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The Column

the interactive e-publication for the global separation science industry

Cover Story

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Inside The Museum

Classifying museum artefacts using SPME–GC–MS

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The Art of Museum Conservation Using Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry

The Column **spoke to Katherine Curran, a lecturer in Sustainable Heritage at the UCL Institute for Sustainable Heritage in London, UK, about her work classifying museum artefacts using volatile organic compound (VOC) analysis and solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS).**

—Interview by ***Kate Mosford***

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Q. Your group has been classifying polymeric museum artefacts using volatile organic compound (VOC) analysis and solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS) (1). What led your group to begin this study?

A: We know that analysis of VOCs is a very powerful technique that has been widely researched for medical applications. Many of the features that make it useful in medicine are extremely relevant to heritage, such as the fact that VOC analysis is noninvasive and that you can obtain useful information, such as the diagnosis of a particular disease, by analyzing complex mixtures of components. These are both really important features when working with museum artefacts because the more you can make your analysis noninvasive the better. Museum artefacts are also really complex, so a technique that can deal with this complexity is very useful in this context also.

There had been some previous work in our research group on the study of VOCs from a historic paper and it was shown that VOC emissions could be linked to material properties and material degradation (2). The conservation of plastics in museum collections is a much less advanced field than the conservation

of older materials such as paper, stone, or wood. However, plastics in museums can be some of the most fragile materials present, and some of the earliest and most experimental plastics are now in museums. Some of these have been found to degrade quite rapidly and dramatically. So there is a lot of research needed to understand degradation processes and how best to conserve such objects.

Bringing all of this together, we felt that using VOC analysis could provide an important insight into the degradation of polymeric museum artefacts such as plastics, identifying evidence of degradation while acting as a noninvasive method.

Q. What kind of polymeric artefacts have you investigated?

A: At Tate in London we analyzed three sculptures, made of either cellulose acetate or cellulose nitrate, two of the earliest and most problematic plastics found in museum collections. These were works by the artists Naum Gabo and Antoine Pevsner.

In the laboratory, we analyzed over 200 objects. These were chosen to include the types of polymers that are known to be found in museum and archive collections and comprised combs and accessories from around the 1930s, vinyl records, plastic toys, jewellery, crockery, and some newly produced polymer samples.

Q. Can you tell us about the chromatographic method you chose for this research and why?

A: We used SPME–GC–MS. SPME was developed in the early 1990s by Janusz Pawliszyn from the University of Waterloo in Ontario, Canada. We used it for analysis of VOCs in the headspace of our objects. It uses a fibre with an adsorbent coating to collect VOCs from the headspace, which are then desorbed in the injector port of the GC–MS system.

We chose it because it is straightforward to use and can be used noninvasively for direct analysis of artefacts on-site in the museum stores or galleries.

Q. Were there any particular precautions that had to be undertaken when analyzing these valuable artefacts?

A: This was all done with the support of collaborators at Tate. We worked with Joyce Townsend, who is a Senior Conservation Scientist, and Deborah Cane, who is a Conservation Manager. They needed to be present when we placed the SPME fibres in the cases with the artefacts

to supervise our work. SPME–GC–MS is a very noninvasive technique, so all we needed to do was place the fibre alongside the object and leave it in place for 1 week. Photos of this can be seen in the paper (1).

Q. What is novel about your approach or findings?

A: No one has used VOC analysis to understand the degradation of plastic museum objects before. There has been research on the characteristic VOCs that certain types of plastic artefacts emit, but our approach looks at how you can use statistical analysis to identify mixtures of VOCs that indicate degradation. This builds on previous research in medicine where mixtures of VOCs are used for disease diagnosis.

Q. Can steps be put in place to halt polymer degradation in museum artefacts?

A: At present, the best approaches are to control the environment in which artefacts are stored or displayed. For plastics, some objects are put in cold storage to slow down the rates of chemical degradation. In many museums, relative humidity is also controlled so that chemical reactions based on hydrolysis are inhibited. Some of the problematic plastics emit acids as they break down, which can accelerate deterioration and have an effect on other objects. Therefore, some museums put absorbents in museum storage containers to absorb these acids. However, it's not always clear that these methods are useful for all objects, and more research needs to be done to identify when such measures are helpful and when they are not.

There has also been very little research done on the best way to clean or treat damaged plastic museum objects. There is research going on to develop nanomaterials for conservation of contemporary art works, as part of a big European project called Nanorestart: http://www.nanorestart.eu/

Q. What other chromatographic techniques have you used for museum conservation?

A: We use gel permeation chromatography (GPC) to identify the molecular weight of polymeric materials. In general, chromatography is hugely important in museum conservation. There is a conference dedicated to it called MaSC "Users' Group for Mass Spectrometry and Chromatography": https://mascgroup.org/. People use pyrolysis (Py-GC–MS), GC–MS, liquid chromatography (LC)–MS,

high performance liquid chromatography (HPLC), and many other techniques for identifying the components of complex mixtures, such as in historic paints.

References

1. K. Curran *et al.*, *Angew Chem. Int. Ed. Engl.* Feb 5 (2018).
2. M. Strlic, J. Thomas, T. Trafela, L. Csefalvayova, I. Kralj Cigic, J. Kolar, and M. Cassar, *Anal. Chem.* **81**, 8617–8622 (2009).

Katherine Curran has worked as a Lecturer in Sustainable Heritage at the UCL Institute for Sustainable Heritage since 2013. She is Assistant Course Director for the MSc in Sustainable Heritage and the Principal Investigator for the ERC Starting Grant funded project "COMPLEX: The Degradation of Complex Modern Polymeric Objects in Heritage Collections: A System Dynamics Approach" which will develop new approaches to understanding and modelling the degradation of modern polymeric materials in collections.

Katherine joined the Institute for Sustainable Heritage as a Research Associate in November 2011, to work on the AHRC/EPSRC Science & Heritage Programme project "Heritage Smells".

From 2010–2011, Katherine received a Fulbright Scholarship from the Fulbright Commission in Ireland and worked with Jeffrey Moore at the University of Illinois in Urbana-Champaign, USA, on the synthesis of macrocyclic compounds via alkyne metathesis.

Katherine obtained her Ph.D. in polymer chemistry ("Pd(II)- and Zr(IV)-catalysed Olefin Addition Polymerisation of Cyclobutene-based Monomers") in 2009 from University College Dublin (UCD), Ireland, and her B.Sc. (chemistry) in 2004, also from UCD.

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News

Agilent Announces Genohm Acquisition

Agilent Technologies (Santa Clara, California, USA) has announced an agreement to acquire Genohm (Lausanne, Switzerland), a developer of highly differentiated, on-premise, and cloud-based software solutions for laboratory management.

Genohm's main laboratory software automation suite, SLIMS, is a digital platform that provides laboratories with a rapidly deployable laboratory information management system (LIMS) and electronic lab notebook (ELN) environment that is used in biobanks, research labs, and next generation sequencing facilities. The platform tracks data and samples, tests and users, results, and workflows. In addition, Genohm also has an application marketplace with preconfigured workflows to enable rapid system implementation across a broad range of industries and scientific workflows.

"We were impressed with the team and the technology," said John Sadler, Vice President and General Manager of Agilent's Software and Informatics Division. "The modern architecture of SLIMS is perfectly aligned with the values of Agilent's OpenLab products. By integrating this technology with our broad and diverse instrument portfolio, we are in a unique position to support and enhance the operations of modern laboratories—truly helping our customers to do more with their data."

"We are very excited to join the Agilent team and believe that together we can accelerate development of the digital lab to help our customers advance science and discovery while ensuring compliance and traceability," said Frederik Decouttere, founder and CEO of Genohm.

For more information, please visit: **www.agilent.com** or **www.genohm.com**

Josef Heiland Receives Eberhard Gerstel Award

Josef Heiland has been awarded the 2018 Eberhard Gerstel Prize for his publication "Temperature Gradient Elution and Superheated Eluents in Chip-HPLC" (1), which was published in the journal *Analytical Chemistry.*

The Eberhard Gerstel Prize is awarded by the Working Group Separation Sciences of the Analytical Division of the German Chemical Society for an outstanding publication in the field of separation sciences and is handed out in honour of the late Eberhard Gerstel Sr., founder of Gerstel.

In his work, Heiland presented a novel concept of temperature programmed microchip HPLC. The paper explains that because of the relatively low mass of the lab-on-a-chip hardware, it is well suited for temperature programming. This means that isocratic separations can be performed at a speed not previously achieved even using pure water and it opens up the possibility of using other environmentally friendly eluents such as supercritical CO_2 and ethanol.

In order to be considered for the prize named after the Gerstel company founder, Eberhard Gerstel (1927–2004), scientists can apply in person or their candidature can be proposed by others. The selection of the prize winner is performed by a jury of five internationally renowned scientists. The next round of applications for the 2020 Eberhard Gerstel Prize will be opened in 2019.

For more information, please visit: **www.gerstel.com**

Photo Credit: Gerstel GmbH & Co.KG

Reference

1. J.J. Heiland, C. Lotter, V. Stein, L. Mauritz, and D. Belder, *Anal. Chem.* **89**(6), 3266–3271 (2017).

LabConnect Expands

LabConnect LLC (Seattle, Washington, USA), a global provider of central laboratory and support services for biopharmaceutical, medical device, and contract research organization, has completed phase one of a 11,000 square foot facility expansion in Johnson City, Tennessee, USA. The new space will significantly increase the capacity for peripheral blood mononuclear cell (PBMC) processing and allow LabConnect to better serve its clients.

Following the completion of phase one of its expansion LabConnect will proceed with stages two and three, which include the addition of more offices and project management space, and building out the PBMC laboratory capabilities.

"We have grown every year since our founding in 2002, and we are committed to maintaining our facilities with cutting-edge technology," said Eric Hayashi, president and CEO of LabConnect. "It is an exciting time to be building our business, constantly improving sample testing services to meet clients' needs, and helping to save lives while supporting clinical trials."

The expansion is scheduled to be complete in September 2018. For more information, please visit **www.labconnectllc.com**

Investigating the Flavour Profiles of E-Cigarettes

Researchers from Gdansk University of Technology, Poland, have investigated the flavour profiles of e-cigarette refill solutions using gas chromatography–tandem mass spectrometry (GC–MS/MS) (1).

The massive rise of e-cigarettes has partly been fuelled by the wide variety of flavours available to smoke. One publication found that 81.5% of young interviewees use e-cigarettes "because they come in flavours I like" (2). Despite EU tobacco regulations stipulating that flavoured cigarettes are prohibited, this does not apply to e-cigarettes with over 7700 unique flavoured e-liquids being sold as of 2014 (3). The sheer quantity of additives across the liquids has led to concerns regarding their safety, with numerous studies finding adverse effects to e-cigarette use (4,5). Therefore, researchers wanted to carry out a wide-ranging chemical analysis to ascertain the unknown nature and impact of these additives on human cells, particularly the lungs (6,7). They also wanted to document and produce data on the compounds that are responsible for specific e-liquid flavours. The first step in this process was the development of a sensitive method capable of analyzing e-cigarette refills.

Using GC–MS/MS researchers evaluated the compounds responsible for five of the most popular flavours (menthol, apple, tobacco, strawberry, and cherry) from five different brands.

The developed methodology successfully quantitated 90 flavour additives and categorized the flavour chemicals for the evaluation of the taste profiles. "I believe the methods would be suitable to analyze more flavours," said Pawel Kubica, Gdansk University of Technology. "Everything depends on the physiochemical properties of compounds and how they interact with the stationary phase and with the detector," he continued.

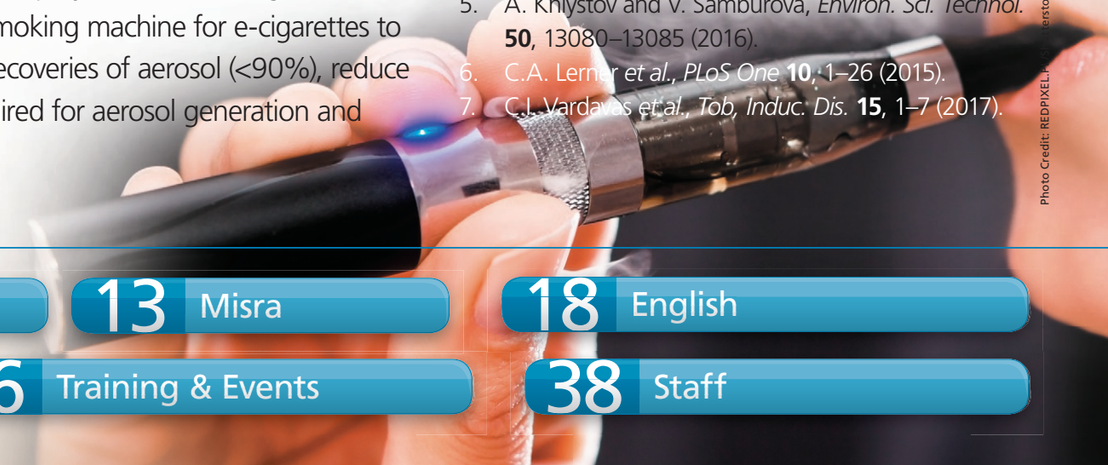
Future studies are likely to follow on the subject because of the enormous variety of e-cigarette flavours, providing a wealth of interesting compounds for study. "We have finished a project to generate and collect aerosol from e-cigarettes," said Kubica. "The main purposes of this project were to design and construct a smoking machine for e-cigarettes to obtain high recoveries of aerosol (<90%), reduce the time required for aerosol generation and collection to below 5 min, to choose the proper solid sorbent to "trap" aerosol efficiently, and to desorb with simple solvents," said Kubica. Previous studies in the area have reported little information on an aerosol generation process, which the researchers from Gdansk University of Technology hope to remedy. "I hope it will be published soon", added Kubica.

Further to this study, researchers have also developed a method to determine flavours together with nicotine in collected aerosol samples using GC–MS/MS—L.B.

For more information, please visit **https://chem.pg.edu.pl/kcha/main-page**

References

1. J. Aszyk *et al.*, *J. Chromatogr. A* **1547**, 86–98 (2018).
2. B.K. Ambrose, B. Rostron, and N. Borek, *JAMA* **314**, 1871–1873 (2015).
3. S. Zhu *et al.*, *Tob. Control* **23**, iii3–iii9 (2014).
4. U.S Department of Health and Human Services, *E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General* (2016)
5. A. Khlystov and V. Samburova, *Environ. Sci. Technol.* **50**, 13080–13085 (2016).
6. C.A. Lerner *et al.*, *PLoS One* **10**, 1–26 (2015).
7. C.I. Vardavas *et al.*, *Tob, Induc. Dis.* **15**, 1–7 (2017).



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Peaks of the Month

- **The *LCGC* Blog: Where Are the Young Investigators in Separation Science?**—One of the initiatives that the SCSC oversees is the nomination process and awarding of the Satinder Ahuja Award for Young Investigators in Separation Science. Where are all of the young investigators in separation science? Certainly, those that have been honoured to date have been worthy; however, there must be more eligible parties out there. **Read Here>>**

- **New HPLC Systems and Related Products Introduced in 2017–2018: A Brief Review**—This instalment describes high performance liquid chromatography (HPLC) and related products introduced at Pittcon 2018 in Orlando, Florida, USA, and in the year prior. It highlights new HPLC and mass spectrometry (MS) systems, modules, chromatography data systems, and other HPLC-related software and provides brief descriptions of their significant benefits and innovative features. **Read Here>>**

Photo Credit: Rost9/Shutterstock.com

- **The Role of Chiral Chromatography in Antiepileptic Drug Development and Epilepsy Therapy**—*The Column* spoke to Arcadius V. Krivoshein, Assistant Professor of Chemistry at the University of Houston–Clear Lake, USA, about his work developing EPP, an experimental anticonvulsant that can help to stop convulsions during epileptic seizures, and the role of chiral high performance liquid chromatography (HPLC) in this research. **Read Here>>**

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- **Chemical Fingerprinting of Mobile Volatile Organic Compounds in Soil by Dynamic Headspace–Thermal Desorption–Gas Chromatography–Mass Spectrometry**—A dynamic headspace–thermal desorption–gas chromatography–mass spectrometry (DHS–TD–GC–MS) method for the fingerprinting analysis of mobile volatile organic compounds (VOCs) in soil is described and tested in this article. **Read Here>>**

Photo Credit: mythja/Shutterstock.com

- **A Nontargeted Metabolomic Approach for Organic Food Fraud**—Researchers from the University of Almería, in Almería, Spain, have developed a nontargeted metabolomic approach to differentiate organic and non-organic tomato crops using high performance liquid chromatography–high-resolution accurate mass spectrometry (HPLC–HRAMS). **Read Here>>**

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News In Brief

Phenomenex (Torrance, California, USA) has announced the formation of a direct sales and service team to serve Portugal. In addition to direct purchasing, customers will have access to rapid product delivery, lower prices, helpful technical support, and educational training seminars. "This new team will enable the company to serve the growing research and development communities in Portugal," said Corinna Jones Rockenbach, Phenomenex regional manager for Spain and Portugal. For more information, please visit: **www.phenomenex.com**

Spark Holland B.V. (Emmen, The Netherlands) has announced an OEM deal with Axel Semrau (Sprockhövel Germany), a long-time distributor of Spark Holland products and services in Germany, Switzerland, and Austria. "We are very pleased to add Axel Semrau as our OEM customer. Its team is highly experienced in Spark Holland products, and Axel Semrau's own advanced software now connects our technologies directly to most of the MS software packages," said Rob van der Knapp, Spark Holland President. The formal commencement date of the OEM contract is 1 January 2019. For more information, please visit: **www.sparkholland.com and www.axel-semrau.de/en/**

Tips & Tricks: Aqueous GPC/SEC: Influence of Salt and pH

Wolfgang Radke, PSS Polymer Standards Service GmbH, Mainz, Germany

While gel permeation chromatography/size-exclusion chromatography (GPC/SEC) of uncharged molecules in organic eluents in most cases is a straightforward task, aqueous GPC/SEC of polyelectrolytes usually requires more parameters to be considered and optimized. This instalment of Tips & Tricks explains more.

Gel permeation chromatography/ size-exclusion chromatography (GPC/SEC) is the workhorse in macromolecular science when it comes to determination of molar masses and molar mass distributions. Once a suitable method is established, the simple methodology, the low workload needed for sample preparation, and the possibility to automate the analysis allows large numbers of samples to be analyzed unattended, thus making the process cost-effective.

However, while identifying appropriate combinations of stationary and mobile phases is often straightforward when using organic eluents, method development of aqueous GPC/SEC—particularly for macromolecules bearing ionizable groups (polyelectrolytes)—is usually more demanding.

Why do Polyelectrolytes Require the Addition of Salts?

Polyelectrolytes are classified as polycations or polyanions. In an aqueous environment, they dissociate to different degrees, depending on pK, pH, and salt concentration. If a polyelectrolyte is dissolved in pure water, only a certain fraction of the repeating units will be dissociated, resulting in charges along the polymer chains and free counter ions (1 in Figure 1). However, complete dissociation will rarely occur because it would require building up a huge charge around the macromolecule.

The charges along the chain will repel each other, resulting in stretching and coil expansion of the polymer chain (2 in Figure 1), which is associated with a

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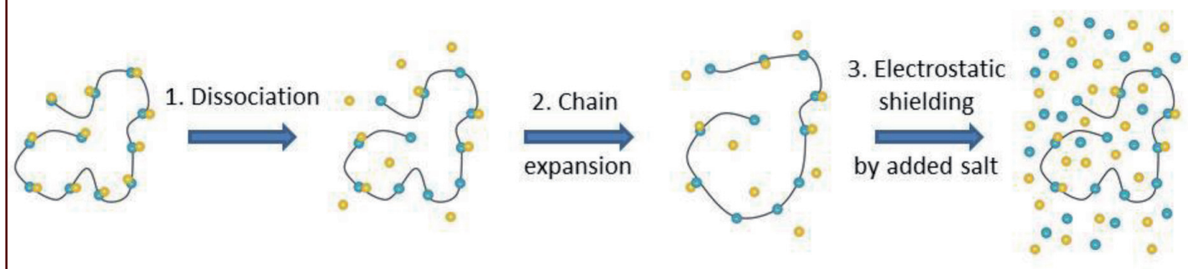
Figure 1: Effects of dissociation and electrostatic shielding by salt addition on polyelectrolyte dimension.



considerable increase of the viscosity of the solution. This is known as the "polyelectrolyte effect".

In GPC/SEC experiments the large size, resulting from increased coil expansion of a polyelectrolyte in a salt-free eluent, will prevent the polyelectrolyte chain from entering smaller pores. Consequently, the polyelectrolyte exhibits a much lower elution volume as a non-ionized sample of similar chain length. If the molar mass of a polyelectrolyte is derived from a calibration curve established using uncharged water-soluble polymers (for example, polyethylene glycols or pullulans) very different molar masses will result.

To counteract the expansion of the polyelectrolyte chain, low molar mass salts are usually added to shield the electrostatic interaction along and among the chains (3 in Figure 1). Upon addition of a salt, the electrostatic repulsion along the chain is shielded and the polyelectrolyte chain shrinks strongly, resulting in a severe change in elution volume.

An example is shown in Figure 2 where the chromatograms of low molar mass polyethylene imines (PEI) are compared using salt-free and salt-containing eluents. PEIs can be conveniently analyzed using acetic conditions. In aqueous trifluoroacetic acid without the addition of NaCl the sample elutes at a significantly lower elution volume compared to the salt-containing eluent. In contrast, the elution volumes of pullulan standards are not altered significantly by the addition of salt. Therefore, if the two chromatograms are evaluated using a pullulan-based calibration curve, large differences in molar mass will result. For the example shown, the molar mass derived in the salt-free eluent exceeds the molar mass in the salt-containing solution by approximately one order of magnitude.

Figure 2: Comparison of chromatograms of PEI. Red: 0.1% trifluoroacetic acid in water, Blue: 0.1% trifluoroacetic acid in 0.1 n NaCl.

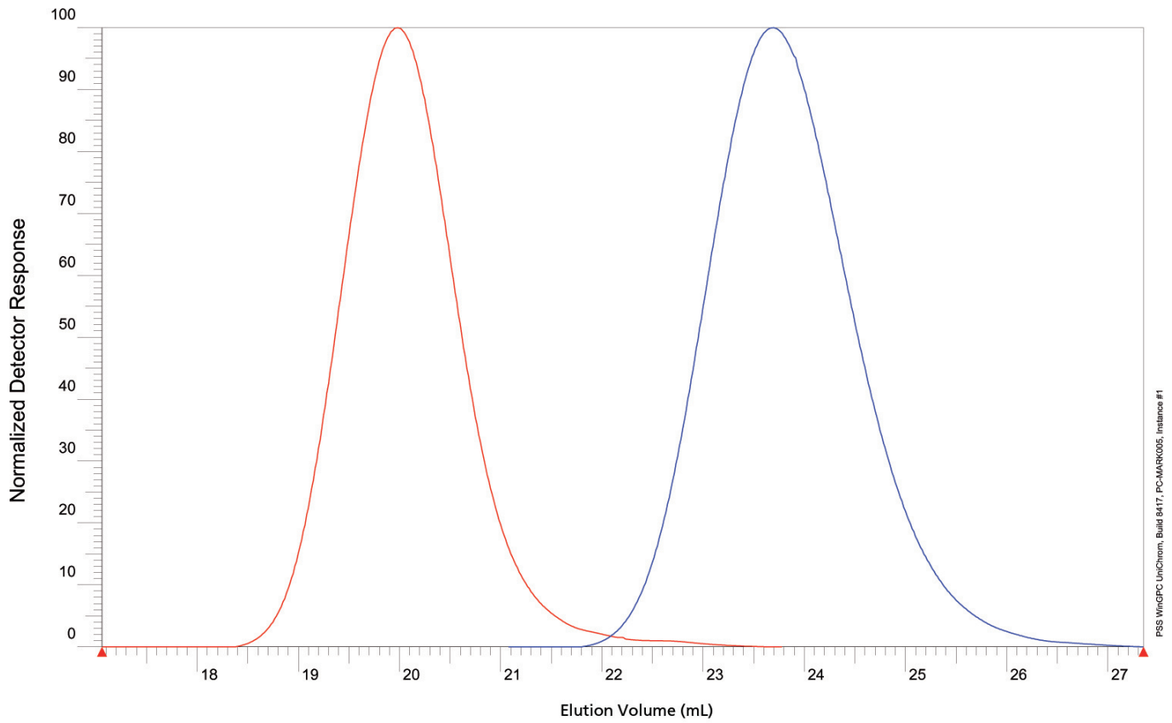
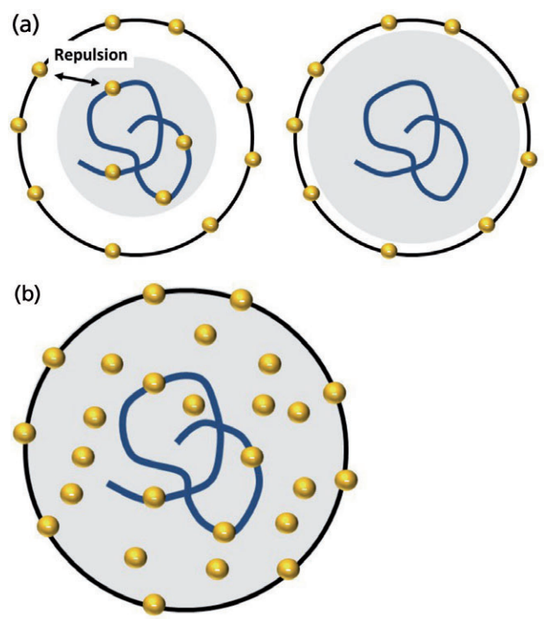


Figure 3: (a): Comparison of accessible pore volume for charged and uncharged macromolecules of the same chain length in a pore of an ionizable stationary phase. Counterions are omitted. (b) Electrostatic shielding of electrostatic repulsion by salt addition. Counterions are omitted.



It should be mentioned that even small salt concentrations significantly alter the size of polyelectrolyte chains. It is therefore hard to establish stable chromatographic conditions for polyelectrolytes in the absence of salt and so it is advisable to add salt to aqueous eluents to obtain stable results.

In most aqueous applications salt addition is necessary to shield electrostatic interaction along and among the macromolecules as well as between the macromolecule and the stationary phase. In addition to coil expansion from electrostatic interaction of charges along the polymer chains, interactions of charges with the stationary phase could also occur. Such ionic interaction will be shielded by the addition of salt as well. Therefore, pure water is rarely applied as eluent in aqueous GPC/SEC.

Why is the pH Important?

The pH of the eluent is another major factor that influences elution in aqueous eluents. The extent of dissociation and, thus, the polarity of the macromolecule, for example, of a poly(acrylic acid), depends on pH. At high pH a significant fraction of the carboxylic acid groups is neutralized resulting in a copolymer containing carboxylic acid and carboxylate groups, while at low pH, dissociation is strongly suppressed, resulting in a less polar poly(acrylic acid). Depending on the polarity of the stationary phase, nonpolar interaction between the less polar poly(acrylic acid) and the stationary phase could occur in aqueous eluents, resulting in late or even no elution at all from the column. This explains why polyacrylic acid in phosphate buffer at pH 9 elutes with high reproducibility as a well-shaped peak, while no elution from the same column is observed in phosphate buffer at pH = 5. Consequently, a suitable pH is usually required to properly elute charged polymers.

Changing pH does not only change dissociation of the polyelectrolyte, but could also alter the charge state of the stationary phase itself if the stationary phase contains ionizable groups. The pH of the eluent applied, which in turn depends on the chemical structure of the polymeric analyte, influences the type of column material to be chosen.

If at a given pH the macromolecule and the stationary phase contain similar charges, the macromolecule will be repelled

from some part of the pore. This might ease counterbalancing undesired nonpolar interaction with the stationary phase. However, the repulsion of the macromolecule from the stationary phase results in an apparent reduction of the pore size of the stationary phase. Despite that, one might still observe typical GPC behaviour for the given polyelectrolyte structure, that is, larger polyelectrolytes eluting before the smaller ones. However, as neutral molecules of the same size have a larger accessible pore volume because of the absence of the above described repulsive forces, the separation is not strictly based on size as indicated in Figure 3(a). To reestablish a true size-based separation, the electrostatic repulsion between the macromolecule and the stationary phase needs to be shielded, for example, by the addition of a low molar mass salt (Figure 3[b]).

Additional Recommendations for Method Development

Finally, if the water-soluble macromolecule contains additional nonpolar structures, the addition of organic modifiers might be required to reduce or eliminate undesired nonpolar interaction of the macromolecule and the stationary phase.

Summary

- Successful GPC/SEC in aqueous eluents requires careful consideration of the interaction of stationary and mobile phase with the macromolecule.
- pH changes the charge state of the analyte molecule, thereby influencing polarity of the macromolecules. As a result, adsorptive nonpolar interaction with the stationary phase might become dominant resulting in late or incomplete sample elution.
- In salt-free conditions, ionizable groups along the macromolecular chain repel each other, resulting in undesired coil expansion, giving the impression of the existence of very high molar mass products.
- Repulsive interaction of charges along the macromolecule as well as between the macromolecules or between the macromolecule and the stationary phase can be shielded by the addition of low molar mass salts.

Wolfgang Radke studied polymer chemistry in Mainz, Germany, and Amherst (Massachusetts, USA) and is head of the PSS application development department. He is also responsible for instrument evaluation and for customized trainings.

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Resources for Careers in Metabolomics for Early Career Researchers

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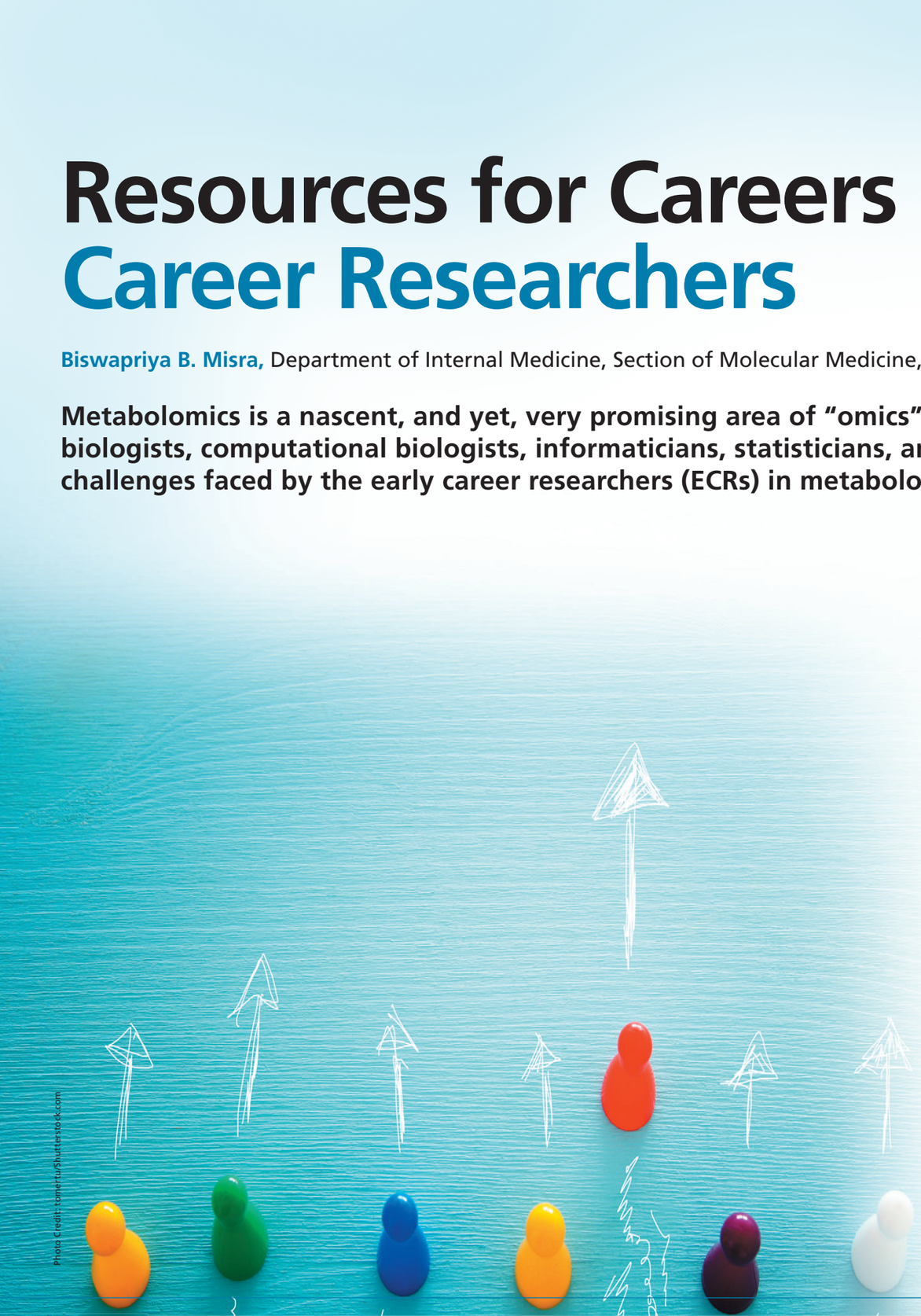
Metabolomics is a nascent, and yet, very promising area of "omics" research, which combines interdisciplinary efforts from analytical chemists, biologists, computational biologists, informaticians, statisticians, and engineers. This article looks at the current trends, future opportunities, and challenges faced by the early career researchers (ECRs) in metabolomics research.



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Metabolomics, a word coined in 1998, is the study of small molecules (that is, organic chemicals with a molecular weight of 50–2000 Daltons) present in a given biological fluid, tissue, organ, organism, and environment at a given time. Using the power and high throughput of analytical platforms, such as mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy, metabolomics promises to be a cornerstone in innovations and discoveries in the areas of medicine, agriculture, biomedical, space, and environment. Thus, science, technology, engineering, and mathematics (STEM) graduates and doctorates who interface with analytical chemistry need to prepare for the challenges of academia or industry as metabolomics research takes a centre stage in this big data era.

A recent market research study published by Crystal Market Research reveals that the metabolomics market is projected to be around $2.5 billion by 2025 (1). Early career researchers (ECRs), loosely defined as those who obtained their PhDs within the last 10 years (at times including PhD students, postdocs, and assistant professors), hold the key to future success in this area of research, and to drive the innovations in the present day. They form an important human resource who have the potential to contribute to the advances in technological innovations. With open science (OS), increased citations, media coverage, a rigorous and transparent peer-review process, better archiving options, low or no cost options, data sharing (2), and open access (OA) (3) models of science and research becoming more popular, there is even more research touching even more lives. I point to some resources below that help in career development to enable a metabolomics or "omics" researcher to realize the avenues available to allow them to perform impactful research as a young investigator in academia or industry.

Resources in Career Development

Several societies, agencies, and publishers, for instance publishers like *Public Library of Science (PLOS)*, *eLife*, and *Accelerating Science and Publication in Biology (ASAPbio)*, promote ECR activities through blogs, travel awards, and building resource centres. Several on-line ECR award funding and resources are made available for ECRs themselves (3). More importantly, applying for these awards are more important than not getting the experience and one does not get an award without applying for one! The skill sets needed to explain science that does not need content expertise to capture a big picture implication of a "metabolomics" study outside of academia can be very demanding. No matter what the scope of the impact is, preparation to excel in one's own field of research is important, and to this end, a diverse array of on-line resources is available to help researchers to develop skill sets, such as communication and leadership skills.

On-Line and Off-Line Courses: A command of data analysis and visualization warrants skill sets in programming and coding, which encompass learning several computer languages. Massive open on-line courses (free on-line courses) offered by the top universities and colleges in a wide range of subjects from programming in R to learning Python and MATLAB on-line are available and are less time-intensive, self-paced, and helpful in crafting a career skill. The most popular ones are: Stanford Online (5), Coursera (6), Khan Academy (7), Data Camp (8), and Lynda.com (9) among others. The above-mentioned resources allow one to learn the "ABCs"—the basics of languages—to even advanced training such as tool or software development. More specifically, courses on deep learning, machine learning, neural networks, data science, big data, bioinformatics, data visualization, statistics with R, Python, and Java are deemed useful for data analysts.

The National Institutes of Health (NIH)'s Metabolomics Common Fund programme (https://commonfund.nih.gov/metabolomics/training) lists and points to an array of resources for metabolomics training. For more focused hands-on training in analytical techniques directed towards metabolomics research, excellent courses are available from West Coast Metabolomics Center (WCMC) and UC Davis on both the data and instrument side, such as those listed here, http://metabolomics.ucdavis.edu/courses-and-seminars/courses. The Metabolomics Workbench provides a lot of materials, resources, and links to metabolomics training through their portal: http://www.metabolomicsworkbench.org/training/online.php. One prominent course is Metabolomics in Medicine (http://metabolomicsinmedicine.org/) supported by the NIH Common Fund Metabolomics Award developed by Martin Kohlmeier at the University of North Carolina, USA. The Southeast Center for Integrated Metabolomics (SECIM) at the University of Florida, Gainesville, USA, (https://ctsi-secim.sites.medinfo.ufl.edu/) lists a host of informative videos, tutorials, and lectures as well.

Metabolomics-Specific Self-Taught Resources: For metabolomics-specific training in resources such as those mirroring advancements in analytical and theoretical methods, on-line resources such as metabolomic tools catalogues are available: OMICtools (10,11), Fiehn Lab resources (12), metabolomics society's resource pages (12), metabomatch catalog (13), and software repositories such as Comprehensive R Archive Network (CRAN) (14), Bioconductor (15), and GitHub (16). In addition, several recent efforts have listed all tools, resources, software, and databases that have been introduced in 2015–2017 (17,18). Using the above resources one can venture into the ever-expanding universe of software, databases, resources, and tools to download, go through the manual, install, and play along for their utility in metabolomic dataset analysis—either for real world data or the trial data that come with the tools.

Metabolomics Courses: There are several courses and workshops available across the world at many institutions and universities and those known as centres for excellence in metabolomics research, training, and education.

Figure 1: ECRS in metabolomics.

For instance, the Birmingham University's Metabolomics Data Processing and Data Analysis is a free on-line course organized by the Birmingham Metabolomics Training Center (19) that is very popular and the only registered MOOC in metabolomics (20) to date. Similarly, the European Bioinformatics Institute (EBI), European Molecular Biology Laboratory (EMBL) organizes several courses in metabolomics (21) all year round, as does Metabolomics Workflows (22) and PhenoMeNal's accessing metabolomics workflows in Galaxy (23). In the USA, courses are organized by the University of Alabama (UAB) (24) and in Canada by the Wishart group at The Metabolomics Innovation Centre (TMIC) (25). Some of these involve bring-your-own-data (BYOD) sessions as well, thus allowing the participants to bring their data and address some of the challenges and workflows on-site. In Europe for instance, the European Metabolomics Training Coordination Group (EmTraG) (http://www.emtrag.eu/) has been launched to implement a metabolomics training strategy and it collaborates closely with ELIXIR (and the ELIXIR Training Platform) at a European level and the Metabolomics Society at an international level.

Tools, Software, and Databases: For increased impact, sharing of the metabolomics datasets is vital to allow peers to gain confidence in the datasets generated by researchers for meta-analysis among others. These public archives are growing and are developed keeping in mind the metabolomics community; they are available at UK-based MetaboLights (26) and USA-based MetabolomicsWorkbench (27), the two most prominent resources for archiving. In addition, open access MassBank (28) and PRIMe (Platform for RIKEN Metabolomics), RIKEN, Japan (29) are other resources where data retrieval is possible. Sharing of large datasets at the DRYAD (30,31) and FigShare (32,33) has provided free resources for obtaining large-scale metabolomics datasets. More recently, the Global Natural Products Social Molecular Networking (34), an open-access knowledge base for community-wide organization and sharing of raw, processed, or identified MS/MS-based metabolomics data (35) hosted at University of California San Diego (UCSD) Center for Computational Mass Spectrometry (CCMS) (36), has gained immense popularity. In addition, for mass spectrometry imaging data, MetaSpace (37,38) is a go-to database. Moreover, The Konstanz Information Miner (KNIME) (39, 40) -based workflows that incorporate steps from chromatographic pre-processing to statistical analysis and visualization of "omics"-scale data are starting to be incorporated into metabolomics pipelines. During the analysis of data, GitHub also welcomes fruitful discussions and helps reproducibility efforts across the globe.

Beyond innovative, impactful, and cutting-edge scientific accomplishments, to build that near perfect curriculum vitae (CV), outstanding scientists in the early stages of their careers must apply for a lot of competitive awards and fellowships dedicated to ECRs by many scientific societies and organizations. Moreover, opportunities for mentoring an undergraduate or a graduate student, judging a school science fair, or the opportunity to speak at a local school should all be accommodated into one's busy schedule to be able to add that extra edge to the CV. This serves multiple purposes: (i) polishing the science communication and public engagement skills, (ii) taking the science out of the confines of the laboratory walls to the masses, and (iii) honing the mentoring and oratory skills that would be necessary while writing manuscripts and presenting in meetings, symposia, workshops, and conferences. Readers are suggested to consult Blank *et al.*, who stress the need for modernizing the PhD and postdoc training across more institutions and more disciplines (41).

A lot of popular resources in metabolomics can be easily tracked on social media platforms such as Twitter with hashtags #metabolomics in addition to those made available to ECRs using #ecr, #ecrchat, #phdchat, and #acwri among others. An on-line presence through blogs and social media such as Twitter, Facebook, and LinkedIn allow networking with peers, potential hirers, and future colleagues. For instance, the International Metabolomics Society hosted forum (http://www.metabolomics-forum.com/) allows a platform for exchange of ideas, troubleshooting, and resolving issues ranging from analytical and data science challenges in metabolomics to career development and growth.

Activities from metabolomics societies from specific countries provide resources to

get ECRs trained and involved, for example, the Scottish Metabolomics Network (http://scottishmetabolomics.net/), Australia New Zealand Metabolomics Network (www.anzmn.org), RFMF (Réseau Francophone de Métabolomique et Fluxomique) (http://www.rfmf.fr/), Korea Metabolomics Society (KoMetS) (www.komets.or.kr), and Swiss Metabolomics Society (http://www.swiss-metabolomics.ch/) among others.

MetaboNEWS serves as the sole platform to showcase the ongoing activities of the early career member network (EMN) committee of the International Metabolomics Society, such as organization of monthly webinars and workshops in the annual metabolomics conference among others. This year's annual gala metabolomics event, the 14th International Conference of the Metabolomics Society, is scheduled to be held in Seattle, Washington, USA, 24–28 June 2018 http://metabolomics2018.org/. Attending these meetings are immensely useful for ECRs to showcase their work to the attendees in the society, as well as networking for future collaborations and job opportunities.

Conclusions

By choosing a research and career path in metabolomics, the opportunities as an ECR abound, but they need to be grabbed in a timely fashion with polished and ready skill sets, supportive mentorship, and readiness to hop onto opportunities. Moreover, the research efforts of ECRs are what is going to shape the future innovations and applications of mass spectrometry and spectroscopy-based metabolomics research efforts.

Conflicts of Interest

The author declares no conflicts of interest.

References

1. Metabolomics Market by Technique and Application - Global Industry Analysis and Forecast to 2025: http://www.crystalmarketresearch.com/press-releases-details/global-metabolomics-market Accessed on 20 November 2017
2. E.C. McKiernan, P.E. Bourne, C.T. Brown, S. Buck, A. Kenall, J. Lin, D. McDougall, B.A. Nosek, K. Ram, C.K. Soderberg, and J.R. Spies, *Elife* **5**, 16800 (2016).
3. B.C. Björk and D. Solomon, *BMC medicine* **10**(1), 73 (2012).
4. ECR Funding sources: https://docs.google.com/spreadsheets/d/1H1aj--VUYr7eMFk_T7x0Oh985LqbyyscXg2wAAevDnU/edit#gid=0 Accessed on 20 November 2017
5. Stanford Online: thttp://online.stanford.edu/ Accessed on 20 November 2017
6. Coursera: https://www.coursera.org/ Accessed on 20 November 2017
7. Khan Academy: https://www.khanacademy.org/ Accessed on 20 November 2017

8. DataCamp: https://www.datacamp.com/ Accessed on 20 November 2017
9. Lynda.com: https://www.lynda.com/ Accessed on 20 November 2017
10. V.J. Henry, A.E. Bandrowski, A.S. Pepin, B.J. Gonzalez, and A. Desfeux, *Database (Oxford)* **14**, 2014 (2014).
11. OMICTools: http://omictools.com/ Accessed on 20 November 2017
12. Metabolomics Society, Metabolomic Databases: http://metabolomicssociety.org/resources/metabolomics-databases Accessed on 20 November 2017
13. Metabomatch: http://www.metabomatch.com/softwares/ Accessed on 20 November 2017
14. CRAN: https://cran.r-project.org/web/packages/available_packages_by_name.html Accessed on 20 November 2017
15. Bioconductor: https://www.bioconductor.org/ Accessed on 20 November 2017
16. GitHub: https://github.com/ Accessed on 20 November 2017
17. B.B. Misra and J.J. der Hooft, *Electrophoresis* **37**(1), 86–110 (2016).
18. B.B. Misra, J.F. Fahrmann, and D. Grapov, *Electrophoresis* **38**(18) 2257–2274 (2017).
19. Metabolomics Data Processing and Data Analysis: http://www.birmingham.ac.uk/facilities/metabolomics-training-centre/courses/Metabolomics-Data-Processing-and-Data-Analysis.aspx Accessed on 20 November 2017
20. Metabolomics: Understanding Metabolism in the 21st Century (FutureLearn): https://www.mooc-list.com/tags/metabolomics Accessed on 20 November 2017
21. EMBO Practical Course on Metabolomics Bioinformatics for Life Scientists: http://www.ebi.ac.uk/training/events/2018/embo-practical-course-metabolomics-bioinformatics-life-scientists-4 Accessed on 20 November 2017
22. Metabolomics Workflows: https://www.ebi.ac.uk/training/events/2017/metabolomics-workflows Accessed on 20 November 2017
23. PhenoMeNal: accessing metabolomics workflows in Galaxy: http://www.ebi.ac.uk/training/online/course/phenomenal-accessing-metabolomics-workflows-galaxy?platform=hootsuite Accessed on 20 November 2017
24. UAB Metabolomics: http://www.uab.edu/proteomics/metabolomics/workshop.php
25. Informatics and Statistics for Metabolomics (2017): https://bioinformatics.ca/workshops/2017/informatics-and-statistics-metabolomics-2017 Accessed on 20 November 2017
26. N.S. Kale, K. Haug, P. Conesa, K. Jayseelan, P. Moreno, P. Rocca-Serra, V.C. Nainala, R.A. Spicer, M. Williams, X. Li, and R.M. Salek, *Current Protocols in Bioinformatics* **14–13** (2016).
27. M. Sud, E. Fahy, D. Cotter, K. Azam, I. Vadivelu, C. Burant, A. Edison, O. Fiehn, R. Higashi, K.S. Nair, and S. Sumner, *Nucleic acids research* **1042** (2015).
28. MassBank, http://www.massbank.jp/?lang=en Accessed on 20 November 2017
29. PRIMe: http://prime.psc.riken.jp/ Accessed on 20 November 2017
30. H. White, S. Carrier, A. Thompson, J. Greenberg, and R. Scherle, In *Dublin Core Conference* 157–162 (2008).
31. Dryad Digital Repository – Dryad: http://datadryad.org/ Accessed on 20 November 2017
32. M. Thelwall and K. Kousha, *Online Information Review* **40**(3), 333–346 (2016).
33. Figshare: https://figshare.com/ Accessed on 20 November 2017
34. GNPS: http://gnps.ucsd.edu Accessed on 20 November 2017
35. MassIVE: https://gnps.ucsd.edu/ProteoSAFe/index.jsp Accessed on 20 November 2017
36. M. Wang, J.J. Carver, V.V. Phelan, L.M. Sanchez, N. Garg, Y. Peng, D.D. Nguyen, J. Watrous, C.A. Kapono, T. Luzzatto-Knaan, and C. Porto, *Nature Biotechnology* **34**, 828–837 (2016).
37. Metaspace2020: http://annotate.metaspace2020.eu/#/datasets Accessed on 20 November 2017
38. A. Palmer, P. Phapale, I. Chernyavsky, R. Lavigne, D. Fay, A. Tarasov, V. Kovalev, J. Fuchser, S. Nikolenko, C. Pineau, and M. Becker, *Nature Methods* **14**(1), 57–60 (2016).
39. KNIME: https://www.knime.com/ Accessed on 20 November 2017
40. M.R. Berthold, N. Cebron, F. Dill, T.R. Gabriel, T. Kötter, T. Meinl, P. Ohl, K. Thiel, and B. Wiswedel, *AcM SIGKDD explorations Newsletter* **11**(1), 26–31 (2009).
41. R. Blank, R.J. Daniels, G. Gilliland, A. Gutmann, S. Hawgood, F.A. Hrabowski, M.E. Pollack, V. Price, L.R. Reif, and M.S. Schlissel, *Science* **358**(6369), 1388–1389 (2017).

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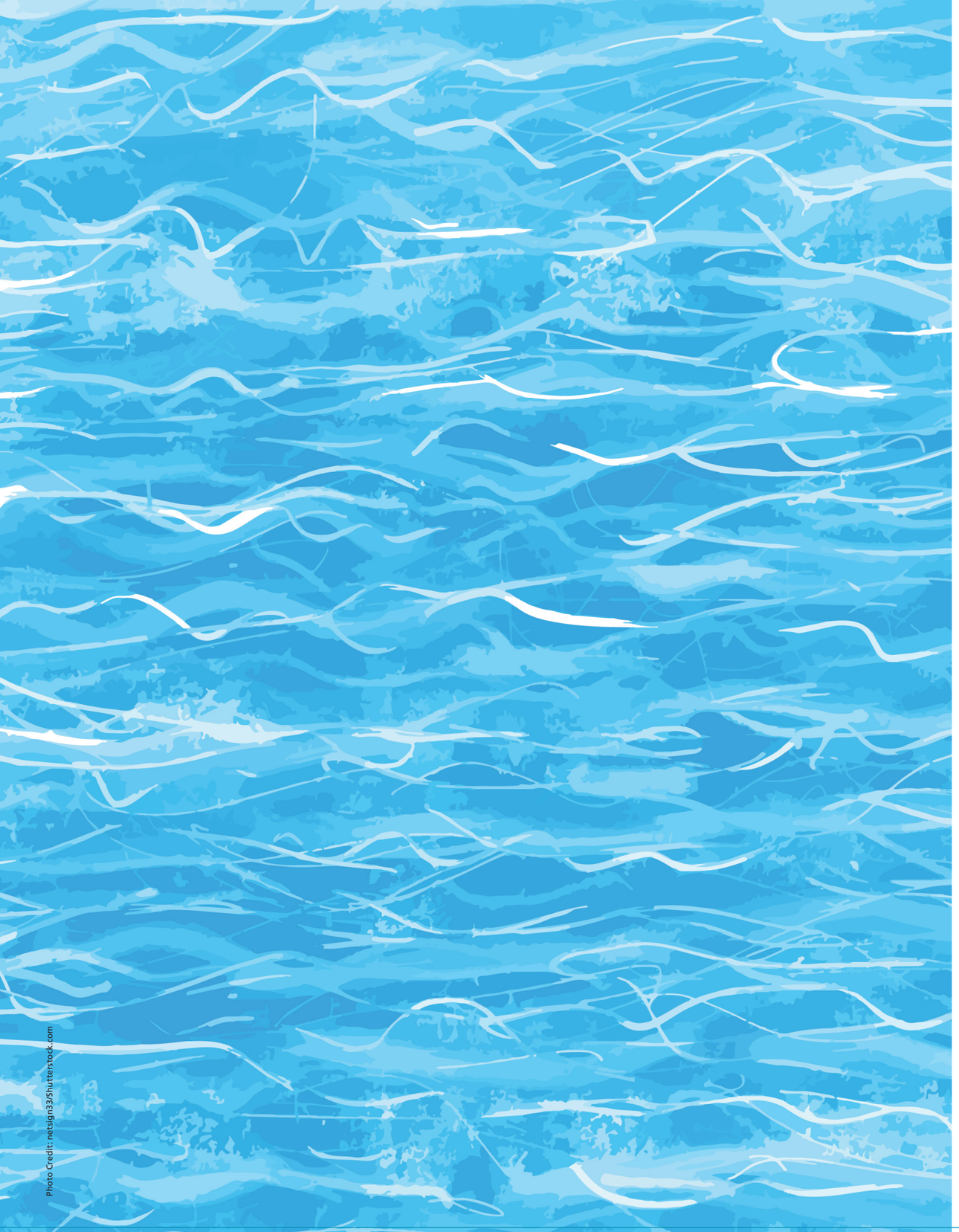

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Injecting Water onto a GC Column: Solving the Mystery of Poor Chromatography

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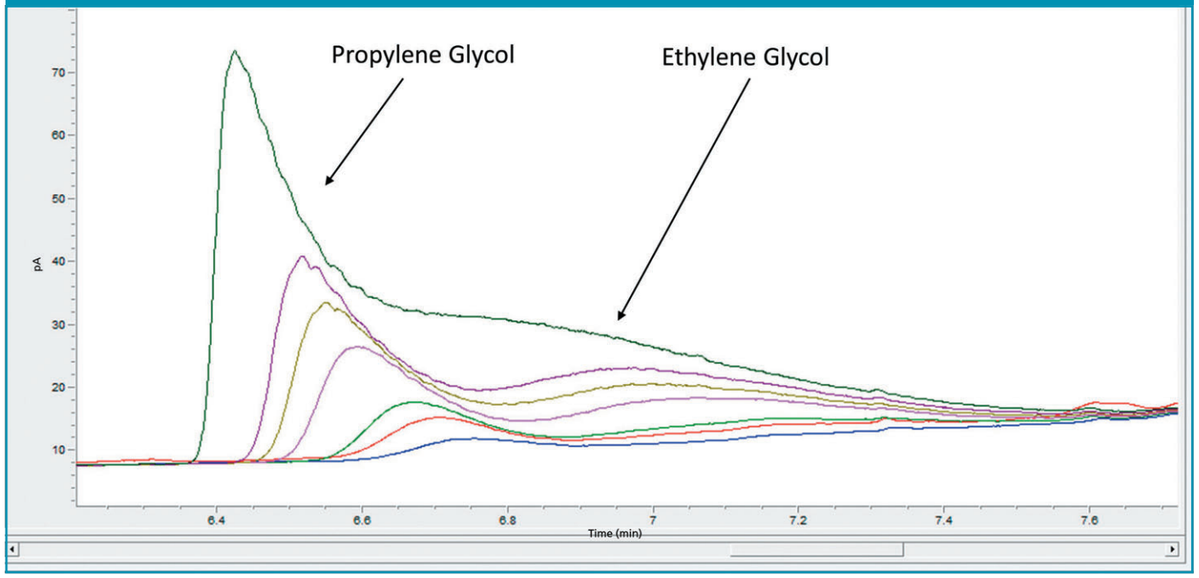
Ethylene glycol is a particularly difficult compound to analyze because it is not easily extracted from water. Many environmental samples originate from water runoff at airports, where ethylene glycol is used as a de-icing agent for airplanes during winter months. Hydraulic fracturing is a technique where pressurized fluid and sand or other solids (proppant) are used in gas drilling to allow gas extraction. Glycols are a common ingredient in most hydraulic fracturing fluid and play a key role in preventing emulsifications and stabilizing the solutions. The direct aqueous injection of ethylene glycol is challenging because it can be difficult to attain reproducibility and good peak shape. The large expansion volume of water can cause backflash, carryover can cause inconsistent results, and excess water can extinguish the flame ionization detection (FID) flame. This article describes a robust approach to analyze glycols in aqueous samples, which reduces downtime and maintains sensitivity.

Many analysts have struggled with aqueous injections; especially to meet the required detection limits. Polar analytes are particularly challenging because alternative methods of analysis require derivatization or complex sample extraction procedures that may introduce a new set of challenges. One of the most notorious pairs of compounds to analyze are propylene (PG) and ethylene glycol (EG). Exposure to glycols can occur

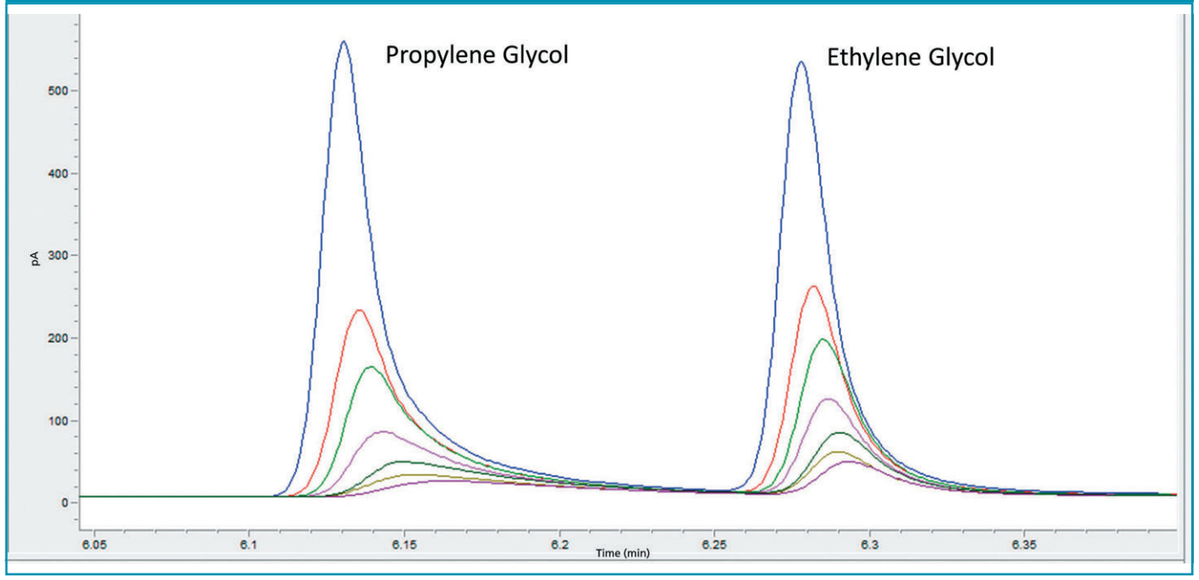
Figure 1: Seven overlaid chromatograms of propylene and ethylene glycol at 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 100 ppm in water by splitless injection, following 80 water samples using a polyethylene glycol (PEG) stationary phase. Samples and standards were made with 100% water.



through environmental contamination of water with automobile antifreeze, aircraft de-icing liquids, and hydraulic fracturing fluid. These compounds have a significant difference: just 2–4 ounces of ethylene glycol can be fatal if ingested, whereas propylene glycol is commonly used in foods. The toxicity of ethylene glycol and potential for exposure from a wide range of sources makes reliable low-level quantification critical.

When I worked in an environmental laboratory, I struggled with tailing peaks, retention time shifts, and nonlinear calibration curves. Peak tailing was so severe that manual integration was necessary. After repeated injections, propylene and ethylene glycol would merge together making identification impossible. Maintenance was sometimes performed after only ten samples and required changing the liner and cutting and baking the column. The method called for a 1-µL splitless injection of standards and samples in water, which were introduced into a 4.0-mm internal diameter (i.d.) single gooseneck liner without wool. This approach assumes most of the sample will be transferred onto a

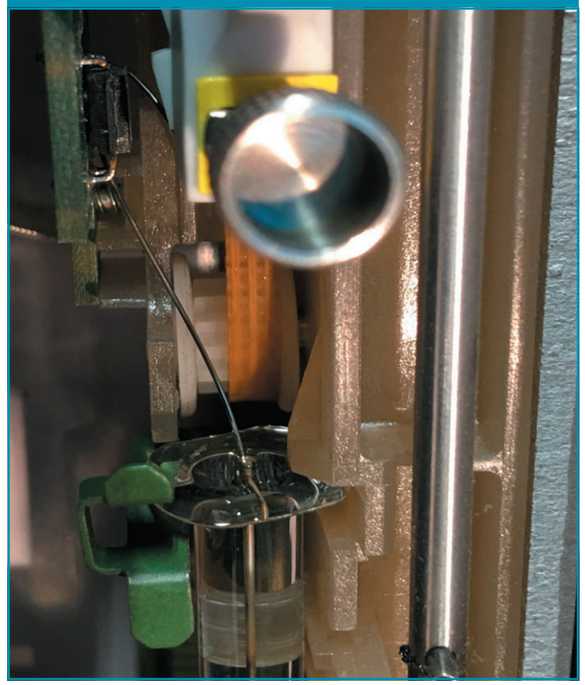
Figure 2: Seven overlaid chromatograms of propylene and ethylene glycol at 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 100 ppm in water by splitless injection, after two hours at 250 °C using a polyethylene glycol (PEG) stationary phase. Samples and standards were made with 100% water.



30 m × 0.53 mm, 1.0-µm polyethylene glycol (PEG) stationary phase. Using a large internal diameter liner allows for the rapid expansion of water vapour and is meant to reduce flashback; however, under our conditions water expands to 1867 µL in a liner with an effective internal volume of 493 µL. This means that not only are we losing a significant amount of sample, but water is also backflashing into the inlet lines and leaving sample residue throughout the injection port. This contamination can, over time, bleed out resulting in ghost peaks, carryover, and an elevated baseline.

Transferring all of the sample onto the column requires slow flows through the liner while active analytes are exposed to the high temperature of the injection port and compounds have longer contact time with active surfaces. Even stable compounds have wider peak widths compared to split analysis using this slow transfer. In the environmental laboratory, a dual column was used with one injection port and a guard column attached to a press-tight "Y" connector that split to a PEG column and a confirmation column, a trifluoropropyl phase.

Figure 3: Bent syringe plunger from 100% water samples, which causes build-up of residue in the syringe barrel. Adding a cosolvent to the samples, standards, and rinse solvent will prevent syringe damage.

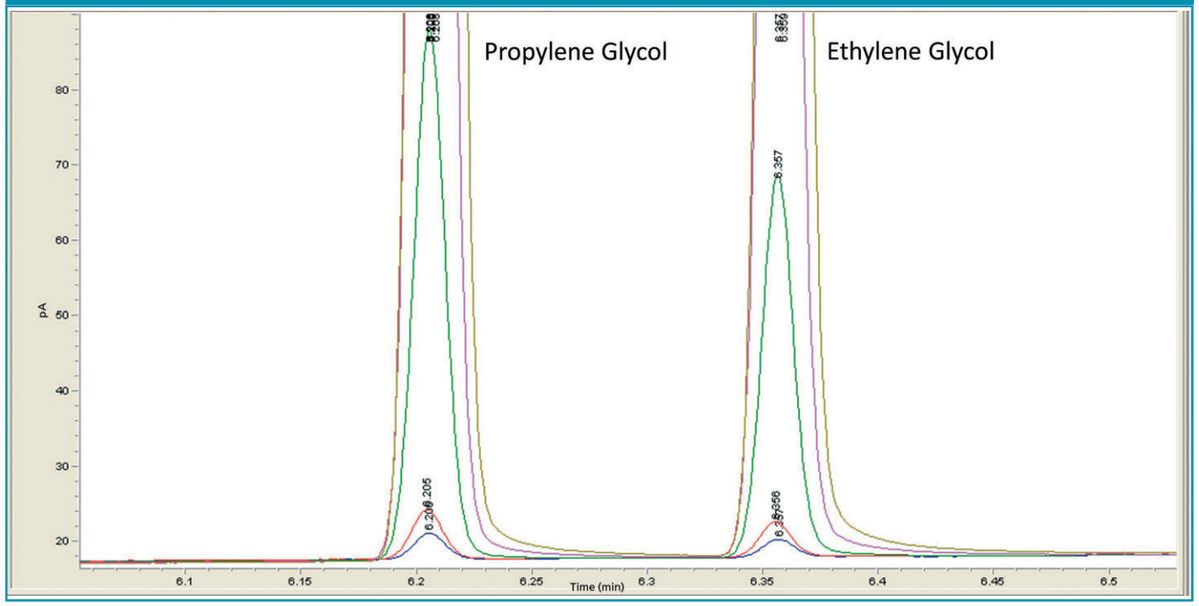


This second phase is considered nonpolar relative to a PEG column and did not perform as well with water. Water does not properly focus on this phase and forms droplets at the head of the column, which can result in split peaks, shifting retention times, and in extreme examples may extinguish the flame ionization detector (FID). When everything was running smoothly our reporting limit was 10 ng on-column and subsequent work using a direct injection (uniliner) technique cut the reporting limit in half.

We revisited this analysis with the goal of reproducing some of the chromatographic struggles of my past and developing a new approach that will extend column lifetime, reduce maintenance, and achieve lower detection limits. Returning to splitless injection, we followed the same conditions as above. Initial chromatography using a PEG phase resulted in acceptable peak shape initially, but following 80 water injections in splitless mode produced those familiar broad peaks that over time move together into an indiscernible hump (Figure 1).

Our theory is that the hydrophilic nature of the PEG column and hydrogen bonding of the phase with water allows the column to retain moisture even at temperatures of 250 °C. The oven conditions used a final oven hold time of 250 °C for 2 min. After conditioning the column for two hours at 250 °C the peak shapes and retention times were restored to original performance (Figure 2). We were able to run 100% water samples and degrade the chromatography again over the course of 20 injections. An additional problem with 100% aqueous samples is the build-up of residue in the barrel of the syringe causing the syringe plunger to either become bent during injection or stuck causing an autosampler error (Figure 3). Another interesting trick

Figure 4: Five overlaid chromatograms of propylene and ethylene glycol at 0.5 ng, 1 ng, 10 ng, 50 ng, and 100 ng on-column concentration in 10:90 methanol–water by split injection, using a polyethylene glycol (PEG) stationary phase and a liner with wool.



other laboratories have used to maintain performance is to add methanol because it can assist in removing water from the column and can prevent autosampler syringe problems. In our next experiment we used 10:90 methanol–water to standards and water samples without baking out the column and maintained the gas chromatography (GC) program with a 2-min hold at 250 °C. Again, the column performance was restored with performance similar to Figure 2.

My colleague, Corby Hilliard, set out to develop a comprehensive in-house method development study by evaluating a variety of conditions that would result in reliable symmetrical peaks and stable retention times (1). A lifetime study was conducted on three PEG columns to determine if the method would hold up to repeated water injections and maintain chromatographic performance. The experimental design consisted of ten injections of 100% water (1 µL splitless) followed by an injection of a 50 µg/mL glycol standard (50:1 split injection, 1 ng on-column). The injections were made in splitless mode to increase column exposure to water, creating a more severe test; the standard injection was made in split mode.

Peak symmetry was evaluated after every ten water injections and the procedure was repeated until the three columns had reached 600 water injections. The most notable departure from our previous work was the use of a high split ratio. It may seem counter-intuitive to vent 98% of the sample and expect better sensitivity, however, the study showed that a 1 µL 50:1 split injection into a liner containing wool eliminated injection port effects, decreased backflash, increased reproducibility, and produced better sensitivity. Using a high split ratio also allows the use of more nonpolar stationary phases. A trifluoropropyl phase column can be used as a confirmation column in split mode because the amount of water on-column is 1/50th of a µL. Since flows through the liner are 50 times higher than a splitless injection, the use of wool in a 4.0-mm-i.d. liner was critical for good reproducibility because the wool allows the sample to vaporize rapidly and completely. The standards were analyzed using split injection across a calibration range that went from 0.5-ng on-column to 100-ng on-column (Figure 4). While carryover is closely associated with splitless injections and the injection port in general, it can also be caused by sample residue in the syringe being transferred from one injection to another. If the syringe is not properly cleaned between analyses or if 100% water is used as the rinse solvent carryover will cause inconsistent results and may permanently damage the syringe plunger (Figure 3). Rinsing the syringe with 50:50 methanol–water three to six times between each injection will eliminate most sample residue and minimize the possibility of carryover. Preparing standards and samples in 10% methanol is recommended for routine analysis because it helps prevent the syringe needle from seizing up and more importantly reduces build-up of water on the PEG phase.

Conclusions

Analyzing highly polar analytes in water samples using split injection provides several advantages over the typical splitless approach: it reduces the risk of backflash, allows for better sensitivity, and also prevents problems with peak tailing and shifting retention times. By using this split injection method with a PEG column, very consistent chromatographic performance was obtained, even following the injection of 600 water samples. Peak symmetry measured after every 100 water samples averaged 0.95 for propylene glycol and 0.99 for ethylene glycol with percent relative standard deviations of 1.2% and 0.7%, respectively. A calibration curve with 0.5 ng, 1.0 ng, 10 ng, 50 ng, and 100 ng on-column had correlation coefficients of 0.9999 for both glycols. Split injection on PEG delivers fast, consistent sample transfer with less water on-column. The method produces symmetrical peaks that elute at stable retention times, which improves reproducibility and sensitivity for low-level aqueous samples.

Reference

1. C. Hilliard and C. English, Reliable Low-Level Analysis of Glycols in Water Using Split Injection, Application Note EVAN2873-UNV (2018).

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Enhancing the Sensitivity of Atmospheric Pressure Ionization Mass Spectrometry Using Flow Modulated Gas Chromatography

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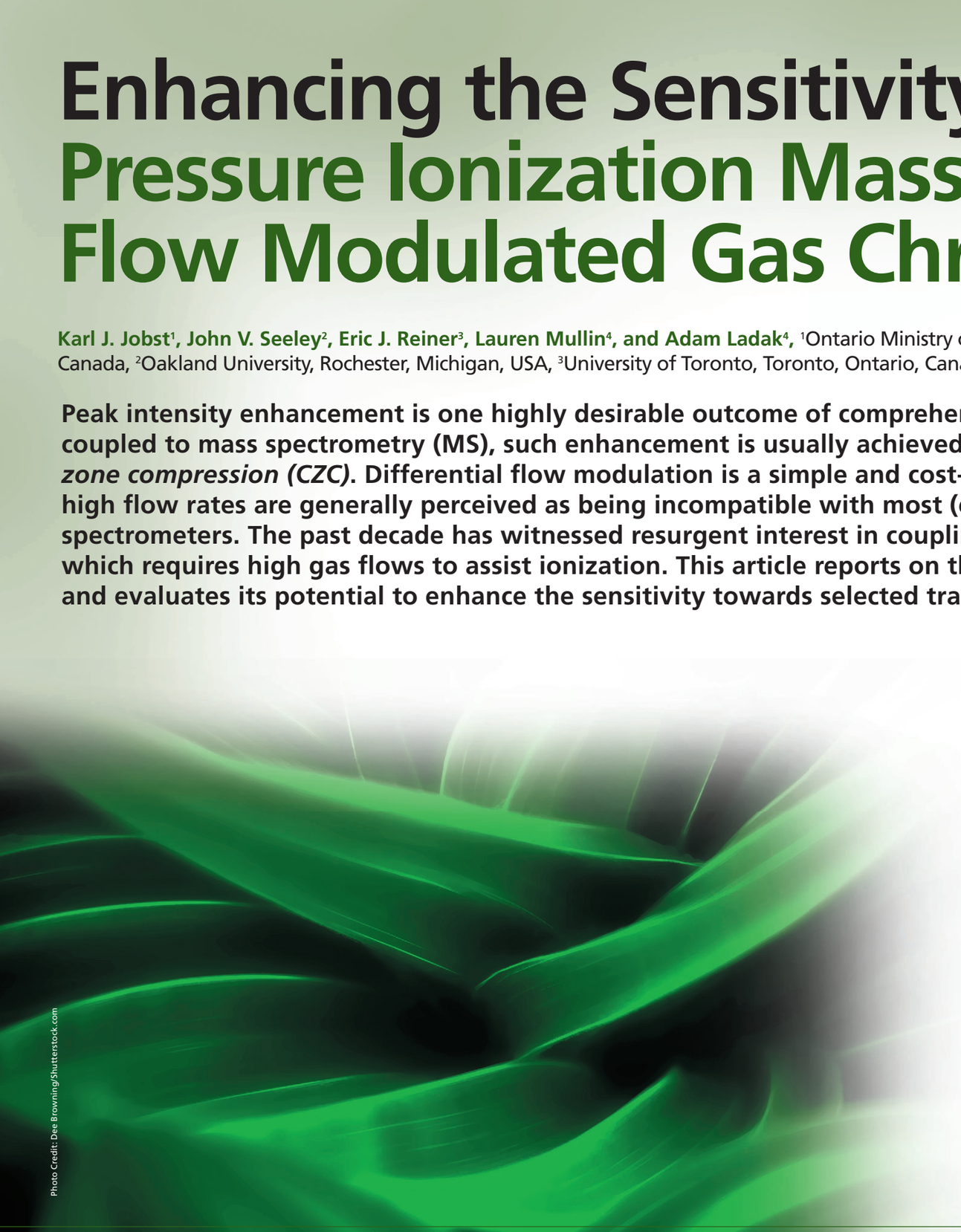
Peak intensity enhancement is one highly desirable outcome of comprehensive two-dimensional gas chromatography (GC×GC). When coupled to mass spectrometry (MS), such enhancement is usually achieved with a thermal modulator using a technique called *cryogenic zone compression (CZC)*. Differential flow modulation is a simple and cost-effective alternative to thermal modulation, but the requisite high flow rates are generally perceived as being incompatible with most (electron ionization [EI] and chemical ionization [CI]) mass spectrometers. The past decade has witnessed resurgent interest in coupling GC to atmospheric pressure chemical ionization (APCI), which requires high gas flows to assist ionization. This article reports on the modification of a GC–APCI system with a flow modulator and evaluates its potential to enhance the sensitivity towards selected trace organics.



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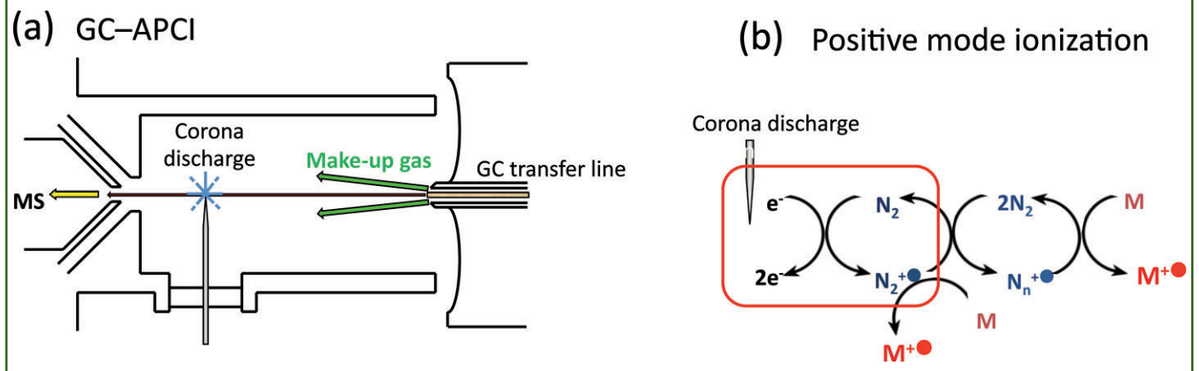
The exposome represents all environmental exposures over the course of a lifetime (1). The identities of most environmental contaminants and their roles in causing chronic diseases are unknown. This is partly a result of the analytical challenges associated with biomonitoring trace levels of environmental toxicants in small sample quantities of serum or tissue. It is estimated that standard gas chromatography–mass spectrometry (GC–MS) and liquid chromatography (LC)–MS platforms are not sufficiently sensitive to identify approximately 90% of unknown contaminants (2).

Inspired by advances in comprehensive two-dimensional gas chromatography (GC×GC) (3), Patterson and co-workers elegantly showed that (targeted) sub-femtogram detection of environmental pollutants is achievable using a technique termed *cryogenic zone compression (CZC)* (4). With CZC, the 3–8 s width of a GC peak is compressed to approximately 200 ms, resulting in a significant enhancement in signal intensity (4–6). This has opened the door to the possibility of monitoring environmental toxicants using less invasive and inexpensive sampling techniques, such as collection of dried blood spots (7). The cost of requisite hardware however, *viz.* a thermal modulator, and associated cryogens and refrigeration is a drawback.

Signal enhancement is also possible using a valve-based flow modulator, a technology

Figure 1: (a) The APGC (atmospheric pressure gas chromatography) ion source; (b) general scheme for positive mode ionization in dry ion source.



pioneered by J.V. Seeley (Oakland University, Michigan, USA) (8,9). In contrast to thermal modulation, a flow modulator requires a high carrier gas flow (>10 mL/min) to transfer the column effluent from the modulator into the mass spectrometer. The potential of flow modulation has not been fully realized because of the perception that high column flows are not compatible with most (electron ionization [EI] and chemical ionization [CI]) mass spectrometers. This is usually addressed by effluent splitting at the cost of sensitivity. Improved pumping capacity of modern turbomolecular pumps can mitigate the problem, but still this hyphenation is generally considered difficult (10,11).

GC coupled to atmospheric pressure chemical ionization (APCI) (12–16) is by contrast ideally suited to high flows. In GC–APCI, the GC effluent is swept into the ion source, which is held at atmospheric pressure, by a high flow of nitrogen gas (>100 mL/min). There is also the additional benefit that the elevated pressures promote collisional cooling of the incipient ions. Consequently, very little fragmentation occurs, resulting in further signal enhancement of the molecular ion. With widespread adoption of atmospheric pressure ionization techniques, such as electrospray ionization (ESI) and APCI, there is growing interest in coupling GC–MS using atmospheric pressure ionization. This article reports on proof-of-concept experiments using a simple, cost-effective flow modulator (17) coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS). The results indicate that environmental contaminants can be detected at low

Figure 2: The flow modulator used in this study.

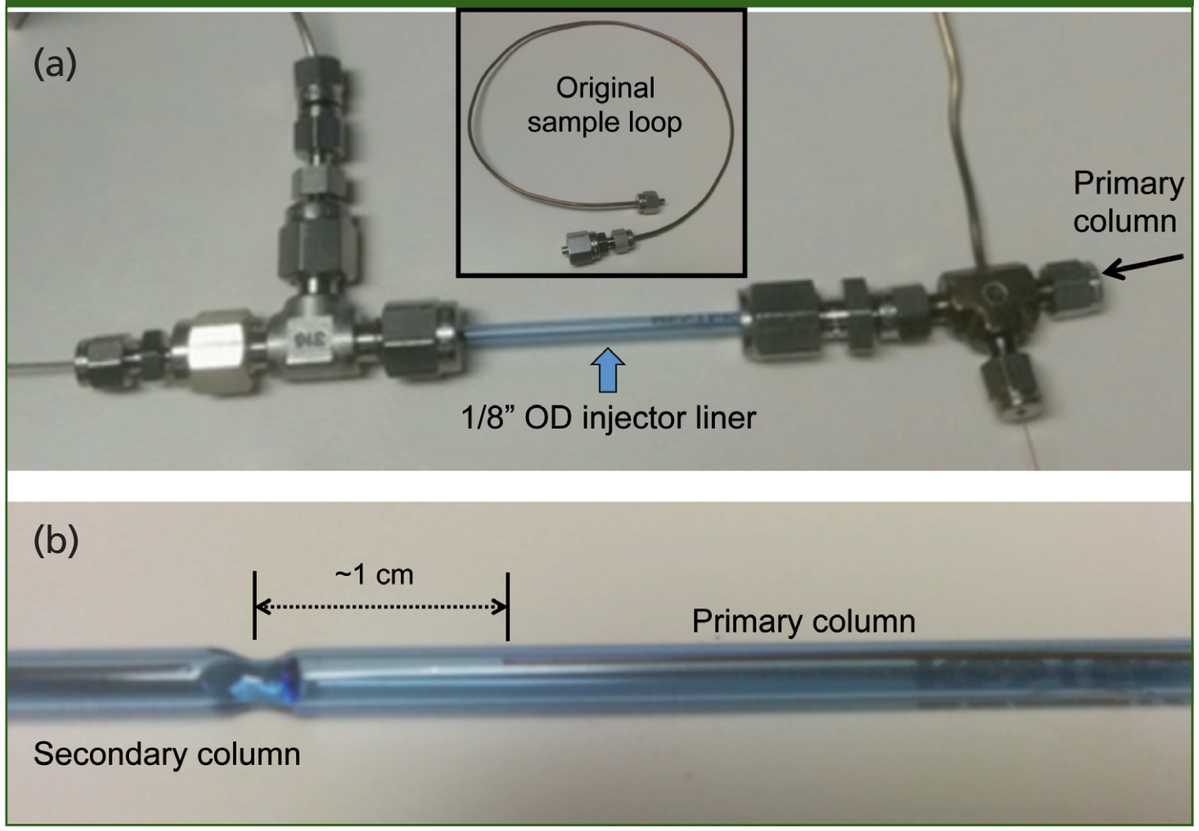
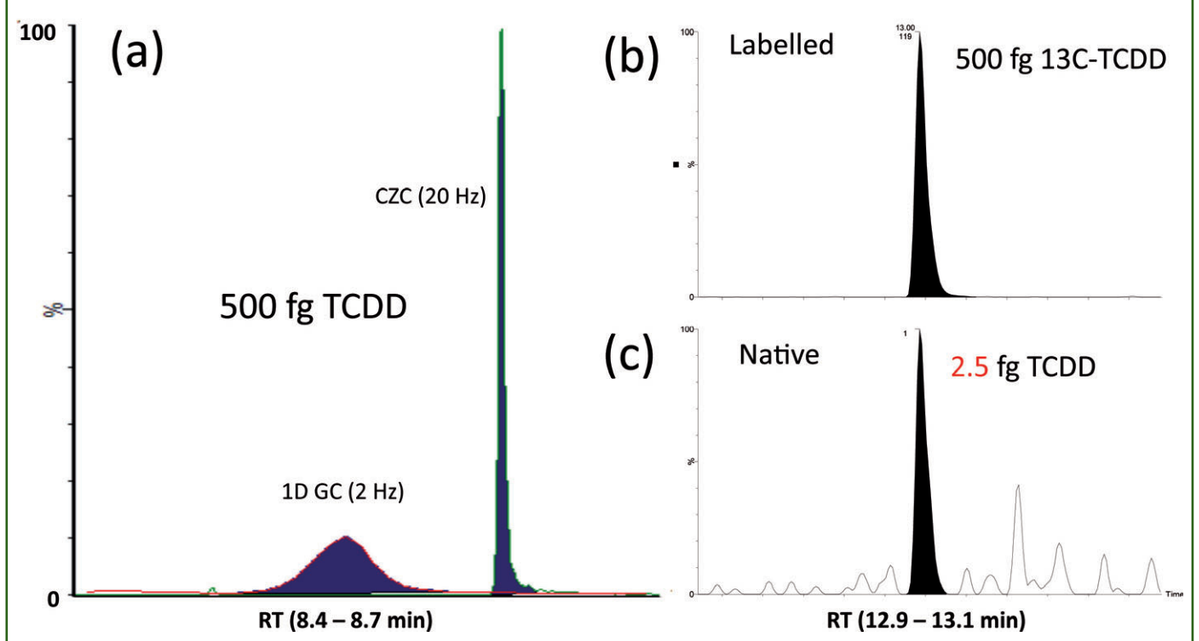


Figure 3: (a) Comparison of pre- and post-modulation of 500 fg of TCDD; (b) CZC of 500 fg of 13C-TCDD and 2.5 fg of TCDD. Experiments were performed using a Zoex ZX2 thermal modulator. Columns: 15 m × 0.25 mm, 0.1-µm db-5 (Agilent) and 0.18 mm × 0.18 µm, 40-m db-5 (Agilent) were employed for experiments (a), and (b) and (c), respectively.



femtogram levels using a full-scanning instrument capable of nontargeted analysis.

Experimental

The experiments were performed using a Xevo G2-XS quadrupole time-of-flight mass spectrometer (Waters) coupled to a modified 7890B gas chromatograph (18,19) (Agilent). In GC–APCI, the GC effluent is swept into the ion source by a 350 mL/min make-up flow (N2). A schematic of GC–APCI is shown in Figure 1(a).

Ions formed by a corona discharge needle set to 3 µA were transferred into the mass spectrometer, aided by 20 V potential and cone and auxiliary gas flows of 175 L/h and 100 L/h, respectively. In the charge transfer mode (Figure 1[b]), ionization was typically initiated by the formation of $N_2^{\bullet+}$ and $N_n^{\bullet+}$ cluster ions, which may undergo charge exchange with analyte molecules (M) provided the ionization energy (IE) of M is lower than the recombination energy (RE) of $N_2^{\bullet+}$. Non-reactive collisions between the incipient ions and the surrounding N_2 results in collisional cooling that significantly reduces fragmentation and therefore also the detection limits for the molecular ions ($M^{\bullet+}$). Other ionization schemes are also possible by introducing dopants to the ion source. For example, H_2O can be used to promote protonation. $O_2^{\bullet-}$ is the primary charge carrier in negative ion mode APCI, which can result in selective ionization and structure diagnostic fragmentation of halogenated compounds (18,20). The technique of ionizing a sample of molecules by gas-phase ion-molecule reactions is well studied (20), and since its inception an enormous number of studies have appeared on its applications in analytical MS.

Figure 4: The home-built flow modulator and its operation in the (a) collection and (b) injection states.

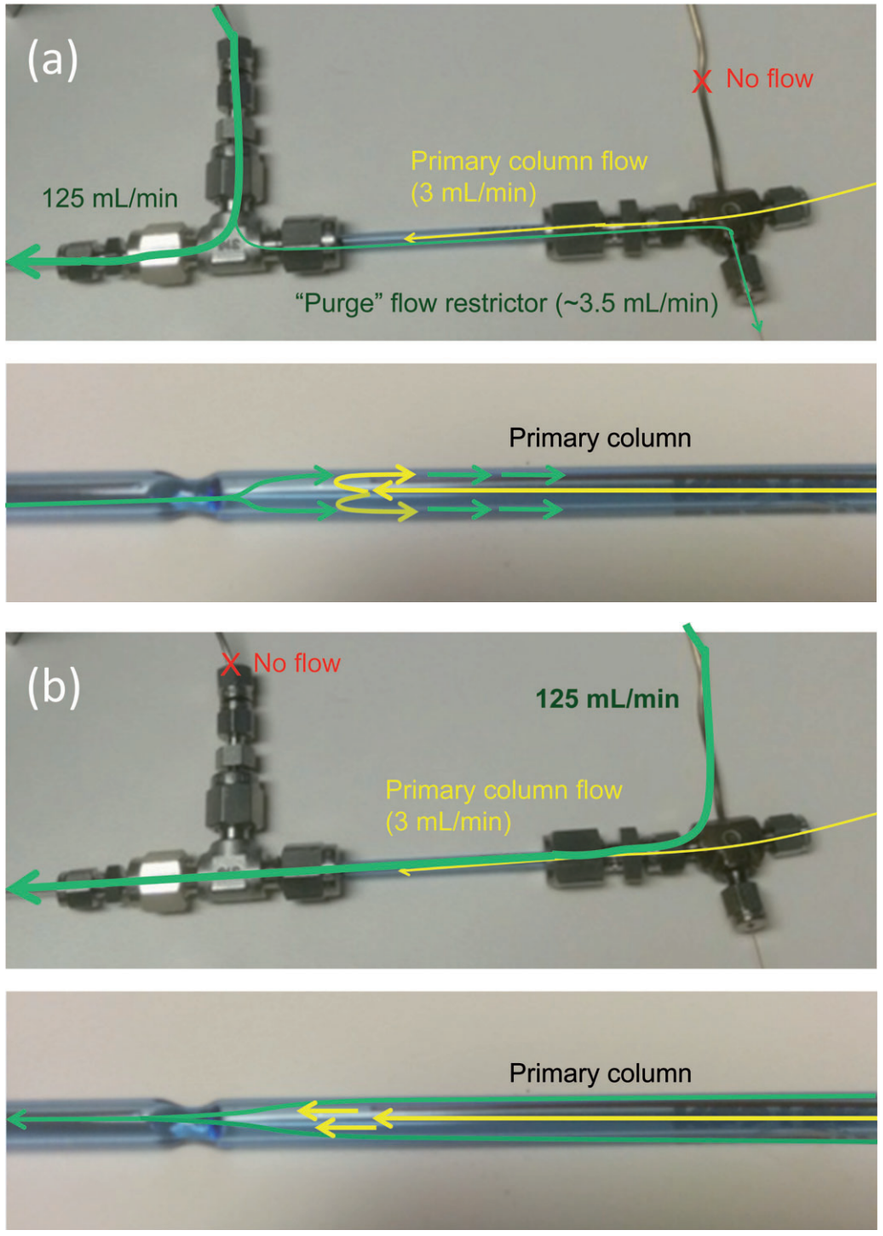
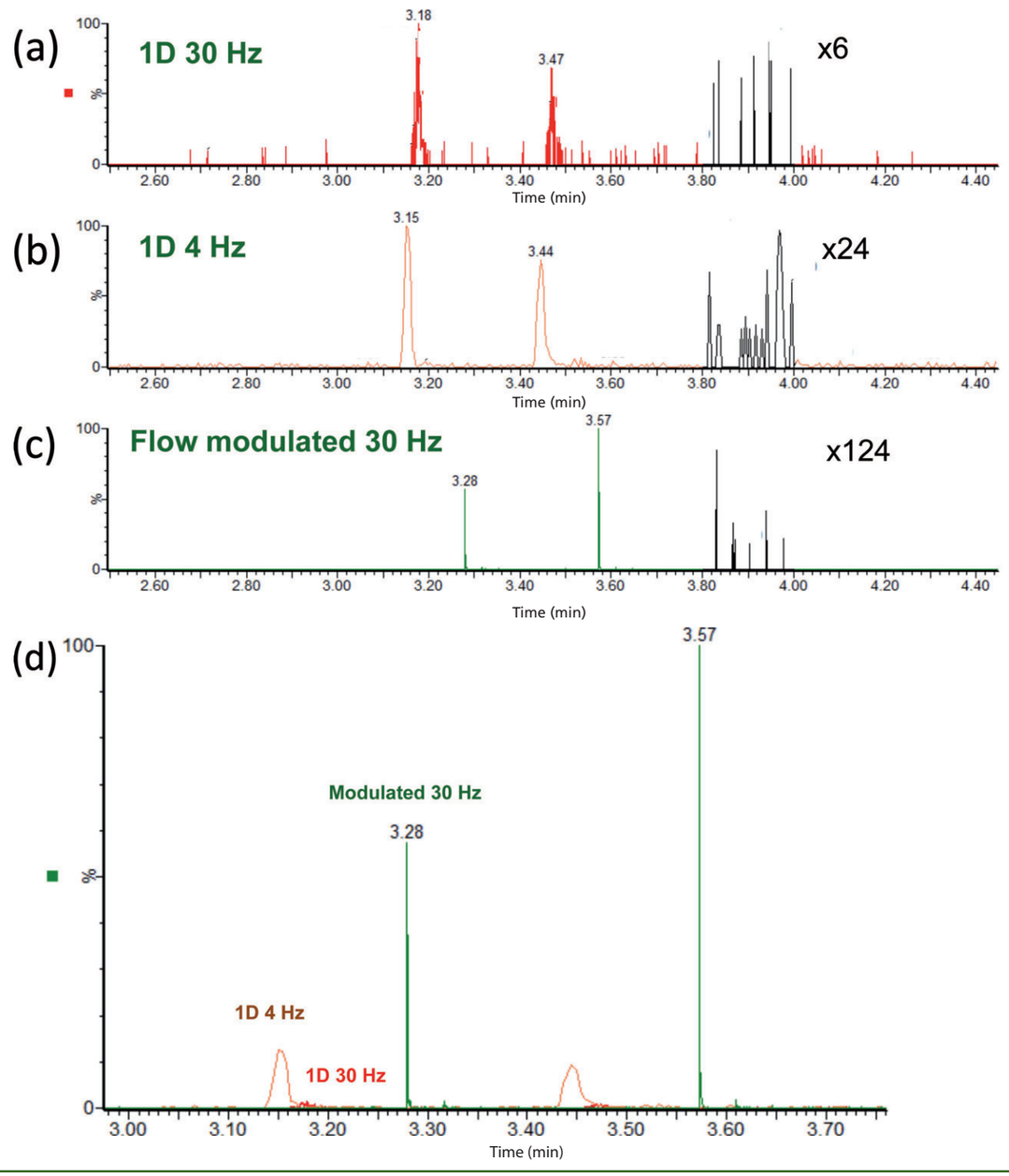


Figure 5: Extracted ion chromatograms of PCB-101 and PCB-118 without modulation (a) 30 Hz and (b) 4 Hz; and (c) with modulation; (d) overlaid chromatograms (a–c).



A ZX2 thermal modulator (Zoex) was used for CZC experiments. A multimode modulator (17) designed and constructed by J.V. Seeley was useds to explore the potential of coupling flow modulation to GC–APCI. The construction, operation, and performance of this device has been described recently (17). Briefly, the multimode modulator (17) of this study was constructed around a 1/8″ outside diameter (o.d.) liner (standard PTV injector liner) (Figure 2).

A piece of tubing or capillary can also be used for primary effluent collection, but the 1/8″ o.d. liner provided the volume required (~200 µL) to accommodate a 4 s modulation period and 3 mL/min carrier gas flow. One end of the joining liner was attached to a cross union, or as shown in Figure 2, a modified tee. The exit of the primary column (15 m × 0.25 mm, 0.1-µm db-5 [Agilent Technologies]) was inserted through the cross and then into the joining liner so that the column exit sat approximately 1 cm from the gooseneck of the liner. The opposite end of the liner was attached to a tee union. A 0.5 m × 0.53 mm Rxi Guard column (Restek) that served as the transfer line to the MS was inserted into the tee and butted against the gooseneck. Auxiliary flow (125 mL/min) was delivered to the tee and cross unions via a two-way, three-port solenoid valve (not shown). A flow restrictor was connected to the cross connection. The operation of the multimode modulator for the purpose of signal enhancement is described below.

Results and Discussion

CZC relies on the same principles of operation as GC×GC, whereby a pulse of cooled nitrogen gas is used to condense and focus GC effluent (primary peak), followed by a pulse of heated gas for re-injection (secondary peak). The timing of the two pulses (modulation period) is configured so that the width of a GC peak is compressed. This is illustrated in Figure 3(a), which displays a ~3 s wide peak prior to modulation and a narrow (250 ms wide) peak post modulation. The 10-fold enhancement in signal intensity (Figure 3[a]) enables low femtogram detection of 2378-TCDD (tetrachlorodibenzo-*p*-dioxin) using QTOF, see Figure 3(c). Note that the identity of the native compound (Figure 3[c]) is confirmed by comparison with the corresponding 13C-labelled compound (Figure 3[b]).

A flow modulator does not condense or focus the GC effluent. Instead, the effluent is trapped in a sample loop of sufficient volume to accommodate the primary flow. The width of the secondary peak can still be controlled by the secondary flow. Figures 4(a) and 4(b) show the flow modulator of this study in the collection and injection states, respectively. In the collection state, effluent

from the primary column (3 mL/min) backfills into the liner as primary carrier gas escapes via the flow restrictor. In the injection state (Figure 2[c]), the secondary flow (125 mL/min) is diverted through the sample loop, flushing the accumulated contents into the mass spectrometer.

From the ratio of the primary and secondary flows, one anticipates a signal enhancement of approximately 40×. As shown in Figure 5(a), the signal-to-noise ratios (S/N) of two polychlorinated biphenyls (101 and 118) are approximately 6:1. When the modulator is activated, this S/N increases by a factor of 20 to 124:1 (see Figure 5[c]). Note that the acquisition time of the mass spectrometer is normally set to acquire ~10 data points across the peak. As shown in Figure 5(b), the S/N of the primary peaks increases to 24:1 when the acquisition rate is adjusted from 30 Hz to 4 Hz. Still, signal enhancement of approximately 10:1 is evident from the overlaid chromatograms in Figure 5(d).

Summary

A flow modulator was constructed and evaluated using QTOF-MS. Like CZC, flow modulation produces sharp peaks (<200 ms wide) with a significant improvement in signal intensity compared to unmodulated peaks. Aside from the obvious implications to quantitative analysis, the enhanced sensitivity also helps address the critical need for nontargeted identification of unknown environmental toxicants. The analytical platform described in this study is capable of acquiring high quality, full scan mass spectra of femtogram level toxicants and as such may be a promising tool in environmental monitoring, biomonitoring, and exposomic research (20).

References

1. C.P. Wild, *International Journal of Epidemiology* **41**, 24 (2012).
2. S.M. Rappaport, D.K. Barupal, D. Wishart, P. Vineis, and A. Scalbert, *Environmental Health Perspectives* **122**, 769 (2014).
3. L. Ramos, Ed., *Comprehensive Two Dimensional Gas Chromatography* (Elsevier, Amsterdam, Netherlands, 2009).
4. D.G. Patterson Jr., S.M. Welch, W.E. Turner, A. Sjodin, and J-F. Focant, *J. Chrom. A* **1218**, 3274 (2011).
5. B. L'Homme and J.-F. Focant, *Analytical Methods* **8**, 249 (2016).
6. C. Brasseur, B. L'Homme, and J.-F. Focant, *Organohalogen Compounds* **74**, 55 (2012).
7. R. Guthrie and A. Susi, *Pediatrics* **32**, 338 (1963).
8. J.V. Seeley, N.J. Micyus, J.D. McCurry, and S.K. Seeley, *American Laboratory* **38**, 24 (2006).
9. J.V. Seeley, *J. Chrom. A* **1255**, 24 (2012).
10. R. Shellie, *LCGC Europe* **21**(11), (2008).
11. M. Poliak, M. Kochman, and A. Amirav, *J. Chrom. A* **1186**, 189 (2008).
12. E.C. Horning, M.G. Horning, D.I. Carroll, I. Dzidic, and R.N. Stillwell, *Anal. Chem* **45**, 936 (1973).
13. D-X. Li, L. Gan, A. Bronja, and O.J. Schmitz, *Anal. Chim. Acta* **891**, 43 (2015).
14. A. Ballesteros-Gomez, J. de Boer, and P.E.G. Leonards, *Anal. Chem.* **85**, 9572 (2013).
15. C.N. McEwen and R.G. McKay, *J. Am. Soc. Mass Spectrom.* **16**, 1730 (2005).
16. C.N. McEwen, R.G. McKay, and B.S. Larsen, *Anal. Chem.* **77**, 7826 (2005).
17. J.V. Seeley, N.E. Schimmel, and S.K. Seeley, *J. Chrom. A* **1536**, 6–15 (2018).
18. S. Fernando, M.K. Green, K. Organtini, F. Dorman, R. Jones, E.J. Reiner, and K.J. Jobst, *Anal. Chem.* **88**, 5205 (2016).
19. D. Megson, M. Robson, K.J. Jobst, P.A. Helm, and E.J. Reiner, *Anal. Chem.* **88**, 11406–11411 (2016).
20. A.G. Harris, *Chemical Ionization Mass Spectrometry* (CRC Press, Boca Raton, USA, 1983).
21. J.R. Sobus, J.F. Wambaugh, K.K. Isaacs, A.J. Williams, A.D. McEachran, A.M. Richard, C.M. Grulke, E.M. Ulrich, J.E. Rager, M.J. Strynar, and S.R. Newton, *J. Exposure Sci. Environ. Epidemiol.* (2017) DOI: 10.1038/s41370-017-0012-y.

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Gas Chromatography with Vacuum Ultraviolet Detection for Pharmaceutical Analysis

A Q&A

Jinjian Zheng
Principal Scientist
Merck. and Co., Inc.

Vacuum ultraviolet spectroscopy enables new capabilities in the analysis of volatile compounds.

Flame ionization and thermal conductivity detectors for gas chromatography have limitations in the types of compounds they can detect and lack the qualitative spectral information. Vacuum ultraviolet (VUV) detectors can detect almost any compound except for hydrogen and some noble gases along with spectral information comparable to a photodiode array detector for liquid chromatography. The versatility and qualitative spectral information offered by GC-VUV offers potential for substantial improvements on conventional gas chromatography (GC) analysis.

LCGC: What do you see as the most significant challenges faced by analytical researchers in the pharmaceutical industry today?

Zheng: There are several challenges in pharmaceutical analysis. One is the regulatory requirements. Samples are analyzed for active pharmaceutical ingredients, trace level impurities, degradation products, mutagenic impurities, metabolites, and more. The method has to be specific, accurate, and precise. The second is the sample's complexity. There are a wide variety of samples, ranging from raw material, intermediate, and drug substance to drug product. There are also in-process control samples that are often reactive and contain a large number of impurities. The third is the time constraint. Analysts are often required to handle a large number of samples in a short period of time. Fast analysis and high-throughput is often essential for pharmaceutical analysis.

LCGC: What are the limitations of detectors that have been used in the past to analyze volatile compounds in pharmaceutical applications, and what unique capabilities does Gas Chromatography-Vacuum Ultraviolet (GC-VUV) spectroscopy offer in contrast?

Zheng: Volatile compounds are commonly analyzed by gas chromatography (GC) instruments equipped with a flame ionization detector (FID). FID is very sensitive and has a wide dynamic range—about seven orders of magnitude. One limitation of FID is that it can only detect compounds with carbon–hydrogen bonds. A thermal conductivity detector (TCD) can be used to detect almost all compounds, but the sensitivity is much lower than FID. An electron capture detector (ECD) can only be used to detect halogenated compounds. The other limitation is that these detectors do not provide qualitative spectral information. VUV detection can be used to detect almost all compounds except for hydrogen and a few noble gases. Of course, the sensitivity is dependent on the compound's structure. But, in general, the absorption seems to be strong in the VUV range. The biggest benefit is that it provides VUV spectral information, which is not available in common GC detectors such as FID, TCD, or ECD. A similar detector, the photodiode array detector (PDA) has become a standard configuration in almost all ultra-high performance liquid chromatography (UHPLC) systems because of its ease of use and the spectral information it provides. I think a VUV detector for GC analysis is similar to a PDA detector for LC analysis, and should become a powerful tool for GC analysis.

LCGC: You recently used the GC-VUV method to measure the water content in organic solvents. What advantage does this new approach provide relative to Karl Fischer titration?

Zheng: I think VUV offers high sensitivity for water detection because water has a strong absorbance at 168 nanometers. We're able to detect water in organic solvents down to the parts per million (ppm) level, which is helpful for our application as the analyte is reactive to water. The other benefit of GC-VUV is the ease of use. All samples were injected from an autosampler and quantified using a water standard. Oven Karl Fischer titration also allows us to automate the analysis, but it's not suitable for the analysis of water in volatile organic solvents.

LCGC: Can you describe how impurities that co-elute with diluents are typically handled and what you were able to accomplish using your VUV detector?

Zheng: For GC-FID, it is very difficult to detect a peak co-eluting with an overwhelmingly large diluent peak. GC-MS may not help as the MS detector will be saturated by huge diluent. For known compounds with authentic markers available, we can change the diluent to achieve the separation. However, there is no good way to handle unknown compounds. Columns with different selectivity may be used to separate the analytes from the diluent, but it can be difficult to track the peaks and identify appropriate conditions to achieve separation. With the VUV detector, we can choose a wavelength to minimize the signal of the solvent and detect the analyte peaks selectively. Alternatively, we can track peaks and make the right decisions to achieve the desired separation.

LCGC: How does the selectivity of VUV compare with other GC detectors?

Zheng: Since traditional GC detectors such as FID only provide two-dimensional chromatograms, selectivity is largely dependent on the level of separation achieved by the GC column. VUV offers an additional flexibility to choose the wavelength to selectively detect compounds of interest. VUV data is three dimensional (i.e., time, absorbance and wavelength) and provides qualitative information that is specific to compounds.

LCGC: What advantages does GC-VUV present in identifying peaks and assessing their purity?

Zheng: With GC-FID, peak identity is confirmed by comparing the retention time of the peak in the sample with that in a standard. With the VUV detector, we can match not only the retention time, but also the VUV spectrum. The spectrum at VUV range (i.e., 120–180 nanometers) is quite distinctive, and has been successfully used to differentiate *cis*- and *trans*-isomers. I believe that peak identification using a combination of retention time and VUV spectrum is more reliable than that based on retention time only.

Peak purity assessment is often used to detect if there is any interference in the main peak. With the VUV detector, it is possible to compare a spectrum at different positions of a peak to determine whether co-eluting peaks are present and to confirm the identities. The extracted spectra will vary if the spectra of the impurity and the main component are different and they are partially separated. This cannot be done using GC-FID analysis because there is no spectral information available. Peak purity assessment using LC-PDA is quite common. However, compared to purity assessment by LC-PDA, GC-VUV has the advantage of high resolving power inherent to gas chromatography and the distinctive VUV spectrum of gas analysis because they do not suffer from the solvation effect of solutions.

LCGC: In your opinion, which applications show the greatest potential for widespread adoption of GC-VUV within the pharmaceutical industry?

Zheng: In my experience, the major advantage of GC-VUV is that it provides qualitative spectral information. This can be used to track peaks, confirm peak identity, assess peak purity, or detect compounds of interest selectively. GC-VUV could become a useful tool for the development of methods for impurity profiling, stability indicating, or the analysis of complex reaction mixtures. The fact that GC-VUV is user-friendly and does not require a steep learning curve should aid its uptake for pharmaceutical analyses.

GC-FID is widely used for the analysis of volatile compounds such as residual solvents due to its excellent sensitivity and wide dynamic range. I don't expect VUV to replace FID in this area. However, for compounds without carbon–hydrogen bonds, GC-VUV could be a good alternative detector for detecting a wide range of compounds.

A Fast, Robust, and Reliable Method for Sensitively Screening Drugs of Abuse in Human Urine for Forensic Toxicology

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Analyzing drugs of abuse (DoA) in human bodily fluids is crucial for clinical research and forensic toxicology. In these routine analyses, a large number of samples must be investigated, with a potentially high laboratory cost for each sample. As such, a reliable and affordable method is required for analysis. In this article, a fast, robust, and reliable method is presented for routine, high-throughput drug screening of urine samples.

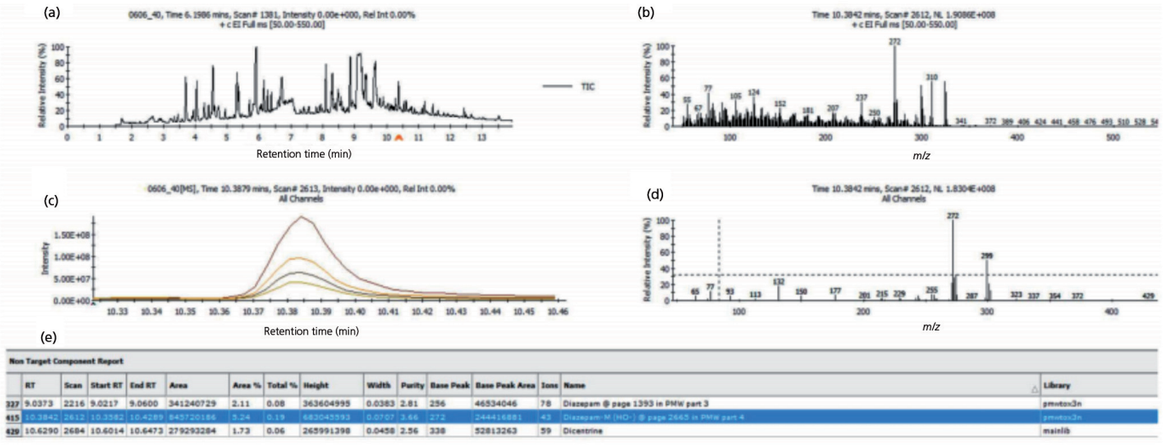
Photo Credit: lokkis/Shutterstock.com

One of the most important requirements for analyzing drugs of abuse in human bodily fluids is that the method must be sensitive enough to selectively detect major drug groups—amphetamines, opiates, synthetic cannabinoids, and others—in one single method, and at very low levels. The challenge that modern laboratories face is that in addition to being sensitive, the process must also have a simple, cost-effective sample preparation, and a robust analytical method that is easy to implement.

For drugs of abuse, the matrix screened is usually urine, within which drugs of abuse (DoA) can be detected for approximately up to one week after use. Urine, like all human bodily fluids, is biologically complex and can vary with the metabolism and the lifestyle habits of the subjects. Consequently, drug substances and their metabolites will often be present at low levels within the sample, which can make accurate selective detection challenging. Therefore, additional sample preparation steps, such as solid-phase extraction (SPE), can be worthwhile to reduce the chemical background and to concentrate the analytes of interest. Gas chromatography–mass spectrometry (GC–MS), operated in electron ionization (EI) mode, is often used for this application because the spectra are easily searchable against existing commercial and private libraries (1).

This article presents a robust method that uses both SPE and EI in combination to produce a technique that can sensitively screen DoA in urine for use in high-throughput and routine laboratories.

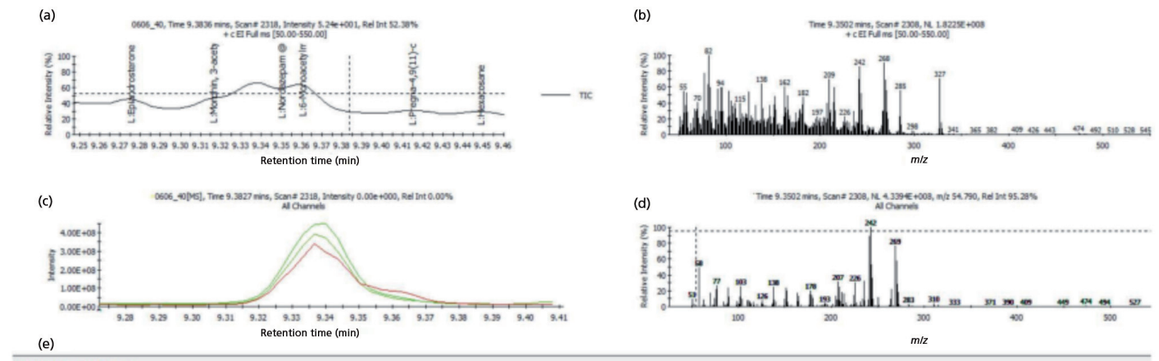
Figure 1: (a) Chromatogram of urine sample D in full-scan mode, (b) raw spectrum at the retention time of diazepam metabolite, (c) extracted ions of the analyte, (d) deconvoluted spectrum, (e) name and RT of the identified compound, in this case diazepam metabolite.



Non Target Component Report

	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name	Library
327	9.0373	2216	9.0217	9.0600	341240729	2.11	0.08	363604995	0.0383	2.81	256	46534046	78	Diazepam @ page 1393 in PMW part 3	pmvtox3n
415	10.2842	2612	10.2582	10.4289	3437200184	5.24	0.19	6820435[illegible]	0.0707	3.68	272	244415681	43	Diazepam-M (HO-) @ page 2665 in PMW part 4	pmvtox3n
429	10.6290	2684	10.6014	10.6473	279293284	1.73	0.06	26599139[illegible]	0.0458	2.56	338	52813263	59	Dicentrine	mainlib

Figure 2: (a) Chromatogram of urine sample D in full-scan mode, zoomed in at RT of nordazepam, showing its coelution with other drugs and compounds, (b) raw spectrum at the retention time of nordazepam metabolite, (c) extracted ions of the analyte, (d) deconvoluted spectrum, (e) name and RT of the identified compound.



Non Target Component Report

	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name	Library
350	9.3162	2298	9.2954	9.3344	2025037373	12.54	0.46	2054567496	0.0390	13.05	82	118207967	147	Morphin, 3-acetyl-	mainlib
351	9.3502	2308	9.3047	9.3767	7660535878	47.44	1.73	5854727439	0.0720	20.16	242	584074961	198	Nordiazepam @ page 1301 in PMW part 3	pmvtox3n

Table 1: Results of drugs screening in samples A–D. Detected compound name, chemical class, as well as retention time and library search scores (SI, RSI, probability, and confidence) are shown.

Urine	Compounds	Class	RT	SI	RSI	Probability	Confidence
A	Clonazepam metabolite	Benzodiazepine	10.6	703	824	96.7	73.9
	Anhydro Ecgonine methyl ester	Opiates	4.1	689	796	98.5	72.2
	Meconin	Opiates	5.9	856	878	94.7	86.3
	Methadon metabolite EDDP	Opiates	7.4	746	776	96.4	75.5
B	Clonazepam metabolite	Benzodiazepine	10.6	762	813	97.3	77.7
	Ibuprofen	NSAID	5.3	818	846	96.7	82.6
	Methadon	Opiates	7.8	658	683	92.4	66.6
	methadon metabolite EDDP	Opiates	7.3	804	840	97.8	81.5
	Nordazepam	Benzodiazepine	9.1	732	784	96.8	74.8
C	Benzoyl ecgonine	Opiates	9.6	709	781	97.7	73.1
	Codeine	Opiates	8.8	741	756	83.6	74.6
	methadon	Opiates	7.8	831	867	96.4	84.2
	methadon metabolite EDDP	Opiates	7.3	883	924	97.0	89.5
	Morphine	Opiates	9.1	870	882	88.1	87.4
	Papaverine	Opiates	10.6	654	689	84.9	66.5
	Paracetamol	analgesic	6.7	614	655	90.3	62.6
D	acetylcodeine	Opiates	9.3	612	694	95.2	63.7
	Anhydro Ecgonine methyl ester	Opiates	4.0	911	916	98.1	91.3
	Benzoyl ecgonine	Opiates	9.6	848	851	98.1	84.9
	Coca ethylene	Opiates	8.3	743	821	97.4	76.6
	Codeine	Opiates	8.9	816	819	51.1	81.7
D	Diazepam M	Benzodiazepine	10.4	710	732	83.6	71.7
	ecgonine methyl ester	Opiates	4.5	895	900	94.6	89.7
	Ibuprofen	NSAID	5.3	646	687	67.8	65.8
	Meconin	Opiates	5.9	899	904	97.2	90.1
	Methadon	Opiates	7.8	631	736	71.0	66.3
	Methadon metabolite EDDP	Opiates	7.3	697	782	97.9	72.3
	Mirtazapine	Antidepressant	9.8	741	761	98.6	74.7
	mono acetyl morphine	Opiates	9.3	583	608	7.4	59.1
	Morphine	Opiates	8.9	665	716	25.6	68.0
	Nordazepam	Benzodiazepine	8.8	789	901	98.1	82.3
	Noscapine	Opiates	10.0	855	864	80.9	85.8
	Oxazepam	Benzodiazepine	7.5	644	769	91.8	68.2
	Papaverine	Opiates	10.4	733	855	87.1	77.0
	Temazepam	Benzodiazepine	8.4	622	632	91.4	62.5
	Temazepam artefact 1	Benzodiazepine	8.5	845	901	93.9	86.2
	Temazepam artefact 2	Benzodiazepine	9.9	588	765	93.4	64.1

Experimental

Sample Preparation: Over 700 urine samples containing various low-level drug traces underwent an SPE procedure. A 30-µL measure of β-glucuronidase was added to 3 mL of urine and incubated for 60 min at 56 °C. A HyperSep Verify AX cartridge (Thermo Fisher Scientific), 6 mL/200 mg, was conditioned with 3 mL methanol followed by 3 mL 0.1% formic acid. Urine was mixed with 3 mL of 2 M acetate buffer at pH 4.8. Urine was checked for accurate pH. The sample was added to the cartridge and sucked through very slowly. Interference elution was performed with a mixture of 1 mL water + 0.1% formic acid, total volume 3 mL, followed by a mixture of 1 mL 50:50 methanol–water + 0.1% formic acid, total volume 3 mL. The cartridge was dried after interference elution with a strong vacuum. Elution was performed

Table 2: Results of drugs screening in samples E–J. Detected compound name, chemical class, as well as retention time and library search scores (SI, RSI, probability and confidence) are shown.

Urine	Compounds	Class	RT	SI	RSI	Probability	Confidence
E	Benzoyl ecgonine	Opiates	9.7	832	852	98.4	83.8
	Bromazepam	Benzodiazepine	9.8	740	779	98.2	75.2
	Carbamazepine	Benzodiazepine	8.6	574	619	90.2	58.8
	Cocaine	Opiates	8.1	584	792	39.5	64.6
	Codeine	Opiates	8.7	758	760	47.1	75.9
	Diazepam M	Benzodiazepine	9.0	685	751	77.7	70.5
	Methylecgonine	Opiates	4.5	868	942	95.8	89.0
	Ibuprofen	NSAID	5.6	643	704	93.0	66.1
	Levomepromazine-M (nor-HO-)	Neuroleptic	10.1	605	669	87.6	62.4
	Levomepromazine-M/A (sulfoxide)	Neuroleptic	10.7	675	701	97.4	68.3
	Meconin	Opiates	5.9	925	926	98.8	92.5
	Methadon metabolite EDDP	Opiates	7.3	664	753	96.9	69.1
	Morphine	Opiates	9.1	619	619	75.1	61.9
	Oxazepam	Benzodiazepine	7.6	735	780	95.6	74.9
	Papaverine	Opiates	10.4	545	607	94.8	56.4
	Quetiapine	Neuroleptic	11.7	876	882	87.5	87.8
F	Amphetamine	Amphetamines	2.1	752	829	63.9	77.5
	Codeine	Opiates	8.9	928	930	63.2	92.9
	Heroin-M (6-acetyl-morphine)	Opiates	9.4	740	835	96.6	76.9
	Hydrocotarnine	Opiates	6.3	793	872	82.5	81.7
	Morphine	Opiates	9.1	910	918	91.3	91.2
G	Chlorprothixene	antipsychotic	10.6	856	861	98.5	85.8
	Carbamazepine	Benzodiazepine	9.9	775	826	98.1	79.0
	oxycodon	Opiates	10.7	883	890	98.4	88.5
	Tilidine metabolite	Opiates	7.4	825	871	97.7	83.9
	Tilidine-M (bis-nor-)	Opiates	7.3	689	756	83.0	70.9
	Tilidine-M (bis-nor-HO-)	Opiates	8.3	588	636	83.3	60.2

H	Diphenhydramine	Antihistamine	9.3	680	696	96.6	68.5
	Venlafaxine	Antidepressant	8.5	621	738	87.4	65.6
I	Amphetamine	Amphetamines	2.1	760	771	79.6	76.3
	Pipamperone	Neuroleptic	11.1	898	908	92.2	90.1
J	Benzoyl ecgonine	Opiates	9.6	687	860	95.9	73.9
	Cocaine	Opiates	8.1	920	927	49.1	92.2
	Codeine	Opiates	8.8	878	878	53.8	87.8
	ecgonine methyl ester	Opiates	4.5	851	852	97.4	85.1
	Levomeprazine	Neuroleptic	10.6	763	787	98.2	77.0
	Meconine	Opiates	5.9	928	940	98.6	93.2
	Methadon	Opiates	7.8	881	893	98.0	88.5
	Methadon metabolite EDDP	Opiates	7.3	917	955	97.5	92.8
	Mirtazapine	Antihistamine	8.3	920	927	89.4	92.2
	Morphine	Opiates	9.2	667	668	68.5	66.7
	Pregabaline	Anticonvulsant	4.3	731	750	97.8	73.7

by adding a mixture of methanol and 5% ammonia solution at pH 9 with a volume of 0.5 mL. For complete elution, this step was repeated. The sample was evaporated under nitrogen until dry. It was then placed in 100-µL mass spectrometry (MS) certified vials and 50 µL of methanol added. It was subsequently centrifuged to precipitate the particles before placement in the autosampler.

The DoA excreted in urine are in the form of glucoronidate conjugates. Therefore, beta-glucuronidase was used for enzymatic hydrolysis, to cleave off glucuronides and sulphate esters prior to GC–MS analysis.

Analytical Conditions: Compound separation and detection was achieved using a Trace 1310 GC system (Thermo Fisher Scientific) coupled with an ISQ 7000 single quadrupole GC–MS system (Thermo Fisher Scientific), which features an advanced electron ionization (AEI) source. Sample introduction was performed using a TriPlus 100 LS autosampler (Thermo Fisher Scientific), injecting 1 µL on the instant connect split/splitless (SSL) injector module (Thermo Fisher Scientific).

DoA Identification: Data were acquired in full-scan mode and processed using Chromeleon Chromatography Data System (CDS) software (Thermo Fisher Scientific). The data subsequently underwent automated spectral deconvolution and peak detection, followed by library searching for putative compound identification with AnalyzerPro software (SpectralWorks).

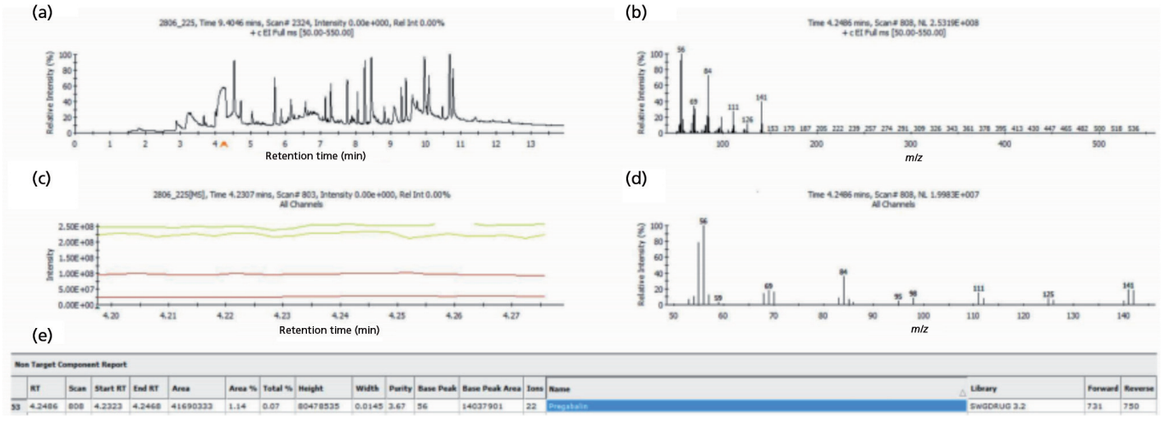
Results and Discussion

In excess of 700 urine samples were analyzed as part of this study. Representative results were selected to show the capacity of this method to detect and quantify challenging drug metabolites in urine, as well as some typical urine sample profiles obtained from drug addicts. Ten urine samples (A to J) were found to be the most interesting, and data for these samples are summarized in Tables 1 and 2. Figures 1 to 4 provide detailed views of some of the more challenging metabolites. One of the examples shown is pregabalin, a drug that can be prescribed for the treatment of conditions such as neuropathic pain and fibromyalgia. However, when consumed in larger quantities this drug has similar effects to classic drugs of abuse. There is a recent trend in Germany of pregabalin prescriptions being sought out by drug abusers. The other examples provided are benzodiazepines, a class of drugs that undergoes significant metabolism and is difficult to analyze at low levels. Even so, the metabolites are identified relatively easy.

The results collected during this study highlight the potential this method holds in the detection of DoA in urine for criminal and forensic investigations and clinical research. The developed methods are not only applicable to classic drug compounds, such as heroine and amphetamines, but also suited for the detection of new psychoactive substances such as fentanyl, the NBOMe family of drugs, and various Spice-type drugs.

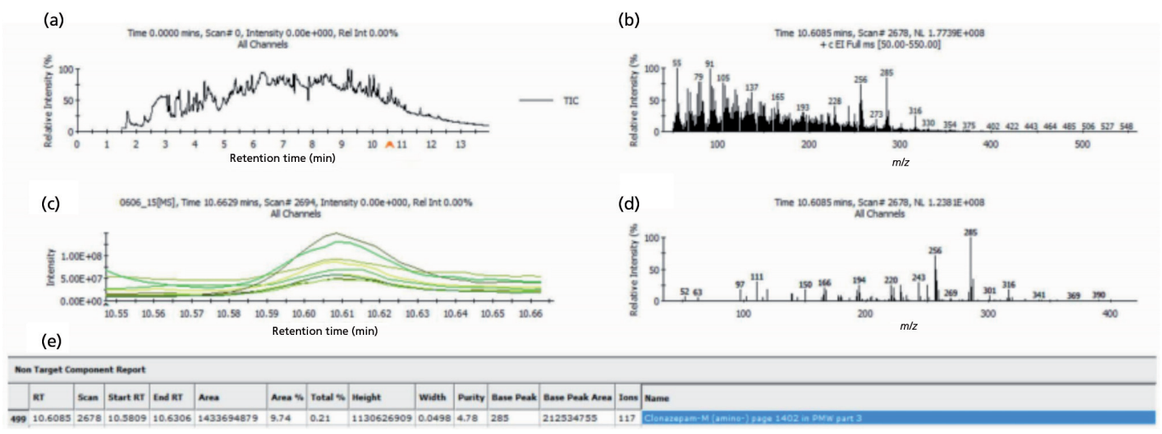
Observable trends can be revealed from the analyses of the subjects' urine profiles. For instance, urine sample D shows a rapid ingestion of all available drugs before the start of the forensic investigation; whereas urine sample J could present a subject going to multiple doctors, attempting to get a prescription for pregabalin, which in sufficient quantities can give a feeling of euphoria. Sample H shows a person taking medication to cope with withdrawal symptoms. Interestingly, in almost all the urine samples, markers for smoking, such as cotinine and nicotine, were found, which could be a reflection of smoking as a coping mechanism for withdrawal (2).

Figure 3: (a) Chromatogram of urine sample J in full-scan mode, (b) raw spectrum at the retention time of pregabalin, (c) extracted ions of the analyte, (d) deconvoluted spectrum, (e) name and RT of the identified compound, in this case pregabalin.



	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name	Library	Forward	Reverse
33	4.2486	808	4.2323	4.2468	41690333	1.14	0.07	80478535	0.0145	3.67	56	14037901	22	Pregabalin	SWGDRUG 3.2	731	750

Figure 4: (a) Chromatogram of urine sample A in full-scan mode, (b) raw spectrum at the retention time of the clonazepam metabolite, (c) extracted ions of the analyte, (d) deconvoluted spectrum, (e) name and RT of the identified compound, in this case clonazepam.



	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name
499	10.6085	2678	10.5809	10.6306	1433694879	9.74	0.21	1130626909	0.0498	4.78	285	212534755	117	Clonazepam-M (amino-) page 1402 in PMW part 3

The data demonstrate that the method presented provided a rapid and robust method for the general unknown screening of drugs of abuse. This method can be used for routine analysis of DoA in runs with a sample throughput of more than 12,000 samples a year.

The concentration factor of 60 gained through sample preparation, and the improved sensitivity of the ion source, made it possible to reach low detection limits. These low detection limits are much more sensitive than the results obtained in immunological testing, which could be especially beneficial for analysis of new psychoactive substances. This would also enhance the integrity of the data by reducing the variability that is commonly seen in immunoassays.

The data provide reliable, library-searchable spectra and can indicate which group of drugs the new compound belongs. It is possible by fragmentation rules to see to which group the analyte belongs, without a complex collection of drugs. When hundreds of substances show up in the chromatogram, chromatographic deconvolution is an incredibly useful tool for this kind of analysis. In contrast, a manual data investigation would require a highly experienced scientist and would be a lengthy and laborious process.

These complete methodologies provided a rapid, reliable, and robust method of screening for drugs of abuse in urine samples in routine and high-throughput laboratories.

References

1. C.A. Hammett-Stabler, A.J. Pesce, and D.J. Cannon, *Clin. Chim. Acta* **315**, 125–135 (2002).
2. J.J. Prochaska, K.H. Delucchi, and M. Sharon, *J. Consult. Clin. Psychol.* **72**, 1144–1156 (2004).

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