, ii) how to avoid the undesirable band distortion caused by enhanced viscous fingering and diluent-to-eluent mismatch effects, and iii) how to minimize the increased inter-column dispersion when using wider connecting tubes (i.d. × 2) and higher flow rates (×5). Scaling-up the HR-SP-LC process will likely mean applying larger numbers of cycles to achieve the same purity level – but, overall, production rates are still expected to increase.

We have focused specifically on pharmaceutical applications, but there are other areas where this approach could bring significant value. The problem we have tackled here is a very general one and is frequently encountered in other separation fields: trace impurities are everywhere and they need to be unambiguously identified for safety/quality controls. One interesting application besides pharma applications is the unambiguous identification of aggregated forms of monoclonal antibodies (mAb), which nearly co-elute with their monomeric form and require complimentary spectroscopic techniques beside conventional separation techniques. This can be done by recycling size exclusion chromatography (2). In addition, the plastics industry would benefit greatly from analyzing the composition of their synthesized polymers by coupling a HR-SP-LC system with NMR spectroscopy. Indeed, for any application where the separation and preparation of a sufficiently large amount of pure impurity remains the limiting step, HR-SP-LC could be of great benefit.

Fabrice Gritti is a Principal Research Scientist at Waters Corporation, Milford, Massachusetts, USA.

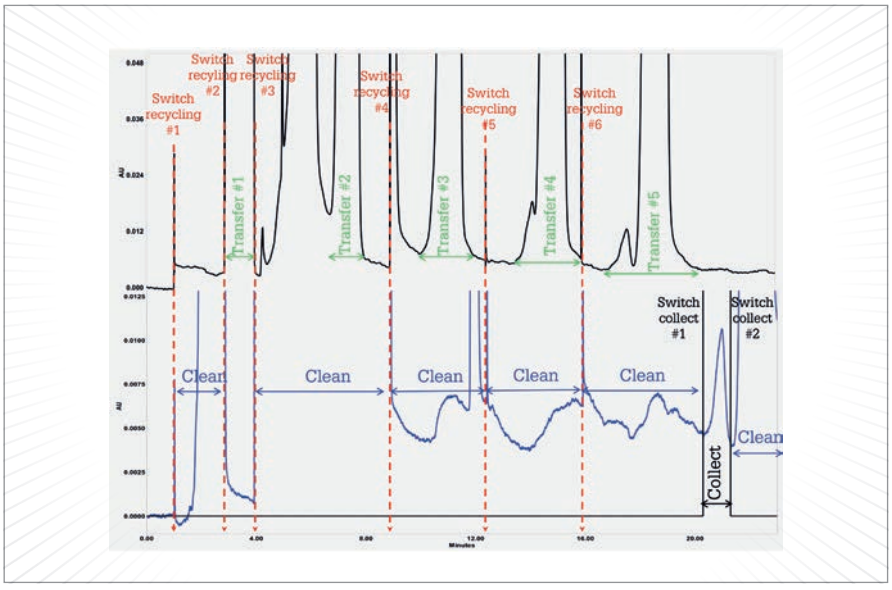


Figure 5. Example of isolation of a trace impurity (left band) from a highly concentrated solution of API (100 µL of estradiol solution at 10 g/L is injected, right band). Top chromatogram: monitoring the progression of the separation from cycle 1 to cycle 5. Bottom chromatogram: complete isolation of the trace impurity after six cycles immediately before collection between t=20.3 and t=21.3 min.

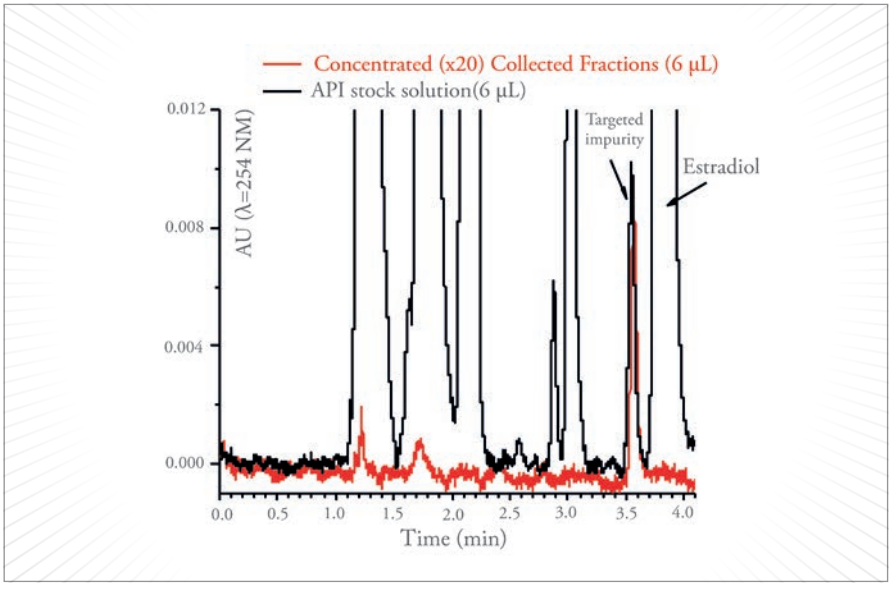


Figure 6. Enrichment of the targeted unknown impurity (x 5000) with respect to the main API compound (estradiol) in the sample mixture after the HR-SP-LC process.

Acknowledgements

The author would like to thank Mark Basile, Sylvain Cormier, Dennis DellaRovere, Michael Fogwill, Martin Gilar, Thomas McDonald (Waters, Milford, MA, USA), Frank Riley and Qi Yan (Pfizer, Groton, CT, USA) for their constant technical and material supports to this research project.

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Profession

Leadership
Talent Development
Career Planning

Those Who Can, Teach: Pharma Stars

In the fourth part of our series on innovative education, a group of academic scientists from Norway, Denmark and the Czech Republic tell us how their new post-graduate bioanalytical course will produce the pharmaceutical scientists of the future.

By Øystein Skjærvø, Veronika Pilařová, Maria Khalikova, Leon Reubsaet, Trine Grønhaug Halvorsen, Astrid Gjelstad, Elisabeth Leere Øiestad, Elsa Lundanes, Steven Wilson, and Stig Pedersen-Bjergaard

In recent years, the complexity of molecules and delivery technologies used in the pharmaceutical industry has increased dramatically. Antibody–drug conjugates, polymer-conjugated small and large molecules, PLGA (poly(lactic-co-glycolic acid)) and hydrogel long-acting delivery products are just a few examples that illustrate this trend. For analytical chemists employed in the pharmaceutical industry, measurements related to new drug candidates and delivery technologies are becoming increasingly complex, and the challenges are even greater when the measurements need to be performed reliably in biological fluids. It is clear that the pharmaceutical industry of the future will demand analytical scientists with training at the highest level.

The scientific literature is rich in technical reports on new drug substances, new delivery technologies, and new analytical tools to support drug discovery and development – but discussions on how to teach future analytical scientists to navigate this constantly evolving landscape are notably lacking. The increasing use of highly complex medicinal products will also complicate bioanalysis outside the pharmaceutical industry, such as in hospital laboratories, analytical contract laboratories, forensic toxicology laboratories, and doping laboratories.

Our new course in advanced bioanalysis – "Bioanalysis: Forefront technologies and applications" – organized at the University of Oslo, Norway, has multiple objectives: to

emphasize the importance of high-level teaching in analytical chemistry, to share our philosophy and experiences, and to encourage the development of similar activities elsewhere. Here, we present the perspectives of both teachers and students to shed light on the theory, practice and reality.

Why Oslo?

Although we have a pharmaceutical industry in Norway, Oslo is not considered a "pharma hub" – nevertheless, we have been actively involved in bioanalysis-related research for decades, and in addition have extensive experience of writing international textbooks in the field of pharmaceutical chemical analysis, bioanalysis, and chromatography (1–3). Our bioanalysis course is in the cross-section between research and teaching, and experiences from both are important to maintain a high quality.

Creating the course

The course was designed in close collaboration between teachers at the School of Pharmacy and the Department of Chemistry, both of which have a focus on bioanalytical research; by involving teachers across institutional barriers, we were able to build a highly experienced team. The course was organized as a 5 ECTS credit program (60 ECTS credits corresponds to one year of full study) with full-day lectures and discussions. Most of the 17 participants were PhD students, but postdocs and senior engineers also took part. As teaching

"Our bioanalysis course is in the cross-section between research and teaching, and experiences from both are important to maintain a high quality."

Box 1. Learning outcomes.

The course will give the students in-depth knowledge on:

- Trends in bioanalytical chemistry
- New technologies in bioanalytical chemistry
- New applications in bioanalytical chemistry
- How to present bioanalytical research in scientific papers
- How to present bioanalytical research in oral communication

Box 2. Course framework.

- Focus on sample preparation, liquid chromatography, and mass spectrometry
- Cutting-edge technologies
- New applications
- Scientific presentation skills
- Flavor of the state-of-the-art
- Teaching is based on scientific papers not older than one year
- Small technical details are not important
- Papers are presented by students and teachers
- The course is suitable for international participation

was open for national and international students, we decided to organize a one-week course rather than spread it over a more extended time period.

First, we defined the learning outcome (see Box 1). Defining a framework keeps teaching relevant and focused, especially when several teachers are involved (see Box 2). Although many different analytical techniques are used in the pharmaceutical industry, we focused on liquid chromatography-mass spectrometry (LC-MS) – the workhorse for bioanalysis. We also covered sample preparation, which plays a vital role in LC-MS-based bioanalysis. Within the cross-institutional team of teachers (senior staff), we have substantial in-house research experience in sample preparation, LC, and MS.

At such a high level, there are no textbooks available, so teaching has to be anchored in scientific papers and experience; selecting appropriate and high quality papers was therefore critical. We discussed all papers within the team before they were included in the final program, ultimately selecting 26 papers for the 2017 course as part of five hot topic areas (see Box 3). The papers were chiefly from 2017, but included some from 2016. The senior

staff presented 12 of the hot topic papers, while the remaining 14 papers were presented by the students (see Assessment below).

Communication skills

Each presentation was 20 minutes, followed by a 10-minute critical discussion of the paper. For some sessions the students discussed scientific questions relevant to the day's hot topic in groups, giving them valuable experience in more informal scientific discussions. We discussed the technical details – after all, we were aiming for an understanding of the principles and major pros and cons of new technologies and applications, hopefully better equipping the students to critically evaluate technology for potential implementation in the pharma industry. But, perhaps more importantly, we focused on giving a flavor of recent progress in the field, which gave it a "conference feel."

We emphasized that following the scientific literature is the best way to stay up-to-date in analytical chemistry at the highest level. Therefore, we critically appraised the technical aspects of the papers, but also focused on clarity of presentation – both orally and in writing – emphasizing the responsibility of scientists to contribute to the scientific literature. We organized three one-hour panel discussions on the following subjects, the first focused on oral presentations, and the latter two on scientific publication:

- How to prepare a good lecture
- How to write a good scientific paper
- How to communicate with editors and reviewers

Box 3. Course topics.

For the course syllabus we selected papers across hot topics:

- Liquid chromatography
- Applied mass spectrometry/ ionization
- Sample preparation
- Small-molecule applications
- Protein applications
- Scientific presentations

Several of the senior staff have journal editing responsibilities, and were able to discuss the peer review process from both author and editor perspectives. Group work was linked to the panel discussions, where the students reviewed several research papers.

Course assessment

To pass the course, the students have to fulfill the following requirements in addition to their active participation during the one-week course in Oslo:

- Prepare a 20-min oral presentation of a hot topic paper (prior to course)
- Prepare a 10-min oral presentation about their PhD research (prior to course)
- Complete one of the following duties after the course (post-course work):
  - o Write a short bioanalysis-related article for a national chemistry or pharmacy journal
  - o Give a bioanalysis-related lecture at a national meeting
  - o Review a research paper for one of the other course participants, prior to journal submission

The 10-min oral presentations on each students PhD research were scheduled for the first day of the one-week course, to get to know each of the students and their work. The post-course work is organized as an alternative to an exam, and is mandatory for the 5 ECTS credits.

Student feedback

Feedback from students is always important when evaluating a teaching program. Each day, we asked students to submit any feedback on post-it notes. Based on this feedback, we made adjustments during the course to optimize the learning outcome and atmosphere. In addition, we asked three students (Øystein Skjærvø, Veronika Pilařová, and Maria Khalikova) to contribute their opinions to this article, in accordance with the feedback notes. They summarized the positive points about the course as follows:

"Very friendly atmosphere, not focusing too much on differences between students and teachers."

"We found discussion – not only with teachers, but also between students – to be extremely beneficial as part of the learning process."

"This course was arguably different (from other university courses) as it was more focused towards innovation and the recent advancements within biological analyses."

"The Chinese proverb 'I hear and forget, I see and remember, I do and understand' explains why many theoretical courses are less productive and less effective and why we are so impressed by the Bioanalysis course at the University of Oslo."

The cons and recommendations for potential improvements were chiefly related to lectures on techniques students were not familiar with, and the intense workload during the one-week course:

"Preparing an oral presentation about a scientific article was a very challenging task for those of us who do not work in this particular field."

"Papers might be selected more carefully; allowing students to receive new knowledge but also giving opportunity to share their own experiences, tricks and tips."

"The timing of this particular course was exceptionally tight."

What's next?

Our first PhD course in advanced bioanalysis was an experiment, and we believe a successful one; the need for training at the highest level in bioanalysis is continuously increasing and there was definite interest in the course. We have therefore decided to arrange it biannually, adjusting according to student feedback.

"Several of the senior staff have journal editing responsibilities, and were able to discuss the peer review process from both author and editor perspectives."

Though it will remain the same in terms of the course framework and learning outcomes, papers for student presentation will be selected more carefully and in agreement with each student, and the time schedule will be a little less compacted.

Although the majority of students this time were Norwegian, in future, we will welcome international students in addition to the local ones – to emphasize that, in the field of bioanalysis, there are no national borders. We prioritize a friendly atmosphere, because this stimulates learning and collaboration.

For further information, see the course webpage: https://www.uio.no/studier/emner/matnat/farmasi/FRM-KJM9930/

Acknowledgements
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Affiliations
Øystein Skjærvø – School of Pharmacy, University of Oslo, Oslo, Norway
Veronika Pilařová – Department of Analytical Chemistry, Charles University, Hradec Kralove, Czech Republic
Maria Albertovna Khalikova – Department of Analytical Chemistry, Charles University, Hradec Kralove, Czech Republic
Leon Reubsaet – School of Pharmacy, University of Oslo, Norway
Trine Grønhaug Halvorsen – School of Pharmacy, University of Oslo, Norway
Astrid Gjelstad – School of Pharmacy, University of Oslo, Norway
Elisabeth Leere Øiestad – School of Pharmacy, University of Oslo, Norway
Elsa Lundanes – Department of Chemistry, University of Oslo, Norway
Steven Wilson – Department of Chemistry, University of Oslo, Norway
Stig Pedersen-Bjergaard – School of Pharmacy, University of Oslo, Norway, and Department of Pharmacy, University of Copenhagen, Denmark

Reproducibility with Pyrolysis-GC-MS and Thermally Assisted Hydrolysis and Methylation

Reproducibility of thermal techniques is addressed using the latest version of the Pyroprobe from CDS Analytical.

By Tom Wampler and Karen Sam

Analytical value depends on the reproducibility of the technique. Reproducibility with a thermal technique depends greatly on temperature accuracy, as well as sample related issues like homogeneity, and consistent sample placement. Pyroprobe filaments are calibrated using optical pyrometry. Using this technique, a series of 20 firings at 1100°C produced an average measured temperature of 1100.15°C with a relative standard deviation of only 0.04 percent. This ensures that Pyroprobe instruments perform with the highest precision.

Samples in the following examples were prepped in DISC tubes, which are precisely sealed at the proper location to ensure correct sample placement within the coil filament (Figure 1). Because of this, it can easily handle both liquid and solid samples. Figure 2 shows five runs of rubber cement diluted in hexane, at 600°C using a Pyroprobe 6000 Series Autosampler. For each run, 0.5µl of the solution was added to a quartz DISC tube, for a sample weight of 5 µg. This produced an RSD for the monomer to dimer ratio of 1.5 percent.

Thermally assisted hydrolysis and methylation (THM) is a useful method for characterizing polyesters, using heat and a reagent to fragment them into recognizable building blocks of methylated polyols and polyacids. In the next example, thermally assisted hydrolysis was performed with tetramethyl ammonium hydroxide and a polyester resin. Approximately 10mg of polyester was dissolved directly into 0.25mL of TMAH (25 percent wt/wt in methanol). Two microliters of solution was added to a DISC sample tube containing a small pad of quartz wool, and run at 540°C. Low peak area ratio RSDs were computed for 4 identified compounds. Replicates are shown in Figure 3. Reproducibility of the Pyroprobe using this technique with its complex sample matrix ranged from 1.65 to 4.33 percent for these compounds.

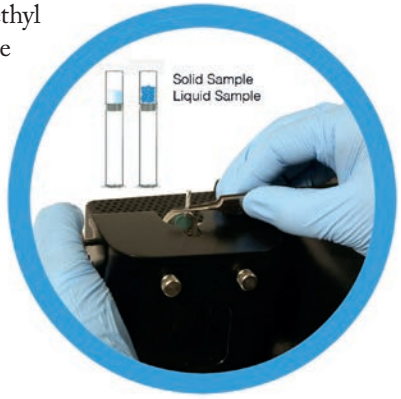


Figure 1. CDS 6000 Series DISC and sample tubes.

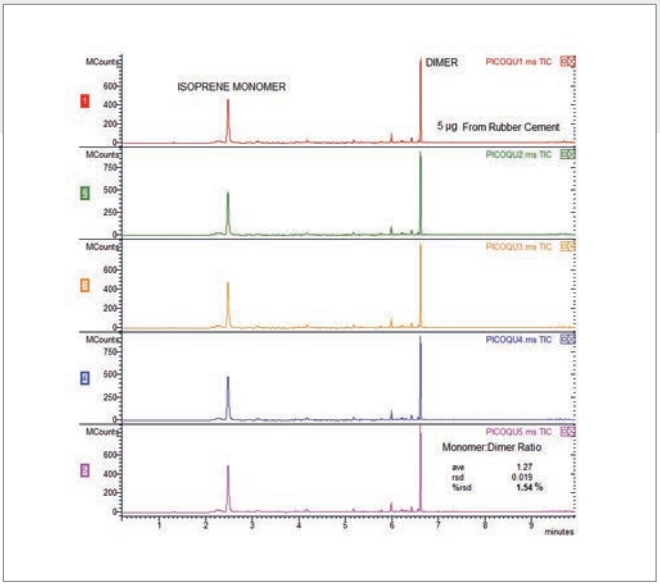


Figure 2. Five runs of rubber cement diluted in hexane, at 600°C using a Pyroprobe 6000 Series Autosampler

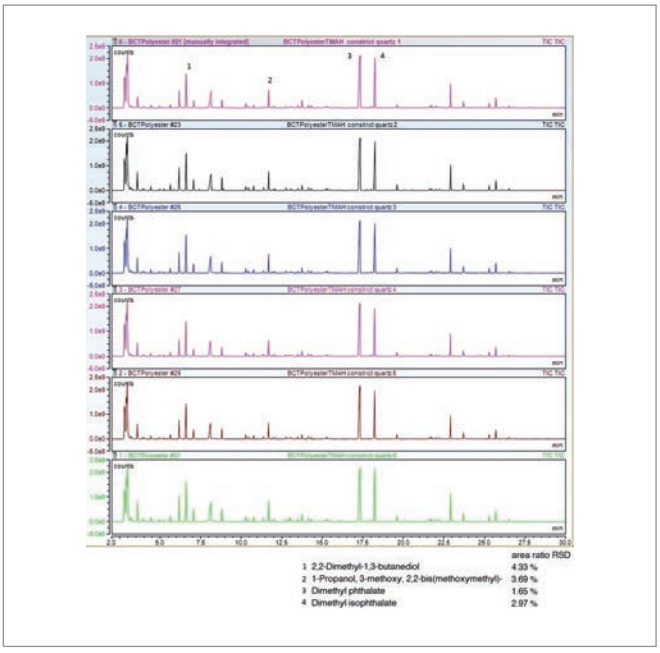
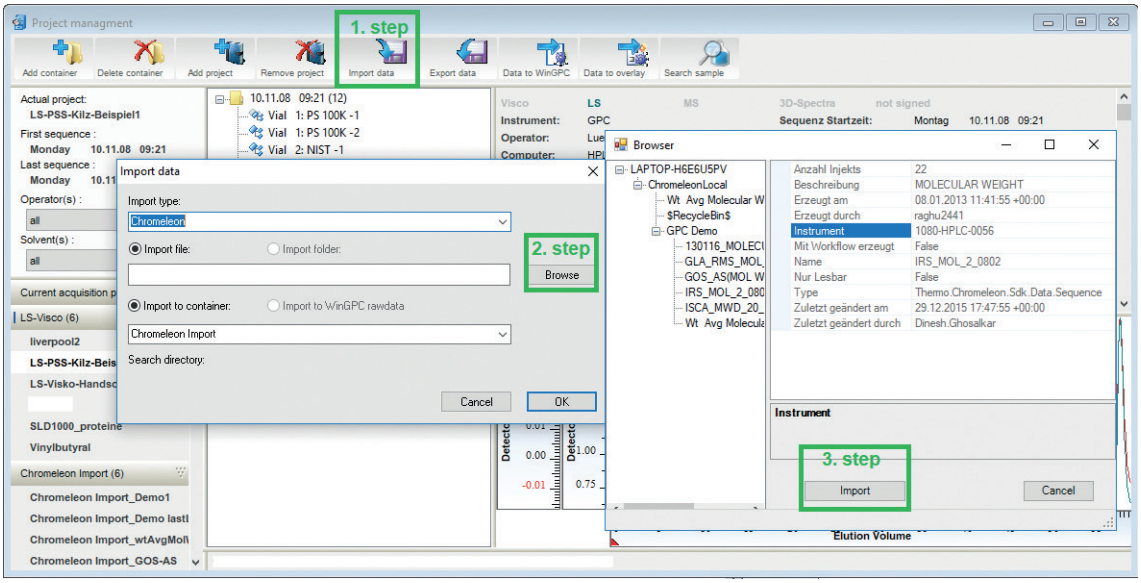


Figure 3. Replicates of polyester with TMAH.

The latest version of the Pyroprobe from CDS Analytical provides easier sample preparation and introduction. The new DISC and sample tubes and an add-on autosampler module ensures an improved user experience, as well as repeatable, reliable results.



Reliable GPC/SEC results by integrating Chromeleon software and WinGPC UniChrom

Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) combines chromatography (HPLC, IC, GC) and routine quantitative MS analysis workflows. PSS WinGPC UniChrom MCDS offers comprehensive solutions for the characterization of macromolecules with GPC/SEC, IPC, SFC and CE in 1 and 2-dimensional setups.

WinGPC is a comprehensve suite of software which has been designed from the ground up to address the special requirements of large molecule analysis. It provides access to distributions and offers all calibration options as well as support of important techniques such as light scattering, viscometry or mass spectrometry. It offers e-workflows and support for many different applications from copolymer analysis and membrane analysis to regulated USP and EP methods for Heparin or Dextran analysis.

To enable Chromeleon CDS users to process GPC/SEC data a native WinGPC- Chromeleon CDS data connector has been developed by Thermo Fisher Scientific and PSS.

WinGPC connects directly to the Chromeleon 7 Data Vault and loads complete sequences into the proven WinGPC project structure. This procedure ensures that all WinGPC evaluation options (with the exception of e-flows that require real-time data acquisition) are fully functional. Even advanced features of WinGPC modules can be used.

The highest transparency and traceability are ensured by the optional WinGPC Compliance Pack. With it, all entries and changes are automatically documented in the sample audit trail and are therefore completely traceable.

For the output of all results, WinGPC standard reports are available. With the optional ReportDesigner, custom report layouts can be designed as desired reporting only the required results. Export functions include the powerful ASCII-MWD report, which also provides a convenient link to a LIMS.

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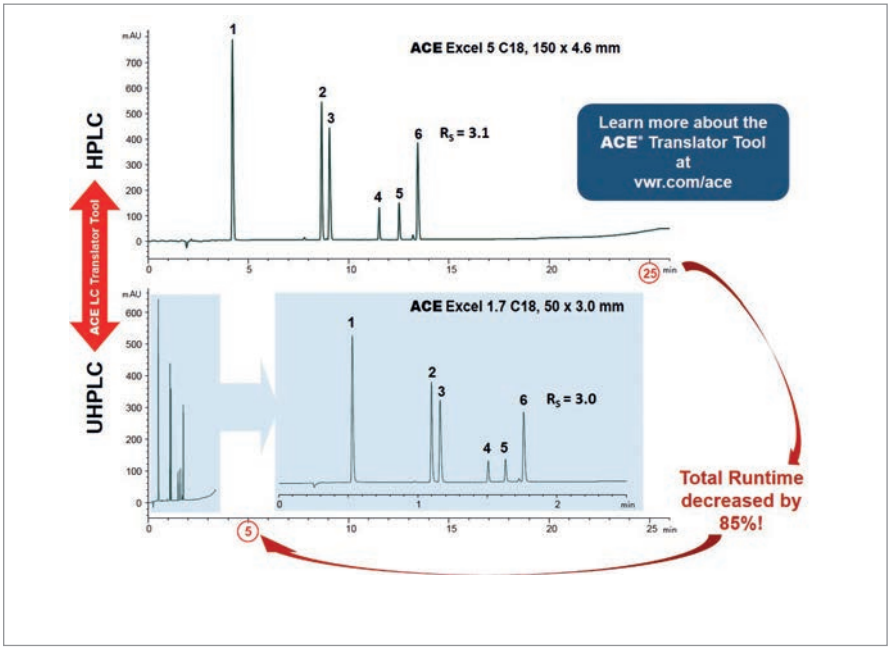

Top: Separation of 6 peptides on an ACE Excel 5 C18 HPLC column installed on an HPLC instrument. Bottom: The same separation translated to an ACE Excel 1.7 C18 UHPLC column installed on a VWR Hitachi Chromaster UltraRs UHPLC system. The method translation was achieved using the ACE LC Translator Tool. Mobile phase: A=0.05% TFAin H_2O, B=0.05% TFA in MeCN. Temperature: 60°C. Detection: UV, 220nm. Sample: 1.Gly-Tyr,2. Tyr-Tyr-Tyr, 3.Val-Tyr-Val, 4.Oxytocin, 5.AngiotensinII, 6.Leu-enkephalin.

For more information on ACE products, please contact our technical support team by emailing info@ace-hplc.com.

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The Sweet Smell of Success

Sitting Down With… Jean-François Focant, Professor, Chemistry Department, University of Liège, Belgium.

Congratulations on receiving the GC×GC Lifetime Achievement Award in Riva del Garda earlier this year!

I must admit receiving a "lifetime achievement" award makes me feel quite old! But it's great to receive recognition of the work my group has been doing over the past few years. In particular, it was a special moment to be presented the Award by John Dimandja – the man who first introduced me to GC×GC 20 years ago and is still a friend and mentor today.

What was your route into analytical chemistry?

I did my Master's thesis in organic chemistry and, when it came time to choose my PhD, an opening came up in analytical chemistry. I wasn't immediately enthusiastic – my perception of analytical chemistry at the time was through student labs, which I found very dull. So why did I go for it? First, the project involved mass spectrometry, which is a cool technique. And second, the practical application of the work caught my attention – developing GC-MS methods to measure toxic dioxins in food. As interesting as fundamental science can be, research for the sake of research doesn't excite me. I need to see how my measurement will be used. It didn't take long to find out just how relevant these particular measurements were for human health – I started my PhD in 1998 and the Belgian dioxin crisis exploded in 1999! Polychlorinated biphenyls were found in Belgian chicken and eggs, and accusations of a cover-up led to a huge political scandal and panic amongst the public. There was a great deal of pressure to get the analyses right, and the tools I developed back then are still used in routine labs 15 years later.

How did GC×GC come into the story?

I traveled to the USA to learn from an expert in dioxins, Don Patterson, then at the US Centers for Disease Control and Prevention (CDC). Through Don, I met and worked with John Dimandja, a real pioneer of GC×GC, and was instantly struck by the potential of the technique. I came home from that trip with a single chromatogram… And I was delighted to have even achieved that! Now, things have moved on and producing modulated traces is easy, but as we look at bigger sample sets, the challenge has shifted to data processing.

What are some of the current projects your team is working on?

We work in a wide variety of applications using GC-MS and GC×GC. Though we still do some work with persistent organic pollutants (POPs), we have an increasing focus on forensics, cancer research and petroleum. In forensics, our work centers on the volatile organic compound (VOC) composition of cadaveric odor, to help police investigate suspicious deaths and to aid search and rescue teams in locating bodies in disaster zones.

We also work on headspace analysis for the diagnosis of a range of cancers. Working with our partner hospital to collect samples of healthy and diseased tissue from the same colorectal cancer patients, we compare the VOC profiles of the samples. We hope to "sniff out" cancer in the same way that medical detection dogs can be trained to do.

Another interesting recent project has been looking at the composition of e-cigarette and heated tobacco vapor. These are very complex, challenging samples and there is an urgent need for more information on their potential health effects.

Tell us about the Multidimensional Chromatography Workshop you are helping to organize.

For 2019, the workshop will be at the University of Liège; it is completely free to attend so it's a great opportunity to bring together this specialized community. There will be a full program of educational sessions, but also a strong emphasis on networking. After all, a lot of the most important interactions at any conference occur over lunch or a glass of wine. Register at http://www.multidimensionalchromatography.com.

How do you fit all of your responsibilities in?

That is my major challenge – in between teaching, research projects and my role as head of the department, it's a delicate equilibrium! My first priority is my family, so I make a rule never to bring work home – I may work late sometimes, but once I'm home, I'm home. With such varied research projects underway, I cannot be on top of all of them at all times, so I rely on my senior scientists to run things day-to-day. It can be frustrating to stand in the lab surrounded by instruments I've been dreaming of for years and know I won't get the chance to use them myself, but seeing my team putting them to excellent use gives me great satisfaction.

What is your focus for the future?

Now that I'm a full professor and my situation is stable, I'm fortunate to be able to spend most of my energy on the talented young people that come through my lab. By training and mentoring others, you can multiply your successes and achieve far more than you could as an individual. In 10 or 15 years from now we could see some of the biomarkers from our research in routine use for detection of cancer – that would be amazing. Perhaps later in my career, when my kids are older, I might take a sabbatical and go back to the lab.

And spend some quality time with the instruments?

Yes, exactly!