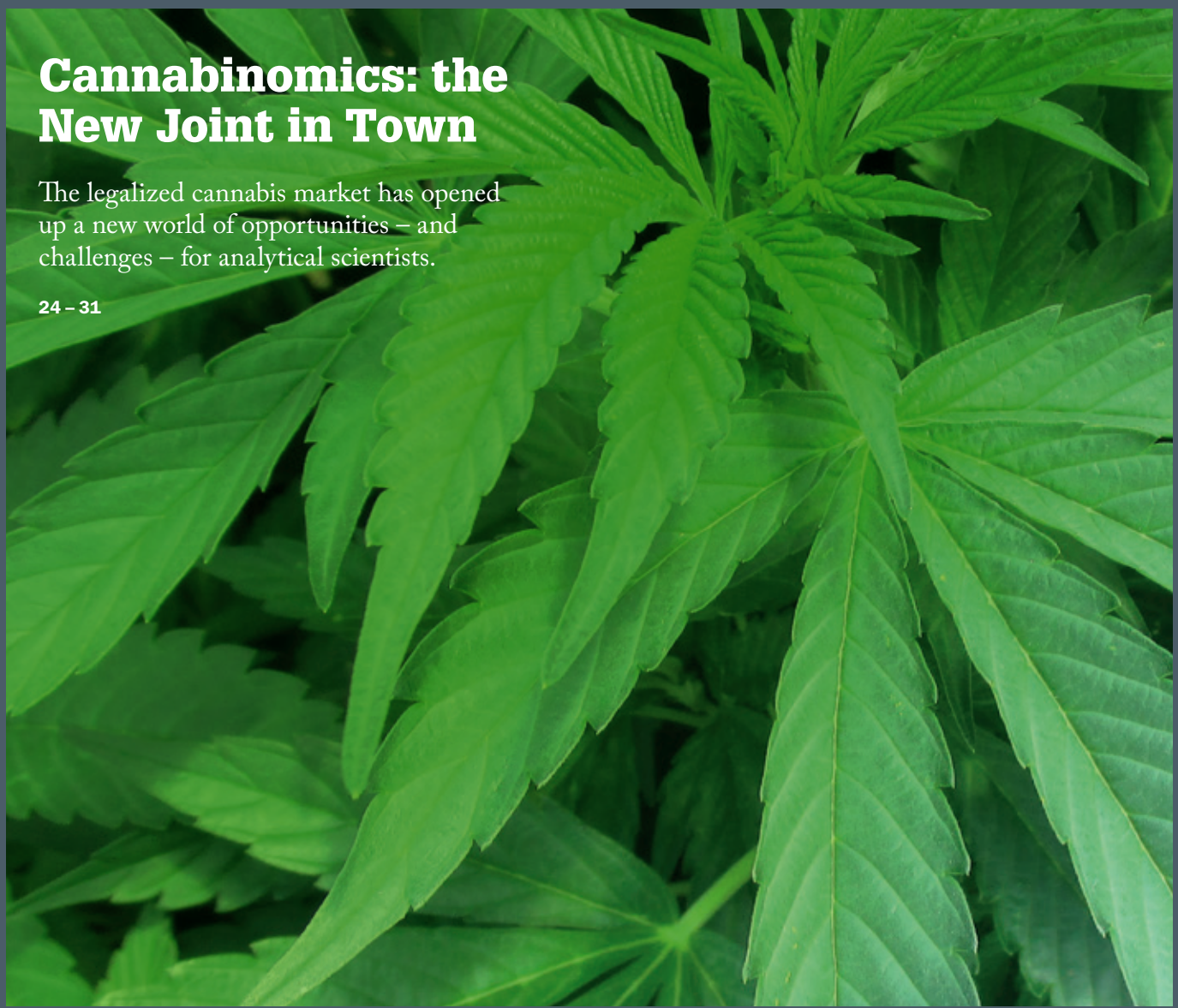
SEPTEMBER 2015 # 32

the Analytical Scientist



Cannabinomics: the New Joint in Town

The legalized cannabis market has opened up a new world of opportunities – and challenges – for analytical scientists.

Thermo SCIENTIFIC

A Thermo Fisher Scientific Brand

built

Remodelling existing design inevitably means compromise. That is why we designed the new **Thermo Scientific™ Vanquish™ Flex UHPLC System** from the ground up to meet the specific challenges of biopharmaceutical characterization, without concessions. Engineered for the analysis of protein aggregates, charge-variants, glycans, and peptides. Achieve confident, robust results from a system that is simple, reliable and fun.

for biopharma

• All the pieces • thermoscientific.com/BuiltForBiopharma

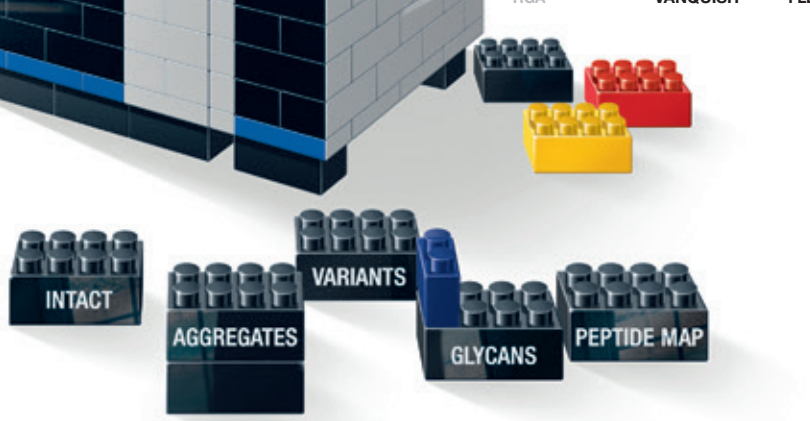
QVQLKQSGPG
PGKGLEWLGV
KMNSLQSNDT
SIMPLICITY
NSGALTSGVH
TKVDKRVEPK
KPKDTLMISR
YNSTYRVVSV
ISKAKGQPRE
DIAVEWESNG
GLYCANS
LSVSPGERVS
ASESISGIPS
GTKLELKRTV
PREAKVQWKV
VYACEVTHQG

CONFIDENCE
IWSGGNTDYN
AIYYCARALT
APSSKSTSGG
TFPAVLQSSG
SPKSCDKTHT
TPEVTCVVVD
AGGREGATES
PQVYTLPPSR
QPENNYKTTP
SVMHEALHNH
FSCRASQSIG
RFSGSGSGTD
AAPSVFIFPP
DNALQSGNSQ
LSSPVTKSFN

LVQPSQSLSI
TPFTSRLSIN
YYDYEFAYWG
TAALGCLVKD
LYSLSSVVTV
VERSATILITY
VSHEDPEVKF
LTVLHQDWLN
DELTKNQVSL
PVLDSDGSFF
YTQKSLSLSP
TNIHWYQQRT
FTLSINSVES
PEPTIDE MAP
ESVTEQDSKD
RGA

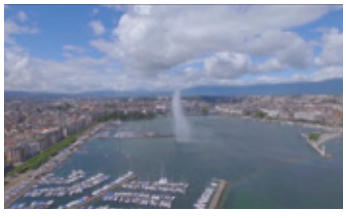
TCTVSGFSLT
INTACT
QGTLVTVSAA
YFPEPVTVSW
PSSSLGTQTY
CPPCPAPELL
NWYVDGVEVH
GKEYKCKVSN
WORKFLOWS
LYSKLTVDKS
GK
NGSPRLLIKY
EDIADYYCQQ
SDEQLKSGTA
STYSLSSTLT
VANQUISH

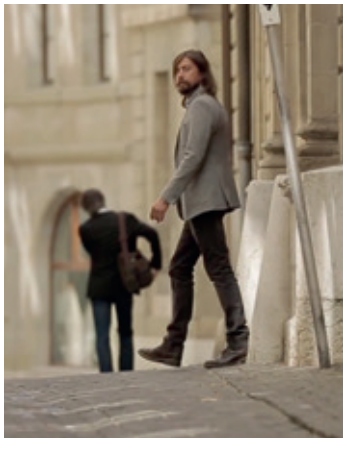
NYGVHWVRQS
KDNSKSQVFF
STKGPSVFPL
PERFORMANCE
ICNVNHKPSN
GGPSVFLFPP
NAKTKPREEQ
KALPAPIEKT
TCLVKGFYPS
RWQQGNVFSC
DILLTQSPVI
VARIANTS
NNNWPTTFGA
SVVCLLNNFY
LSKADYEKHK
FLEX UHPLC

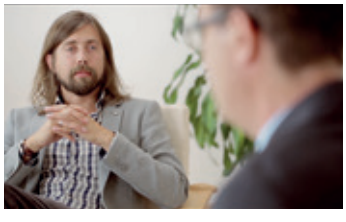
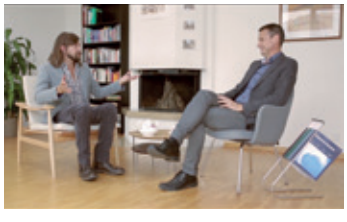
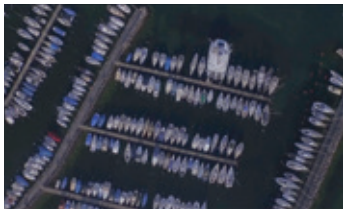


Online this Month

Coming soon...



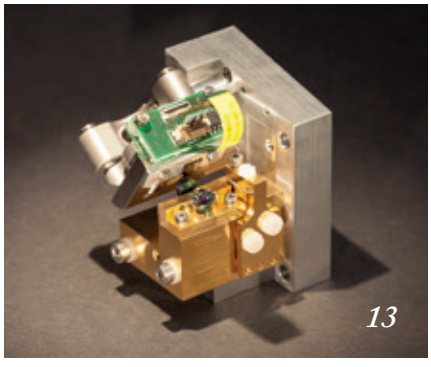


Tea With Rich...at HPLC 2015 in Geneva.

Watch the trailer now: tas.txp.to/0715/TeaWithRich

Contents



On The Cover

An explosion of green from legally grown cannabis plants
Credit: Scott A. Kuzdzal

Upfront

In My View

Experience the quintessence

Milli-Q® Integral system
pure and ultrapure water at your fingertips

- Dual POD (point-of-delivery) concept saves space and increases convenience
- Lower running costs and water waste with exclusive Elix® technology
- Millitrack® embedded e-Solutions for optimized laboratory performance and remote access

Experience more www.merckmillipore.com/ultrapure

Merck Millipore is a division of MERCK

TOSOH

YOUR SPECIALIST IN SEPARATION

POWERED BY 40 YEARS OF GFC EXPERIENCE: TSKgel UP-SW3000

- HIGHER RESOLUTION FOR mAb AGGREGATE ANALYSIS
- SHORT RUNTIMES IN QUALITY AND PROCESS CONTROL
- COMPATIBLE WITH ALL UHPLC INSTRUMENTS
- SEAMLESS TRANSFER OF HPLC METHODS

FIND OUT MORE ABOUT OUR NEW GEL FILTRATION COLUMNS FOR UHPLC ON HTTP://BIT.LY/1LOJS3N

TOSOH BIOSCIENCE

36

Features

Reports

Departments

Sitting Down With

the **Analytical Scientist**

ISSUE 32 - SEPTEMBER 2015

Change of address:
tracey.nicholls@texerepublishing.com
Tracey Nicholls, The Analytical Scientist, Texere Publishing Limited, Booths Hall, Booths Park, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

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

Distribution:
The Analytical Scientist (ISSN 2051-4077), is published monthly, by Texere Publishing Ltd and is distributed in the USA by UKP Worldwide, 1637 Stelton Road B2, Piscataway, NJ 08854. Periodicals Postage Paid at Piscataway, NJ and additional mailing offices
POSTMASTER: Send US address changes to The Analytical Scientist, Texere Publishing Ltd, C/o 1637 Stelton Road B2, Piscataway NJ 08854
Single copy sales £15 (plus postage, cost available on request tracey.nicholls@texerepublishing.com)

SHIMADZU
Excellence in Science

CHANGES **EVERYTHING**

Shimadzu's new LCMS-8060 makes a real difference to working better and faster. The LCMS-triple-quadrupole combines all UF technologies and pushes the limits of LC-MS/MS quantitation for applications requiring highest sensitivity and robustness.

World's highest sensitivity
based on the new UF Qarray technology, delivering new limits of MRM sensitivity and impacting full-scan sensitivity

Unmatched speed
due to data acquisition with scan speed of 30,000 u/sec and polarity switching time of 5 msec

Outstanding durability
achieving peak area response RSD of 3.5 %*, thus showing high robustness

UFMS
ULTRA FAST MASS SPECTROMETRY

www.shimadzu.eu

*2,400 samples of femtogram levels of alprazolam spiked into protein-precipitated human plasma extracts over a 6 day period (over 400 samples were injected each day).

How Do You Measure Success?

Opening Pandora's box with a seemingly simple question.

As Amy Herr notes wryly in the final pages of this issue: "A lot of analytical scientists are intrigued by measurements." Hard to argue with that point, but the art of measuring extends beyond the numbers generated by analyses. Indeed, Amy also says that, as an engineer, conferences are important because they allow her to look behind solutions to understand the "metrics of performance" for a particular problem. Put another way, she is interested in how success is measured. And that strikes me as a powerful concept.

In fact, it opens up a Pandora's box of additional questions – especially as success is very much dependent on your perspective. Is your new analytical method successful because it pushes the boundaries of what's possible? Or is it unsuccessful because the extra sensitivity it provides is unnecessary and burns through precious resources? Ad de Jong raises an interesting point on page 36 when it comes to the method development for non-targeted analyses. How do we know that our methods are truly optimized?

Has your analytical laboratory been successful this year because it hit all (financial) targets? Or have unnecessary – and unidentified – mistakes occurred because of corner cutting or inadequate training? Are you successful because you are now "Director of Important-Corporate-Division" or because you invest your time in others, sharing hard-earned knowledge with the next generation? Perhaps you volunteer at your local school to help inspire the success of the next, next generation (see page 42).

Is your nation successful because it has assured the safety of its citizens with high-quality food and drugs (or simply shelter) or because it is rolling out more charitable and advanced programs to help resource-poor nations do the same?

Yes, indeed. Success is not black and white, but an ever-shifting palette of grey.

As we enter the autumn months of The Analytical Scientist's third year, we are not immune to the question. I certainly hope that we have stayed true to our mission: celebrating, recording and scrutinizing the world of analytical science – but only you, our readers, can measure our success.

One thing is certain: we are all in a better place when we start asking the questions "what is success and how do we measure it?"

So... how do you measure success?

Rich Whitworth
Editor

Upfront

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

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

Alzheimer's Analytics

Predictive biomarkers in saliva may yield a simple, noninvasive diagnostic test

The baby boomer population is aging and the incidence of Alzheimer's disease on the rise, but a definitive diagnostic test for the condition has been elusive. Currently, Alzheimer's is diagnosed by a series of cognitive tests, with biological confirmation possible only by postmortem examination of the brain. But cognitive tests aren't always reliable, and postmortem examinations aren't much help to patients.

New research from the University of Alberta, Canada, suggests that the answer may lie in saliva. At the recent Alzheimer's Association International Conference, neuroscience student Shraddha Sapkota reported that metabolomic analysis of salivary samples by liquid chromatography-mass spectrometry allows clear discrimination between patients with Alzheimer's, those with mild cognitive impairment, and those with normal cognitive aging (1). The researchers were also able to identify the top metabolites for distinguishing between conditions. Most importantly, the study results revealed directional associations between certain metabolites and cognition states – that is, biomarkers upregulated in patients with known cognitive impairments were predictive of episodic memory problems and slow neurocognition when elevated in those with normal aging. Detecting such biomarkers in saliva tests may eventually allow doctors to identify Alzheimer's patients before they become symptomatic. Not only that, but gaining this knowledge before cognitive issues become evident allows patients to take part in decision-making processes while still able, and allows researchers to identify potential clinical trial participants for Alzheimer's disease therapeutics or preventatives.

The benefits of the research extend beyond identifying predictive biomarkers. The saliva test itself is a new approach to Alzheimer's diagnosis and has advantages of its own. Saliva testing is easy, painless and noninvasive, and the fluid itself is easy to transport – ideal given the likelihood of repeated testing over a long time span. The work is still in its early stages, but salivary biomarkers appear to show clear diagnostic promise. *MS*

References

1. *S Sapkota, et al., "Metabolomics analyses of salivary samples discriminate normal aging, mild cognitive impairment, and Alzheimer's disease groups and produce biomarkers predictive of neurocognitive performance". Presented at the Alzheimer's Association International Conference; July 21, 2015; Toronto, ON, Canada. Abstract ID: 4782.*

Do Bears Also Eat in the Woods?

Laser ablation and ICP-MS has the answer

Grizzly bears have a somewhat elusive lifestyle, but researchers at the Washington State University Bear Research, Education and Conservation Center, hope that laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS, see page 20 for more) could plug some of the knowledge gaps as far as diet goes. Some analytical methods, such as stable isotope analysis of hair, are already used to study a bear's reliance on salmon and other foods, alongside traditional observational

analysis, but the group believe that LA-ICP-MS could have a much bigger impact.

As LA-ICP-MS had not been previously used on animal hair, the researchers had to fine tune and adjust the laser settings for optimal analysis. Appropriate internal and reference material standards were also required. Jennie Christensen, an environmental scientist at Stantec who collaborated on the study (1) explains, "For internal standards, we opted for sulphur due to its high and consistent signal across the length of the hair. For reference materials, we initially attempted to produce a pressed pellet of human hair reference material, but they were friable and created dust, contaminating the analyses." The group settled on DOLT 2 (dogfish liver powder pressed into a pellet) a well established reference material that provided consistent results for the elements of interest. "Where we deviated from a conventional approach to LA-ICP-MS was in our quality assurance/quality control," says Christensen. "Though we conducted the typical background runs, reference standard runs, and internal standards, we also included some additional tests for quality to ensure accurateness and effectiveness of our approach and calculations."

The results? Christensen confirmed that hair certainly does record a highly accurate and detailed account of dietary intake of certain metals, most prominently copper, zinc and mercury. "The information provided by LA-ICP-MS was temporally more detailed than conventional analysis using blood sampling," says Christensen. She adds that it is also immeasurably safer for the researcher, who no longer requires actual access to the bear; hair is easy to obtain through hair-snagging programs or collection from "rubbing trees".

The analysis also highlighted some potentially worrying conclusions about dietary impact on bear health. "We were able to ascertain improved risk assessment for bears that may have access to metal-laden foods. In our study, the fish had higher concentrations of mercury than other terrestrial-borne foods and it was easy to see when and how much fish individual bears consumed," says Christensen. "While conventional analysis and past research has never identified any mercury-associated health risks to salmon-eating grizzly bears, using LA-ICP-MS to get a more accurate temporal record of exposure clearly indicates that some bears exceeded toxicity thresholds for mercury for up to 76 consecutive days."

Monitoring trace elements in wildlife using LA-ICP-MS is in its infancy. And although this study is useful for understanding exposure to metals in grizzly bears, Christensen says that every wildlife species will require modification of the approach. But the researchers have already been evaluating the technique for a few other mammalian species and are also expanding the scope to humans – as a non-invasive technique to evaluate exposure to various metals, deficiencies in essential elements and potential health risks. Christensen is also evaluating the use of other non-invasively collected tissues for biomonitoring metal exposure and health. *SS*

Hair analysis continues on page 14.

Reference

1. *M Noël, et al., "Using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to characterize copper, zinc and mercury along grizzly bear hair providing estimate of diet," Science of The Total Environment, 529 (1), (2015). DOI: 10.1016/j.scitotenv.2015.05.004*

(Don't) Fade to Grey

Synchrotron-based FTIR, XRF, and XANES probe Matisse's jaded yellow

"The Joy of Life" by Henri Matisse, 1905–06, The Barnes Foundation, Philadelphia

Research into the degradation of cadmium sulfide (CdS) yellow pigments on the works of the early modern masters began when Barbara Buckley, the director of conservation at the Barnes Foundation, observed the declining condition of Henri Matisse's Le Bonheur de vivre (The Joy of Life, 1905-60, The Barnes Foundation) – a "monumental" work that defined his signature style and kick started a long-lasting rivalry with Picasso.

The yellow paint beneath the central reclining figures was fading to a dirty ivory color; simultaneously, the upper layer was flaking away to reveal the original bright-yellow color beneath. Jennifer Mass, a senior scientist at the Scientific Research and Analysis Laboratory Conservation Department, Winterthur Museum, Delaware, USA, led the international research project that followed. "The code of ethics of the American Institute for Conservation of Artistic and Historic Works (AIC), challenges chemists in the field of art conservation to do our work either noninvasively using standoff methods or in as minimally invasive a manner as possible," she says. To that end, the team initially chose to examine the painting using portable X-ray fluorescence spectroscopy (pXRF), which revealed that both cadmium yellow and chromium yellow pigments had been used, but only the cadmium yellow paints were undergoing a physical and chemical breakdown.

Next, microsamples of paint were removed to probe composition as a function of depth, using synchrotron-based FTIR, XRF, and XANES (X-ray absorption near-edge structure) imaging. "The analyses revealed that the off-white crust was composed predominately of cadmium carbonate, cadmium sulfate, and cadmium oxalate," says Mass. Studying the composition of the paint as a function of depth helped identify the compounds as photo-oxidation products rather than residual starting reagents or fillers. The resulting cadmium compounds are all white, which explains the fading of the paint from yellow to ivory – and, as the pigment degrades, the sulfates seem to cause acid hydrolysis of the oil binder, resulting in the crumbling and flaking.

Sadly, it's not an uncommon problem. "The phenomena described above are observed predominately in cadmium yellow paints prepared between the 1880s and the 1920s, which unfortunately means that degradation of cadmium yellow paints is found in the works of the impressionist, post-impressionist, and early modernist masters, including Claude Monet, Pablo Picasso, Vincent Van Gogh, and Henri Matisse. Literally billions of dollars of global cultural heritage is at risk," says Mass.

On the bright side (pun intended), the new knowledge gained is likely to lead to recommendations for preservation. Next, the group is interested in detecting at-risk paintings before the degradation of occurs. "Preliminary work has revealed that cadmium yellow pigments that are beginning to degrade have unique fluorescence properties. Ongoing work will attempt to reproduce these early findings, and determine under what conditions they can be predictive," concludes Mass. *RW*

Reference

1. *E Pouyet, et al., "2D X-ray and FTIR micro-analysis of the degradation of cadmium yellow pigment in paintings of Henri Matisse", Applied Physics A (2015). DOI: 10.1007/s00339-015-9239-4.*

Quantum Leap in Real-time Spectroscopy

Continuous monitoring of chemical products with an innovative matchbox-sized laser

Product quality monitoring is essential in pharmaceutical and chemical production, but often performed manually. Researchers from two Fraunhofer Institutes (Applied Solid State Physics in Freiburg and Photonics Microsystems in Dresden, Germany) may have found the ultimate solution. The team has developed a matchbox-sized laser module that can be rapidly tuned over a wide spectral range, opening the door to spectroscopic identification and quantification of chemical substances in real-time. Ralf Ostendorf, project manager at the institute in Freiburg, says the original goal was simply to develop a miniaturized laser source.

"Next, we decided to build a compact laser source with the aim of demonstrating the capability of laser-based mid infrared spectroscopy in different applications," says Ostendorf. "We used a small silicon chip that integrates an optical diffraction grating in a micro-optical-electric mechanical system (MOEMS) scanner and combined this with a quantum cascade laser (QCL) chip. Both chips independently measure only a few millimetres – but the potential is enormous."

QCLs emit light in a very broad spectral range in the mid infrared but the MOEMS diffraction grating can be continuously tilted (with frequencies of up to several kHz) using electrostatic forces, which in turn rapidly "tunes" the wavelength of the laser to a narrow band that can "scan" a wide spectral range. "At this point, we realized that we are able to perform very fast spectroscopy – even in real-time," says Ostendorf.

Real-time spectroscopy allows continuous monitoring, which can be used to optimize chemical reactions or measure product composition. Moreover, Ostendorf says, "The spectral brightness of our laser also makes it possible to measure aqueous solutions. Traditional techniques like FTIR can only measure water film solutions up to 10 µm. We were able to identify caffeine dissolved in water (25 mg/L) through a 150 µm water film – and that's very attractive for sensing solutions that are operated in a bypass flow cell."

Ostendorf has high hopes for the device: "The system could be used for quality monitoring in the food industry, for the measurement of trace gases in environmental control, or even in the medical sector," he says.

"We're currently working on our first real-time spectroscopic measurements to demonstrate the capabilities. And we want to further miniaturize the laser source and simplify the alignment procedures of the grating and micro optics."

Clearly, there is a great deal of work ahead, but widening the spectral tuning range could open up access to even more applications – at which point, the term quantum leap seems entirely justified. *RW*

Splitting Hairs

Despite the recent bad press, cutting-edge analytical techniques are transforming the field of hair analysis

By Bryn Flinders, post-doctoral researcher, Maastricht MultiModal Molecular Imaging institute (M4I), The Netherlands.

Forensic scientists adopted microscopic hair analysis way back in the 1950s, examining the characteristics of forensic hair samples in terms of pigment distribution or the scale pattern in order to compare it with hair taken from a suspect.

However, the validity of the technique came under heavy fire earlier this year. An article in The Washington Post reported that US Federal Bureau of Investigation (FBI) examiners gave flawed testimony regarding microscopic hair analysis, resulting in several wrongful convictions (1). But in a way, that's old news. The introduction of DNA profiling completely changed the game, and convictions based on microscopic hair analysis have been overturned as a result.

Moreover, hair analysis continues to evolve and it can provide a great deal of useful information. For example, toxicologists and forensic scientists use it to detect drugs of abuse. Hair analysis is highly advantageous as it can provide a historic record of ingested chemicals versus a transient detection in biological fluids, and it can reveal chronological information about drug intake. This approach uses chromatographic techniques coupled with mass spectrometry to analyze 1-cm long hair segments. As good as this technique is, sample preparation is complex and also consumes the sample, making repeat analyses difficult.

Recently, the use of mass spectrometry imaging (MSI)-based techniques, such as matrix-assisted laser desorption/ionization (MALDI) and secondary ion mass spectrometry (SIMS), have been used to provide visual, chronological information on the distribution of pharmaceutical compounds or drugs of abuse in hair samples (see Figure 1). The improved spatial resolution results in a more accurate drug usage history, using micrometer versus centimeter lengths of hair, and in hours versus months.

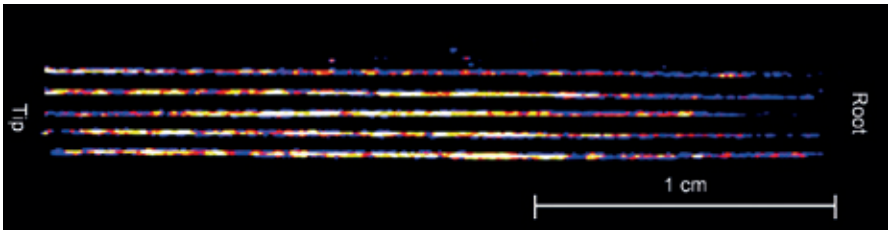


Figure 1. MALDI-MS image showing the distribution of cocaine throughout five hairs.

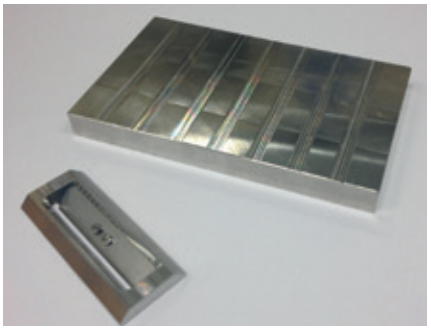


Figure 2. Innovative apparatus (cutter and block) used to literally split hairs.

One of the issues often raised is whether detected compounds are present because of external contamination or actual abuse. The use of decontamination protocols and the detection of unique metabolites is usually enough to discern this. An innovative apparatus (developed by FOM-AMOLF/M4I, see Figure 2) to prepare longitudinal sections coupled with high spatial resolution imaging techniques provides more detail for the consequences of decontamination protocols (2).

Mass spectrometry imaging has potential in the future to provide information about a suspect from single hair samples found at a crime scene, because the presence of pharmaceuticals, drugs of abuse, or other compounds could be unique to a suspect. If a DNA profile is unobtainable due to the absence of the hair root, or links need to be made to a particular chemical exposure, the technique offers real advantages. Chemical analysis of hair samples could potentially provide valuable information on proteins, metabolites or small molecules, for example, that together with other evidence could help to identify a suspect.

References

1. *http://goo.gl/UMn9cI*
2. *B Flinders, et al., "Preparation of longitudinal sections of hair samples for the analysis of cocaine by MALDI-MS/MS and TOF-SIMS imaging", Drug Testing and Analysis, first published online (2015).*

Simpler Blood Sampling

hemaPEN aims to rewrite the textbook for disease screening, therapeutic drug monitoring and medical research

What? hemaPEN is a prototype blood collection and storage device, and the first product concept from ASTech (www.astech.org.au) – the ARC Training Centre for Portable Analytical Separation Technologies, which is part funded by the Australian Research Council, the University of Tasmania (UTAS), and

Trajan Scientific and Medical. hemaPEN aims to harness the benefits of dried blood spot (DBS) sampling in an intuitive, safe, and portable format.

Who? hemaPEN is the result of research by Florian Lapierre, an ASTech post-doctoral research fellow from UTAS with a PhD in the field of sophisticated microfluidic device design. As with many good ideas, Lapierre was inspired by something really quite simple: "I was clicking my pen and thought, why have a retractable pen? So you can click when you need it, and not get ink everywhere when you don't. hemaPEN takes the same form, collecting blood when you want to and safely storing it inside."

Lapierre says that working at ASTech has given him greater insight into developing thought processes beyond the traditional academic setting. "I am undertaking my industry placement at Trajan and have a senior mentor who encourages me to think about commercialization, which has changed the way I approach my research."

Why? hemaPEN allows people to collect an uncontaminated and precise volume of their own blood from a fingertip at home, eliminating the need to travel to a medical clinic. It's easier for the clinic and less stress for the patient.

How? After a simple finger prick, hemaPEN collects and stores an accurate microsample of blood using microfluidic technology that can be placed in the mail to an analytical laboratory.

Next? Chief Executive Officer of Trajan (www.trajanscimed.com), Stephen

Tomisich said, "Whilst this first iteration of hemaPEN provides a DBS format ready for Liquid Chromatography-Mass Spectrometry (LC-MS) analysis, we are now working on future versions with various interfaces, potentially with in-built sensing technology. Excitingly, we will soon commence trialling the device with patients to determine the impact of self-sampling to their lifestyles and wellbeing."

In My View

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

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

Contact the editors at
edit@texerepublishing.com

There's More to Life Than DNA

Genomics often steals the headlines, but could metabolomics be the true deliverer of personalized medicine?

By Kévin Contrepois, post-doctoral research scientist, and Michael Snyder, Professor, Department of Genetics, Stanford University, California, USA.

The promise of personalized medicine is to accurately predict, diagnose and treat diseases for each individual by taking into account their biological uniqueness. To date, personalized genomes have been the main focus of such efforts, allowing the identification of disease-causing and drug-interacting variants and thus informing potential therapeutic decisions. This approach has been widely successful in the diagnosis and treatment of cancer and rare Mendelian and idiopathic diseases.

Although successful, the personal genomics approach is limited. Why? Because it does not capture biological information from the patient's environment or lifestyle. Most common diseases, including metabolic and mental disorders such as diabetes and autism, are influenced by a variety of factors and cannot be predicted from DNA sequence only. For these reasons, there is significant value in including additional personalized information derived from transcriptomics (gene expression), proteomics (protein expression), and metabolomics (metabolite profile), which together help monitor biochemical changes reflective of genetic, environmental and lifestyle influences. Despite the fact that these technologies provide independently more accurate measurements of a current physiological state than genome sequencing, their combination is essential in unraveling the molecular mechanisms contributing to clinical outcomes, which could reveal more efficient patient-tailored therapeutic targets.

As a proof-of-principle, we have published a study integrating detailed molecular information from RNAs, proteins and metabolites with genomic sequences of a single individual monitored over a 14-month period (1). This multi-omics approach uncovered dynamic molecular and pathway changes across healthy and pre-diseased states, and resulted in the actionable diagnosis of medical risks. The same approach is currently being applied to a larger cohort of patients at risk for type 2 diabetes (2) and is expected to reveal integrative multi-omic signatures associated with the development of insulin resistance and glucose misregulation.

Metabolomics has a number of advantages over other established 'omics'. First, it measures the closest molecules to biochemical phenotypes in a fast, inexpensive and unbiased way. Along with the realization that most common health problems (diabetes and cancer, for example) are associated with unique metabolic abnormalities/signatures, the field of metabolomics is attracting increased interest and holds great promises for understanding and diagnosing disease. In addition, metabolites are not only regulated by the host genome but also by RNA and protein activities, which means metabolomics contains integrated

multi-level information. Importantly, when performed on body fluids (blood or urine), metabolomics captures information not only from the host, but also from their microbiome (the commensal microbes present in or on the host). This is critical, because the gut microbiome is now recognized as a major modulator of human health and a crucial player in regulating health/disease states. For instance, the gut microbiome is essential in digestion and synthesizes hundreds of essential metabolites, such as vitamins.

Despite its great potential, we feel metabolomics has been understudied compared with other 'omics'. Indeed, we do not even know how large the human metabolome really is... Part of the challenge is that metabolites have highly diverse chemical properties, making it difficult to assess the complete metabolome of an individual with a single technology or experiment. In this context, we recently developed an optimized liquid-chromatography coupled to mass spectrometry (LC-MS) analytical platform to robustly profile urine and plasma metabolites (3). Such state-of-the-art LC-MS experiments can now routinely generate robust data sets that contain more than 10,000 metabolic features derived either from the host or the gut microbiota, among which most are still uncharacterized. Hence, a major remaining challenge in the field rests in the accurate identification of non-annotated peaks.

Thanks to its unique ability to monitor metabolites that not only contain dynamic information from the host, but also from their microbiome, metabolomics will undoubtedly become a key player for enabling personalized medicine. It will most likely enable the discovery of useful biomarkers for the onset and pathogenicity of human disease, but will also help us better understand human health and treat disease. Importantly, small molecule measurements are relatively tractable to adapt for clinical assays. For example, rapid home-test measurements of glucose and sodium levels are now possible, and several continuous monitoring assays now exist for the former.

In addition, information from many of these devices can be monitored directly on smartphones, enabling rapid feedback on metabolic state. In our view, metabolomics will lead to novel, useful biochemical tests that will profile critical components in individuals in real-time. Moreover, the integration of metabolite profiles with genomic, transcriptomic and proteomic data will produce a much clearer picture of health and disease states and significantly improve personal health management.

References

1. *R Chen, et al., "Personal omics profiling reveals dynamic molecular and medical phenotypes," Cell 148, 1293–1307 (2012).*
2. *"The integrative human microbiome project: Dynamic analysis of microbiome–host omics profiles during periods of human health and disease," Cell Host & Microbe 16, 276–289 (2014)*
3. *K Contrepois, L Jiang and M Snyder, "Optimized analytical procedures for the untargeted metabolomic profiling of human urine and plasma by combining hydrophilic interaction and reverse–phase liquid chromatography – mass spectrometry," Molecular & Cellular Proteomics 14: 10.1074/mcp. M114.046508, 1–12, (2015).*

Future Food Forensics

It's time to introduce gold standard analytical science in the fight against food fraudsters.

By Dietmar Knopp, Professor of Applied Biochemistry and Head of Group 'Bioanalytics' at the Chair of Analytical Chemistry, Institute of Hydrochemistry, Technische Universität München, Munich, Germany.

Scared by spectacular news stories in the media, my friends and I are paying much more attention to the quality of our food. Our most serious concern is the risk of food poisoning by harmful microorganisms such as E. coli O157:H7 or Salmonella, or by natural toxins, such as mycotoxins. The second important health issue we've recognized is the misuse of animal drugs, especially antibiotics. Who wants unauthorized medical treatment leading to the cultivation of multidrug-resistant bacteria? And let's not forget the third risk of allergic reactions – or even death – caused by poor or incorrect food labeling...

Beyond worrying about our health, other food safety and authenticity issues can affect food quality and contravene ethical (vegetarians) or religious (halal) choices. Disregarding accidental mislabeling, such issues are becoming increasingly common as fraudsters try to profit from selling food to unsuspecting victims. Globalization and rapid distribution systems have made food fraud a problem of international scale.

Fraud is very simple: take money for a high-quality product but provide a lower-grade item. Fraudsters swap the main ingredient with a similar product that is cheaper or they may extend or adulterate the food with cheaper material (you may remember the horsemeat scandal). They may not declare all of the ingredients and may lie about the manufacturing process/location or geographical origin.

As an analytical scientist, should I have blind trust in existing and new methodologies? Are they robust and reliable enough to ensure honest and accurate food labeling? I have my doubts – after all, each analytical method has its pros and cons. Traditional analytical methods for food authentication determine components using wet chemistry and chromatography or measure stable isotope ratios and trace elements. Wouldn't it be more reliable to measure several components (the more the better) in one single analysis, making it non-targeted?

There are several emerging non-targeted approaches that aim to create a profile (the 'metabolome') of as many metabolites as possible in the sample. Typically, they employ 'fingerprinting' detection methods such as non-invasive vibrational spectroscopic techniques (NIR, MIR, FTIR, and Raman), nuclear magnetic resonance (NMR) spectroscopy and an increasing range of mass spectrometry (MS)-based techniques. However, larger databases and improved multivariate data analysis tools need establishing to ensure success. That said, I am somewhat optimistic about the prospects of these new developments.

Targeted approaches focus on a defined group of key biomarkers, and can certainly indicate the presence of a specific type of food and/or adulterant. The current gold standard here is performed using robust and highly discriminating MS-analysis (proteome analysis) using ESI and MALDI or the rapidly evolving genetic methods that apply a set of different specific DNA molecular markers followed by highly specific amplification through PCR techniques.

I've always been inspired by the outstanding properties of biomolecules, especially antibodies. Different formats of immunoassay, such as ELISA, immune 'dot blot', Western blot coupled with lateral flow devices constitute fast and cost-effective tools for routine screening of allergens, GMOs, seafood and fish, meat, milk and dairy products, feedstuffs and fruit juice. To date, the main disadvantages are the limited availability of commercial antibodies and kits, problems in the analysis of highly processed or complex food matrices, and unsatisfying parallel identification and reliable quantification of multiple biomarkers. Automated antibody (or lectin) chip arrays or bead-based immunoassays are promising assay formats and may provide the necessary flexibility, sample throughput and robustness for routine screening in the future.

Although the art of food authentication focuses primarily on consumer safety, the economic impact on genuine producers and processors is becoming increasingly important. Though food fraud and other illegal activities in the industry are hampered by different national and international regulatory requirements, it is analytical chemistry that will provide the winning solutions. And I'm sure bioanalytical methods will increasingly help in our response to the challenges of food forensics.

Organoarsenical Seafood Cocktail

Are we analyzing the right arsenic species or are organoarsenicals actually wolves in sheep's clothing?

By Jörg Feldmann, Chair in Environmental and Analytical Chemistry Trace Element Speciation Laboratory (TESLA), College of Physical Sciences, Aberdeen University, Scotland, UK.

Inorganic arsenic – arsenate or arsenite – is a Class I carcinogen that is heavily regulated in potable water. To monitor the concentration of these arsenic species, the determination of the total arsenic concentration of 0.01 mg/L is considered sufficient, since only traces of other arsenic species occur in water. However, arsenic forms rather stable carbon bonds and there are more than 100 organoarsenicals known to occur in biological tissues – mainly in marine organisms.

For example, the major organoarsenical in seafood is arsenobetaine, which is deemed non-toxic; while for most other organoarsenicals insufficient or no toxicological data exist. The determination of the total arsenic concentration in food is not relevant to establishing the toxicological potential of the food commodity, which complicates the analytical question, since inorganic arsenic needs to be determined solely amongst a plethora of organoarsenicals.

In June 2014, the United Nations' World Health Organization (WHO) and Food

"Seafood could contain a hundred times more total arsenic than rice. But so far, it is unregulated."

and Agriculture Organization (FAO) recommended national authorities to implement regulations for a maximum level of inorganic arsenic rice and in June 2015 the EU implemented maximum level for different rice products, such as polished rice (0.2 mg/kg) or rice intended for baby food (0.1 mg/kg); this was the first regulation on that level for any food commodity. The inorganic arsenic contribution is relatively large and only a three organoarsenicals, such as dimethylarsinic acid (DMA), exist in rice. This makes the determination relatively easy and the analytical community was able to determine the inorganic arsenic concentration in rice samples with confidence.

A worldwide proficiency test (1) showed that determination is method independent; however, the most commonly used technique was ion exchange chromatography coupled to inductively-coupled plasma mass spectrometry (IC-ICP-MS). Recently, more affordable methods, such as species-specific hydride generation coupled to ICP-MS (2) or atomic fluorescence spectrometry (AFS) (3), have shown to give complementary data without the use of chromatography. This is crucial since small laboratories don't have the capability of hyphenating chromatography with ICP-MS.

However, seafood could contain a hundred times more total arsenic than rice. But so far, it is unregulated. The reason is the generally low contribution of inorganic arsenic amongst many known and some unknown organoarsenicals. However, it is expected that in the next few years the analytical community will develop reliable methods for inorganic arsenic based on the experience taken from the rice trials.

But are we really focusing on the right arsenic species? Should we be neglecting organoarsenicals? Clearly, complete monitoring would not be possible because of the vast number of different organoarsenicals species and the lack of a single method. But surely we must at least start making steps in the right direction.

We have recently started working in the growing field of lipid-soluble organoarsenicals, which contains more than 50 new compounds, such as arsenic containing fatty acids (AsFA), hydrocarbons (AsHC), phospholipids (AsPL), and fatty alcohols (AsFOH). Typically, the arsenic is dimethylated and occurs as an end-standing moiety of a longer carbon chain. Arsenolipids are present in the mg/kg range in commercial fish products, fish oils (4) and in algae products (5). And though many arsenolipids have been identified, determining those that may hydrolyze during extraction is still an analytical challenge (6, 7).

Two recent toxicology papers became the major justification for doing such sophisticated identification of novel lipid-soluble arsenic compounds. The Schwerdtle group (University of Potsdam, Germany) first discovered that highly purified, synthesized AsHC standards are cytotoxic in the micromolar range to two human cell lines in an in-vitro test (8) – mirroring the cytotoxic concentration range of arsenite, though through a different mode of action. In the group's second paper, AsHC was shown to be detrimental to the late development stages of larvae of Drosophila (fruit flies) – once again toxicity was through a different mode of action but effective in the same concentration range as arsenite (9).

Ever since regulations were introduced worldwide for inorganic arsenic in rice, work on arsenic speciation in biological samples has become more important and rewarding. But don't worry – there is still much more to be done by analytical chemists; for example, we need to establish robust methods that can be used routinely for inorganic arsenic in all food commodities. We also need to be vigilant for other 'benign' organoarsenicals that could turn out to be as toxic as inorganic arsenic. To conclude, we must continue developing tailored methods that make it possible to determine the real toxicological potential of organoarsenicals in food.

References

1. *MB de la Calle, et al., "Does the determination of inorganic arsenic in rice depend on the method? Trends Anal. Chem., Vol. 30, No. 4, 641–651 (2011).*
2. *AH Petursdottir, et al., "Hydride generation ICP–MS as a simple method for determination of inorganic arsenic in rice for routine biomonitoring", Anal. Meth., 6, 5392–5396 (2014).*
3. *B Chen, et al., "Accurate fast screening for total and inorganic arsenic in rice grains using hydride generation atomic fluorescence spectrometry (HG–AFS)", Anal. Meth., 6, 7554–7558 (2014).*
4. *E Schmeisser, et al., "Direct measurement of lipid–soluble arsenic species in biological samples with HPLC–ICPMS", Analyst, 130, 948–955 (2005).*
5. *A Raab, et al., "Comprehensive analysis of lipophilic arsenic species in a brown alga (Saccharina latissima)", Anal. Chem, 85, 2817–2824 (2013).*
6. *KO Amayo, et al., "Identification of arsenolipids and their degradation products in cod–liver oil", Talanta, 118, 217–223 (2014).*
7. *MS Taleshi, et al., "Arsenolipids in oil from blue whiting Micromesistius poutassou – Evidence for arsenic–containing esters", Sci. Rep., 4, 7492 (2014).*
8. *S Meyer, et al., "In vitro toxicological characterisation of three arsenic–containing hydrocarbons", Metallomics, 6, 1023–1033, (2014a).*
9. *S Meyer, et al., "Arsenic–containing hydrocarbons are toxic in the in vivo model Drosophila melanogaster," Metallomics 6, 2010–2014 (2014b).*

The Road to Complete Chemical Analysis

New analytical technology combinations promise simultaneous solvent-free measurement of every element on the periodic chart. And that's something worth shouting about.

By Richard E. Russo, senior scientist, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

Can one technology or instrument measure every element and isotope on the periodic chart? Yes, it can.

Our laser technologies research group has found that such capability exists by combining laser ablation sampling with optical and mass detection in a single instrument. Laser induced breakdown spectroscopy (LIBS) provides light elements, halogens and major concentrations, while inductively-coupled plasma mass spectroscopy (ICP-MS) simultaneously provides trace elemental and isotopic analysis. The approach makes it possible to measure, for example, hydrogen, nitrogen, fluorine and carbon at the same time as uranium and thorium isotopes.

Another beautiful aspect of the single instrument approach is the ability to predict interferences in each of the technologies and develop correction factors. For example, identifying the elemental contaminants measured by LIBS that lead to isobaric interferences in the mass spectrometer.

We are working on a technology platform that advances LIBS called laser ablation molecular isotopic spectroscopy (LAMIS). It measures molecular spectra appearing after the ablation laser pulse. With LAMIS, the isotopes of light elements (for example, carbon, nitrogen, hydrogen, chlorine) become part of the analysis package. Therefore, we can eliminate the need for multiple analytical technologies to characterize samples fully using this new approach: tandem LA-LIBS/LAMIS-ICP can do the job of glow discharge (GD), X-ray fluorescence (XRF), carbon, and mercury analyzers, optical emission spectroscopy (OES) and MS at the same time.

Rapid chemical imaging, analysis and depth profiling with high spatial resolution at atmospheric pressure are additional benefits that underlie the complete elemental/isotopic analysis capabilities that we have studied and developed. Notably, performance (for example sensitivity) is driven by the application; the instrument can be optimized for a specific element or isotope or optimized for best sensitivity over the entire periodic chart of elements.

Another benefit is that there is no need for solid sample digestion, eliminating the use of hazardous and costly acid or other solvent dissolution and purification procedures before analysis – laser ablation does all the sampling you need. We have found the technology is also suitable for liquid and gas analysis, but the real value is in changing the solid-sample analysis paradigm away from conventional dissolution. What about heterogeneity? Well, instead of being a challenge it becomes a feature, measurable through spatial mapping or averaged to present a bulk analysis.

The LIBS/LAMIS combination is an all-optical elemental/isotopic analytical technology that is suitable for real-time standoff measurements. For example, NASA has proven that LIBS can work on Mars with a standoff distance of about 7 meters – a very successful demonstration of real-time elemental analysis in a challenging environment. And we think that many industrial applications can benefit from standoff in-line elemental analysis; for example, raw material feedstock analysis, pharmaceutical inspection, Li-ion batteries, steel, polymers, food.

Something that I find puzzling is the fact that laser ablation has been studied and developed for more than 50 years, has been adopted in many applications (surgery, cutting, welding, nanomaterials, and so on) – and yet, for chemical analysis, it is not a mainstay approach. In my view, the main impediment is the need for a paradigm shift in thinking.

Reliable commercial instruments with data analysis software are available, making the value proposition for this technology compelling. Does the ability to measure every element and isotope with a single instrument, no sample preparation and rapid turn-around time not sound good to you? Certainly, paradigm shifts require risk takers – just like giving up a typewriter to use a computer. You don't see many typewriters these days...

I think another hindrance to laser ablation adoption for chemical analysis has been the component approach to applications, which has led people to ask the wrong question: "What is the best laser and or detector?" Although components are critical to the application, the real question is "What are the requirements of the analysis?" In this way, performance metrics are guaranteed. I think it is only a matter of time before industry will realize the benefits of this rapid, flexible, single instrument approach.

DataApex

Clarity™

DataApex
www.dataapex.com

CHROMATOGRAPHY SOFTWARE

500+ Controlled instruments

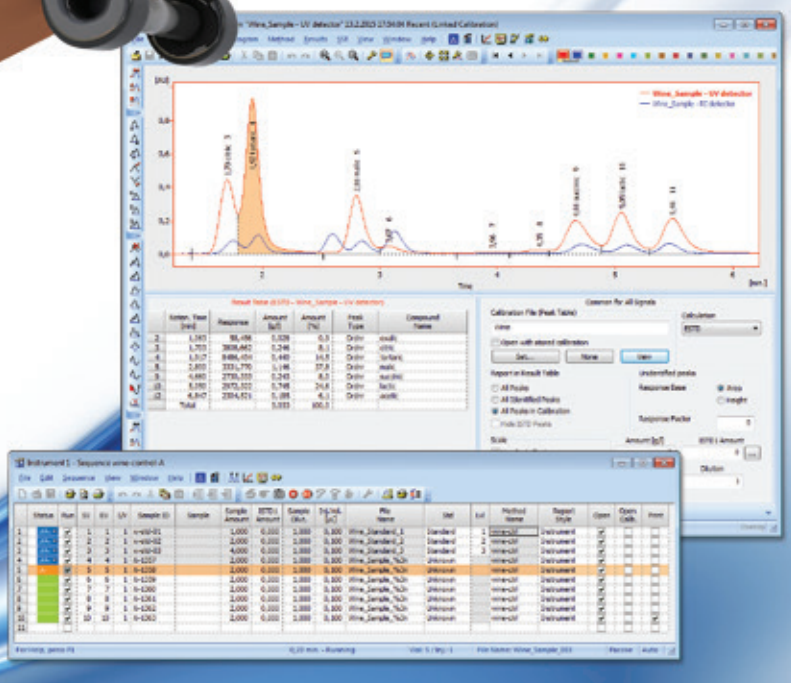
Optional extensions
(PDA, MS, GPC, GCxGC…)

Free software updates

Clarity2Go – monitoring app for smartphones

FREE DEMO VERSION

EASY
RIDE



The Virtues of Graphene

In what should be our constant endeavor to improve sample preparation, we must look to new materials – and focus on bending them to our own needs.

By Constantine Stalikas, Professor of Analytical Chemistry, Ioannina University, Greece.

I'm certainly not the first to say that sample preparation is extremely important in chemical analysis (and I doubt I will be the last). It eliminates unwanted matrix components, enriches or preconcentrates analytes, influences the quality of the obtained results and the total analysis time. Leading scientists in the field do give sample preparation the attention it deserves; the saying "if you fail to prepare, prepare to fail" is really not an overstatement (https://theanalyticalscientist.com/issues/0513/fail-to-prepare-prepare-to-fail/).

The marriage of materials and analytical chemistry is an important development in sample preparation. Today, there is a plethora of useful materials available to analytical scientists for sample processing. At the top of the list – or pretty close to the top – are carbon-based nanomaterials (including graphene in its various forms) for sorbent-based extraction procedures, such as classical solid-phase extraction (SPE), dispersive SPE, stir-bar sorptive extraction, and miniaturized solid-phase microextraction.

Graphene (a term recommended by the International Union of Pure and Applied Chemistry commission to replace the older term "graphite layers") is a two-dimensional (actually one atom thick) carbon nanomaterial with a honeycomb pattern. Graphene's exceptional properties – ultrahigh specific surface area (2630 m^2g^{-1}), hydrophobicity, chemical versatility and tunability (especially in its oxygen-functionalized derivative, graphene oxide) and high chemical stability – make it a superior adsorbent candidate for many different sample preparation methods.

Other features that make graphene desirable as sorbent are its planar geometry of nanosheets and wrinkly surfaces that can interlock well with adsorbed targets, and its large delocalized π–π electron system, which can form a strong π–π stacking interaction with organic molecules.

Graphene oxide (also called graphitic oxide or graphitic acid) can be used for the extraction of metal ions and analytes exhibiting polar functionalities, such as hydroxyls, carbonyls, amines, heteroatoms (O, N, S, P), under normal-phase SPE conditions. In contrast, metal chelates and analytes exhibiting non-polar functionalities, such as aromatic, alkyl, alicyclic functional groups, can be extracted using graphene in reversed-phase SPE conditions.

Graphene is an ultra-light material, so it is typically hard to recover from suspension, even by high-speed centrifugation. But imbuing graphene with magnetic properties can solve this problem. Our group developed and used – for the first time – an iron oxide-oxyhydroxide/graphene composite material as a sorbent for dispersive SPE of polychlorinated biphenyls, polyaromatic hydrocarbons and phthalates. The material capitalizes on the adsorption features of both elements and the magnetic properties of iron oxide (1).

In the same context, we functionalized cotton fibers to produce a web of aminosilica-graphene cotton microfibrils for a novel and straightforward cotton-based extraction mode. The extraction of analytes on cotton fibers was followed by facile collection of cotton pieces, and the elution and subsequent injection into a gas chromatograph. To test the applicability of the functionalized cotton and to figure out the mechanism of extraction, several groups of pollutants were employed successfully, including polycyclic aromatic hydrocarbons, phthalates, musks, phenolic endocrine disrupters and haloacetic acids (2).

But it's not all plain sailing. Although graphene is a highly efficient adsorbent, it usually shows little analyte specificity. To increase extraction selectivity, graphene (or graphene oxide) must be modified, which can be achieved through functionalization or hybridization with other groups, units, or materials that have specific affinity for target analytes. For example, aptamer-conjugated graphene oxide has been developed for the selective enrichment and ionization of cocaine and adenosine in human plasma (3).

It's clear to me that we need more research focused on enhancing the selectivity and extraction efficiency of graphene-based sorbents. Another challenge will be the development of simple and environmentally friendly preparation methods to obtain high-quality graphene with homogeneous lateral size and shape. Nevertheless, in my view, we are only just starting to realize the full potential of graphene as sample preparation material.

References

1. *AA Karamani, et al., "Zero-valent iron/iron oxide-oxyhydroxide/graphene as a magnetic ssorbent for the enrichment of polychlorinated biphenyls, polyaromatic hydrocarbons and phthalates prior to gas chromatography–mass spectrometry", J Chromatogr A, 1271, 1–9 (2013).*
2. *I Montesinos, et al., "Graphene-coated cotton fibers as a sorbent for the extraction of multiclass pesticide residues from water and their determination by gas chromatography with mass spectrometry", J Sep Sci, 38, 836–84 (2015).*
3. *B Gulbakan, et al., "A dual platform for selective analyte enrichment and ionization in mass spectrometry using aptamer-conjugated graphene oxide", J Am Chem Soc, 132 (49), 17408–17410 (2010).*

HUMANITY IN SCIENCE AWARD

phenomenex® ...breaking with tradition℠

&

the Analytical Scientist

2015 Winners
Andreas Seidel-Morgenstern (left)
and Peter H. Seeberger (right),

Who will be the winner in 2016?

Nominations for the 2016 Humanity in Science Award are now open

Analytical science has the power to change human lives for the better, but rarely receives the same fanfare as other scientific disciplines. The Humanity in Science Award was launched to recognize and reward a recent breakthrough in analytical science that has truly made the world a better place.

The 2016 award will be presented on May 10 in Munich, Germany.

Could the grand winner be someone you know? Nominate an analytical science project with a humanitarian impact now at www.humanityinscienceaward.com

Why enter?

- *$25,000 grand prize*
- *All-expenses paid trip to Analytica 2016*
- *Opportunity to tour Phenomenex headquarters in Torrance, California*

Nominations close on November 27, 2015 - *Good luck!*

YouTube humanityinscienceaward @humanityaward humanityinscienceaward@gmail.com

UNRAVELING THE CANNABINOME

Legalized cannabis is an exploding market that offers exciting opportunities – and challenges – for analytical scientists. Here, we offer an overview of the fast-emerging field of cannabinomics and the analytical methods being employed.

By Scott Kuzdzal and William Lipps

The leaf of the Cannabis plant contains more than 500 unique compounds; we have a long way to go before understanding the health benefits of all of them – or appreciating the synergies between cannabinoids, terpenoids and flavonoids. For more than 80 years, cannabis chemistry has been suppressed, and the industry itself needs to recover. In the USA, there are currently 24 states (and the District of Columbia) that have legalized medical marijuana and another four states that have legalized recreational marijuana. The analytical upshot? Cannabis sold for either medicinal or recreational use needs testing for potency and to reduce the risk of contamination. Indeed, cannabis-testing laboratories are sprouting up across the USA; they are vital to accurate cannabis product labeling, as well as cannabis quality control. Cannabis testing also serves to determine peak harvesting times for growing operations.

Tools of the trade

The important psychoactive component in marijuana is tetrahydrocannabinol (THC), making it the primary focus of potency testing. Natural Cannabis plant material contains THC-A, the non-psychoactive, carboxylic acid form of THC. Since THC-A is converted to THC upon heating, high-performance liquid chromatography (HPLC) is the method of choice for testing because it can distinguish THC from THC-A. Gas chromatography (GC) can also determine total THC.

A big challenge in potency testing is the complexity of the cannabinome; there are at least 80 cannabinoids present in cannabis, each with unique therapeutic properties (1). Most cannabis analytical testing, however, includes values for less than a dozen cannabinoids. Cannabidiol (CBD) – another major component of cannabis (2) – is a phytocannabinoid that accounts for 35 to 40 percent of cannabis extracts. THC-A, THC and CBD, cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabinol (CBN), Δ9-tethrahydrocannibinol (Δ9-THC), Δ9-tethrahydrocannibinolic acid (Δ9-THCA) and cannabichromene (CBC) are commonly measured cannabinoid (CBx) profiles. These cannabinoids are all separated and analyzed using conventional HPLC (not UHPLC) in under two minutes (3). More work is needed to develop analytical conditions for rapid and accurate separation and detection of the 70-plus other cannabinoids.

The rise of vaping and edibles

Smoking dried plant material is the most common technique for taking cannabis. However, the growing use vaporizers, together with the market in electronic cigarettes, means the demand for "vaping" oils has exploded.

There are various ways to extract cannabis oil from dry products. For example, a highly concentrated form of cannabis oil utilizes butane as an extraction solvent (butane hash oil or BHO). Heptane, propane, glycerin, and ethanol, and even rubbing alcohol, have been used to extract cannabis oil. This is clearly a concern and, in May 2014, the state of Colorado issued a new regulation requiring all solvent-extracted cannabis concentrates to be tested for residual solvents.

See online database at www.Leafly.com.

Figure 1. Health disorders with proposed cannabinoid impact.

The Dark Side

Outside of the natural cannabinome, cannaceuticals and other therapeutic efforts, cannabis and cannabinoids are illegally manipulated, synthesized and consumed. The World Health Organization states that cannabis is the most widely cultivated, trafficked and abused drug, accounting for 50 percent of drugs seized by law enforcers worldwide. It is estimated that around 147 million people globally consume the drug. Consequently, the research focus in much of the world is on forensics and toxicology. Here, we present a selection of recent papers that aim to track and assess the illicit – and often synthetic – world of cannabis.

Determination of a selection of synthetic cannabinoids and metabolites in urine by UHPSFC-MS/MS and by UHPLC-MS/MS.

The researchers used ultra-high performance supercritical fluid chromatography-tandem mass spectrometry (UHPSFC-MS/MS) and reversed phase ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for determining two synthetic cannabinoids and eleven metabolites in urine. Sample preparation included a liquid-liquid extraction after deconjugation with ß-glucuronidase. MS/MS detection was performed with positive electrospray ionization and two multiple reaction monitoring transitions. Deuterated internal standards were used for six of the compounds. Elution order obtained by UHPSFC-MS/MS was almost opposite to that obtained by UHPLC-MS/MS, making this instrument setup an interesting combination for screening and confirmation analyses in forensic cases.

T Berg, et al., Drug Test Anal, Aug 25 (2015). PMID: 26304456.

In vivo detection of the new psychoactive substance AM-694

Another, safer form of hydrocarbon extraction is CO2 (gas or liquid) extraction. Yet another method involves fat-based concentrates using infused butters or edible cooking oils. In addition to their use in vaping oils, cannabis concentrates play a very important role in a fast-growing edible industry. Concentrated oils and butters are applied to a wide variety of cannabis edibles, ranging from beverages and confectionery to pizza (4). And the majority of medical marijuana patients prefer edibles and sublingual drops over smoking. Sublingual dosing ensures that maximum natural cannabinoids, including THC-A, are bioavailable; potency testing is important to ensure accurate dosing.

What are you smoking?

In addition to cannabis potency profiles, cannabis testing also identifies a wide variety of contaminants, such as residual solvents, pesticides, microorganisms, mycotoxins (such as aflatoxin) and heavy metals. Such contaminants are especially troublesome to medical marijuana patients with compromised immune systems.

Residual solvent testing is performed commonly with headspace GC-MS, which is similar to US Pharmacopeial Convention "USP <467> for Residual Solvents". The enormous number of pesticides available makes it impossible to test for all of them , but one again GC-MS is the preferred instrumentation platform for pesticide residue testing. GC-MS instruments with twin-line capabilities allow labs to run residual solvent (or terpene) analyses on the same instrument as pesticides. While there are currently no guidelines for residual pesticide screening in cannabis, most labs test for the most common pesticides employed during cannabis cultivation: organophosphates, carbamates, pyrethroids and avermectins. MRX Labs in Portland, Oregon, USA, is leading the way in pesticide analytical testing services, offering a panel of 40 pesticides.

The ideal conditions for cannabis growth are also ideal for the growth of potentially harmful bacteria and fungi including yeast and molds, therefore microbial contamination poses health risks to consumers and immunocompromised individuals. Microorganisms are monitored using analytical techniques ranging from simple petri film tests to genomics testing. Quantitative polymerase chain reaction (qPCR) will likely replace the current practice of testing for molds and bacteria using petri dishes.

Matrix-assisted laser desorption/ionization (MALDI) microorganism identification is commonplace for clinical microbiology and could be used as a qualitative technique to certify the presence or absence of various microorganisms in cannabis samples. MALDI could also compete with genomics testing for cannabis strain typing. And mycotoxins can be detected using LC-MS/MS. Finally, heavy metals (lead, mercury, cadmium, and arsenic), can be tested by atomic absorption, inductively coupled plasma ICP-MS.

Therapeutic terpenes

Terpenes are a very important class of cannabis compounds and are analyzed using headspace GC-MS. They are produced in cannabis trichromes (as is THC) and give cannabis its distinctive flavor and aroma. Terpenes also act as essential, medicinal hydrocarbon building blocks, influencing the overall homeopathic effect. The roles of terpenes in personalized therapies is becoming

[an agonist for cannabinoid receptors] and its metabolites.

A 25-year-old man was hospitalized following a major trauma after ingestion of alcohol and an unknown pill. Urine and blood samples were investigated for possible substance abuse. A general unknown screening of biological samples, extracted by liquid-liquid extraction (ethylacetate and dichloromethane) in basic, acidic and neutral conditions, was achieved to verify the presence of drugs of abuse and/or their metabolites, both in gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). For the quantification of AM-694, urine was extracted by solid phase extraction (SPE). Quantification of AM-694 (internal standard AM-2201), midazolam and α-hydroxymidazolam (internal standard halazepam) was performed by LC-MS/MS analysis in multiple reaction monitoring. Qualitative information about the AM-694 metabolites was obtained by LC-MS/MS in selected-ion monitoring.

E Bertol, et al., Forensic Sci Int, Jul 17, 256:21–27, (2015). PMID: 26295909.

Comprehensive review of the detection methods for synthetic cannabinoids and cathinones.

A number of N-alkyl indole or indazole-3-carbonyl analogs, with modified chemical structures, are distributed throughout the world as synthetic cannabinoids. Cathinone analogs are also abused and cause serious problems worldwide. Acute deaths caused by overdoses of these drugs have been reported. Various analytical methods that can cope with the rapid changes in chemical structures are required for routine analysis and screening of these drugs in seized and biological materials for forensic and clinical purposes. Although many chromatographic methods to analyze each drug have been published, there are only a few articles summarizing these analytical methods. This review presents the various colorimetric detections, immunochemical assays, gas

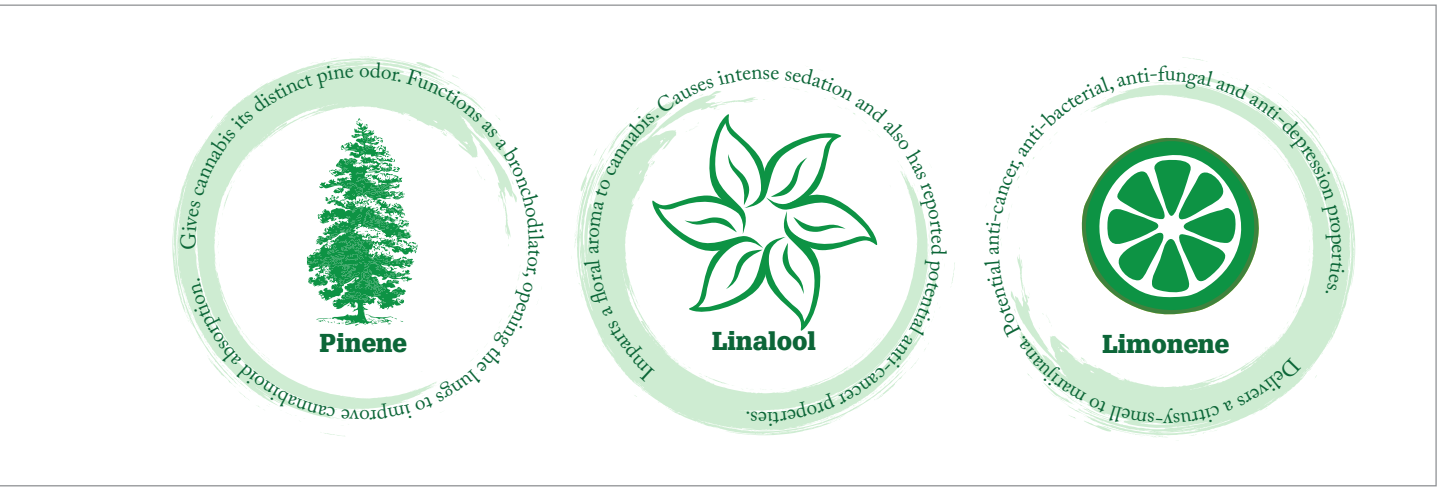


Figure 2. Three terpenes and their impact on aroma and health benefits.

increasingly important (see Figure 2). For example, pinene acts as a bronchodilator, increasing THC absorption. Linalool can impart sedative effects, and limonene, like most terpenes, has its own therapeutic effects. It is likely that flavonoids will also play an increasingly important role in understanding cannabinoid synergies and the therapeutic effects of cannabis.

Sample prep and GLP

The analytical techniques used in cannabis testing (Figure 3), have a long history of application in clinical, pharmaceutical, food safety and environmental markets. Analytical instrument manufacturers are, therefore, assisting cannabis testing laboratories with sample preparation protocols, method development and establishing good laboratory practices (GLP) to ensure that they meet or exceed state laws for environmental and food safety testing laboratories. Whereas established industries have longstanding guidelines and standards, the cannabis industry is feeling its way as it develops. Consequently, there is a widespread lack of standards, reference materials, proficiency testing and laboratory certification. There are, however, experienced laboratory directors with analytical,

chromatographic-mass spectrometric methods, and liquid chromatographic-mass spectrometric methods proposed for the analysis of synthetic cannabinoids and cathinones.

A Namera, et al., Forensic Toxicol, 33(2):175–194 (2015). Epub 2015 Mar 6. PMID: 26257831.

Quantitation of synthetic cannabinoids in plant materials using high performance liquid chromatography with UV detection (validated method).

The authors present a validated, broadly applicable HPLC-UV method for the determination of synthetic cannabinoids in plant materials using acetonitrile extraction and separation on a commercial phenylhexyl stationary phase. UV detection provides excellent sensitivity with limits of quantitation (LOQ) less than 10 µg/g for many cannabinoids. The method was validated for several structural classes (dibenzopyrans, cyclohexylphenols, naphthoylindoles, benzoylindoles, phenylacetylindoles, tetramethylcyclopropylindoles) based on spike recovery experiments in multiple plant materials over a wide cannabinoid contents range (0.1-81 mg/g). Average recovery across 32 cannabinoids was 94 percent for marshmallow leaf, 95 percent for damiana leaf, and 92 percent for mullein leaf. The method was applied to a series of case-related products with determined amounts ranging from 0.2 to >100 mg/g.

LA Ciolino, J Forensic Sci, Jul 14 (2015). PMID: 26175160

Pressurized liquid extraction for the determination of cannabinoids and metabolites in hair: Detection of cut-off values by high performance liquid chromatography-high resolution tandem mass spectrometry.

In this study a fast, accurate and sensitive method for the determination of cannabinol, cannabidiol, THC and THC-COOH in hair has been developed. The extraction of analytes from hair (50mg) is based on an automated pressurized liquid extraction (PLE) using water

sample preparation and data processing skills, who are interacting and beginning to develop cannabis testing quality standards.

Although marijuana has been legalized by several states, it has yet to be legally recognized by the US federal government. Since there are no US Food and Drug Administration (FDA) criteria for cannabis testing, individual states have had to establish regulations on their own. The FDA has recently ruled that cannabidiol products don't fit under the legal definition of dietary supplements and therefore can't be marketed as such under the federal Food, Drug and Cosmetic Act (5).

Personalized cannabinomics

The next frontier for cannabis analytical testing resides in personalized cannabinomics. Ken and Melodie Kovash operate a biomedical farm in Oregon (GI Grow – www.gigrow.us) in accordance with Oregon Medical Marijuana Program rules; they are pioneering a personalized cannaceutical approach. The company has a holistic approach to cannaceutical development, focusing on quality rather than yield, to produce blends of CBx oils in response to patient outcomes and tailoring personalized cannabinomics strategies for each individual patient. Cannabis produces different chemicals due to multiple environmental/physical stresses and needs.

As we learn more about the health benefits of cannabis, we must gain a much better understanding of the synergies of cannabinoids, terpenes and flavonoids. This represents a great opportunity for analytical chemists; the potential to understand and unlock the interactions of cannabinomics on a larger, disease-specific scale. Taking it a step further, fine-tuning of cannabis components for maximum therapeutic effect requires more research and analytical testing.

Improving quality controls

Additional opportunities exist in developing automated sample preparation and analytical testing methods. Technologies like online SFE-SFC-MS/MS integrated with LC and MS address cannabis sampling deficiencies, allowing fully automated extraction, separation and detection of all components in cannabis.

As the need for better quality control continues and standardization is introduced in this emergent industry and market, lower limits for the various cannabis contaminants will be established and regulations will be introduced. Mass spectrometry will play a greater role in quantitation as detection levels are lowered and confirmatory tests are required.

With an increase in cannabis consumption comes an increase in public safety concerns, such as drugged driving. Law enforcement authorities need new, low-cost methods for rapid salivary, breath-based and/or finger-stick screening of individuals that appear to be under the influence of marijuana. Also, better product packaging and labeling is needed to reduce accidental infant exposures, especially for confectionery-like, medicinal marijuana edibles. One particular difficult challenge in cannabis testing for public and workplace safety is the need to identify better biomarkers that indicate accurate timelines of cannabis exposure. Currently, it is difficult to distinguish between recent cannabis usage and long term, chronic marijuana exposure.

modified with the surfactant sodium dodecyl sulphate as eluent phase. PLE extract is then cleaned up by SPE using polymeric reversed phase cartridges Strata XL before the injection in the HPLC-HRMS/MS system. Chromatographic conditions obtained with a fused-core column allowed a good separation of the analytes in less than 4min. The whole procedure has been validated according to SWGTOX guidelines.

C Montesano, et al., J Chromatogr A, Aug 7, 1406:192–200 (2015). PMID: 26118805.

Comprehensive monitoring of the occurrence of 22 drugs of abuse and transformation products in airborne particulate matter in the city of Barcelona.

Measuring drugs of abuse (DA) in airborne particulates could be an additional tool to evaluate drug use patterns in time and space, and identify potential emission sources. The researchers monitor the occurrence of 22 licit and illicit DAs and transformation products belonging to six different chemical groups in airborne particulate matter (PM10) in the city of Barcelona. Samples were collected from 12 different selected locations on one weekday (Wednesday) and one weekend day (Saturday), during five consecutive weeks. A previously developed analytical methodology, based on pressurized liquid extraction (PLE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination, was adapted for analyzing the target compounds. Cannabinol, cocaine, and methamphetamine were found to be the most ubiquitous and abundant compounds in PM10.

N Mastroianni, et al., Sci Total Environ, Nov 1, 532:344–52 (2015). Epub 2015 Jun 14. PMID: 26081737.

Separation of cannabinoids on three different mixed-mode columns containing carbon/nanodiamond/amine-polymer superficially porous particles

The researchers used three mixed-mode high-performance liquid chromatography

Figure 3. Common cannabis analytical testing methods.

A stronger integration between testing labs and growing operations, extraction operations, dispensaries, customers, clinical labs and physicians will ensure that cannabis product information is accurate and more readily available. Meanwhile, cloud technology and vertical supply chain integration can improve operational efficiency. All-in-one business management software solutions, such as those provided by Viridian Sciences (Vancouver, Washington, USA) enable 'cannabusiness' to run efficiently with automated inventory tracking, seed-to-sale reporting, financial accounting, management, and quality control.

On your doorstep

Cannabis testing is not just a growing US market. Sativex – a synthetic, pharmaceutical version of cannabis – has been approved for use in 25 countries as a treatment for muscle spasm pain in multiple sclerosis patients. Marinol (a synthetic THC product) has been FDA approved to treat nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional treatments. The FDA also approved Marinol to treat appetite loss associated with weight loss in people with AIDS. Idrasil is a physician prescribed "medical cannabis in a pill". Unlike Marinol, Idrasil is an all-natural cannabis plant extract containing the full spectrum of naturally occurring cannabinoids from cannabis.

As more cannabis-based or synthetic cannabinoid drugs and homeopathic medicines enter the marketplace, and as more states legalize medical and/or recreational marijuana, the need for cannabinoid testing will continue to increase. Therefore, all aspects of analyses, from instrumentation to testing protocols

columns packed with superficially porous carbon/nanodiamond/amine-polymer particles to separate mixtures of cannabinoids. Columns evaluated included: reversed phase (C18), weak anion exchange, 4.6 × 33 mm, 3.6 µm, and 4.6 × 100 mm, 3.6 µm, reversed phase, strong anion exchange (quaternary amine), 4.6×33 mm, 3.6 µm, and hydrophilic interaction liquid chromatography, 4.6 × 150 mm, 3.6 µm. Different selectivities were achieved under various mobile phase and stationary phase conditions. Efficiencies and peak capacities were as high as 54 000 N/m and 56, respectively. Fast separations were achieved in less than five minutes. A real world sample (bubble hash extract) was also analyzed by gradient elution.

CH Hung, et al., J Sep Sci, Jun 15 (2015). PMID: 26075936.

Determination of XLR-11 and its metabolites in hair by liquid chromatography-tandem mass spectrometry.

XLR-11 (a cyclopropylindole [synthetic cannabinoid]) has been widely abused in South Korea recently. Identification of metabolites in hair can be an important proof of synthetic cannabinoids use because it can exclude the possibility of passive smoke exposure. The researchers describe a quantitative analytical method of XLR-11 and its metabolites in hair by liquid chromatography with ESI-MS/MS. The target analytes were extracted with methanol from washed and cut hair samples and the extracts were evaporated, filtered and analyzed by LC-MS/MS with electrospray ion source in positive-ionization mode. JWH-018-d9 and JWH-018 N-5-hydroxypentyl metabolite-d5 were used as internal standards. Chromatographic separation was completed within 15 minutes. No interferences were detected in 10 blank hair samples. The validation results proved that the method was selective, accurate and precise with acceptable linearity within calibration range.

M Park, et al., J Pharm Biomed Anal, Oct 10, 114:184–9 (2015). PMID: 26070160.

and even proficiency testing, will expand with this emerging industry. Clearly, opportunities abound for all levels of analytical scientists, from graduating students to senior scientists. Modern labs, such as Steep Hill Labs, ProVerde Laboratories, MRX Labs, G.O.A.T. Labs, Trace Analytics and Rose City Labs are leading the way in cannabis testing. They've gone beyond mere cannabis analysis to include components of biotechnology, research and development, and education. Analytical instrument manufacturers will continue to develop new LC, GC, LC-MS, GC-MS and ICP-MS protocols that support cannabis testing, including completely automated and standardized methods.

The US cannabis industry is projected to be an $8 billion industry by 2017 (6), and retail/dispensary sales of cannabis is predicted to exceed $7 billion by 2019, with a total additional economic value of the marijuana industry estimated to be between $23 to $29 billion (7). Cannabis is big business – and with big business comes challenges and opportunities; analytical scientists also have a big role to play.

To learn more about the science of cannabis, please visit CANNCON (www.canncon.org), a scientific organization and annual conference that is devoted entirely to cannabis testing and research. CANNCON'S hands-on "CANNA BOOT CAMP" teaches all aspects of cannabis, from cultivation and extractions to laboratory testing.

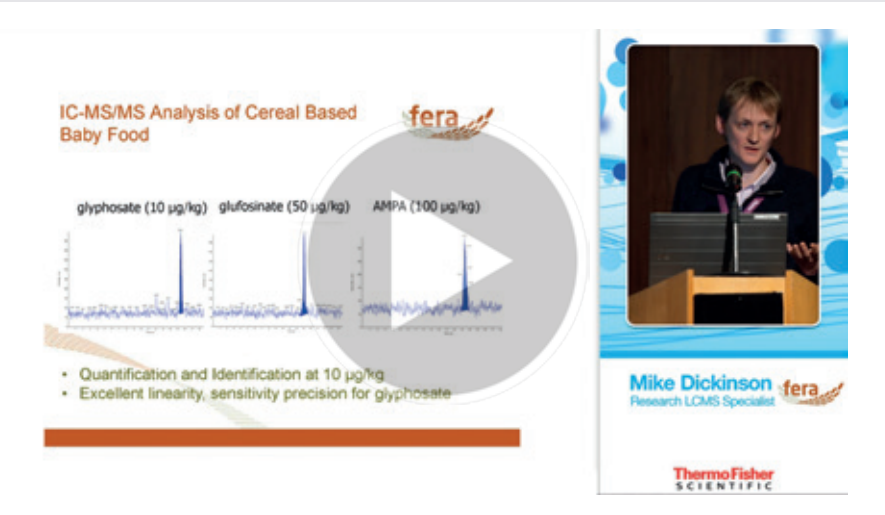
Scott Kuzdzal is Life Science Business Manager, and William Lipps is Business Unit Manager - Environmental/Chemical, both at Shimadzu Scientific Instruments, Columbia, Maryland, USA.

References

1. *L Hanus, "Biogenesis of cannabinoid substances in the plant", Acta Universitatis Palackianae Olomucenesis Facultatis Medicae 116: 47-53 (1987).*
2. *LM Borgely, et al., "The pharmacologic and clinical effects of medical cannabis", Pharmacoteherapy (Review) 33 (2):195-209 (February 2013).*
3. *K Kovash, et al., "Medicinal cannabis and the need for enhanced cannabinoid profiling", poster presentation at MSACL 2015.*
4. *E Rosenthal and D Downs, "Beyond Buds", ISBN:978-1-936807-23-9 (2014).*
5. *MJBiz Daily article, "FDA says CBD products don't qualify as dietary supplements", May 15, (2015).*
6. *M Ferner, "U.S. legal pot sales to hit $8 Billion a year in 2018: Report", Huffington Post (04/11/2014 15:02 EDT)*
7. *2015 Marijuana Business Factbook statistics, a division of Anne Holland Venures Inc., Marijuana Business Daily (www.MJBizDaily.com).*

Marching Forward with Food Analysis

The analytical aspect of the food industry has changed significantly over the last decade – mainly because of advances in technology. From pesticide residues to metabolomic approaches, the field is now more exciting than ever.



To watch the online presentation, visit http://tas.txp.to/0915/pesticide

By Mike Dickinson, Research LCMS Specialist, Fera Science Ltd., Sand Hutton, UK.

I've been with Fera (in its various guises) for 12 years now. I joined for what I thought would be a single year of good experience after finishing my BSc in Biology at the University of York. But I never left – I was hooked. After two years, I'd made it into a research team, and was lucky enough to work with food analysis research scientist Richard Fussell (who recently joined the team at Thermo Fisher Scientific).

My time at Fera so far has given me an excellent and broad view of the field. In the early years I was involved in the application of emerging mass spectrometry techniques for the multi-residue determination of pesticides and veterinary medicines in food. I also looked at the fate and behavior of pesticides, veterinary medicines and pharmaceuticals in the environment. Twelve years isn't such a long time in terms of analytical chemistry, but during that time, mass spectrometry has advanced at a furious pace. In more recent years, high-resolution (HR)-MS systems started to appear in our laboratories and, as luck would have it, I began working on fate and behavior projects that could benefit from HR-MS. Now, I find myself working almost entirely in the world of HR-MS, including TOF, QTOF, and Orbitrap instruments. I lead metabolomics at Fera in Adrian Charlton's section and essentially bring LC- and GC-MS expertise alongside NMR-spectroscopy for biochemical profiling. We aim to exploit fully the complementary nature of the three approaches; NMR gives excellent reproducibility and you can quantify without analytical standards – but sensitivity can be an issue. LC- and GC-MS systems offer additional coverage, especially in terms of sensitivity. We're currently involved in a EU project called ABSTRESS (www.abstress.com), which is looking at dual stress in legumes. There have been many studies addressing resistance mechanisms in drought-stressed and disease-stressed plants individually, but with dual stress the biochemistry in the plant can be different. We're using metabolomics and transcriptomics to identify the hub genes that are crucial for dual stress resistance, working with 12 national and international partners in the EU, including a state-of-the-art plant phenotyping platform at INRA (the French National Institute for Agricultural Research). It's exciting work.

The IC-MS story

In addition to cutting-edge research that addresses global food challenges, one of the big goals at Fera is to improve analytical throughput and efficiency. Back in 2008, Richard Fussell and I embarked on a project to try and combine single residue methods for polar ionic pesticides (PIPs), such as glyphosate, chlormequat, mepiquat, ethephon – the project continues today with Stuart Adams, Senior analytical chemist at Fera. I recently gave a lecture that summarizes the project and outcomes at the 1st International Symposium on Recent Developments in Pesticide Analysis called "IC-MS Multi-residue pesticide methods, fantasy or reality?" – the video is available here: http://tas.txp.to/0915/pesticide

In pesticide analysis, multi-residue methods are king; they enable higher throughput and increase laboratory efficiency. Over the years, analytical chemists have done a fantastic job of shoehorning a high number of pesticides into such multi-residue methods. Unfortunately, several stubborn stragglers refuse to be constrained – either they don't quite fit with the chromatography chemistry or the MS analysis is not ideal. Not only are PIPs difficult to separate,

they need to be quantified and identified at low concentrations (for example, 0.1 µg/L in drinking water). And the truth is that even the single residue methods we do have are not particularly successful for certain compound-matrix combinations; for example, glyphosate at low µg/kg concentrations in maltodextrin products.

In fact, glyphosate was a big reason why we decided to investigate the potential of ion chromatography (IC) in pesticide residue analysis. Glyphosate is the most used pesticide throughout the world – people have been splashing it over their gardens for year (as an aside, renewed interest in its toxicity has been hitting the headlines recently). Glyphosate is also one of the most difficult compounds to analyze. Fortunately, it is amphoteric (can exist in different ionic forms) and that triggered a question: why not use IC?

One hurdle was the perceived difficulty in hyphenating IC with MS; MS systems don't get along with salt mobile phases (potassium hydroxide, in our case). The introduction of robust and reliable membrane electrolytic suppressors to convert potassium hydroxide to water on exit from the column was a game changer. Nevertheless, we still had our concerns, and kicked off the project using a relatively old MS instrument, primarily to protect the expensive MS systems – against damage should the suppressor fail. Despite the antiquity of the MS system, we started getting really promising results for retention of glyphosate and glufosinate (another systemic herbicide) using our Dionex ICS-3000. More important were the findings of transformation products – AMPA (from glyphosate) and MPPA and N-Acetyl glufosinate (from glufosinate) – all of which are in the residue definitions for either food or water, or both. We had to load 5-ml samples into the system using an inline concentrator pre column; you can probably imagine the state of the MS source after a series of injections, given that we were not only concentrating our compounds of interest but also matrix co-exatractives. Maltodextrin products proved especially challenging.

The pesticide lab of tomorrow

In terms of IC-MS for pesticide analysis, we've learnt a number of tricks along the way (for example, writing a script that shut down the pump in the case of a consistent offscale detector response, which can be indicative of a suppressor failure emergency) – and we benefited from the work of Anastassiades et al., in particular the QuPPe method (1). Indeed, Stuart has continued to develop the method, extending it to other pesticides of interest as well as some other stragglers that don't fit particularly well into multi-residue methods. Notably, we've also moved onto much more advanced triple quadrupole MS systems and we now inject only 100 µl samples because of advances in sensitivity. The use of internal standards was also another big step forward, and I suspect another area where we can expect to see further benefits.

Fera has an upcoming collaborative research project with Thermo Fisher Scientific that will employ a state-of-the-art system – a Dionex ICS-5000 coupled to a TSQ Quantiva triple quadrupole mass spectrometer. It's a combination that promises to give us the highest possible levels of sensitivity, and also offers the potential to enter into the world of 2D-LC. The ability to use conventional reversed-phase chromatography on the first dimension column and IC on the other would certainly help separate some of the trickier compounds from the matrix. We could also run two columns in IC mode, using the second column as a concentrator before MS analysis. Stay tuned for further developments!

Looking to the future makes me think about how quickly things have changed. Walking through the laboratories at Fera is very different today than when I started just 12 years ago; there is less wet chemistry with fewer "hands on" analysts, and the instrumentation is much more sophisticated, which has increased efficiency immensely. Technicians no longer need to spend hours concentrating samples because the equipment provides the sensitivity we require, especially with the revolution in triple quadrupole instruments.

We now have more than 35 MS systems that deal with routine analyses (the majority are LC-triple quads, with about five HR-MS instruments). And though the triple quadrupole instruments are very sensitive, reliable and ideal for quantification and targeted methods, it wouldn't surprise me if hybrid quadrupole high-resolution instruments start to take over at some point in the future. Instruments like the Q Exactive offer good sensitivity for targeted analysis alongside the potential for full-scan, high-resolution data acquisition.

Looking further into the future, there will no doubt be a continuation in the development of hardware, but software is likely to result in breakthroughs with the biggest impact in my opinion. Indeed, software companies and developers must focus on providing user-friendly, efficient, fully capable solutions. I also expect to see scaling down of instruments to drive towards portable equipment that can be operated by non-experts. We've already seen the beginnings of a revolution in NMR benchtop devices like the Picospin, so why not with MS instruments? The core technology may not be there quite yet, but it will come. Miniaturized Hybrid Quadrupole-Orbitrap anyone? I'll take two.

Reference

1. *M Anastassiades et al., "Quick method for the analysis of numerous highly polar pesticides in foods of plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPPe Method, Version 8.1 www.eurl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf, 2015)*

Making Solutions Routine

The Pesticide Explorer Collection offers complete workflow-driven solutions to meet the challenges of modern pesticide residue analysis. Are you ready for a premium solution?

Last month, we introduced the Pesticide Explorer Collection and described the "Standard Quan" package (see tas.txp.to/0915/standardquan). Here, we speak with Dipankar Ghosh, Global Director for Environmental, Food Safety & Industrial Markets at Thermo Fisher Scientific, to discuss the motivation behind the Collection and to reveal the second solution – "Premium Quan".

What inspired the Pesticide Explorer Collection?

In the food and environmental markets, pesticides are the most common – and the most widely analyzed – contaminants. Despite pesticides being relatively well controlled in developed countries, the global nature of the food chain has increased the risk of a wide range of pesticides appearing in quantities that are higher than regulations allow. Our aim was to save analysts valuable time in the method development process. We provide the sample extraction method, the chromatographic method (with the most suitable column) as well as the MS method and data evaluation tools. In the past, we've offered systems and methods independently, but we realized that in routine analytical laboratories there was a real need and demand for pre-developed, robust solutions.

For targeted screening, we offer two triple-quadrupole solutions and a high-resolution accurate mass (HRAM) solution using our Q Exactive Focus platform, which also features in our non-targeted workflow. Importantly, our collection offers solutions for all levels of analysis and for all budgets, which is where we really differentiate ourselves from the competition.

Laboratories that are branching out into pesticide analysis or new to the area of mass spectrometry will particularly benefit from our Pesticide Explorer Collection, which makes the process as smooth as possible. Likewise, laboratories that are expanding to meet growing demand now have a solution that scales up very well indeed.

How was the Collection developed?

Our lab in San Jose, USA, spearheaded the process, but the Special Solutions Center in Dreieich, Germany, was also heavily involved from a method development point of view. We also had solid support from our facilities in Runcorn, UK, and benefited from our HPLC team in Germering, also in Germany. It was a real international effort! Clearly, for such an offering, robustness and reproducibility were of great importance; much of the development time was spent testing the methods on multiple systems with different analysts to ensure that we had made all the right choices, from sample extraction to data evaluation.

What differentiates the Premium Quan solution?

Our second triple-quadrupole-based solution – "Premium Quan" – benefits from the same QuEChERS start-up kit and chromatographic separation (using specially selected columns and the UltiMate 3000 LC system) as the Standard Quan package. The big difference? The TSQ Endura is upgraded to our highest-end triple quadrupole mass spectrometry system – the TSQ Quantiva – to offer the ultimate in sensitivity. In many ways, the Premium Quan solution is for users who wish to make a longer-term investment with a view to not only meet the detection limits of today, but also those of the future.

CMC STRATEGY FORUM

ADVANCING BIOPHARMACEUTICAL DEVELOPMENT

JAPAN 2015

9-10 November 2015 • Tokyo Marriott Hotel, Tokyo, Japan

SCAN to browse CASSS for program updates at casss.org

Organized by CASSS and Supported by the Pharmaceuticals and Medical Devices Agency, Japan and the Japan Pharmaceutical Manufacturers Association

pmda JPMA CASSS www.casss.org

Optimizing the Search for the Unknown

Non-targeted profiling is progressing in many application areas, including food, drugs, environmental, proteomics and metabolomics – but how can we measure the suitability of our own methods?

By Gerhardus(Ad) de Jong, Biomolecular Analysis, Utrecht University, The Netherlands.

There is a growing interest from various application fields of modern analytical chemistry for efficient profiling, which falls into two categories: targeted and non-targeted. The purpose of targeted analysis is the (semi-)quantitative determination of known compounds, while the non-targeted approach aims to obtain a comprehensive view of sample composition. It is possible to limit profiling to certain groups or types of compounds. Examples of profiling are impurities in drugs and food, and toxic components in the environment. Notably, the relatively new areas of proteomics and metabolomics require even more powerful profiling methods.

Non-targeted profiling is an undirected search for unknown compounds, so there isn't a clear method optimization goal, making a difficult task even harder. However, there is potential to use advanced technologies to make the job easier. In this article, I hope to start a debate about potential and limitations of different approaches and procedures for the optimization and use of non-targeted profiling.

Making safer drugs and the rise of metabolomics

I have a great deal of experience and knowledge in impurity profiling of drugs, which I began gathering some 25 years ago while working in the pharmaceutical industry. Today, impurity profiling is an important step in the development and quality control of pharmaceuticals (1, 2). The US Food and Drug Administration (FDA), for example, requires impurity profiling to be conducted using modern analytical and chemometrical methods. Synthesis- and process-related compounds, including residual solvents, and decomposition products discovered while the drug is being developed, can all affect safety. Therefore, extensive impurity profiling is essential, especially if there are changes in the synthesis method and if product stability is investigated. Prior knowledge of the synthesis and possible decomposition is also necessary and this is where support from chemistry colleagues is required, see Figure 1 (3). The non-targeted detection of impurities is considered the first step in the entire "impurity fate mapping framework". The concentration levels with respect to the main compound are often below 0.1 percent, and generally, if they are above this limit, they must be identified. For (geno)toxic impurities the limits for reporting and identification are lower. Different complementary analytical methods are applied and the information is combined, based on the results and toxicological data specifications are chosen for quality control.

In clinical analysis (and in other application fields such as food quality and forensics), the application of non-targeted metabolomics is increasing (4, 5). It's exciting because it compares the profiles of samples from, in the case of clinical analysis, healthy and unhealthy individuals, with the hope of discovering potential biomarkers. Clearly, the process is much more complex than drug impurity profiling because it requires the analysis of biological samples, that contain much larger numbers of compounds. You can see an example of a liquid chromatography-mass spectrometry (LC-MS) analysis of rat urine in Figure 2 (6), which helps to illustrate the complexity. Moreover concentrations can differ from trace (ppb) levels to much higher concentrations. So the big question is: how can we optimize separation and MS data extraction? There is no easy answer; the entire analytical workflow is complex and demands optimization of all steps, from sampling and sample preparation through to analysis and data interpretation. Data preprocessing (alignment of peaks and normalization of peak areas) and chemometrical procedures are also needed when it comes to comparing profiles. Moreover, biological variance has to be taken into account to interpret the data correctly. If discriminatory masses (potential biomarkers) are found, they need identifying. Subsequently, these compounds require biochemical interpretation. A word of caution: you simply can't do all this

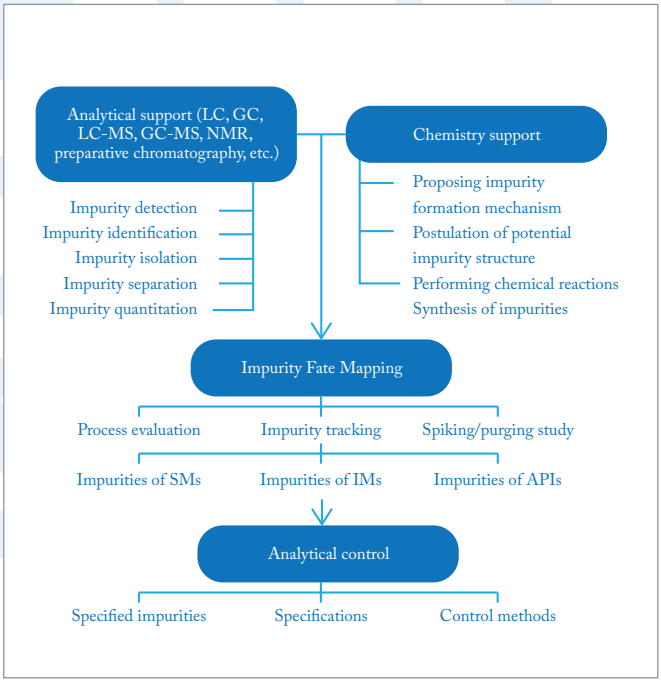


Figure 1. General outline of the impurity fate mapping framework (3).

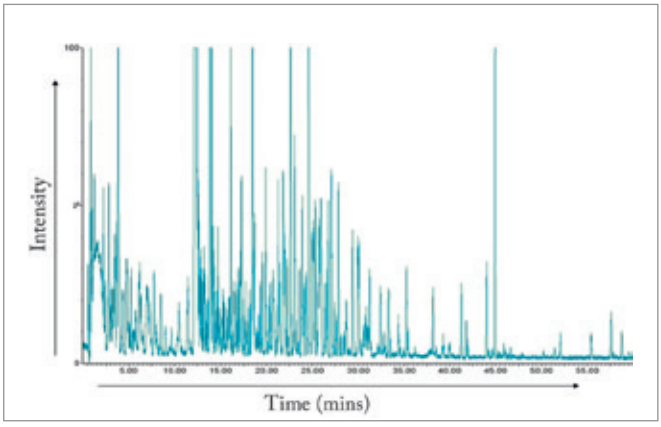


Figure 2. LC-MS (total ion current) analysis of rat urine (6).

alone, so it is important to collaborate with biochemists and other complementary disciplines, such as chemometrics.

Optimized?

Ultimately, optimization should result in a useful, suitable method for the analytical goal. Unfortunately, in non-targeted analysis, you never really know if you have reached the end of the process! It's a complex and challenging topic, and for about 10 years I have been a member of an international impurity profiling group with representatives from six pharmaceutical companies. The group comprised analytical chemists from industry who worked closely with their product quality colleagues – and some of the members had quality responsibilities too. We met every six months to discuss the issues and also exchanged many ideas about procedures for each stage of drug development.

One of the important questions that often comes up for discussion is: "When do we need to identify an impurity?" No doubt, new knowledge about the product is always important, but it can be a difficult discussion when it comes to efficiency and economics. Certainly, there are official concentration limits above which identification is needed. And if you know that the compound is toxic, the identification limit is lower. Clearly, there is a grey area – and it raises an important (and ethical) analytical question.

Analytical choices

Analytical procedures for non-targeted profiling should be suitable for different types of compounds and, therefore, should also be based on universal methods. However, from my experience, there isn't a single method that can offer all the information required, so you need multiple "analytical windows". Sample-relevant test compounds are very useful for method optimization in this regard, and help keep it on track. Ahead of real analysis, sampling and sample preparation are important steps that have an impact on selectivity. Clearly, the main goal is sample clean-up, which aims to remove compounds that can disturb the analysis. However, for reliable profiling, you also need high recovery of the compounds of interest. If liquid-liquid and liquid-solid extraction are used, the recovery of some known impurities (metabolites) and/or test subtances can be measured to assess suitability of your choice.

A number of analytical techniques prove useful for profiling because they pump out a lot of information. Nuclear magnetic resonance (NMR) spectroscopy, for example, is a powerful universal technique that even makes direct analysis of urine samples possible. However, the limited sensitivity can prevent the detection of important compounds. Gas and liquid chromatography (GC and LC), especially coupled with MS, are also highly suited to profiling. Generally, a derivatization procedure is combined with GC "to widen the window". LC can separate both polar and apolar compounds; the choice of stationary and mobile phase (mainly gradient elution) can increase the flexibility. And new stationary phases can offer increased retention of relatively polar compounds. Capillary electrophoresis (CE) coupled with MS for separation of ionogenic and very polar compounds looks promising for profiling.

In MS-based metabolomics, the complexity of the analytical system can be reduced by eliminating the separation step all together. Samples may be injected directly into the mass spectrometer, but ionization can be influenced by the matrix, and isomeric and isobaric compounds cannot be distinguished. Separation by GC, LC, or CE before MS offers a powerful analytical system, and the use of two-dimensional separation systems can further enhance information.

MS with total-ion detection can be applied for universal detection. High selectivity and sensitivity can be obtained using extracted-ion chromatograms/electropherograms. But a critical point is that detection of unknown impurities and metabolites is based on the total-ion signal, so the results depend on the

separation and sensitivity of the system.

Software plays an increasingly crucial role in data evaluation (feature extraction) as it helps uncover useful information. Identification can be gained through accurate mass data and databases, or through MS/MS spectra. Of course, spiking samples with reference compounds also helps confirm identity of new compounds. Beyond analysis, chemometrical approaches are also fast gaining traction.

Jumping the method validation hurdle

Generally after optimization, an analytical method must be validated. The aim is clear: to show that a method meets the analytical goal. Unfortunately, there are no clear guidelines for validating non-targeted profiling. So, what are the requirements and how can we do it? The main analytical goal is to get a complete "picture" of the compounds of interest and not (in the first stage) quantitative analysis. First of all, the effects of system parameters on the picture (spectrum or chromatogram) must be investigated. Second, if test compounds (known impurities, metabolites) are available, they can be used to demonstrate reproducibility and detection limits. Accuracy (recovery) can be critical for unknown compounds, as has been stressed before.

In conclusion, non-targeted profiling is complex, which means that optimization is in no way straightforward. To simplify the process, the application goal(s) should determine the best (or most appropriate) analytical method(s). Speed of analysis could be another important aspect depending on the scale of the study and the number of individual samples. A balance must often be found between throughput and the amount of information that is required.

The main challenge facing optimization of non-targeted methods is the very nature of the approach. How do we know where the edge is or how much further we need to go before we uncover another layer of valuable information?

References

1. *S Görög, "The importance and challenges of impurity profiling in modern pharmaceutical analysis", Trends Anal Chem, 25, 755–757 (2006).*
2. *S Görög (Ed.), "Drug-impurity profiling", Trends Anal Chem, 25, 755–820 (2006).*
3. *D Jain and PK Basniwal, "Forced degradation and impurity profiling: recent trends in analytical perspectives", J Pharm Biomed Anal, 86, 11–35 (2013).*
4. *G Theodoridis and ID Wilson (Eds.), "Hyphenated techniques for global metabolite profiling", J Chromatogr B, 871, 141–382, (2008).*
5. *GJ de Jong and R Ramautar (Eds.), "Separation techniques in metabolomics", Trends Anal Chem, 61, 156–235 (2014).*
6. *RS Plumb, et al., "Generation of ultrahigh peak capacity LC separations via elevated temperatures and high linear mobile-phase velocities", Anal Chem, 78, 7278–7283 (2006).*

Attaining Accurate Authentication

As fraudsters become increasingly knowledgeable and adept, authentication of food and beverages becomes more and more challenging. I was keen to see if GC-Orbitrap technology represented a new tool in the defense of food safety and quality – and Scotland's centuries-old whisky industry.

By Jana Hajšlová, Professor and Laboratory Head, Department of Food Chemistry and Analysis, University of Chemistry and Technology, Prague, Czech Republic.

My father graduated from the same university as me – the Institute of Chemical Technology Prague – and specialized in inorganic chemistry, so it wasn't too difficult to decide how I wanted my career to develop. But my father had set the academic bar very high; he was a guru in several weighty fields, including semiconductor research, and also worked for the United Nations on geological research projects. I decided to take a different route through chemistry and joined the faculty of food and biochemical technology. In the beginning, my father was a little disappointed by my choice as he considered it "university cooking", but it didn't take him long to realize that food chemistry and analysis was an exciting and cutting-edge field. Indeed, food analysis presents some of the most complicated matrices, which makes trace analysis very challenging at times (see page 32 for a perfect example). I too realized that I'd made an excellent choice and never regretted it.

Bitten by the technology bug

In the early days, I remember using gas chromatography instruments manufactured in Czechoslovakia; currency issues and availability prevented us from exploring imported options. The instruments were complex with many buttons and functions, but worked very well. More importantly, they allowed me to discover a great fondness for separation science – and technology. Even back then, I was doing sensory analysis on GC by removing the FID on repeat experiments and inhaling the scents from the peaks. Later, I moved more firmly into food safety because environmental issues were beginning to drive the industry towards change. I remember using a single chromatograph (funding was still challenging) connected to four selective detectors and an electronic printer; it was high technology at the time and very exciting. I knew I always wanted to be at the cutting-edge in terms of analytical instrumentation.

In the mid-1980s, I did a couple of years as visiting scientist at the Free University of Amsterdam working on very advanced techniques under two renowned chromatographers: Roland Frei and Udo Brinkman (who was head of the Royal Netherlands Chemical Society). Michel Nielen (now at RIKILT Wageningen UR) was my peer and remains my good friend and colleague. We are co-chairing the 7th International Symposium on Recent Advances in Food Analysis (RAFA 2015, www.rafa2015.eu) in November.

When I returned to the Institute in Prague, we started working on many more international collaborations and advanced instrumentation was more readily available. Our strategy was to focus on advances in mass spectrometry – something we continue to do today. We have a huge interest in assessing novel instruments and techniques from all the major companies. When I was asked to evaluate GC-Orbitrap technology ahead of its launch at ASMS 2015, I of course responded positively.

GC-Orbitrap technology – a true novelty

The pace of technological innovation has been startling, but the analytical challenges have also changed tremendously; the two aspects are part of the same cycle. Over the years, technology, such as automated sample injection and the sensitivity increase delivered by triple quadrupole MS (in both GC and LC), have constantly strived to answer the analytical questions of the moment. I was telling my students recently that the current challenges in food analysis are most likely to be addressed by high-resolution MS (HR-MS), which offers so many advantages compared with unit resolution MS/MS.

In the past, I've worked with medium resolution time-of-flight (TOF)

instruments with a maximum resolving power of about 10,000 FWHM, and then moved onto improved TOFs with about 30,000 FWHM. Orbitrap technology coupled to LC was a real breakthrough, offering resolution up to 60,000 FWHM with high mass accuracy – and further developments increased resolving power in some variants up to 450,000 FWHM (at m/z 200).

Today, Orbitrap is available for GC instrumentation in the Q Exactive GC, which is yet another key advance. I consider myself impartial when it comes to technology, but I can say that GC-Orbitrap technology offers several real benefits. I was particularly impressed with the linearity range of the instrument, which is a limitation of TOF instruments. In 'fingerprinting' style studies, relative ratios of responses for features are also diagnostic, so linearity plays a very important role. In our studies, we saw good linearity over six or seven orders of magnitude.

For me, two challenging areas stand out as real opportunities for Orbitrap technology to differentiate itself against triple-quadrupole instrumentation. The first is non-targeted screening, where you wish to confirm whether or not a sample is contaminated with unknown compounds – mycotoxins or other natural toxins using LC-Orbitrap, for example. Here, the combination of full scan and accurate mass is unparalleled, as discussed in my recent lecture 'Effective Food Safety Control: Pesticide Residues and More within a Single Run' at the 1st International Symposium on Recent Developments in Pesticide Analysis – you can watch the video here: LINK The second area is food authentication, which I believe is even more challenging. Traditionally, several markers have been used to answer questions of authentication, but with little in-depth knowledge of the matrices and other potential clues. Comprehensive MS fingerprinting using full-scan HRAM data coupled with advanced chemometrics can offer surprising insights into authenticity and classification of samples – something that was not before possible in a single analytical run.

Whiskey or Whisky?

When I tested the Q Exactive GC ahead of its launch, I was keen to benchmark it in three main areas: linearity, sensitivity and selectivity. But more than that, I wanted to assess its potential in the aforementioned area of food authenticity, which is why we focused on several whisky samples in addition to pesticide analysis. I was quite surprised to find that many compounds were identified automatically in both sets of samples, which proved to me that the deconvolution function was working well.

Analyzing the very important food commodity that is whisky seemed like a good idea given the fact I was in the UK. In particular, we were interested to see if we could authenticate whiskies in terms of age, geographical origin, brand and raw materials by building up databases and statistical models from samples of known origin. The end game is to use the data and models generated to assess unknown samples using HRAM fingerprints to gain a probability of authenticity. In our early work with GC-Orbitrap technology, we were fine tuning the method and found that ethyl acetate extraction gave us a good signature in terms of the compounds derived from the oak casks used in the aging process for whisky. As I hinted earlier, I was especially impressed with the linearity across major and minor compounds and the ability to identify ions that could be used to discriminate between whiskies.

A growing wish list of recent advances

Having spent time with GC-Orbitrap technology, what is my conclusion? Well, the Q Exactive GC is on my wish list! Especially as we have plans to establish a center of excellence in food and nutritional science – and that means we need great instrumentation. GC-Orbitrap technology represents the current pinnacle of innovation in that space right now, and would complete my collection – after all, I already have four TOF instruments, including a GC×GC-TOF-MS system.

Over the next few weeks, Michel Nielen and I – along with the rest of the team – will be conducting the strict selection process of oral abstracts for RAFA 2015. We started the conference 14 years ago to place an emphasis on excellence – and, as the name indicates, recent advances in the field – the two aspects that drive our selection process. Notably, we made a decision right from the beginning to separate presentations from independent (academic or industry) scientists and instrument company researchers – though certainly not in terms of quality. Richard Fussell is a perfect example of a quality scientist who will command attention and respect on both sides of the divide. Indeed, vendor lunchtime seminars are always packed and I am sure we will learn more about the Q Exactive GC this November. I will also be very interested to see if anyone will independently present work based on their experience with GC-Orbitrap technology – I'm quite confident we will...

When I was invited to Thermo Fisher Scientific's laboratory in Runcorn, UK, to test drive GC-Orbitrap technology, I was very curious to learn what added value or extra features it could offer. I can say that it certainly fills a gap – especially in metabolomic style approaches. I also suspect it will have a disruptive impact on certain areas of the mass spectrometry market. My independent advice? Take Orbitrap technology for a spin and decide for yourself.

Video interview with Jana Hajšlová: tas.txp.to/0915/Jana
To find out more: thermoscientific.com/HRAMGCMS

Profession

Leadership
Talent Development
Career Planning

Your Local School Needs You

Professionals in the fields of science, technology, engineering, and mathematics – STEM – have great potential to boost scientific literacy in schools. Can you make time to pass on years of experience and educate the next generation?

By Robert Thomas

Having worked in the field of laboratory instrumentation for almost 25 years, I now make my living from writing about analytical chemistry topics. If you've read any of my publications or textbooks, you know that my main reason for writing is not only because I have a passion for this field of science; I also want to pass on the knowledge I've acquired over the past 40 years. I believe as scientific professionals, we all have a duty to use our knowledge to improve the world in any way we can. Moreover, there are too many people in the world who have no real understanding of even basic scientific principles – public debates on global warming, genetically modified crops, evolution... and even the Loch Ness monster, are all testaments to this fact (1).

Time for fun (work)

Seven years ago, I decided to do something about the general level of scientific ignorance. I wanted to participate in the education of my two daughters and also contribute to the scientific literacy of the general public. I thought about teaching, but the US system does not encourage retired/experienced scientists to go into teaching – the certification process is time consuming, cumbersome, and expensive.

Anyway, I came across the AAAS (American Association for the Advancement of Science) affiliated Senior Scientists and Engineers STEM volunteer program. The program places retired scientists, engineers, and physicians into elementary, middle and high schools in the Washington, DC area with the objective of applying their experience to help teachers make science more interesting to learn by using creative and innovative methods. Each volunteer commits to an entire school year by dedicating a few hours of his or her time each week.

I began volunteering in 2008, but I wasn't sure where it would take me! Today, I support three chemistry teachers at Sherwood High School in Sandy Spring, Maryland. I volunteer at the school for one day a week and talk about real-world applications of chemistry in relation to the curriculum.

Over the years, we've expanded the program to include discussions about events in the media that have a chemistry "flavor". This includes the TV CSI crime series, drug testing, fracking for natural gas, toxic effects of lead and mercury on the environment, the Fukushima nuclear disaster, conflict minerals, Curiosity science experiments on Mars and many, many other interesting topics.

We have discussion periods at the end of every class to get the students' input and to see if they've been paying attention (headphones in ears are often a give-away).

We have also organized field trips to the local water authority lab where

they saw analytical chemistry applied to something they could relate to – clean drinking water. We've had trips to the Montgomery County Crime Lab to learn all about drug testing; to the National Institute of Science and Technology (NIST) Center for Neutron Research, which provides neutron measurement capabilities to the U.S. research community… and to the USA Science and Engineering Festival, held on alternate years in Washington, DC.

In addition, I was fortunate enough to acquire an atomic absorption instrument donated by a local lab, so we have performed some basic trace metal studies in drinking, river and pond water samples. I'm also hoping to get additional equipment, such as basic IR, UV, GC and LC instrumentation – donations are gladly accepted! There is no question about it; the program has definitely put a spark (no pun intended) into the chemistry classes. The teachers see the benefits and the students did not realize that chemistry was so much a part of everyday life – or that it could be so much fun to learn.

Volunteering to raise standards

Our volunteer program began in 2004 and currently has more than 160 active volunteers. The volunteers are mainly retirees, but for the past few years, we have started to recruit working people who are encouraged by their employers (public and private sector) to volunteer in their local communities. I lead the volunteer team in Montgomery County, Maryland, where I have more than 50 STEM professionals contributing to classroom activities in elementary, middle and high schools.

If I could select one common reason why we all do this, it's because we are all saddened by the falling science standards in our public schools. There is ample evidence of this in a recent study by the Organization for Economic Cooperation and Development (OECD). The study focused on the understanding of science, math and reading demonstrated by 15-year olds in 70 countries (2). Worryingly, the USA's ranking fell to 23rd for science and 29th for mathematics. For me, it is clear that improving our K-12 (primary through secondary) STEM education standards is high priority, if the USA wants to maintain a leading industrial and technological position in the world.

President Obama is also aware of the shortcomings of the US science educational system. One of the suggestions of the 2014 PCAST (President's Council of Advisors on Science and Technology) report was for every middle and high school to collaborate with a STEM professional so that students are in contact with real-world scientists and engineers to provide them with insights into the outside world (3).

Finally, the Next Generation Science Standards, a joint project by the US National Research Council and the Achieve Group (and supported by 26 states), has seen a real need to change the way science is being taught (4). This direct quote from the report validates what our STEM volunteer program is about: "K–12 science education should reflect the real world interconnections in science". No doubt your country could also benefit from similar schemes.

STEM professionals in the classroom

Our program shows clearly that placing experienced, real-world scientists and engineers in the classroom is a cost-effective approach to enhancing learning and motivating students to pursue technical and scientific careers. Indeed, in many parts of the USA, STEM volunteer programs are gaining a great deal of momentum and are making a significant impact on demonstrating to students that these can be compelling subjects to learn.

In the USA alone, there are more than one million scientists and engineers over the age of 60, who have at least a Bachelor's degree. But there are only a few hundred actively involved in STEM

By Argonne National Laboratory

volunteer programs, so we have barely tapped into a huge pool of potential volunteers. We firmly believe that a volunteer program like ours can make a huge impact to help children find a passion for science and mathematics.

Do it for the students

As scientists, we continually have to remind the general public that the benefits of a STEM education are far more than just learning about complex scientific topics. Students also learn to think more critically, which better prepares them for the problem-solving activities they will require later in their professional life. So, if you can spare the time, I strongly encourage you to get involved. If you live in the Washington, DC area, feel free to check us out at www.seniorscientist.org, where you will find details about our program, together with volunteering opportunities in other parts of the country.

In return, I can promise that your expertise and experience will bring enormous benefits to the classroom. Our program is a strong testament to this fact, which came about because three concerned scientists responded to an editorial in Science magazine in 2003 about the general public's lack of science literacy (5). Because of that initiative, twelve years later, we will have more than 200 STEM professionals going into the 2015–16 school year, reaching approximately 20,000 students every week… And that's no mean achievement for a group of retired scientists and engineers who thought their professional contributing days were over...

Robert Thomas is a Principal Consultant at Scientific Writing Solutions, Gaithersburg, Maryland, USA.

References

1. *How American fundamentalist schools are using Nessie to disprove evolution, Glasgow Herald, June, 24, (2012). www.heraldscotland.com/news/education/how-american-fundamentalist-schools-are-using-nessie-to-disprove-evolution.17918511*
2. *Organization for Economic Cooperation and Development (OECD) Program for International Student Assessment (PISA) report, (2010). www.oecd.org/dataoecd/34/60/46619703.pdf*
3. *Prepare and Inspire: K-12 Education in Science, Technology, Engineering, and Math (stem) for America's Future: Report to the President (September 2014). www.whitehouse.gov/administration/eop/ostp/pcast*
4. *Next Generation Science Standards: For States, by States (April 2013). http://www.nextgenscience.org/*
5. *Public Engagement with Science: Science Magazine Editorial: Alan Leshner, Volume 299 (February 2003). www.sciencemag.org/content/299/5609/977.full.pdf*

Your Standards Organization Needs You

Analytical scientists play an important role in the standard setting process. Are you involved?

By Taco van der Maten

Allow me to lay my cards straight on the table: I am a director of ASTM International, a standards development organization that serves as an open forum for the development of international standards. I also chair the ASTM F40 committee on declarable substances in materials, which has about 10 analytical scientists out of 200 members – I am also an active member on several other committees.

Standards are extremely important for society and industry; as an analytical scientist you should be on the inside, giving your valuable input. And you don't have to be selfless about it – getting involved in ASTM can help you grow professionally too. You can simply join up and move through the ranks of the committees you're interested in.

The insider

Being on the inside allows you to become involved in pertinent discussions before they are common knowledge – and that's good for business because you can keep one-step ahead. It also means you gain

new perspectives on what is happening around the world. For example, the interest in shale gas has led to developing methods for determining the quality of the air compared to that extracted from conventional wells. The insight gained can be invaluable; whenever a new analytical technique is discussed by the committee and people are beginning to work on standards for test methods, you know you should be taking it seriously. Of course, we only discuss open topics and we ensure confidentiality by adhering to our anti-trust statement (which is read out at every committee meeting).

As a member of a standards committee you assist with checking test methods that are written or proposed by other members – you can also submit your own ideas based on your own analytical knowledge and skills. For analytical scientists, this is more straightforward because you are likely an expert on beginning with the scope of the method, deciding on analytical instrumentation and chemicals, evaluating the results and writing scientific reports. Analytical scientists also understand that quality checks must be incorporated within the method; it is from these we can develop a standard.

From a personal point of view, I've been in the driving seat of a test method "F2617 Standard Test Method for Identification and Quantification of Chromium, Bromine, Cadmium, Mercury and Lead in Polymeric Material Using Energy Dispersive X-ray Spectrometry." Given the "technical context", I had to organize everything, deal with the feedback and build it into the method – and convince people that it had merit! Finally, after a year and a half of debating, discussion, meetings and testing, the method was finalized, which gave me great feeling of achievement. I think this example proves my point about growing professionally; you have the opportunity to take on responsibilities that may extend well beyond your day job.

A standard advocate

Let's be clear on standards: we need them! They provide an impartial way to check quality and, in many ways, underpin commerce. Indeed, standards help to establish a sense of clear trust between sellers and buyers.

Standards development began in earnest when early American railroad engineers sought assurance of the quality of the rails they were buying to build the network. It was a big demand in the late 1800s

Credit: Heinrich Pniok (www.pse-mendelejew.de); license FAL (artlibre.org/licence/lal/de)

Lutetium – a lanthanide.

because the field needed to establish acceptable tests that could ensure all rail manufacturers used the appropriate type of steel. Of course, over the years standards have extended to many other materials, such as gold and even rare earths.

Rare earths are an interesting area because they provide essential elements – neodymium and praseodymium – that are in high demand for manufacturing the powerful magnets used in mobile phones, hybrid motorcars and wind turbines. China is the biggest rare earth producer – and though the resources still exist in certain parts of the USA and Europe, the rare earth ore refining process uses environmentally unfriendly chemicals to extract the elements of interest. Let me explain the "rare" aspect more visually: if you plot all the elements on the horizontal axis and the numbers of atoms respective to a million atoms of silicon on the vertical axis, it produces a nice logarithmic plot. The graph would, in fact, also highlight the fact that all rare earth elements (lanthanides) have the same level of occurrence as nickel and lead – clearly, those aren't particularly rare. The rarity stems from the difficulty in separating them. Because they have similar chemical properties, they react in much the same way, so complex chemistry is needed for extraction.

For rare earths, as with all things, analytical scientists are essential for the process of establishing quality standards. We help decide how quality will be checked, the method to be used, the concentration levels, security, speed, certainty and reproducibility – all the usual aspects. As far as I am aware, only ASTM and China are currently developing methods for determining quality parameters for rare earths; we're working with colleagues from the magnet industry to help them determine the quality of the raw material they receive from their suppliers.

ASTM is industry driven; people come to us for methods to determine quality, and we have experts who can respond accordingly. In reality, the methods can be quite broad, involving analytical chemistry and physics. In the case of the magnet example, we wrote the method to not only establish concentration of the elements but also to assess magnetic parameters of the material.

How clean is 'green'?

If you're remotely interested in the environment, it could act as an additional draw to getting involved with a standards organization. As I noted, extracting and processing rare earths has environmental consequences, but there is actually a growing interest in "green mining". Once again, the analytical scientists in our committee are involved in helping determine the level of waste (for example, lead) produced by green mining operations. Of course, legislators expect zero waste, but someone has to step in and ask how mines are measuring "zero" and which analytical techniques are being used.

The Regulation of Hazardous Substances (RoHS) is another example where legislators demand minimal levels. Here, the level of bromine, cadmium, chromium, lead and mercury in electrical products – including plastics, printed circuit boards – is under scrutiny. So, if the European Union decides it wants no more than 100 mg of cadmium per kg in a computer, we establish whether the level refers to components or to the computer as a whole. Regulators are very good at passing laws, but it's down to analytical scientists to assess how the rules apply and to devise an acceptable standard method. We investigate the options and choose the best method and techniques to achieve the end goal – which is why x-ray fluorescence (XRF) was chosen as the preferred method for checking toxic heavy metals in computers.

Being involved in ASTM is exciting and I believe there is room for many more analytical scientists. We want you to bring your experience to the table and help create the very important standards that society needs to ensure our world is as clean, safe and fair as possible. It's easy to join and very rewarding; a small annual fee makes you a fully paid-up member with the right to vote, so long as you are the only member from your organization (if you are one of many members from the same company, then we only allow one vote to ensure fairness).

Anyone interested in finding out more should see the membership page on our website: http://www.astm.org/MEMBERSHIP/index.html

Taco van der Maten holds several positions within ASTM International as Director of the board, chairman of Committee F40 on declarable substances in materials and a member of committees D02 on petroleum products and D20 on plastics. In PANalytical, Enschede Area, the Netherlands, Taco holds the position of market segment manager for x-ray fluorescence for oils, fuels, petrochemicals and polymers.

Spectroscopy's New Role in Document Security

Even in today's digital world, some of our most valuable documents are still printed on paper. Banknotes, passports and professional licenses have high value and are at risk for counterfeiting, tampering and forgery. The security printing industry has responded to the challenge by developing a multitude of new anti-counterfeiting and protection techniques, many of which are based on incorporation of novel optical features enabled by spectroscopy.

By Cicely Rathmell, M.Sc.

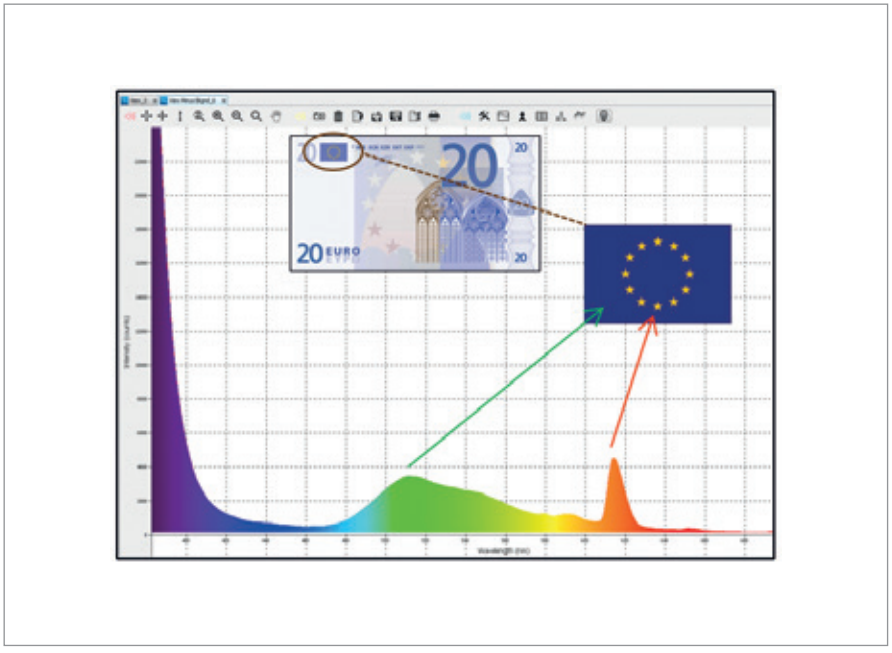


Figure 1: Fluorescence from a security feature on a 20 Euro bill.

Introduction:

Inks and pigments with optically unique properties can provide quick visual verification of authenticity, and can be difficult to replicate. These range from iridescent or color-shifting inks containing tiny flakes of mica that cause a change in color with viewing angle to inks that are poorly read by the illumination sources in traditional scanners used for copying.

Fluorescent inks are often used to overlay documents with special words or images that appear only when illuminated by UV light or light of another specific wavelength. Fluorescent pigments may also appear as fibers incorporated into the paper itself.

To test this, we assembled an Ocean Optics spectrometer system to look at fluorescence from the European Union flag security feature on a 20 Euro note. When viewed with UV light, fluorescence from the security feature should cause the blue background on the flag to appear green, while the yellow stars should appear orange (Figure 1).

Experimental Conditions:

We configured a modular fluorescence system consisting of a reflection probe, a 385 nm LED for excitation and a high sensitivity spectrometer for detection. With the probe pointed at the flag, a broad fluorescence peak was observed from ~490-560 nm, corresponding to the expected green color for the background when viewed with UV light. Fluorescence from the stars was visible in the same spectrum as a strong peak from 610-620 nm.

Results:

While a counterfeit bill might be able to replicate some Euro security features accurately enough to fool the eye at a glance, a full spectral measurement compared against an authentic Euro fluorescence spectrum could very quickly discern a fake.

Conclusions:

Optical security features and analysis techniques are an increasing part of safeguarding and evaluating document security. Compact spectroscopy systems are an important part of every step in the authentication process, from development to analysis in the field. Ocean Optics technologies can be adapted for integration into robust analysis systems and complemented by user-friendly pass/fail software to help professionals in this burgeoning field that protects our economic systems, our possessions and even our identities.

References

1. *Optical Document Security. Optical Document Security, n.d. Web. 21 July 2015.*

UHP-SEC Analysis of Immunoglobulins with TSKgel UP-SW3000

Antibody therapeutics are dominating the biopharmaceutical market. In 2013, six of the top ten global drug brands were monoclonal antibodies (mAbs). The characterization of these complex biomolecules is a major challenge in process monitoring and quality control. The main product characteristics to be monitored by chromatography are aggregate and fragment content, glycosylation pattern and charged isoforms.

The standard QC method for mAb aggregate and fragment analysis is size exclusion chromatography (SEC). A new series of 2 micron silica based UHPLC columns with 25 nm pore size can be applied to either increase speed or improve resolution of the separation of antibody fragments, monomers, and dimers.

Experimental:

Columns:	TSKgel UP-SW3000, Competitor Protein SEC 200 column
Column size:	4.6 mm ID x 15 cm
Eluent:	100 mmol/L phosphate buffer (pH 6.7) + 100 mmol/L sodium sulfate + 0.05% NaN3
Flow rate:	0.35 mL/min
Temperature:	25 °C
Detection:	UV @ 280 nm, micro flow cell

Sample (Calibration):

1. thyroglobulin, 640,000 Da (1' thyroglobulin dimer);
2. γ-globulin, 155,000 Da (2' γ-globulin dimer);
3. ovalbumin, 47,000 Da;
4. ribonuclease A, 13,700 Da;
5. p-aminobenzoic acid, 137 Da; Inj. Volume 5 µL

Sample (mAb Analysis):
therapeutic mAb (mouse-human chimeric); 1: trimer; 2: dimer; 3: monomer; 4: fragment ; Inj. Volume 10 µL

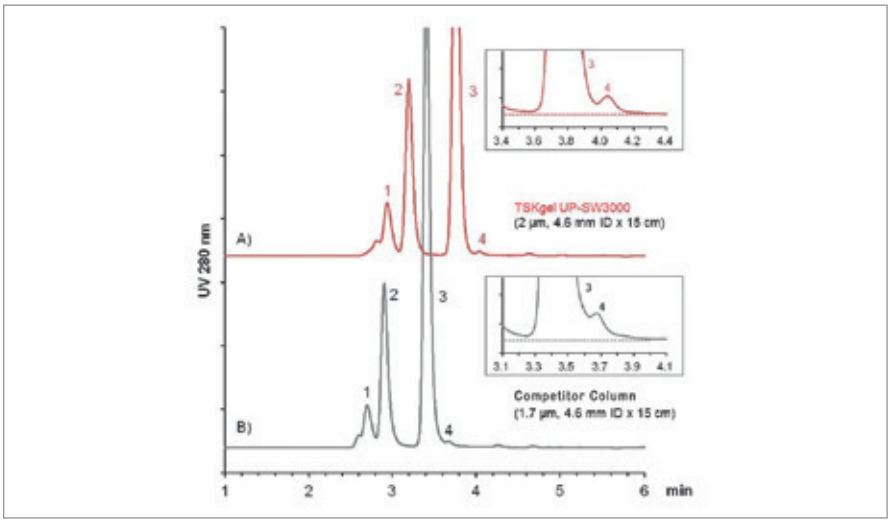


Figure 2: Comparison of Antibody Analysis

Results

The calibration of TSKgel UP-SW3000 shows a slightly shallower slope in the region of the molecular weight of γ-globulin than the one of a competitor 1.7 micron UHPLC column (figure 1). These differences in the separation range and steepness of the curves are related to a slight difference in pore size (25 nm for TSKgel versus 20 nm for the 1.7 µm material).

The separation of an antibody on the new 2 µm packing compared to the competitor column is depicted in figure 2 . The difference in pore sizes results in a slightly better separation in the mass range of antibodies, fragments and aggregates. Based on the wider separation window the resolution is slightly higher with TSKgel UP-SW3000.

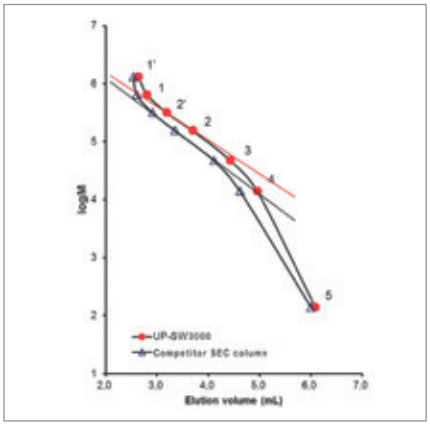


Figure 1: Comparison of Calibration for TSKgel UP-W3000 and a Competitor Column

Conclusion

TSKgel UP-SW 3000 is ideally suited for the analysis of the aggregate and fragment contents of antibody preparations. It features the same pore size as the renowned TSKgel G3000SWXL column while improving resolution through a smaller particle size. Based on the optimized pore size and the high degree of porosity the resolution in the range of IgG is even superior to a competitive UHPLC column with slightly smaller particle and pore size.

TOSOH

Aroma profiling of cheese

Using dynamic headspace sampling and thermal desorption for rapid profiling of cheese aroma

The aroma profile of cheese is an important part of the consumer experience, with a range of compounds responsible for the wide variation of cheese odours. This presents analysts with a substantial challenge when wishing to identify key aroma components, many of which are present at trace levels and have low odour thresholds. Here we show how Markes' technologies can be used to characterize both the high- and low-concentration components of cheese aroma profile:

- Dynamic headspace sampling using Markes' Micro-Chamber/ Thermal Extractor™ flushes the organic vapours from the cheese onto a sorbent-packed tube.
- Thermal desorption (TD) using Markes' fully-automated TD-100™ thermal desorber is used to pre-concentrate the volatiles before delivery into the gas chromatograph.

Headspace sampling on 5 g of grated cheddar was carried out at 40°C, to generate a full VOC profile reflecting that produced in the mouth. Samples were collected on to multi-sorbent tubes in order to handle the full range of analytes expected to be present in the VOC profile. The use of multiple sorbents in this way is only possible because Markes' TD systems are designed so that analytes enter and leave the tube (or trap) at the end with the weakest sorbents. This ensures that low-volatility 'sticky' analytes are retained on the weakest possible sorbent, so that when the gas flow is reversed, they desorb easily.

Analysing high- and trace-level components in a single run

Markes' TD splitting technologies offer a particular advantage for aroma profiling because of their ability to run a single sample twice, using different split ratios to accurately measure trace-level and high-concentration compounds in the same sample (Figure 1). In this case, the sample was desorbed using a 'high split' (51:1), with a small volume being sent to the GC. This allowed the high-concentration components to be analysed without overloading the analytical system. The remainder was re-collected onto a fresh sorbent tube, and then desorbed as before but using a 'low split' (6:1) – sending a higher proportion of the sample to the GC. This allowed the trace-level components to be quantified more accurately.

Overall, this approach facilitates the rapid and straightforward sampling of volatiles from cheese, including the side-by-side comparison of samples under identical conditions. An additional benefit – described in the accompanying full Application Note 101 at http://bit.ly/AppNote101 – is gained when the technique is used with time-of-flight mass spectrometry (TOF MS), for screening of targets and unknowns in a single run.

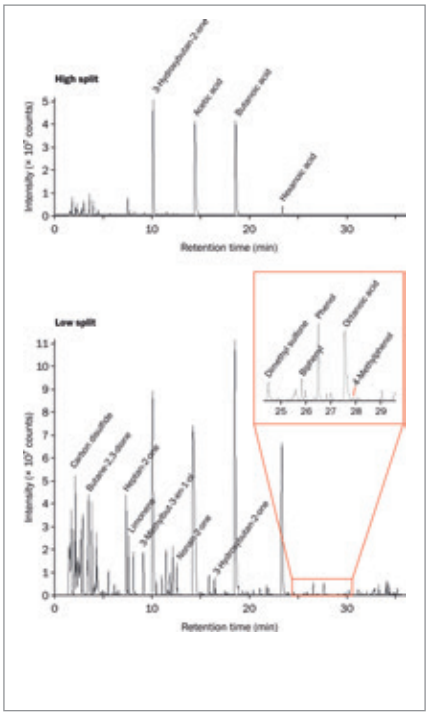


Figure 1: Analysis of the VOC profile of extra-mature Cheddar. (A) High-split (51:1) conditions provide an indication of the quantities of high-concentration components. (B) Low-split (6:1) conditions aid identification of trace-level components (see inset). Example peaks are highlighted; for a full peak listing, please see Markes Application Note 101.

MARKES international

Bioengineering a Quantitative Future

Sitting Down With... Amy Herr, Lester John & Lynne Dewar Lloyd Distinguished Professor of Bioengineering, University of California, Berkeley, USA.

How did you get into analytical chemistry?
I'm actually an engineer by training and not a chemist or life scientist, which I guess is a little unusual in the world of analytical chemistry, though becoming increasingly common as the field of bioengineering matures. After all, there is a large overlap between the two disciplines; engineers are interested in understanding problems and then applying fundamental approaches – in physics, chemistry and biology – to solve them. And it just so happens I'm intrigued by measurements – a lot of analytical chemists are also intrigued by measurements!

Why engineering?
I grew up in Florida – about 40 miles south of Kennedy Space Center – so I've seen tens, if not hundreds, of space shuttle and rocket launches from my front yard. Given the links to NASA, our middle and high schools had exceptional 'science research' programs. This enriching environment sparked my life-long interest in science, technology, math and engineering.

But you didn't end up a rocket scientist...
I chose Caltech because it's one of the homes of aeronautical engineering, but I was given some great advice that widened my focus; I ended up going to Stanford where I discovered that my understanding of materials and mass transport could be useful in devising new measurement approaches. From there, the biggest measurement challenges came to light; bioscience and biomedicine have particularly ambiguous and important unanswered questions. It's a field where measurement scientists can make a big difference.

And what are the biggest challenges?
One area that has gained a lot of attention from bioengineers, chemists, and others is diagnostics; I've been thrilled and humbled by the progress of others. The NIH has now recognized the importance of bringing engineers and analytical scientists into that area – and the impact of the Gates Foundation cannot be overstated. The shift of focus has legitimized these tough global questions as being valuable academic and educational pursuits for engineering. As an educator, it's important for me to see students understand that contemplating and tackling these questions is not out of their reach – even at the start of their careers.

What's driving your research right now?
I'm most interested in the layer below diagnostic devices – on the validation of promising biomarkers. The nucleic acid space is really moving forward, thanks in large part to the Human Genome Project and the resultant technology. But in the equally important (if not more so) protein measurement space, we've been struggling.

What is your approach to the problem?
Paralleling the 'scientific method', engineers are trained to use an 'engineering design process' – a formal practice that enables us to break down the messy, ambiguous world around us into tractable questions. I'm really passionate about making measurements of potential protein biomarkers that i) make sense because they dynamically reflect what's happening in a disease process through protein signaling, and ii) may be inaccessible to current, macro-scale tools. For us, that means creating high-specificity measurements at the single-cell and small sample volume level. Here, an engineer's view of transport, materials, and quantitation, coupled with a focus on good design really helps.

How do you measure your own success?
When I was younger, I gravitated to long-distance running rather than sprinting. I recognized that if I put the effort in, over time I could really improve – and I did. Being involved in the diagnostics space is my way of trying to change the world. But – like my running – I know I've got to be in it for the long haul. Our focus for the foreseeable future will be on developing quantitative, precision tools suitable for many thousands of individual samples – at the bench – to help fill in significant gaps in biomolecular measurements, with an applied view to solving unmet needs.

How does it feel mixing with 'traditional' analytical chemists?
One reason I love to go to forums like the HPLC conference is to learn – not just the solutions to problems but also what the important metrics of performance are (how success is measured) and how it fits in the bigger picture. It makes sense for analytical chemists and engineers to collaborate with each other – and not just to tackle other people's problems. The analytical chemists (and other engineers) I've met are welcoming, curious and motivated to keep advancing. It's a great community.

Tell us about your induction into the Georges Guiochon Faculty Fellowship...
It was a complete shock and a true honor – and quite humbling. The Fellowship was put in place as a memorial to both Georges' monumental (and, for me, overwhelming) contributions and his passion for bringing 'new blood' into the field. I certainly hope to carry the torch in terms of reaching out to others and welcoming them into this vigorous, technical community. It was an honor to be presented with the award by Lois Ann Beaver (Georges' widow), who I had the great pleasure of speaking with at length about his work and motivations – that was a sparkling highlight of the experience for me. In researching more about Georges, I was inspired to learn that he too was a *bone fide* engineer.

Heavy workload?
– of course!

I can handle it - and so can you …

with intelligently automated GERSTEL Solutions for GC/MS and LC/MS. The GERSTEL MPS handles Your Sample Preparation and Introduction efficiently, accurately and reliably, automated to your specifications. Setup by mouse-click and your MPS works day and night, using less solvent and without anyone watching over it.

GERSTEL – More Productivity and Less Stress for Laboratory Staff, the Workplace and the Environment.

What can we do for you?

ISO 9001

Agilent Technologies
Premier Solution Partner

GET GERSTELIZED!

GERSTEL

www.gerstel.com