LCGC™
INTERNATIONAL

DECEMBER 2024

Ion Mobility Mass Spectroscopy

Efficient PFAS Feature Prioritization

Agilent 6475 LC/TQ

PFAS Screening and Characterization

Methods, Tools, and Environmental Insights

This custom ebook is sponsored by Agilent Technologies and presented in partnership with *LCGC International*.

Agilent

From Pesticides to PFAS:

Using Ion Mobility Spectrometry in Non-Targeted Exposomic Analyses

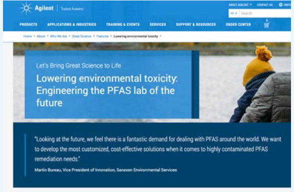
Adding ion mobility spectrometry optimizes chromatography-mass spectrometry workflows for PFAS characterization among complex sample environments. *By LCGC Staff*

INTRODUCTION

With growing evidence that surrounding environments can impact human health, the role of chemical exposure is receiving greater scrutiny. Poly- and perfluoroalkyl substances (PFAS) is one such class of molecules receiving attention recently for their potential ill effects and are of note for how pervasive PFAS are in consumer products and correspondingly being leached into the environment.

Cover: Evodigger - stock.adobe.com; 김대수 김대수 - stock.adobe.com

Characterizing PFAS in both the organisms and their surroundings are key for assessing what PFAS are in the natural environment, but also for determining PFAS presence in living organisms and their associated health impacts. For full chemical characterization, the combination of ion mobility spectrometry with chromatographic separations and mass spectrometry analysis provides a valuable reservoir of high dimensional chemical data for thorough exposomics. This multi-modal platform creates a wealth of datapoints to facilitate enhanced chemical specificity regarding chemical species beyond what can be gleaned from chromatography and mass spectrometry alone. Workflows such as these have proven valuable for deeper characterization of additional PFAS species in a range of sample types, including those from the environment. Assessing samples for xenobiotic molecules like PFAS from the environment and animals can inform how chemical exposure impacts human health.



Let's Bring Great Science to Life

Lowering environmental toxicity: Engineering the PFAS lab of the future

"Looking at the future, we feel there is a fantastic demand for dealing with PFAS around the world. We want to develop the most customized, cost-effective solutions when it comes to highly contaminated PFAS remediation needs."

Martin Bureau, Vice President of Innovation, Sanexen Environmental Services

FEATURE

Lowering environmental toxicity:
Engineering the PFAS lab of the future

Human Health Impacts and PFAS Exposure

There is recent thought that the environmental exposure and chemical exposure may be a source of risk for some diseases. From the human genome project, over 90% of diseases were determined to be due to causes outside of genetics. Pesticides, plastics, and other xenobiotic chemicals are present that may be contributing to ill health. PFAS are highly fluorinated example chemical species that are present in a wide variety of different products including but not limited to firefighting foams, clothing, paint, toilet paper, and food packaging that are starting to be implicated in negative health effects.

Considering how pervasive PFAS are in the environment and daily life, the question remains regarding how these compounds are impacted by living organisms, and what kind of harmful changes may come from contact with these compounds. Many of these compounds can bioaccumulate and can impact endogenous molecules, so there is a need to assess these effects. To make progress toward answering these questions, there is a need to identify what compounds are present and directly measure the quantities of PFAS in not only the human body, but also within larger ecosystems where humans live.

Big Data Analysis via Chromatography, Ion Mobility, and Mass Spectrometry

When selecting analytical techniques for a range of 'omics realms, the typical approaches selected include chromatographic separations and mass spectrometry.

Liquid and gas chromatography methods typically leverage difference in polarity or boiling point as a mode to separate each analyte before mass analysis. With mass spectrometry, a multi-stage approach using a fragmentation stage for monitoring both precursor and fragment species permit solving the puzzle of what PFAS are present, and even determine if new PFAS are present.

However, chromatographic separations alone can be insufficient for teasing apart highly similar molecules, notably isomeric chemical species that have comparable physical properties but differ in shape. Ion mobility spectrometry offers a gas-phase option for size-to-charge ratio differentiation of analytes and is a technique that can be readily incorporated with chromatography and mass spectrometry. In drift tube ion mobility spectrometry, a neutral buffer gas such as nitrogen fills the drift tube chamber that also has an electrical gradient applied. As ions are pulsed into the drift tube, the ions collide with the buffer gas in a low energy, tumbling manner that does not result in fragmentation. For two isomeric molecular species with the same m/z with distinct arrangement of atoms in their structures, the more compact molecule will have fewer collisions with the buffer gas and arrives sooner at the mass spectrometer inlet than the larger structure that will more collisions with the buffer gas. Correspondingly, the differences in drift time between these species creates another dimension of information in addition to chromatographic separation and mass analysis, which allows for the two distinct isomers to be revealed. Because IMS separations occur on a millisecond time scale, ion mobility spectrometry can fit readily between minute timescale chromatography and sub-millisecond mass spectrometric analysis. Moreover, the ion-neutral collision cross section (CCS) can be obtained from IMS separations, providing essentially a surface area for molecules. Data resulting from chromatography, IMS, and MS used in combination are notoriously complex. With retention time, drift time, and m/z, there can be thousands of distinct features in even one sample run. Varying analysis parameters to optimize results will continue to multiply the data, causing significant complexity when extracting information during post-run sample processing.

Ion mobility spectrometry offers a gas-phase option for size-to-charge ratio differentiation of analytes...

Plotting CCS as a function of m/z can yield the IMS and MS trendlines to identify molecular classification and features. Additionally, CCS databases provide another property dimension for identifying unknown species. Halogenated molecules such as PFAS are higher density xenobiotics and correspondingly have resulting trendlines that have a distinctly shallower slope relative to the biological molecule space on CCS and m/z plots. This provides additional means to distinguish PFAS analytes from the copious nucleic acids, proteins, lipids, sugars, and other endogenous species in biological samples. Distinguishing xenobiotics from endogenous species is particularly crucial within non-targeted analytical workflows wherein the CCS versus mass-to-charge ratio (*m/z*) trendline features provide an important route of distinction between endogenous molecules and xenobiotics. A non-targeted approach aims to identify chemical species with properties consistent with intended analyte classes for detection that can then be followed with tandem mass spectrometry in combination with CCS data to elucidate structural information. With sufficient species resolution in the drift time domain, isomers can be subjected to fragmentation according to drift time and tease out distinct fragmentation mass spectra for the isomeric species.

This non-targeted multidimensional approach will be invaluable for characterizing the 14,000 PFAS from the EPA master list and countless others not yet characterized.

PFAS Identified in Environmental Water and Fish Samples

Recent work applying chromatography, ion mobility, and mass spectrometry to PFAS analysis in environmental samples assessed the presence of PFAS within lakes and rivers in North Carolina, US. In essence, passive aquatic sampling was accomplished via stationary phase material attached to floating rubber embroidery hoops. The hydrophobic stationary phase can attract nonpolar molecules like PFAS present in the water systems, deployed both below a chemical manufacturing plant at King Bluffs, NC and above the manufacturing plant at Jordan Lake in NC. The upriver site at Jordan Lake was found to have 20 different PFAS, while the downriver King Bluffs site had the same 20 PFAS plus an additional 57 fluorinated species. Of the additional 57, 36 were known PFAS species and the remaining 21 were unknown PFAS compounds that were investigated further with nontargeted characterization. A total of 14 unique molecular formulas based upon retention time, CCS, and m/z data gathered, and additional tandem MS data yielded 11 different proposed structures. The remaining 3 structures with identifiable molecular formulas had insufficient tandem MS data for proposing corresponding structures. Some examples of the novel PFAS identified that were not present on the US Environmental Protection Agency (EPA) PFAS master list include PFBESA and PFPeESA, which are two members of a homologous series differing by a

CF_2 unit within the perfluoroalkyl chain on the core structure. The characterization was aided by the linearity with which retention time versus *m/z* and CCS versus *m/z* trendlines fell across the series and were correlated further on fragmentation spectra from tandem MS. This non-targeted multidimensional approach will be invaluable for characterizing the 14,000 PFAS from the EPA master list and countless others not yet characterized. Moreover, only 6 of the known PFAS have had regulations proposed, leaving a large gap between with known and potential PFAS.

Within the same locations for water sampling, filets obtained from fish living in those bodies of water were also analyzed for PFAS. Among the fish samples, 22 PFAS were identified but only 4 of those species overlapped with 13 PFAS found via targeted analysis in water samples. The primary trend observed between species identified in water and those in fish was that the longer carbon chain PFAS species tended to be found within the fish tissue, while the shorter carbon chain PFAS were found in water. Comparison with additional sources like sediment or plants may reveal even more PFAS species.

Alligators as Sentinel Species for PFAS Exposure and Health Impacts

In collaboration with NCSU and Clemson, the chromatography, IMS, and MS platform was used for characterizing PFAS in alligators. Alligators can act as sentinel species for human exposure due to alligators' long lives of about 60 years or more, their role as apex predators, and their proximity to human habitats. Alligators sampled in a catch and release manner were assessed for their external health appearance including looking for wounds and culturing wounds, checked for microchips, assessed for length and girth, blood samples collected, and marked with an orange dot to clarify that a given one has been sampled already before release. Use of a portable laboratory permitted blood sample preparation such as centrifugation and flash freezing for endogenous molecule studies. Alligator sampling was performed at a range of sites including a known PFAS near a PFAS manufacturer, a presumably clean reference site that is not directly connected via body of water, a known contaminated site that is connected via body of water, coastal North Carolina, and Florida. In the last four years of sampling, 191 plasma samples have been obtained. In total, there were 37 PFAS identified within the North Carolina alligators that fell within 6 different subclasses of PFAS compounds. With a focus on sites A, B, and C–which are the clean reference, the site near a chemical manufacturer, and the known contaminated site, respectively–site A formed a distinct


Guide to Targeted Quantification and Screening of PFAS Compounds in Environmental Matrices

Agilent

PCA cluster while sites B and C tended to cluster together. Moreover, sites B and C had notably higher quantities of PFAS overall relative to the clean reference site A.

A focus on the perfluorooctanesulfonic acid (PFOS) sub-class sampling results can provide some interesting comparisons to a study conducted on people living in the Wilmington, NC area. This study was known as the Gen X study because of the high level of a PFOS compound called Gen X that was released into the nearby Cape Fear River. In the alligators sampled at the clean reference location, site A, the average PFOS concentration in blood was 34.6 ng/mL, whereas at site B, the location with known contamination from the factory upriver, had alligators with an average PFOS blood concentration of 205 ng/mL. The roughly 6-fold increase in PFOS content in blood from alligators living in the two distinct environments correlates well with the sites' respective locations regarding presence versus absence of connection via body of water to the PFAS manufacturer upriver. In comparison, human blood samples from the Wilmington area had an average PFOS concentration of 9.4 ng/mL. Although humans are not actively living in the exact same circumstances as alligators, knowing about the exposure levels in alligators can be informative for humans encountering the river itself, consuming fish caught from the river, and other exposures.

...knowing about the exposure levels in alligators can be informative for humans encountering the river itself, consuming fish caught from the river, and other exposures.

Conclusions

As questions are beginning to arise regarding potential connections between chemical exposure and ill health, developing analytical workflows to identify xenobiotics like PFAS is crucial. Past approaches employing just chromatography-mass spectrometry have come to be realized as insufficient for complete chemical characterization but introducing IMS into these platforms opens new avenues for parsing highly similar analytes. Even for non-targeted qualitative detection of unknown molecules, use of IMS allows for comparison to emerging CCS libraries that offer additional angles to assist in molecular identification. Chromatography, ion mobility, and mass spectrometry coupled together have proven effective for identifying distinct PFAS species within not only water samples, but also amongst greater matrix complexity found in fish tissue and alligator blood. Analytical workflows of this sort incorporating IMS are readily applicable to a broad selection of xenobiotic analytes that may be present amongst even high background samples.

lightpoet - stock.adobe.com

Efficient PFAS Feature Prioritization

in Non-Target HRMS Data – Application to Contaminated Soils

Leveraging mass defects and isotopic patterns from HR-MS for effective PFAS screening.

By LCGC Staff

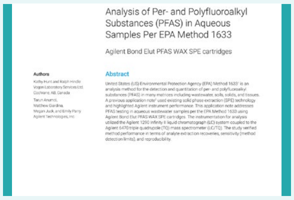
INTRODUCTION

Poly- and per-fluorinated substances (PFAS) have been receiving attention from the scientific community as evidence has grown regarding potential negative impacts from PFAS on human health and the environment. With PFAS used in the manufacturing of a wide range of industrial and consumer products, detection of PFAS reaching the surrounding ecosystem has become even more pertinent. Identifying and quantifying PFAS in soil samples is a critical aspect of environmental PFAS analysis as a method of tracking PFAS contamination, but soil matrices have many background species that can interfere with identification of known and novel PFAS.

High resolution mass spectrometry (HR-MS) can be leveraged for nontargeted PFAS screening approaches using mass defects and isotope patterns via the MD/C-m/C approach to identify and prioritize PFAS in HR-MS spectra, a method that is compatible with application to real environmental samples. Furthermore, a Python-based data processing and visualization platform, PFAScreen, can combine these post-processing workflows into one streamlined procedure. An example of how the MD/C-m/C approach can be applied to analysis of PFAS contamination in soils will be discussed in addition to outlining each component behind the overall method.

PFAS and Relation to Health and the Environment

The primary motivation for method development intended for PFAS analysis lies in the need to detect and characterize PFAS contamination present within environmental samples. One of the main issues of the PFAS class is environmental persistence of the highly fluorinated carbon chains, and many of these species can eventually transform within their surroundings into other molecules. Depending upon the physical and chemical properties of the transformation products, there may be additional substances that have increased mobility ecologically or are capable of bioaccumulation in flora and fauna. Direct identification of PFAS with reference standards can be a barrier to complete analysis since the actual number of authenticated PFAS reference materials is limited, even though there are thousands or even millions of PFAS species that could be present in the surrounding environs.



Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples Per EPA Method 1633

Agilent Bond Elut PFAS WAX SPE cartridges

Authors

Kathy Hunt and Ralph Hindle
Vogon Laboratory Services Ltd.
Cochrane, AB, Canada

Tarun Anumol,
Matthew Giardina,
Megan Juck, and Emily Parry
Agilent Technologies, Inc.

Abstract

United States (US) Environmental Protection Agency (EPA) Method 1633[1] is an analysis method for the detection and quantitation of per- and polyfluoroalkyl substances (PFAS) in many matrices including wastewater, soils, solids, and tissues. A previous application note[2] used existing solid phase extraction (SPE) technology and highlighted Agilent instrument performance. This application note addresses PFAS testing in aqueous wastewater samples per the EPA Method 1633 using Agilent Bond Elut PFAS WAX SPE cartridges. The instrumentation for analysis utilized the Agilent 1290 Infinity II liquid chromatograph (LC) system coupled to the Agilent 6470 triple quadrupole (TQ) mass spectrometer (LC/TQ). The study verified method performance in terms of analyte extraction recoveries, sensitivity (method detection limits), and reproducibility.

APPLICATION NOTE
Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples Per EPA Method 1633

For an analytical challenge posed by untold numbers of uncharacterized and unknown PFAS molecules, non-targeted methods of detection are a preferred. However, non-targeted screening can be a laborious process requiring many manual steps such as manual verification of analyte species class due to high false positive rates in sensitive workflows, or the often-proprietary software tools that can create difficulty in reproducing workflows. Correspondingly, a highly efficient approach is necessary to decrease false positive rates while also ensuring use of open-source techniques to increase reproducibility of the workflows.

Single-Stage HR-MS for PFAS Identification in Non-Targeted Screening

To describe the single stage HR-MS approach for PFAS screening, a comparison of hydrocarbon and PFAS molecules will be discussed regarding their respective mass defects and isotope patterns. In hydrocarbon species, there are often large positive mass defect because of the many hydrogen atoms present, each of which individually have a positive mass defect. By contrast, PFAS, which are mainly dominated by fluorine atoms, often have a small negative mass defect. The chemical mass defect has been used in many previous approaches to differentiate PFAS from other chemicals, but there are still many compounds that overlap with the mass defect range of most PFAS.

The two properties described above can be combined into a method that leverages both mass defect and isotopic information to improve PFAS screening overall.

As an addition to the mass defect method, isotope patterns in high-resolution mass spectrometry data can be used to differentiate PFAS from other species. Fluorine has only one naturally occurring isotope, which creates difficulties for detection via the isotope pattern, but there are still discernable differences between hydrocarbons and highly fluorinated substances. In an example hydrocarbon containing a chain 27 CH_2 units in length, there are 27 carbon atoms to reach a nominal mass of around 400 Da. By contrast, an example perfluorinated species with a nominal mass around 400 Da, PFOA, has only eight carbon atoms. The relative abundance of the M+1 isotope within the isotope patterns is typically much lower in PFAS relative to hydrocarbon compounds. In this example, there is a 30% relative abundance of the M+1 isotope for the hydrocarbon and only 8.7% relative abundance of PFOA. This permits direct estimation of the carbon number for all the detected features in our non-targeted screening workflow by using the natural abundance of the carbon-13. This calculation can be completed directly using only the abundance of the M+1 isotope.

The two properties described above can be combined into a method that leverages both mass defect and isotopic information to improve PFAS screening overall. Normalization of mass-to-charge ratio to the carbon number (m/C) plotted versus the mass defect normalized to the carbon number (MD/C) is an additional technique to distinguish PFAS from other molecular classes. From the PFOA example described earlier with eight carbon atoms and using isotope abundances detected in mass spectrometry, the carbon number can be estimated. MD/C is then determined to be -0.003 and m/C calculated at 52, both of which were as anticipated because the molecule is almost entirely made up of CF_2 units. However, for the hydrocarbon molecule, which is basically made up of CH_2 repeating units, there is an MD/C of +0.015 and an m/C of 15. The higher m/C, the greater quantity of heavy elements are likely present, while the MD/C is smaller and likely negative for high heteroatom content such as found in PFAS.

When the above method is applied to soil measurements, the initial MD/C versus m/C plot included roughly 12,000 features that were detected from four soil samples. Different groups of molecules can be then identified as clusters within these plots, with each cluster generally including molecules of similar properties and structures. Typical hydrocarbons cluster around an m/C in the range of 10 to 30, while PFAS often fall within an m/C range exceeding 30. In the initial soil extractions, most of the compounds extracted had an m/C around 20, which is unlikely to include PFAS. By focusing on species with m/C greater than 30, the number of features can be reduced by 92%, since only 8% of features are present within the typical PFAS region of over 30.

Another approach used is Kendrick mass defect analysis, which enables identification of homologous series for a predefined repeating unit, such as CF_2. Compounds found around a Kendrick mass defect near zero typically are dominated by repeating units, whereas those with Kendrick mass defects on the extreme ends are likely false positives that are not part of a homologous series. By simply using an m/C range of 25 to 70 and an MD/C range of -0.01 to 0.0005, the Kendrick mass defect analysis combined with the MD/C versus m/C post filtering approach can nearly eliminate false positives in the soil sample PFAS screening.

Non-Targeted Tandem MS for Detection of PFAS

Along with single stage mass spectrometry prioritization in PFAS, tandem MS methods exist to further facilitate non-targeted screening methods. In PFAS analysis, there are typically common repeating units such as perfluoro alkyl chains or perfluoro ether chains. Fragmentation performed in tandem mass spectrometry can result in distinct molecular dissociation patterns that can generate diagnostic fragment ions, including fragmentation along different portions of carbon chains. Identification of PFAS polymers can be performed by monitoring the CF_2 unit mass difference between fragment ions. The main advantage of this approach is that even if the specific fragment ion masses are unknown, PFAS polymers can still be recognized according to the mass differences. Examination of all fragment ion mass differences—not simply the difference between the precursor species and fragment of interest—can efficiently identify characteristic mass differences and diagnostic fragment ions for characterizing a broad span of PFAS species. The Mass Bank database can then be used to detect false positive rates of this approach. This non-targeted PFAS screening workflow as a whole has been combined into an open-source Python-based platform, PFAScreen, that can generate feature lists for samples. All the data can be directly visualized in the tool, so a homologous series can be visualized with extracted ion chromatograms correlated to detect associated fragments. Adducts and theoretical isotope patterns can also be matched with measured isotope patterns, thereby streamlining non-targeted screening workflows.

Streamlined HR-MS Workflow for PFAS Analysis of Contaminated Soil

As example applications of the above methods, two cases were applied to extractions of highly contaminated soils obtained from Germany. The first case is in Northwestern Germany, where PFAS were detected in surface and drinking water. A prior study had located the main contamination source to a small 10-hectare agricultural field site. Although previous studies had analyzed this soil, non-targeted screening techniques had not yet been used, and therefore risked missing novel or unanticipated PFAS. The case in Northwestern Germany applied to in-depth non targeted screening, carboxylic and sulfonic acids that were considered in previous studies. Nearly all the compounds identified were sulfonic acids, which were then semi-quantified to demonstrate that large fractions of PFAS at that site were previously unknown. The other site investigated in detail was located in Southwestern Germany where, more than 10 years ago, the last contamination occurred due to PFAS-containing paper sludge that was brought out to agricultural fields. The PFAScreen tool was used in the analysis and revealed that the two soil samples from the south have distinctly different contamination patterns relative to those from the north. Although the carboxylic acids were found as transformation products from precursors in both sites, the northern site had several previously unknown species identified that were likely microbial transformation products of PFAS. On the more southern site, the materials used for paper coating seem to be the main contaminants and appear to have simply degraded slowly over time. While the contaminants have largely remained immobile, transformation products resulting after some intermediate carboxylic acids may be capable of leaching into aquifers and eventually to the groundwater.

> **While the contaminants have largely remained immobile, transformation products resulting after some intermediate carboxylic acids may be capable of leaching into aquifers and eventually to the groundwater.**

Conclusions

The MD/C-m/C approach is an efficient workflow that can be used to distinguish highly probable PFAS species from matrix components. A main advantage is that for this workflow does not require any molecular formula determination beforehand, which is often a challenge for PFAS species due to fluorine's highly simplistic isotope pattern. With the many possible chemical formulas that can include fluorine, there are some limitations. Detector saturation with higher abundance species may have carbon numbers overestimated, while low abundance species may have limitations in detection of the M+1 isotope species sufficient for a robust carbon number estimation. However, this approach overall offers an effective route for data prioritization and reduction of false positives in downstream workflows. Moreover, the MD/C-m/C method can be applied broadly across datasets in an open-source manner in PFAScreen where data can be processed and visualized in one platform.

vladim_ka - stock.adobe.com

Agilent 6475 LC/TQ Performance Highlights with EPA Method 1633

for Per and Polyfluoroalkyl Substances (PFAS) in Solid Samples

Authors

Emily Parry,
Matthew Giardina,
Tarun Anumol, Linfeng Wu, and Patrick Batoon

Agilent Technologies, Inc

ABSTRACT

This application note details the performance of the Agilent 6475 triple quadrupole LC/MS (LC/TQ) coupled with the Agilent 1290 Infinity II liquid chromatograph (LC) for the analysis of per and polyfluoroalkyl substances (PFAS) in accordance with EPA 1633.[1] Method transfer between the Agilent 6475 and 6470 B triple quadrupole LC/MS systems was seamless, and the new intelligent source optimization algorithm was tested, as the EPA 1633 analyte list includes thermally sensitive compounds. Calibration linearity and reproducibility were verified, and the results met all method requirements. The same soil extracts were analyzed on both systems (6470 B and 6475 LC/TQ) to demonstrate comparable performance. Finally, instrument robustness was challenged by >1,000 noninterrupted injections of extracted fish samples while monitoring calibration verification standards after every 50 injections of matrix.

Introduction

PFAS are ubiquitous environmental contaminants that have been found in drinking water, wastewater, soil, and food.[2] The U.S. EPA has two well-established methods (EPA 537.[1] and 533) for the analysis of 29 PFAS compounds in potable water. EPA 1633 details analysis of 40 PFAS compounds in nonpotable water, biosolids, solids, and tissue.[1] It uses isotope dilution quantitation and has matrix-specific extraction protocols. Wastewater testing in accordance with the method has previously been detailed on the 6470 B LC/TQ.[3]

This application note shows EPA 1633 method performance in soil using the 1290 Infinity II LC system coupled to a 6475 LC/TQ. The 6475 LC/TQ maintains the robust performance that defined the Agilent 6470 B LC/TQ, while including new intelligence features to simplify tuning and method optimization, such as artificial intelligence (AI)-based tuning, maintenance monitoring and method development automation, and so on.

Specific highlights included here are:

- Ease of method transfer from the 6470 B LC/TQ, and new intelligent optimization features for the 6475 LC/TQ
- Performance of the 6475 LC/TQ with the EPA 1633 analyte list
- Soil quantification comparison between the 6470 B and 6475 LC/TQ systems
- Instrument robustness of >1,000 injections of fish tissue

Experimental

Samples and calibration standards

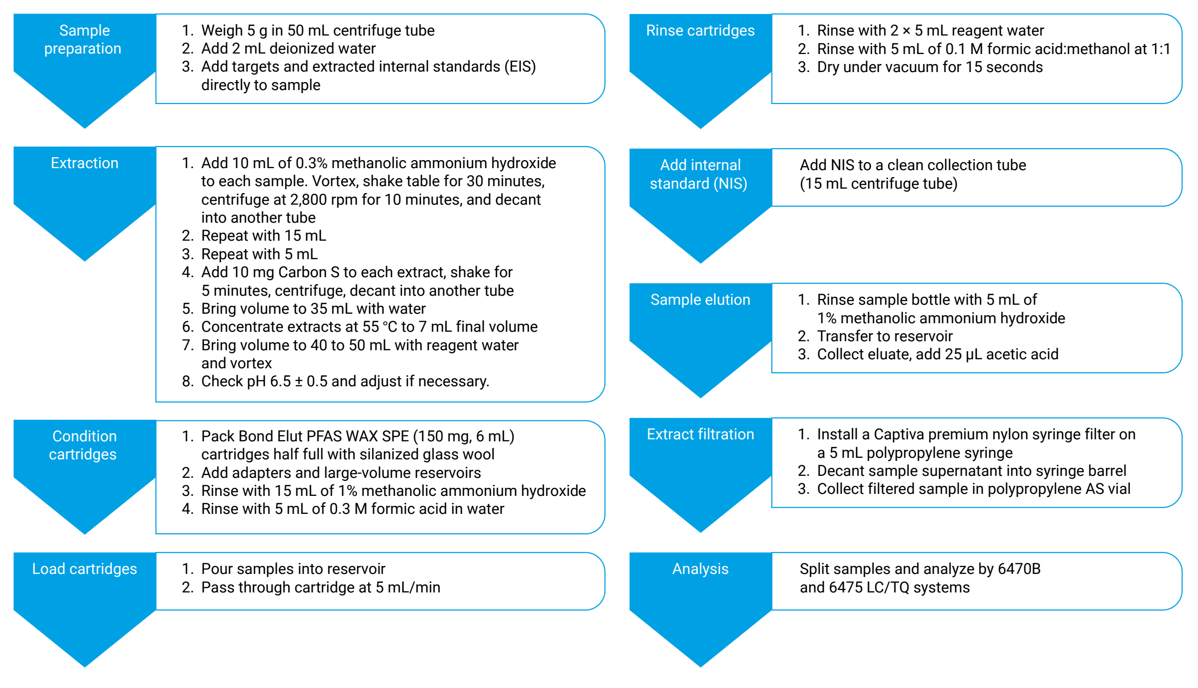
Standard mixes were purchased from Wellington Labs (ON, Canada). Seven standards with concentrations similar to those listed in EPA 1633 were prepared. Soil quantification comparison between the 6470 B and 6475 LC/TQ systems are from standards prepared separately.

Soil extracts were spiked with Ottawa sand and extracted in accordance with the methods[1] listed in **Figure 1**. The Agilent consumables and supplies used for the extraction are listed in **Table 1**.

The spiking concentrations for the target compounds ranged from 2 to 40 ng/g, extracted internal standard (EIS) ranged from 1 to 20 ng/g, and nonextracted internal standards (NIS) ranged from 1 to 4 ng/g (Appendix A). Four replicate samples were spiked, extracted, and analyzed on both the 6470 B and 6475 LC/TQ systems.

Salmon tissue was sourced from a local supermarket and extracted as described

FIGURE 1: Extraction scheme.



Sample preparation

1. Weigh 5 g in 50 mL centrifuge tube
2. Add 2 mL deionized water
3. Add targets and extracted internal standards (EIS) directly to sample

Extraction

1. Add 10 mL of 0.3% methanolic ammonium hydroxide to each sample. Vortex, shake table for 30 minutes, centrifuge at 2,800 rpm for 10 minutes, and decant into another tube
2. Repeat with 15 mL
3. Repeat with 5 mL
4. Add 10 mg Carbon S to each extract, shake for 5 minutes, centrifuge, decant into another tube
5. Bring volume to 35 mL with water
6. Concentrate extracts at 55 °C to 7 mL final volume
7. Bring volume to 40 to 50 mL with reagent water and vortex
8. Check pH 6.5 ± 0.5 and adjust if necessary.

Condition cartridges

1. Pack Bond Elut PFAS WAX SPE (150 mg, 6 mL) cartridges half full with silanized glass wool
2. Add adapters and large-volume reservoirs
3. Rinse with 15 mL of 1% methanolic ammonium hydroxide
4. Rinse with 5 mL of 0.3 M formic acid in water

Load cartridges

1. Pour samples into reservoir
2. Pass through cartridge at 5 mL/min

Rinse cartridges

1. Rinse with 2 × 5 mL reagent water
2. Rinse with 5 mL of 0.1 M formic acid:methanol at 1:1
3. Dry under vacuum for 15 seconds

Add internal standard (NIS)

Add NIS to a clean collection tube (15 mL centrifuge tube)

Sample elution

1. Rinse sample bottle with 5 mL of 1% methanolic ammonium hydroxide
2. Transfer to reservoir
3. Collect eluate, add 25 µL acetic acid

Extract filtration

1. Install a Captiva premium nylon syringe filter on a 5 mL polypropylene syringe
2. Decant sample supernatant into syringe barrel
3. Collect filtered sample in polypropylene AS vial

Analysis

Split samples and analyze by 6470B and 6475 LC/TQ systems

TABLE 1: Agilent consumables and supplies used for extraction.[4]

Consumables and Supplies	Part Number
Bond Elut PFAS WAX Solid Phase Extraction (SPE) Cartridge, 150 mg, 6 mL	5610-2150
Bond Elut Carbon S SPE Bulk Sorbent, 25 g Bottle	5610-2093
Centrifuge Tubes and Caps, 50 mL	5610-2049
Centrifuge Tubes and Caps, 15 mL	5610-2039
Bond Elut Empty SPE Cartridges, 60 mL	12131012
Bond Elut Adapter Cap for 1, 3, and 6 mL Bond Elut Cartridges	12131001
Glass Wool, Silane Treated, 50 g, for GC	8500-1572
Captiva Disposable Syringe, 5 mL	9301-6476
Captiva Premium Syringe Filter, Polypropylene Housing, Nylon Membrane, 25 mm Diameter, 0.2 µm Pore Size	5190-5092
Vac Elut SPS 24 Manifold with Collection Rack for 10 × 75 mm Test Tubes	12234003
Collection Rack and Funnel Set for 12 or 15 mL Conical Tubes, for Vac Elut SPS 24 Manifold	12234027
Stopcock Valve (20 pack)	12234520
Polypropylene Screw-Style Vials, 2 mL	5191-8150
Screw-Style Cap, 9 mm, with Polypropylene/Silicone Screw Septa	5191-8151

TABLE 2: LC instrument conditions.

Parameter	Value
LC	Agilent 1290 Infinity II LC System, consisting of: – Agilent 1290 Infinity II high-speed pump (G7120A) – Agilent 1290 Infinity II multisampler (G7167B) – Agilent 1290 Infinity II multicolumn thermostat (G7116B)
Analytical Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Delay Column	Agilent InfinityLab PFC delay column, 4.6 × 30 mm (p/n 5062-8100)
Column Temperature	50 °C
Injection Volume	2 µL
Mobile Phase	A) 2 mM ammonium acetate in water B) 95:5 acetonitrile:water
Gradient Flow Rate	0.4 mL/min
Gradient	Time (min) % B 0.0 15 0.5 15 1.5 25 7.0 60 10.0 100 12.0 100 12.1 15.0
Stop Time	12.5 min
Post-Time	2.5 min

in a previous application note.5 Extracts were postspiked with a midlevel concentration of PFAS mix. The extract served to represent a challenging matrix to determine the robustness of the 6475 LC/TQ. Fish extract was analyzed with a 5-minute gradient. After 50 injections of fish sample, a midlevel calibration sample was analyzed using the full gradient in **Table 1**.

Triple quadrupole liquid chromatography/mass spectrometry conditions

LC/TQ analysis was performed with an Agilent 1290 Infinity II LC system coupled to the Agilent 6475 triple quadrupole LC/MS system. The system was controlled by Agilent MassHunter acquisition software 12.0 (LC/TQ), which has new intelligence features and maintains method compatibility with prior versions. The acquisition method was based on the previously published parameters.[3] Full LC parameters are shown in **Table 2**. MS conditions are displayed in **Table 3**. The Agilent PFAS MRM Database for triple quadrupole LC/MS (product number G1736AA), which is developed and optimized for the 6470 B LC/TQ, was directly imported into the 6475 LC/TQ with MassHunter acquisition software 12.0.

The 6475 LC/TQ offers new intelligence features, including a fully automated method development algorithm, which updates method settings with the best value without user intervention. Parameter selection for ion source optimization has two algorithm models: extracted ion chromatogram (EIC) and weighted EIC models. The EIC model maximizes total signal response, while the weighted EIC model favors low-responding analytes in a complex mixture with adjustable weights. The 6470B LC/TQ ion source values for EPA 1633 have been previously optimized and published.6,3 Comparing results from the previous manual process to the newly intelligent software provided an interesting test case. The PFAS in the EPA 1633 analyte list include compounds that are sensitive to source temperature (e.g., NFDHA).

TABLE 3: MS conditions.

Parameter	Value
MS	Agilent 6475 LC/TQ with Agilent Jet Stream Electrospray ion source (p/n G6475A)
Source Parameters	
Gas Temperature	230 °C
Gas Flow	8 L/min
Nebulizer	20 psi
Sheath Gas Temperature	355 °C
Sheath Gas Flow	10 L/min
Capillary Voltage (Negative)	2,500 V
Nozzle Voltage (Negative)	0 V

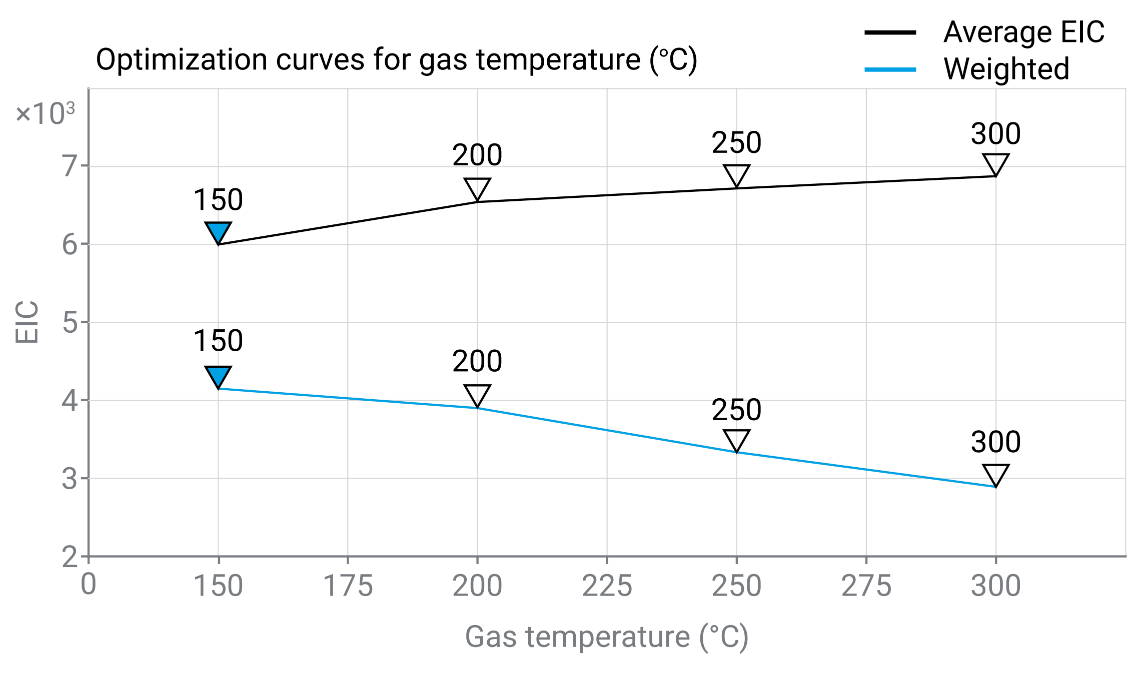
Output can be viewed overall or on an individual compound basis. **Figure 2** shows the differences between the two models for drying gas temperature.

Ion source optimization models started to diverge around 225 °C. Divergence indicated that while the overall EIC went up at the higher temperature, some of the low-abundance compounds showed lower abundance. After considering 13% individual compound results, we confirmed that the best compromise was a temperature of 230 °C, which was selected in our original method.[3] Interestingly, this was the approximate temperature at which the two models diverged, which was helpful for verifying the optimal gas temperature 8% for analysis.

EPA 1633 analyte performance with the 6475 LC/TQ

Calibration standards were injected with seven replicates. Linear or quadratic curves with 1/x weighting were fit for all compounds. The instrument linearity calculation described in EPA 1633 method section 10.3.3.3 was performed, and RSDs for the tested range (**Table 4**) were all under 14%. The RSDs of the seven

FIGURE 2: Source optimization curves.



replicate injections at the low calibrator were also evaluated. The two compounds with the highest RSDs, near 15%, were MeFOSA and MeFOSAA.

One important consideration for achieving good calibration fits for the fluorotelomer sulfonate (FTS) compounds is the selection of the quantifying transition for the labeled extraction standards. In EPA 533, it is noted that M+2 isotope of the sulfur compound has the same nominal mass as the 13C2 labeled isotope.[7] At the lower concentrations examined in EPA 533, this showed minimal interference; however, EPA 1633 has wider concentration range, so it is important to select the lower-abundance product ion: *m/z* 81.

Soil quantification comparisons between the 6470 B and 6475 LC/TQ systems

Extraction recoveries from the four spiked soil extractions were compared between the 6470B and 6475 LC/TQ systems. For all compounds on both instruments, the average recoveries were within 70 and 130% with RSDs less than 20%, as shown in **Figures 3** and **4**. The overall average recoveries for all targets were 103.2 and 97.7%, with average RSDs of 3.4 and 2.0% for the 6470 B and 6475 LC/TQ systems respectively. This demonstrates excellent and comparable performance between the two instruments.

Robustness of 6475 LC/TQ

An advantage of the 6475 LC/TQ is its robust performance with matrix samples. Figure 5 shows the performance of PFBA, PFOA, and PFBS across 1,300 injections

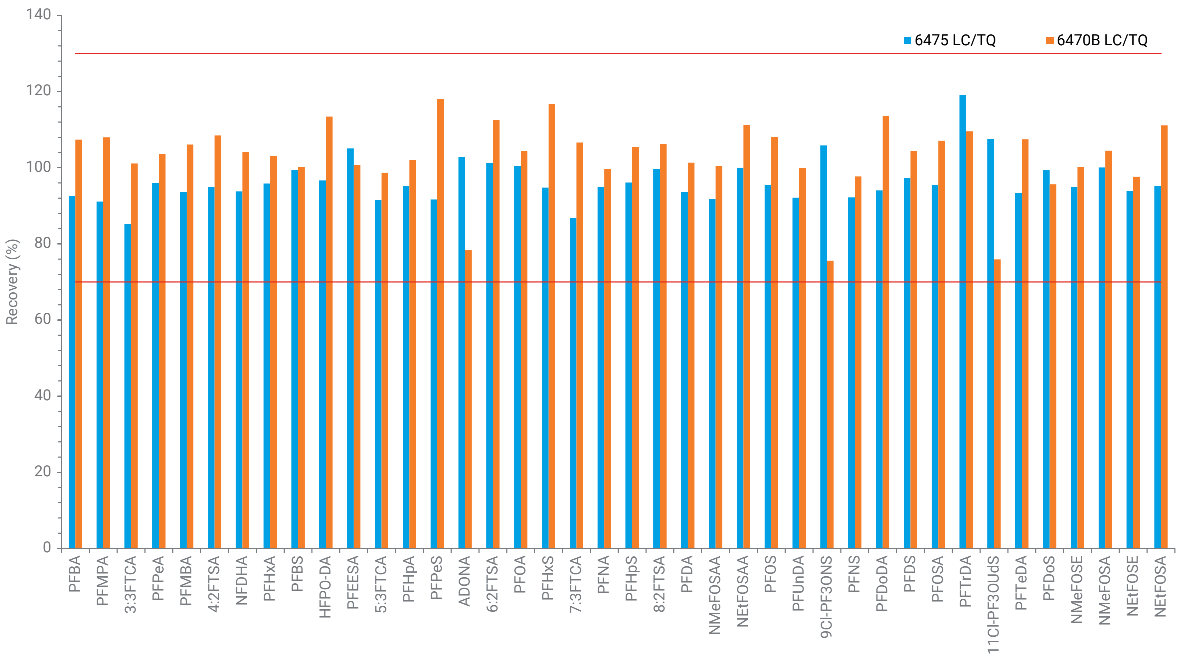
TABLE 4: Method performance data (continued on next page).

Compound	%RSD of Calibration (Option 1)	%RSE of Calibration (Option 2)	Low Calibration (ng/mL)	High Calibration (ng/mL)	R^2	% RSD at Low Level
11Cl-PF3OUdS	8%	7%	0.20	100.0	0.998	3.2%
3-3 FTCA	10%	10%	0.40	200.0	0.997	5.2%
4:2 FTS	10%	10%	0.38	187.5	0.995	11.8%
5-3 FTCA	10%	10%	2.00	1,000.0	0.998	3.6%
6:2 FTS	9%	11%	0.38	190.0	0.997	6.3%
7-3 FTCA	11%	10%	2.00	1,000.0	0.998	7.1%
8:2 FTS	8%	10%	0.38	192.0	0.996	8.1%
9Cl-PF3ONS	8%	8%	0.20	100.0	0.998	4.2%
ADONA	7%	9%	0.20	100.0	0.998	1.6%
HFPO-DA	9%	10%	0.20	100.0	0.998	4.2%
NEtFOSA	9%	12%	0.10	50.0	0.996	9.5%
NEtFOSAA	13%	13%	0.10	50.0	0.996	11.3%
NEtFOSE	8%	9%	1.00	500.0	0.997	2.5%
NFDHA	9%	10%	0.20	100.0	0.998	8.1%
NMeFOSA	10%	12%	0.10	50.0	0.996	14.1%
NMeFOSAA	12%	14%	0.10	50.0	0.996	15%
NMeFOSE	8%	10%	1.00	500.0	0.997	2.1%
PFBA	8%	10%	0.40	200.0	0.997	3.1%
PFBS	9%	10%	0.09	44.4	0.997	7.3%
PFDA	10%	12%	0.10	50.0	0.997	6.8%
PFDoA	9%	11%	0.10	50.0	0.997	8.3%
PFDoS	9%	11%	0.10	48.5	0.997	10.8%
PFDS	9%	11%	0.10	48.3	0.997	12.8%
PFEESA	8%	8%	0.20	100.0	0.998	2.5%
PFHpA	9%	12%	0.10	50.0	0.996	5.8%
PFHpS	9%	12%	0.10	47.7	0.997	10.4%
PFHxA	9%	11%	0.10	50.0	0.997	7.5%
PFHxS	10%	11%	0.09	45.7	0.997	3.5%
PFMBA	8%	10%	0.20	100.0	0.998	3.1%
PFMPA	8%	10%	0.10	50.0	0.998	1.7%
PFNA	10%	12%	0.10	48.1	0.996	9.7%
PFNS	8%	10%	0.10	50.0	0.997	12%

TABLE 4: Method performance data (continued on next page).

Compound	%RSD of Calibration (Option 1)	%RSE of Calibration (Option 2)	Low Calibration (ng/mL)	High Calibration (ng/mL)	R^2	% RSD at Low Level
PFOA	9%	12%	0.10	50.0	0.997	11.3%
PFOS	7%	9%	0.09	46.4	0.997	6.7%
PFOSA	8%	10%	0.10	50.0	0.997	3.4%
PFPeA	8%	10%	0.20	100.0	0.997	3.1%
PFPeS	9%	13%	0.09	47.1	0.996	7%
PFTeDA	9%	11%	0.10	50.0	0.996	9.6%
PFTrDA	11%	12%	0.10	50.0	0.996	7%
PFUnA	11%	12%	0.10	50.0	0.996	10.2%

FIGURE 3: Average target recoveries for four replicate extractions analyzed on the Agilent 6470B and 6475 triple quadrupole LC/MS systems.



of salmon matrix. The solid line represents the performance of the CV standards injected every 50 salmon samples, while the colored dots are the replicated injections of the postspiked salmon matrix. For the entire compound list, CV abundances varied between 3 to 22%. Most of the compound list (86%) abundance RSDs were under 10%. The FTS and FOSAA compounds had abundance RSDs higher between 16 and 22%, which is normal for these two classes.

FIGURE 4: Target %RSDs for four replicate extractions analyzed on the Agilent 6470B and 6475 triple quadrupole LC/MS systems.

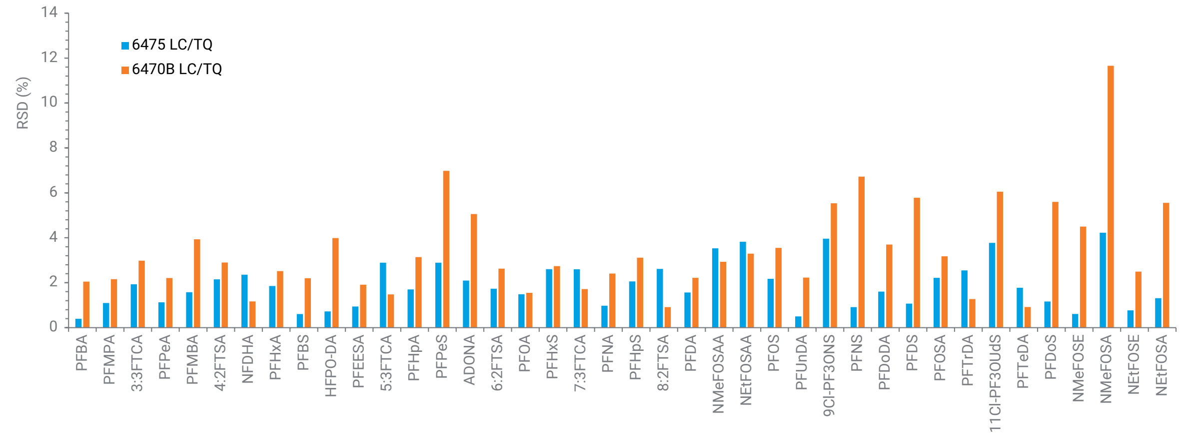
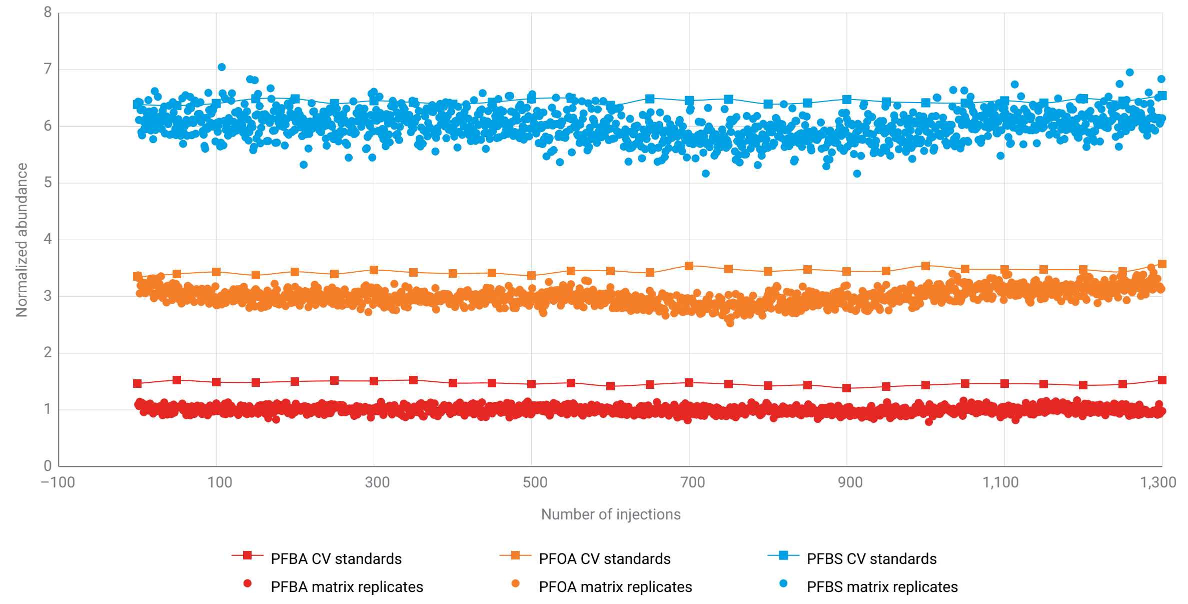


FIGURE 5: Instrument robustness across 1,300 injections. Abundance was normalized and scaled for visualization. The solid line represents the continuing calibration verification standards with the individual points of the matrix injections.



Conclusion

This application note demonstrates the excellent analytical sensitivity, precision, accuracy, and robustness of the Agilent 6475 triple quadrupole LC/MS system for PFAS analysis. This PFAS analysis of soil samples shows comparable results between the Agilent 6470 B and 6475 LC/TQ systems.

REFERENCES

1. Office of Water and Office of Science and Technology, Engineering and Analysis Division, United States Environmental Protection Agency. EPA 821-D-21-001. Draft Method 1633, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. Last revised August **2021**. https://www.epa.gov/system/files/documents/2021-09/ method_1633_draft_aug-2021.pdf
2. United States Environmental Protection Agency. Our Current Understanding of the Human Health and Environmental Risks of PFAS. Last updated March 16, **2023**. https://www.epa.gov/pfas/our-current- understanding-human-health-and-environmental-risks- pfas
3. Hunt, K. *et al.* Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples Per EPA Draft Method 1633. *Agilent Technologies application note,* publication number 5994-4926EN, **2022**.
4. Per- and polyfluoroalkyl substances (PFAS) analysis for environmental samples. Agilent Technologies consumables ordering guide, publication number 5994-2357EN, **2023**.
5. Yang, X. A Fast Sample Preparation Workflow for Veterinary Drugs Analysis in Salmon. *Agilent Technologies application note*, publication number 5994-1124EN, **2019**
6. Giardina, M. Analysis of Per- and Polyfluoroalkyl Substances in Soil Extracts. *Agilent Technologies application note*, publication number 5994-2999EN, **2021**.
7. Rosenblum, L. and Wendelken, S.C., United States Environmental Protection Agency. Method 533: Determination of Per- and Polyfluoroalkyl substances in drinking water by isotope dilution and anion exchange solid phase extraction and liquid chromatography/ tandem mass spectrometry, **2019**. https://www.epa.gov/ sites/default/files/2019-12/documents/method-533- 815b19020.pdf

Agilent
Trusted Answers

Your Perfect Partner for PFAS Analysis

Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals with diverse industrial and consumer uses. However, they pose a high risk to ecosystems and human health, leading to strict regulation and monitoring. Testing for PFAS compounds is crucial for risk reduction and potential eradication. Since their inception, Agilent has been supporting PFAS labs by providing start-to-finish solutions for diverse industry challenges. From consulting to implementation, sample preparation to measurement and reporting Agilent delivers solutions that meet regulatory needs.

For more information, scan the code or visit
www.agilent.com/en/solutions/environmental/pfas-testing

PFAS analysis applications

Environmental
- Water
- Soil
- Air
- Industrial emissions

Food
- Food and feed
- Food ingredients
- Food contact materials

Materials
- Consumer products
- Semiconductor manufacturing
- Battery manufacturing

Pharma
- Extractables and leachables

Translational research
- Exposure assessment studies

DE-004226

Published in the USA. December 1, 2024
5994-8323EN