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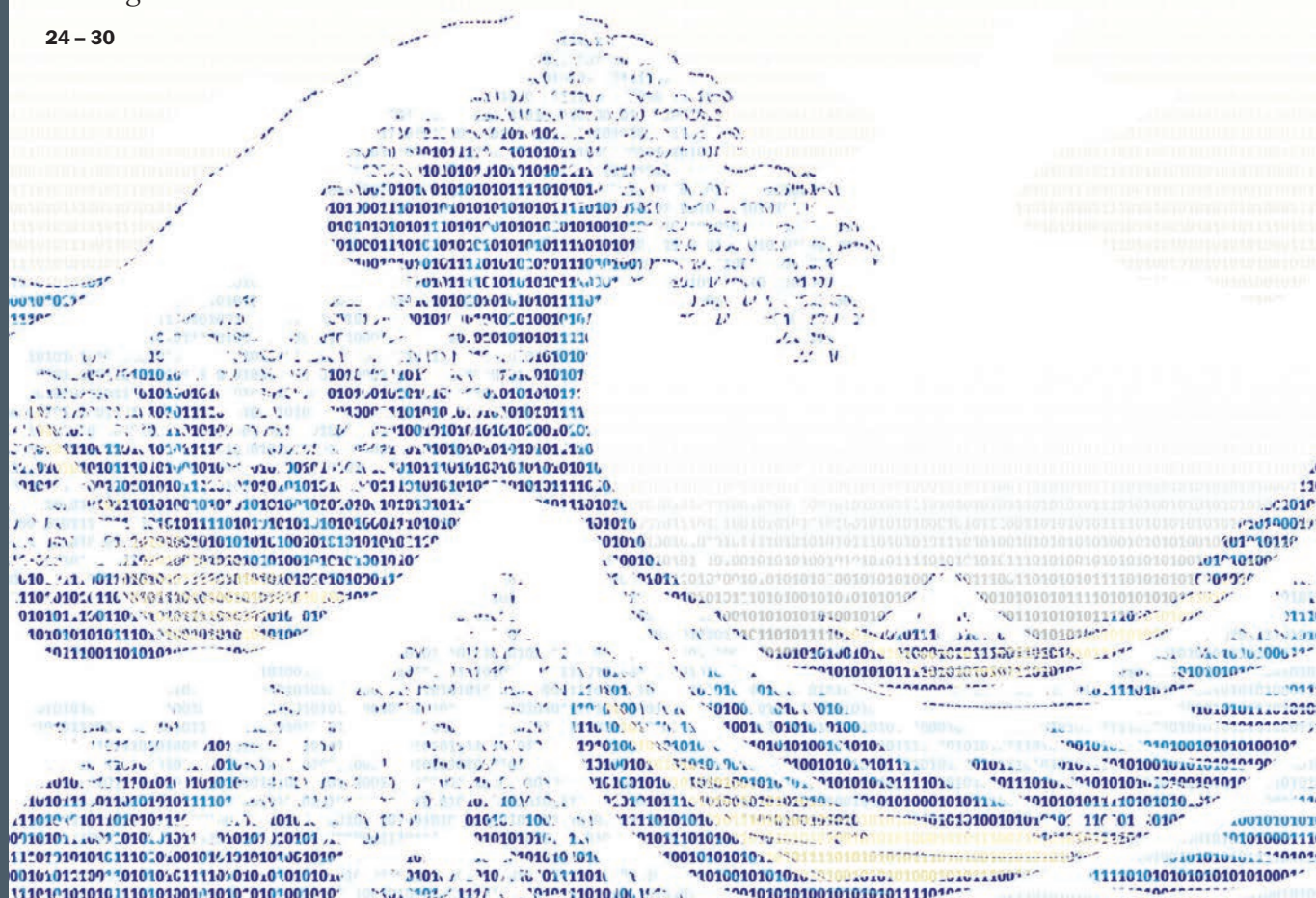
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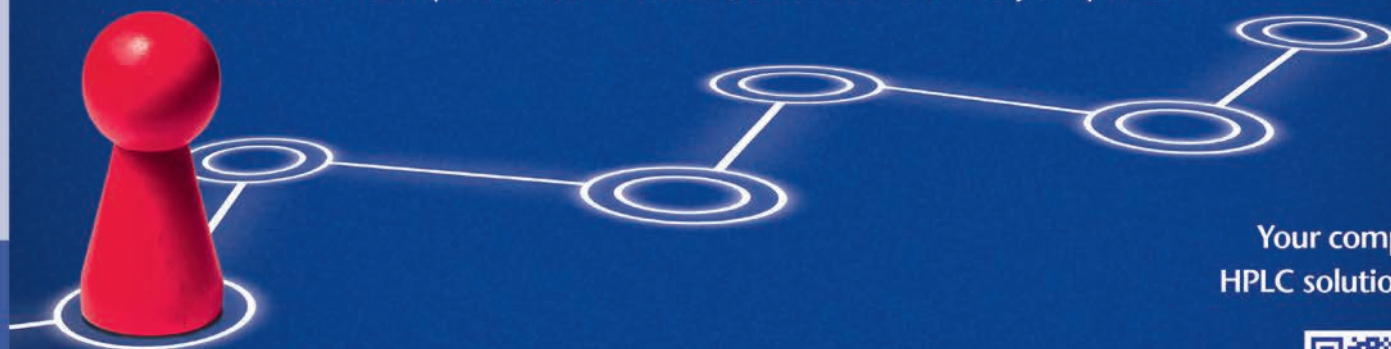


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# Online this Month

## Is Print Dead?

Clearly not. You're reading this... But that's not to say there isn't room for some exciting digital publishing, as proved by The Analytical Scientist iPad app. Here, we take you on a whistle-stop tour of the navigation features.



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*Congratulations to Colin Stone.*

Colin will no doubt be enjoying our app on his brand new iPad mini after winning the online *Chromatography Needs and Preference Survey* prize draw.



## Immune System at Room

...eds of extracellular signaling proteins secreted  
... immune responses were discovered – and what it tells  
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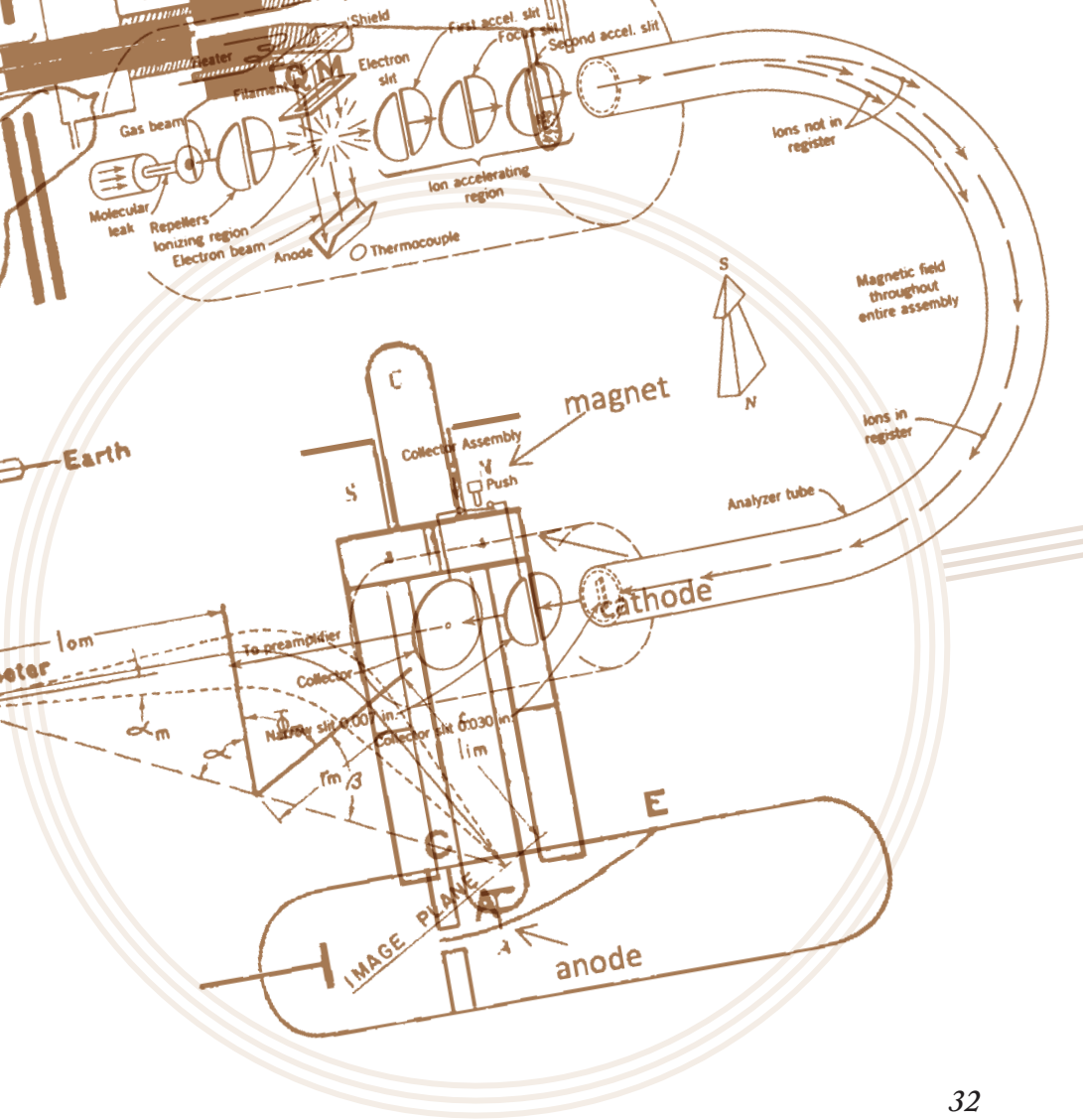
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## the Analytical Scientist

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**Editor** - Rich Whitworth

rich.whitworth@texerepublishing.com

**Editorial Director** - Richard Gallagher

richard.gallagher@texerepublishing.com

**Scientific Director** - Frank van Geel

frank.vangeel@texerepublishing.com

**Graphic Designer** - Marc Bird

marc.bird@texerepublishing.com

**Managing Director** - Andy Davies

andy.davies@texerepublishing.com

**Director of Operations** - Tracey Peers

tracey.peers@texerepublishing.com

**Publishing Director** - Lee Noyes

lee.noyes@texerepublishing.com

**Audience Development Manager**

- Tracey Nicholls

tracey.nicholls@texerepublishing.com

### Editorial Advisory Board

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### General enquiries:

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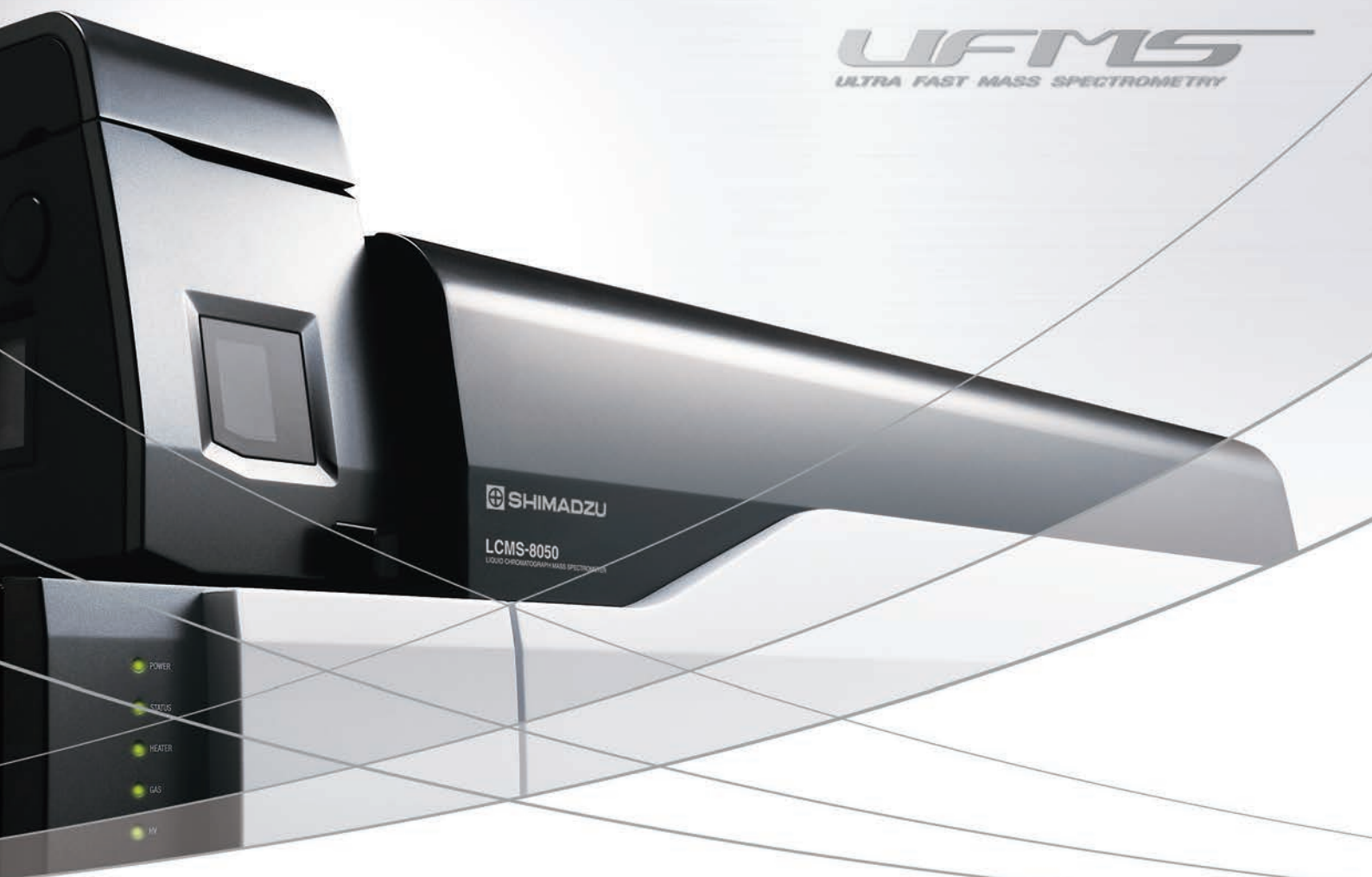
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## Comment Is King

*The discussion thread on an opinion article on chemometrics has been incredibly insightful – and spurred our commissioning strategy.*

Editorial



The cover feature in this issue is a strident call to arms for chemometricians, written by a leading light in the field, Lutgarde Buydens. In it, Buydens sets out the challenges that mega datasets present to traditional chemometrics and offers a radical blueprint to address these challenges. It's stirring stuff.

At the risk of getting too much into the minutiae of the editorial process, the story behind commissioning the Buydens feature is also noteworthy.

Back in March, The Analytical Scientist published Ewa Szymańska's thoughtful *In My View* article entitled "Why Isn't Chemometrics Center Stage?". We knew it was interesting, but I was startled by the volume and passion of the debate that it generated online. Some of the comments, for example, showed just how much the late Bruce Kowalski inspired his students:

*"Nearly 40 years after being the first PhD out of Bruce Kowalski's group, chemometrics has changed very little. People are still treating the technology as 'black magic' and forget that chemometrics is a useful tool only when you have lots of data."* – Doug Dierdorf

*"Bruce Kowalski, my post-doc supervisor 1994-6, always said that chemometrics would only impact analytical chemistry when (properly) being incorporated in software of instruments. I think he was right all the time. Specifically, the software should flag whether certain data requirements are not being met."* – Klaas Faber

Others contended that automation is essential, despite the obvious misgivings about how the inexperienced might end up using it:

*"Speeding up the acquisition of a 'clean' data set is a great place to start. Model maintenance and updating is another opportunity for automation. This requires exquisite capture of metadata from controlled vocabularies; a foundation for automating the creation of large multivariate data sets."* – James Roberts

Given the deluge of commentary, we knew that a provocative and expansive piece on chemometrics was required, hence the Lutgarde Buydens article. Call it Reader Power; Comment is King!

Charles Prestwich Scott, editor of the Manchester Guardian (now the Guardian), famously wrote *"Comment is free, but facts are sacred"*, in reference to his editorial values. While this is certainly true, and nowhere more so than in the analytical sciences, in the Internet era insightful comment has the potential to question, to challenge, and to influence.

Buydens' feature is not the end of the discussion about chemometrics by any means. Both the author and I invite you to pile in.

---

*"Why Isn't  
Chemometrics  
Center Stage":  
tas.txp.to/0313-  
chemometric*

*"Towards  
Tsunami Resistant  
Chemometrics":  
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tsunami*

Rich Whitworth  
Editor



### Lutgarde Buydens

“During my PhD on drug activity, I realized that much more information is hidden in experiments than is obvious at first sight,” says Lutgarde Buydens. As a student of Désiré Luc Massart, one of the founders of chemometrics, data analysis and interpretation quickly became her main focus. After postdocing in the US, Buydens joined Radboud University in the Netherlands, where she is now chair in Analytical Chemistry – Chemometrics. Her interest in better data analysis and interpretation is as strong as ever. “To help discover new knowledge in so many different fields, from food science, medical sciences to industrial processes still excites me every day,” she says.

Read Buydens’ formula for a bright future for chemometrics on page 24.



### Giuseppe Astarita

Giuseppe Astarita works at the intersection of biology and technology. He is principal scientist in lipidomics and metabolomics at Waters Corporation (Milford, Massachusetts, USA) and serves as an adjunct professor at Georgetown University (Washington, DC, USA). An expert in lipid mass spectrometry and metabolomics for translational medicine and biomarker discovery, his interests are in the role of lipid metabolism in health and disease, including inflammation, Alzheimer’s disease, metabolic syndrome, cardiovascular disease, aging, and drug addiction. In 2010, The International Society for the Study of Fatty Acids and Lipids named him Top New Investigator Award in Biochemistry of Lipids.

For an assessment of omega-3 analysis, see page 22.



### Dave Strong

Dave Strong graduated with a PhD in analytical chemistry from the University of Bristol, UK, in 2012 and currently performs toxicological testing on human samples at HFL Sports Science. “My work entails extensive research and method development in both biological and man-made matrices, which includes substantial wet chemistry, such as solid phase extraction”. Dave has developed numerous methods from scratch, such as the extraction of caffeine from saliva, the extraction of octopamine from human urine... and the extraction of synthetic cannabinoids from blood and saliva (see page 44).



### Frank Neely

After choosing chemistry as a major largely on the premise that “if it looks hard, it must pay well”, Frank Neely ultimately received a PhD in analytical chemistry from Georgia Tech. Since then, he has been employed by Eli Lilly & Company and several Johnson & Johnson medical device companies. “It may sound corny”, Frank says, “but I feel an amazing sense of accomplishment when I see a product for which I had some involvement sitting in someone’s medicine cabinet or hear patients describe how something improved their life”. He also advises people to exercise discretion about what they keep in their medicine cabinets.

Discover Frank’s survival strategy on page 16.



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# Upfront

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

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

*rich.whitworth@texerepublishing.com*

## Microbe Probe

**Want to mine for precious molecules in living microorganism colonies? Combine surface sampling and electrospray ionization mass spectrometry, and hey presto!**

Pieter Dorrestein's research aims to understand at the chemical level how microbes interact with each other, with their surroundings, and with their host. "We are interested in the specialized molecules that microbes produce to curate their specific environmental niche," he explains, with the goal of "repurposing them for clinical applications," citing the examples of antibiotics (Penicillin, erythromycin, daptomycin), immunosuppressants (rapamycin), and anti-cancer agents (epothilones).

Photo of flow probe and living *Streptomyces coelicolor* A3(2) colony. For video, visit: [youtube.com/watch?v=1R8DpdZgC6Q](https://youtube.com/watch?v=1R8DpdZgC6Q)

To achieve their goal, researchers in Dorrestein's lab at the University of California, San Diego are directly sampling analytes of living microbe colonies ahead of electrospray ionization (ESI) and tandem mass spectral analysis (1). The key component is a liquid microjunction surface-sampling probe (LMJ-SSP). It is based on a device developed by Van Berkel at Oak Ridge National Laboratory, which comprises two co-axial capillary tubes that simultaneously deliver and aspirate solvent (see Figure 1).

"Current abilities in sequencing allow us to discover the genetic capacity of microbes to make such specialized metabolites, but the analytics to detect them is far behind. Traditionally, it is done one molecule at a time." By removing the need for sample preparation and studying living colonies, the new technique could open doors to high-throughput studies or be used to probe novel microbiome interactions.

When asked about sensitivity, Dorrestein replies: "The method is not as sensitive as LC-MS because we only detect the most ionizable or abundant molecules. But it turns out that many of the specialized molecules that alter the environmental niche of microbes are produced in fairly large quantities". In fact, it is not uncommon that the compounds of interest are produced in tens or even hundreds of micrograms per ml in a Petri dish, ranking them among the most concentrated molecules in the sample. "The localized nature of the sampling method further enhances observed concentrations because the molecules are often highly localized as well," he says.

Given the diverse nature of the chemical landscape uncovered by the approach and the complexity of the datasets produced, intelligent data analysis is essential. By considering compounds and their spectra



First we made them  
multi task...then we  
conquered space.

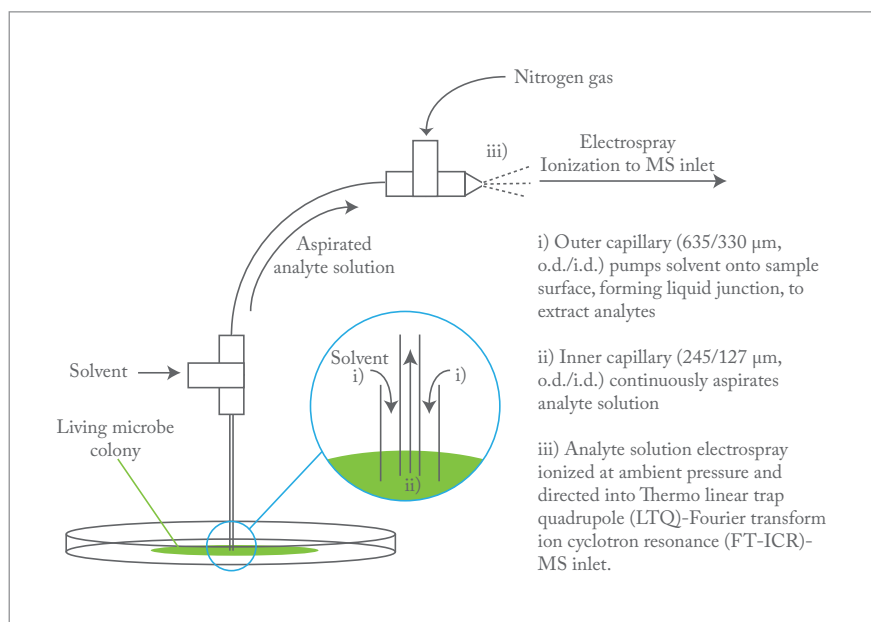


Fig 1. Real-time MS analysis of microbial colonies using coaxial flow probe and ambient electrospray ionization.

in the context of a network of chemically similar molecules, “molecular families” emerge, which are represented by a tight cluster within the molecular network. Such molecular networking can be used to form a system-wide picture or to characterize individual compounds. Furthermore, combining molecular family information with gene cluster data generates tools, such as peptidogenomics, that makes high-throughput analysis a real possibility.

An earlier version of the technology – nanoDESI – combined with molecular networking (3) piqued the interest of Roberto Kolter and Matt Traxler back in 2012 (4). They lined up potential question-based applications, including “What chemical ecology underlies the microbiome associated with human skin? What signals potentiate relationships between hosts and symbionts? How do soil bacteria, long known to make a variety of antibiotics, deploy their arsenals in the presence of competitors?”

The Dorrestein approach has some

bugs: capillary clogging needs to be reduced and depth recognition for automation must be added. When these issues are addressed (solutions are in the pipeline), progress on answers to Kolter and Traxler’s questions, and more besides, will become a real possibility. *RW*

For more on research at the Dorrestein Lab’s, visit: [chem-faculty.ucsd.edu/dorrestein](http://chem-faculty.ucsd.edu/dorrestein)

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4. M. Traxler and R. Kolter, “A massively spectacular view of the chemical lives of microbes”, *PNAS*, 109 (26), 10128–10129 (2012).



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## Seeing Cyanostars

**A new star-shaped macrocycle has potential applications in perchlorate remediation, biosensing, and separation science**

High yield, one-pot reactions for macrocycle synthesis are rare. So chemists at Indiana University Bloomington have surprised everyone, including themselves, by easily producing a spectacular new star-shaped cyclic macromolecule, in 10-gram batches (1). According to Amar Flood, associate professor in the Department of Chemistry and corresponding author on the paper, “We performed computer modeling prior to the synthesis – and hoped that the carbonate base might act as a template as much as a catalyst (but) we did not expect such a remarkable yield”.

While macrocycles might be common – even “a dime a dozen” as Flood has noted in the past – cyanostars are special because they capture large charge-diffuse anions previously thought to be only weakly coordinating. “We show that cyanostars bind these ‘weak’ anions with as much tenacity as most ligands bind metals,” Flood explains.

“It’s also noteworthy that their five-fold symmetry is rare and it introduces ideas of exotic materials, like quasicrystals.”

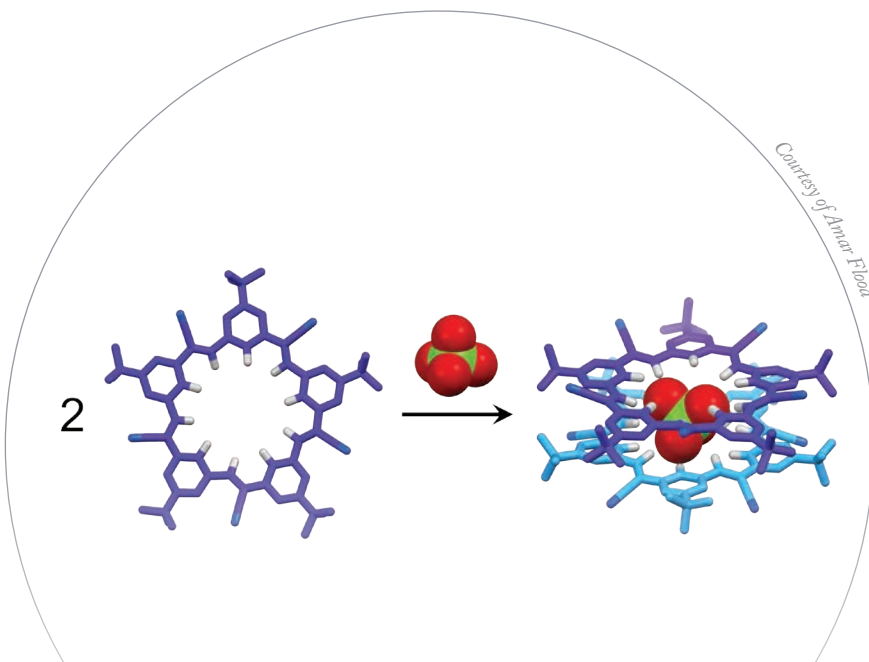
Given the ease of cyanostar synthesis and their interesting binding characteristics, commercial use in analytical and preparative separation seems plausible. “Cyanostars may be incorporated into the stationary phase of column support materials to enhance the separation of compounds with negatively charged moieties, such as pendant phosphate, sulfates and acetates,” he says. But he predicts that more likely applications include environmental perchlorate remediation and biological phosphate sensing. “A form of the cyanostar would be capable of binding biological phosphate such as AMP, ADP and ATP, some DNA and RNA, and phospholipids.” To that end, the group

is currently examining the cyanostar’s affinity for biological phosphates, after which further customization of the molecular design will be required. “We have experience collaborating with other groups to incorporate anion receptors into polymer membranes for ion-selective electrodes,” Flood says, “and we expect cyanostars to serve as ionophores within such devices to detect environmental or bio-relevant anions.” *RW*

For more on Flood’s research, visit:  
[www.indiana.edu/~floodweb](http://www.indiana.edu/~floodweb)

### Reference

1. Semin Lee, Chun-Hsing Chen & Amar H. Flood, “A pentagonal cyanostar macrocycle with cyanostilbene CH donors binds anions and forms dialkylphosphate [3]rotaxanes,” *Nature Chemistry* 5, 704–710 (2013). doi:10.1038/nchem.1668



## Joined-Up Data?

**Accelrys makes big claims for the new Experiment Knowledge Base. We look into who in the analytical sciences world might be interested, and**

**what value might be derived from it.**

R&D can be an uphill struggle when scientific data is produced at a greater rate than useful knowledge can be derived from it. According to Accelrys, they have a solution: a new laboratory informatics system called Experiment Knowledge Base (EKB). According

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The Analytical Scientist quizzed Accelrys senior director Ted Pawela to find out more.

Why is the ability to search and explore multiple data sets so important?

R&D data, and analytical data as a specific case, generally do not reside in a single system, but in a variety of systems. Instruments often have their own data management solution; electronic lab notebooks capture important observations and derived results that are not a part of raw data; scientific data management systems (SDMS) fail to store and associate calculated results, and the most widely used tool in science – MS Excel – is a disconnected island. Until scientists can leverage the complete body of knowledge that their organizations have invested to create, they cannot learn anything from what's been done in the past. They are "doomed to repeat the past" – and all of its experiments.

Is EKB a bespoke or 'off-the-shelf' product?

EKB is not a bespoke solution, but a product with a number of baseline configurations to fit different application environments, for example, catalyst development, chocolate formulation, bio-fermentation, chemical/petrochemical processing.

What about future-proofing against new data streams?

Future data streams do not require upgrades to the product, because EKB uses the Accelrys Enterprise Platform (AEP) and Pipeline Pilot to integrate with new data sources. Customers can use the Pipeline Pilot visual authoring environment to tap into and mine data from virtually any source using standard technology.

"Virtually any source" is a big claim. What are the limits?

This extends to any non-binary data system. If the raw data is accessible without using a proprietary application programming interface (API) or hidden formats, EKB can utilize the data.

What about further mining of data at a later stage, as one would do with high-res mass spec?

While it provides multiple capabilities for planning and managing experiments, EKB's "big picture objective" is to help researchers mine experimental data from all sources in order to learn, and accelerate scientific innovation [...] AEP is already being used by Accelrys customers to extract and use data from many analytical instruments and databases, including high resolution mass spectrometry systems.

Who is most likely to benefit from EKB? The perfect candidate laboratory is any R&D facility in which flexibility is important. Labs that do many kinds of experiments in support of finding new or improved product performance are a great fit due to EKB's flexibility and modifiable workflow management. Formulations development, bioprocessing, and flavor development labs are good examples of such environments. Conversely, labs whose primary mission is to run relatively few experiments over and over in an effort to measure manufacturing quality or compliance are not a good fit.

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The Berkeley Open Infrastructure for Network Computing (BOINC) now gives Android operating system users the potential to donate excess computing power to diverse and fascinating research initiatives with a new app.

What?

Current projects include Einstein@Home's search for astronomical phenomena, such as radio pulsars (340,000 global participants have helped discover almost 50 to date), and FightAIDS@Home, which allows the Olson Laboratory at the Scripps Research Institute in California to use community computational power to identify new candidate drugs with the right shape and chemical characteristics to block HIV's essential enzymes.

Why?

Data analysis and processing is a major bottleneck in research, especially when access to funding or computer infrastructure is limited. By properly harnessing the growing number of powerful and energy-efficient personal devices, research can be accelerated. As a solid testament to the clout of distributive computing, the World Community Grid, which runs on BOINC, has provided researchers with nearly 775,000 years of free computing runtime since its launch in 2004.

How?

The new BOINC app for Android OS (version 2.3 or higher) acts as



Max Planck Institute for Gravitational Physics/B. Knispel (photo), NASA (pulsar illustration)

the conduit between your device's unused processing capability and the researchers that need it. Download it at the Google Play store. But don't worry; even if you're not an Android phone or tablet user, you can still download BOINC software to use the idle time of your regular computer.

Who?

FightAIDS@Home is just one of many active projects hosted by the World Community Grid. Others include research into green energy and clean water, as well as cures for other diseases. Past projects have included human proteome folding, genome comparisons, and malaria research. *RW*

Links

BOINC: [boinc.berkeley.edu](http://boinc.berkeley.edu)

World Community Grid:

[www.worldcommunitygrid.org](http://www.worldcommunitygrid.org)



## Off the Menu

### Chasing the dragon – with poppy seed bagels

In 2010, Pennsylvania mother Elizabeth Mort had her newborn taken into protective custody after testing positive for opiates in a routine hospital test. The cause? A single poppy seed bagel.

Fast forward to July 2013, and Mort's story is splashed across the media after Lawrence County's child welfare agency and Jameson Hospital agreed to settle the suit filed on Mort's behalf by the American Civil Liberties Union

of Pennsylvania (1). According to the lawsuit, the federal standard for opiates is 2000 ng/ml but Jameson Hospital, which has since changed its policies, alerted authorities after finding 300 ng/ml.

But this story is not so unusual. Popular myth debunking website Snopes (2) lists four cases of unfair dismissal involving the dreaded poppy seed. These – and the subsequent law suits – perhaps prompted the change in threshold introduced in 1998 (3): “[DHHS] amendments raised the initial and confirmatory test opiate thresholds from 300 [ng/ml] to 2000 ng/ml. The DHHS amendments also established a new requirement to test for 6-acetylmorphine (6-AM), a metabolite

that comes only from heroin, using a 10 ng/ml confirmatory level, for specimens that have tested positive for morphine on the confirmatory test at the 2000 ng/ml level.”

Quite why the hospital chose to report 300 ng/ml as a positive result two years after the law had been changed is unclear, but such a high-profile false-positive case certainly gives pause for thought: are there other serious miscarriages of justice out there? *RW*

#### References

1. <http://buff.ly/164bGu8>
2. [www.snopes.com/medical/drugs/poppyseed.asp](http://www.snopes.com/medical/drugs/poppyseed.asp)
3. <http://tas.txp.to/gpogov>



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# In My View

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

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

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## Sidestepping Obsolescence

**How analytical scientists can enjoy a satisfying and intellectually stimulating career**



*By Frank Neely, engineering fellow of Advanced Technologies & Engineering at Johnson & Johnson Medical Devices and Diagnostics, Somerville, NJ, USA.*

Over a century ago, Lord Kelvin proclaimed: “If you cannot measure it, you cannot improve it.” Indeed, the ability to make accurate measurements is critical to progress, so one might expect analytical scientists to be highly revered – their counsel sought out as a prerequisite to embarking on any technical project. Yet, more frequently, analytical scientists are relatively silent players on the project team.

Why does this apparent paradox exist? Partly, it’s a logical consequence of advances in instrumentation. Once the exclusive province of basement-dwelling gurus, nuclear magnetic resonance systems sit on the bench top adjacent to fume hoods, and mass spectrometry systems compete with ultraviolet detectors for popularity, all thanks to dramatic improvements in reliability, cost, and size. Similarly, high-performance liquid chromatography and gas chromatography autosamplers produce unprecedented levels of precision, and basic laboratory automation can

perform most elements of sample preparation. The next step will be for advances in microfluidics to shrink the chromatography lab itself down to the size of a microscope slide! To make matters worse, routine analytical testing has become an outsourced commodity. Given all these changes, how can today’s analytical scientist stay inspired or attain more recognition? I believe the key lies in providing true value to one’s organization, which all begins with understanding the company, its products, and its core technologies.

The workplace has changed dramatically since I started three decades ago. The rigid silo-oriented organization has been replaced with a more collaborative model, creating opportunities that were not apparent in the past. But to collaborate effectively, we must have a broad understanding of the business and function as an ambassador of analytical science to others, particularly non-analytical staff. Whether in research and development or quality assurance/control, problems happen and they need to be solved – quickly. We must remember that we are scientists first and that our instruments are simply the tools of the trade; just as we expect our auto mechanic to diagnose a mechanical problem without bogging us down in a detailed treatise on the merits of a ratchet wrench, we should understand when our colleagues

*“We must have a broad understanding of the business and function as an ambassador of analytical science.”*



need detail or an overview. Debating over theoretical plates and producing data without recognition of its use or consequence do us no favors.

*“Who better to contribute to the design of experiments or mentor the project team on data interpretation than the analytical scientist who understands the method intimately?”*

Often, experiments are designed without consideration of measurement uncertainty. As a result, all subsequent mathematical analysis of the data may be utterly meaningless. Sometimes the wrong parameter is measured, leading to a similarly useless outcome. Albert Einstein once said, “Not everything that counts can be counted, and not everything that can be counted counts”. Who better to contribute to the design of experiments or mentor the project team on data interpretation than the analytical scientist who understands the method intimately?

Beyond the more obvious contributions above, many other paths can help us add value. Technical support, process development, and regulatory affairs may at first glance seem like a departure from analytical science, and I would agree they are a step away from the traditional scientific career path; however, they offer noteworthy contributions to the company and

provide a different perspective of the business. Experience and training as an analytical scientist are invaluable. For me, a two-year development assignment in regulatory affairs was an absolute eye-opener. It gave me a much greater appreciation of the challenges faced by regulatory affairs personnel and their response to seemingly simple questions from regulatory agencies. Similarly, stepping out of my insular analytical laboratory into a cross-company technical support department has allowed me to gain experience in a much broader variety of products and technologies, and exposed me to many more analytical technologies than if I had focused solely on chromatography. Knowledge gained in one project has been subsequently useful in other projects, underscoring the need to continue to grow intellectually. I urge analytical scientists to embrace the learning of new skills and take advantage of in-house training opportunities. For example, becoming Six Sigma and LEAN certified has enabled me to communicate more effectively with my engineering counterparts – though I already knew much of the material, scientists and engineers often speak different languages.

Stepping outside the bounds of analytical science is not for everybody, and I’m not suggesting that we must leave the field to advance our careers, but we must recognize and acknowledge that the practice of analytical science is changing. The first step on the path to extinction is choosing not to evolve. There is no job security in performing a routine, easily outsourced task. By using our analytical science background in its broadest sense, we can enhance our value to our organizations, lose our cloaks of invisibility, and even enjoy a greater sense of career satisfaction as a result. ■

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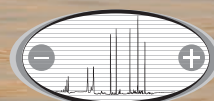
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# Multi-dimensional Marvel

**GC×GC provides simple improvements today and will be key to the personalized miniature measurement devices of tomorrow.**



*By Robert A. Shellie, Australian Centre for Research on Separation Science (ACROSS), University of Tasmania, Hobart, Australia.*

Following one of my recent presentations, an observer commented that while she enjoyed the talk and agreed that GC×GC is especially suited to analysis of complex mixtures, she felt generally overwhelmed by its complexity. She is not alone: while improved resolution sounds good to just about everybody, there is a widespread perception that multidimensional separations are more complex than “regular chromatography”. I want to take a few moments to debunk this complexity myth.

GC×GC is short-speak for comprehensive two-dimensional gas chromatography. Put simply, GC×GC uses two GC columns and harnesses the resolving power from both columns to provide improved separation. Recently, my team has been working on an approach that uses multiplexed detection and three separation columns. We configure the modulator in such a way

that the instrument essentially comprises two parallel GC×GC column sets. What sets this apart is that both GC×GC column sets use the same detector.

A decade ago, this would have been complicated; in fact, any GC×GC was. Many users relied on homemade modulation systems and it could be a real battle to interpret results: there are stories of PhD students manually counting peaks and throwing in the towel after reaching a couple of thousand. Luckily, instrumentation and software advances mean that neither construction nor data interpretation are challenging today. Modern instruments are essentially walk-up: the most complex step is column installation.

Of course, a few lessons on GC×GC theory never go astray. I recall grappling with the concept of modulation for about a week in my early learning days, but using GC×GC to tackle new challenges has given me a great deal of satisfaction for more than a decade now and I don't intend on stopping soon.

A non-exhaustive review of GC×GC papers published so far this year reveals a broad spectrum of applications that benefit from GC×GC analysis. Food/flavor/fragrance continues to be a field heavily using GC×GC, accounting for more than 30 percent of the published papers. Those associated with environmental contaminants account for 23 percent, with the combined areas of fuel and industrial chemistry making up a further 24 percent. Importantly, around 12 percent of the papers already published this year are associated with GC×GC theory or instrumentation and software advancement. This is a good indicator of a healthy field. The balance of papers can be classified as forensics, geochemistry and metabolomics/biomarker discovery, plus one fascinating investigation looking at the effect of the “shark necronome” on feeding behavior. As a resident of

Australia, I will follow this study, which reports the potential discovery of a shark repellent, with keen interest!

Regarding our own recent investigations into multiplexed detection for parallel GC×GC, there is great benefit in obtaining three independent sets of retention data (or retention indices) in a single chemical analysis. GC×GC is good but it cannot separate everything, so the possibility of adding additional stationary-phase chemistry to further tease out resolution is inviting. Hyphenating GC×GC with fast mass spectrometry (MS) is very common, but having two MS instruments connected to a GC is not a viable option for most laboratories. Multiplexed detection makes our three-column system MS-compatible.

It has been reported in the literature that comparing three complementary retention indices against tabulated data for an “unknown” analyte can provide strong enough evidence to assign identity – even without recourse to mass spectrometry. We are enthusiastically exploring the GC×GC / GC×GC-MS nexus using our new multiple-column approaches. Thus, while our colleagues in MS laboratories claim to be moving ever closer to eliminating chromatography, we shall continue our investigations, which we believe will alleviate our dependence on mass spectrometry.

It is not that I dislike mass spectrometry, but if we are aiming for a future filled with personalized measurement devices, multidimensional separation offers great potential for miniaturization. Chemical analyses performed by hyphenated techniques are structured so they alleviate the need for high resolution in any single analysis dimension. In a way, we are shrinking the chromatographer's triangle of compromise, achieving speed without loss of separation. ■



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## Ears Wide Open

**The best clinical diagnostic tools must balance accuracy with cost and ease-of-use. Listen, and get a full understanding of the needs in your field: it's not just about the science.**



*By Yaroslav Faybisenko, co-founder of Pixie Scientific, New York, NY, USA.*

Have you ever wondered how you can use what you know as an analytical scientist to make an impact on people's lives? I have and, fortunately, I found that the first step is easy: listen to people in need. Better yet, befriend a professional who speaks with many such people regularly. In my case, this was a clinician who told me stories of trial and error for analyses and therapeutic decisions that made my head spin. Clinicians have batteries of tests at their disposal to guide decisions and to monitor patients, it's true, but conversations invariably raise complaints about test inadequacies, poor ergonomics, and high lab fees.

A typical scientist's reaction to hearing this is to claim, "I can do the same thing more accurately" or "I can do the same thing cheaper." But before you rush to develop new analytical technology that improves things on one axis, delve more deeply into the real world; listen to your clinician friend a little more intently.

Many of today's practices are

products of circumstance. A diagnostic technology that once offered a big leap in accuracy can become a gold standard for treatment decisions, but as new risk factors are uncovered by research and the at-risk population balloons, the same technology may well be too expensive and unwieldy to use as a screening method.

A great example of this is colon cancer, where colonoscopy is the gold standard for detecting both cancer and pre-cancerous lesions. Colonoscopies are expensive (and only a fraction of those at risk comply). Home screening tests do exist – the fecal occult blood test (FOBT) and fecal immunochemical test (FIT) – but they detect late-stage cancers, not pre-

*"But before you rush to develop new analytical technology that improves things on one axis, delve more deeply into the real world"*

cancers. The correct diagnostic – the real gold standard – is a cheap, easy and accurate test for precancers. It would help to significantly reduce mortality and the cost of treatment.

A different problem is exemplified by prostate and breast cancers. For these disease, cheap and easy-to-use biomarker-based screening tests are widely-available. Unfortunately, in too many cases they are unlikely to result in care that changes outcomes. More accurate tests are costlier and more difficult to perform as they require tissue samples.

*"An approach that has not yet been well explored is putting screening and monitoring tests in hands of the people."*

There are tests in development for these cancers that will provide a better balance between accuracy, usability, and cost. Throughout medicine, the same needs apply: to catch signs of disease or acute injury earlier, when progression may still be arrested, and to do so without burdening the healthcare system's already stretched resources.

An approach that has not yet been well explored is putting screening and monitoring tests in the hands of the people. Until the recent advent of portable separation and analytical technologies, testing products for consumers were seen simply as sample acquisition (at best, sample preparation) devices ahead of the lab. Additionally, clinicians hold a legitimate fear that personal testing would lead to people making their own treatment decisions, misinterpreting results and stampeding into the healthcare system, or, worse still, misinterpreting results and gaining a false sense of security.

By fully exploring the world of clinicians, we are able to learn more than just the need for better testing; we can discover the fears and anxieties that come with the territory of medical practice. Learning as much as we can about the clinical world is the key to a truly successful contribution. ■



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## Omega-3 Odyssey

**Are the health claims for these compounds warranted and, if so, can their measurement be streamlined?**



*By Giuseppe Astarita, Principal Scientist,  
Metabolomics & Lipidomics at Waters  
Corporation, Millford, MA, USA.*

You have probably noticed during a regular visit to your local grocery store that many common food products, such as bread, cereal, milk, and eggs, are 'rich in omega-3s'. And perhaps you have a friend who is taking omega-3 supplements?

Omega-3s are polyunsaturated fatty acids (PUFAs), a class of lipids characterized by a carboxylic group, an aliphatic chain, and multiple double bonds. They are named according to the position of the first double bond in the carbon chain, starting from the terminal carbon atom of the molecule (called the 'omega carbon' because omega is the last letter of the Greek alphabet). The other type of PUFAs are omega-6s.

The importance of PUFAs to human health is that the overall balance between omega-3 and omega-6 species seems to modulate many biological processes including the relaxation and contraction of smooth muscle tissue, blood coagulation and, most notably, inflammation. Some long-chain omega-3s are found to be particularly enriched in the brain and retina, playing a major role in cognition and vision.

Various methods have been adopted to measure omega-3 levels in biological and food samples. Historically, gas chromatography-mass spectrometry (GC-MS) has been the technique of choice. Analysis by GC, however, requires a multi-step procedure for the hydrolysis and derivatization of the PUFAs to fatty acid methyl esters. Alternatively, liquid chromatography (LC)-MS and tandem MS analysis allows the direct measurement of both free and esterified PUFAs without the need for hydrolysis or derivatization. The less abundant oxygenated PUFAs are usually measured by LC-tandem MS after a solid-phase extraction purification step.

Supercritical fluid chromatography-MS and, more recently, ultra performance convergence chromatography-MS have been adopted for the analysis of PUFAs. These technologies have the advantage of reducing the use of toxic organic solvents and facilitating the purification process. Other non-chromatographic techniques utilized for PUFA analysis include desorption ionization-MS, ion mobility-MS, Raman spectroscopy and nuclear magnetic resonance (NMR). These solutions, although not as sensitive, do offer the potential for real-time analysis.

Within each omega family, there is a subclass distinction: short-chain and long-chain PUFAs. The human body cannot manufacture short-chain PUFAs and must rely entirely on dietary intake for these essential nutrients. Long-chain PUFAs, on the other hand, can be made by the body starting from a shorter chain or can be absorbed directly through diet.

The short-chain omega-3 fatty acid that is most abundant in food is alpha-linolenic acid (ALA), which is present at high levels in leafy green vegetables and flaxseeds. The most abundant dietary long-chain omega-3 PUFAs are eicosapentanoic acid (EPA) and

docosahexaenoic acid (DHA), which are present in oily fish and fish oil supplements. Omega-6 PUFAs mainly include the short-chain linoleic acid (LA) and to a lesser extent the long-chain arachidonic acids (ARA), which are abundant in vegetable oils, such as corn, soybean, safflower and sunflower oils (<http://ndb.nal.usda.gov/>).

Once absorbed in the intestines, PUFAs are processed in the liver and transported through the blood to all tissues. Here, PUFAs can be found as free fatty acids, esterified to complex structural lipids, such as phospholipids, or metabolized into bioactive oxygenated species. Overall, from just a handful of omega-3 and omega-6 precursors, there are hundreds of derived species in our tissues.

Most Western diets are deficient in omega-3s and abundant in omega-6s. Current nutritional research shows that a diet enriched in omega-3s offers health benefits and anti-inflammatory properties, whereas an excess of omega-6s might contribute to the pathogenesis of many chronic inflammatory diseases, including cardiovascular and autoimmune diseases. This evidence is gradually impacting dietary habits, food production and marketing strategies. In the future, food labels may be required to display detailed information about the content of omega-3 versus omega-6 PUFAs, as well as short-chain versus long-chain PUFAs, to facilitate the comparison of food products and help consumers in their food choices.

Consequently, the development of rapid and inexpensive assays for screening omega-3s and omega-6s represents an opportunity, not only for labeling foodstuffs but also to assess nutritional deficiencies or imbalances. Personalized nutritional interventions aimed at balancing omega-3 and omega-6 might help improve the overall health. ■





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# Towards Tsunami-Resistant Chemometrics

Despite a fall from grace in recent years, chemometrics has a key role to play in the interpretation of mega-variate datasets. Here, I diagnose the problems that the field currently faces and propose that developing a theory of chemometrics offers a way forward.

*By Lutgarde Buydens*





The ever-increasing volume of information generated by hyphenated analytical platforms requires science – and scientists – to adapt or be drowned by the deluge of data. One development, actually a new science paradigm, that puts the analytical sciences in the driving seat of scientific research is so-called ‘data driven’ research: increasingly, analytical measurements are performed to generate hypotheses rather than to confirm them. However, to live up to the promise of data-driven research, powerful chemometric data analysis is essential and, at the moment, this is sadly lacking.

In an era where tsunamis of data are flooding the scientific world, it is painfully apparent that the development of data analysis methods has not kept pace. The standard workhorses of chemometrics, such as principal component analysis (PCA), which were designed to cope with multivariate data, are cracking at the seams under the pressure of mega-variate datasets originating from comprehensive molecular profiling, biobank samples, sensor technologies and so on. Chemometricians are not coming up with sensible answers to questions about these kinds of data.

Another striking shortcoming of chemometric data analysis is the lack of underlying generic strategies for workflow. In practice, each individual dataset currently requires its own analysis research project to cope with its peculiarities, which originate in measurement methodologies as well as in sample or data types.

These issues have brought us to an unprecedented state of affairs. Chemometrics, which has always been in demand for the study of larger and more complex datasets, is now inadequate and underappreciated, floundering in the wake of the data tsunamis. It is high time that action was taken to keep the field afloat. Here, I analyze and diagnose the situation, and then propose a plan of action.

## Diagnosis

Let’s first note that chemometricians are not the only ones struggling with the data tsunami. Computer scientists, too, have ‘big data’ problems and are working furiously on solutions for managing and sharing large amounts of scientific data while maintaining data integrity. We must follow their efforts closely.

On analyzing the current state of chemometrics, a few immediate conclusions can be drawn.

First, explorative analysis must be upgraded. We have always taken for granted the explorative power of PCA, our basic tool, but it is just not up to the task of exploring mega-variate datasets.

Second, we cannot assume linearity, the very assumption that enabled us to develop the powerful and robust methods to analyze moderately complex nonlinear behavior. While the assumption remains a valid approach to ‘classical’ multivariate

datasets, whether it applies to mega-variate data over broad scales is, at best, an open question.

Third, there is an urgent need for new methods and strategies that combine data from different sources; an example would be the association of images and molecular profiles measured over different timescales. Data from a whole host of disparate platforms, including unstructured data, such as text, need to be integrated.

Fourth, we need to develop a ‘chemometric theory’. This is urgent. Chemometrics evolved, for various reasons, as one hundred percent empirical science so, unlike (applied) statistics we don’t have an underlying theory to fall back on. However, we now need one, or at the very least a general strategy, to make chemometrics tsunami-resistant.

## Explorative analysis

The first step of data analysis is explorative analysis. If we can’t reveal the essence of the data in a simple plot and access easy tools to explore further ideas, we can forget about the generation of new hypotheses. PCA is the jack-of-all-trades of chemometrics for explorative analysis. Its basic principle is simple and powerful: that which causes the largest variation in a dataset is most relevant, and identifying it reveals the essence of the data.

With mega-variate data, however, this principle stumbles. Measurements are now often performed to search for the so-called ‘needle in the haystack’. In biomarker discovery, for instance, the principle of ‘largest variance is most important’ has no value; rather, most of the variation is due to uninteresting causes and an explorative PCA plot reveals nothing of value, as can be seen in Figure 1a.

To extract the needle in the haystack, projection techniques that use alternative criteria, such as independent component analysis (ICA), have been explored (for an overview, see Reference 1). While sometimes successful, these criteria are artificial and often computationally-intensive, and have never truly taken off. A recent and interesting approach is sparse PCA (2), in which one tries to find a loading vector with many zeros that still explains a large part of the variance; this makes interpretation much easier. However, it is clear that no general projection method will reveal the essential structure of information in all cases.

A much more sensible approach would be to focus first on removing the uninteresting variation. This requires knowledge of at least some of the sources that causes the irrelevant variation, which brings up a key point that has been recognized for some time in chemometrics: incorporating prior knowledge is a key issue in exploring huge datasets. The principle is, however, easier to advocate for than to apply. Yes, application of constraints in analysis is a well-known approach; in curve resolution techniques, for constraints of non-negative concentrations, for



the shape of chromatographic/kinetic profiles and for many other factors, it is well established (3). But for explorative analysis hardly anything has been achieved so far.

While explorative analysis implies that not much is known about the data, additional knowledge is often available in the form of gender, disease state, batches, instrument or measurement conditions, and so on. Recently, a promising approach has emerged that exploits this kind of knowledge in explorative analysis: the combination of PCA with analysis of variance (ANOVA). ANOVA is a basic statistical technique that separates variation in data that is caused by different sources (called 'main effects' in the ANOVA jargon) and their possible influence on each other ('interaction effects' in ANOVA jargon). The basic idea is simple: first, separate the variation in the data according to the different known sources, with ANOVA, and then analyze the interesting parts, using PCA.

*ANOVA models the total variation in the dataset:*

*Total Variation = Mean +  
Variation due to the different main  
effects (sources) +  
Variation due to the different interaction  
effects +  
Residual Variation (not due to any of the  
known sources)*

This approach allows separate analysis of interesting parts of the variation; alternatively, non-interesting variation can be removed before PCA analysis (see Figure 1b). It is especially attractive because it combines cornerstone methods from chemometrics and statistics. Several variants have been proposed which differ mainly in the specific aim of the analysis (4-6).

When more structured information is available this too can be used. For example, the critical information for biomarker discovery is a change from a basic state, in which there is normal but wide variation, to a state in which specific variation is added or removed due to a change, such as in metabolic activity. The detection of changes is open to many discriminant methods although the large amount of 'normal' variation can hamper discovery of the sought-for differences. One recent method, orthogonal partial least squares (O-PLS) (7) aims to solve this problem by removing the uninteresting data on the basis that that it is not correlated with, and is thus orthogonal to, class membership. This approach is meeting with some success.

*"Measurements are now often performed to search for the so-called 'needle in the haystack'. In biomarker discovery, the principle of 'largest variance is most important' has no value"*

Another new and promising idea models the basic state variation by means of PCA and subsequently projects the other interesting data into this model (8, 9). Part of the variation will be explained by the basic state PCA, but it is the residual, non-explained part that contains the information on the differences from the basic state. This is a powerful approach for detecting minor differences in explorative situations. It is actually the application of a principle that has been used for a long time in the field of industrial process control. Here, the basic, normal state of a multivariate industrial process is modeled into the so-called normal operating conditions (NOC) with PCA. During operation, the process is monitored by projecting the actual state vector into the NOC-space. When this state vector fits nicely into the NOC, everything is okay; when the fit decreases and the residuals increase, the process is 'out of control' and the residual

variation provides clues to the possible process faults. In the same way, the normal operating conditions (NOC) of the comprehensive -omic profile of healthy (basic state) people, cells or any similar thing can be modeled by PCA. Analysis of the residuals contains clues to the differences in the diseased state. This approach has been successfully applied to detect and diagnose rare metabolic diseases in children (8). The idea of analyzing residuals from well-described states in a focused way is quite new to our field and there is ample room to further elaborate upon it or to generalize it for more complex situations (9).

The above approaches work best when the data are obtained in a well-designed way. While interesting results can be obtained in 'dirtier' situations, it requires further research. Current approaches are far from perfect but they do illustrate that one of the keys to breakthroughs in explorative analysis is exploitation of prior knowledge. Much research is still required and novel methods will be welcomed for data that are not well designed.

### Nonlinear behavior

Linear models are attractive because their behavior is well studied and understood, and because confidence intervals can easily be constructed to validate their performance in different situations. They also require relatively few data to construct them robustly. Even when the data do not follow linear behavior perfectly, the use of linear models is often preferred for these reasons. In practice, the domain of interest is often split up into smaller parts,

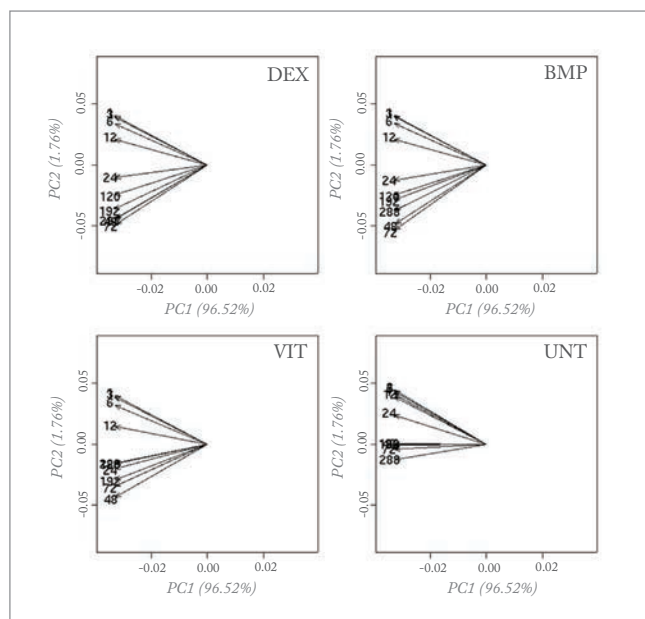


Fig 1a: Loading plot of a PCA analysis on a genomics (osteogenesis time series) dataset, investigating a time effect under different treatments of stem cells; the four planes represent four different treatments and the arrows represent the time points. No differences can be seen in the four treatments when analyzing the whole dataset.

where a linear approximation is valid, allowing the use of safe and well understood linear models. Linear regression and PLS are therefore used in the majority of chemometric research studies.

However, linear models are simply not sufficient for the analysis of large datasets. This has been recognized for some time and a good deal of research has been devoted to developing better systems (10). At one point, neural methods were considered to be the nonlinear method of the future but the realization that they behave unstably has made them considerably less attractive. Among the most powerful methods today are the so-called kernel methods, such as support vector machines (SVM) and Kernel PLS. In these methods the data are transformed in a 'feature' space, usually of higher dimension, in which linear separation is possible (11). The distance methods, successfully applied in social studies and only recently brought to the attention of chemometricians (12), can be considered as kernel methods too. In these analyses, it is not the data themselves but a distance matrix calculated from the data that is analyzed, using a linear method such as PLS. Walczak showed that with a simple Euclidian distance, noteworthy nonlinear separation problems can be solved.

The major drawback of kernel methods is that they are 'black box' models in which information on the important variables is lost. This makes them useless for projects such as biomarker discovery. While samples can be projected into the model and classified, and properties accurately calculated, exactly which variables contribute to the classification or the value of the property under investigation remains unknown. Until this problem is resolved these otherwise powerful kernel methods

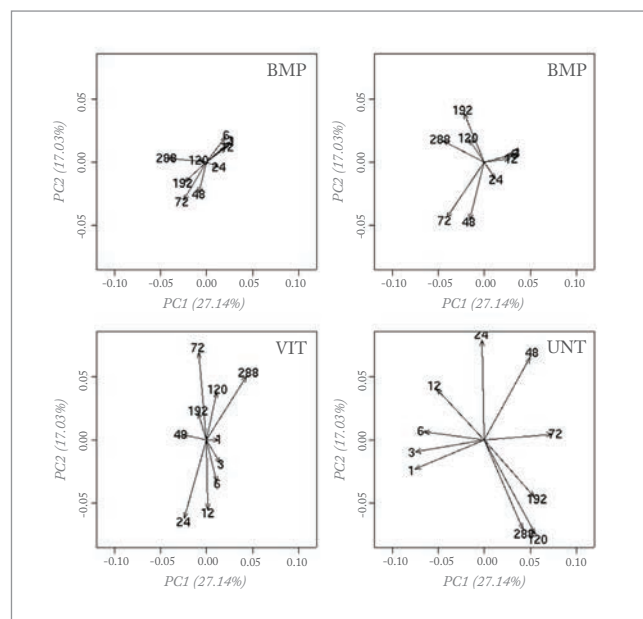


Fig 1b: Loading plot of a PCA analysis of an interesting interaction effect obtained by an ANOVA analysis of the Fig 1a dataset. In this analysis, clear differences between the treatments can be seen; for more explanation, see reference 6.

will not have the utility that they deserve in chemometrics. Recently, an earlier idea from Gower (13) has been exploited to disclose variable information from the kernel model (14, 15). This is the concept of the pseudo-samples or 'spy samples', which are artificial samples that carry all their weight in one variable, with the other variables being set to average. When these pseudo-samples are projected in the kernel method they reveal the behavior of the variable for which they carry weight. This can be visualized in a very intuitive way and, while the approach is still its infancy, it has already been applied in complex metabolomics studies (see Figure 2) (16).

## Fusion of data

To acquire a comprehensive molecular picture of a complex system such as the metabolome, a combination or hyphenation of multiple analytical techniques is needed; no single measurement principle can capture all of the molecular diversity and concentration range of the components. There is therefore an urgent need for data analysis approaches that integrate data across platforms and modalities (such as images and profiling methods) and that can even incorporate text data. Several strategies have been proposed for this, which can be divided into low-, mid-, and high-level fusion.

In low-level fusion the different datasets are simply concatenated. For high-level fusion, a separate model is constructed per dataset and it is the outputs of these models that are combined. In mid-level fusion the most interesting features, extracted from each dataset separately, are combined to build the final model. A further approach to data fusion, focused on

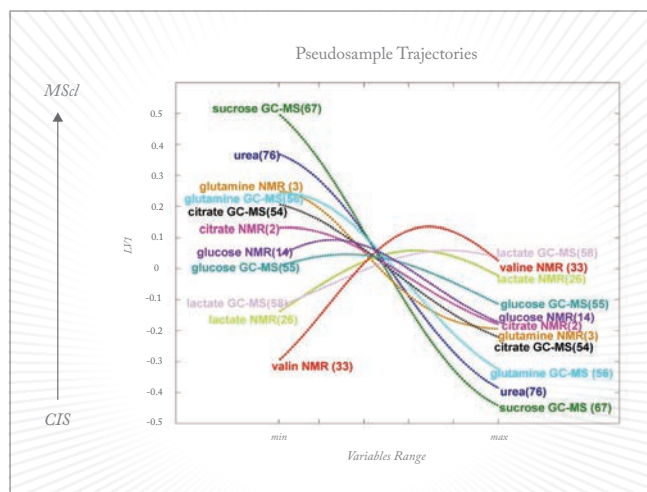


Fig 2: Pseudosample trajectories in a metabolomics study to distinguish clinically isolated syndrome of demyelination (CIS) from multiple sclerosis (MScl). Each pseudosample trajectory reveals the behavior of one metabolite in the two diseases; for more explanation, see Reference 15.

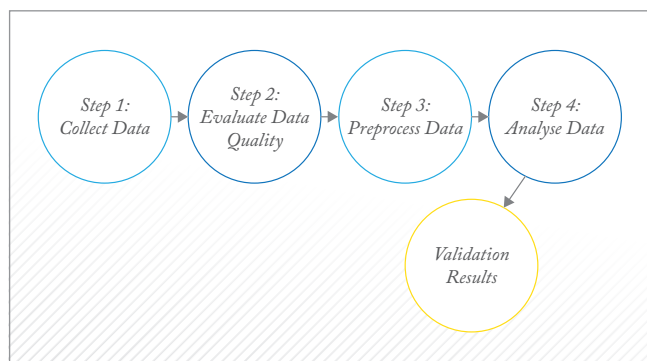


Fig 3: Chemometric workflow.

regression problems, is the so-called multi-block method.

The success of data fusion can be quite variable: sometimes it outperforms the individual datasets while on other occasions the results can be disappointing. This unpredictability in outcome is down to two major factors. First, fusion strategies are almost always linear and, as noted above, linearity cannot be assumed a priori. Some efforts have been made to combine data nonlinearly, by combining kernels of the data rather than the full datasets themselves. This is a powerful method for prediction but the 'black box' aspect is a drawback. The concept of pseudo-samples too is also quite promising, as demonstrated in a recent complex study of multiple sclerosis (16). Second, it is apparently impossible to predict or foresee which combination of datasets will be successful. The obvious criteria – correlation with the property under investigation and the amount of mutually

exclusive information in both datasets – are not always adequate; in some cases, a dataset uncorrelated with the property under investigation and highly correlated with the first dataset can unexpectedly and drastically improve performance. This imparts to the whole procedure a sense of trial-and-error, and makes it cumbersome and time consuming. Methods to overcome these issues are urgently required.

## Chemometric Theory

Unlike (applied) statistics, chemometrics has no underlying theory: it evolved as a fully empirical science in which each dataset is almost considered to be a separate project. There is a recognizable workflow for chemometrics, which is illustrated in Figure 3. Within each box, however, preprocessing or methods of analysis are selected largely based on previous experience and their performance for the problem at hand. Ideally, the problem at hand should be related to a more general situation and from there the strategy or workflow should be streamlined.

Sometimes, methods such as preprocessing are specially designed for a specific situation. This does not imply, however, that they will automatically work optimally in similar situations because artifacts, such as base lines, are often instrument- or even environment-dependent, turning each dataset into a unique problem. This leaves no other choice than an empirical trial and error approach for each dataset. But the consequence of the myriad methods developed, each solving a specific problem, is that inexperienced users are totally confused. Taking the example of preprocessing, this chaos is described in work by Engel et al. (17): for what is a straightforward classification problem based on a simple spectroscopic dataset, there are several thousand reasonable preprocessing methods available, all of them published for a similar dataset and problem setting. When these methods are applied by inexperienced but scientifically-sound users, the results are truly astonishing, as shown in Figure 4. Each dot represents a specific 'reasonable' preprocessing according to two performance criteria: the classification performance and the model complexity. What might be described as 'reasonable' or 'previously successful' is no guarantee of success with the problem at hand. This chaos is, I venture, the main reason that chemometrics does not get the consideration that it deserves from analytical scientists.

The complexity of the situation does not, however, exempt us from trying to find structure in this apparent chaos. If we, as chemometricians do not succeed in at least partially solving this problem, we cannot expect to survive the data tsunami. The good news is that there is already one part of the data workflow in which progress has been made, namely validation. We can rightfully be proud of our achievements and attitude towards the thorough and



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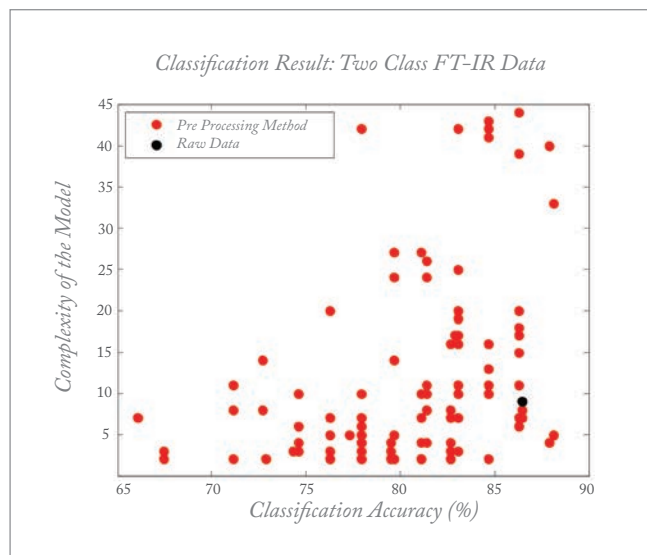


Fig 4: Effect of different preprocessing methods on performance (classification accuracy and model complexity) in a simple discrimination problem. Each dot represent a reasonable preprocessing method; the black dot represents analysis on the raw data (without preprocessing) For more explanation, see Reference 16.

independent validation of results. We must stay focused on this, especially on the validation of explorative methods.

The main reason that chemometrics has ended up in a tricky situation is that chemometricians have dared to tackle the difficult 'dirty' problems that don't fit nice statistical distributions or theories. Chemometrics emerged where statistical theory was no longer applicable, a fact that has been recognized by statisticians (18).

This is not a unique situation; I would make the comparison with medicine (as with all comparisons it hobbles, but it is thought-provoking). Underlying biochemical and physiological theories provide the basis of medical sciences. A clinician, however, has to treat individual patients whose symptoms are unique. These symptoms are probably related to an underlying biochemical or physiological problem but are co-influenced by a myriad of internal and external factors, making the exact appearance of illness specific for every patient. Despite this complexity, medical diagnosis and treatment have emerged as medical sciences.

A similar kind of chemometric theory will allow a much more structured and logical approach to the analysis of complex data. Better diagnosis and understanding of the underlying issues will enable the selection of a more efficient treatment. Moreover, better understanding of data and their peculiarities will help in one other aspect that is of increasing importance, namely the prevention of scientific fraud. The analysis of data is especially prone to fraud. I am convinced that with a chemometric theory and with our attitude to validation, we can contribute to the

development of a general strategy for fraud prevention.

The development of a chemometric theory will be an important, if not the most important, step towards tsunami-worthy chemometrics.

*Lutgarde Buydens is at Radboud University Nijmegen, Institute for Molecules and Materials, Analytical Chemistry, in Nijmegen, The Netherlands.*

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# The Origins of Mass Spectrometry

Between 1900 and 1940, the development of the positive ray analyzer led to the study of isotopes for the first time, setting the stage for the development of mass spectrometry. Here, a series of brilliant experiments from the “Era of the Physicist” is revisited.

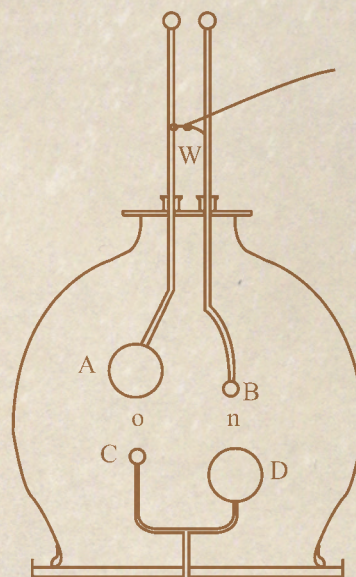
*By Mike Grayson*

This year, we celebrated the centenary of J.J. Thomson’s publication of a monograph entitled “Rays of Positive Electricity and Their Application to Chemical Analysis”. Thomson was most definitely a visionary, but the chemical community was not quite ready to embrace the positive ray analyzer, what we now know as the mass spectrometer. However, his colleagues in the physics community saw that the instrument was critically important in the investigation of the elements and their isotopes; a concept that only became accepted in the early 1920’s. Of course, much groundwork had been done by a variety of researchers prior to 1913, providing Thomson with a foundation to base his research on. Here, I examine significant developments both before and after the publication of Thomson’s monograph.



## *In the Beginning: The Gas Discharge Experiment*

The origins of mass spectrometry began with the earliest gas discharge experiments. These were conducted in a partially evacuated glass envelope in which electrodes were inserted. At an appropriate gas pressure and voltage across the electrodes, a variety of glowing light phenomena were observed. While frequently used as an entertaining display of the curious properties of electricity, a number of serious researchers were intent on better understanding the various phenomena. Originally, only qualitative observations could be recorded; how the glow in the tube changed with pressure, nature of the gas, and magnitude of the voltage.

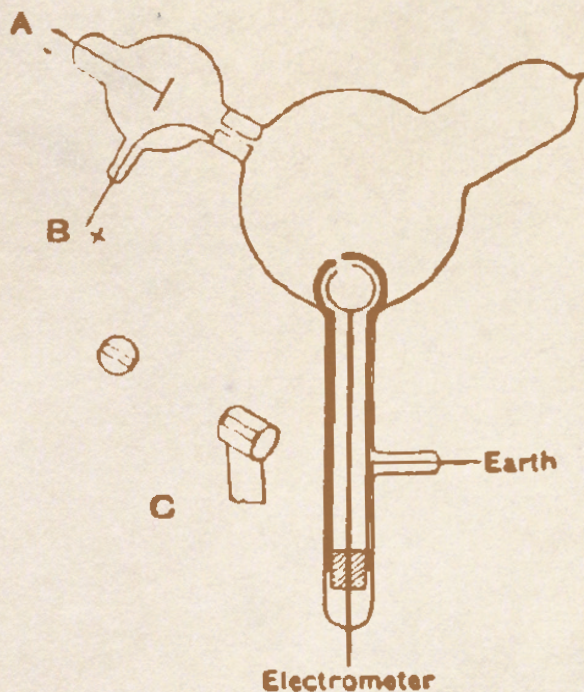


One of Faraday's apparatus for gas discharge experiments circa 1838. Adapted from: M. Faraday, "Experimental Researches in Electricity", Thirteenth Series. Philosophical Transactions of the Royal Society of London, 4 (1837-1843) 54-56 (1838).

## *Thomson and the properties of the electron*

One of the earliest scientific puzzles posed by the gas discharge experiment was whether or not charged particles were involved, or only light was produced. By experimenting with magnets and additional electric fields, researchers such as Goldstein, Hertz, Lenard, Crookes, Perrin and Wien, determined that 'corpuscles of negative electricity', or electrons, were present.

Thomson was awarded the Nobel Prize in Physics in 1906 "in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases".



Two of Thomson's apparatus, circa 1897, to determine if cathode rays were 'negatively electrified particles'. This was a refinement of Perrin's earlier work in which he observed that negatively charged particles were influenced by the presence of a magnetic field. Ref: J. J. Thomson, "Cathode Rays", Philosophical Magazine, 44(5) 293-316 (1897).



## Kanalstrahlen in the gas discharge experiment

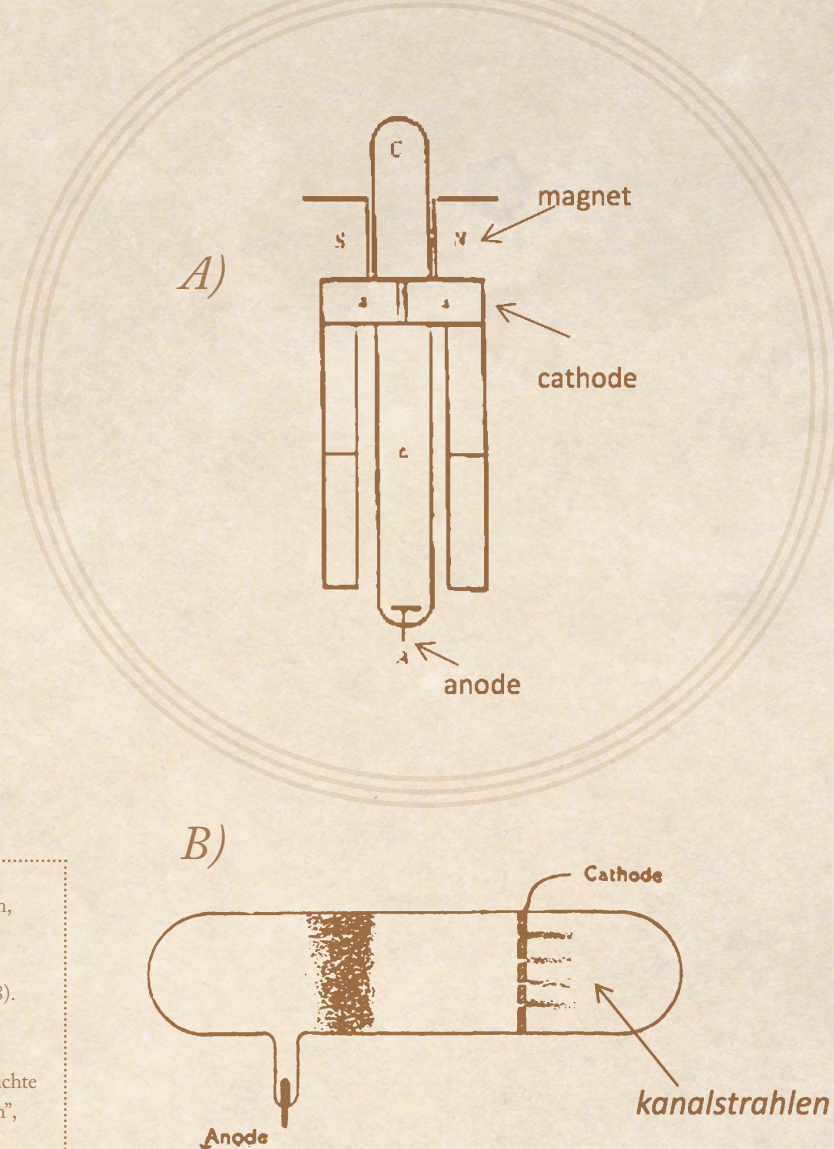
Many of the same researchers who investigated cathode rays also turned to the study of kanalstrahlen, or positively charged particles, produced in the gas discharge experiment.

At first Goldstein thought they were unaffected by magnetic fields.

Later Wien and Thomson showed that kanalstrahlen could be deflected if the magnetic field was strong enough.

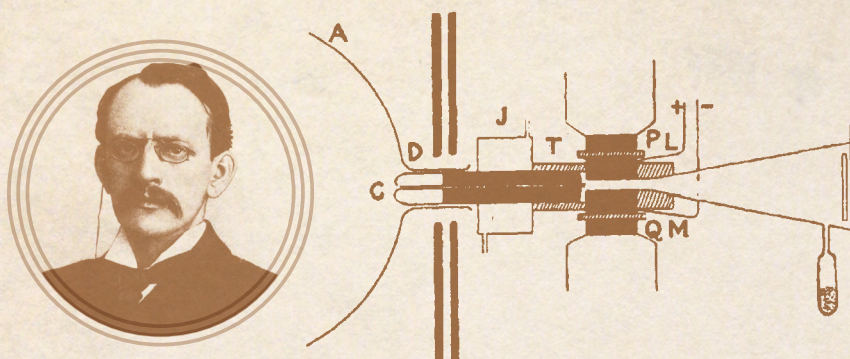
A) Apparatus Goldstein used to observe kanalstrahlen, rays of positive electricity, circa 1886. Ref: W. Wien, "Untersuchungen über die electrische Entladung in verdünnten Gasen" *Annalen der Physik* 65, 440 (1898).

B) Wien's apparatus to investigate kanalstrahlen, circa 1898. Ref: E. Goldstein, "Über eine noch nicht untersuchte Strahlungsform an der Kathodeinducirter Entladungen", *Berlin Akademie Monatsbericht*, II, 691 (1886).



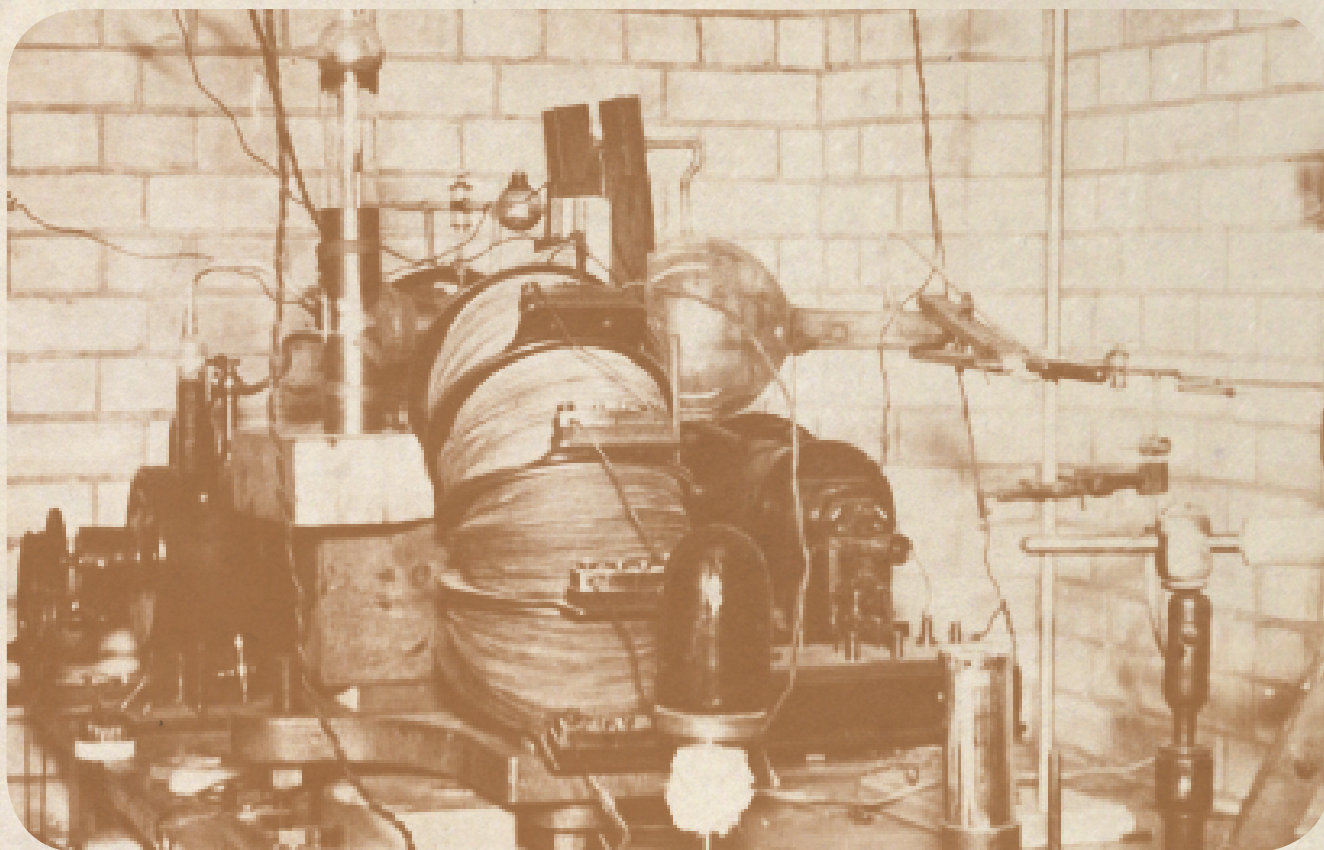
## Thomson's Positive Ray Analyzer

Thomson was able to obtain mass spectra of various gases and compounds with this apparatus in the early 20th century. While demonstrating proof of principle, it was not an easy tool to use, despite his advocacy of it in his 1913 monograph, "Rays of Positive Electricity and Their Application to Chemical Analysis".

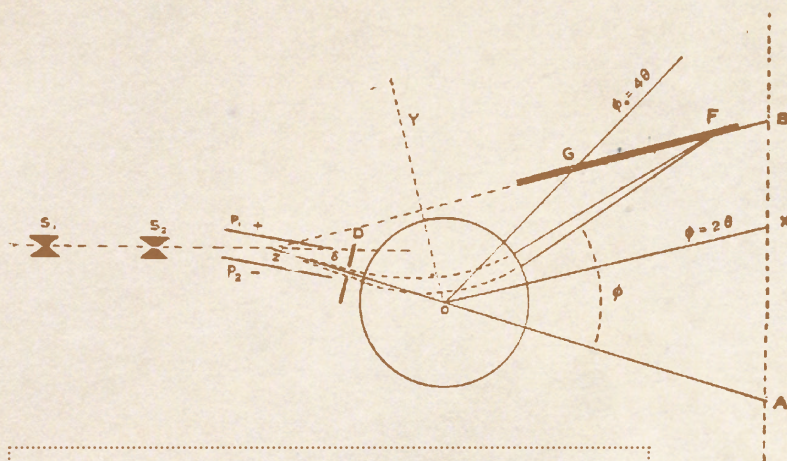


Thomson's positive ray analyzer, circa 1910. Ref: J. J. Thomson, "Rays of Positive Electricity", *Proc. Roy. Soc. London*, (A), 89, 1-20 (1913).





Thomson's positive ray analyzer in the Cavendish Laboratory in Cambridge. The glass volume in the center is where the gas discharge took place.



Schematic diagram of the elements of Aston's first mass spectrograph circa 1919. Ref: F. W. Aston, "A Positive-Ray Spectrograph", *Philosophical Magazine*, 38, 707-715 (1919).

### *Aston's mass spectrographs*

Francis W. Aston studied under Thomson and quickly realized that Thomson's mass analyzer was limited in both resolving power and mass range. He began to develop a new mass analyzer which he would improve over several decades. He still relied on the gas discharge experiment to produce ions for analysis, but was able to obtain much better mass spectra.

Aston's work with this instrument earned him the 1922 Chemistry Nobel Prize "for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"



Table of Elements and Isotopes

Element.	Atomic number.	Atomic weight.	Minimum number of isotopes.	Masses of isotopes in order of intensity.
H	1	1.008	1	1.008
He	2	4.00	1	4
Li	3	6.94	2	7, 6
Be	4	9.1	1	9
B	5	10.9	2	11, 10
C	6	12.00	1	12
N	7	14.01	1	14
O	8	16.00	1	16
F	9	19.00	1	19
Ne	10	20.20	2	20, 22, (21)
Na	11	23.00	1	23
Mg	12	24.32	3	24, 25, 26
Si	14	28.3	2	28, 29, (30)
P	15	31.04	1	31
S	16	32.06	1	32
Cl	17	35.46	2	35, 37, (39)
A	18	39.88	2	40, 36
K	19	39.10	2	39, 41
Ni	28	58.68	2	58, 60
As	33	74.96	1	75
Br	35	79.92	2	79, 81
Kr	36	82.92	6	84, 86, 82, 83, 80, 78
Rb	37	85.45	2	85, 87
I	53	126.92	1	127
X	54	130.2	5, (7)	120, 132, 131, 134, 136, (128, 130 ?)
Cs	55	132.81	1	133
Hg	80	200.6	(6)	(197-200), 202, 204

Dempster's later results (V. p. 148)

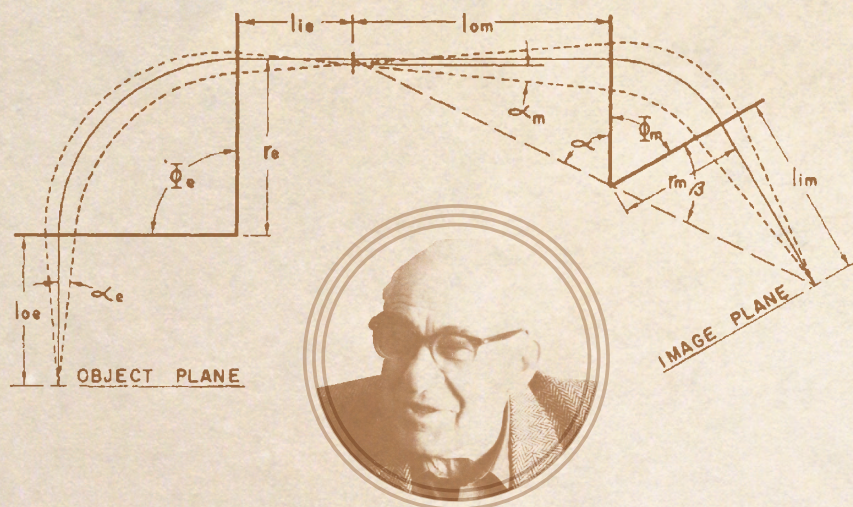
Ca	20	40.07	(2)	(40, 44 ?)
Zn	30	65.37	(4)	(64, 66, 68, 70)



### Exploring the elements for isotopes

Beginning in the early 1920s, Aston began a life-long investigation of the elements and their isotopes, determining their precise mass and relative abundance. During his career, he published almost 70 journal articles and several books on the subject. While others in Europe and America made some contributions, Aston essentially owned the field.

Taken from: F.W. Aston, "Isotopes" 1st ed.  
1922, London: Edward Arnold & Co.



The double focusing instrument provided higher resolving and improved mass measurement accuracy for the study of the elements. Commercial variations of this mass analyzer design were successfully marketed up until the 1990s. Ref: E. G. Johnson and A.O. Nier, "Angular aberrations in sector-shaped electromagnetic lenses for focusing beams of charged particles", Physical Review, 91, 10-17 (1953).

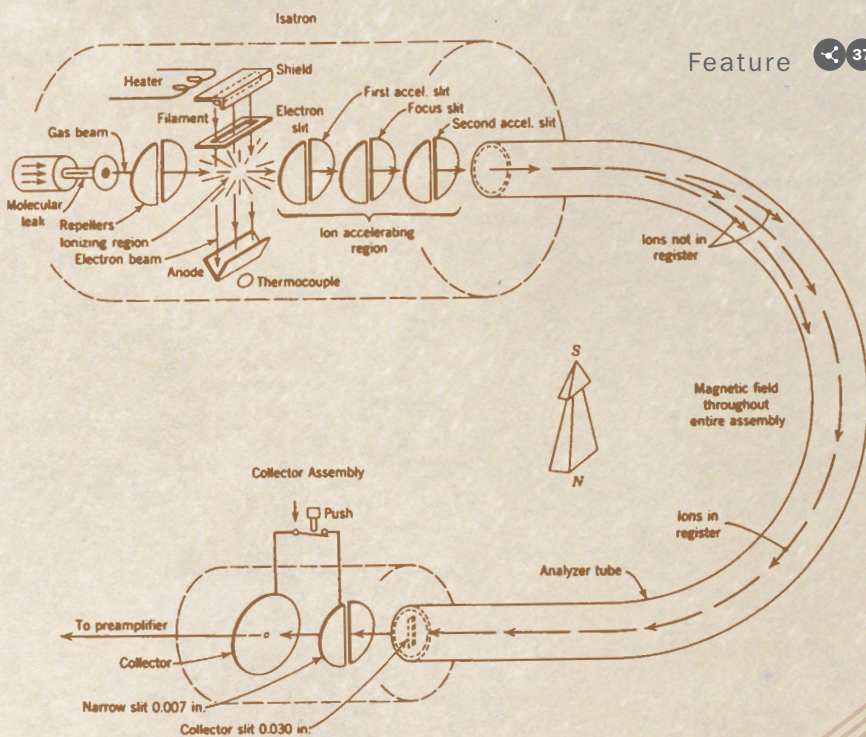
### Nier: Mass Spec Pioneer

Alfred O. C. Nier, perhaps more than any other physicist, helped to spread the application of mass spectrometry in many different scientific fields. With a strong background in electrical engineering and excellent experimental skills, he specialized in creating inexpensive, reliable instruments suitable for specific applications (isotope ratio analysis, leak detection, process control, general analytical, upper atmosphere analysis, planetary atmosphere analysis, respiratory gas analysis). He gave away his instruments and expertise to colleagues both inside and outside the physics and University of Minnesota communities.



## Dempster's mass spectrometer

A. J. Dempster, working at the University of Chicago, built a positive ray analyzer similar to that of Thomson and he too decided that a new mass analyzer was needed. His approach, the 180° magnetic sector instrument, was operational before Aston had finished his first mass spectrograph. Dempster also abandoned the gas discharge experiment as a means of producing ions and developed an ion source to produce 'slow canal rays', thus simplifying the requirements for the mass analyzer.



Schematic of Consolidated Engineering Corporation's 21-103 mass analyzers based on Dempster's design circa 1945. Taken from the Collection of instrument manuals at the Chemical Heritage Foundation, Philadelphia PA)



## Second Generation Mass Spectrometrists

A new generation of physicists rose to prominence in the early 1930s, among them Kenneth Bainbridge, Josef Mattauch, and Walker Bleakney. They developed more powerful instruments, refined the accuracy of measurements of the relative abundance and masses of the elements and their isotopes, and broadened the application of mass spectrometry to areas outside the realm of physics.

Photos: (left): Kenneth Bainbridge, Harvard University, Massachusetts, USA. (Right) Josef Mattauch, University of Vienna, Austria.

Mike Grayson is Archivist for the American Society for Mass Spectrometry (ASMS), Santa Fe, New Mexico, USA. For more information, see "Critical Mass: A History of Mass Spectrometry" ([tas.txp.to/0813-CHF](http://tas.txp.to/0813-CHF)), taken from the joint ASMS-CHF Oral Histories project ([tas.txp.to/0813-ASMS](http://tas.txp.to/0813-ASMS)).



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# Personalized Patents

## Business

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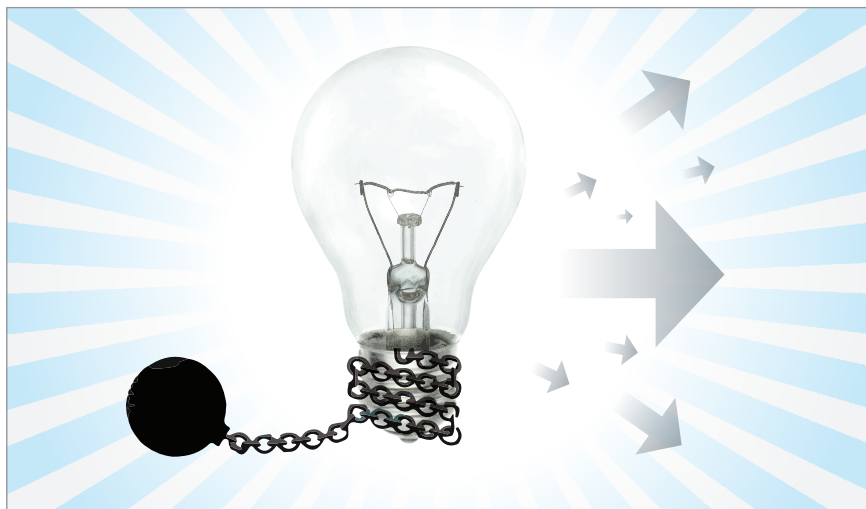
As medicine advances towards analyzing patient biomarkers to predict response to specific drugs, the centuries-old European patent system is adapting to keep pace.

*By Simon Kiddle and Hilary van der Hoff*

Patent offices across the globe are struggling to decide whether and how patents should be granted. Here, we present some patent guidelines for analytical companies involved in personalized medicine development in Europe and discuss potential challenges.

Once upon a time, it was straightforward to protect new therapeutic compounds using the patent system. Applicants applied for patents claiming new drugs or new medical uses of known drugs, and attempted to obtain regulatory approval to sell their drug for the patented medical use. The patents and any extensions provided by supplementary protection certificates then expired and generics manufacturers were free to compete with the innovator company.

Nowadays, it is common for research or clinical trials involving a therapeutic product to spark further discoveries about the group of patients in which a drug works or in which it works best. Examples of this type of personalized medicine abound in the scientific literature and patients can be defined by genotype, single-nucleotide polymorphism, epigenetic, or protein markers. Such stratification allows therapies to be selectively prescribed to patients who respond best to the treatment while avoiding treating those



who do not respond or in whom the treatment produces adverse effects. In some cases, defining the patient group means the difference between clinical trial success and failure, enabling rational design of smaller trials with high success rates in patients defined by one or more biomarkers. Drugs may be approved in conjunction with companion diagnostics (fast becoming known as “theranostics”) to better control identification of patients who will benefit most.

Patenting lab-based methods of stratifying patient groups provides one form of patent protection for such advances, but the burning question for many innovator companies is whether

they can extend patent protection for the therapeutic product itself. It's a vexed question – especially if the drug has already been described for use in an overlapping or similar population of patients compared with the patients newly identified through the personalized approach. And if this subject matter is patentable, then what protection will be available to companies and what are the real world consequences?

Under the European Patent Convention it has long been recognized that finding a further medical use of a known therapeutic agent is patentable over the earlier known medical use. This acknowledgment led to case law allowing



“Swiss” form patent claims directed to the use of the therapeutic agent “for the manufacture of a medicament” for treating the newly identified condition.

Over time, a line of increasingly liberal European Patent Office (EPO) Board of Appeal cases developed these ideas to the point where identifying a new class of patient treatable using a known drug or a new clinical situation constituted patentable subject matter. Notably, the Board in decision T1020/03 explicitly recognized that the investment in clinical trials to find new applications of therapies needed the reward of patent protection to justify it on economic grounds.

After the T1020/03 decision was delivered in 2004, the European Patent Convention was revised and one of the changes was to enshrine the basis for medical use claims in the convention. The EPO chose to do this using different claim wording (“composition for use in a method for treatment”) though publicly stated that it was intended to match as closely as possible the scope of protection provided by a Swiss-type claim.

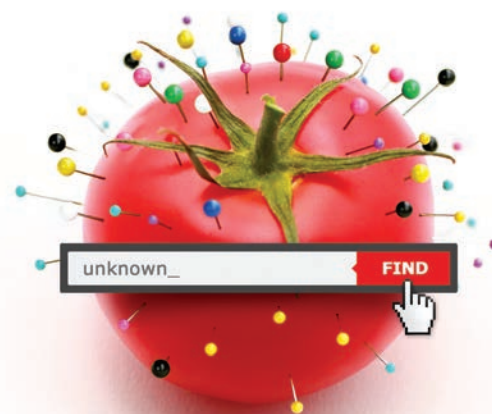
Together, the effect of these changes has been subtle, but favorable to European patent applicants in the field of personalized medicine. The new claim format defines a method of treating patients rather than obliquely referring to manufacturing drugs, and can be easily adapted to “test and treat” claims in which results from analyses identify patients as being eligible for treatment. So the change in law and the development of case law can be used to an applicant’s advantage for patent claims directed to personalized medicine by defining markers or other clinical criteria by which patients can be selected for treatment. If the markers define a subset of patients who would not have been treated without the insight obtained through the analytical step, then there is a good chance

that the method will be considered new and inventive, allowing a patent to be granted (subject to meeting all the other normal requirements for patentability).

Things get complicated where the analytical step identifies a group of patients that overlaps fully or partially with the group of patients that the drug was already intended to treat. For example, a drug may already have received regulatory approval for treating a certain disease and may have been administered to a diverse population of patients with the disease. Subsequent identification of a biomarker linked with drug response may subsequently allow more intelligent prescription of the drug by distinguishing between patient subgroups who respond best, those who do not respond, and those in whom the treatment produces adverse effects. Companies naturally wish to obtain patents reflecting this advance, including claims directed to the drug for use in treating the disease in a group of patients specifically characterized as having the biomarker linked with good response. The EPO has struggled with the question of whether treatment of the same disease in a newly-defined patient subgroup represents a genuinely new medical use of the drug, or whether such claims are merely the old medical use described in a different way and, therefore, should be rejected for lack of novelty.

Where a drug was previously described for possible use in treating a disease but no patients were actually treated, or where there was some limited use in patients but with a poor or unknown response rate, then use of the drug for treating patients who have a biomarker indicative of good drug response may be considered novel. In this situation, a patent disclosing a new and non-obvious link between a particular biomarker and a good response to the drug may be able to claim the drug for use in treating the disease in patients who have the biomarker. For example:

## Whatever is there, find it!



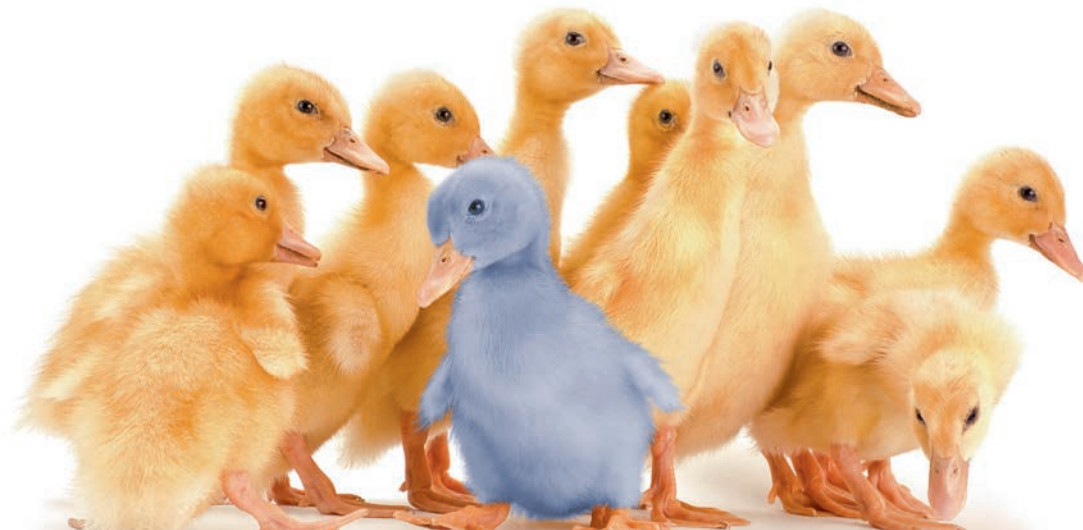
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*Composition X for use in a method for treating leukaemia in a patient, wherein the leukaemia is associated with amplification or overexpression of gene Q, the method comprising administering X to the patient.*

Recent developments at the EPO also indicate that including an active step of determining a patient's genotype (or other markers used in personalized medicine) and treating the patient on the basis of the genetic/biomarker result will avoid objections of lack of novelty. The step of testing the patient to determine the presence of the biomarker is new, even if the drug was previously used successfully for treating the same disease in the same type of patients. Claims in the following format may be allowable:

*Resveratrol for use in a method for preventing cardiovascular disease in a patient, wherein the patient has genotype Z, and wherein the method comprises identifying that the patient has genotype Z by genotypic analysis and administering resveratrol to the patient.*

And so, the age of personalized

medicine claims has been ushered into the patent system in Europe.

For innovator companies this matters a great deal, given the economic costs of drug development. It can mean that effective patent term is lengthened if the personalized medicine applications are filed some years after an initial patent covering the product or its first medical use. In many cases, the types of claim that are granted fit in well with the prescribing information that accompanies the therapeutic agent, and indeed the drug approval. This makes it harder for generics companies to escape infringement. Even by taking advantage of "skinny labeling", which allows the product label of a generic medicine to omit patented medical uses, generics will still be wary of infringing such patents. Although it is generally believed that in the UK at least, sale of a drug is only likely to be found to infringe a medical use patent if the drug is packaged with an insert mentioning the patented use, this has not been tested in the courts and there are concerns that generics companies might

still be caught by "indirect" infringement, if it is known that the drug will be used (either on- or off-label) for the patented use. Therefore, the advent of personalized medicine may allow innovator companies to push back the effective "off-patent" date of their therapeutic products.

With increasing numbers of patents being filed for medical uses based on analytical steps such as biomarker determination, epigenetic analysis and genotype profiling of patient populations, we expect personalized medicine to continue to be at the forefront of the drug patenting battleground for years to come.



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*Simon Kiddle and Hilary van der Hoff are both partners and patent attorneys at Mewburn Ellis LLP, UK ([www.mewburn.com](http://www.mewburn.com)).*

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2. Learn how a new column designed to improve SCD stability can extend the life of sulfur detector ceramic reaction tubes.
3. Profit from a review of applications that demonstrate improved capability.



Speaker  
Daron Decker,  
*Gas Chromatography Technical Specialist*



Moderator  
Rich Whitworth,  
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# Tracking Designer Drugs

Combining solid phase extraction with high-resolution accurate mass LC-MS increases sample throughput and improves detection and identification of novel synthetic cannabinoids.

By Dave Strong and Simon Hudson

## The Problem

The rapid increase of novel and variant synthetic cannabinoids designed to circumvent regulation has resulted in many unknown analytes and metabolites that must be detected in forensic and toxicological urine samples. How can we more capably detect trace amounts of these drugs in complex matrices, such as urine and blood?

## Background

In recent years, many designer drugs have become available for purchase online and in specialist shops. A subset of these compounds – synthetic cannabinoids – are marketed as herbal smoking blends under the generic name ‘Spice’. Others are marketed under the guise of incense or room odorizers but, despite often being marked as “not for human consumption”, are smoked as an alternative to cannabis. Spice products tend to profess a long list of plant or herbal ingredients and when previously analysed were not found to contain tobacco or cannabis. However, more detailed investigation of these herbal mixtures (1, 2) have identified unlisted active ingredients such as JWH-018 and CP47,497 (see Figure 1). Such drugs are classified as cannabinoids because they invoke similar physiological and psychological effects to active ingredients in cannabis, for example

tetrahydrocannabinol (THC). Many synthetic cannabinoids were originally developed by John W. Huffman's group in Clemson University, South Carolina, in the 1980s-90s to explore drug-receptor interactions targeting the CB1 and CB2 cannabinoid receptors (2, 3), but most have never been tested on humans. Whereas the side effects of cannabis are well documented, there is a general lack of information regarding the exact ingredients, purity, potency and long term effects of synthetic cannabinoid mixtures. In fact, the use of synthetic cannabinoids has been shown to cause severe withdrawal, psychotic episodes, convulsions, and life-threatening conditions in some individuals (4, 5).

As a consequence of these health risks, the sale of cannabinoids has become increasingly controlled by authorities worldwide (6). However, almost as quickly as legal restrictions are placed

upon drugs, many isomers, derivatives, and novel variants are developed to replace them, with just small changes in structure required to put molecules outside of the scope of standard detection. With a constant influx of novel molecules, there is a clear need for the ability to detect trace levels of both known and novel compounds for forensic and toxicological purposes. But how can we go about doing this?

Synthetic cannabinoids can be resolved and detected using routine gas chromatography (GC)-mass spectrometry (MS) and liquid chromatography (LC)-MS, but identification and quantitation is limited by the availability of pure reference materials. Tandem LC-MS coupled with analyte specific transitions can assist with detection where metabolites are known and reference materials are available to optimize transitions, but where reference materials are not available and metabolism

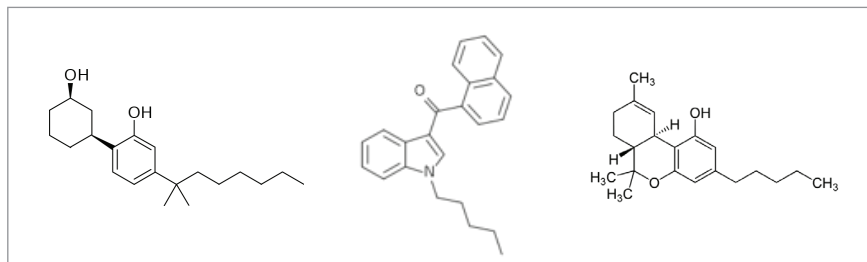


Fig 1: Structures of the synthetic cannabinoids CP47,497 (left) and JWH-018 (centre), and the structure of tetrahydrocannabinol (THC), the major active compound in cannabis (right).

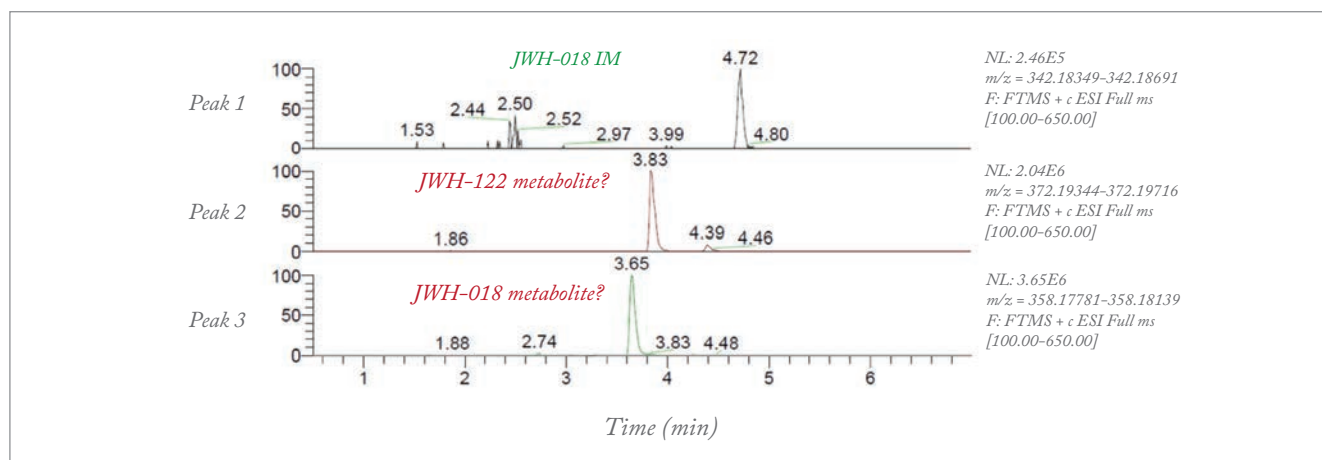


Fig 2: ToxID output from a hospital patient urine sample, suggesting two synthetic cannabinoids are present alongside the internal marker

is unknown, tandem LC-MS approaches are of less use.

#### The Solution

To combat the rapid increase in variants and novel cannabinoids that fall outside the current legislation, we have employed high resolution accurate mass (HRAM) LC-MS (see the „The System“, page 46). The use of this technology greatly improves the speed, selectivity and capacity of drug detection in urine samples from suspected users. Whereas numerous compounds of interest with differing chemical formulae may have the same nominal mass, their accurate masses are different and resolvable by HRAM LC-MS. High selectivity in full scan mode, coupled with low retention time, potentially allows hundreds of compounds to be accurately identified. MS/MS can then be used to elucidate structures of isomers, further increasing selectivity and reducing the need for reference materials to set up a screen.

Although selectivity is greatly improved by HRAM LC-MS, injection of unextracted urine results in substantial ion suppression and matrix interference. In this case, trace levels of cannabinoids may remain undetected so we developed a solid phase extraction (SPE) procedure

to purify and concentrate samples prior to analysis. The specific interaction between target analytes and the chosen sorbent bed permits various ‘washes’ of chosen polarity, acidity, and aqueous/organic constituents to remove as much matrix as possible before elution of the target compounds. Synthetic cannabinoids generally undergo extensive phase I and II metabolism, resulting in glucuronic acid-conjugated hydroxylated metabolites. Therefore, the SPE procedure was preceded by an enzymatic hydrolysis step using  $\beta$ -glucuronidase to cleave sugars from the drug molecule.

To prepare urine for extraction, five synthetic cannabinoids (JWH-018, JWH-073, JWH-200, JWH-250 and AM-694) were spiked at 50 ng/ml into blank urine. Additionally, an unextracted standard of the five cannabinoids in 10% MeOH in  $H_2O$  was prepared to determine sample recovery during extraction.

Samples were extracted by SPE and analysed using full scan HRAM LC-MS, with data processing to screen for known compounds. Follow-up on suspect analytes was then conducted using the same sample but with MS/MS.

Cannabinoid recovery was highly consistent between different urine samples, (80–90 percent), with highest

recoveries of 96 percent compared with the unextracted standard. Peak responses were strong and we decided that the spiking level in subsequent analyses could be dropped by a factor of 20, giving a new reporting level of 2.5 ng/ml.

The assay was then tested in parallel on urine samples from a hospital patient suspected of consuming herbal smoking mixtures. As administration samples would contain predominantly phase I and II metabolites, it was decided that any of the tested compounds could be used as an internal marker. Therefore, a 2 ml aliquot of the urine was spiked with JWH-018, hydrolyzed, and extracted by SPE.

The output from the ToxID software library suggested detection of a hydroxylated metabolite of JWH-122 and one of JWH-018, alongside the JWH-018 internal marker (see Figure 2). MS/MS data confirmed the structures were most likely those suggested by the library (see Figure 3a and 3b). However, interrogation of the full-scan MS data uncovered the presence of further suspect compounds. MS/MS data confirmed these as one metabolite of AM-2201 (see Figure 3c) and two metabolites of MAM-2201 (see Figure 3d and 3e).

The five metabolites initially suggested that four compounds were present in the

## The System

**Hardware:** Thermo Accela LC system with Phenomenex Luna C18 (2) column, interfaced to Thermo LTQ Orbitrap, providing 6-minute run time.

**Software:** Full scan HRAM LC-MS data processed and analyzed by ToxID (Thermo Fisher). ToxID's database is regularly updated and includes information on protonated monoisotopic masses of known cannabinoids. Narrow range mass filters (typically  $\pm 1$  ppm) can be employed to selectively filter data for reporting. Analysis is further aided by entering retention times for analytes for which reference material has been obtained or for which prior detection has occurred. Such processing allows retrospective screening of prior-run samples once new compounds are detected and added to database.

urine sample. However, hydroxylation of cannabinoid alkyl side chains can also occur (7). So, while it is possible that the sample contained all four compounds, it is far more likely that they are instead all metabolites of AM-2201 and MAM-2201. Previous work on the metabolism of JWH-018 has demonstrated multiple hydroxy metabolites and similar metabolism has also been observed for JWH-122. Our sample had only one metabolite for both JWH-018 and JWH-122, which is more consistent with them being derived from AM-2201 and MAM-2201 respectively, where hydroxylation is occurring via substitution of the fluorine atom. For AM-2201, substitution was also detected on the indole moiety. MAM-2201 had hydroxylation on both the naphthoyl and the indole moieties.

Identification and reporting of unknown metabolites in a case like this would be virtually impossible without HRAM LC-MS. The method employed is selective and sensitive, and provides

affordable, rapid turnaround times for synthetic cannabinoid screens of both blood and urine samples.

### Beyond the solution

Following the identification of five new metabolites of AM-2201 and MAM-2201, the ToxID library database was updated to include the compounds for future testing – the database currently screens for over 180 cannabinoids and their metabolites. It also makes retroactive screening of previously analyzed samples possible. Most importantly, it offers broad coverage and the ability to respond rapidly to new compounds because the database can be regularly updated with new, internationally reported compounds.

*Dave Strong is a scientist and Simon Hudson is a technical manager at HFL Sport Science, Cambridgeshire, UK.*

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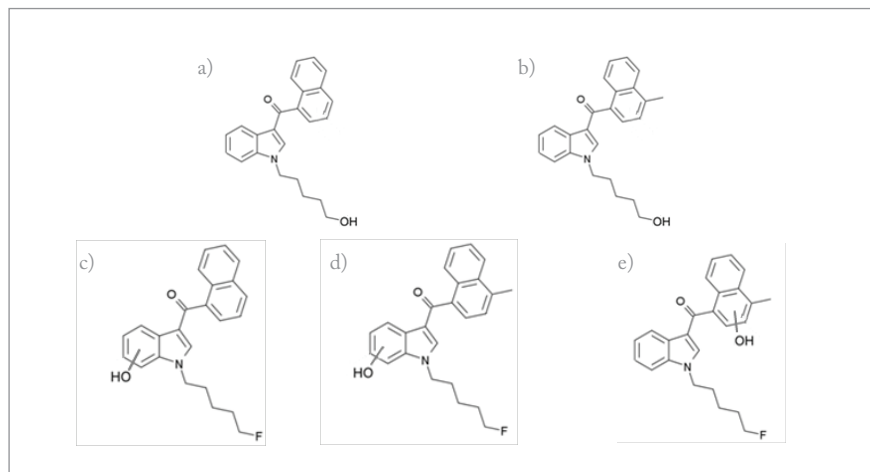


Fig 3: Structures of five metabolites detected in a hospital patient urine sample. (a) A structure identified by the ToxID library as hydroxy JWH-018, but is likely to be a metabolite of AM-2201, with hydroxylation occurring by substitution of the fluorine atom on the alkyl side chain. (b) A structure identified by the ToxID library as hydroxy JWH-122, but, as with 3(a), it is likely to be a metabolite of MAM-2201. (c) Hydroxy metabolite of AM-2201 with hydroxylation on the indole moiety. (d) Hydroxy metabolite of MAM-2201 with hydroxylation on the indole moiety. (e) Hydroxy metabolite of MAM-2201 with hydroxylation on the naphthoyl moiety.



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## A toolbox of amino acids for mAb separations

*Size exclusion chromatography (SEC) is well established for mAb aggregate analysis. As the technique has been used since the early days of monoclonal antibody (mAb) development for pharmaceutical purposes, various method improvements have evolved. For instance, the benefits of arginine on analytical SEC of mAb aggregate samples are well-known. A new application note shows how SEC of mAb aggregate samples may take advantage of other amino acid additives in the mobile phase.*



Recently, various approaches to improve analytical SEC have focused on reducing the analysis time. This can be achieved by staggered injection protocols or increased linear flow rates. The mobile phase composition itself leaves less room for method improvement, compared to other chromatographic modes. As soon as a certain ionic strength (inhibiting electrostatic interactions without causing hydrophobic interactions) and the pH of the mobile phase (ensuring structural integrity of proteins and the stationary phase) are set, one might think that the analysis solely depends on the particle size, packing quality and column length. However, the mobile phase composition is not at the end of its rope, in case the mentioned parameters have been set. For example, arginine and other amino acids can be added to the mobile phase to affect the aggregate recovery in SEC.

A mAb was aggregated by incubation at 75 °C for 5 min. Subsequently, the sample was analyzed via TSKgel UltraSW Aggregate 7.8 mm ID x 30 cm L with

different mobile phases, all of them using virgin columns. 0.2 M lysine, arginine, proline, glutamine or sodium sulfate were added to 0.1 M sodium phosphate buffer, pH 6.7, respectively. A flow rate of 1 mL/min was applied, and 20 µl (100 µg) of the aggregated mAb samples were injected onto equilibrated columns. Figure 1 illustrates the results on aggregate recovery. Glutamine and proline show a similar behavior: the aggregates are hardly recovered for the first two injections, while the aggregate peak suddenly appears for injection #3 and #4. The rise is not as sudden for sodium sulfate, but the aggregate peak will only achieve its full size for injection #10. In opposition to these results, lysine shows an even improved aggregate recovery compared to arginine. The inter-injection variability is low, depicting the complete aggregate content for all of the injections.

Besides aggregate recovery, resolution of the different sample components, namely the monomer and the different

aggregates, is crucial for accurate analysis. Clearly, there is motivation to increase resolution. Figure 2 depicts the separation profile of an aggregated mAb sample on TSKgel UltraSW Aggregate using 0.1 M sodium phosphate buffer, pH 6.7 with an addition of 0.2 M arginine. Ten injections with the arginine buffer were followed by ten injections applying sodium phosphate buffer with an addition of 0.2 M sodium sulfate, in order to compare the two buffers. Monomer aggregate resolution, as well as monomer fragment resolution is slightly improved for the amino acid buffer.

Besides arginine, both proline and glutamine provide slightly increased monomer aggregate resolution. For arginine, the fragment monomer resolution is also improved. Although these increases in resolution are not drastic, they confirm that increased resolution due to the use of an advanced mobile phase is possible and that mobile phase testing can contribute to a more reliable and robust aggregate analysis.

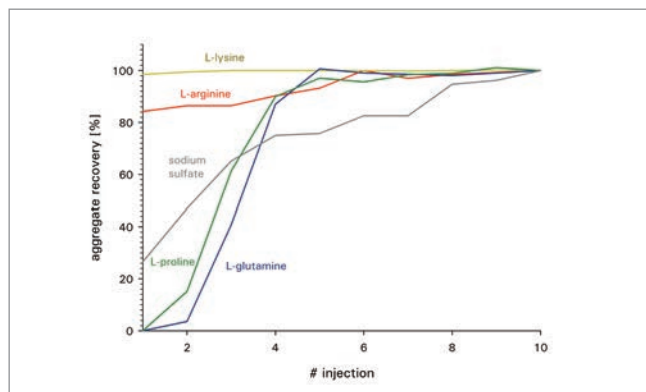


Fig. 1: Aggregate Recovery on New TSKgel UltraSW Aggregate Columns  
The mobile phases contain different amino acids: lysine (yellow), arginine (red), proline (green) and glutamine (blue). Sodium sulfate was added instead of an amino acid as a reference. Lysine and arginine allow almost complete aggregate recovery starting with injection #1, while proline and glutamine lead to reduced aggregate recovery compared to sodium sulfate.

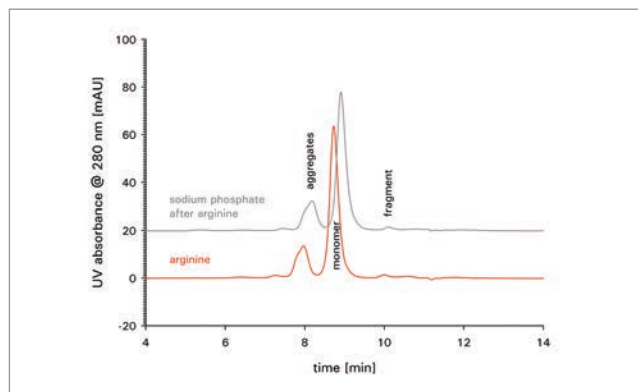


Figure 2: Separation profile of an aggregated mAb sample on TSKgel UltraSW Aggregate. Mobile phase: 0.1 M sodium phosphate buffer containing 0.2 M arginine (red). After 10 injections, the mobile phase was switched to sodium phosphate buffer with an addition of 0.2 M sodium sulfate (grey). For both mobile phases, injection #10 is shown.

## Determination of phthalates in water using the VWR-Hitachi ChromasterUltra Rs new high-resolution column

*The ChromasterUltra Rs together with LaChrom Ultra II high resolution column delivers excellent performance for the separation of similar compounds.*

Phthalates are present in a vast range of consumer products owing to their use as plasticisers, dispersants, gelling agents and emulsifying agents to name but a few.

They are known, however, to cause malformations of the reproductive tract in mammals and there is also reported genotoxicity in humans. It is therefore important to monitor these within consumer products. This causes some problems for the Chromatographer as separation of such compounds using RP HPLC can be troublesome in that full base line separation is not achieved between a number of peaks. Therefore, some methods are developed with needlessly complicated gradients, which, of course, reduce the robustness of the method.

Using the ChromasterUltra Rs together with the LaChromUltra II column (250 mm x 3 mm, 1.9  $\mu$ m) achieves good separation for all 17 phthalate compounds.

The Chromatogram shows a good separation of 17 Phthalate esters (100 ng/ $\mu$ l each in hexane) in drinking water, especially between such similar compounds as 7. DIBP and 8. DBP (see Figure 1).

The ChromasterUltra Rs has been designed with exactly this application in mind – the separation of similar and related compounds. With very low dwell volume and a newly designed, patented gradient mixing technology, the superior performance of the system complements the LaChromUltra II C 18 column. This high resolution column with grafted organic/inorganic silica is not only highly pressure stable to 1400 bar but is also highly resistant to alkali mobile phase as a result of the polymeric surface modification;

therefore, mobile phases between pH 1 and pH 12 can be used.

The ChromasterUltra Rs Diode Array Detector offers very low noise and drift, and a newly designed total internal reflection capillary flow cell. Therefore, high sensitivity with low noise is achieved for much lower limits of detection.

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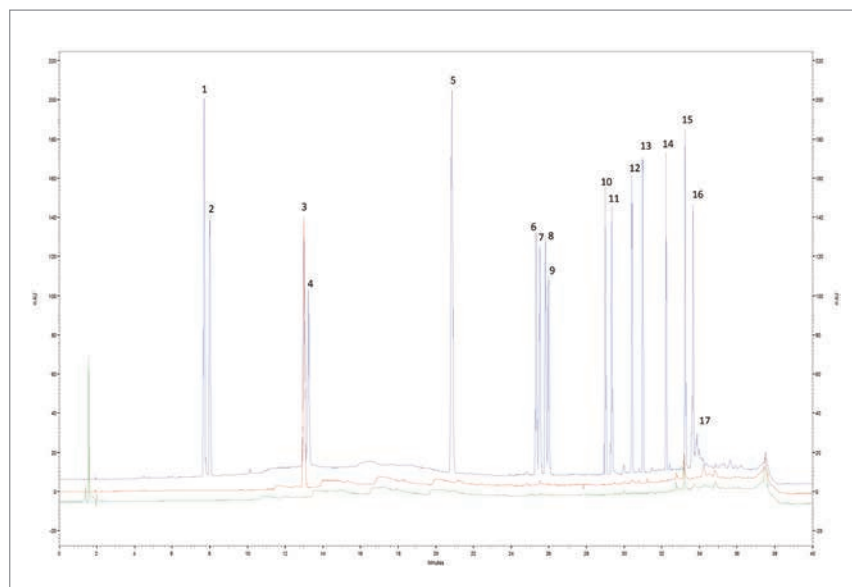


Figure 1. Black trace: Tap Water. Blue Trace: Tap Water spiked with 100 ng 17 phthalate esters mix. Red Trace: Tap Water spiked with 300 ng 17 phthalate esters mix. (Phthalate esters: 1.DMP, 2.BMEP, 3. DEP, 4. BEEP, 5.BABE, 6. BBP, 7. DIBP, 8. DBP, 9. BBEP, 10. DAP, 11. DCP, 12. BMPP, 13. DHP, 4. DEHP, 15. DNOP, 16.DNP, 17. HEHP)





# We Are Family

Sitting Down With Alexandra Knauer,  
Owner, and Alexander Buenz, CEO, of Knauer.



How has Knauer changed over the last 50 years and since you both took senior positions?

Alexandra Knauer: My parents founded the company in 1962. My father built the instruments – osmometers back then – in the kitchen while my mother used a typewriter to create the manuals.

Nowadays, we must look at things much more thoroughly. Innovation and corporate social responsibility (CSR) are a much stronger focus. And the activities we engage in are more structured so that we are able to better measure progress.

Alexander Buenz: I can speak of the last seven years: yes, we have increased the size of the company but we have also changed the way we do business. It takes much longer to develop new products – or rather solutions – compared with the early days, when Alexandra's father could do the whole job on his own.

What keeps you both motivated?

AK: I started working when we were not doing so well, so my first motivation was to preserve the company. I realized it was the right thing for me and now cannot imagine doing anything else.

AB: My main driving force is to change things and to help people grow. I enjoy working with people who are as enthusiastic as the two of us – people who want to bring real value to our customers.

AK: And we enjoy working together. We've been working together for almost seven years now. We share lots of ideas to make progress because we both know that things cannot stay the same. It's a very good fit – we are both pulling on the same rope!

And how does that partnership work?

AB: I don't believe we had it figured out at the beginning, it developed over time. Maybe I'm more focused on technology and development, but Alexandra is very active in those areas too and we both

meet regularly with colleagues from each department. Sharing our points of view always leads to a better solution, maybe because we are of different genders.

*“My parents did manufacturing, packing, selling – everything – in the family home. We always felt that the company was the first child!”*

Knauer seems to be a very family-oriented business...

AK: Yes, it's like a big family. I think it is important to care not only that projects are finished well but also that everyone is okay in their personal life. I am convinced that work is more fun when you really know the people and understand how everyone's feeling.

Alexandra, what was your experience of growing up with the company?

AK: I was the third child, born four years after the company was founded. My parents did manufacturing, packing, selling – everything – in the family home. We always felt that the company was the first child! Our grandmother took good care of us, but children prefer to spend time with their parents and that was something we missed at times. I did not always strive to be the leader of this company because I saw from an early age what hard work it was.

The two of you decide to host a dinner party for four others – who would you invite from the field of analytical science and why?

AK: Jean Pierre Chervet from Antec. He's a very vivid personality. We've had a very long relationship but we don't see him very often. Also, Ira Cohen – our American distributor and representative. He has worked with Knauer for many years but he's seldom in Germany.

AB: Rainer Blair, CEO of AB Sciex. I like the way he does things business-wise but I also like his way of approaching individuals with a very human touch. Of course, there's also the intention of getting him as a customer! I'd also invite an excellent lab scientist who can give us solid insights into new applications.

Which developments or trends have caught your eye recently?

AK: Looking at the worldwide HPLC market, nutrition is a focus in many countries.

AB: The open software environment that enables integration of instruments from multiple vendors is a great development. One day, customers will be able to make an instrument choice without regard to the software.

What lies ahead for Knauer?

AK: We are quite small but still have ambitions to be known throughout the world. One clear goal is high quality, eco-friendly systems; another is continuous chromatography, where we have many new products.

You have won many awards – why?

AB: Juries have admired the unique things that only Knauer offers, for instance, the concept of cooking with each other to build better working relationships or our active involvement in child education. Also, Alexandra has proved that being successful is about more than just profit. She has a very personal approach and really takes care of her employees like a family. We all enjoy working with her.



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