Evidence of self-medication in dolphins

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Industry legend Tony Edge

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There’s a debate in philosophy about whether science produces real knowledge – perhaps to the surprise of many scientists! The predictive power of scientific theories would seem to suggest that they are offering accurate descriptions of the world. Yet theories do change over time – scientists used to be confident about the existence of the “ether.” Will the unobservable entities and best-explanations we posit today stand the test of time?

What about the amyloid hypothesis? In 2006, Sylvain Lesné, a researcher working at the University of Minnesota, discovered a previously unknown oligomer species, dubbed $A\beta^{56}$, which when isolated and injected into the brains of mice, caused Alzheimer’s-like symptoms (1). The paper boosted the hypothesis that amyloid buildup was a primary cause of Alzheimer’s. And, since then, many millions – perhaps billions – of dollars have been spent developing anti-$A\beta$ therapies, with little to no success. Now, a six-month investigation led by Science magazine has found that the 2006 study may contain fabricated results (2).

If true, the findings would cast doubt on 16 years of research – consider that more than 2300 papers cite the original study. As Thomas Sudhof, Nobel laureate and expert on Alzheimer’s, pointed out in the article, besides the wasted funds, the most obvious damage is “wasted thinking in the field because people are using these results as a starting point for their own experiments.”

Given popular “debates” over the efficacy of vaccines and the causes of climate change, confidence in science is not at its highest. And though it may be tempting to pour scorn on “science deniers,” we ought to be wary of overconfidence. Do scientists, on the whole, put too much stock in the findings of individual papers and the strength of the peer review process? (Take a look at the pair of opinion pieces beginning on page 09 about academic integrity and peer review problems in analytical science.) Regardless of any fabrication, other researchers have struggled to replicate Lesné’s results, so should the paper have been cited more than 2300 times? What proportion of these researchers cast a truly skeptical eye over the findings? Not only do we have epistemological reasons to doubt (especially where less well-validated theories are concerned), but we must also must remember that scientists are only human – and foul play is always possible.

Rather than producing a “true” body of knowledge, perhaps science simply paints an increasingly more refined picture of the world over time. Things may be clearer today than in the past, but we shouldn’t get too attached to our current world-image. Healthy skepticism about papers and processes can help avoid wasting money – and perhaps more importantly – time and effort.

James Strachan
Editor
In My View

08  The microplastics problem is snowballing, says Damia Barcelo, and we need standardized analytical techniques to dissect the issue.

09  It appears some journal editors giving articles preferential treatment for a fee, says Victoria Samandiou.

10  Yet some of the issues raised by Victoria are “frankly child’s play” compared to what one anonymous contributor has seen…
33 Chromatography: Caroline West casts a critical eye over the current state of supercritical fluid chromatography

37 Spectroscopy: The most exciting development in spectroscopy today? For Juergen Popp, it’s photothermal IR microscopy and recent developments in IR spectroscopy.

41 (Bio)Pharma: Eliza Lee introduces the trendsetting techniques of biotherapeutic molecule characterization

34 Features

14 Frontline Forensics

We examine the critical role analytical scientists play across the forensics field, from keeping the public safe from chemical warfare agents and improvised explosive devices, to developing new technologies in the war against synthetic cannabinoids

29 Mass Spec: Rick Yost shares his thoughts on the evolution of ion mobility spectrometry–mass spectrometry

50 Sitting Down With

Tony Edge, Site Director (Production & R&D), Avantor Sciences, UK

California at Berkeley, USA
Gary Hieftje, Indiana University, USA (Retired)
Hans-Gerd Janssen, Unilever Research and Development, The Netherlands
Ian Wilson, Imperial College London, UK
Jenny Van Eyk, Director of the Advanced Clinical Biosystems Research Institute, USA
Luigi Mondello, University of Messina, Italy
Martin Gilar, Waters, USA
Michelle Reid, Cristal Therapeutics, The Netherlands
Monika Dittmann, Independent Analytical Scientist, Germany

Peter Schoenmakers, University of Amsterdam, The Netherlands
Robert Kennedy, University of Michigan, USA
Ron Heeren, Maastricht University, The Netherlands
Samuel Kounaves, Tufts University, USA

33 Chromatography: Caroline West casts a critical eye over the current state of supercritical fluid chromatography

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Just What the Dolphin Ordered

Dolphins are rubbing themselves on metabolite-rich coral – could they be self-medicating?

Indo-Pacific bottlenose dolphins have been observed queueing up to rub themselves on corals and sponges in coral reefs – but, until now, no one quite knew why. In search of an answer, a group of researchers sampled three of the dolphins’ preferred coral and sponge species and found that they were releasing mucus. This led them to hypothesize that the behavior may be linked to active metabolites in the coral (1).

To test their theory, the researchers analyzed the mucus using a combination of high-performance thin-layer chromatography with on-surface assays and high-resolution mass spectrometry (2). “We found 17 biologically active substances with antimicrobial, antioxidant, hormonal, and toxic properties across all three invertebrate species,” says lead author Gertrude Morlock, Full Professor of Food Science at Justus Liebig University Giessen, Germany.

Gram-negative and Gram-positive bacterial bioassays revealed that the three invertebrate species were releasing antibacterial compounds that act against both types of bacteria. The authors also used well-known mammalian hormonal receptors in the bioassay to detect hormonal effects. Although all three species demonstrated antimicrobial properties, there were also clear differences – for example, the leather coral produced more hormonally active compounds.

“As a result of these findings, we dared to hypothesize that the bioactive molecules can have an effect upon skin contact,” says Morlock. “This may provide evidence of self-medicating in dolphins.”

Why the hyphenated bioanalytical technique? According to Morlock, combining chemistry and biology on the same planar surface is extremely helpful in prioritizing important compounds among the thousands present in a natural sample. “This technique is also very matrix-tolerant, so the raw sample extract can be used,” she says. “It requires minimal sample preparation steps and is fast and cost-efficient.” The technique also calls for minimally invasive sampling; any other technique would have required a much larger sample volume to achieve the same results.

The researchers called for further research on vertebrate-invertebrate interactions in coral reefs and highlighted the need for interdisciplinary and hyphenated bioanalytical thinking, as well as the importance of conserving this essential habitat for marine life.

Reference

Good As Gold

Could a biosensor spun from gold be the future of wearable sensing technology?

Wearable sensors can provide researchers and clinicians with 24/7 insights – and the more accurate, sensitive and specific the data, the better. Now, a group of researchers from the University of Tokyo, Japan, have developed a new ultrathin biosensor, spun from gold, that uses surface-enhanced Raman spectroscopy (SERS) (1). Importantly for the wearer, the sensor can be applied directly to the skin with no irritation or discomfort.

The new technology is easy to fabricate, highly scalable, and low-cost, and it enables the identification of diverse analytes at low concentrations – including sweat biomarkers, drugs of abuse, and microplastics. Given that previous wearable SERS sensors – produced via complicated fabrication processes and offering only limited sensing capabilities – have not typically been suitable for widespread use, the gold biosensor really stands out from the crowd as a significant step towards generalizability and practicality. And the team say they are looking to push both the sensitivity and the specificity even further in the future, with an eye on virus detection or glucose monitoring.

Reference
Deep Visual Proteomics

How a new method could transform our understanding of cancer evolution – potentially exposing tumor vulnerabilities

A combination of artificial intelligence (AI) image analysis, laser microdissection, and ultra-high sensitivity mass spectrometry – dubbed “Deep Visual Proteomics” (DVP) – is allowing researchers to characterize thousands of proteins from single cell types while preserving spatial information (1).

“It is useful for a wide range of purposes – from better understanding the function of cell heterogeneity in basic research to diagnosing and treating disease processes in the context of clinical pathology,” says lead author of the study, Andreas Mund, who is an associate professor at the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

When examining the spatial distribution of a primary melanoma, DVP revealed how the proteome of normal melanocytes evolved as they become invasive melanoma cells within the same tissue slice. According to the authors, the study is paving the way for planetary-level, close-to-real-time biodiversity monitoring.

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around the world

To protect ecosystems from biodiversity loss, we need accurate and internationally comparable monitoring methods. Ideally, they should be cost effective and able to access the most remote of areas – but that’s exactly where current methods struggle. Now, two researchers from the University of Zurich and the University of Montreal have developed a new method to monitor biodiversity from space; specifically, they showed that changes in plant species’ composition can be reliably assessed using imaging spectroscopy at the beta-diversity scale. According to the authors, the study is paving the way for planetary-level, close-to-real-time biodiversity monitoring.

References

Tweet of the Month

“Rage Against the Machine never specified what machine they were raging against but I bet it was a mass spectrometer”

@zzzytty
The increasing amount of plastic litter in our seas, coastal waters, freshwaters, and agroecosystems is truly concerning. To give you a brief idea of how widespread this problem is, we are producing around 350 million tons of plastic each year. River plastic input, at a global scale, has been recently estimated between 0.8-2.7 million tons per year. In Europe alone, microplastic (MP) input through rivers is 5,000 tons per year. Estimates suggest that we use one million plastic bags worldwide each minute.

As for the consequences for human and animal health, there are ongoing investigations. There is increasing evidence of MP bioaccumulation in fish and other biota resulting in oxidative stress, increased acetylcholinesterase activity, behavioral alterations, transfer across trophic webs, and hormesis. Globally, humans intake over 4,000 MP particles per year via drinking water. For countries in the South of Europe, where a lot of fish is consumed, humans may accumulate over 11,000 MP particles each year. MP particles have also been observed in the atmosphere, specifically in Chinese megacities where plastics fibers from textile, construction, and industrial materials contribute to airborne particles.

The COVID-19 pandemic has substantially increased the plastic problem. Around four billion masks are being used daily around the globe and, in the UK alone, an Olympic size swimming pool will be filled every month with plastic waste from COVID-19 tests. Once the pandemic is over, we will need to conduct additional reconnaissance studies to measure the amount of plastic generated during the pandemic and how it has impacted our global environment. This is our duty as environmental chemists, as scientists, and also as citizens of the world.

Unfortunately, there are very few papers reporting interlaboratory studies for MP analysis. And one of the major challenges associated with MP research is that, at present, there are no standard methods for MP analysis. Currently, for a comprehensive quantification of MP, FTIR or Raman spectroscopy is first used to measure the particle size, distribution, and number of MP particles, followed by pyrolysis GC-MS for identification of polymers – as well as any additional chemicals absorbed by the MP. But the truth is that MP analysis is still in the
early stage of method development and so we are not yet in a position to compare methods developed in one laboratory with those of another. Moreover, quantitative data is difficult to compare on a global scale. Clearly, we need to do more work on interlaboratory programs, such as QUASIMEME and the former BCR program in the EU.

Nevertheless, reliable analytical methods are the first step in performing further ecotoxicological investigations. And so, as a community, we must work on improving our analytical capabilities to measure MP in a standardized way.

Going forward, I would like to see standard, well-established protocols using three different techniques: FTIR, Raman, and pyrolysis GC-MS. And, given the ultimate aim, we should be aiming for the “greenest” analytical methods possible. We should also be working towards portable devices that allow us analysts to quickly identify and quantify the MPs content in a variety of samples, especially in the context of real-time data with health implications – for drinking water treatment plants or in food inspection services.

However, in the meantime, change is required – and urgently. I personally would like to urge a change from single use plastics to biodegradable ones, replacing PET with PEF (polyethylene furanoate) and using new polymers based on PLA (polylactic acid). I also want people to be aware that, at present, only around 2–3 percent of manufactured plastic is biodegradable. As such, an important area to focus on is plastic recycling, which is, at the moment, only approximately 10 percent effective. The construction of depolymerization plants to break down PET instead of increasing the number of incineration or landfilling facilities for plastic waste disposal is one way forward – and I am aware that the first steps have been already established.

Analytical scientists have an important role to play in solving this huge and growing problem by establishing standardized analytical methods and addressing the challenges of MP characterization. And with analytical science consistently advancing by leaps and bounds, I am confident these are achievable goals – hopefully in the near future.

In two previous In My View articles, I raised ethical concerns in academia and academic publications about authorship (1) and citation issues (2). Many readers will be familiar with some of the issues raised in the piece on citation ethics – ever noticed how extra citations requested from reviews are from the same journal or, worse, the same author. I also described how to navigate self-citation – and my advice was to defer to the Committee Of Publication Ethics (COPE) guidelines (3). But a recent experience of my own suggests that corruption is mutating.

To make a long story short, I received an email from a so-called press agency (who will remain unnamed) in relation to my role as editorial board member and as editor in chief of a SCI journal. They informed me that they have many articles – from their clients (!) – waiting to be published. Their proposal was for me to evaluate their articles to help accelerate acceptance in return for a fee, determined by the impact factor of the SCI journal. In other words, pay for favorable handling!

“I didn’t continue the correspondence to gather more details about the scale of the Academic Integrity Volume 3: An Indecent Proposal

Are some journal editors giving articles preferential treatment for a fee?

By Victoria Samanidou, based at the Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

“Our proposal was for me to evaluate their articles to help accelerate acceptance in return for a fee, determined by the impact factor of the SCI journal.”
Are We Enabling Abusive Reviewers?

In response to Victoria’s piece on citation ethics, an anonymous reader wrote in to share their experiences with what they called “abusive” reviewers.

By Anonymous

Some of the issues raised by Victoria Samanidou’s recent piece (1), while worrying, are frankly child’s play compared to what I have seen. It can be so much worse.

Abusive reviewers may become aware (by early citation, arXiv, word of mouth) that a “target” paper or researcher is under review, and then write to the journal’s editor demanding to be made a reviewer. Associate editors may accede to this demand, fearing escalation to the Editor in Chief, and the potential loss of their status as associate editor (and consequent loss of academic status).

When decision letters for contributed papers are sent, it’s common practice to send all reviews to all the reviewers, which seems a very reasonable thing to do, but sadly it can open the door to abuse in unexpected and unprincipled ways.

Scathing anonymous reviews will damage the paper’s authors in the eyes of the other unnamed Reviewers. In a blind review process, abusive reviews amount to character assassination – what is said cannot be unsaid. And when a paper is accepted pending revision, reviewers in the first round are often asked to review the revision. If this happens, in the second round, a reviewer may stridently attack another two to grow in its place? Who will be our Heracles? Perhaps it is up to all of us to do what we can, stay true to the principles of academic integrity, and speak out about these new schemes.

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“It’s common practice to send all reviews to all the reviewers, which seems a very reasonable thing to do, but sadly it can open the door to abuse in unexpected and unprincipled ways.”
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Reference
Diving Deeper into the Depths of the Proteome

With advancements in mass spectrometry technology, proteomics has opened the door to a new wealth of possibilities to evolve how we understand and treat diseases.

Capturing the complexity of the proteome is not easy – especially across tens of thousands of proteins with never-ending variations in amino acid sequence and post-translational modifications. Despite the challenges, the proteomics field has made great strides in recent years – in areas ranging from single-cell analyses to high-throughput processing of hundreds, if not thousands, of samples in the same day. Technological advances continue to open new doors and innovation does not seem to be slowing down. For instance, in the sample collection and preparation stages of plasma, tissues or single cells, the research community has seen advances in labeling techniques and robotics that allow automation of the entire process. Another example exists at the sample inlet – which usually involves liquid chromatography – where increasingly high-throughput proteomics is being made possible by “locked-down” systems (defined gradients and methods) that ensure workflow consistency across multiple labs.

One of the most rapidly evolving technologies for proteomics researchers is mass spectrometry (MS). “We have seen improvements in sensitivity, dynamic range and ease of use through changes to the overall workflow and the data independent acquisition (DIA) approaches,” says Jose Castro-Perez, Senior Director of Market Development at SCIEX. “These advances are often paired with new software tools that deal with complex data sets using artificial intelligence. In short, researchers can dig even deeper into the proteome.”

In the pursuit of progress in the proteomics field, SCIEX launched the ZenoTOF 7600 system, a quadrupole time-of-flight (QTOF) mass spectrometer, in 2021, which offered new fragmentation capabilities and increased sensitivity levels. The ZenoTOF 7600 system was ranked first in The Analytical Scientist’s 2021 Innovation Awards for “huge leaps” in speed and sensitivity. SCIEX achieved this through a new trap and release setup. At the end of the collision cell, ions are trapped in the Zeno trap, a short linear ion trap; voltages are then applied, causing the ions to be released so that lower m/z ions entering the TOF accelerator catch up with heavier ions at higher m/z. This process allows all ions to meet before being pushed into the TOF extraction region. The result is a substantial gain in sensitivity (≥90% duty cycle) without losing mass resolution or accuracy, leading to improvements in MS/MS spectral quality. In addition, the new electron activation dissociation (EAD) technology offers an alternative, reagent-free fragmentation strategy, which preserves labile modifications.

“The ZenoTOF 7600 system can quantify up to 40 percent more proteins and analyze samples five times faster for large biobank studies,” says Castro-Perez. “Moreover, EAD fragmentation is allowing researchers to better understand how proteins are post-translationally modified – an important but challenging area in biomarker research.”

Exploring the depths of the unknown, faster

When scientists speak about their proteomics needs and what they desire from innovative technologies, sensitivity and throughput come first. Researchers want to integrate new and deeper levels
of the proteome, while running several samples simultaneously — without sacrificing protein load. And that is where another MS-based technique comes into its own: data independent acquisition mass spectrometry (DIA-MS).

DIA-MS has recently received a great deal of attention because it allows researchers to conduct analyses without any prior knowledge of the sample in question (1,2,3). The method can be set up if the required mass range is known. At the same time, the mass of a given precursor will be recorded with greater selectivity across the entire mass range. The straightforwardness of the setup, as well as a high degree of accuracy and high-throughput multiplexing capabilities, makes DIA a good fit for proteomic applications.

Considering all these points, SCIEX recently launched Zeno SWATH DIA. They have managed to reduce runtimes by more than half—from 60-minute gradients down to 30- or even 20-minute gradients, without seeing a drop in the performance of the mass spectrometry system. “In essence, Zeno SWATH DIA provides researchers with an even simpler workflow that allows them to detect and quantify more proteins than ever before, maximizing the amount of information extracted from the samples,” says Castro-Perez.

Exploring next-generation proteomics to advance drug discovery and precision medicine

These gains in sensitivity and throughput empower researchers to investigate new outcomes for proteomics research.

Single-cell workflows, for example, are on the rise in the research community. Although present in quantities an order of magnitude greater than mRNA, cellular proteins cannot be amplified. However, the sensitivity of the ZenoTOF 7600 system, combined with Zeno SWATH DIA, can help overcome this challenge and allow researchers to unlock samples with more limited cellular protein content. The advances could significantly impact drug discovery — especially in cancer research, where there is a great degree of heterogeneity across different cell types. By delving deeper into the proteomic profile of individual cancer cells, scientists may understand, in greater detail, how certain drugs exert their effect by modifying the proteome. Such knowledge could be especially valuable for personalized medicines, including cell and gene therapies. If researchers know how drugs work within individual patients, physicians may be able to tailor treatments based on the patient’s response to therapy. MS-enabled proteomics will be an attractive complementary technique to RNA sequencing by offering a previously unavailable layer of information.

Single-cell proteomics could also enable new advances in our understanding of cell differentiation and evolution, and it could accelerate biomarker discovery for disease diagnosis and prognosis.

“I am confident that the latest iteration of the Zeno trap will be genuinely transformational across many application areas,” says Castro-Perez. “Zeno SWATH DIA will enable researchers to identify increasingly large numbers of proteins expressed within a greater number of individual cells, with higher sensitivity, accuracy and speed. This will help them apply proteomics analysis to application areas previously thought impossible.”

For more information, visit sciex.com/ZenoSWATHDIA

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We examine the critical role analytical scientists play across the forensics field, including: keeping the public safe from chemical warfare agents and improvised explosive devices, developing new technologies in the war against synthetic cannabinoids, and gleaning evermore comprehensive information about a person from their fingerprints – including whether they’ve recently ingested cocaine or heroin. Welcome to forensics on the frontline.
The Art of Chemical Warfare Agent Analysis

Philipp Sulzer, Science Manager at IONICON, talks us through the challenging, potentially perilous, and (unfortunately) increasingly relevant field of chemical warfare agent analysis.

In 2017, Kuala Lumpur airport, Malaysia, had to be decontaminated after a deadly attack on Kim Jong-nam, the North Korean leader Kim Jon-un’s brother. He was killed using the toxic liquid nerve agent VX, which is classified by the United Nations as a weapon of mass destruction.

Then the following year, Sergei Skripal – a former Russian military officer and double agent for British intelligence – and his daughter Yulia were poisoned in Salisbury, UK, by means of an even more potent nerve agent, Novichok. Both Sergei and Yulia Skripal spent several weeks in hospital in critical condition, before being discharged. A few months later, a British woman Dawn Sturgess died after spraying a perfume bottle containing the poison – found seven miles north of Salisbury – on her wrist.

These two incidents show that in spite of international agreements prohibiting their use, chemical warfare agents (CWAs) are a real and present danger.

Here, we ask Philipp Sulzer, Science Manager at IONICON: what role do analytical scientists play in confirming whether a CWA has been used or whether an environment is safe following an attack? What precautions must be taken? And how is research regulated in such a sensitive field?

How did you end up specializing in chemical warfare agents?

I was born, grew up, and still live in the western part of Austria, where I also studied physics at the University of Innsbruck. I first came into contact with the world of what might be summarized as “hazardous substances” during my PhD studies at the Institute for Ion Physics and Applied Physics, when we investigated low-energy electron interactions with explosives. What really fascinated me were the reactions of not only science colleagues, but also friends and family whenever I talked about our results. Simply put, the results of basic research on “boring” compounds often meet with limited interest – but, as soon as the work involves explosives, people start asking questions.

Literally the day after my PhD defense in 2008, I joined IONICON Analytik as head of the applied science department. IONICON, now a medium sized enterprise, was then a small university spinoff founded by the inventors of proton-transfer reaction mass spectrometry (PTR-MS). Because this extremely sensitive real-time trace gas analysis technology is ideal for the investigation of hazardous substances, I was more than happy to join international research collaborations and projects on the detection and analysis of explosives, psychoactive substances, toxic industrial chemicals (TICs), and CWAs. One early example was a project named SPIRIT, funded by the European Commission, in which we built a PTR-MS instrument for extremely sensitive and selective real-time monitoring of building HVAC systems for terrorist TIC/CWA attacks. This was highly successful, so we were invited to join more and more TIC/CWA-related projects.

What happens immediately after a CWA attack?

This is when first responders – highly trained professionals (who typically do not have a scientific background) with easy-to-operate detectors – are needed.

Analytical scientists are required later in the process for two main tasks. First, we can provide detailed laboratory analysis of samples from the site of attack to get as much information as possible. With this information it might even be possible to track down where and when the CWA was synthesized. Second, we can perform the research needed to develop and improve the technology and algorithms of CWA detectors.

Did the Kuala Lumpur airport and Salisbury incidents change the CWA field?

What makes these two incidents stand out is that the use of CWAs has been proven. Additionally, there are numerous reports of alleged assassination attempts on politicians and other public figures, as well as about incidents in armed conflicts where the involvement of CWAs is ambiguous because confirmative analysis was performed too late or was not possible at all.

However, one huge analytical challenge rarely discussed in the media is how to confirm that a (public) space is “clean and safe” after a CWA attack. There are standardized cleaning procedures using dedicated decontamination agents but there should also be analytical confirmation that the floor, handrails,
“One huge analytical challenge rarely discussed in the media is how to confirm that a (public) space is ‘clean and safe’ after a CWA attack.”

Door pulls, and so on are safe to touch. Obviously, this can be done by taking a wipe test, bringing the swab to a lab, and performing a GC-MS analysis. But repeating this process over and over to cover only part of an airport is extremely time-consuming and therefore expensive.

Where is CWA research usually performed?
Access to live agents for research purposes is extremely limited and, in some countries, even impossible. Thus, most research is done using analogues. These have the obvious advantage that they are readily available and can be handled in common analytical labs. Analogues typically mimic certain CWA parameters very well – for instance, vapor pressure or persistence – but can show considerable differences concerning the ion chemistry, which is a crucial property for chemical ionization-based analyzers. In any case, it is essential to verify the results obtained with analogues by using live agents.

For several years we have been collaborating with CBRN Protection, a Chemical, Biological, Radiological, Nuclear, and high yield Explosives consulting company located in Austria. They organized measurement campaigns at certified facilities, where we got access to tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX, sulfur mustard (HD), nitrogen mustards (HN), and lewisite (L). Based on these results, we now know that our technology works very well with live agents and also have ideas for further improving our instruments.

Are there risks to analytical scientists working in the field – or in the lab?
In my experience, there is no possibility that an analytical scientist would even come close to live agents during CWA research in a lab. Certified facilities have extremely strict rules to ensure that any CWA handling is performed only by their own trained professionals. Everything involving live agents is done by these experts within a special fume hood in which the scientist operates the analytical device from a safe distance. Thus, the risk is negligible.

Some facilities also offer field testing in which scientists could indeed come into contact with CWAs. Therefore, they have individual safety protocols involving medical pre-clearances, full body protection including respiratory filters, and so on. I imagine that this could make extended research quite tedious, especially for someone not used to wearing such equipment.

However, one risk that must be taken into account is contamination of the analytical equipment during measurements. This is obviously highly instrument-specific but, with PTR-MS, we are in the fortunate position of having one of the most sensitive real-time detectors in existence. It can even analyze its own exhaust gas for any CWA contamination within the device.

What are the main analytical challenges associated with detecting CWAs – and which techniques are usually used?
For rapid, on-site detection, ion mobility spectrometry (IMS) is generally used. IMS devices are typically handheld, very robust, and can be operated by non-scientific personnel at the push of a button. However, they are built for detecting very high CWA concentrations typically present immediately after an attack. Our measurement campaigns indicated that these devices’ sensitivities are insufficient for detecting residual traces on surfaces, which can still present a considerable risk.

The ideal analytical instrumentation has a LoD below contamination levels that could cause any health effects, gives results in real-time, detects a broad range of CWAs (and TICs), and has a substance library that can be easily updated when new compounds of interest arise. Moreover, it should be easy to operate, robust, reliable, low-maintenance, and allow operators to use them autonomously in the field. To my knowledge, such a “Swiss Army knife” device is not currently available, but an IONICON PTR-TOF QB instrument gets pretty close.

How is IONICON working to overcome challenges in these areas?
We have come a long way, from basic research on analogues over a decade ago to live agent tests with a dedicated PTR-MS hazardous material monitor (HMM). Let me give you
just two examples of major challenges we had to overcome within this timespan.

In 2013, J.M. Ringer from the German Bundeswehr Research Institute for Protective Technologies and CBRN Protection published a paper on nerve agent studies performed using two different IONICON PTR-MS instruments. They found that the common reagent ion H3O+ causes heavy fragmentation of the CWA molecules, which reduces sensitivity and selectivity. Outstanding sensitivity and selectivity can be achieved using NH4+ but, for the production of these reagent ions, some form of toxic and corrosive ammonia is required as a consumable, which is why it was hardly ever used until recently. In 2017, we developed and patented a novel method to produce NH4+ solely from water and nitrogen/air, without any ammonia at all. Ever since, PTR-MS studies using ammonium reagent ions have experienced a renaissance because no additional source gas is necessary for NH4+ production.

Developed at a university, PTR-MS is traditionally a tool tailored for analytical experts. Using it for routine CWA analysis, in which operators often have no analytical background, required a complete redesign of the instrument’s interface. As a result, we created the Automated Measurement and Evaluation (AME) software, offering one-button simplicity. With AME, the PTR-MS instrument automatically changes the ion chemistry, compares the measured results with patterns in a database, and displays the corresponding compound concentrations in real time. Whenever an additional compound needs to be detected and quantified, a simple database update is sufficient.

Combining these and many other technological advancements, we can now offer the HMM for real-time quantification of minute CWA and TIC traces.

Overall, how might technological advances change the CWA detection field?

The CWA detection field is already quite advanced, with readily available solutions for most (though not all) analytical tasks. However, there is still plenty of room for improvements that will make detection faster, more sensitive, and more selective. With the PTR-MS based HMM, we are convinced that we also have a solution to the formerly unsolved problem of efficient decontamination control.
Frontline Analysis in the War Against Spice

How MANDRAKE gathers intelligence, shares information with the police and public, develops new technologies – including a portable benchtop NMR enabled by a unique pattern recognition algorithm – to combat the UK’s synthetic cannabinoid epidemic

By Oliver Sutcliffe

A few years ago, “Spice” – a drug containing one or more synthetic cannabinoids – rose to prominence in the UK after images of people (often homeless) stood frozen or collapsed in the street were published on the front pages of national newspapers. The city of Manchester, where I work, has been referred to as the “spice capital” of the UK, but, in truth, the abuse of synthetic drugs is endemic in every major city in the UK and in many cities throughout the world.

Let me take you back to Manchester in 2013; the police were aware of psychoactive substances circulating in what were referred to as “head shops” – specialized shops selling “legal highs” that are structurally similar and mimic the effects of controlled drugs. In 2016, the UK government passed the Psychoactive Substances Act, which made the supply and production and import of new psychoactive substances illegal. The head shops shut overnight, but it wasn’t long before a black market was established.

With the birth of a robust black market, individuals on the streets started exhibiting much more severe effects than had been seen with the “legal” synthetic cannabinoids, including withdrawal and the catatonic states featured in the headlines. The problem was compounded by the fact that many police officers were uncertain as to the legal status of the synthetic cannabinoids and struggled to identify them on the street – moreover, prevalence and potency varied widely. Violence and collapses in the city surged. One report claimed that 95 percent of the homeless community in Manchester were using spice regularly (1); and a 2016 survey found that 33 percent of inmates in UK prisons had used spice in the last month (2).

As part of a series of initiatives to combat the problem, we at Manchester Metropolitan University joined forces with Greater Manchester Police to co-create MANchester DRug Analysis and Knowledge Exchange (MANDRAKE). The aim was to expand our understanding of what is actually in the products people were taking, as well as exploring the human effects through our academic collaborations. Ironically, the ban on legal highs meant that many academic groups could no longer purchase and test these drugs. Indeed, many groups across the country stopped looking for these compounds because in some cases required specific Home Office licenses – an expensive and time-consuming process – to conduct their research on the now-illegal substances.

Criminologists in other parts of the country had spoken to users, asking about drugs on the street, what they’d been using, whether the drugs were getting stronger or weaker. But it was all subjective. We needed a way to identify and monitor what people were taking. Our lab was fully licensed to hold controlled drugs, and to even make new drugs to use as reference materials. So, what began as an informal knowledge sharing arrangement grew into a formal drug testing facility, running continuously and covering the whole of Greater Manchester. And it was unique in the UK.

The power of NMR

There are three main components to MANDRAKE: reducing harm, gathering intelligence, and developing new technologies that can be used on the front line staff in the police or prison service. If, for example, the police seize a batch of pills and their presumptive tests (normally a colorimetric test) are inconclusive, these samples can be potentially sent to MANDRAKE for some initial presumptive tests, using infrared spectroscopy or nuclear magnetic resonance (NMR) spectroscopy, which are followed up by confirmatory tests and quantification using gas chromatography-mass spectrometry. The final results are usually disseminated within two-and-a-half hours of us receiving the initial sample.

The benchtop NMR device we use is interesting. We were originally approached by an instrument manufacturer, Oxford Instruments, who thought we might be interested in purchasing a benchtop NMR to use when teaching. They demonstrated how it worked by testing different alcohols. I asked whether they’d thought about using it for forensics; they hadn’t. But they had explored meat speciation during the UK’s horsemeat scandal (an exciting decade!) using an algorithm that could fingerprint different leads, which we thought could work in drug-testing context. So, they funded us to develop a similar algorithm for forensics using our in-house library of substances. In the resulting paper, we presented a 93 percent accuracy rate (3) – excellent for a single component for our patented approach.

The main advantage of our pattern recognition algorithmic NMR approach is that it’s extremely easy to use, which is vital if we want prison guards or police officers to use it. Frontline responders aren’t scientists and need a simple discriminator they can interpret – a red light or a green light so to speak, which was our assumption from the beginning and informed how we developed the algorithm and user interface. In addition, by removing any complicated spectral analysis from the process, you...
also remove any potential operator bias – which we know exists. NMR also has some advantages over Raman spectroscopy – though I will say that Raman has advanced since we first started working with NMR. We found that when firing Raman at a tablet or a piece of paper we would get results that just didn’t make sense because it was picking up the excipients or something in the background. With NMR, you extract the drug out of whatever matrix is in there, which produces a better match.

And because the device was portable, easy to use and rapid in terms of its turnaround time, we were able to trial frontline analysis. We decided to focus on MDMA initially because it is the most common controlled drug in the nighttime economy. And because the algorithm allows us to identify a drug without using a reference standard material (meaning we didn’t have to carry around a sample of MDMA, which would be illegal!) we were able to deploy at a nightclub in Manchester. We get the results back in 2.5 minutes and are also able to detect anything dangerous, both in terms of potency or another substance cut with a pill. During this pilot project, we found the world’s strongest MDMA tablet – four to five times the usual dose. We issued a warning on social media, which allowed individuals to dispose of the tablets before taking them and putting themselves in serious danger.

The work that we do to identify substances and rapidly issue warnings feeds into our broader role as an intelligence gathering service, which is crucial to fighting the spice epidemic. We are able to provide quantitative data on the changes in spice potency over time, which the police can use to paint a more comprehensive picture of the market. For example, we’ve seen sudden increases in potency after manufacturing errors and decreases following the arrest of major players. We now know which strains are on the street, how their potency is changing over time. So, if we hear reports of sudden increases in potency or of new strains, we can test those, issue warnings and create reference materials to use in the future. Overall, it allows the police to be more proactive in dealing with drugs.

In one notable example, an individual, who had smoked spice and then felt very unwell, decided to submit the rest of it to find out what it was – a legal move. We were able to synthesize the compound to confirm the structure – a completely new cannabinoid that had not been seen in the UK before (it had only been found in the US). Such information feeds into the national and international surveys on drugs and can help the police understand the wider situation.

In addition to testing batches of drugs, we’re also involved in developing new ways of finding out whether someone has taken spice. For example, last year we – alongside the University of Bath – received a £1.3 million research grant to help refine a portable device based on a simple fluorescence-spectral-fingerprinting-based saliva test, developed by the Bath group in 2019 (4).

**The battles to come**

Fortunately, the legislative changes we’ve seen to combat new psychoactive substances – both in the UK and in China – have reduced the number of new drugs on the market. A few years ago, we were running samples and seeing completely new substances on a regular basis, but that has subsided. The main challenge now is understanding local variations in potency so that we can identify unusually strong batches on the streets and issue warnings in a matter of hours.

Many of the devices out there are black and white in terms of “is it or isn’t it there?” For example, we got a sample from the police that they believed was MDMA, but the infrared kept returning a negative result. It turns out it was 6-bromo-MDMA, but it has an additional bromine on the aromatic ring in the drug, so it didn’t initially match exactly what was in the database. Our system was able to say that, although it is a new substance and isn’t in the database, it is an MDMA derivative. So, if people are collapsing at an event or on the street, we can pass that information to welfare teams or paramedics who can treat people in a similar way to how they might treat someone who took the derivative drug. If we can increase the number of field-deployable devices that are able to do this, we could access more robust quantification and therefore more effective harm reduction and knowledge gathering.

I’ve also had conversations with people looking at forensics. In the UK, we have major backlogs in forensic services, which leads to a lot of additional police time. Hypothetically, if a police officer arrests someone because they’ve found a bag of white powder in their possession. Instead of waiting days or weeks for the results of testing the sample, they could run it through the NMR and get a result the same day – and potentially find out that their suspect was actually selling crushed paracetamol, for example.

There are many ways we can use rapid, portable, easy-to-use analytical tools.

Oliver Sutcliffe is a Senior Lecturer at Manchester Metropolitan University and Director of MANDRAKE

References available online
What Does Your Fingerprint Say About You?

Sex, age, pathology, and now, with imaging mass spectrometry, whether you’ve recently taken cocaine or heroin...

With Catia Costa, Research Fellow at the University of Surrey, UK

What inspired your research into differentiating between contact and ingested cocaine metabolites?

Initially, we were excited to detect drugs and their respective metabolites in the fingerprints of someone who had taken cocaine. But during conference presentations, people would often ask the crucial questions: “Can you be sure the donor took cocaine? What if they had just come into contact with the drug by handling a banknote?” This was a fair question and initially we hypothesized that detecting both the parent drug and its metabolite could help make that distinction, but we needed evidence of that.

To answer that question, we traveled to the forensic service provider in Ireland – Forensic Science Ireland (FSI) - to (literally) get our hands on some cocaine seized by the police. We performed a lot of dermal contact experiments with cocaine under several different scenarios. One of the key findings in this study was that cocaine’s main metabolite – benzoylegonine, which is normally produced in the body as the drug is metabolized – was also present in the seized cocaine powder. This meant that finding the metabolite was not strictly indicative of cocaine ingestion.

We needed a way of distinguishing between a fingerprint containing the drug/metabolite after ingestion and a fingerprint containing the drug/metabolite after coming into contact with the substance...

So how did you distinguish between the two?

One of the main challenges is that cocaine is very sticky – it tends to hang around on the fingers for 24 to 48 hours. You could wash the cocaine from the fingers to detect the metabolites, but if investigators are looking at fingerprints left at the crime scene, the circumstances of deposition are unknown, and it is unlikely the fingerprint donor washed their hands. Additionally, the handwashing process is subjective and does not always remove all traces of cocaine.

We went on to study the “contact or ingestion” problem using mass spectrometry imaging; specifically, with three approaches: desorption electrospray ionization (DESI), matrix assisted laser desorption ionization (MALDI) and time of flight secondary ion mass spectrometry (ToF-SIMS), which allowed us to visualize fingerprints at different length scales, ranging from the whole fingerprint pattern down to the individual pore structure. We found that if someone had touched cocaine, we would see less of the metabolite and the distribution was very spotty because, as you can imagine, people aren’t deliberately spreading the drug uniformly over their fingers! But if someone had ingested the drug, we saw more of the metabolite and a uniform distribution over the fingerprint area.

How far away are you from having something that could be used in a real-life investigation?

Quite far! There’s still a great deal of research that needs to be done to cover the breadth of illicit drugs out there and the complexity of using fingerprints as a drug testing matrix (e.g. quantitation, robustness, etc). For example, we’ve looked at heroin, and found that detecting heroin and respective metabolites in fingerprints works well with traditional liquid chromatography-mass spectrometry (LC-MS) approaches. We were able to distinguish between contact and ingestion of heroin without the need for mass spectrometry imaging – we simply used morphine, a secondary metabolite of heroin, to identify the cases where heroin was ingested. Techniques like LC-MS are mature and routinely used for drug testing, but for mass spectrometry imaging, we’re still far from having a mature, standardized test that can be done in a forensics lab using commercially available instruments.

What are the advantages and disadvantages of the various techniques you’ve used in your research?

LC-MS is arguably the best technique we’ve used so far. It is the most sensitive and extremely repeatable. The main problem is sample preparation – ideally, you must collect the fingerprint on a piece of paper, extract the sample, dry it down and reconstitute it. That might take an entire day. Part of my PhD was to look at faster methods, and we came across a technique called paper-spray mass spectrometry. Here, the samples are collected on a little triangle of chromatography paper, which we put into the source, apply high voltage to the back of the triangle, a touch of solvent, and the ions are extracted into the mass spectrometer. With that technique, you could get a (yes/no) result in one minute, which is much quicker than LC-MS – but it is also less sensitive.

The DESI, MALDI, and ToF-SIMS we used for MS imaging...
were great because you can see how the drug is distributed over the fingerprint, but the sensitivity of those techniques is not as good as LC-MS or even paper spray. It all depends on what you want to target, whether you need spatial distribution, a yes or no type test, or a more quantitative analysis.

What are your plans for future research?
Ultimately, we are keen to develop a usable technology – we really think it has potential. But given the levels of certainty you need in forensics, as well as the limited funding and regulatory hoops you have to jump through to conduct research in the area, we think therapeutic drug monitoring (TDM) might be a more realistic application! Hospitals need ways to monitor whether a patient is complying with their medication. And post COVID-19, remote methods are becoming more popular. You can’t easily ask patients to produce blood or urine samples and send them in the post. But what if they could just send a fingerprint? Admittedly, this work is also quite challenging because it’s difficult to tell how much fingerprint you’ve collected; we’re working on how to measure the mass of a fingerprint, which would make quantification of the drugs and metabolites possible for TDM.

What other challenges have you faced during your fingerprint research?
For both criminal investigations and clinical settings, investigators/doctors want to know not only whether someone has taken a drug, but also how much of it they’ve taken – and we have some way to go before we nail the quantification aspect. But to be honest, simply conducting the research is quite tricky; we hold a finger to the paper for 30 seconds, at roughly the same pressure, but the results can vary depending on how sweaty the donor is – and there’s a range of additional factors and potential investigator biases. These are all potentially solvable issues, but more research is needed! I suppose if it were easy, everyone would be doing it…

What other things can we learn from a person’s fingerprint?
Ah! That’s another major challenge: getting people to believe the fingerprint is more than just identification. You can tell a lot about a person from their fingerprint! Simona Francese, Professor at Sheffield Hallam University, and other researchers around the world have done great work to explore the determination of sex, age and even physiological or pathological states from a person’s fingerprint. Professor Francese’s work looked at detecting and mapping hemoglobin variants in blood fingermarks and blood stains and identifying a bunch of different condom lubricants from fingerprint samples.

One of the next steps in fingerprint research is to be able to understand more about the conditions under which a fingerprint was deposited — that’d be a significant step as it could give us a better understanding of the crime scene and how things unfolded. Methods to find and develop fingerprints found at crime scenes have seen great improvements over the last few years, but if the suspect isn’t on a database, it’s usually a dead end. With the chemical analysis of a fingerprint, you can learn more about the person — what we call donor profiling — to narrow down your list of suspects.
Ionoptika were involved in the University of Surrey project – they used water cluster SIMS to differentiate between ingested and noningested cocaine on a person’s fingertips.

**Can you give me an introduction to the project?**

Established in 1994, Ionoptika is one of the leading providers of high-performance ion beam technologies for surface analysis and nanofabrication applications and has expertise in cluster ion beams for secondary ion mass spectrometry (SIMS). Ionoptika had previously collaborated with the University of Surrey and the Ion Beam Centre to design a tool for single ion implantation (now sold as the Q-One system). We had already established a strong relationship when Mel Bailey got in touch about our water cluster SIMS technology. The goal was to see if we could help with their fingerprint imaging project.

**What was the outcome of the study?**

The results were really exciting. We could see the cocaine in the fingerprints, but we could also see the metabolite produced by the body when the donor ingested the drug. That means we now have a way of telling whether someone has taken cocaine or merely come into contact with it.

**What is the biggest analytical challenge in forensics today?**

As we start looking for smaller and smaller amounts of material in these samples, sensitivity becomes the biggest challenge. That’s why we strive to push the boundaries with techniques such as water cluster SIMS. It’s great to see the impact technologies like these can have.

These types of analytical techniques are helping us understand more and more about evidence as simple as a fingerprint. In this study, we looked at drug use – but there are many more markers that could be investigated. Eventually, they could help forensic scientists more easily identify an individual or even understand their state of mind (check out Simona Francese’s work at the University of Sheffield in this area). That’s really powerful.

**Reference**

Bomb Track

The chemical profiles of smokeless powders – a frequently used material in improvised explosive devices (IEDs) – are often the only link between the offense and the offender. Can we profile faster and with greater precision?

By Ids Lemmink

The misuse of explosive materials by criminals and terrorist groups threatens public safety in all countries. In previous decades, there have been thousands of bombings around the world using improvised explosive devices (IEDs) – and Western cities, such as Brussels, Boston, Madrid, and London, have not been immune. The sole purpose of IEDs is to hurt and kill innocent people – changing the lives of the victims and their loved ones within a fraction of a second. Smokeless powders (SPs) are used as their main explosive material in 10 to 20 percent of all IEDs. SPs are energetic materials used as propellant in ammunition, as well as in pipe bombs and pressure cooker IEDs. Over 10 million pounds of SPs are produced each year and sold without restrictions – causing serious problems.

Often in IED cases, highly discriminative evidence, such as DNA or fingerprints is absent, which makes chemical profiling of SPs one of the only forensic options available to help the investigating authorities find a link between the offense and the offender. The more characteristic features included in the chemical profiling, the more discriminative the results will be. When these characteristics are independent, their evidential value in the forensic comparison can be multiplied.

Currently, the chemical profiling of smokeless powders is centered around identifying and comparing the additive composition of two SP samples. The additive composition is determined in a qualitative and quantitative manner using GC-MS or LC-MS. The additives present in SPs are mostly stabilizers, deterrents, plasticizers, and flash inhibitors, added by the manufacturer to improve the SP performance. Manufacturers often keep their additive mixture a secret, but tend not to change it much over time once they are happy with the performance. This makes the additive profiles between SP samples originating from different manufacturers very distinctive, but the profiles originating from the same manufacturer can be difficult to tell apart.

Nitrocellulose (NC) is the main component in all smokeless powders. Until recently, NC had been largely ignored – as the elephant in the room – because it was too complex to analyze for chemical profiling purposes. NC is a high-molar-mass polymer that is produced by the nitration of plant-based cellulose using nitric acid and concentrated sulfuric acid. Recent research in our group by Rick van den Hurk has shown for the first time that SPs can be differentiated based on their molecular-weight distribution (MWD) using size-exclusion chromatography (SEC). In contrast to additive composition, MWD varies significantly between SP samples from the same manufacturer; for each production round a new batch of cellulose can be obtained from a variety of suppliers.

The degree of nitration of NC in SPs differs between 2.32 and 2.76 with batch-to-batch variation, due to slight differences in reaction conditions during production. This variation could make the degree of nitration of NC a good characteristic to include in the chemical profiling of SPs. The current method to determine the degree of nitration of NC in SPs uses a multistep solvent extraction, followed by an alkaline hydrolysis, and ion-exchange or CE separation. The total analysis time of this method is roughly three hours, requiring intensive manual labor and at least 20 mg of SP. No research on the potential of using the degree of nitration of SP-samples for a forensic comparison has been performed.

With this in mind, I set out to develop a quick and accurate method that could differentiate SP samples based on their degree of nitration. The aim was to develop something application focused, which could be widely adopted by the forensic field. That meant placing a high premium on accuracy and precision, while reducing analysis time, equipment cost, and the degree of manual labor required.

By combining SEC, alkaline hydrolysis, and an ion-exchange separation, we determined the degree of nitration in real SP samples. Pair-wise statistical comparison demonstrated that 80 percent of the SP sample set could be differentiated with statistical significance solely based on the degree of nitration. The total analysis time was only 12 minutes and required minimal sample preparation taking less than five minutes. A publication is in progress.

I hope that as a result of our work, determining the degree of nitration of NC will become standard practice in the forensic field when there is the need to chemically profile and compare SP samples. We are not trying to replace assessments of the additive composition or MWD but rather to introduce an additional independent parameter to refine understanding in forensic comparisons. Given that the MWD, additive composition, and the complete degree of nitration of NC are not dependent on each other, the overall evidential value of the individual features can be multiplied.

In our research, we observed that aging and different storage conditions can influence the chemical profile of the SPs. The effect of different storage conditions can present an opportunity, because SP samples originating from the same batch can, after a certain amount of time, be differentiated based on their chemical profile; but this also brings risk – storage can increase the chance of false negative results. For example, a SP-based IED stored in a humid environment might obtain a different chemical profile to the SP stock stored in a dry garage. The effect of aging and different storage conditions should be investigated before it can be applied in an actual forensic comparison and finally make its way to court.

The chemical profiling of SPs might not be able to prevent the production and usage of IED-based SPs. However, it can help identify those persons responsible and possibly prevent future attacks.

Ids Lemmink is a Masters Research Intern (graduated Cum Laude), Analytical Chemistry at the University of Amsterdam, the Netherlands.
Michelle Wood on the Future of Forensics

Michelle Wood has headed up the Forensics and Toxicology R&D team at Waters for more than two decades; here, she talks us through some of the biggest challenges and trends in the forensics field today.

What's the main challenge facing the forensics field today?
Forensic science (and forensic toxicology in particular) is continually evolving, so new challenges come along all the time. Whether it's a new drug or analogue, a need to respond to new legislation, interest in a different specimen type – every day brings something new.

If there's a “main challenge,” it's the all-encompassing challenge at the analytical bench level and includes all the work that is required to keep up the service for both current and new drugs. It requires efforts on many fronts:

• A finger on the pulse of drug use and emerging trends within the service area and beyond (drugs that are already in, and those that may be coming, into the region)
• Continual development of new methods or expansion of current panels
• Validation studies to assess the effect of the method changes or instrument upgrades
• Maintenance of current analytical technology and continual assessment of the scope of these methods to avert false, but forensically significant, false positives and false negatives
• Assessment and implementation of newer technologies that may expand the scope of testing
• Automating high volume methods to improve sample throughput and lab efficiency
• The forensic defensibility and certainty of the first level screening and the second level confirmatory test that ultimately leads to a reportable finding
• Maintaining compliance with forensic standards of practice and the appropriate regulatory agencies that oversee the laboratory's testing services

How will modern tools and analytical breakthroughs drive forensic science?
Thankfully, analytical tools improve all the time – and there are some great companies out there with the know-how to deliver some fantastic innovations. The company that I work for has been innovating for over 60 years and is a leader in mass spectrometry (MS)-based technologies. In this regard we, and other manufacturers who are active in this field, are continually making improvements in the specificity, as well as the sensitivity, of the instruments, but also in ease-of-use, which can really make this technology accessible to a wider audience. As an example, the screening of seized drug material used to be performed by low-tech methods, such as chemical color tests or thin layer chromatography (TLC); nowadays, very simple mass detectors can provide a much more accurate result in less than a minute – that’s less time than the color test but with more specificity.

As mentioned, any competition between vendors/manufacturer's is healthy – and ultimately drives the development of better products for the end users (usually, analytical scientists). For example, improved analytical sensitivity is always welcomed as it can translate into an ability to make use of more simplified sample preparation procedures for some labs (such as sample dilution where previously extensive sample clean up may have been required) or it might open up an ability to analyze other types of specimens, such as saliva/oral fluid or hair. These alternative specimens can be very informative as they may extend the detection window of drug substances – detecting drugs earlier after usage and for a longer period after usage – or even capturing a single use/or administration of a substance, instead of multiple or repeated usage of drugs.

Are there any researchers you admire in the forensics space?
I'm excited by the work of Olof Beck (Professor at the Karolinska Institute, Sweden) and his coworkers on breath analysis – specifically the use of exhaled breath for determination of drug substances. We are all very familiar with the use of alcohol breathalyzers at the roadside as a preliminary indication of whether the proportion of alcohol in the breath is likely to exceed prescribed limits. However, Professor Beck, and other investigators, have demonstrated that it's also possible to use exhaled breath to measure drugs and metabolites. As the concentrations of drugs in breath are relatively low, there is a need for highly sensitive analytical systems, such as mass spectrometers, to accurately detect the substances.

The potential benefits of developments in this area are wide-ranging; as a specimen, the investigators have demonstrated that breath can reflect very recent use/exposure of drugs and therefore...
Feature offers potential for screening drivers suspected of driving under the influence of drugs at the roadside or for testing overdosed or intoxicated patients in a hospital setting. Devices that capture the exhaled breath have now been developed, and also offer ease and convenience of collection for this very interesting specimen.

Overall, how might analytical science (and scientists) change the forensics field over the next decade?

In the next decade, I believe that we will see significant advances in two key areas: i) an increased use of automation and robotics to improve laboratory efficiency, and ii) the development of more field-based mass spectrometry solutions. Automation is increasingly applied in forensic laboratories facing an ever-increasing volume of cases. We are seeing this trend in all forensic areas – from forensic chemistry labs, who receive hundreds of seized drug materials to analyze, to forensic toxicology labs with an increasing number of biological samples to analyze and turnaround. Unfortunately, there is not always a commensurate increase in laboratory staff; automating tasks frees up personnel to focus on other activities.

I also believe that we will see the development of more mobile, and more transportable mass spectrometry systems. There is an increased need to take this highly specific, highly sensitive technology closer to the action and outside of the traditional laboratory environment. The present limitations are related to the requirement of a high vacuum within the mass spectrometer for optimal sensitivity; currently this is easier to achieve within a conventional laboratory environment; however, if we can develop highly sensitive systems that can reach the appropriate operating vacuum very quickly (minutes instead of hours), such systems can be fitted into mobile units, transported where they are needed, and quickly operational.

Is there a particular technology that you wished you could have used earlier in your career?

One technology that has increased in popularity over the last 20 years or more is time-of-flight mass spectrometry – and high-resolution mass spectrometry in general. Of course, high resolution MS systems were around more than 20 years ago, but they were not ready for “prime time” – in other words, they were not mature enough (nor simple enough) to be used in a routine toxicology laboratory. This has changed completely. The technology is now accessible to all and allows determination of masses to four-decimal places, which analytically provides exceptional high specificity (reduced ambiguity) for identification of drug substances and so much more besides! I wish that I’d had this technology years ago…
Bruker’s Bavarian Benchtop NMR

Joerg Koehler explains how Bruker is working with Bavarian police to develop new spectroscopy-based tools for forensic narcotics analysis.

What are the main analytical challenges faced by law enforcement agencies?

Law enforcement agencies face several challenges when it comes to analyzing suspicious drug substances – either with the analytical methods themselves or with the substances being tested.

For greater quantification and identification, agencies in most countries have to apply at least two analytical methods, whether that be nuclear magnetic resonance (NMR), mass spectrometry (MS), infrared, Raman spectroscopy, or X-ray diffraction. And that slows down the time to results and causes bottlenecks in the testing facility. When using chromatographic methods coupled to MS to perform analyses, coelution can make separation and identification difficult. With MS specifically, you first have to know what you want to quantify and then a compound specific reference substance has to be available – and for new psychoactive substances (NPS), you’re on your own!

Even if there is no legal requirement for orthogonality, authorities still want comprehensive testing methods that capture the widest range of narcotics at the lowest possible cost of ownership. Enter benchtop NMR coupled with sophisticated software solutions to automatically interpret the data.

What challenges did you face during development?

Bruker implemented a new algorithmic approach to match NMR spectra to existing database entries to quantify the detected substances. The new solution had to be able to deal with a variety of degrees of freedom, such as chemical shift, concentrations, pH value, and knowledgebase expansion in an NMR non-expert operation environment.

What is your aim for the collaboration with BLKA?

The Bavarian State Criminal Police Office in Germany (BLKA) is able to measure real-world samples – such as NPS and other substances not yet known to authorities, as well as classic narcotics – taken from the streets. By collaborating with BLKA, Bruker is able to gain access to these samples, which normally wouldn’t be available. The samples are then tested by NMR to detect substances within the mixtures, and the results are added to the database and software, to be cross-checked against future samples. The intense and frequent exchange strongly supported the development of the new solution and ensured it was easy to use in this specific environment.

What other projects in the forensics space would you like to highlight?

None for the public domain at the moment, but we are continuing to closely work with our customers and partners in the forensic segment. There are over 100 Bruker NMR systems running at authorities in more than 40 countries. These long-standing relationships foster exchange and understanding of both new developments from an instrument point of view, and new trends and needs from an analytical perspective.
What’s the single biggest analytical challenge facing the forensics field today?

NPS are by far the biggest one. NPS have become a global phenomenon, with 134 countries and territories from all regions of the world having reported one or more NPS (1). NPS rates are increasing at an exponential rate, with around 50-100 occurring per year, globally. These substances are designed to mimic the effects of classic drugs but have minor differences in the chemical structure to pass detection methods. And because each NPS has a unique molecular structure, it can be almost impossible for drug enforcement officers to detect and analyze a sample correctly without applying NMR.

Another challenge is time-to-result – especially as the reports generated are usually key pieces of evidence in legal proceedings. With our solution, reports are generated minutes after a sample has been analyzed.

Overall, how could analytical science change the forensics field over the next decade?

The compact size of benchtop instruments means that many more forensic laboratories, law enforcement agencies, and border forces are able to access detailed information provided by NMR, without the need for a complex installation. The widespread availability of NMR could lead to better analysis of substances – known and unknown – and contribute towards the monitoring, assessment, and control of new and potentially threatening narcotic and psychotropic drugs.

The inherent advantages of NMR, including identification of structural isomers, quantification without specific reference material, and quick time-to-result typically require spectroscopic specialists to run the analysis and interpret the data. By removing this burden and providing comparable, robust, and operator independent final results, the analytical landscape has been changed forever.

Joerg Koehler is Head of Business Unit Industrial at Bruker BioSpin

Reference
The advantages of helium as a carrier gas for mass spectrometers have long been known, but it is a limited natural resource with varied supplies and expenses. On the other hand, hydrogen gas generators can be installed in any lab for a constant supply and, conveniently, increased chromatographic speeds.

Learn from experts how LECO’s Pegasus® BT can use hydrogen as a carrier gas without breaking its stride.

https://knowledge.leco.com/fast-gc
Bone of contention. In our top pick of the papers this month, researchers have used Zooarcheology by mass spectrometry (ZooMS) to uncover the origins of some Mesolithic pendants – and the results are somewhat surprising. Previously, it was assumed all of the pendants were made from animal bones – even though a few of them appeared to be a little different to the rest. Now, thanks to the ability of ZooMS to distinguish the bones based on their peptides, Kristiina Mannermaa and her team have discovered that 12 of the 37 samples were human in origin. This is the first evidence of the use of human bone for making pendants in Northeast Europe.

The researchers note that there is no reason to believe this is evidence of cannibalism, and instead focus on the fact that there seems to be no distinction between the human bones and animal bones use. “The fact that the use of human bones was not emphasised in any way and that the objects are indistinguishable and similar to objects made of animal bones may indicate the intertwining of animals and humans in the Stone Age worldview,” said Mannermaa.

You are what you eat? Pieter Dorrestein’s research team have developed a new reference-data-driven method for untargeted metabolomics analysis that helps identify the many metabolites produced when we eat. “Untargeted mass spectrometry is a very sensitive technique that allows for the detection of hundreds to thousands of molecules that can now be used to create a diet profile of individuals,” said Dorrestein in a press release. The approach is based on matching metabolomics tandem MS data to metadata-annotated source data to create a pseudo-MS/MS reference library. This is then applied to food data, increasing the MS/MS spectral usage 5.1-fold over conventional approaches.

The hope is that this new and improved approach will enable researchers to better understand how what we eat has an impact on our health, linking diet to clinical outcomes. “It is now possible to link molecules in diet to health outcomes not one at a time but all at once, which has not been possible before,” added Dorrestein.

Probing the pan-cancer proteome. Using data-independent acquisition MS, researchers have analyzed the proteomes of 949 cancer cell lines across 28 tissue types and created a monster of a resource: the ProCan-DepMapSanger pan-cancer proteomic map. With 8,498 proteins analyzed, the map captures a large amount of data about cell-type and post-transcriptional modifications. Some of their findings include single-cell origins of cell lines, drivers of protein expression patterns, and potential drug sensitivities. As the authors state, “this dataset represents a major resource for the scientific community, for biomarker discovery and for the study of fundamental aspects of protein regulation that are not evident from existing molecular datasets.”

IN OTHER NEWS

MALDI-TOF MS approach shows potential for rapid detection of SARS-CoV-2 virus and its variants from wide ranging sources.

Zoltan Takats and his team show how desorption electrospray ionization (DESI) can be used to overcome some of the shortcomings of current DESI approaches for MS imaging of biological tissues.

GC-MS analysis of essential oils reveals new targets for safe and effective mosquito control.

New ion trajectory software, SimELIT, provides simulations from the ion source to the detector to improve understanding of ion dynamics and instrument development.

Nikolai Slavov and colleagues propose best practices, quality controls, and data reporting guidelines to assist adoption of reliable quantitative workflows for single-cell proteomics using tandem MS.
The Rise of IMS-MS

Rick Yost, Head of Analytical Chemistry at the University of Florida, past president of ASMS, and co-inventor of triple quad mass spectrometry technology, shares his thoughts on the evolution of IMS-MS

Give us some background on your interest in ion mobility, particularly when interfaced to mass spectrometry. Since its commercial adoption in the 1940s and 1950s, mass spectrometry has continued to progress in new and exciting ways. And I’m particularly excited about any tools or techniques that accelerate scientific research. The marriage of ion mobility (to separate ionized compounds in the gas phase) with mass spec (to identify and detect them) nicely complements the capabilities of chromatographic separation (GC-MS and LC-MS). One advantage is that IMS separation is faster than chromatographic separation by a factor of >1000.

Can you offer some perspective on the history of IMS and IMS-MS? E. W. McDaniel performed fundamental studies on ion mobilities and ion molecule reactions in gasses during the 1950s and 1960s at Georgia Tech – and these were key to the development of IMS. Francis Karasek at the University of Waterloo first employed atmospheric pressure drift tube ion mobility for analytical applications in the 1970s, and IMS came into widespread use for homeland security and military use for detecting explosives and chemical warfare agents. But the real leader in developing IMS and IMS-MS for analytical uses was Herb Hill at Washington State University (who was previously a postdoc for Karasek). These early IMS systems all employed ion mobility at one atmosphere, so they didn’t require vacuum pumps. Even still, as we entered the 21st century, all commercial IMS were designed for homeland security and military applications.

The first commercial integrated IMS-MS instrument used a traveling wave IMS interfaced to a Q-ToF mass spec, and was introduced by Waters in 2006; the first commercial IMS-MS using a classic drift tube, also interfaced to a Q-ToF, was first offered by Agilent in 2014. Both of these instruments perform ion mobility at reduced pressure (typically at <1 percent of atmospheric pressure) to make it easier to interface the IMS with the high vacuum required by the mass spectrometer (about a billionth of atmospheric pressure). But that inevitably compromises the separation that can be achieved compared with operating the IMS at one atmosphere.

Ching Wu, one of Herb Hill’s PhD students in the 1990s, founded Excellims in 2005 to develop atmospheric pressure high performance IMS (HPIMS) systems for new application areas. In 2006, Excellims introduced an atmospheric pressure drift tube IMS with electrospray ion source (the GA2200) as well as one that could be added to the front of Thermo and other mass spectrometers in 2014 (the MA3100). Excellims also introduced an integrated system that interfaced IMS to their own compact linear ion trap MS/MS instrument in 2019 (the MC3100).

Because of my interest in combining IMS with MS, I followed developments at Excellims, getting to know Ching and his team. I saw a lot of potential in moving IMS-MS into clinical and point-of-care applications, and therefore agreed to serve as a member of their scientific advisory board in 2018.

What are the advantages of atmospheric pressure IMS-MS? Clearly a big advantage of performing IMS at one atmosphere is the improved separation possible with a reasonable length drift tube (hence the term HPIMS or high-performance IMS), as well as eliminating the need for vacuum pumps for the IMS. And that’s why atmospheric pressure IMS is widely used in passenger screening at airports, but not used in other commercial IMS-MS systems.

By interfacing an atmospheric pressure IMS with a mass spectrometer, you end up with a much more powerful analytical system than IMS alone – you’re able to differentiate compounds that have the same drift time by different masses, as well as identify unknown IMS peaks by their mass spectra. And adding tandem mass spectrometry (MS/MS) to the mix, as on the MC3100, further increases the selectivity as well as the
ability to identify unknowns.

The third advantage of this approach is that it makes two complementary analytical instruments possible: a small, low-cost, stand-alone IMS for field use or rapid screening in point-of-care clinical applications, and then a larger (and more expensive) tandem IMS-MS system, with the same IMS front end, but interfaced to a mass spectrometer, for laboratory studies, method development, and validation of screening performed with the standalone IMS system.

**What are the benefits of HPIMS in clinical diagnostics and disease research?**

In my lab at the University of Florida, we have used the Agilent 6560 IMS-MS instrument (with a meter-long drift tube IMS operated at 1/200 of an atmosphere interfaced to a Q-ToF mass spec) for studies in metabolomics, lipidomics, and clinical analysis. We’ve found that the instrument provides many advantages, including rapid analysis (eliminating or dramatically reducing the need for LC separation), reducing interferences, helping identify unknowns, and, in particular, resolving isomers that cannot be resolved by LC or MS alone. For example, we’ve found that IMS can resolve many steroid isomers of interest in antidoping studies of athletes; it can also remove the interference of a Vitamin D isomer in its clinical analysis. But that instrument is big (I like to say 10 foot long and 10 foot tall!) and is priced proportionally. Moving those analyses onto a smaller and less expensive IMS-MS platform, such as the MC3100, would be very attractive. In fact, we have evaluated the Vitamin D assay on the Excellims MA3100 interfaced to a Thermo Orbitrap mass spec.

Another intriguing application area for IMS-MS is breath analysis for clinical applications (everything from routine diabetes monitoring to screening for diseases, such as COVID). And this is clearly an application where the availability of both a stand-alone IMS for rapid point-of-care screening and a laboratory IMS instrument interfaced to a mass spectrometer for method development and validation would be a very powerful combination.

**What future developments do you foresee for IMS technology?**

I see a lot of potential for IMS for rapid analyses in a variety of application spaces, from quality control to clinical analysis. The availability of a complementary IMS-MS system for developing and validating those applications, and then troubleshooting them when problems arise, could be game changing – particularly if the IMS and IMS-MS systems were well integrated in terms of hardware and software platforms. For many of these applications, front end sampling capabilities (for breath analysis, for instance) and back-end software integration will be key to the widespread acceptance of such technology.
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**Parsing parosmia.** The first early reports identified a loss of smell as one of the cardinal symptoms of COVID-19. With time, it became apparent that some went on to experience parosmia, a condition in which familiar smells become distorted and disgusting. In order to gain insight into the mechanisms involved, researchers from the University of Reading used gas-chromatograph olfactometry to separate out the chemicals that make up the smell of instant coffee and let several people with parosmia after infection smell them one at a time. They were able to identify 15 molecular triggers. The research could aid in the development of diagnostics and therapies for this condition in the future.

**The wonderful wizards of MS.** Data security is a hot topic these days and digital encryption keys have become commonplace. In recent years, molecular encryption strategies have emerged, with researchers using DNA chains and polymers to store and transport encryption keys. Now, a team of US-based researchers have encoded a 256-bit cipher key into the sequences of eight 10-monomer-long oligourethanes. The molecular-based key was embedded in the ink of a personal letter, mailed to a third party, recovered using the same sequential analysis method (which involved high-resolution LC-MS), and then used to successfully decrypt The

**It’s not a bug, it’s a feature.** Using four unrelated strains of C. elegans originating from different parts of the world, a group of worm biologists have developed a model system to study individual differences in metabolism. The team carried out a series of experiments including gas chromatography-mass spectrometry, high performance liquid chromatography-mass spectrometry, and metabolic network analysis to identify differences and variations in metabolites between the four strains.

**Scrutinizing Hemp Strains.** Industrial hemp is hot, garnering the increased attention of several industries – from pharma to food. But not all hemp strains are alike – and the genetic diversity at play can have a significant impact on the resulting metabolite profile, which in turn may affect its application. And that’s why researchers from the University of Duisberg-Essen, Germany, have developed a comprehensive two-dimensional liquid chromatography method, which, when combined with a new demodulation process, is capable of identifying differences between the two varieties of industrial hemp in terms of their cannabinoid and phenolic profiles.

**IN OTHER NEWS**

New linear relative response factor model-based liquid chromatography method validated as alternative for THC content determination.

Tube plasma ionization open-air source for GC-MS shows improved analysis of wider range of compounds compared to APCI and APPI, with lower limits of detection.

Researchers develop a sensitive and specific LC–MS/MS assay for thebaine – key to distinguishing between heroin use or poppy seed consumption in a positive urine morphine test result.

Nested polymerase chain reaction coupled with LC-MS enables improved wastewater surveillance of SARS-CoV-2 variants for early warning.

Damaged catnip produces iridoid nepetalactone, a mosquito repellent, GC-MS analysis finds – explaining why cats instinctively rub and roll against the plants.
Who’s Afraid of SFC?

Supercritical fluid chromatography has come a long way in terms of versatility and ease of use, so there’s no reason to be scared, says Caroline West. But we need another round of innovation from manufacturers to take SFC to the next level.

Overall, how would you characterize the current state of SFC?

Overall, there has been a good deal of interest in applying SFC in a variety of ways, but there remains a degree of resistance. To be honest, I think that is largely due to people not wanting to change. If someone has been using GC or LC for years, it’s not easy to convince them to learn a completely new technique and invest in a (more) expensive instrument. In my experience, the younger generation are more willing to give SFC a go – largely because they’re less likely to be completely invested in another technique. If SFC is to reach its significant potential, it will almost certainly be the next generation of scientists driving it forwards.

Shimadzu’s instrument, which came out a few years later, is also worth noting for the simplicity it brought to the hyphenation of supercritical fluid extractions, which made methods more reproducible in general.

I’d say the vendors did a great job – and their efforts generated plenty of excitement around the technique. Unfortunately, for whatever reason, we’re still using pretty much the same instruments 10 years later... And that’s a shame because I believe there is a great deal of scope to improve SFC instrumentation given what we’ve learned about the technique in the past 10–15 years.

So what have we learned about SFC over the past decade or so?

Quite a lot actually – and it was overdue. In the beginning, SFC was used only with pure fluids – mostly CO2 in the mobile phase. Over time, we progressively introduced small portions of co-solvents, to improve analyte solubility and peak shape, but also to have more flexibility on method optimization. Co-solvents are now used in large quantities. For example, we can now do very wide gradients starting with 100 percent CO2 and finishing in 100 percent co-solvent conditions, especially to analyze complex samples with a wide range of polarities. Admittedly, we still need to better understand diffusion coefficients and how they relate to changes in viscosity in the system. But our overall understanding of the technique is much improved and we’ve seen it applied in a number of new application areas. I don’t want to be too hard on the instrument developers, but there’s certainly room for improvement!

What would be on your wishlist for a new SFC instrument?

Besides the UV sensitivity issue that I mentioned, I would like more simplicity in terms of the design and function. From an outsider’s perspective, SFC can look complicated – there are certainly more parameters to optimize than with GC. If the instruments operated more akin to an LC system, I think that would help adoption – especially, as an SFC instrument already looks like the typical LC “hifi tower.” I’d also like more flexibility. It isn’t particularly easy to change from one mode to another. Let’s say I want to do a very wide gradient going from 100 percent CO2 to 100 percent co-solvent, I can push the instrument to achieve that goal – but it isn’t how the instrument is designed to be used. When the co-solvent is a mixture (e.g. solvent and water), it also isn’t possible to change the proportion of solvent and water during the gradient, because the pumps are only two way, not three way. MS hyphenation also needs to be simplified.

Such wishlist requests would expand what’s possible in a single experiment (rather than needing two separate instruments), opening up new possibilities in terms of applications. For example, there’s a great deal of interest in applying the technique to biomolecules, such as proteins or nucleic acids, but this is tricky with the current generation of instruments.

Where is SFC most widely applied right now?

The number one application for SFC today is natural products, which has overtaken small molecule pharmaceuticals. The main drivers are the rise of Chinese traditional medicine and foodomics, which generally don’t require the same sensitivity or resolution as pharmaceuticals. We also see many applications in bioanalysis – this wasn’t the case 10 years ago and is a result of better hyphenation to mass spectrometry, which aids in the detection of minor metabolites. Again, this relates to the issue of the wider gradient; if you’re looking for polar metabolites, sensing with 100 percent CO2 won’t work...
– you’ll need something else in your mobile phase. Environmental analysis is another growing area – again, this is an application area that generally isn’t constrained by sensitivity or method validation to the same extent as pharma.

**What are the main benefits of SFC?**

SFC is very versatile. You can use it to detect non-polar, chiral, achiral, small molecules, “big” molecules, such as synthetic polymers – though we are restricted when it comes to the large biomolecules. It’s also very complementary to other techniques. Even if your reverse phase LC is working well, you may need to confirm your results with orthogonal analysis. And in areas such as traditional Chinese medicine, the aim is to gain a comprehensive fingerprint of the product, which requires several techniques.

**What are the common misunderstandings?**

There is a common misconception that, because we mostly use CO2, SFC won’t work for polar molecules. But, as we’ve discussed, advances over the past decade have overcome this issue. I also think that people often overestimate the complexity of SFC, fearing that they won’t be able to manage the system and get it working properly – especially if they’re looking for a system suitable for non-expert users (so they can simply place their sample in an automatic injector and leave it to run). In fairness, I used to think this too – that you need some knowledge to use the technique properly – but I’ve been proven wrong in recent years. If you have some standard methods established, it really is possible for a non-expert to use SFC today. So my main message is to reassure people: SFC isn’t so frightening!

**How optimistic are you about the future of SFC?**

I’m realistic. SFC is never going to replace the better-established (notably, they’re not actually much older!) techniques of GC and LC. My hope is that SFC will be better accepted as a useful complement. Personally, I think it’s brilliant – that’s why I’ve been promoting it for the past two decades! It wasn’t so long ago that few people were listening. The instruments 10 years ago really changed that. And I think it’ll take innovation on the instrument side to kickstart SFC again.

Caroline West is an Associate Professor at the Institut de Chimie Organique et Analytique (ICOA), CNRS UMR 7311, University of Orleans, France.
SciX Sneak Preview

KEYNOTE SPEAKER
“The Future of Space Exploration: Earth-based, Deep Space-based, Robotic and Human”
Amanda Hendrix
Senior Scientist, Planetary Science Institute
Boulder, Colorado, USA

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- Sensitive and Selective Bioanalysis using SERS and SESORS; Karen Faulds
- Advances in Interfacial and Voltage-gated Two-dimensional Infrared Spectroscopy; Martin Zanni
- Pushing the Frontiers of Stimulated Raman Imaging for Complex Subcellular Bioanalysis; Lu Wei
- Raman Spectroscopy and Machine Learning for Medical Diagnostics and Forensic Purposes; Igor Lednev
- Stimulated Raman Scattering Imaging: From Label-free to Metabolic to Super-multiplex and to Single-molecule Imaging; Wei Min
- Multifaceted Laser Induced Plasma: Spectroscopy and Beyond; Igor Gornushkin
- Process Analytical Utility of Raman Microspectroscopy for Cell Therapy Manufacturing Validation; James Piret
- Mass Spectrometry Au Naturel: A Tool for Structural Biology; Joseph Loo
- Nonlinear Electrophoresis of Colloidal Particles; Aditya Khair
**Beating back bacteria.** With the threat of antibiotic-resistant bacteria growing, researchers from the University of Agriculture and the University of Education in Faisalabad, both in Pakistan, developed a surface-enhanced Raman spectroscopy (SERS) method to identify and characterize colistin-resistant and susceptible Escherichia coli strains. Comparing spectral features from three susceptible strains with those of three resistant strains, the researchers were able to distinguish between the two with 100 percent specificity, 99.8 percent sensitivity, and 100 percent accuracy. The authors concluded that SERS, alongside multivariate data analysis techniques, is highly suitable for the identification and discrimination of colistin-resistant strains in a reliable, fast, and cost-effective manner.

**First-of-its-kind Biofinder?** Researchers from the University of Hawai‘i at Manoa recently developed a highly-sensitive “Biofinder” tool to detect organic fluorescence signals. Using biofluorescence imaging, they were able to accurately detect bio-residue in fish fossils from the 34–56-million-year-old Green River formation. The authors corroborated these results with Raman and attenuated total reflection Fourier-transform infrared spectroscopies, scanning electron microscopy, energy dispersive X-ray spectroscopy, and fluorescence lifetime imaging microscopy. Results confirmed once more that biological residues can survive millions of years and that using biofluorescence imaging can efficiently detect these traces now. The authors hope that this technology may aid NASA’s “search for life”, one of the major goals of NASA’s planetary exploration missions.

**SERS unmasking high-risk biomarkers.** Researchers from China’s Hefei Institute of Physical Science have developed novel highly sensitive biosensors based on SERS technology that can detect PD-L1 biomarkers to help monitor tumor growth in cancer patients. These aptasensors, according to the paper, measure PD-L1 on circulating malignant exosomes “by making it ‘sandwich’ between CD63 targeting magnetic probes and PD-L1 targeting SERS tags.” In the mouse model, this technique also demonstrated much higher sensitivity than the standard ELISA method. Overall, this SERS-aptasensor revealed itself as a versatile and ultrasensitive tool that could be useful for monitoring patient health as they undergo PD-1/PD-L1 immunotherapy.

**IN OTHER NEWS**

- **Bruker launches novel NMR test for molecular phenomics research on “long COVID” patients’ blood samples for multi-organ risk assessment.**
- **New NIR spectroscopy algorithm suitable for high-throughput identification of crop varieties’ authenticity could potentially improve crop breeding efficiency.**
- **SERS chip-based analysis method demonstrates fast, reliable sensing of trace THC and cannabino – two active cannabis compounds.**
- **Japan Aerospace Exploration Agency, NASA, and European Space Agency collaborate to investigate the X-ray universe using high-resolution imaging and spectroscopy.**
- **Applications open for the Association of British Spectroscopists’ Edward Steers bursary aimed at promising early-career scientists using analytical spectroscopic techniques.”**
Exciting Times For... Spectroscopy: With Juergen Popp

We’re asking leading spectroscopists what they think the single-most exciting development in spectroscopy is today. Here, Juergen Popp, Scientific Director at the Leibniz Institute of Photonic Technology, highlights photothermal IR microscopy and the many recent developments in IR spectroscopy.

Tell us a little about your background in spectroscopy...

Spectroscopy – and Raman spectroscopy in particular – has accompanied me my whole scientific life. I first came into contact with laser spectroscopy more than 30 years ago during my diploma thesis, where I worked with Wolfgang Kiefer – a pioneer in the field of Raman spectroscopy. Since that time, I have been fascinated by both laser and Raman spectroscopy.

My current research group investigates new chemically sensitive linear and nonlinear spectroscopic contrast mechanisms, laser technologies, and detection techniques for multi-contrast or multi-parameter imaging of biological and biomedical targets (such as pathogens and their antibiotic resistances, tumor cells, tissue samples, cell organelles, organs, and marker molecules). My research covers the entire range from basic optical/spectroscopic research to translation into clinically applicable methods.

All this work is driven, in particular, by a vision to use spectroscopic approaches – particularly Raman-based methods – for improved medical diagnostics and therapy. We have made significant headway, having developed several compact clinically applicable systems with a high technology readiness level for preclinical and prospective clinical studies.

What are the biggest developments in the spectroscopy field over the past decade or so?

A comprehensive answer to this question is difficult – or even impossible – because the field of optical spectroscopy has developed rapidly in recent years. Therefore, I would like to focus here especially on the development of Raman spectroscopy, which, in my opinion, has become one of the most important optical analytical methods in the last 10-20 years – next to fluorescence spectroscopy.

Though Raman spectroscopy was still reserved for specialists in the early 1990s, it has, in recent years, become a fairly routine method, with applications extending into all areas of the natural sciences – and also into unexpected disciplines like art history. Raman spectroscopy has even left the earth and is flying to Mars!

The main reasons for this are rapid advances in instrumentation, the availability of small, easy-to-use lasers (mainly diode lasers), which no longer require special electrical connections or cooling, the development of the CCD camera as a powerful multi-channel detector, and especially the existence of efficient filters to suppress the elastically scattered Rayleigh light. These advances in instrumentation have led to the availability of easy-to-use, commercial Raman instruments and have greatly expanded the range of applications.

Furthermore, an increased interdisciplinary dialogue between spectroscopists and end users, such as clinicians, has resulted in Raman spectroscopy entering a new era. This application push of Raman spectroscopic bioanalytics over the past 10 years has led to both new hardware and software advances that include new Raman fiber probe designs, field-deployable easy-to-use Raman microscopes, and, most importantly, novel data processing techniques that exploit artificial intelligence (AI) for automated analysis of data sets.

The latter is very important – but does not only apply to Raman spectroscopy. The success of spectroscopic methods for medical diagnosis and therapy (and other applications, such as in the life sciences, process analytics, pharmaceuticals, or environmental analysis) is closely related to the development of tailored data evaluation algorithms. In short, measurement data must be translated into qualitatively and quantitatively usable information for the end user – and significant progress has been made in this area in recent years. In fact, we have developed a universally applicable Raman data analysis software called RAMANMETRIX (see: https://docs.ramanmetrix.eu/). This software allows robust and reliable data analysis of Raman spectroscopic data with the click of a button.

Over the past 10-20 years, Raman spectroscopy has evolved from a purely scientific research method to a mature analytical tool with a wide range of potential applications.

What is the single-most exciting development happening in spectroscopy today – and why?

In my opinion, the latest developments in IR spectroscopy (the sister method to Raman spectroscopy) are particularly exciting. For many years, a major problem in IR spectroscopy was the lack of suitable IR excitation sources with a high photon density. This problem has since been
solved with the introduction of quantum cascade lasers as highly brilliant light sources; indeed, using these lasers as an excitation source for IR spectroscopy or imaging in the spectral range from 950 to 1800 cm\(^{-1}\) can be seen as a significant milestone. For one thing, they partially compensate for the appearance of strong IR water absorption bands in the IR spectra of biomedical and biological samples that mask other relevant bands. And as the intensity of quantum cascade lasers is several orders of magnitude higher than that of thermal emitters in FTIR spectrometers, large-scale and uncooled microbolometer arrays can be used as detectors instead of the smaller MCT-based liquid nitrogen-cooled FPAs. A spectrometer or interferometer for spectral information acquisition is not needed because quantum cascade lasers are tunable.

Another highly exciting development is photothermal IR microscopy, which is based on the non-radiative transformation of absorbed energy into heat. The use of tunable quantum cascade lasers for IR-excitation causes absorbed heat to locally expand and thereby change the refractive index of the sample, which can be detected with optical systems in the visible range. This allows IR images of aqueous samples (such as living cells) to be acquired with submicrometer spatial resolution. This feat has not been possible before and will significantly expand the application range of IR spectroscopy/microscopy, especially as it relates to biomedical issues.

Finally, I would like to highlight the exploration of a completely new method of IR absorption spectroscopy: field-resolved infrared spectroscopy. In contrast to conventional FTIR spectroscopy, this novel method measures the coherent field emitted by the vibrationally excited molecules after excitation with an ultrashort MIR pulse of a few optical cycles. The detection of this field allows a significant lowering of the detection limit compared with FTIR spectroscopy and thus the analysis of low-concentration molecules in strongly absorbing mediums.

Is there an application area that will benefit most from these developments?
Clinical diagnostics will benefit most – and, in fact, are already benefitting – from all of the above-mentioned developments. The sharp increase in cancer (aging society) and the rapid spread of life-threatening infectious diseases and antibiotic-resistant germs (increasing global mobility and ill-considered use of broad-spectrum antibiotics) represent areas of unmet medical need.

There is a great need for new methods that enable earlier diagnosis of these diseases and, therefore, allow initiation of targeted therapy as soon as possible. In recent years, spectroscopic methods have shown their potential to provide clinicians with relevant information to address these medical challenges.

What about major challenges for the field?
A major challenge is the clinical translation of spectroscopic approaches for routine clinical diagnostics. Although research on clinically suitable compact light sources, ultra-sensitive detectors and their linkage with modern concepts for AI continues, translational research in Europe is facing major challenges, especially with regard to the EU Medical Device Regulation (MDR) 2017/745.

Currently, this EU regulation significantly hinders the testing of spectroscopic approaches on patients in the form of preclinical or clinical studies. For example, Raman spectroscopy has proven its potential in proof-of-principle studies for certain diagnostic and therapeutic questions, but the actual performance has not yet been demonstrated under routine clinical conditions in the form of comparative studies in a large cohort of patients.

New types of funding are urgently needed to build on proof-of-concept research to establish follow-up studies according to the above-mentioned MDR guidelines. Special infrastructures that offer open user platforms – for example, under the umbrella of a university hospital – and bundle the expertise of renowned players from science and industry are necessary to accelerate the translation of new diagnostic and therapeutic procedures in the long term. The Leibniz Center for Photonics in Infection Research, Jena, Germany – which was recently included in the national roadmap by the German government – shows what such translational research could look like in concrete terms.
With more happening in the world of mass spectrometry than ever before, we decided it was high time we launched a dedicated newsletter to help better serve the community – a space for #TeamMassSpec to flourish! Mass Spec from The Analytical Scientist will not only keep you up-to-date with the latest advancements in technology and the most exciting applications, but also bring together (and amplify) all the different voices in the field.

SIGN UP HERE
Putting COVID-19 on a diet. It turns out SARS-CoV-2 loves fat. Like, a lot. Based on observations that some people with a higher body-mass index were more sensitive to COVID-19, researchers from Oregon Health & Science University and the Department of Energy’s Pacific Northwest National Laboratory decided to use nontargeted lipidomics to look a little closer at how the virus alters lipid levels in cells. What they saw was a massive shift, with some fats increasing 64-fold. Further research revealed the virus completely takes over the fat-processing system in the body. Based on this discovery, the team decided to put the virus on a diet – pumping it with weight-loss drugs (small-molecule glycerolipid biosynthesis inhibitors) to cut off its fuel supply and stop it replicating. Crucially, the authors found this inhibition works across the main variants of concern, meaning this approach could continue to work as the virus evolves.

Combating hunger. Talking of diets, have researchers just discovered the latest, greatest diet pill? Perhaps, Jonathan Long and his team published a study showing that exercise induces the production of Lac-Phe – an “anti-hunger” molecule that appears to suppress appetite post-exercise (at least in mice). Using MS, the team studied how the metabolome shifted and changed during exercise, and eventually honed in on one peak in particular with a mass of 236. Thanks to some collaborative work with a team at Stanford University, they were able to decipher that this molecule was a combination of lactate and phenylalanine. They even went further and fed this molecule to obese mice, finding that their food intake dropped by about 30 percent. The next steps are to elucidate the mechanism behind this activity.

A boon for drug discovery. Drug discovery just got a new best friend in the shape native ambient MS. By applying this technique to the analysis of non-covalent protein-drug complexes in vivo, researchers have managed to gather more information about drug-target interactions in their native physiological environment. “Using mass spectrometry on proteins is often compared to making an elephant fly,” said lead researcher Helen Cooper. “What we’ve done is add an unsecured hat – the drug molecule – to the elephant, and measured the whole process. It’s exciting because it opens up the possibility of being able to follow the route of a drug through the body. By identifying which proteins it interacts with, scientists will be able to predict at an earlier stage whether or not it will have the desired therapeutic effect.” The hope is that this same technique can next be applied to human tissues, and that it will help guide drug discovery efforts in the future.

IN OTHER NEWS

IMS-MS approach elucidates higher order structures of monoclonal antibodies.

Cyclic ion mobility MS reveals localization of aspartic acid isomerization to isoaspartic acid in therapeutic peptides.

Quantitative chemical proteomics assay reveals off-target landscape of histone deacetylase (HDAC) drugs.

Quantitative MS-based succinylproteomics analysis of SARS-CoV-2 infection reveals host protein post translational pathways that could provide potential antiviral drug targets.

Open-access research article explores application of sub/supercritical fluid chromatography for biomolecules and how it can fingerprint complex peptides.

Sapient receives US$9.2 million grant from the Bill & Melinda Gates Foundation to use its rapid liquid chromatography-mass spectrometry platforms for population-scale discovery metabolomics analysis.
Meet the Trendsetting Techniques of Biotherapeutic Molecule Characterization

Advanced analytical technologies now offer the detailed assessments of structure and function that developers need to bring their molecules to market.

Producing accurate analytical assessments of complex biological molecules places a heavy demand on lab workers and managers—and an even heavier demand on capabilities and operational resources. The demands are non-negotiable; developers absolutely need detailed functional and structural data to advance their programs. Assessing the therapeutic activity of today’s biotherapeutic molecules requires sensitive analytical technologies and systems that perform two key roles: i) characterizing the increasingly complicated protein structures, and ii) closely observe pharmacokinetic activity.

Analytical assessments of complex biological molecules rely on an array of functional assays to help determine whether the molecule under scrutiny elicits an appropriate cellular and immune response that correlates to potency in vivo. Fortunately, analytical technologies are evolving; breakthroughs in tech are rendering assessments more efficient and providing for as-yet unmet needs in biopharmaceutical analysis. Here, we examine the use and evolution of three key analytical technologies.

Hello, ELISA
One challenge biopharma developers face is the question of how to evaluate the potential functionality of the molecule in vivo. During early phase development, researchers often begin assessments using an enzyme-linked immunosorbent assay (ELISA) to determine the binding potency of their biological molecule. As binding potency assays typically require less time for development and qualification, this approach can accelerate the Investigational New Drug (IND) filing process.

ELISA is a plate-based assay technique designed for detecting and quantifying soluble substances, such as peptides, proteins, antibodies, and hormones. This popular early-development phase assay—also referred to as an enzyme immunoassay (EIA)—often serves as a jump-off point for biomolecular analysis (1).

In an ELISA, the target macromolecule is immobilized on a microplate and then complexed with an antibody linked to a “reporter” enzyme. By measuring the activity of the reporter enzyme via incubation with the appropriate substrate, a measurable detection is observed. In most cases, the most critical aspect of ELISA analysis is that it identifies a highly specific antibody–antigen interaction.

Because it can yield fundamental and critical data, ELISA is generally considered an early development tool. In some cases, a cell-based assay may be needed to assess proper function; for example, if the molecule has multiple functional domains that may interact with several molecules to produce the desired effect.

There are several types of cell-based assays that can be used as a model to assess a molecule’s activity in vivo. Cell viability assays determine the ratio of live to dead cells. Cell proliferation assays assess the biological process of cells as they proliferate over time through cell division. Other varieties of assay include cytotoxicity, cell signaling, and cell apoptosis. Cell-based assays have a number of utilities—for example, certain assays can measure anticancer drug effects, and others can yield data to support CMC development and technical transfer (2).

A prize-winning approach: PCR DNA amplification
Polymerase chain reaction (PCR) is recognized as one of the most important scientific advances in the history of molecular biology. It revolutionized the study of DNA so completely that its creator, Kary B Mullis, was awarded the Nobel Prize for Chemistry in 1993. Described by the National Institutes of Health’s National Human Genome Research Institute (NHGRI) as molecular “photocopying,” PCR assays have proved to be an efficient means of “amplifying” or copying small segments of DNA (3).

According to the National Human Genome Research Institute, because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without PCR amplification. Once amplified, the DNA produced by a PCR assay can be used in many different laboratory procedures. Lastly, PCR also supports a number of laboratory and clinical techniques including DNA fingerprinting, detection of bacteria or viruses, and diagnosis of genetic disorders.

But PCR is also an essential analytical tool for biologics. For example, quantitative PCR is necessary for amplification of residual host cell DNA (HCD) – an upstream process-related impurity resulting from cell culture processes. HCD can elicit oncogenicity, infectivity, and possible immunomodulatory effects. Demonstration of viral clearance is also needed prior to clinical trials to ensure the purification process steps have reduced viruses that may have been introduced during production. During viral clearance

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studies, reverse-transcriptase PCR (RT-PCR) can be used to confirm the presence of viruses and determine their identity.

**Glycan analysis: Not so sweet**

Glycan analysis can be challenging because glycosylation occurs heterogeneously; multiple glycan structures are present on a single site but can also result from branching. Relative quantitative glycosylation analysis provides better information with more reproducible and robust results; however, such methods are time-consuming and laborious to accomplish in commercial settings. In contrast, high-throughput glycan analysis can save time but usually only offers qualitative results.

Currently, appropriate glycan analysis method(s) may be chosen based on selection criteria, information needed, and suitability relative to the process stage at which the data is needed. However, an understanding of how glycan structure affects the protein activity of the molecule will help determine which method is appropriate.

**Evolving analysis: capillary electrophoresis**

At the turn of the 21st century, release and stability analytical analysis for size variants was typically performed using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), a discontinuous electrophoretic system commonly used to separate proteins with molecular masses between 5 and 250 kDa. Similarly, isoelectric focusing (IEF) gels were used to assess and correlate charge variants.

Both of these techniques are time-consuming and expensive because processing the gel slabs and processing/imaging the gels can be labor and material intensive. The result is a higher than necessary cost of goods (CoG) profile that is not economically sustainable in the long term, especially for labs purchasing pre-cast SDS-PAGE and IEF gels.

Since then, capillary electrophoresis (CE) has become more ubiquitous and central to CE-SDS and (i)cIEF analysis. Capillary electrophoresis allows for separation of the molecule based on size or charge within a capillary, which enables more robust and reproducible results.

Increasingly complex molecules such as bi/multi-specific antibodies, fusion proteins, or recombinant proteins are posing new challenges because they require novel techniques and don’t quite fit into the traditional analytical “box.”

Evaluating and assigning the critical quality attributes (CQAs) for complex molecules can be difficult. For example, consider the characterization of multi-specific antibodies; traditional size exclusion high-performance liquid chromatography (SE-HPLC) and CE methods may not have the sensitivity to resolve minor differences in protein product variants, such as chain mispairings. For optimal results, the above-mentioned platform methods should be optimized to ensure that the intricacies of more complex molecules (product-related variants, impurities, and post translational modifications) can be resolved in a reproducible and robust manner.

**Advanced analytical methods lead the way ahead**

Industry experience tells us that when manufacturers combine fit-for-purpose cell line development platforms with advanced structural and functional analytical methods, it can optimize development and accelerate project timelines.

Incorporating phase-appropriate analytical platform methods along with high-throughput techniques to accurately characterize molecules in development will play an increasingly crucial role, helping biologics developers bring safe and efficacious drugs to patients faster.

References

Ideal Choice for Challenging Phosphorothioate Oligonucleotides: New YMC-Accura Triart Columns

Several substances are able to interact with common “inert” column hardware materials such as stainless steel or titanium. Critical substances in particular are oligonucleotides. The adsorption on metallic surfaces typically leads to peak tailing, loss of recovery and sample carry over. This behaviour can especially be seen when a new column is used without any conditioning before the actual analysis.

For these challenging substances, various bioinert options are offered for YMC-Triart columns. The new YMC-Accura Triart (U)HPLC columns are characterised by a bioinert surface coating on the column body and frit. YMC-Accura columns are ready to use without any preconditioning, unlike the analysis of sensitive substances using standard columns. The sensitivity is significantly enhanced and precious samples are recovered without any loss. Doubled peak heights and peak areas are provided for phosphorothioate oligonucleotides analyzed using a YMC-Accura Triart Bio C18 column compared to those for a regular stainless-steel column.

Full method details can be accessed here: https://ymc.eu/d/brDnU

Experts in Reproducibility

- Robust Bio-RP (U)HPLC
  Extremely inert particles for sharp peaks of proteins/peptides, oligonucleotides or mAbs.
- High Recovery IEX
  Low adsorption and excellent resolution in proteins, mAbs and oligonucleotides analyses.
- Highly Efficient HIC & SEC
  Different selectivities for fast and reliable analysis of proteins, mAbs and ADCs.

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Solutions

Significantly increased peak heights and areas are obtained using a YMC-Accura Bio C18 column for the challenging analysis of phosphorothioate oligonucleotides.
Nicotine analysis in e-liquid without glycerin interference - BaySpec portable mass spectrometer

Here is a comparative analysis between various concentrations of nicotine in electronic cigarette liquid (e-liquid) and a nicotine analytical standard measured at the same concentrations. Glycerin is a major base component in e-liquid used for electronic cigarettes, and with certain ionization techniques, is known to interfere with the ionization of compounds, including nicotine. The comparative analysis will be between the e-liquid samples with glycerin as a major component of its base and a nicotine analytical standard that does not have glycerin present. If a significant level of ion suppression from the glycerin is taking place, the intensity of the nicotine peak from the e-liquid is expected to be significantly smaller than the analytical standard at the same concentration. Conversely, if ion suppression from the glycerin is not taking place or the suppression is negligible, the intensity of the nicotine peak from the e-liquid should be comparable to the analytical standard at the same concentration.

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Extractions of Illicit Drugs from Wastewater using Empore™ Syringe-type Membrane SPE Cartridges

Illicit drugs and their metabolites are frequently detected in wastewater. The type and concentration of illicit drugs detected often provide insight into drug use and trafficking patterns within a region, but also the adverse effects on aquatic life, farm animals, and humans, by spreading into soils and waterways.

This application note utilizes a novel method for extractions of illicit drugs using the Empore™ EZ-Disk, which is a syringe-type SPE cartridge fitted with the Empore™ solid phase extraction (SPE) membrane. The syringe-type filter contains a 1.2µm glass filter fiber membrane, a 0.22µm PTFE microporous filter membrane, and the Empore™ mixed phase cation SPE membrane, all 25mm in diameter.

50mL of wastewater sample was extracted with the syringe-type SPE cartridge mounted the Empore™ EZ-Trace SPE workstation. Analytes were eluted with 2mL of methanol. Extracts were analyzed using LC-MS/MS. Recoveries of 10 illicit drugs were between 88 and 113% with 1-9% RSD when extraction is performed at a 10mL/min flow rate (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>morphine</td>
<td>94.7</td>
<td>8.7</td>
</tr>
<tr>
<td>ketamine</td>
<td>100.4</td>
<td>0.9</td>
</tr>
<tr>
<td>cocaine</td>
<td>103.4</td>
<td>1.9</td>
</tr>
<tr>
<td>amphetamine</td>
<td>90.1</td>
<td>4.8</td>
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<tr>
<td>methamphetamine</td>
<td>88.4</td>
<td>3.6</td>
</tr>
<tr>
<td>MDA</td>
<td>112.5</td>
<td>1.7</td>
</tr>
<tr>
<td>MDMA</td>
<td>100.4</td>
<td>2.1</td>
</tr>
<tr>
<td>non-ketamine</td>
<td>95.8</td>
<td>3.2</td>
</tr>
<tr>
<td>O6-monoacetyl-morphine</td>
<td>107.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>93.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table 1. Average percent recovery of 10 analytes for a 10mL/min extraction flow rate (n=6).

Email address: info@cdsanalytical.com

Empore™ EZ-Disk Syringe-Type SPE Cartridges

- Flexibility for use with Different Sample Volumes
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- 10 mL/min Flow Rate
Food Analysis with Confocal Raman Microscopy

In the food industry, various ingredients and additives such as emulsifiers, stabilizers or thickeners are commonly used to optimize the texture or flavor of food. Their distribution and microstructure strongly influence the properties of the final product. Therefore, research and development, as well as quality control, require powerful analytical methods for studying the distribution of compounds in food.

This survey shows how Raman imaging can characterize food samples to help understand products and production processes with confocal measurements, scans guided by an integrated profilometer and investigations that employ a Raman spectral database. It describes experiments on white chocolate, fat spreads, a sugar bar, a squashed banana pulp sample and a honey pollen grain. It also features 3D Raman imaging of conventional and spreadable butter, topographic Raman imaging of frosted gingerbread, and Raman-based automated particle analysis of a mixture of baking ingredients.

Learn more about Raman microscopy for Food Analysis

3D Raman Imaging

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Let your discoveries lead the scientific future. Like no other system, WITec’s confocal 3D Raman microscopes allow for cutting-edge chemical imaging and correlative microscopy with AFM, SNOM, SEM or Profilometry. Discuss your ideas with us at info@witec.de.
Selecting the Optimal Column for Native SEC-MS of Monoclonal Antibodies

Characterization of monoclonal antibodies (mAbs) is essential for product safety and efficacy. Size exclusion chromatography (SEC) coupled with mass spectrometry (MS) is increasingly used to identify the accurate molecular mass of mAbs and their impurities. However, traditional SEC generates high particle shedding, which decreases ionization efficiency over time. To avoid shedding for MS and multi-angle light scattering (MALS) applications, Tosoh Bioscience developed TSKgel® UP-SW3000-LS U/HPLC size-exclusion columns. In this application note, the column was coupled with an MS instrument for the analysis of a mAb standard. Data demonstrate that the TSKgel UP-SW3000-LS column surpasses competitive UHPLC columns and a dedicated low shedding column for SEC of proteins in terms of particle shedding observed by MS. Moreover, the column helps maintain ionization efficiency in the electrospray ionization (ESI) source >90% compared to the initial injection over >50 injections, thus increasing data quality and reducing ion source cleanings.

Read the full application note here: bit.ly/SEC-MS-of-mabs

SEC-MALS/MS FOR ACCURATE SAMPLE CHARACTERIZATION

A Mass spectrum of mAb sample

B Ionization efficiency

A column optimized for MALS/MS and biotherapeutic analytics
The TSKgel® UP-SW3000-LS U/HPLC SEC column offers unique noise suppression, resulting in increased sensitivity of advanced detection.

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Sitting Down With…
Tony Edge, Site Director (Production and R&D), Avantor Sciences, UK
Did you always want to be a scientist?
The blunt answer is no. I genuinely had aspirations of playing for Manchester United – holding the FA cup aloft! But those dreams were dashed fairly early on when I realized that I didn’t quite have the talent to play professionally. Fortunately, science took over by my teens. I wanted to be a physicist originally – all of my university applications were for physics degrees. That didn’t quite materialize and I ended up developing a passion for chemistry, especially the physical underpinnings of the field.

Initially, I was looking at reaction kinetics and fluid dynamics, which led me to chromatography. And I found the perfect combination; if you look at some of the underlying equations that govern chromatography, it’s all fluid dynamics; and if you consider chromatographic separations, it’s all reaction kinetics. Since then, I’ve been separation science mad.

You’ve had a wide range of roles throughout your career. Any major lessons learned?
There are many lessons I’ve learned from a technical perspective. But one key overarching lesson is: Don’t be afraid to try stuff! I remember early in my career, my PhD supervisor asked me for my advice on something he was a world-leading expert on. I remember thinking, you’ve written hundreds of books on this and you’re asking me? But it taught me that everyone’s opinion should be respected. Often we don’t put our hands up because we’re too frightened, but a wide range of perspectives challenges dogma. So put your hand up, try something out, you might love it! Most people reflecting on their careers only regret the things they didn’t try.

What’s the biggest development in chromatography over the course of your career?
There’s been a myriad of developments given the length of my career! One of the key ones has to be the introduction of ultra-performance LC and the sub-2 micron particle. It got everyone thinking about what they could do with this kind of sensitivity – and it also inspired the launch of superficially porous materials. Too often we become fixed in our ways because things work relatively well, so why bother? Sometimes it takes a technological leap forward to give everyone a wakeup call. Sub-2 micron was a fulcrum point for the field of separation science – and it is still having ramifications today.

What about the future of chromatography?
I often hear people say things like, “Chromatography is an older, more robust technology.” Though I don’t disagree, there are a number of exciting applications on the horizon. One thing COVID-19 has taught us is that mass testing at a scale of billions of tests every week is something that the general populace will do. And if we look at the technology involved, that’s not so far removed from a chromatography column. We already have smart watches that can tell our heart rate, temperature, or glucose levels. What information would we glean if separation science was involved?

From a technical perspective, multidimensional chromatography and the work coming out of the University of Amsterdam is incredibly exciting. The ability to create 3D printed columns is something I’ve always been fascinated by, and I can see that coming to fruition in the very near future.

Finally, in terms of chemistry, if we look at the space industry, they have had to develop chemistries that are stable in very extreme environments. Could we apply some of those technologies to the field of chromatography? The fact that we can now send a GC-MS to the planet Mars suggests that chromatography has got an important role in the future – perhaps discovering life on distant planets!

Is there a lack of appreciation, especially among students, of the importance of chromatography?
Absolutely. I think there are two challenges. First, not everybody knows what chromatography is. Second, even if they do do the blotting paper experiment at high school and gain some understanding, they never find out how powerful chromatography is.

And because the technology is more robust, you don’t need to know how it works to use the machine – you just need to hit the big green button. And that’s a shame because we need gifted people who understand the fundamentals to develop next-generation technologies that we’ll need to improve our health, ensure our environment is stable, or make sure the food that we eat is healthy and safe. There’s so much more we can do with chromatography in these areas so it is frustrating when people think of it as a “green button” technology.

Have we accepted mediocrity?
Some have, yes. There is an attitude of “that’ll do,” which I strongly reject. Instead of running with a bit of kit that’s a meter high, weighs 500 kg and costs hundreds of dollars per sample, we could have a device that sits on your wrist and monitors your health day to day. We need to keep moving chromatography forward.
Be Bold
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