The Column

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Cover Story

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When Less is More

CE–MS for metabolomics

Cover Story

Features

Regulars

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Capillary Electrophoresis–Mass Spectrometry for Metabolomics: Extracting Chemical Information from Less

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This is the second in a series of articles exploring current topics in separation science that will be addressed at the HPLC 2019 conference in Milan, Italy, from 16–20 June.

Metabolomics has become an important tool for addressing biological and clinical questions (1). The analytical techniques commonly used for metabolomics studies often require relatively large amounts of biological material, notably for sample preparation and injection. As many studies have not been focused on limited amounts of sample material, relatively little effort has been paid to downscale the analytical workflow for metabolomics.

However, more and more biological questions are dealing with small sample amounts. For example, microfluidic three-dimensional (3D)-cell culture models, which can mimic physiological tissues by arranging different cell types

in a 3D environment within a proper micro-environment, are increasingly being used to address biological questions. These microfluidic cell culture systems inherently deal with relatively low numbers of cells, for example, those in the range of hundreds to thousands of cells. Another example concerns the unravelling of the behaviour of a single cell within a population of cells, and, as such, obtaining a better understanding on the role of cell heterogeneity in tumour biology. To address these questions with a metabolomics approach, the development of new microscale analytical techniques and workflows is needed.

Capillary electrophoresis–mass spectrometry (CE–MS) is an attractive microscale analytical technique for addressing biological questions inherently dealing with low amounts of material. In CE, nanolitre injection volumes are often employed from just a few microlitres of sample. As such, CE–MS is well-adapted for the profiling of especially polar and charged metabolites in tiny sample amounts, as demonstrated for mouse cerebrospinal fluid (CSF) (2,3).

CSF can only be obtained in a few microlitres under proper experimental conditions. Using only a 1:1 dilution of CSF with water, and therefore fully retaining sample integrity, more than 300 compounds could be observed. As only 45 nL of the sample were consumed from a vial containing only 2 µL of a 1:1 diluted CSF, the proposed CE–MS approach allows multiple analyses on a single highly valuable mouse CSF sample, enabling repeatability studies and the analysis of the same sample at different separation conditions to further enhance metabolic coverage. Performing multiple analyses on a single scarcely available biological sample is not possible with conventional analytical techniques used in metabolomics.

Alongside the low sample and solvent requirement of CE, the separation mechanism of CE, in which compounds are separated on the basis of their charge-to-size ratio, is fundamentally different from chromatographic-based separation techniques, thereby providing a complementary view on the composition of endogenous metabolites present in a given biological sample. In comparison to chromatographic-based methods, the separation efficiency of CE is very high because there is no mass transfer between phases, and under well-designed experimental conditions only longitudinal diffusion contributes to band broadening. An overview of the analytical features of CE–MS for metabolomics studies, especially for those dealing with limited sample amounts, will be given during the short course "Advanced CE–MS approaches for

metabolomics" on Sunday 16 June at HPLC 2019 in Milan, Italy.

Until now, various research groups have developed CE–MS approaches for metabolic profiling of limited sample amounts (4,5), and also for single cell analysis (6–9). Concerning the latter, the metabolomics studies were often focused on the analysis of a relatively large single non-mammalian cell with a diameter in the range of 100–1000 µm and a cellular sample content ranging from 100 to 500 nL. To profile metabolites in a single mammalian cell is clearly an enormous analytical challenge; for example, the content of a single HepG2 cell is only around 3 pL and a diameter of ~12 µm. In my group, low-flow CE–MS approaches utilizing a sheathless porous tip interface are examined for metabolic profiling of low numbers of mammalian cells using HepG2 cells as a model system (10). The aim is to be able to profile a wide range of endogenous metabolites in just a few cells and ultimately a single cell; the latter will really enable the effect of cell heterogeneity to be studied—a subject that really matters in key fundamental biological questions. In my keynote lecture at HPLC 2019, I will address the current state-of-the-art of our CE–MS platform for metabolic profiling of low numbers of mammalian cells by presenting results obtained for HepG2 cells.

Metabolomics studies dealing with small amounts of biological sample have to critically consider preanalytical steps because adsorption effects, notably with sample volumes far below 1 µL, may result in significant analyte losses. Moreover, another challenge is how to effectively get the compounds or the fraction of interest from ultra-small sample amounts into the CE–MS system. Various strategies that have been explored in our laboratory and by others will also be outlined during HPLC 2019.

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Rawi Ramautar obtained his Ph.D. on the development of capillary electrophoresis–mass spectrometry methods for metabolomics from Utrecht University, The Netherlands, in 2010. In 2013 and 2017, he received the prestigious Veni and Vidi research grants from the Netherlands Organization for Scientific Research for the development of CE–MS approaches for volume-restricted metabolomics. Currently, he is a principal investigator at Leiden University, The Netherlands, where his group is developing microscale analytical workflows for sample-restricted biomedical problems.

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News

Markes Receives Two Queen's Awards

Markes International (Llantrisant, Wales, UK) has been awarded two Queen's Awards for Enterprise in the categories of "innovation" and "international trade". The awards stemmed from Markes' new valving technology, which is incorporated into all its thermal desorption instruments, as well as its continued growth in overseas trade.

The switch-valve that is the subject of the innovation award was developed and patented by the company and allows routine monitoring of a wide range of volatile and semivolatile organic compounds, by virtue of its tolerance of temperature extremes, small size, quick operation, and construction from inert materials.

"Precise, reproducible control of gas flows is vital for the successful operation of any thermal desorption instrument," said Alun Cole, Founding Director of Markes International.

This is the second time Markes has been awarded a Queen's Award for "international trade"; they received their first in 2015.

"Looking back at the 20 years Markes has been in business, it's fair to say that innovations have been central to our achievements. We always have been, and continue to be, an innovative company, and are therefore very proud to have received these Queen's Awards. These stand as testament to our excellence in analytical chemistry—both in our technical innovation, as well as our success in satisfying our customers globally," said Cole.

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

Sourcing Prebiotic Oligosaccharides from Vine Shoots

Following decades of research, evidence of the human microbiome's importance in maintaining overall health and well-being has become evident, spawning a multimillion-dollar industry. A wide range of products aimed at repairing and maintaining a healthy microbiome are now available, with many focusing on prebiotics.

Described as selectively fermented ingredients that allow specific changes, both in composition and in activity of the gut microbiota, prebiotics pass through the stomach and small bowel intact before reaching the large bowel. They are fermented here by microbiota, such as *bifidobacteria* and *lactobacilli*, generating short-chain fatty acids (SCFA). SCFAs are involved in many important physiological events, such as bowel function, calcium absorption, lipid metabolism, or the reduction of colon cancer risk (1).

Most commercialized prebiotics will contain inulin, lactulose, galactooligosaccharides (GOS), and fructooloigosaccharides (FOS) as there is enough scientific evidence to give them credibility, however, there are other food ingredients, such as fructans, galactans, xylooligosaccharides, β-glucans, or arabinoxylands that are gaining attention because of their prebiotic properties.

Xylooligosaccharides, which are formed by xylose units linked through β-1,4 glycosidic bonds, are receiving particular attention because they can be obtained from lignocellulosic residues, such as hardwoods, hulls, brans, corn cobs, and corn stover, or from industrial by-products, such as brewery spent grains and shells (2). The low cost combined with renewability and high availability makes them a promising source of probiotics, however, research on their viability as prebiotics in limited. To address this, researchers investigated the prebiotic activity of oligosaccharides obtained from vine shoots—one of the most abundant by-products produced by wineries—with 2–4 tons being produced per hectare per year (3).

The vine shoots were subjected to hydrothermal treatment before purification and prebiotic evaluation using a range of techniques, including high performance liquid chromatography (HPLC), matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF), high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), and ultrahigh-performance liquid chromatography–diode array detector-electrospray ionization-mass spectrometry (UHPLC–DAD-ESI-MS).

Results confirmed the suitability of a refined mixture of xylooligosaccharides as a prebiotic ingredient. Structural characterization provided insight into the compound, and a fermentation test using human faeces confirmed the generation of SCFAs, which are the desired product of prebiotics and, which have many positive health benefits.—L.B.

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Thermo Fisher Opens New Indian Customer Solution Center

Thermo Fisher Scientific (Waltham, Massachusetts, USA) has announced the opening of its Customer Solution Center in Delhi, India. The analysts will focus on development, training, and support for next-generation workflows and integrated solutions across chromatography and mass spectrometry.

"The opening of the Food Safety Customer Solution Center in India demonstrates our unwavering commitment to support the food and beverage industry in providing safer, more nutritious, and better-informed choices for its customers," said Mitch Kennedy, President, Chromatography and Mass Spectrometry, Thermo Fisher Scientific.

The Food Safety Customer Solution Center will work in collaboration with the Food Safety and Standards Authority of India (FSSAI), and will serve as a regional hub for scientists, partners, and the FSSAI to collaborate with subject matter experts. "We welcome the establishment of this world class center and see it as a tremendous benefit in the capacity building of food analyst and technical staff of the food safety authorities, both at the government of India and at the state level," said Rita Teaotia, Chairperson, FSSAI.

For more information, please visit: **www.thermofisher.com/foodandbeverage**

Alaskan Lake Analysis Suggests Early Human Migration to North America

As the final land mass to be colonized by *Homo sapiens*, the Americas represent a major landmark in the history of mankind, the fulfilment of a journey from near extinction to world colonization, and the successful accession to evolutionary dominance across the globe. However, the timeline and pathway of that arrival remain a contentious issue even after decades of debate (1,2,3).

At the heart of the issue lies the Beringia, the corridor of human migration to the Americas, which appeared between eastern Siberia and the western edge of the Laurentide ice sheet during the last ice age. The traditional theory posits a "swift peopling" of the Americas via the Bering land bridge from Asia. However, more recent genetic data and archaeological finds have challenged this view.

The alternative theory is known as the *Beringian standstill hypothesis* (BSH), in which a population of proto-Americans migrated from Asia during or even prior to the point in time when glacial ice sheets were at their largest. The period of separation is the crucial part of this theory with evidence of thousands of years of isolation needed to prove the BSH theory to be viable (4,5).

Archaeological, genetic, and paleoenvironmental evidence so far has offered complementary, but also conflicting timelines, with the lack of direct physical evidence of human occupation in eastern Beringia being a major obstacle to determining the timing and manner of human migration to the Americas (1,6).

In an attempt to correct this evidential deficit, researchers analyzed sediment core from a lake located in the northern foothills of Alaska's Brooks Range. The lake is located on an older glacial landscape that escaped glaciation during the last ice age, allowing it to archive environmental changes from that time period.

Sediment core samples were obtained from the lake and analyzed using traditional paleoecological methods to analyze charcoal and pollen, and gas chromatography–mass spectrometry (GC–MS) to analyze polycyclic aromatic hydrocarbons (PAHs) and sterols. Transient climate model simulations were then used to contextualize the findings and the ecological interpretations (7).

Results support the theory of human presence in the region. This was demonstrated by the existence of faecal biomarkers, and evidence of elevated burning during the period, which suggests human ignition as a likely culprit.

The data indicate that humans occupied eastern Beringia at the same time they inhabited Siberia, and they affected the Arctic landscapes during the height of the last ice age, confirming the theory that founder populations of the Americas were present in the Arctic regions well before full expansion into the Americas occurred.—L.B.

Photo Credit: Kovalenko I/stock.adobe.com

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Peaks of the Month

- **The *LCGC* Blog: A Decision Tree for Gas Chromatography Method Development**—To answer the question "Is there a good flow diagram I can use for gas chromatography (GC) method development?" please read on for our first attempt at something suitable, in the form of stepwise decision trees and flow diagrams. **Read Here>>**

- **Hydrophilic Interaction Liquid Chromatography: An Update**—This article is an update on the technique of hydrophilic interaction liquid chromatography (HILIC) and covers recent ideas on the mechanism of separation, and how it may be manipulated to suit the separation of particular sample types. **Read Here>>**

- **Tuberculosis Diagnosis Using GC×GC–TOF-MS**—*The Column* spoke to Jane Hill from Dartmouth College, USA, about her group's development of a breath analysis method for tuberculosis (TB) diagnosis using multidimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS). **Read Here>>**

- **Recent Advances in Solid-Phase Microextraction, Part 1: New Tricks for an Old Dog**—In this instalment of Sample Preparation Perspectives, Doug Raynie provides an overview of some of the major advances related to the traditional practice of solid-phase microextraction (SPME). **Read Here>>**

- **Analysis of Dioxins in Foods and Feeds Using Gas Chromatography Tandem Mass Spectrometry**—In the early days of dioxin analysis, applied methods were laboratory- and time-consuming. Only GC–HRMS, which is complicated, was used. Nowadays, GC–MS/MS is suitable for control proposes. Using GC–MS/MS means that solvent consumption for sample preparation can be reduced by a factor of 10 and the purity of the obtained fraction can be enhanced, indicating that GC–MS/MS is appropriate for dioxin analysis. **Read Here>>**

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News In Brief

Agilent Technologies (Santa Clara, California, USA) has announced that Antoni Ribas has been awarded an Agilent Thought Leader Award in support of his genomics and immunotherapy research. His research focuses on implementing next-generation genomics solutions to identify mechanisms and biomarkers associated with resistance and response to immunotherapy in patients with melanoma. "I'm very honoured to receive this award and to be given the opportunity to further my research on the deadliest form of skin cancer. By partnering with Agilent, my laboratory will be able to intensify our research efforts in developing new treatment strategies for the many patients who still do not respond to current therapies," said Ribas. For more information, please visit: **www.agilent.com**

Knauer (Berlin, Germany) has been ranked as one of the top employers in Germany for 2019, according to a study by 3.works GmbH, a Düsseldorf-based employment research institute.This is the second time Knauer has been honoured as one of the best employers in Germany. The study is based on a comprehensive employer evaluation system in Germany, which has evaluated the data of more than 70,000 companies. Criteria included independent certifications, ranking and awards, employee satisfaction ratings, and student surveys, as well as activities in the areas of corporate social responsibility, diversity, and family friendliness. For more information, please visit: **www.knauer.net/**

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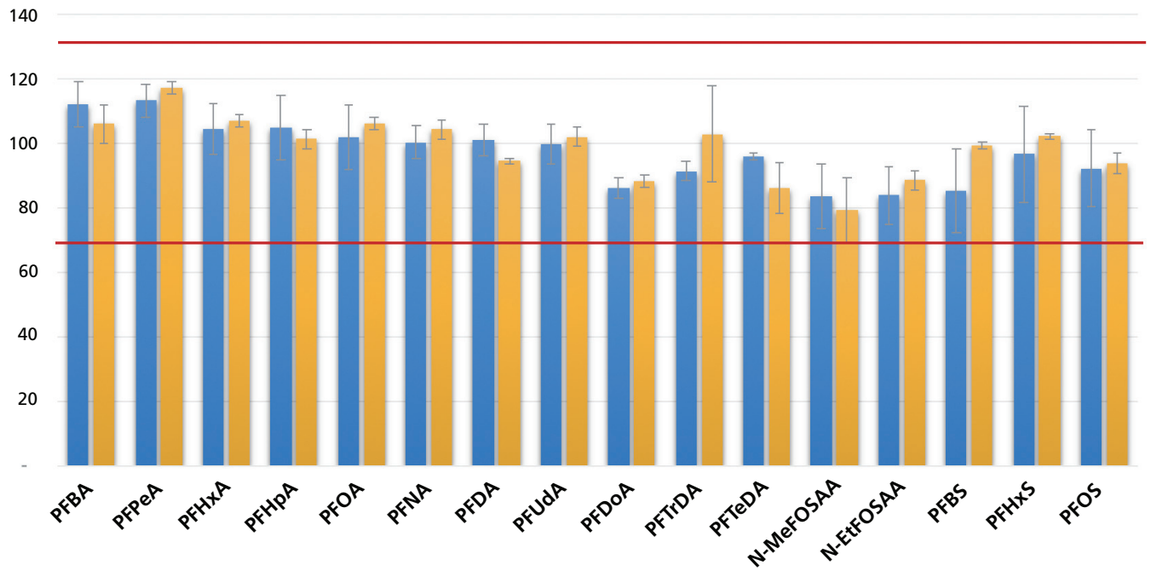
Quantitative Analysis of PFAS in Drinking Water Using Liquid Chromatography Tandem Mass Spectrometry

Emily Parry and Tarun Anumol, Agilent Technologies, Inc., Little Falls, Delaware, USA

Per- and polyfluoroalkyl substances (PFAS) are chemicals found in fire-fighting foams and consumer products requiring water-resistant and stain-repellent properties. As a result of their unique chemical properties and long-term widespread usage, these chemicals are an emerging human health concern. US Environmental Protection Agency (EPA) released analytical methods for PFAS measurement in 2009 and most recently in November of 2018. In this article, data generated using these methods with allowed analytical modifications is presented and demonstrates robustness and reproducibility while achieving low level detection limits in drinking water.

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made compounds widely used in industry and manufacturing because of their uniquely desirable chemical properties. These compounds are used in non-stick cookware, food

Figure 1: The average spike recoveries of PFAS in ultrapure and finished drinking water using SPE.



contact materials, fire-fighting foams, surfactants, and many other applications. Their chemistry makes these compounds extremely persistent, bioaccumulative, and potentially toxic to animals and humans (1). As a result of their widespread usage over the last few decades, they are now ubiquitous in the environment.

There are more than 4500 PFAS commercially manufactured, but only very few have been monitored in the environment. The most commonly measured PFAS classes in the environment are the perfluorocarboxylic acids (PFCAs), such as perfluorooctanoic acid (PFOA), and perfluorosulfonic acids (PFSAs), such as perfluorooctanesulfonate (PFOS). Some of these PFAS compounds are currently the subject of regulation and much public and research attention (2).

US Environmental Protection Agency (EPA) indicates a drinking water health guidance for PFOA and PFOS at a combined 70 ng/L, while several US states have guidelines for PFOA, PFOS, and other PFAS (PFNA, GenX) at low ng/L levels.

Figure 2: Chromatogram of EPA 537.1 analytes with the addition of PFBA and PFPeA.

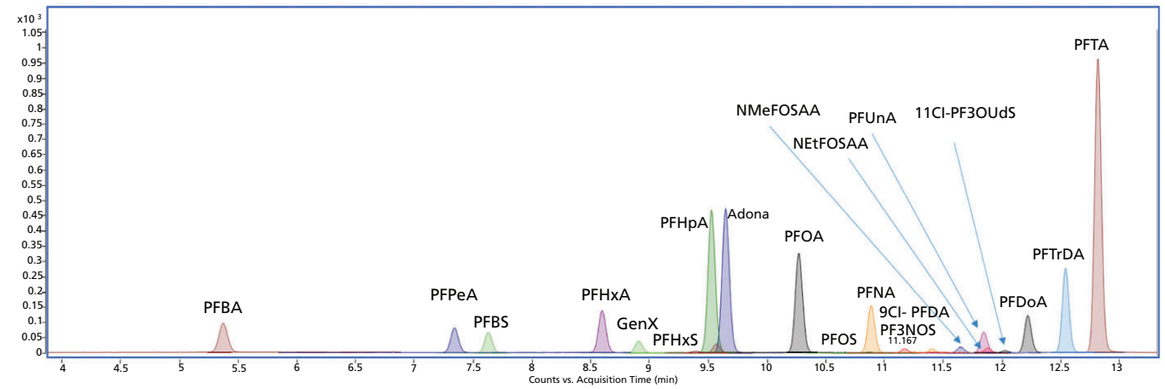
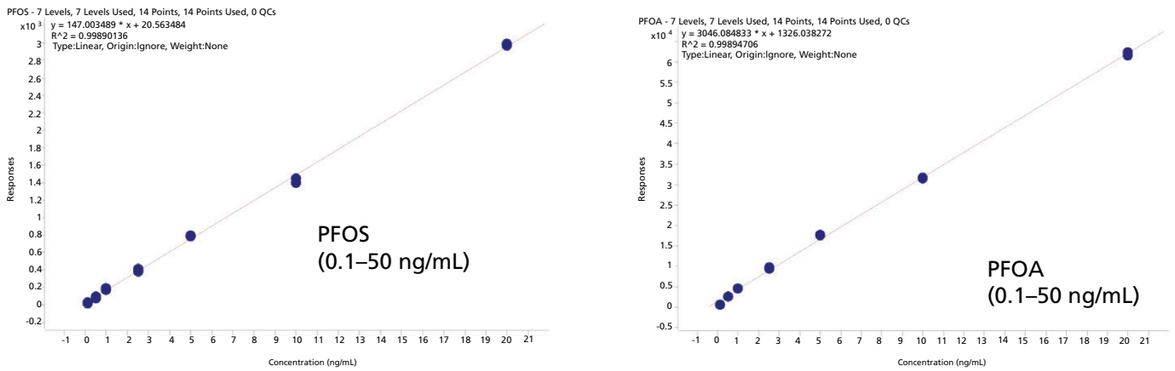


Figure 3: Linear calibration curves for PFOS and PFOS; 7-point calibration curve in duplicate (14 points) from 0.1–50 ppb in the extract.



In Europe, the drinking water directive recommends levels of lower than 0.1 µg/L for individual PFAS, and 0.5 µg/L for total PFAS, while several member countries have guidelines for PFAS in the ng/L range in drinking water. PFOS and its salts have been listed as priority pollutants to be phased out from use under the Stockholm Convention. With PFOA and PFOS banned or in the process of being phased out by manufacturers globally, alternative compounds are being used resulting in "emerging" classes of PFAS now being detected in the environment.

The measurement of these compounds at ng/L levels is quite challenging. Therefore, the need for standard methods to measure them in the environment is critical for establishing baselines and future regulatory decisions. In 2009, the US EPA established EPA Method 537 for the quantification of 14 PFAS in drinking water, using solid-phase extraction (SPE) and liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) (3). In late 2018, the US EPA revised this method (EPA 537.1) to include four emerging PFAS including hexafluoropropylene oxide dimer acid (HFPO-DA aka GenX), ADONA, 9Cl-PF3ONS, and 11Cl-PF3ONS (components of F-53B; replacement for PFOS) (4).

This article aims to provide a simple SPE procedure for the extraction of PFAS in drinking water analyzed in EPA Method 537, along with an LC–MS/MS method for the analysis of PFAS listed in EPA Method 537.1 to achieve the required low ng/L levels in drinking water.

Experimental

Chemicals: Standards were purchased from Wellington Laboratories, Inc. and

Table 1: PFAS compound optimized transitions and estimated limit of detection on the LC-TQ system

Analyte	RT	Transition	Fragmentor	Collision Energy	Estimated Instrument Level of Detection (pg on column)
PFBA[1]	5.4	213 > 168.9	60	6	0.1
PFPeA[1]	7.3	263 > 218.9	60	6	0.06
PFBS	7.6	298.9 > 80.1 298.9 > 99.1	100	34 22	0.11
PFHxA	8.5	312.9 > 119.1 312.9 > 269	70	14 6	0.7
HFPO-DA	8.9	285.1 > 169	100	0	0.44
PFHpA	9.5	362.9 > 169 362.9 > 319	72	9 0	0.18
PFHxS[2]	9.5	398.9 > 80.1 398.9 > 99.1	100 70	37 34	0.31
ADONA	9.65	377.1 > 251.1 377.1 > 84.9	95	0 30	0.04
PFOA	10.2	412.9 > 169 412.9 > 369	69	13 3	0.08
PFOS[2]	10.8	498.9 > 80.1 498.9 > 99.1	100	38 38	1.30
PFNA	10.9	462.9 > 169 462.9 > 418.9	66	13 3	0.26
9Cl- PF3ONS	11.2	531 > 351.1	90	20	1.35
PFDA	11.4	512.9 > 219 512.9 > 468.9	100 81	3 12	1.51
NMeFOSAA	11.7	570 > 482.9 570 > 418.9	115	15 12	0.47
PFUnA	11.6	562.9 > 219 562.9 > 519	100 73	15 4	1.17
NEtFOSAA	11.9	584 > 525.9 584 > 418.9	115	15 15	1.01
11Cl-P3OUdS	12.0	631 > 451	70	30	1.32
PFDoA	12.2	612.9 > 269 612.9 > 568.9	100 79	15 4	0.50
PFTrDA	12.6	662.9 > 169 662.9 > 618.9	100 91	23 7	0.18
PFTA	12.8	712.9 > 669 712.9 > 169	100	7 23	0.11

[1]Not included in EPA Method 537 or EPA Method 537.1
[2]EPA Method 537.1 requires that the 80 *m/z* product ion must be used to reduce bias between linear and branched isomers

calibration standards diluted to a desired concentration in 96:4 methanol–water.

Instrumental: Five µL of the standard–sample were introduced for analysis into the LC–MS/MS system. Instrument sensitivity allowed for the reduction of 10 µL cited in the EPA 537 method. LC separation was performed on an Agilent 1260 Infinity II Prime LC system with a 3.0 × 50 mm, 1.8-µm Zorbax Eclipse Plus C18 column (Agilent). A 4.6 × 50 mm, 3.5-µm Zorbax Eclipse Plus C18 delay column (Agilent) was used after the binary pump to separate background PFAS introduced from the solvent, tubing, and the degasser from the desired analytes.

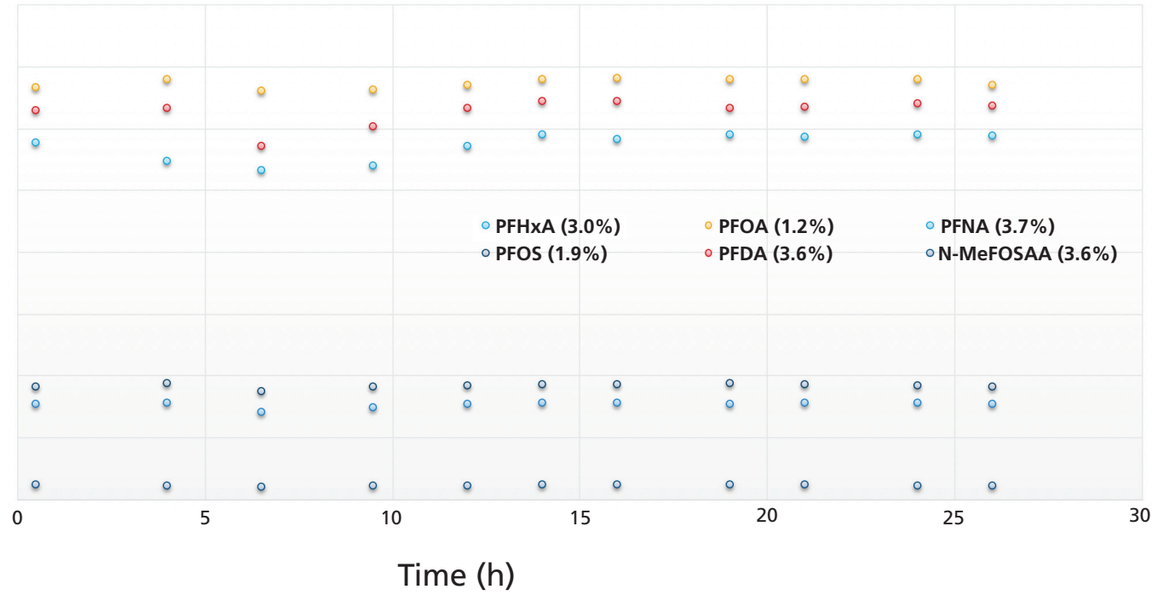
The Agilent Jet Stream Technology Ion Source (AJS) was used for maximum ionization. Source parameters were the same as can be seen in reference 5, with the exception of the increase of drying gas flow to 7 L/min. The Agilent Ultivo Triple Quadrupole LC/MS (LC-TQ) was operated in dynamic multiple reaction monitoring (MRM) mode to optimize sensitivity through maximizing dwell time. For most analytes, two transitions were acquired to provide quantitation and qualification ratios. MRM parameters are noted in Table 1. EPA Method 537.1 now requires the use of 80 mass-to-charge ratio (*m/z*) for PFHxS and PFOS to reduce bias between linear and branched isomers and this was implemented.

Solid-Phase Extraction: Six replicates of 250-mL ultrapure water and finished drinking water samples were spiked at 4 ng/L for each PFAS. The samples were then extracted using a weak anion exchange (WAX, 150 mg, 6 cc) SPE cartridge (Agilent) as in the procedure described in EPA Method 537. Details for the specific SPE procedure can be found in reference 6. The eluate was evaporated to a final volume of 1 mL constituting ~96:4 methanol–water. Figure 1 shows that the extraction recoveries of all PFAS compounds were 70–130% and ranging from 79–112% in both ultrapure and drinking water. The relative standard deviations (RSDs) for all compounds was <15% too—within acceptable parameters for the EPA method—demonstrating that the cartridge is effective at extracting low-level PFAS from drinking water samples with high efficiency.

Results and Discussion

Background Contamination Elimination: In this study, a delay column was installed in between the pump mixer and the injection port to time resolve any background PFAS coming from the solvents or the tubing of the LC system itself.

Figure 4: Raw response deviation for six PFAS in the continuous calibration standards run across a 26-h batch; the number in brackets is the RSD%.



Chromatographic Separation and Method Performance: The analysis and separation of the 18 PFAS in EPA Method 537.1 were performed with all analytes achieving good peak shapes and peak widths between 6–10 s. Figure 2 shows a representative chromatogram of the 14 analytes in EPA Method 537, four of the emerging PFAS (GenX, ADONA, 9Cl-PF3OUdS, and 11Cl-PF3OUdS) added to EPA Method 537.1, and the addition of PFBA and PFPeA. PFBA and PFPeA were added to show the good chromatographic separation and peak shapes of the early PFAS eluters, even though these are not present in the EPA method. The mobile phase was 5-mM ammonium acetate in water and 5-mM ammonium acetate in 95:5 methanol–water, instead of the 20 mM used in the EPA methods. The EPA's method flexibility allows changes in the LC separation. However, the EPA notes that reduced RT stability was observed over time with lower concentrations. Reduced stability at the lower concentration has not been

observed so far. The gradient run time was reduced from 37 min in EPA Method 537 to 19.5 min (14-min gradient and a 5.50-min post time).

Figure 3 shows representative calibration curves for PFOA and PFOS from 0.1–50 parts per billion (ppb) in the final extract. Calibration curves were linear with R2 > 0.99. Complete details of the analytical method including method optimized parameters and method verification along with linearity, robustness, and analysis of real-world drinking water samples can be found in reference 5.

Robustness and Reproducibility: US EPA Method 537 requires sensitive analysis of PFAS and robustness of the data across samples and batches. For example, the method calls for the injection and analysis of a continuing calibration standard in a batch every 10 samples to monitor system performance and variability. In this study, this method was evaluated by following the raw response of the PFAS standards run as continuous calibration standards every 10 samples across a batch of samples over a 26-h worklist. The standards were prepared in drinking water extracts at 1 ppb in the vial (~2.5 ng/L in sample equivalent). All PFAS analytes had response variation less than 5%RSD except N-EtFOSAA (5.6%). Figure 4 illustrates the response stability of the calibration standards across the 26-h batch and shows that the relative response, uncorrected by internal standards (ISs), was stable across the 11 CCV samples analyzed over 26 h.

Conclusions

The analysis of PFAS at extremely low levels in drinking water is required for adequate baseline monitoring and regulatory determination. This article provides a sample extraction protocol for PFAS in the US EPA method that achieves high recoveries in the target matrix, and a robust LC–MS/MS method for excellent separation, low level detection, and reliable and robust quantification of PFAS.

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The 48th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2019)

The 48th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2019) will be held 16–20 June 2019 at the Milano-Bicocca University, in Milan, Italy. This is the first time that this symposium will be held in Italy.

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The **HPLC** symposium series is the largest, most recognized international forum on high performance liquid chromatography (HPLC) and related techniques. It is the ideal location for industrial and academic researchers to exchange information with other colleagues from all over the world. Both fundamental and practical aspects of separation science will be covered during the conference, with the main focus being on new, highly relevant trends emerging in this field, cutting-edge applications, and innovation in the technology.

Among the many topics that will be covered at **HPLC 2019**, particular emphasis will be given to hyphenated techniques, in particular liquid chromatography coupled to mass spectrometry (LC–MS), design and characterization of stationary phases, micro- and nano-fluidics, and supercritical fluid chromatography (SFC) and capillary electrophoresis (CE) and their applications in proteomics, metabolomics, food analysis, and characterization of biopharmaceuticals and biosimilars. **HPLC 2019** will also feature some unique highlights, such as a session entirely dedicated to HPLC for process analytical technology (PAT) and quality assessment in preparative chromatography, and a session focusing on high-performance thin-layer chromatography (HPTLC). A complete list of topics can be found on the HPLC website.

Thanks to its multidisciplinary character, the symposium represents an important source for analytical chemists, biochemists, and engineers seeking practical solutions to their problems. Researchers will be presenting their work as lectures in topical scientific sessions or in exciting poster sessions. We strongly encourage the active participation of younger scientists in the symposium. A

great amount of effort has been made to guarantee low student fees, including some special packages designed to give students the opportunity to attend short courses taught by highly reputed experts. We are offering 14 short courses on different topics, with descriptions found online.

A series of awards will be presented during the conference, including the Csaba Horváth Young Scientist Award, the Best Poster Award, the Uwe Neue Award, and many more. In addition to the traditional awards, **HPLC 2019** will feature new exciting ideas to recognize the scientific activity of young colleagues, which will be announced soon on the website and on social media.

A rich scientific and social programme awaits participants, starting with the opening ceremony in the exclusive location of the "Giuseppe Verdi" Conservatorio di Milano, the largest music academy in Italy and one of the most important worldwide. A unique opening event merging music and science will celebrate the genius of Leonardo da Vinci on the 500th anniversary of his death.

Milan is a cosmopolitan and vibrant centre of Italian culture. The various cultures of northern Europe and Mediterranean countries merge and as such Milan has a lot to offer, including art, museums, history, opera (La Scala), music, fashion, design, and Italian culinary delights.

Milan also offers a wide range of accommodation for all budgets, and with its three international airports it is easily accessible by plane, and also by car or train for those closer by.

The organizers look forward to welcoming participants in Milan in 2019!

E-mail: **hplc2019@effetti.it**
Website: **www.hplc2019-milan.org**


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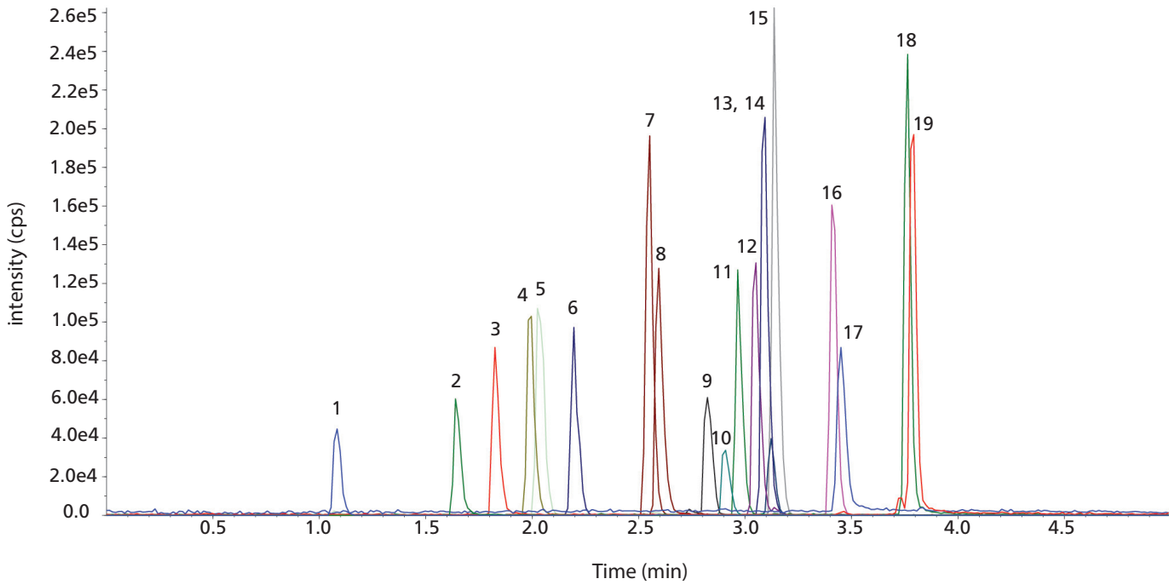
Rapid Extraction and Analysis of PPCPs From Sediments by QuEChERS and Liquid Chromatography Tandem Mass Spectrometry

Scott Krepich, Phenomenex, Inc., Torrance, California, USA

Pharmaceutical and personal care products (PPCPs) are routinely detected in a variety of aquatic environments and these compounds encompass a wide range of chemical and physical properties that contribute to their combined analytical screening challenges. Further examination of their accumulation throughout environmental samples that contain solids, including sediments, adds additional levels of analytical difficulties in their effective extraction and new sample matrix interferences. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) has become a very popular extraction and cleanup technique for the analysis of multiresidue pesticides in agricultural and food samples. Applying the same principles to overcome similar challenges in PPCP analysis in solid environmental samples is demonstrated in this article.

Figure 1: PPCP standard mix – 50 ppb ESI+.



Among the myriad of contaminants of emerging concern (CECs) that are expanding and persisting throughout the environment are a broad group of pharmaceuticals and personal care products (PPCPs) that include steroid hormones, industrial chemicals, and pesticides. These will reach the environment through routine human usage and disposal in wastewater where they can settle within diverse pockets of moist sediments.

A common technique for extracting a wide range of multiresidue pesticides out of both wet and dry solid agricultural and food samples is QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe). This technique is essentially a facilitated solvent extraction with salts into acetonitrile followed by dispersive solid-phase extraction (dSPE). When analyzing hundreds of multiresidue pesticides with diverse chemical and physical properties, it can be difficult, if not impossible, to provide effective sample cleanup through traditional retentive solid-phase extraction. As such, instead of targeting retention of analytes of interest, dSPE is applied to target removal of sample matrix components. Primary secondary amine (PSA) sorbents are routinely used to target and remove excess organic acids, small fatty acids, sugars, and anthocyanins. Endcapped C18 (C18E) sorbents are routinely used to target and remove excess fats, sterols, and other nonpolar interferences. Graphitized carbon black (GCB) sorbents are routinely used to target and remove pigments. These are the routine dSPE sorbents used in a traditional QuEChERS cleanup, and there are also customized dSPE sorbents designed to remove other specific matrix interferences without losing recovery of some analytes of interest.

Because parallel challenges exist for PPCPs in sediment analysis (effective extraction from solid matrices and many analytes with diverse chemical properties), a modified QuEChERS approach can be an elegant solution applied to the same underlying chemistry principles.

Methods

Sample Preparation—Modified QuEChERS Extraction: Two grams of dried sediment were weighed into a 50-mL polypropylene vessel and spiked with internal standard. Two preparations are required for each sample to cover the positive ion-mode and negative ion-mode analyses. The same sample preparation applies to each, and in this article the positive ion-mode case

Table 1: Positive ion-mode transitions and recoveries

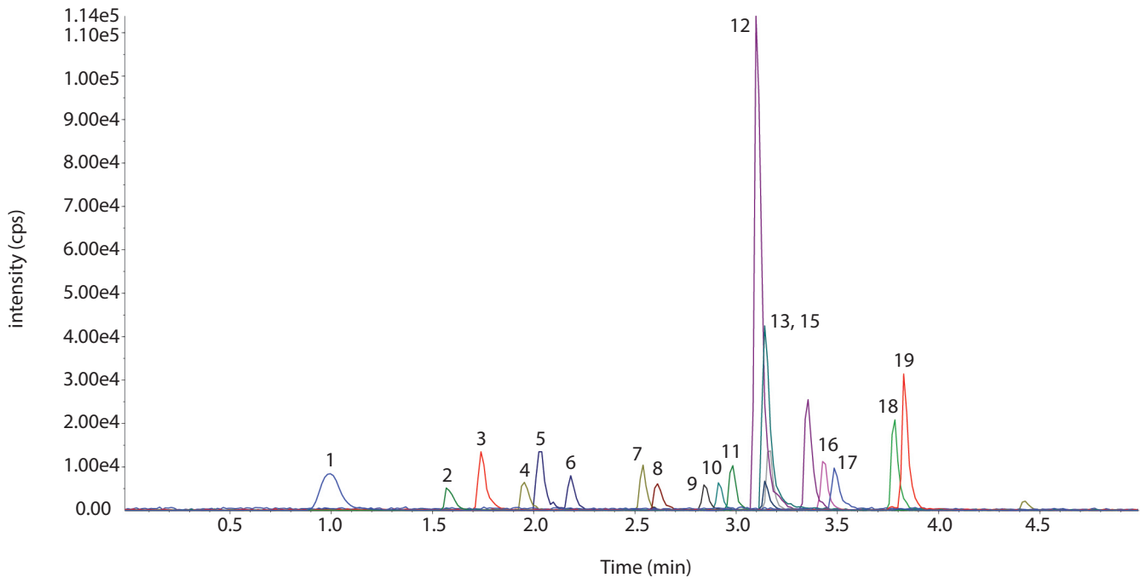
Peak Number	Compound	MRM Transition	Average Recovery (%)	%RSD
1	Atenolol	267.3 → 145.3	107	6
2	Trimethoprim	291.0 → 261.0	103	1
3	Caffeine	195.2 → 138.2	102	9
4	Sulfamethoxazole	253.9 → 156.0	96	1
5	Metoprolol	268.2 → 116.0	108	0
6	Primidone	219.2 → 162.0	110	5
7	Meprobamate	219.0 → 158.0	103	7
8	Propranolol	260.0 → 115.9	103	5
9	TCEP	284.9 → 222.8	106	6
10	Phentoin	253.0 → 182.1	99	9
11	Carbamazepine	237.1 → 194.1	115	5
12	Erythromycin	716.0 → 158.1	120	7
13	DEET	192.2 → 119.0	108	4
14	Fluoxetine	310.2 → 44.1	98	8
15	Carisoprodol	261.0 → 176.0	100	4
16	Diazepam	285.0 → 154.0	101	2
17	TCPP	327.2 → 174.7	98	3
18	Oxybenzone	229.2 → 151.0	92	12
19	TDCPP	431.0 → 99.0	119	7

is covered. Sand was used for the method blanks, and sediment and sand spiked with internal standards and recovery standards were used for the control sample.

A 10-mL measure of deionized water was added and vortexed, followed by 10 mL of 1% acetic acid in acetonitrile, and the subsequent slurry was vortexed.

Sodium Acetate and Magnesium Sulfate Extraction: QuEChERS salts were added and the slurry vortexed, and then the sample was centrifuged for 5 min at 4000 rpm.

Figure 2: PPCP extract – 50 ng/g ESI+.



Samples were then placed in a rack at -20 °C for 2 h to facilitate the extraction of the supernatant.

An 8-mL measure of the acidified acetonitrile supernatant was then transferred to a tube containing QuEChERS dSPE PSA sorbent (Phenomenex). For negative ion-mode analysis, the supernatant would be cleaned up with PSA and C18 dSPE sorbent.

Tubes were then centrifuged for 10 min at 3000 rpm, and the supernatant decanted and filtered through a 0.2-µm syringe filtered before drying under a gentle stream of nitrogen at or below 35 °C.

A 50-µL measure of acetone was then added to the dried sample and vortexed to dissolve any residue; 950 µL of 50% methanol in water solution was then added ready to transfer to an autosampler vial for analysis.

The liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis was performed on a 50 × 2.1 mm, 2.6-µm Kinetex core–shell C18 column (Phenomenex) with a formic acid in water

and methanol gradient in 8 min on a Triple Quad 4500 (Sciex) (Figure 1).

Results and Discussion

Average spike recoveries were within 80–120%, and precision was below 15% RSD (Table 1).

The modified QuEChERS method proved to be a very simple and effective method for the determination of PPCPs in sediments. Sample throughput was improved significantly by reducing the average sample processing time to less than 4 h compared to upwards of 12 h for a typical Soxhlet extraction.

Ion suppression and enhancement challenges were also reduced by removing sediment interferences in the dSPE step of the sample preparation (Figure 2).

Upon sample dry-down, sometimes a brown residue persisted that could hold onto some of the analytes and internal standards and decrease their recovery. Adding a small amount of acetone to initiate the sample reconstitution helped to dissolve this residue and improve recoveries.

Conclusion

PPCPs are detected in many different aquatic environments and aside from analyzing the water source directly, sediments must also be analyzed to understand the fate of these compounds. The outlined modified QuEChERS extraction protocol effectively recovers these diverse range of compounds from the solid and semisolid sediments and eliminates matrix interferences that can disrupt accurate LC–MS/MS analysis.

Acknowledgements

Special thanks to the Sanitation Districts of Los Angeles County – San Jose Creek Water Quality Laboratory for their contributions.

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Enhanced Detection of Oxygenated Polycyclic Aromatic Hydrocarbons Using Atmospheric-Pressure Chemical Ionization-Mass Spectrometry

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Polycyclic aromatic hydrocarbons (PAHs) and their oxygenated derivatives (oxy-PAHs) are highly toxic carcinogens that present a significant hazard to human health. To fully understand the risks associated with exposure to PAHs, robust analytical methods for their detection are required. Mass spectrometry coupled with ultrahigh-performance liquid chromatography (UHPLC–MS) has proven to be a powerful technique for the analysis of these compounds. This article looks at the benefits of using atmospheric-pressure chemical ionization (APCI) in the place of traditional electrospray ionization (ESI) for the detection of oxy-PAHs.

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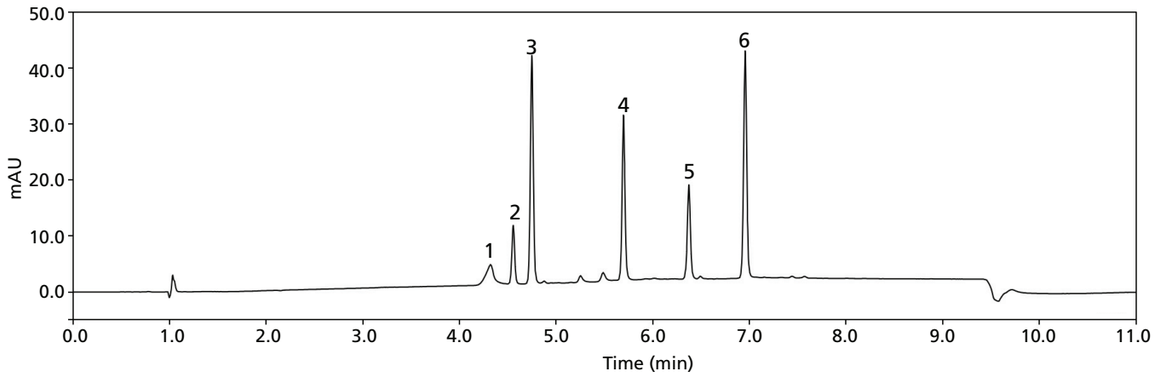
Polycyclic aromatic hydrocarbons (PAHs) are a diverse family of environmental pollutants that are widely recognized as highly toxic and carcinogenic to humans (1). Comprised of multiple fused aromatic rings, these compounds are produced by the incomplete combustion of carbon-based materials, predominantly as a result of human activity, such as the burning of fuel for cooking and heating, vehicle emissions, and power generation, but also from natural phenomena, such as wildfires. PAHs can undergo further reactions in both the gas and condensed phase to produce oxygenated derivatives (oxy-PAHs), which have similar harmful health effects (1).

Individuals may be exposed to PAHs and oxy-PAHs in different ways, with food being a major source (2,3). PAHs can enter the food chain by deposition and transfer from air or soil, or through specific food preparation practices. For example, foods that are grilled, roasted, or smoked during preparation can contain higher levels of PAHs (3). Given the serious health concerns associated with PAHs, and the high risk of human exposure by inhalation and ingestion, the analysis of these compounds in a wide range of materials has been the focus of several environmental and nutritional studies (2–5).

Gas chromatography–mass spectrometry (GC–MS) has proven to be a capable analytical technique for the analysis of PAHs (1,4,5). However, for more polar, functionalized variants, such as oxy-PAHs, reduced volatility and greater

Figure 1: UV chromatogram (254 nm) of oxy-PAH separation (10 ng on column per analyte); peak assignments are shown in Table 1.



potential for adsorption mean these substances often cannot be detected with sufficient sensitivity using GC–MS. While derivatization can sometimes be used to overcome these analytical challenges (5), this process can be time-consuming and derivatization reactions may not always proceed to completion.

Alternative techniques based on high performance liquid chromatography (HPLC) coupled with either optical or MS detection offer a faster and more convenient approach to oxy-PAH analysis (1,6). While electrospray ionization (ESI) is generally the preferred ionization technique in most LC–MS applications, studies have shown that atmospheric-pressure chemical ionization (APCI) offers improved performance when it comes to oxy-PAH analysis (1,7). This article compares APCI-MS and ESI-MS detection for the analysis of six oxy-PAH standards using ultrahigh-performance liquid chromatography (UHPLC) and otherwise identical conditions.

Experimental

Standard Preparation: Standard stock solutions of 2-naphthoic acid, 2-naphthol, benzanthrone, 1-hydroxypyrene, 1,4-anthraquinone, and 1-tetralone were prepared in methanol at a concentration of 1 mg/mL. A working solution for injection into the UHPLC–MS system was prepared by mixing the stock solutions and diluting with methanol to give a final concentration of 10 µg/mL for each analyte.

Figure 2: Comparison of MS peak areas and *S/N* in positive (+) and negative (-) APCI and ESI mode for the six oxy-PAHs.

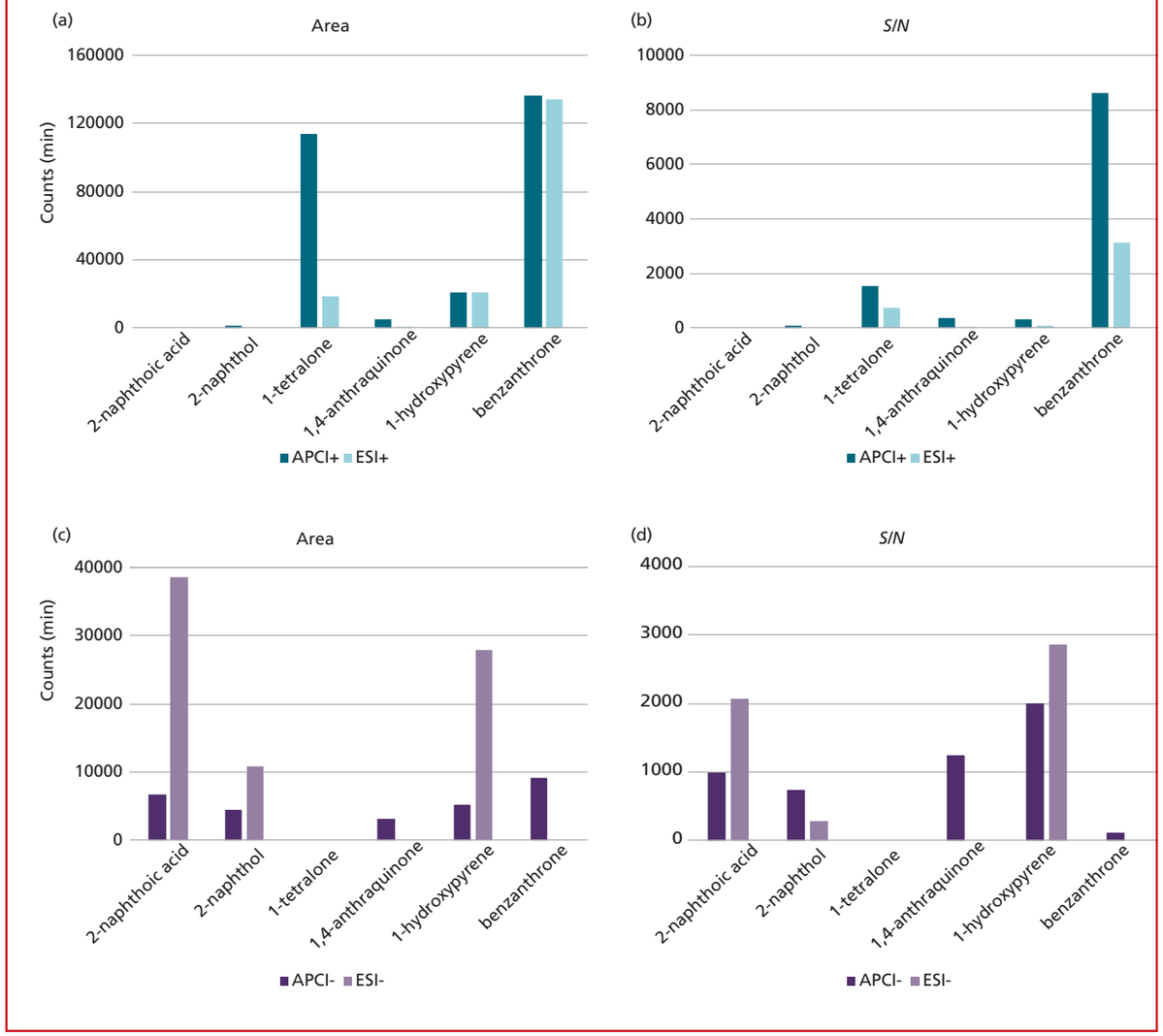


Table 1: Overview of oxy-PAHs retention times (t_R[UV]), average molecular weight (MW), and observed most abundant *m/z* (+/-, positive/negative mode; n.d., not detected). *m/z* values shown in parentheses were hardly detectable from full scans but detectable in selected ion monitoring (SIM) scans. Green shaded fields indicate the best MS detection results for each compound.

	Compound	t_R (UV) (min)	MW (Da)	*m/z* APCI +	*m/z* APCI -	*m/z* ESI +	*m/z* ESI -
1	2-naphthoic acid	4.33	172.2	n.d.	171.0 $[M-H]^-$	n.d.	171.1 $[M-H]^-$
2	2-naphthol	4.56	144.2	(145) $[M+H]^+$	143.1 $[M-H]^-$	n.d.	143.1 $[M-H]^-$
3	1-tetralone	4.75	146.2	147.2 $[M+H]^+$	n.d.	147.2 $[M+H]^+$	n.d.
4	1,4-anthraquinone	5.70	208.2	209.1 $[M+H]^+$	208.0 $[M]^-$	(209.1) $[M+H]^+$	n.d.
5	1-hydroxypyrene	6.37	218.3	219.1 $[M+H]^+$	217.0 $[M-H]^-$	218.1 $[M]^+$	217.0 $[M-H]^-$
6	benzanthrone	6.95	230.3	231.1 $[M+H]^+$	230.0 $[M]^-$	231.1 $[M+H]^+$	n.d.

Analytical Conditions: A Vanquish Flex Binary UHPLC system (Thermo Fisher Scientific) equipped with an ISQ EM single quadrupole mass spectrometer (Thermo Fisher Scientific) was used for the analysis. Chromatographic separation was achieved using a 150 × 2.1 mm, 3-µm Hypersil Green PAH column (Thermo Fisher Scientific), using a water–methanol gradient method and sample injection volume of 1 µL.

Data Analysis: Chromeleon 7.2.9 Chromatography Data System software (Thermo Fisher Scientific) was employed for data acquisition and processing.

Detection of Oxy-PAH Standards Using APCI-MS and ESI-MS

Baseline separation for all six oxy-PAH standards was achieved (Figure 1), as well as small amounts of impurities visible in the UV chromatogram, which may have

resulted from synthesis of the standard compounds.

Switching from ESI to APCI detection could be achieved by changing the source probe and turning the APCI needle to the appropriate position. The molecular masses and most abundant mass-to-charge ratio (*m/z*) values for the six oxy-PAHs detected using the various experimental conditions are shown in Table 1. As a result of the different ionization mechanisms associated with each ionization mode, in some cases the ions generated were not the same.

For both ESI and APCI, when operating in positive mode, most ions formed as the protonated molecular ion $[M+H]^+$. In negative mode, formation of the deprotonated molecular ion $[M-H]^-$ was favoured. Notable exceptions were 1-hydroxypyrene, 1,4-anthraquinone, and benzanthrone. 1-Hydroxypyrene was detected as the molecular ion $[M]^+$ in positive ESI mode. Although 1,4-anthraquinone and benzanthrone were detected as the molecular ion $[M]^-$ in negative APCI mode, these were not detected in negative ESI mode. This difference is due to a process known as *electron capture negative ionization*, which is observed when using APCI for molecules that lack an abstractable hydrogen.

1-Hydroxypyrene was detected under all four experimental conditions. 2-Napthoic acid, 2-napthol, and 1-tetralone were detected using both APCI and ESI, although in just one polarity in full scan mode. Using a selected ion monitoring (SIM) scan of the $[M+H]^+$ ion, 1-napthol was also detected in positive APCI mode. The $[M+H]^+$ ion of benzanthrone was easily detected in both positive ionization modes. However, while APCI generated the $[M]^-$ ion in negative mode, its detection was not possible in negative ESI mode. 1,4-Anthraquinone provided good signals in positive and negative polarity modes using APCI, but was not observed in either of the full scans using ESI. Only by performing a SIM scan could the $[M+H]^+$ be detected under ESI conditions, albeit as a weak signal.

As well as the oxy-PAH molecular ions, additional species attributed to adducts were observed in the ESI spectra. In negative mode, a signal at *m/z* 365.0 was detected for 2-naphthoic acid, while in positive mode, signals at *m/z* 247.1 and 301.2 were detected for 1-hydroxypyrene and at *m/z* 253.1 and 285.1 for benzanthrone. By contrast, when using APCI, just one adduct was observed at *m/z* 179.1 for 1-tetralone.

Detection of Oxy-PAHs Using APCI-MS

The MS peak areas and signal-to-noise ratio (*S/N*) obtained for the most abundant *m/z* signals under all four experimental conditions are compared in Figure 2. Figures 2(a) and 2(b) reveal that the signal responses obtained using APCI in positive mode were comparable or higher than with ESI, while the *S/N* achieved for each of the detected compounds were better using APCI. Figures 2(c) and 2(d) show that the peak areas for the three compounds detected using ESI negative mode were higher than those recorded using APCI negative mode. However, the *S/N* is only higher for two of these analytes (2-napthoic acid and 1-hydroxypyrene). Importantly, APCI operating in negative polarity mode enables the detection of two additional analytes (1,4-anthraquinone and benzanthrone).

Conclusion

Although conventional ESI showed higher detection sensitivity for some analytes when operating in negative polarity mode, APCI enabled convenient detection of all six oxy-PAHs studied. This compares to just one of the standards being barely detectable when using ESI mode. Taken together, these results demonstrate that APCI-MS offers several benefits over ESI-MS, and may be considered the preferred ionization mode for UHPLC–MS analysis of oxy-PAHs.

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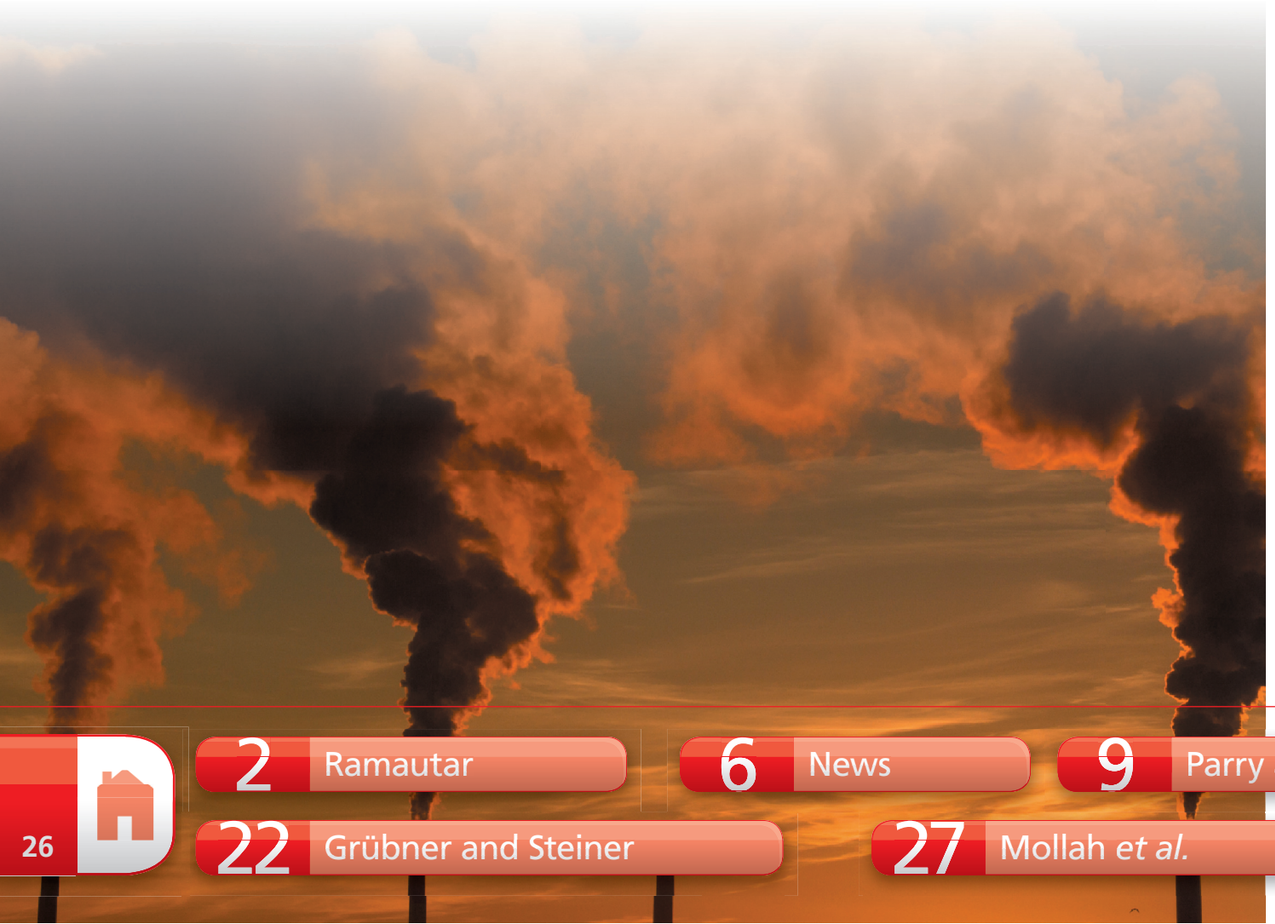
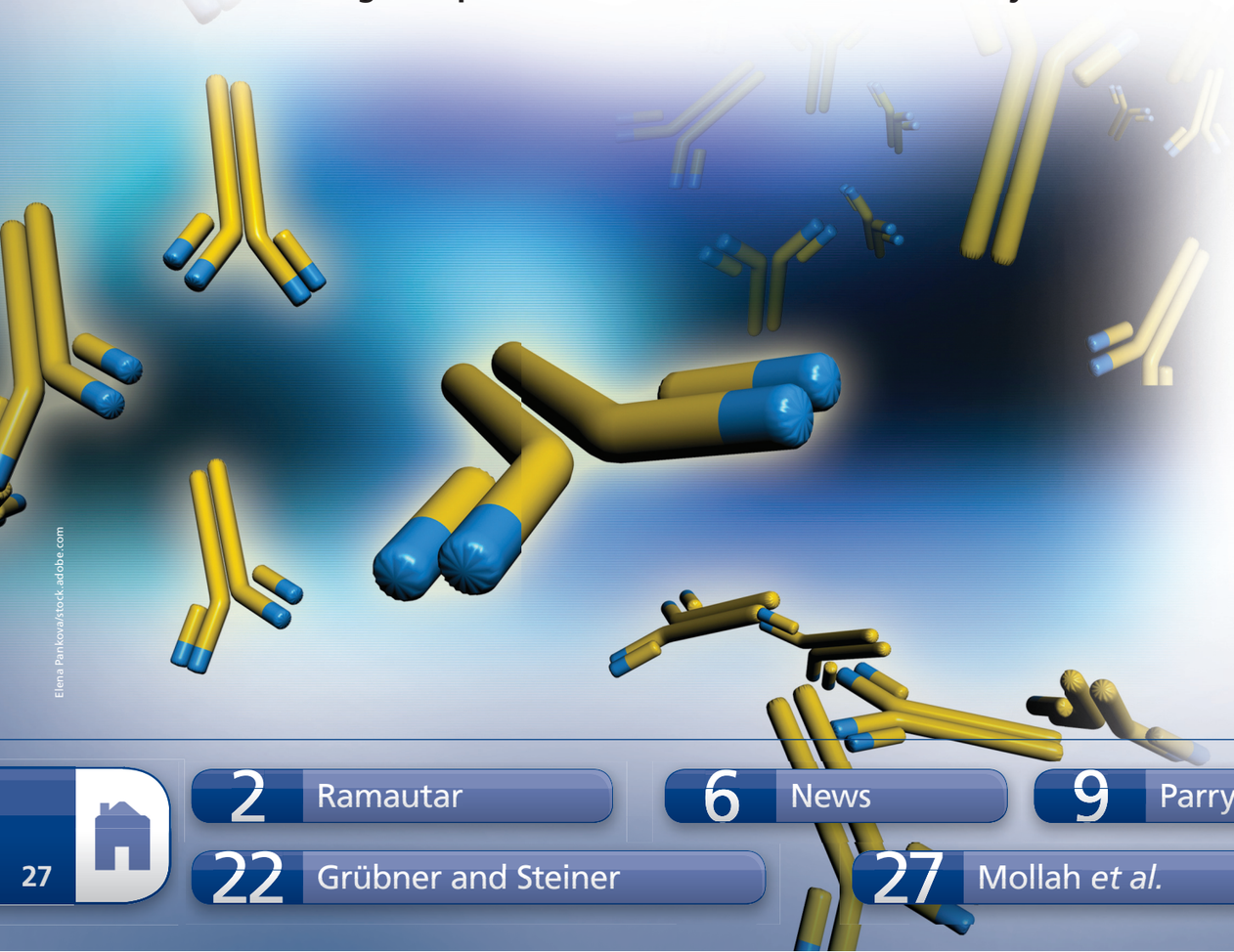


Drug Development: Overcoming Glycan Screening Bottlenecks for Clone Selection and Cell Culture Optimization

Sahana Mollah, Mark Lies, Marcia Santos, Tingting Li, and Kelly May, Sciex, Brea, California, USA

Glycosylation is one of the most common protein post-translational modifications (PTMs), so detecting changes in glycosylation is important for researchers to determine glycan profiles that may affect the function, efficacy, and clearance of novel biologic drugs. As glycans are so important for biological function, screening glycan profiles early in the clone selection and cell culture optimization process of drug development can provide better information for selecting the right clones. Doing this, however, has traditionally meant setting aside an entire day for sample preparation before performing the analysis the next day. In order to reduce the challenges of tedious sample preparation, long separation times, and data analysis, researchers need to accelerate the workflow to allow screening of more glycan samples per day, and obtain visual, actionable data without the need for time-consuming interpretation. This article focuses on ways to accelerate glycan screening data analysis, while keeping high reproducibility.



Elena Pankova/stock.adobe.com

A major focus of the pharmaceutical industry is the production and development of biologics—therapeutics produced via biological means—to provide novel treatments for diseases with unmet clinical needs. A large percentage of biologics under development are proteins, such as monoclonal antibodies (mAbs), fusion proteins, antibody–drug conjugates (ADCs), and enzymes. The structures of these protein drugs are made more complex by post-translational modifications (PTMs).

Glycosylation is a particularly prevalent and structurally complex PTM that results in the addition of carbohydrate-based moieties called *glycans*. Glycan chains markedly affect the protein stability, activity, antigenicity, and pharmacodynamics, and are therefore of major importance to the development of a successful biologic that exhibits optimal therapeutic efficacy (1).

To ensure the safety and potency of commercial biologics before regulatory approval, appropriate glycosylation must be demonstrated (2). It is also necessary to demonstrate that the manufacturing process is robust and can be reproduced for batch release. Glycosylation is likely among the most crucial quality attributes and the most difficult parameter to control (3).

There are a variety of analytical methods available for characterization at the intact protein level, including chromatography, electrophoresis, and mass spectrometry (MS). Recent advances

Figure 1: Fully automatable glycan sample preparation workflow (7).

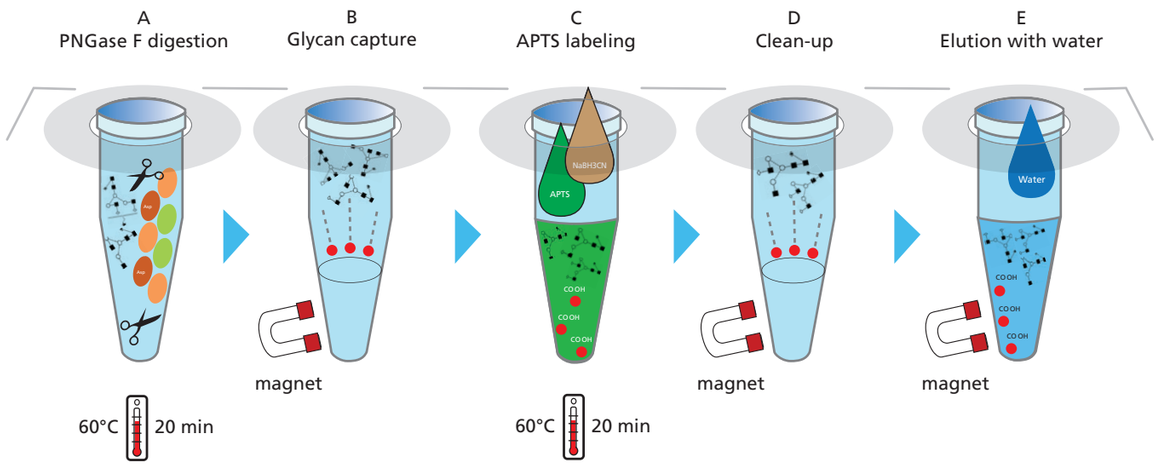
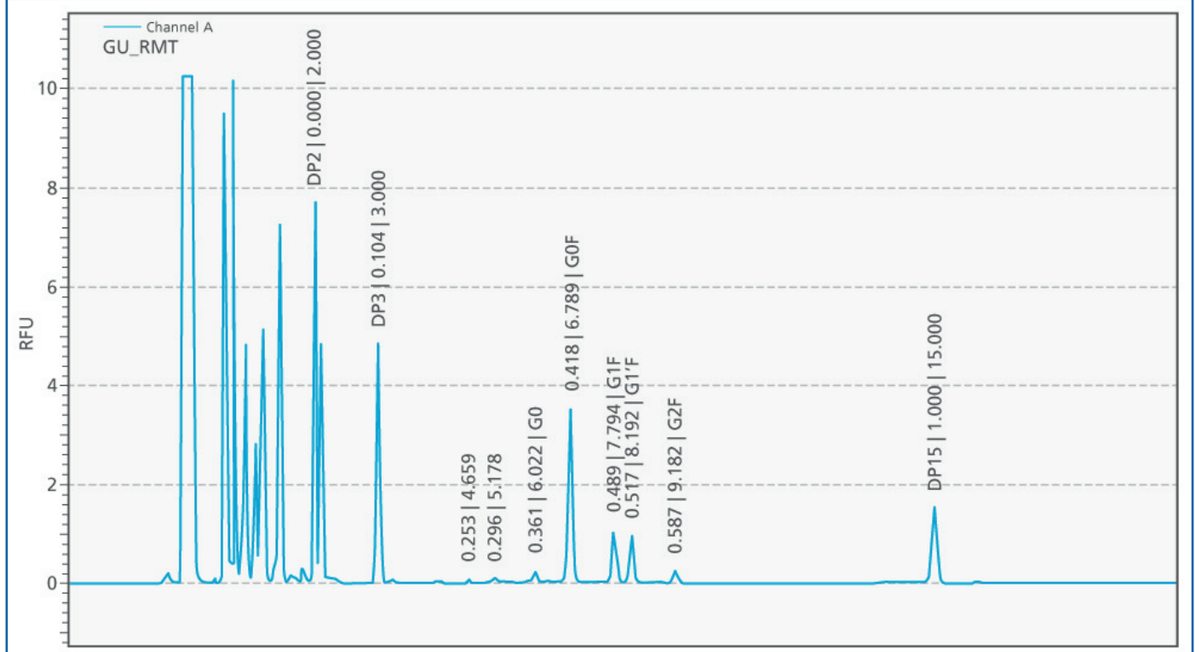


Figure 2: Electropherogram view of the detected glycan profile in the analyzer user interface.



in capillary electrophoresis (CE) techniques have been used for detailed characterization of glycans attached to biotherapeutic proteins. As CE is a versatile analytical platform, it offers a rapid, yet simple method for exhaustive carbohydrate screening (4,5).

The Challenges

Although there have been important advances, glycan screening remains a challenge in the biopharmaceutical industry. The sample preparation can be tedious, labour-intensive, and technique-dependent. Separation of carbohydrates is demanding because of the complex, diversified structure of glycans; they also lack chromophore or fluorophore groups and most glycans have a neutral charge. Furthermore, the complexity of glycans that can be added to a protein contributes to its heterogeneity, leading to questions as to the biological significance of the multitude of glycan peaks that can appear during profiling. Beyond the experimental difficulties associated with glycan analysis, data interpretation and structural identification are perhaps the greatest bottlenecks for streamlined glycan analysis (6,7).

Here we present an automated, high-throughput glycan screening method using qualitative, multiplexed CE. By using a fully automated magnetic bead-based sample preparation, the glycosylation pattern of a therapeutic antibody under investigation was separated and identified by CE.

Acceleration and Reproducibility of High-Throughput Glycan Screening

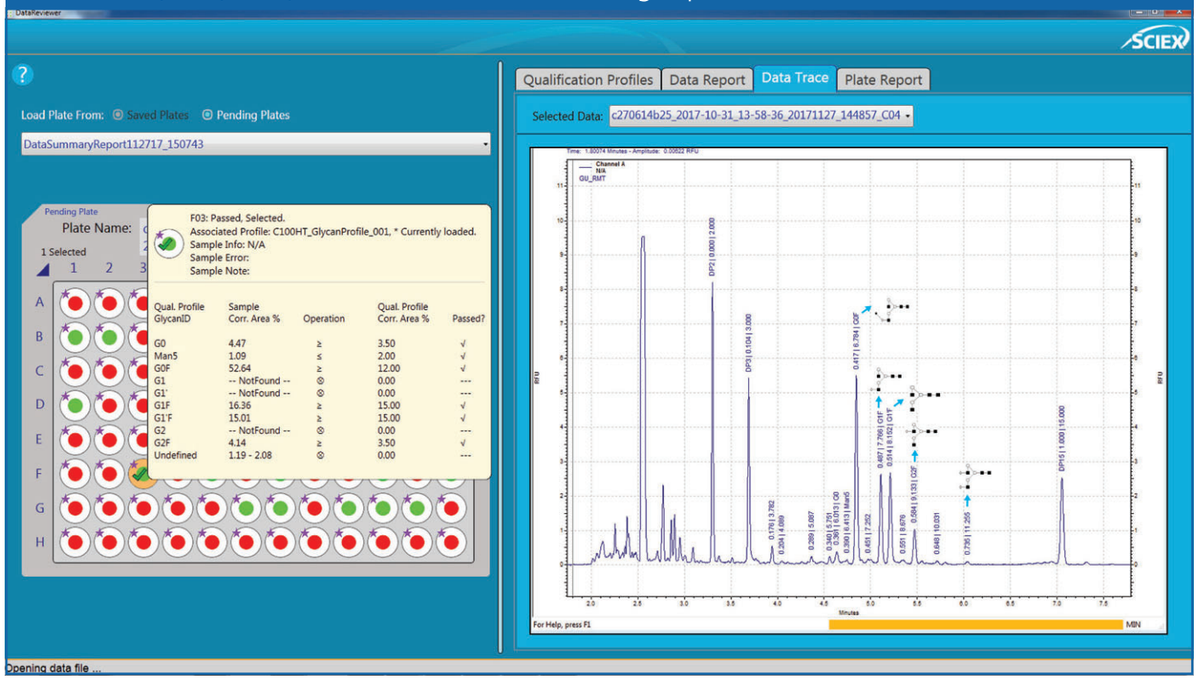
Glycan acceptance criteria need to be quick and intuitive and allow real-time decisions on clone selection and cell-line optimization. Furthermore, automated glycan identification would eliminate the need for tedious glycan database searches, thus minimizing the potential for human error and delivering a more robust workflow.

To test a high-throughput screening method for speed and reproducibility, a multiplexed capillary electrophoresis platform providing the screening of large numbers of candidates quickly enough to allow for real-time adjustments was evaluated (8).

Method

A purified antibody—MAK33—obtained from Roche Diagnostics was diluted to 10 mg/mL before sample preparation. Automated sample preparation including denaturation, digestion, and glycan labelling was performed with a setup for 96-well plates (Beckman Coulter Biomek i5). The antibodies were denatured for 8 min at 60 °C using the denaturing solution master mix (Figure 1, Step A) and digested

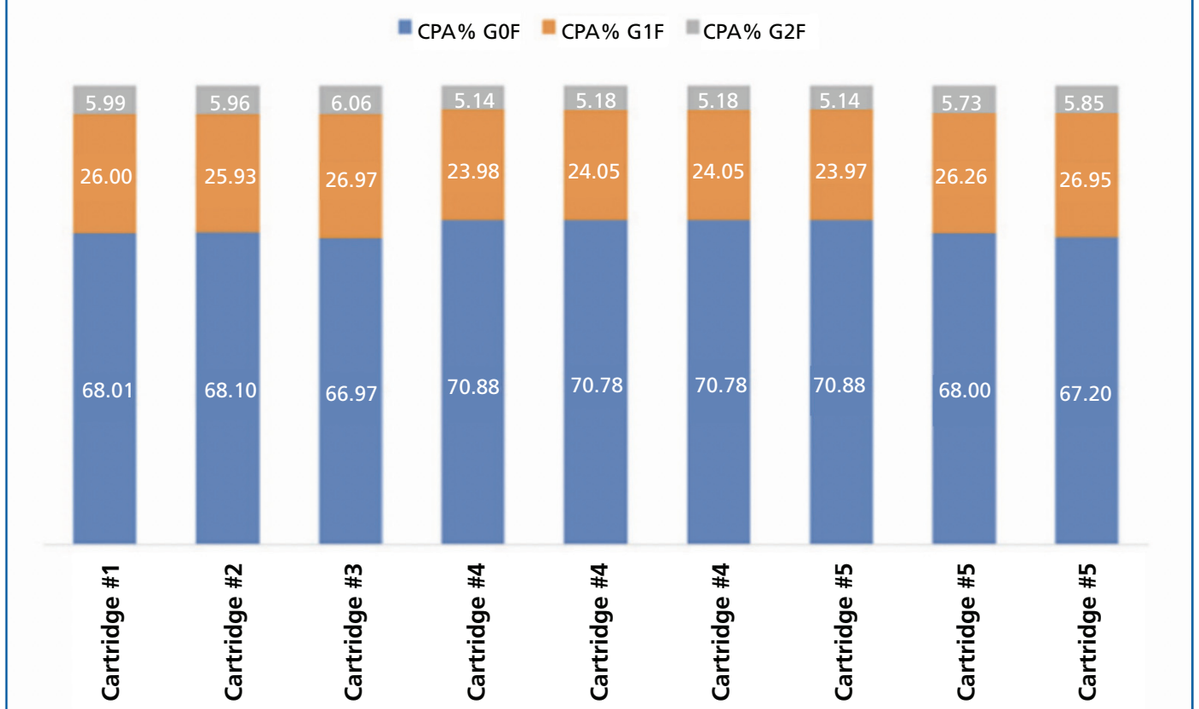
Figure 3: Qualification profile and dynamic pass/fail display of the wells on a 96-well plate. In the left panel of this figure, a plate map in the software illustrates that all 96 samples were selected for data analysis, as indicated by the green check marks. Glycans released from MAK33 were analyzed for corrected peak area percentage between each sample well for G0, G0F, G1F, G1'F, and G2F species. The RSD% for 96 samples is below 9% for G0, and around 6% for G0F, G1F, G1'F, and G2F as shown in the right panel.



for 20 min at 60 °C using PNGase F (Figure 1, Step B). The released glycans were captured by magnetic beads, and then labelled with 1-aminopyrene-3,6,8- trisulfonate (APTS) for 20 min at 60 °C (Figure 1, Step C). Excess APTS was removed from the reaction mixture using a magnetic-bead-mediated series of washes (Figure 1, step D). The labelled N-linked glycans were eluted from the beads by 50 µL of water (Figure 1, Step E).

At this point, the samples were ready to be analyzed by electrophoretic separation. The C100HT Biologics Analyzer (Sciex) was set up with a pre-filled buffer tray, sample plate, and gel-preloaded multicapillary separation cartridge. Following that, the appropriate pre-configured process profile was selected and sample information entered. Analysis of a full 96-well plate took approximately 170 min and the separation and detection of the glycan

Figure 4: The average of corrected area percentage observed during the study with three systems and five cartridges, used for a total of 865 runs performed.



profile was visualized by the anchor peaks in the electropherogram (Figure 2). A qualification profile for each glycan of interest was pre-set in order to define the glycan composition of each sample and presented as pass or fail (Figure 3). DataReviewer Software in the C100HT from Sciex was used to analyze the data (8.9).

Results and Conclusions

An intermediate precision study (Figure 4) was carried out using three systems, five cartridges with total 865 runs to illustrate the robustness and reproducibility of this method. The chart shows the consistency in corrected area percentage of three major glycan species found in MAK33. The method can screen glycan expression profiles for up to five 96-well plates of glycans samples in one day. Automated APTS labelling and cleanup reduces the need for human intervention while providing better precision and minimizing variation between samples.

Producing optimal and consistent glycosylation requires an integrated approach for successful biopharmaceutical drug development with systematic glycosylation analysis throughout the manufacturing processes. A dramatically shortened screening time enables clones to be selected from large sample populations quickly and cell culture optimization to be performed in parallel, thereby increasing the likelihood of selecting the best clones and manufacturing conditions. From a productivity perspective, good quality high-throughput data allow confident decisions on clone selection and cell optimization in real time, early in the process, providing more cost-efficient workflows.

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