Perfluoroalkyl Substances **(PFAS)** Testing Guide

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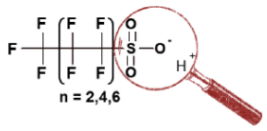
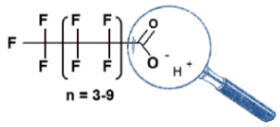
PFAS Structure Complexity

Basic PFAS Structures



Perfluorosulfonic Acids

PFBS, PFHS, PFOS



Perfluorocarboxylic Acids

C_6 acid - C_{12} acid

PFOA C8 acid

Fluorotelomer Alcohols

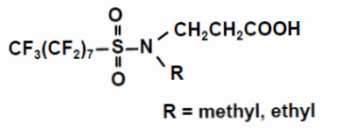
6:2, 8:2 and 10:2

Anionic at environmental pH, water soluble

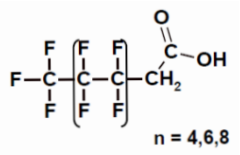
More volatile

Complex PFAS Structures

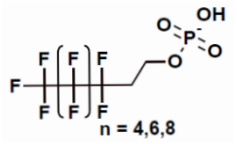
Sulfonamides



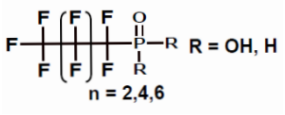
Telomer Acids



Phosphate Esters



Phosphinic/phosphonic

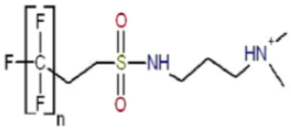


Further Complex PFAS Structures

Perfluoroalkyl Sulfonates

PFBS	n = 4	Qn
PFPeS	n = 5	Ql
PFHxS	n = 6	Qn
PFHpS	n = 7	Qn
PFOS	n = 8	Qn
PFNS	n = 9	Ql
PFDS	n = 10	Qn

Fluorotelomer Sulfonamide Amines

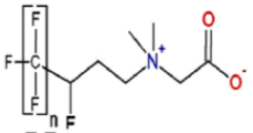


6:2 FtSaAM	n = 6	Sq
8:2 FtSaAm	n = 8	Ql

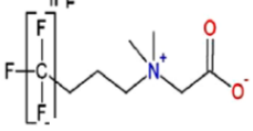
Perfluoroalkyl Carboxylates

PFBA	n = 4	Qn
PFPeA	n = 5	Qn
PFHxA	n = 6	Qn
PFHpA	n = 7	Qn
PFOA	n = 8	Qn
PFNA	n = 9	Qn
PFDA	n = 10	Qn
PFUdA	n = 11	Qn
PFDoA	n = 12	Qn
PFTrA	n = 13	Qn
PFTeA	n = 14	Qn

Fluorotelomer Betaines

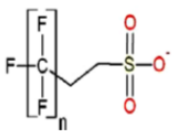


5:1:2 FtB	n = 5	Sq
7:1:2 FtB	n = 7	Sq
9:1:2 FtB	n = 9	Sq



5:3 FtB	n = 5	Sq
7:3 FtB	n = 7	Sq
9:3 FtB	n = 9	Sq

Fluorotelomer Sulfonates



4:2 FtS	n = 4	Qr
6:2 FtS	n = 6	Qr
8:2 FtS	n = 8	Qr

PFAS: The Forever Chemicals

PFAS have been around for 70 years, but are only now being found everywhere and seemingly in everything. Increasing public concern about PFAS is driving unprecedented growth in analytical technology and methodology. Now, explore HPLC column choices and sample preparation options for diverse sample matrices, separation selectivities, and workflows. Here you will find the latest word on PFAS analysis - but certainly not the final - on this rapidly expanding field of investigation.

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Introduction

1. The History and Future of PFAS Analytical Methodology

Introduction

Analytical methods for PFAS have greatly evolved over the past decade. Beginning in 2009, USEPA published EPA Method 537 (1), their first standard method for PFAS in drinking water. EPA 537, which targeted only 17 PFAS analytes, was developed to support the Third Unregulated Contaminant Monitoring Rule (UCMR3) conducted from 2012-2017. From that time forward, the scope an complexity of PFAS analysis has continued to evolve.

EPA Method 537

The original EPA PFAS method, EPA Method 537, utilized Solid Phase Extraction (SPE) to remove chemical interferences and to concentrate the compounds of interest, followed by LC-MS/MS to identify and quantitate the 14 analytes. The sample preparation step of the method specified a strongly hydrophobic SPE sorbent composed of styrenedivinylbenzene polymer (SDVB) or equivalent. The HPLC column used in method development and validation was Phenomenex Gemini® 3u C18. The SPE equivalency specification allowed other SPE sorbents to be used, such as Phenomenex Strata®-X (SDVB co-polymerized with polyvinyl-pyrrolidone).

EPA Method 537.1

The original EPA Method 537 was updated to EPA Method 537.1 (2) in 2018. The new drinking water method recognized the major changes in the PFAS landscape that had been during the intervening nine years. PFOS and PFOA, the compounds originally identified as being of greatest concern, had ceased to be manufactured and had been replaced in commerce by the so-called “Gen-X” family of PFAS which were thought to be less toxic and more environment friendly. Four of the most commonly encountered Gen-X compounds were therefore added to the original analyte list, giving EPA Method 537.1, a total of 18 analytes. The method also included a more restrictive SPE sorbent specification in the sample preparation step. The authors of the method specified that only SDVB SPE Polymers could be used, and included language that disallowed the use of other “co-polymers.” This language was updated in 2020 under Method 537.1, Revision 2.0, as indicated in Section 6.9.1, that “the sorbent may not be modified with monomers other than SDVB.” [See Chapter 2: EPA Method 527.1]

EPA Method 533

In 2019 EPA published the PFAS Action Plan (3) which focused on proactive strategies and policies to address PFAS contamination and protect public health and the environment. This coincided with the release of a new analytical method for PFAS in drinking water – EPA Method 533 (4). This new method increased the total number of analytes to 25 to include eleven short chain PFAS compounds that were more prevalent in the new manufacturing processes. However, four long chain PFAS compounds that had been part of the EPA Method 537.1 were removed from the panel due to 7 years of non-detects in the UCMR program. These additions and subtractions created the need for a new SPE sorbent in sample preparation step of the method because the hydrophobic SDVB sorbent used in EPA Method 537.1 was not able to retain the more hydrophilic short chain PFAS compounds. Consequently, EPA Method 533 specifies the following requirements for the SPE sorbent used:

- Weak anion exchange, mixed-mode polymeric sorbent (polymeric backbone with a diamino ligand)
- Particle size approximately 33 µm
- The SPE sorbent must have a pKa above 8, so it remains positively charged during extraction

EPA Method 533 was developed and validated around the use of Phenomenex Strata-X-AW SPE sorbent which shows excellent recoveries for all 25 analytes. The analytical column identified in EPA Method 533 is still Phenomenex Gemini 3 µm C18. However, there was also the addition of an isotope dilution quality assurance scheme to account for any bias created in sampling, sample preparation or LC system variation. Method 533 will be employed in the UCMR5 cycle which will begin in 2022 (5). [See Chapter 3: EPA Method 533].

DOD QSM 5.3

The DOD Quality Systems Manual 5.3 (6), is not a method per se, but rather a set of quality processes established by the Department of Defense (DOD) to ensure consistency across laboratories that analyze non-drinking water samples (wastewater, soils, sediment, etc.) from military installations. One of the few method criteria specified in DOD QSM 5.3 (in Table B-15) is the use of graphitized carbon black (GCB) media in the final step of the sample preparation process following the SPE step. Initially, laboratories that follow the DOD guidelines used either a separate GCB tube for this second extraction step, or added a small amount of GCB media to the final extract as a dispersive SPE step. However, both of these procedures require additional time and resources, and can decrease the accuracy and precision of the overall analytical method owing to increased sample manipulation.

Introduction (continued)

In response to these procedural deficiencies, Phenomenex – in partnership with a leading PFAS testing laboratory organization - developed a new compliant SPE tube configuration, wherein Strata®-X-AW SPE media is packed on top of the GCB in the same tube. Using this new product - Strata PFAS - laboratories have demonstrated comparable recoveries for all analytes compared with the two step process, but with lower RSD values. They have also demonstrated significant time savings in the sample preparation step, as well as reduction in the rate of samples re-runs due to QC batch failure. [See Chapter 5: Strata® PFAS for DOD QSM 5.3]

Other Multi-Media Methods

All the validated EPA PFAS methods previously described were designed specifically for the analysis of PFAS in drinking water. Analysts who wish to analyze PFAS in other matrices (such as waste water, sediment, leachate, bio-solids, and tissues) are generally free to develop and validate in-house methods for these matrices. These customized "MOD Methods" are usually based upon the analyte lists and LCMS operating conditions of EPA Method 537.1 or EPA Method 533, but with appropriate modifications to the sample preparation step.

For example, waste water samples with high levels of particulates cannot be reliably analyzed by strictly following the drinking water methods because filtration is contra-indicated. Therefore, these difficult samples are often analyzed by an improvised method (based upon EPA method 537.1 or 533) wherein the official method specified SPE sorbent is replaced with a larger, 100u particle sorbent such as Strata-XL, or Strata- XL-AW. In addition, modified methods have been developed for the analysis of PFAS in food products, sediments and other difficult matrices by incorporating the QuEChERS sample preparation procedure, either alone or in combination with weak ion exchange SPE. [See Chapter 6: Determination of PFAS in Sediments, and also Chapter 7: New Concerns about PFAS in Food].

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10. CompTox Chemicals Dashboard | PFASMASTER Chemicals (epa.gov)

Pending Official PFAS Methods

There are several new official method developments in progress. FDA has developed a QuEChERs-based LC-MS/MS method for PFAS in food and feed products that has now undergone single laboratory validation (7). In addition, USDA is developing a UHPLC-MS/MS method for the analysis of PFAS in muscle tissue and plasma (8). Finally, several ASTM methods may be forthcoming soon, as well as the much-anticipated EPA Method 8327 (9)that should prove to be more applicable for ground water, surface water and waste water matrices. However, discussion of these more recent developments will be deferred to the next edition of the PFAS Guide when more operating experience and method validation data will be available. Please refer to the Product Guide for a complete summary of Phenomenex products that are referenced in current official methods or can be applied as equivalent.

The Future of PFAS Method Development

Clearly, the development of PFAS methodology is far from over. With over 9,000 PFAS compounds listed in the EPA PFAS Master List (10), a great deal of advanced method development work will be needed to fully establish the depth and breadth of the PFAS contamination problem. Going forward, Phenomenex intends to maintain its leading role as an analytical chemistry innovator in the field of PFAS analysis.

To that end, we round out this PFAS Applications Guide with two, forward-looking technical notes intended to stimulate future scientific thought and inquiry. [See Chapter 9: pH-Variable LC Mobile Phase Gradient and also Chapter 10: Column Chemistry Considerations].

Conclusion

All of the above considerations point to significant challenges in developing new PFAS analytical methods. **Phenomenex is committed to lead the way with groundbreaking research into the development of unique stationary phases that offer novel PFAS selectivity, as well as new SPE sorbents and configurations for better sample cleanup and improved sensitivity – especially for difficult matrices like food and biota.** While this new PFAS Testing Guide is a significant update to our original 2017 edition, we fully expect that new discoveries in both PFAS chemistry and analytical methodology will quickly result in this new Guide becoming obsolete. We look forward to continuing to provide the tools and techniques to further understand these uniquely challenging pollutants.

Drinking Water

Drinking water has received the majority attention as a primary source of PFAS exposure. Drinking water has also been the most widely studied, notably through the USEPA Unregulated Contaminant Monitoring Rule (UCMR) program, initially using EPA Method 537.1 and more recently using EPA Method 533 with its expanded analyte list and attention to shorter chain PFAS and the GenX compounds. As analytical technology advances, method developers continue to create analytical methods for PFAS in drinking water with expanded analyte lists and advanced techniques, such as large volume direct injection and on-line Solid Phase Extraction (SPE). Although some of these modifications are not considered "official methods" for regulatory purposes, they are widely used for investigation and problem assessment.

2. EPA Method 537.1

PFAS in Drinking Water Using Strata® SDB-L Solid Phase Extraction (SPE) and a Luna® Omega 1.6 µm PS C18 UHPLC Column

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[2]Weck Laboratories, Industry, CA 91745, USA

Overview

This application demonstrates the suitability and advantage of using Strata SDB-L SPE along with a Luna Omega 1.6 µm PS C18 UHPLC column in the performance of EPA Method 537.1, the official SPE liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water in the United States.

Introduction

EPA Method 537 Version 1.1, was first published in 2009 for use in the Third Unregulated Contaminant Rule (UCMR3) nationwide drinking water survey. This original PFAS method specified 14 target PFAS analytes, including Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), the two PFAS compounds of largest historic use. However, both the production and use of PFOS and PFOA were discontinued between 2000 and 2015 and were replaced by shorter chain PFAS compounds (informally referred to as the "GenX" compounds), which were thought to be less persistent in the environment and less toxic. Therefore, EPA Method 537.1 was introduced in 2018 as an update to EPA Method 537 Version 1.1. It included the original 14 PFAS analytes and added 4 of the shorter chain PFAS compounds for a total of 18 analytes. As originally published, EPA Method 537.1 specified that only SPE cartridges based upon SDVB (styrenedivinylbenzene) polymers could be used in the extraction procedure, owing to low recovery of the short chain PFAS compounds on non-SDVB polymers. This stipulation was continued in the most recent update: Method 537.1 V2, published in March, 2020.[1]

In this technical note we present analytical results for the analysis of drinking water by EPA Method 537.1 using Strata SDB-L SPE (based upon a SDVB polymer) and a Luna Omega 1.6 µm PS C18 UHPLC column. The data demonstrates excellent recovery for all 18 PFAS analytes on Strata SDB-L. Likewise, Luna Omega 1.6 µm PS C18 provides outstanding column efficiency and analyte resolution for greater method sensitivity and shorter run times.

Materials and Methods

Solid Phase Extraction Protocol

Following the procedures of EPA Method 537.1, V2, Sections 6.9 - 6.11 and 11.3 - 11.4

Cartridge: Strata SDB-L, 500 mg/6 mL
Part No.: 8B-S014-HCH
Load: 250 mL sample that has been fortified with surrogates
Elution: 2x 3 mL Methanol
Dry Down: With Nitrogen in a heated water bath
Reconstitute: Adjust final volume to 1 mL with 96:4 Water:Methanol (v/v) and add internal standards

HPLC Conditions

Following the procedures of EPA Method 537.1, V2, Sections 6.9 - 6.11 and 11.3 - 11.4

Column: Luna Omega 1.6 µm PS C18
Dimension: 100 x 2.1 mm
Part No.: 00D-4752-AN
Mobile Phase: A: 0.1 % Acetic acid in Water
B: Methanol
Gradient:

Time (min)	%B
0	20
0.5	30
7	90
7.5	100
9	100

Flow Rate: 0.7 mL/min
Injection Volume: 4 µL
LC System: Agilent® 1260 Series HPLC
Detection: Agilent Ultivo™ Triple Quadrupole MS

Data and Results

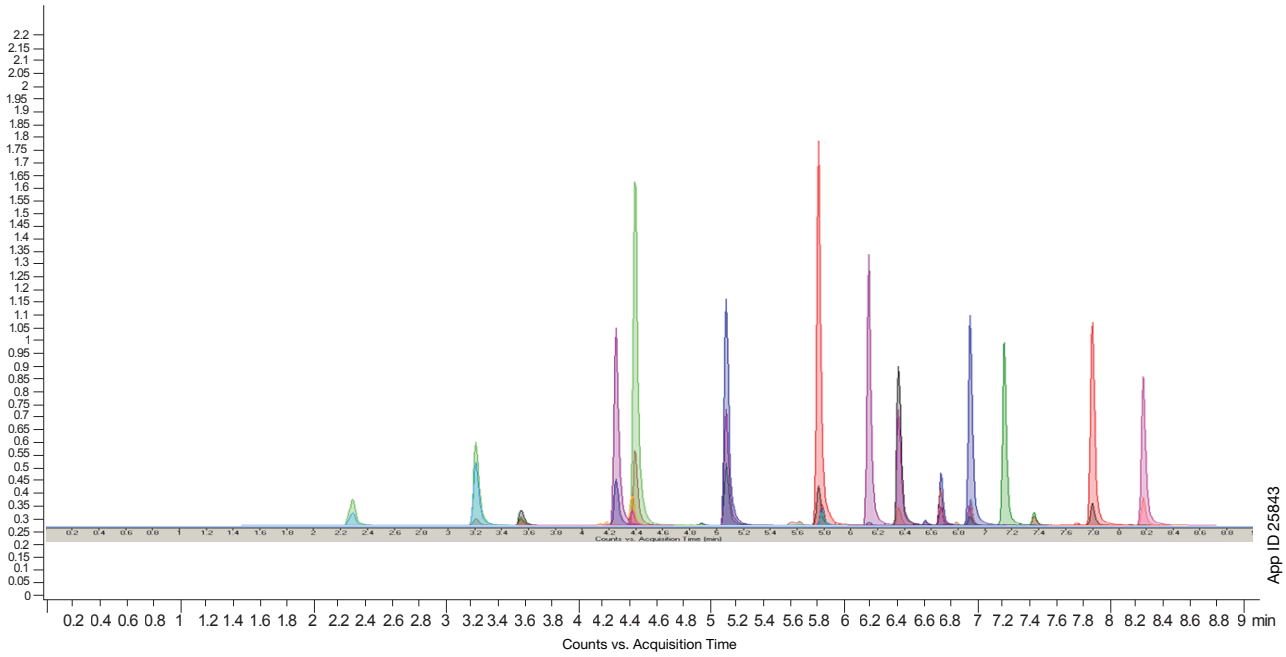
PFAS Target Analytes and UHPLC Retention Times

Analyte	RT (min)	Internal Standard
PFBS	2.29	13C4 -PFOS
PFHxA	3.20	13C2 -PFOA
HFPO-DA	3.55	13C2 -PFOA
PFHpA	4.24	13C2 -PFOA
PFHxS	4.39	13C4 -PFOS
ADONA	4.41	13C2 -PFOA
PFOA	5.08	13C2 -PFOA
PFOS	5.72	13C4 -PFOS
PFNA	5.77	13C2 -PFOA
9Cl-PF3ONS	6.15	13C4 -PFOS
PFDA	6.35	13C2 -PFOA
NMeFOSAA	6.70	d3 -NMeFOSAA
PFUnA	6.83	13C2 -PFOA
NEtFOSAA	6.88	d3 -NMeFOSAA
11Cl-PF3OUdS	7.17	13C4 -PFOS
PFDoA	7.37	13C2 -PFOA
PFTrDA	7.80	13C2 -PFOA
PFTA	8.18	13C2 -PFOA

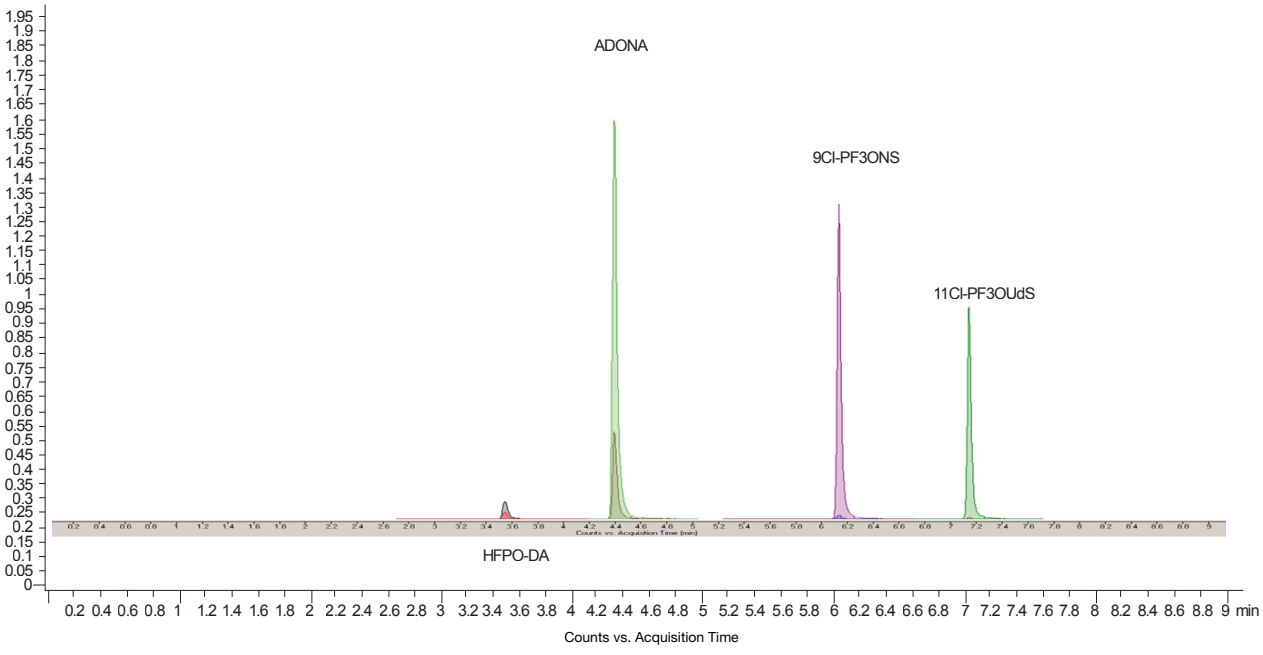
2. EPA Method 537.1 (continued)

Full PFAS Target Analytes

PFAS Replacement Compounds



Short Chain ("Gen X") PFAS Analytes



2. EPA Method 537.1 (continued)

Method Precision and Accuracy from the Analysis of 50 Consecutive Laboratory Control Samples (LCS)

	Mean % Recovery	Standard Deviation
13C2-PFDA	106	15.8
13C2-PFHxA	108	18.2
d5-EtFOSAA	104	19.8
13C2-HFPO-DA	104	17.1
11Cl-PF3OUdS	105	10.9
9Cl-PF3OUNS	104	11.9
ADONA	103	13.6
Et-FOSAA	111	13.8
HFPO-DA	104	15.6
Me-FOSAA	113	18.5
PFBS	104	14.7
PFDA	106	12
PFDoA	105	17.4
PFHpA	111	14.8
PFHxA	109	14.1
PFHxS	108	15.1
PFNA	109	12.7
PFOA	109	12.5
PFOS	111	13
PFTeDA	103	14.6
PFTrDA	104	13.7
PFUnA	107	13.7

Discussion

These results fully demonstrate the suitability of the combination of Strata® SDB-L 500 mg/6 mL and Luna® Omega 1.6 µm PS C18 for use in EPA Method 537.1. Luna Omega 1.6 µm PS C18 provides excellent separation of all analytes, including the shorter chain "Gen X" compounds. The accuracy and precision of the data, as demonstrated by the analysis of 50 consecutive LCS samples, are well within the requirements of the method. However, beyond meeting method requirements, the additional advantage of the Strata/Luna combination is its contribution to environmental laboratory productivity. In the published version of EPA Method 537.1, the run time for the 25 analytes (18 target analytes and 7 internal standards) was 25 minutes. In the data presented here, the equivalent run time is 8.5 minutes. Which represents a nearly 3-fold productivity increase in the chromatographic step compared to the method as originally published. This illustration of EPA Method 537.1 suitability and productivity demonstrates why the combination of Strata SDB-L and Luna Omega 1.6 µm PS C18 has become the environmental testing industry's go-to approach for PFAS drinking water analysis, regardless of the instrumentation platform used.

Conclusion

EPA Method 537.1 is an official US regulatory method to be used by environmental laboratories to quantitate PFAS in drinking water. Health advisory drinking water limits for PFOS and PFOA have been established at 70 µg/L and it is expected that official drinking water limits will be promulgated for these two compounds, and for additional PFAS in the near future. As drinking water utilities and water resource agencies opt (or are required) to routinely test for PFAS in drinking water or water supplies, EPA 537.1 is destined to transition from its prior status as an exotic analytical method to a common environmental laboratory test. The Strata/Luna combination is already widely used in EPA Method 537.1 testing in the United States owing to the combination of high accuracy and precision and reduced analysis time. These properties will allow for higher sample throughput at lower detection levels as PFAS water testing becomes ever more prevalent.

Acknowledgments

We would like to acknowledge the invaluable assistance of Weck Laboratories in demonstrating the suitability of Strata SDB-L and Luna Omega 1.6 µm PS C18 for this essential environmental application.

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3. EPA Method 533

PFAS in Drinking Water

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Introduction

The first official EPA method for Per- and Polyfluoroalkyl Substances (PFAS) was EPA 537 developed in 2009, in part to support the UCMR3 study for drinking water systems in the US. This method included 14 PFAS compounds, including both PFOS and PFOA, which were then considered to have potential impact on human health. In 2018, EPA 537.1 was introduced to include 4 of the "replacement" PFAS compounds which had replaced PFOA and PFOS in many manufacturing processes in the interim.

In 2019, EPA released their PFAS Action Plan, which outlined the steps that the EPA proposed to take to identify and regulate PFAS in the environment. The PFAS Action Plan called for the development and promulgation of new analytical methods that would allow scientists to effectively measure more PFAS compounds, with greater accuracy and precision. Published at the end of 2019, EPA 533 is the first of these new PFAS analytical methods.

EPA 533 is complementary to EPA 537.1. It analyzes 14 of the 18 compounds from EPA 537.1, plus an additional 11 "short chain" (C4-C12) PFAS compounds. Of the original EPA 537 and EPA 537.1 compounds, 4 were not included in EPA 533, since they had been shown not to be present in drinking water during the previous UCMR study. Of the new EPA 533 compounds, PFBA and PFPeA, had been intentionally excluded from EPA 537.1 because they were too polar to be extracted by a styrene divinylbenzene (SDVB) solid phase extraction (SPE) sorbent during the sample preparation step. However, EPA 533 was able to include these 2 compounds, along with the other short chain analytes, because this new method employs a polymeric weak anion-exchange (WAX) sorbent in the SPE sample preparation step which is very selective for the more polar/acidic PFAS analytes. An additional distinction of EPA 533 is that it uses the isotope dilution technique to enhance method accuracy and robustness.

Materials and Methods

The following is a summary of the prescribed experimental conditions taken from EPA 533. It should be noted that Strata®-X-AW and Gemini® 3 µm C18 were the respective SPE sorbent and LC column used in the development of EPA 533 and in its subsequent multi-laboratory validation.

Sample Preparation Protocol

Pre-treatment: 100-250 mL sample is fortified with isotopically labeled analogues of the method analytes
Cartridge: Strata-X-AW 500 mg/6 mL
Part No.: 8B-S038-HCH
Load: Pass pre-treated sample through the cartridge
Wash 1: Aqueous Ammonium acetate followed by Methanol
Wash 2: Methanol
Elute: Ammonium hydroxide in Methanol
Dry Down: Under a gentle stream of Nitrogen in a heated water bath
Reconstitute: Adjust the final volume to 1 mL with 20 % Water in Methanol (v/v) before analyzing by LC-MS

LC Conditions

Column: Gemini 3 µm C18
Dimension: 50 x 2.0 mm
Part No.: 00B-4439-B0
Mobile Phase: A: 20 mM Ammonium Acetate
B: Methanol
Gradient:

Time (min)	%B
0	5
0.5	5
3	40
16	80
18	80
20	95
22	95
25	5
35	5

Flow Rate: 0.25 mL/min
Injection Volume: 2 µL
MS Detection: Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS)

Table 1.
EPA Method Comparison

EPA 537.1	EPA 533
18 analytes	25 analytes (including 14 from 537.1 and 11 new short chain compounds)
SDVB SPE sorbent	WAX SPE sorbent
Isotopic Internal Standards	Isotopic Internal Standards plus Isotope Dilution standards for each analyte

3. EPA Method 533 (continued)

Results

Table 2.
Isotopically Labeled Isotope Performance Standards and Retention Times

Isotopes Analytes	RT (min)
$^{13}C_3$-PFBA	4.14
$^{13}C_2$-PFOA	12.19
$^{13}C_4$-PFOS	13.73

Table 3.
Isotope Dilution Analogues: RTs and Suggested Isotope Performance Standard References

Isotopically Labeled Analyte	RT (min)	Suggested Isotope Performance Standard
$^{13}C_4$-PFBA	4.14	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	6.13	$^{13}C_3$-PFBA
$^{13}C_3$-PFBS	6.62	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	8.12	$^{13}C_4$-PFOS
$^{13}C_5$-PFHxA	8.35	$^{13}C_2$-PFOA
$^{13}C_3$-HFPO-DA	9.06	$^{13}C_2$-PFOA
$^{13}C_4$-PFHpA	10.34	$^{13}C_2$-PFOA
$^{13}C_3$-PFHxS	10.61	$^{13}C_4$-PFOS
$^{13}C_2$-6:2FTS	12.05	^{13}C4-PFOS
$^{13}C_8$-PFOA	12.19	$^{13}C_2$-PFOA
$^{13}C_9$-PFNA	13.70	$^{13}C_2$-PFOA
$^{13}C_8$-PFOS	13.73	$_{13}C_4$-PFOS
$^{13}C_2$-8:2FTS	14.94	$^{13}C_4$-PFOS
$^{13}C_6$-PFDA	15.00	$^{13}C_2$-PFOA
$^{13}C_7$-PFUnA	16.14	$^{13}C_2$-PFOA
$^{13}C_2$-PFDoA	17.13	$^{13}C_2$-PFOA

Table 4.
Method Analytes, Retention Times, and Suggested Isotope Dilution Analogue References

Analyte	Peak No. (Figure 1)	RT (min)	Isotope Dilution Analogue
PFBA	3	4.15	$^{13}C_4$-PFBA
PFMPA	4	4.84	$^{13}C_4$-PFBA
PFPeA	6	6.13	$^{13}C_5$-PFPeA
PFBS	8	6.62	$^{13}C_3$-PFBS
PFMBA	9	6.81	$^{13}C_5$-PFPeA
PFEESA	10	7.53	$^{13}C_3$-PFBS
NFDHA	11	8.01	$^{13}C_5$-PFHxA
4:2FTS	13	8.12	$^{13}C_2$-4:2FTS
PFHxA	15	8.36	$^{13}C_5$-PFHxA
PFPeS	16	8.69	$^{13}C_3$-PFHxS
HFPO-DA	18	9.06	$^{13}C_3$-HFPO-DA
PFHpA	20	10.42	$^{13}C_4$-PFHpA
PFHxS	22	10.62	$^{13}C_3$-PFHxS
ADONA	23	10.73	$^{13}C_4$-PFHpA
6:2FTS	25	12.04	$^{13}C_2$-6:2FTS
PFOA	28	12.19	$^{13}C_8$-PFOA
PFHpS	29	12.28	$^{13}C_8$-PFOS
PFNA	31	13.70	$^{13}C_9$-PFNA
PFOS	34	13.74	$^{13}C_8$-PFOS
9Cl-PF3ONS	35	14.53	$^{13}C_8$-PFOS
8:2 FTS	37	14.94	$^{13}C_2$-8:2FTS
PFDA	39	15.00	$^{13}C_6$-PFDA
PFUnA	41	16.14	$^{13}C_7$-PFUnA
11Cl-PF3OUdS	42	16.70	$^{13}C_8$-PFOS
PFDoA	44	17.13	$^{13}C_2$-PFDoA

3. EPA Method 533 (continued)

Table 5.
Precision and Accuracy Data for Reagent Water

Analyte	Low Fortification (ng/L)	Mean % R_a (n=7)	% RSD_a	High Fortification (ng/L)	Mean % R_a (n=5)	% RSD
PFBA	10	128	8.6	80	98.4	2.4
PFMPA	10	108	4.5	80	98.1	2.2
PFPeA	10	107	4.9	80	99.6	3.6
PFBS	10	102	9.1	80	96.2	2.9
PFMBA	10	111	6.8	80	101	3.4
PFEESA	10	107	10	80	98.8	4.0
NFDHA	10	110	15	80	98.5	5.4
4:2FTS	10	94.4	14	80	100	5.7
PFHxA	10	102	8.0	80	97	7.7
PFPeS	10	99.5	19	80	101	7.8
HFPO-DA	10	102	9.7	80	102	4.7
PFHpA	10	108	7.0	80	104	4.1
PFHxS	10	103	9.0	80	97.7	5.5
ADONA	10	96.3	3.1	80	96.8	5.6
6:2FTS	10	109	15	80	111	11
PFOA	10	108	7.4	80	98.5	6.9
PFHpS	10	98.8	8.9	80	102	7.0
PFNA	10	109	6.2	80	99.6	5.6
PFOS	10	104	8.7	80	98.0	4.3
9Cl-PF3ONS	10	99.7	4.6	80	103	6.8
8:2FTS	10	100	17	80	100	13
PFDA	10	100	4.2	80	100	1.8
PFUnA	10	102	10	80	97.3	8.1
11Cl-PF3OUdS	10	106	5.3	80	102	6.1
PFDoA	10	101	6.2	80	96.3	5.1

3. EPA Method 533 (continued)

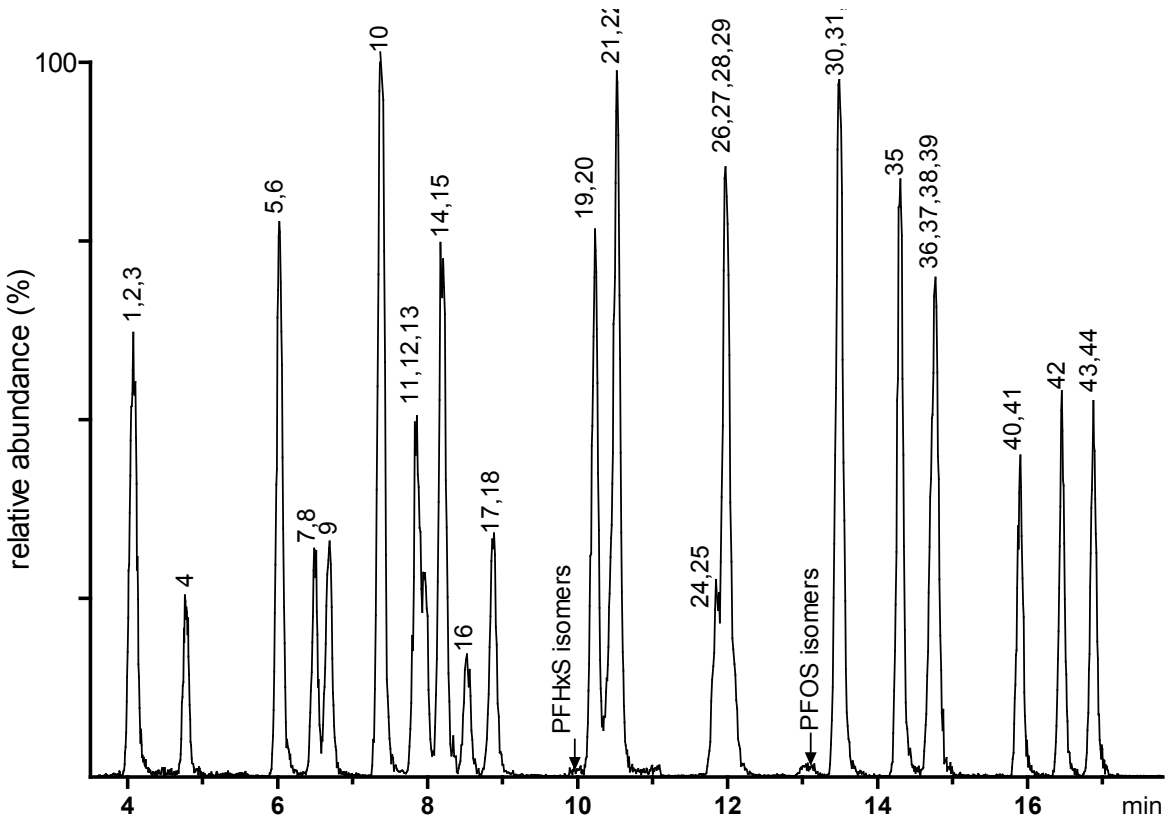
Table 6.
EPA 533 Precision and Accuracy Data from a Commercial Laboratory

Analyte	MS %	MSD %	BS %	BSD %
11Cl-PF3OUdS	85	84	95	86
4-2FTS	113	104	109	100
6-2 FTS	94	96	108	102
8-2 FTS	97	100	89	101
9Cl-PF3OUdS	101	107	99	119
ADONA	118	116	111	99
HFPO-DA	100	97	110	101
NFDHA	117	126	117	114
PFBA	102	116	89	95
PFBS	117	106	97	105
PFDA	102	99	112	104
PFDoA	104	107	108	109
PFEESA	116	109	119	115

Analyte	MS %	MSD %	BS %	BSD %
PFHpA	112	115	94	97 %
PFHpS	119	117	119	114 %
PFHxA	113	107	91	95 %
PFHxS	96	101	108	110 %
PFMBA	106	101	111	118 %
PFMPA	99	100	108	117 %
PFNA	107	104	105	110 %
PFOA	101	104	101	100 %
PFOS	117	115	108	108 %
PFPeA	97	96	92	88 %
PFPeS	86	99	103	104 %
PFUnA	105	103	115	113 %

Continued in next column

Figure 1.
Chromatogram from EPA Method 533



3. EPA Method 533 (continued)

Discussion

In this application, the method is outlined for both the SPE method and the HPLC conditions. In **Table 1**, the EPA methods are compared to show where they differ. **Tables 2-4** outline the specifics for the analytes in EPA Method 533 and then the suggested isotopes in relation to each. Specified retention times (RT) are also mentioned for each of the analytes. In **Table 5**, the acceptable precision and accuracy data is presented. In **Table 6** the data is displayed from an actual laboratory example that displays the results of how a laboratory implements EPA 533 and in **Figure 1** all necessary peaks from the specified method are shown in the example chromatogram. These data demonstrate that EPA Method 533 using Strata®-X-AW SPE for clean-up and a Gemini® C18 column for analysis provide accurate and sufficient results for a commercial laboratory running this method.

Conclusion

EPA 533 is a significant improvement over EPA 537.1 for the analysis of PFAS in drinking water. This new method eliminates the 4 compounds from the EPA 537.1 analyte list that were not detected over the 10 year period that EPA 537.1 was being used to monitor these compounds. However, it also i.e. features the addition of 11 new PFAS compounds that were not included in EPA 537.1 which are believed to be of greater environmental significance. These 11 compounds include many of the "replacement" compounds that are currently being used in the manufacturing of products that utilize PFAS chemistry. This makes EPA 533 a much more relevant environmental method. Furthermore, EPA 533 is a more robust analytical method owing to the use of the isotope dilution technique which provides a means to correct for the loss of analytes during sample preparation step, as well as to offset the potential effects of ion suppression or enhancement arising from matrix variation. Consequently, EPA 533 will play a critical role in the UCMR5 cycle beginning in 2021 to assess the safety of US public drinking water systems. In this way, EPA 533 will play an essential role in the EPA PFAS Action Plan, potentially leading to official PFAS drinking water regulations.

However, there are a few specific requirements in this method that the analyst must carefully follow. The SPE sorbent mass (in mg) must be at least 2x the sample volume (in mL) to prevent potential overloading of the sorbent. To illustrate, a 100 mL sample must be extracted with an SPE mass of at least 200 mg, a 250 mL sample must use an SPE sorbent mass of at least 500 mg and so forth. In addition, the SPE media must meet the following specifications listed in the method:

- Approximately 33 µm particle size
- Employ a mixed-mode polymeric sorbent mechanism (polymeric backbone and a diamino ligand functional group)
- Display a pK_a above 8 so that the SPE media remains positively charged during extraction

Strata-X-AW meets all these requirements and was found to show excellent performance in EPA 533 during routine laboratory operation as demonstrated by the performance data presented above. In addition, as has also been noted, both the Strata-X-AW SPE sorbent and the Gemini 3 µm C18 HPLC column were used in the development of EPA 533 and its validation. Understandably, both products are now widely employed in environmental laboratories for the routine analysis of PFAS by EPA 533.

Acknowledgement

The assistance of Dr. Agustin Pierri, Weck Laboratories, in providing the operational recovery data is gratefully acknowledged.

Reference

EPA Method 533 'Determination of Per- and Polyfluoroalkyl Substances in Drinking Water By Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry' (2019) https://www.epa.gov/sites/production/files/2019-12/documents/method-533-815b19020.pdf

4. Large-Volume Direct Injection

Quantitation of PFAS in Water Samples using LC-MS/MS Large-Volume Direct Injection and Solid Phase Extraction

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Introduction

PFASs are unique chemicals whose physiochemical properties make them important for use in a variety of industrial and consumer products including carpets, cookware, food packaging, fire suppressants, and others (1). Chemically, PFASs are aliphatic structures containing one or more C atoms on which H substituents have been replaced by F atoms. Classification and naming is typically by the particular functional group present, such as carboxylic acids, sulfonates, phosphonic acids, etc., as well as the length of the carbon chain. Desirable in various industrial applications for their chemical stability and low reactivity, these properties also make PFAS highly resistant to degradation in aquatic environments. Typical concentrations of PFASs found in various environmental water sources range from pg/L to µg/L levels (2).

Human exposure to PFAS residues has been implicated in the incidence of cancer, obesity, endocrine system disruption, and other adverse health effects (3-4). In recognition of these potential risks, sources of human exposure to these chemicals (e.g., via drinking water) are receiving public and scientific attention.

PFASs exhibit relatively high aqueous solubility and can be transported and bioaccumulated from contaminated water sources. The US EPA maintains health advisory limits for select PFASs (e.g., perfluorooctanoic acid (PFOA) at a limit of 70 ng/L) in water, but these levels have been exceeded in some areas experiencing extreme point source inputs of these chemicals (5).

Given the tremendous persistence of PFASs in the environment and their known presence in human populations exposed via drinking water and other environmental routes, demonstration of the capability for accurate and precise low-level quantitation is paramount for research and testing laboratories. Robust quantitative analytical methods utilize the specificity and sensitivity of LC-MS/MS with MRM monitoring. However, a primary analytical challenge to this assay is the prevention and reduction of background PFASs originating from the LC system and contamination during sample collection and preparation.

This application note presents two methods for the quantitation of per- and polyfluorinated alkyl substances (PFASs) in water samples. While the MS/MS detection method using the SCIEX Triple Quad™ 5500 System is similar between the two methods, the sample preparation and injection volume differ significantly.

Key features of PFAS methods

- LC-MS detection using a Shimadzu® LC-20ADXR coupled to a SCIEX® Triple Quad 5500 System
- Special modifications to the pumps and autosampler are described to mitigate laboratory-based contamination of PFASs.
- Use of a delay column for separation of a contamination PFAS peak from the analytical peak
- The first method presented here utilizes a weak-anion exchange solid phase extraction (SPE) method to concentrate water samples for analysis using a 7.5 minute HPLC gradient.
- The second method utilizes dilution of a water sample in methanol and direct injection of 950 µL of the diluted sample using a 17.5 minute HPLC gradient.
- Large volume injection of an aqueous sample is intended to achieve method sensitivity while reducing accumulated background during sample concentration steps.
- Both methods achieved accurate quantitation at levels of approximately 1-10 ng/L for more than 17 PFASs.

4. Large-Volume Direct Injection (continued)

Methods

Standards and internal standards (IS): The PFAS standards and internal standards were obtained from Wellington Laboratories (Guelph, Ontario) and were prepared in Baker HPLC-grade methanol. Standard stock solutions were prepared by dilution with 96 % methanol and 4 % water (purified using a Millipore® water purification system).

Sampling and sample preparation: Water samples were obtained anonymously from various sources in the United States. Samples were stored in the dark at 4 °C in 250 mL high density polyethlyene bottles until analysis.

Chromatography: Shimadzu® LC-20ADXR binary pumps with a Shimadzu DGU-20A5 degasser was used for separations. All fluoroethylene polymer (FEP) tubing on the Shimadzu pumps and degasser was replaced with PEEK tubing with similar internal and external dimensions. A Phenomenex Luna® C18(2) column (dimensions shown in **Table 1**) was installed between the pump mixing chamber and the column, outside of a Shimadzu CTO-20AC column oven. This column served as a delay or hold-up column to isolate PFAS contamination originating from the pumps and eluents. A longer and/or larger diameter Luna C18(2) column must be installed on heavily contaminated systems to prevent breakthrough of contamination.

Chromatographic separation was performed using a Phenomenex Gemini® C18 HPLC column at 0.6 mL/min (**Table 1**). The Gemini C18 column was heated to 40 °C in the column oven. A PAL-HTC-xt autosampler with dynamic load-wash (DLW) was modified by replacing all FEP tubing from the rinse solvent lines, the needle seal, and the sample holding loop with PEEK or stainless steel. The autosampler syringe and sample holding loop was rinsed with methanol and 1:1 methanol:acetonitrile between samples.

Table 1. LC columns for methods 1 and 2.

Method	Column	Dimensions
Delay column	Phenomenex Luna C18 (2), 5 µm	30 x 2 mm
Method 1 HPLC Column	Phenomenex Gemini C18, 3 µm	50 x 2 mm
Method 2 HPLC Column	Phenomenex Gemini C18, 3 µm	100 x 3 mm

Method 1: Solid phase extraction and 10 µL injection: A mixture of surrogate standards (25 ng) was added to 250 mL water samples in the sampling bottle, and the entire volume was extracted using weak anion exchange SPE as recommended by ISO standard 251016. The empty sample container was rinsed with 10 mL of methanol with 0.3 % NH_4OH, which was then added to the SPE tube to elute the PFASs. The extract was evaporated to dryness, reconstituted in 500 µL of 80 % methanol/20 % water, and transferred to a polypropylene vial for analysis. All standards and blanks were also prepared at a final methanol concentration of 80 %.

For Method 1, 10 µL injections of the standards and samples were analyzed using a 6.5 min gradient method (**Table 2**) with a 7.5 min total runtime, including the 1 min autosampler injection cycle. Water with 20 mM ammonium acetate was used as the "A" solvent and methanol was the "B" solvent.

Table 2. LC gradient for method 1 at a flow rate of 0.6 mL/min.

Step	Time (min)	A (%)	B (%)
0	0.00	90	10
1	0.10	45	55
2	4.50	1	99
3	4.95	1	99
4	5.00	90	10
End	6.50		

Method 2: Dilution and large volume injection: A 1 mL aliquot of a water sample was added to a 2 mL clear glass autosampler vial with a polyethylene septum cap containing 0.65 mL of methanol and a mix of surrogate standards at a final concentration of 50 ng/L. The final concentration of methanol in the diluted sample was 40 %, and standards, blanks, and quality control samples were all prepared at the same concentration. A PAL HTC-xt autosampler was modified to inject 950 µL of the diluted samples and standards.

For Method 2, samples were analyzed using an extended 15.5 min gradient method (**Table 3**) with a 17.5 min total runtime, including the 2 min autosampler injection cycle. Water with 20 mM ammonium acetate was used as the "A" solvent, and methanol was the "B" solvent.

MS/MS detection: A SCIEX® Triple Quad™ 5500 System with a Turbo V™ Ion Source and ESI probe was used for analysis in negative polarity. The ion source parameters were optimized for the LC conditions using the Compound Optimization (FIA) function in Analyst® Software (**Table 4**).

Table 3. LC gradient for method 2 at a Flow Rate of 0.6 mL/min.

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	1.5	35	65
2	8.0	5	95
3	8.1	1	99
4	12.0	1	99
5	12.5	90	10
End	15.5		

One characteristic MRM transition was monitored for each analyte and internal standard (Appendix **Table 1**). The Scheduled MRM™ algorithm was activated to monitor compounds only during a 60 second expected retention time window to maximize dwell times and optimize the cycle time of the method. As a result, all of the peaks in the calibration contained >12 points per peak.

4. Large-Volume Direct Injection (continued)

Table 4. Ion source parameters for methods 1 and 2.

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	-4500 V
Temperature (TEM)	600 ˚C
Nebulizer Gas (GS1)	50 psi
Heater Gas (GS2)	50 psi

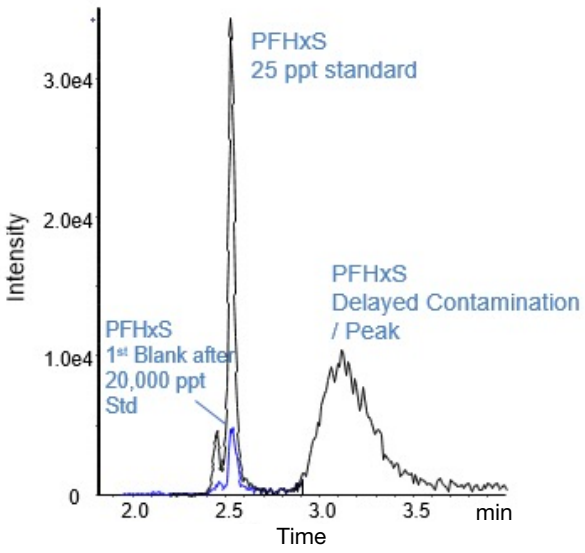
Calibration was performed using a 7-point curve at concentrations of 25, 50, 250, 1000, 2500, 10000, and 20000 ng/L for Method 1 and 1, 2, 5, 20, 50, 100, and 200 ng/L for Method 2. Quantitation was performed using MultiQuant™ Software 3.0.2 using 1.0 Gaussian smoothing and 1/x2 weighted linear regression. PFASs with matched isotopically labeled surrogate standards were quantified using isotope dilution, while PFASs without matched surrogate standards were quantified using internal standard calibration with structurally similar isotopically labeled standards (full analyte and internal standard list shown in Appendix **Figure 1**). A concentration factor of 500 was applied to samples analyzed using Method 1, and a dilution factor of 1.65 was applied to samples analyzed using Method 2.

Method 1 chromatography results

The Gemini® C18 column was selected for both methods based on its strong retention and predictable resolution of PFASs. All of the other columns tested exhibited breakthrough of the short chain acids in the column dead volume during optimization of the 950 µL injection method. The Luna® C18(2) column was selected as the delay column for both methods after initial testing indicated that it provided better separation of PFAS contamination than other columns. For PFASs, blank contamination is a major concern for analysis due to potential contamination during sample preparation or contamination originating from analytical instrumentation. **Figure 1** shows a small carryover peak at 2.5 min for PFHxS in a blank analyzed immediately following the injection of the highest calibration standard of 20,000 ng/L. The area of the carryover peak was only 0.078 % of the highest standard and 21 % of the lowest calibration standard for Method 1 (25 ng/L). The second peak at 3.2 min in **Figure 1** is attributed to delayed PFHxS contamination originating from the HPLC pumps. Without the delay column, this contamination would instead focus on the analytical column and elute at 2.5 min along with the standard and sample peak.

A 50 mm x 2 mm, 3 µm Gemini C18 column was selected for Method 1, which utilized a 10 µL injection volume. The chromatographic separation of 25 PFASs is shown in **Figure 2**. The average peak asymmetry factor for the first 2 eluting peaks (PFBA and PFBS) in the initial calibration standards was calculated to be 1.3 in Method 1 using MultiQuant Software.

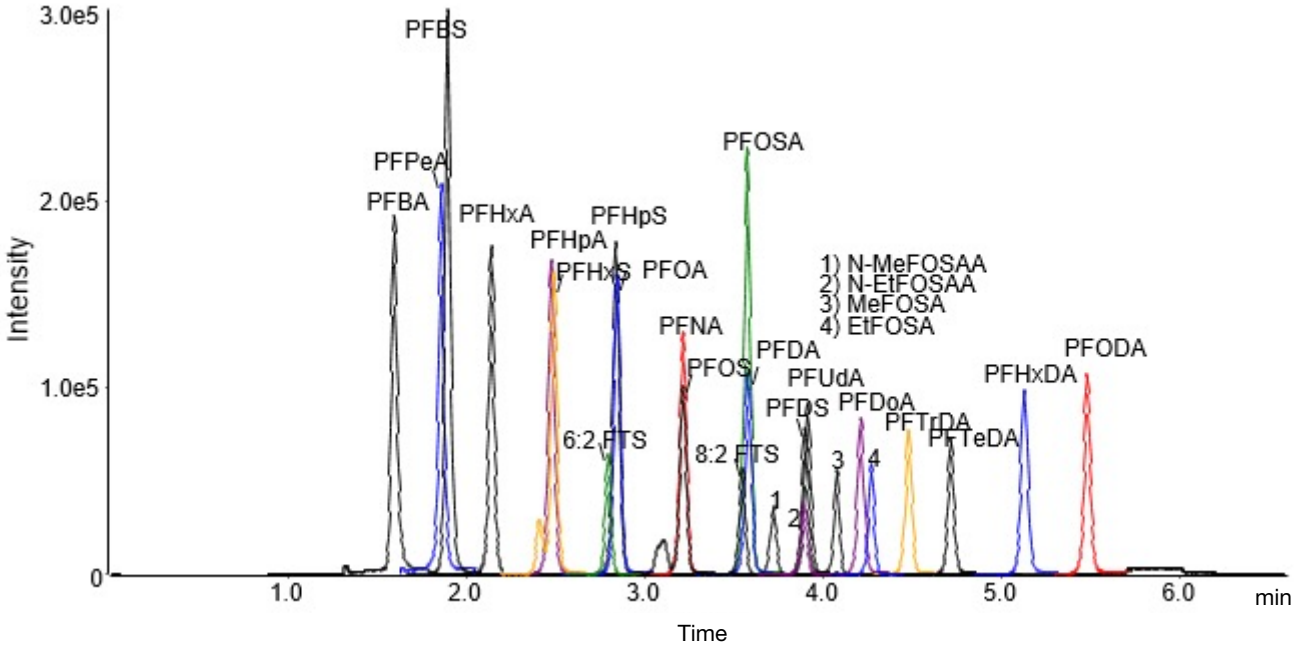
Figure 1.
Evaluating carryover



Overlaid MRM traces for PFHxS in the lowest calibration standard (black, 25 ng/L) and a blank injection (blue) that followed the highest concentration standard (20 µg/L). The delayed peak in the calibration standard trace represents the ambient LC system contamination retained by the delay column.

4. Large-Volume Direct Injection (continued)

Figure 2.
Method 1 chromatography: Weak anion exchange SPE with 10 µL injection



Overlaid Chromatograms of a 1 µg/L Standard Injected using Method 1.

3.0.2. This is within the acceptance criteria set by EPA 537 of 0.8-1.5 7.

Partial resolution of the branched and linear isotopes is necessary for PFAS analysis to distinguish between samples containing only linear isotopes or isotope mixtures. As shown in **Figure 2**, the earlier eluting branched isotopes are clearly distinguishable from the major peak corresponding to the linear isotopes for the 2 compounds that contained both branched and linear isotopes in the standards (PFHxS and PFOS). Most methods recommend that these two peaks are summed for quantitation, which was performed in this method using MultiQuant™ Software 3.0.2.

Method 1 calibration

The initial 7-point calibration for Method 1 exhibited good accuracy within +/- 30 % of the expected values for all points, accuracy within +/- 10 % for the lowest calibrator, and R2 coefficients of >0.990, as shown in Table 5. Based on the S/N ratio of the low calibrator and the linearity of the curve, the calibration range could be extended on both the high and low levels to improve the dynamic range. A water sample analyzed using Method 1 exhibited concentrations of several PFASs ranging from 0.974 to 53.3 ng/L, as shown in Figure 3.

4. Large-Volume Direct Injection (continued)

Method 2 chromatography

Method 2 is a large-volume, direct aqueous injection method designed for drinking, surface, and ground water samples. After the addition of surrogate standards and a simple dilution with methanol, 950 µL of the sample was injected directly onto the Gemini® C18 column. In contrast to Method 1, a longer and larger diameter column was used to improve retention of the analytes in the large volume injection. This resulted in a longer total runtime (17.5 minutes compared with 7.5 minutes), but provided robust results for the large volume injection and minimal retention time shift (**Figure 4**). The only compound that exhibited deteriorated peak shape due to the large injection volume was PFBA. However, the broadened peak shape of PFBA did not affect quantitation accuracy or precision.

Figure 3.
Overlaid chromatograms of PFASs quantified in a water sample using method 1.
This method uses a solid-phase extraction and a 10 µL injection.

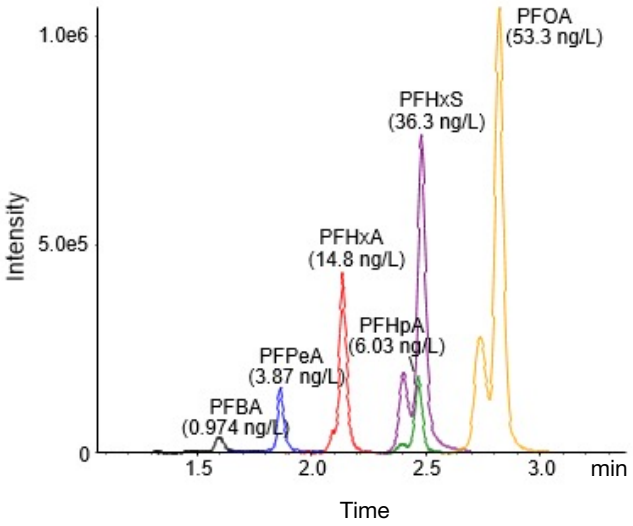


Table 5.
Calibration curves for method 1 and 2.
Sensitivity and linearity from 25 to 20,000 ng/L and 1 to 200 ng/L (coefficient of regression, R2) using Method 1 and Method 2, respectively. S/N calculated using MultiQuant™ Software 3.0.2.

Compound	Method 1				Method 2			
	Calibration range (ng/L)	Linear correlation (R2)	S/N of 25 ng/L standard	Accuracy of 25 ng/L standard	Calibration range (ng/L)	Linear correlation (R2)	S/N of 1 ng/L standard	Accuracy of 1 ng/L standard
PFCAs								
PFBA	25-20,000	0.997	108	104 %	1-200	0.997	328	97 %
PFPeA	25-20,000	0.998	88	103 %	1-200	0.999	137	101 %
PFHxA	25-20,000	0.998	104	93 %	1-200	0.999	284	101 %
PFHpA	50-20,000	0.999	116	101 %	1-200	0.993	267	96 %
PFOA	25-20,000	0.999	117	106 %	1-200	0.999	113	99 %
PFNA	25-20,000	0.990	91	109 %	1-200	0.999	137	101 %
PFDA	25-20,000	0.998	103	105 %	1-200	0.997	176	96 %
PFUdA	25-20,000	0.995	84	101 %	1-200	0.998	168	99 %
PFDoA	25-20,000	0.998	60	101 %	1-200	0.994	127	94 %
PFTrDA	25-20,000	0.998	32	104 %	1-200	0.995	125	95 %
PFTeDA	25-20,000	0.994	15	107 %	1-200	0.998	56	98 %
PFHxDA	25-20,000	0.999	21	103 %				
PFODA	25-20,000	0.999	33	102 %				
PFSAs								
PFBS	25-20,000	0.995	31	92 %	2-200	0.994	1178	100 %
PFHxS	25-20,000	0.999	604	103 %	1-200	0.998	229	96 %
PFHpS	25-20,000	0.997	103	105 %	1-200	0.999	327	99 %
PFOS	25-20,000	0.995	312	105 %	1-200	0.999	251	99 %
PFDS	25-20,000	0.998	88	102 %	1-200	0.999	516	98 %
Other PFASs								
6:2 FTS	25-20,000	0.991	100	98 %				
8:2 FTS	25-20,000	0.992	113	97 %				
PFOSA	25-20,000	0.997	118	104 %	1-100	0.997	1012	96 %
MeFOSA	25-20,000	0.996	96	103 %				
EtFOSA	25-20,000	0.994	90	101 %				
N-MeFOSAA	25-20,000	0.996	109	100 %				
N-EtFOSAA	25-20,000	0.994	61	103 %				

4. Large-Volume Direct Injection (continued)

Similar to Method 1, blank contamination from the instrument was minimized by using a delay column in Method 2. Blank contamination from sample preparation was also minimized in Method 2 by reducing the number of pipetting steps and testing all new batches of solvents prior to use. The integrated areas of the first blank after the highest concentration sample (200 ng/L) were less than 50 % of the lowest calibrator. For example, the area of the first blank analyzed after the 200 ng/L calibration standard was 22 % of the area of the 1 ng/L standard for PFOA as shown in **Figure 5**. The other blanks shown in **Figure 5** exhibited even lower response for PFOA, which could be contributed to laboratory contamination for the method blank and solvent contamination for the instrument blank.

To be compatible with common sampling practices, the Method 2 was not optimized for recovery of the longest chain PFASs, PFHxDA and PFODA, from the sample container or from the autosampler vial. Due to the stronger hydrophobicity of these compounds compared with the shorter chain PFAS, PFHxDA and PFODA appeared to bind to polypropylene containers when the methanol concentration was <40 %. Modifications to this method to improve the recovery and precision of PFHxDA and PFODA analysis may include collecting smaller samples (10-20 mL), diluting the entire sample with methanol in the sampling container, and adding surrogate standard directly to the sampling container.

Direct analysis of water samples is impaired by the presence of 5 g/L Trizma in samples, which is added to drinking water samples as a requirement by EPA method 537. Trizma suppresses ionization of the PFASs and elutes slowly from the column for minutes after the injection. Therefore, Trizma should not be added to samples that will be analyzed using direct aqueous injection, but is fully compatible with SPE as performed in Method 1.

Method 2 calibration

Similar to Method 1, the initial calibration results for Method 2 exhibited good accuracy within +/- 30 % of the expected values for all points, accuracy within +/- 10 % for the lowest calibrator, and R2 coefficients >0.990, as shown in **Table 5**. In the development of Method 2, calibration standards for 6:2 and 8:2 FTS, MeFOSA, EtFOSA, MeFOSAA, and EtFOSAA were not analyzed in the full calibration curve.

4. Large-Volume Direct Injection (continued)

Figure 4.
Method 2 chromatography: Dilution of water sample in methanol and 950 µL direct injection

Chromatogram of a 10 ng/L matrix spike into groundwater that was diluted with methanol and injected according to Method 2.

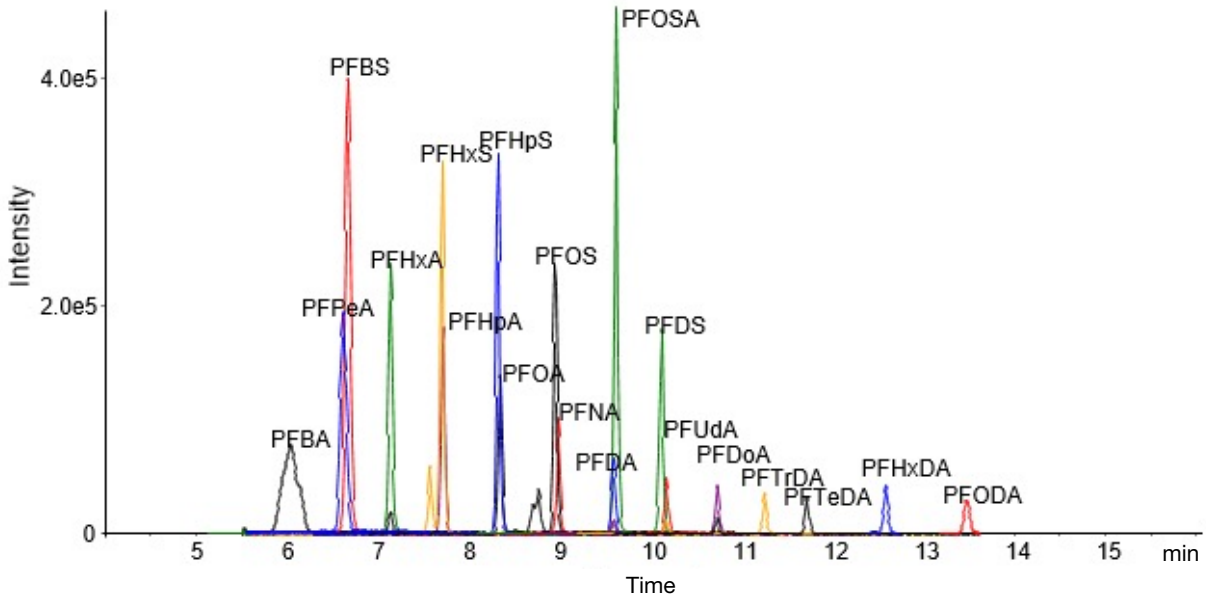
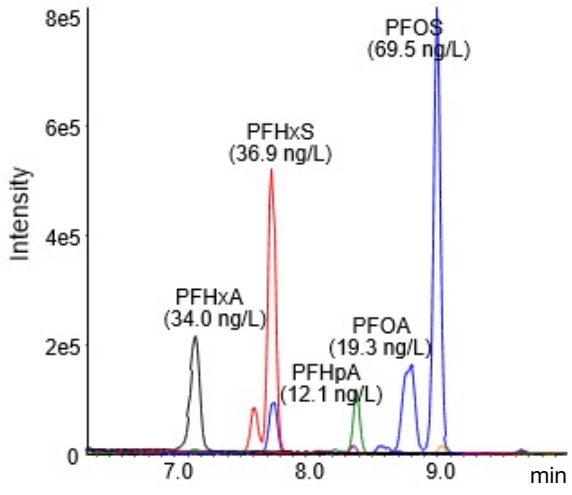


Figure 6
Overlaid MRM traces of PFASs detected in a groundwater sample with the calculated concentrations of each PFAS.

The sample was prepared and analyzed using Method 2.



Method 2 performance

Because large-volume injection methods are less common for PFASs compared with offline extraction methods, this application note reports the recovery and precision of continuing calibration standards over 1 week of continuous water sample analysis to demonstrate the robustness and accuracy of Method 2. The chromatogram and quantitated values for several PFASs in one of these water samples are shown **Figure 6**.

As shown in **Table 6**, a continuing calibration standard at 20 ng/L analyzed 1 week after the initial calibration exhibited quantitative accuracy of 92-99 % for all compounds with the exception of PFTrDA (81 %) and PFBS (84 %). Due to limited availability of surrogate standards, PFBS was analyzed using 18O2 PFHxS as an internal standard, and PFTrDA was analyzed using 13C2 PFDoA. The absence of an exact isotope-labelled surrogate for these two compounds likely contributed to the decreased accuracy of the ongoing calibration standard.

During the 1 week period of full-time water sample analysis, 9 replicates of the 20 ng/L continuing calibration verification (CCV) were analyzed as shown in **Table 6**. The precision (%CV) for all of the PFASs was <5 %, which indicates excellent precision for the large volume injections. The surrogate recovery, calculated as the response of the surrogate standard in the 20 ng/L ongoing calibration standard divided by the response of the surrogate standard during the initial calibration, was within 73-120 % for all of the PFASs analyzed.

4. Large-Volume Direct Injection (continued)

Summary

The 2 methods reported here were designed for optimum robustness using the SCIEX® Triple Quad™ 5500 System as the analytical platform. Both methods may be expanded to include soil, sediment, and biological extracts. Minimum and maximum reporting limits of approximately 1 ng/L to 400 µg/L could be achieved using both methods. These ranges could be expanded by increasing the extracted volume in Method 1 or by further dilutions in Method 2. The example chromatograms shown in this application note also demonstrate that the lower calibration levels than the levels analyzed here could be included in initial calibration curves to further improve the sensitivity of the method.

Method 1 has the advantage of compatibility with EPA Method 537 (7) and allows sample concentration using solid phase extraction. Method 2 has the advantages of minimal sample preparation and fewer steps to introduce lab-based PFAS contamination. With the growing need for PFAS analysis of environmental samples, these versatile methods will be useful for labs aiming to evaluate growing lists of PFASs.

Table 6.
Method 2

Accuracy of a 20 ng/L CCV analyzed 1 week after the initial calibration and precision of 9 replicates of a 20 ng/L CCV analyzed between 5 and 7 days after the initial calibration using Method 2.

Compound	Calculated conc of 20 ng/L CCV	Accuracy of 20 ng/L CCV	Surrogate standard recovery	Precision of 20 ng/L CCVs (%CV)
PFCAs				
PFBA	19.4	96 %	107 %	1.50
PFPeA	19.7	98 %	107 %	1.40
PFHxA	19.7	99 %	108 %	2.26
PFHpA*	18.5	92 %	103 %	3.11
PFOA	19.2	96 %	105 %	2.07
PFNA	19.3	97 %	107 %	1.11
PFDA	19.4	97 %	107 %	2.62
PFUdA	18.8	94 %	109 %	2.90
PFDoA	18.7	94 %	99 %	1.90
PFTrDA	16.3	81 %	99 %	4.77
PFTeDA	18.9	95 %	73 %	1.43
PFSAs				
PFBS	16.8	84 %	112 %	2.65
PFHxS	19.2	96 %	112 %	1.94
PFHpS	19.4	97 %	112 %	3.85
PFOS	18.8	94 %	120 %	2.62
PFDS	18.6	93 %	120 %	2.69
Other PFASs				
PFOSA	19.0	95 %	112 %	0.98

4. Large-Volume Direct Injection (continued)

Applications

Aknowledgements

SCIEX® acknowledges TestAmerica (Sacramento, CA) for collaborating with SCIEX by providing and conducting the analysis of standards for this application note. SCIEX also acknowledges Phenomenex (Torrance, CA) for providing HPLC columns and expertise for this application note and other method development efforts.

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Appendix Table 1.
MRM masses for methods 1 and 2.

Analytes are shown in bold font, and internal standards are shown in italic font.

Compound	Q1	Q3	DP	CE
PFBA	212.9	169	-25	-12
PFPeA	262.9	219	-20	-12
PFHxA	313	269	-25	-12
PFHpA	363	319	-25	-12
PFOA	413	369	-25	-14
PFNA	463	419	-25	-14
PFDA	513	469	-25	-16
PFUdA	563	519	-25	-18
PFDoA	613	569	-25	-18
PFTrDA	663	619	-25	-20
PFTeDA	713	669	-25	-22
PFHxDA	813	769	-25	-24
PFODA	913	869	-25	-26
PFBS	298.9	80	-55	-58
PFHxS	399	80	-60	-74
PFHpS	449	80	-65	-88
PFOS	499	80	-65	-108
PFDS	599	80	-85	-118
6:2 FTS	427	407	-50	-32
8:2 FTS	527	507	-50	-40
PFOSA	498	78	-60	-85
MeFOSA	512	169	-75	-37
EtFOSA	526	169	-75	-37
N-MeFOSAA	570	419	-40	-36
N-EtFOSAA	584	419	-50	-36
13C4_PFBA	217	172	-25	-12
13C5_PFPeA	268	223	-20	-12
13C2_PFHxA	315	270	-25	-12
13C4_PFHpA	367	322	-25	-12
13C2_PFOA	415	370	-25	-14
13C4_PFOA	417	372	-25	-14
13C5_PFNA	468	423	-25	-14
13C2_PFDA	515	470	-25	-16
13C2_PFUdA	565	520	-25	-18
13C2_PFDoA	615	570	-25	-18
13C2_PFTeDA	715	670	-25	-22
13C2_PFHxDA	815	770	-25	-24
1802_PFHxS	403	84	-60	-74
13C4_PFOS	503	80	-65	-108
13C8_PFOSA	506	78	-60	-85
M2-6:2FTS	429	409	-50	-32
M2-8:2FTS	529	509	-50	-40
d3MeFOSA	515	169	-75	-37
d5EtFOSA	531	169	-75	-37
d3-MeFOSAA	573	419	-40	-36
d3-EtFOSAA	589	419	-50	-36

Wastewater, Sediment, and Soil

As the PFAS story continues it is becoming more widely recognized that drinking water is not the only environmental media of concern. From its primary sources in fire suppression foams, industrial discharges and consumer products, PFAS is also widely found to occur in soils, sediments, surface water, groundwater and wastewater discharges, illustrating the widespread dispersion and persistence of this unique class of compounds. These discoveries have required the development and application of more advanced sample preparation, chromatography and mass spectrometry techniques to overcome the challenges of matrix and spectral interferences. In this section, two recent applications have been selected to illustrate the analytical challenges of these more difficult matrices.

5. SPE for DOD QSM 5.3

Per- and Polyfluoroalkyl Substances (PFAS) Extraction by LC-MS/MS Using Strata PFAS for a Stacked Solid Phase Extraction (SPE) Solution

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Overview

PFAS are a class of highly stable synthetic organic compounds used in a wide variety of industrial and commercial applications. They are also highly stable in the environment and strongly bioaccumulate. As a result, they have become ubiquitous throughout the global environment and are often referred to in popular media as "Forever Chemicals". Consequently, PFAS levels need to be tested in drinking water and more recently methods have been developed to measure PFAS in other environmental matrices that require more complex clean-up solutions, such as wastewater, soils and sediments.

The United States Department of Defense (DOD) is dealing with very extensive PFAS contamination owing to the widespread use of PFAS based Aqueous Film Forming Foam (AFFF) used as fire suppression foams at many military installations. As a result, DOD has developed its own PFAS analytical guidelines to deal with the unique environmental monitoring and clean-up challenges found on their installations. These guidelines, contained within the DOD QSM 5.1/5.3 documentation (Department of Defense QSM (osd.mil), feature a unique sample clean-up and concentration approach not found in EPA Methods which are designed only for drinking water application.

DOD QSM 5.1/5.3 specifies the use of a polymeric weak anion exchange (WAX) SPE sorbent in combination with graphitized carbon black (GCB) sorbent for the clean-up of solid samples, soils, biota, sediments, or non-drinking water samples. This can be performed by using two individual tubes of WAX and GCB sorbent that are applied sequentially or the use of dispersive SPE (dSPE) utilizing GCB following the WAX SPE tube extraction. Both methods add time to the clean-up procedure and present the opportunity for loss of analytes and introduction of imprecision. In this communication we describe a significant improvement to the guidelines, Strata PFAS SPE, wherein the two sorbents are contained within a single tube, offering the opportunity for decreased sample processing time and increased accuracy and precision. When comparing recoveries for a small subset of analytes for a WAX SPE and dSPE GCB method vs Strata® PFAS, the recovery is greatly improved for Strata PFAS (**Table 1**).

Strata PFAS is a stacked single cartridge solution with polymeric WAX and GCB sorbents that functions as a traditional Solid Phase Extraction (SPE) cartridge with a built in polishing step to meet the aforementioned DOD guidelines. This SPE product increases lab productivity and reduces the need for multiple extraction tubes when compared to a traditional two tube method.

Table 2 presents typical analyte recovery data from a routine Laboratory Control Sample (LCS) analyzed by a commercial testing laboratory highly experienced with the performance of DOD QSM 5.1/5.3. The LCS had been spiked with all 32 target analytes at 25 µg/L and was analyzed with a batch of field samples to demonstrate method performance and data acceptability. The recovery data show that all 32 analytes were well within method recovery limits with an average recovery of 98.8 % and a mean recovery of 99.0 %, thereby demonstrating acceptability of the use of Strata PFAS in the performance DOD QSM5.1/5.3.

The LCS sample was extracted with Strata PFAS under the conditions shown below and analyzed on a LC-MS/MS system using a Gemini® 3 µm C18 HPLC column under the conditions described below.

SPE Conditions

Cartridge: Strata PFAS (200 mg WAX/50 mg GCB/ 6 mL)
Part No.: CS0-9207
Condition 1: 4 mL 0.3 % Ammonium hydroxide
Condition 2: 4 mL Methanol
Equilibrate: 5 mL Water
Load: Add sample at 4 mL/min
Wash: 2x 4 mL Water
Elute: 2x 4 mL 0.3 % Ammonium hydroxide in Methanol
Evaporate: To dryness and reconstitute to 1 mL with Methanol/Water (96:4)

LC-MS/MS Parameters

Column: Gemini® 3 µm C18
Dimensions: 50 x 2.0 mm
Part No.: 00B-4439-B0
Mobile Phase: A: 20 mM Ammonium acetate in Water
B: Methanol
Gradient:

Time (min)	%B
0	5
0.1	55
4.5	99
8.0	99
8.5	5

Flow Rate: 0.6 mL/min
Delay column: Luna® 5 µm C18(2) 30 x 3.0 mm (00A-4252-Y0) installed between the autosampler and mobile phase pump mixer
Injection Volume: 10 µL

Mass Spec Parameters

Mass Spec Detector: SCIEX® Triple Quad™ 4500
Ion Source Parameters: Samples were ionized using electrospray in negative ion-mode

Parameter	Value
CAD	9
CUR	30
GS1	40
GS2	60
IS Voltage	-4500
TEM	450

MRM Transitions for HFPO-DA

Compound	Q1	Q3	RT	DP	CE
HFPO-DA (Quant)	329	185	3.7	-30	-32
HFPO-DA (Qual)	329	169	3.7	-30	-18
13C3 -HFPO-DA	332	185	3.7	-30	-32

5. Strata® PFAS SPE for DOD QSM 5.3 (continued)

Table 1.
Recovery Comparisons of WAX SPE and dSPE using GCB vs Strata PFAS Single Cartridge Method

Analyte	WAX SPE + dSPE GCB % Recovery	Strata PFAS Stacked Cartridge % Recovery
13C2-PFDoDA	77.0	84.5
13C2-PFTeDA	62.0	84.0
PFODA	38.0	78.3
PFHxDA	63.0	89.3

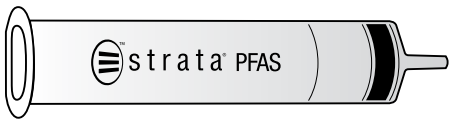

Table 2.
Recovery of QSM 5.3 Target Analytes from a Laboratory Control Sample Using Strata PFAS SPE (WAX/GCB)

Analyte	Actual Concentration	Sample Result	% Recovery	Method Limits	Pass/Fail
PFBA	25.600	22.640	88	84-135	Pass
PFPeA	25.600	22.157	87	75-138	Pass
PFBS	22.640	22.300	99	81-133	Pass
4:2-FTS	23.920	22.078	92	64-134	Pass
PFHxA	25.600	24.644	96	80-137	Pass
PFPeS	24.000	21.699	90	82-132	Pass
HFPODA	25.600	26.336	103	0-130	Pass
PFHpA	25.600	27.018	106	80-140	Pass
PFHpA	25.600	27.018	106	80-140	Pass
PFHxS	24.200	24.713	102	71-131	Pass
DONA	24.120	26.083	108	70-130	Pass
6:2-FTS	24.280	24.217	100	51-155	Pass
PFHpS	24.360	23.015	94	80-129	Pass
PFOA	25.600	25.043	98	83-138	Pass
PFOS	24.480	22.492	92	54-139	Pass
PFNA	25.600	25.872	101	73-140	Pass
9Cl-PF3ONS	23.840	21.863	92	70-130	Pass
PFNS	24.560	21.993	90	71-121	Pass
PFNS	24.560	21.993	90	71-121	Pass
PFDA	25.600	25.047	98	78-137	Pass
8:2-FTS	24.520	22.231	91	62-133	Pass
PFOSA	25.600	25.714	100	73-121	Pass
NMEFOSAA	25.600	30.906	121	53-136	Pass
PFDS	24.640	22.873	93	69-124	Pass
PFUnDA	25.600	26.353	103	70-134	Pass
NEtFOSAA	25.600	28.765	112	59-145	Pass
11Cl-PF3OUdS	24.120	22.625	94	70-130	Pass
PFDoDA	25.600	27.710	108	75-139	Pass
10:2-FTS	24.680	26.626	108	50-124	Pass
PFDoS	24.800	21.509	87	39-121	Pass
PFTrDA	25.600	25.814	101	67-144	Pass
PFTeDA	25.600	25.446	99	79-134	Pass
PFODA	25.600	27.373	107	10-124	Pass

Recovery Range:	87 % - 116 %
Average Recovery:	98.8 %
Mean Recovery:	99.0 %

6. Determination of PFAS in Sediments

Determination of Perfluoroalkyl Substances (PFAS) in Sediments by QuEChERS Extraction and HPLC-MS/MS

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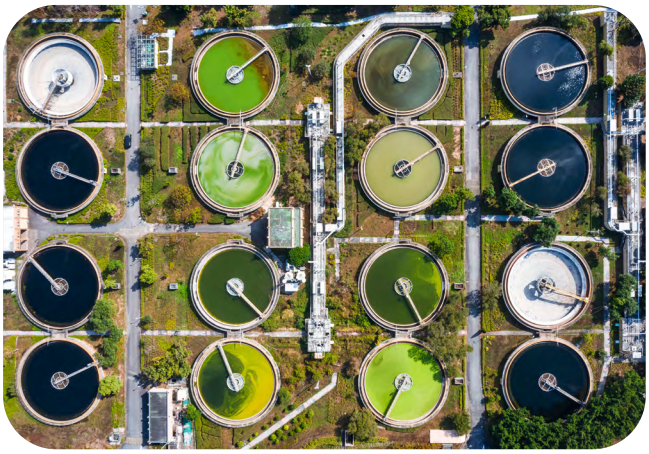
Introduction

Perfluoroalkyl substances (PFAS) are a class of highly stable synthetic organic compounds used in a wide variety of industrial and commercial applications including surface treatment for textiles, packaging materials, and non-stick cookware. PFAS are characterized by a hydrophobic fully fluorinated alkyl chain and a hydrophilic functional group. They are persistent in the environment due to the exceptional stability of the C-F bond. Once released in the aquatic environment, these chemicals will partition between the water phase and the sediment.



Currently, there are no federal regulatory limits controlling the discharge of PFAS compounds into the environment. Looking forward, it is possible that at some point EPA may establish regulatory limits for the various PFAS compounds in drinking water, wastewater and solid waste. In anticipation of such future developments, it is prudent to develop robust analytical methods and begin to better understand the fate and transport of these compounds in both the solid and liquid environmental fractions.

There are several methods available for the extraction and analysis of PFAS in aqueous samples, including the EPA Methods 537.1 and 533 previously described in this Guide (5). However, very few procedures are available for extracting these compounds in solid matrices such as sediments (1). Typical methods used are mechanical shaker and ultrasonic-assisted Solid-Liquid Extractions (SLE) 3, 4, 5). The extracts are then subjected to additional cleanup steps, usually by solid phase extraction, such as in the DoD QSM 5.3 approach previously described in this Guide. These are generally solvent-intensive and time-consuming processes. However, in 2003, an extraction procedure called QuEChERS (Quick-Easy-Cheap-Effective-Rugged-and-Safe) developed by researchers at the US Department of Agriculture was introduced (6). It was originally developed to extract pesticide residues in food matrices but has since found many other applications in the field of environmental analytical chemistry.

Our laboratory (LACSD) previously developed and validated a QuEChERS sediment extraction procedure for emerging contaminants including: pharmaceutical and personal care products, steroids, alkylphenol ethoxylates, and pyrethroid pesticides 7,8,9). We have successfully applied the same extraction method to determine perfluoroalkyl substances in marine and freshwater sediments.

Materials and Methods

Reagents/Chemicals

- QuEChERS Extraction – In a 50 mL plastic centrifuge tube combine 2.0 g of Anhydrous Magnesium Sulfate, and 1.5 g Sodium Acetate or use approximately 3.5 g of AOAC 2007.01 roQ™ extraction packet (part no. AH0-9043)
- QuEChERS dSPE Clean-Up — roQ 15 mL dSPE Kit (part no. KS0-8926)

Sample Preparation

QuEChERS Extraction Protocol

1. Weigh 2.0 g of dried sediment into a polypropylene container and spike with isotopically-labeled internal standards. *PPCPs, Steroids, and Pyrethroids can be extracted concurrently with this method by adding the appropriate internal standard and spiking solutions to the samples and QCs [7,8,9].*
2. Add 10 mL deionized water and vortex. Add 10 mL acidified acetonitrile (1 % acetic acid) to the slurry and vortex.
3. Add the extraction salts (1.5 g Sodium Acetate and 2 g $MgSO_4$) to the sample and vortex for 1 minute.
4. Centrifuge the samples for 5 minutes at 4000 rpm.
5. Place the samples in a rack and freeze at -20° for 30-60 minutes. This freezing step allows for easier extraction of the supernatant.
6. Transfer 8-9 mL of the acetonitrile supernatant into a roQ QuEChERS PSA/C18 dSPE clean-up tube (Part no. KS0-8926) and vortex for one minute.
7. Centrifuge the dSPE tubes for 10 minutes at 3000 rpm.
8. Place an aliquot of the extract in a HPLC vial and dilute 1:1 with deionized water. The sample is now ready for analysis.

HPLC-MS/MS Conditions

Column:	Gemini® 3 µm C18
Dimensions:	100 x 3 mm
Part No.:	00D-4439-Y0
Inline Filter:	Phenomenex Krudkatcher™ Ultra
Delay Column:	Luna® 5 µm C18 (2) 30 x 2.0 mm
Part No.:	00A-4252-B0
Mobile Phase:	A: 20 mM Ammonium acetate in water B: Methanol
Gradient:	**Time (min) % B** 0.0 10 1.5 65 8.0 95 8.1 99 12.0 99 12.5 10
Injection:	90 µL
Flow Rate:	0.6 mL/min
Temperature:	40 °C
Detector:	SCIEX® 5500 QTRAP®
Detection:	MS/MS ESI Negative (sMRM)
Analytes:	1. PFBA 2. PFPeA 3. PFBS 4. PFHxA 5. PFPS 6. PFHxS 7. PFHpA 8. PFHpS 9. PFOA

6. Determination of PFAS in Sediments (continued)

Mass Spectrometer Parameters

Table 1.
MRM Transitions and Compound Dependent Parameters

Compound Name	Q1	Q3	DP	CE
Perfluorobutanoic acid (PFBA)	213	169	-71	-14
Perfluoropentanoic acid (PFPeA)	263	219	-71	-12
Perfluorohexanoic acid (PFHxA)	313	269	-60	-14
Perfluoroheptanoic acid (PFHpA)	363	319	-62	-15
Perfluorooctanoic acid (PFOA)	413	369	-91	-12
Perfluorononanoic acid (PFNA)	463	419	-79	-15
Perfluorodecanoic acid (PFDA)	513	469	-83	-17
Perfluoroundecanoic acid (PFUdA)	563	519	-60	-17
Perfluorododecanoic acid (PFDoA)	613	569	-50	-21
Perfluorotridecanoic acid (PFTrDA)	663	619	-49	-18
Perfluorotetradecanoic acid (PFTeDA)	713	669	-63	-20
Perfluorobutanesulfonate (PFBS)	299	80	-94	-70
Perfluoropentantesulfonate (PFPeS)	349	80	-96	-66
Perfluorohexanesulfonate (PFHxS)	399	80	-92	-75
Perfluoroheptanesulfonate (PFHpS)	449	80	-75	-84
Perfluorooctanesulfonate (PFOS)	499	80	-78	-96
Perflluorooctanesulfonamide (PFOSA)	498	78	-60	-84
Perfluorononanesulfonate (PFNS)	549	80	-87	-100
Perfluorodecanesulfonate (PFDS)	599	80	-55	-100
Perfluoro-n-^{13}C4-butanoic acid (M4PFBA)	217	171.9	-71	-13
Perfluoro-n-^{13}C5-pentanoic acid (M5PFPeA)	268	222.7	-71	-12
Perfluoro-n-^{13}C5-hexanoic acid (M5PFHxA)	318	272.9	-60	-13
Perfluoro-n-^{13}C4-heptanoic acid (M4PFHpA)	367	321.8	-62	-14
Perfluoro-n-^{13}C8-octanoic acid (M8PFOA)	421	376	-91	-12
Perfluoro-n-^{13}C9-nonanoic acid (M9PFNA)	472	427	-79	-17
Perfluoro-n-^{13}C6-decanoic acid (M6PFDA)	519	474	-83	-21
Perfluoro-n-^{13}C7-undecanoic acid (M7PFUdA)	570	525	-60	-17
Perfluoro-n-^{13}C2-dodecanoic acid (M2PFDoA)	615	570	-50	-24
Perfluor-n-^{13}C2-tetradecanoic acid (M2PFTeDA)	715	670	-63	-25
Perfluro-^{13}C3-butanesulfonate (M3PFBS)	302	80	-94	-55
Perfluoro-^{13}C3-hexanesulfonate (M3PFHxS)	402	80	-92	-85
Perfluoro-^{13}C8-octanesulfonate (M8PFOS)	507	80	-78	-100

Note: DP = Declustering Potential
CE = Collision Energy

Table 2.
MS Source Parameters

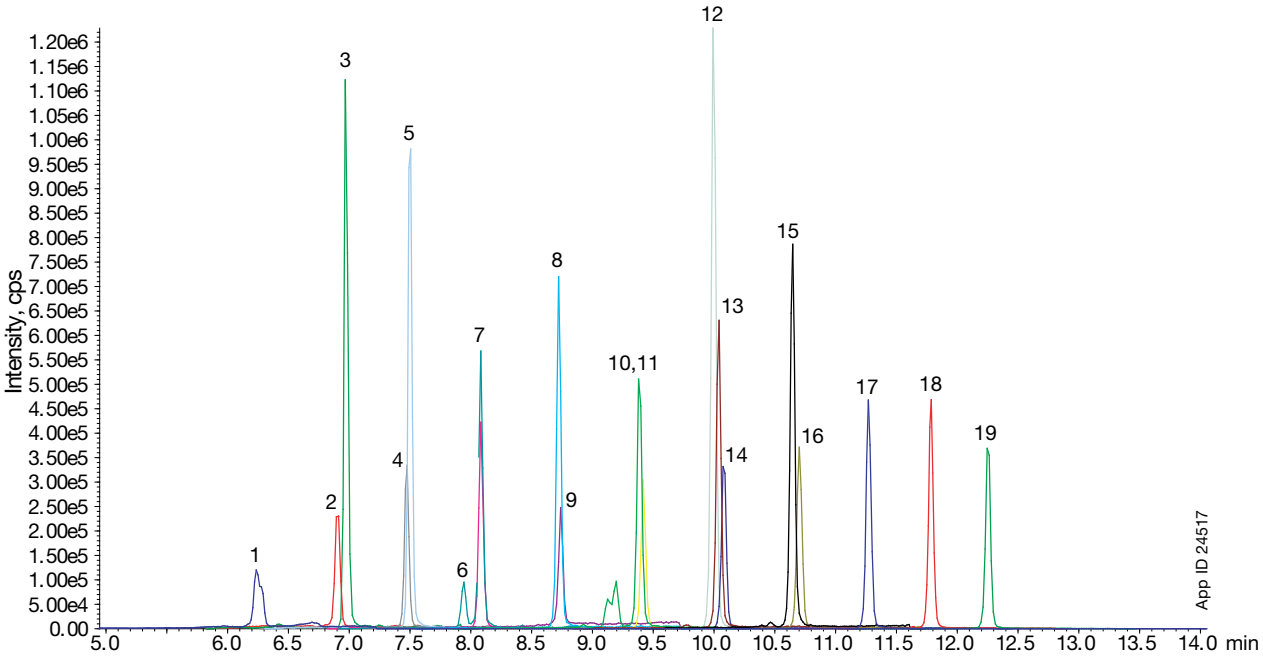
Source Parameters	Settings
Temperature	400 °C
Gas 1	50
Gas 2	50
Curtain Gas	35
Ionization Energies	-4500 V
Collision Gas	High

Table 3.
Method Performance Data for Sediments Spiked at 1 ng/g of the Target Analytes (n=4)

Compound	Average % Recovery	% RSD
PFBA	91.7	0.76
PFPeA	86.3	6
PFHxA	89.4	1.2
PFHpA	93.1	2.9
PFOA	98.3	1.5
PFNA	93	1.6
PFDA	87.7	4.5
PFUdA	92.3	2.1
PFDoA	92.5	4.1
PFTrDA	88.2	2.1
PFTeDA	87.6	2.1
PFBS	86.3	2.1
PFPeS	96.2	3.2
PFHxS	81.3	5
PFHpS	92.3	2.6
PFOS	92.1	2.6
PFOSA	104.5	6.3
PFNS	89.8	6.8
PFDS	87.3	6.7

6. Determination of PFAS in Sediments (continued)

Figure 1.
Extracted ion chromatogram of sediments spiked with 1.0 ng/g of the target analytes



Results and Discussion

QuEChERS is a vortex-assisted solid-liquid extraction procedure that uses acetonitrile, salts, and buffering agents for extraction, phase-separation, and pH adjustment respectively. Extracts are subsequently transferred to a dispersive solid phase extraction (dSPE) tube containing a drying agent ($MgSO_4$) and SPE sorbents such as C18 or PSA for sample cleanup.

The modified QuEChERS method presented here is a simple, efficient, and cost-effective method for determining PFAS levels in sediments. Accuracy and precision were assessed using four replicates of sediments spiked with the target analytes. Average % recoveries are all within the 80-120 % range and % RSDs for all analytes are below 10 % (**Table 3**). Reporting limits were set at 0.05 ng/g dry weight based on a 2.0 g initial sample weight.

SANITATION DISTRICTS OF LOS ANGELES COUNTY

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Acknowledgements

Special thanks to Syljohn Estil and to the Sanitation Districts of Los Angeles County – San Jose Creek Water Quality Laboratory for contributing this method.

Food and Food Packaging

It was recognized fairly early that PFAS compounds used in food packaging materials (such as pizza boxes and microwave popcorn bags) could migrate into consumable food products and contribute to increased PFAS body burden. In addition, as PFAS contamination has continued to spread throughout the environment it has been more recently recognized that these materials can also enter the human food supply chain through animal consumption of PFAS contaminated water and feed, thereby further increasing our PFAS body burden. Regardless of source, the analysis of PFAS in food and food packaging materials – and their myriad complex matrices - features additional difficult analytical challenges.

7. New Concerns about PFAS in Food

The Convergence of Environmental Contamination and Food Safety

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Abstract
Per- and Polyfluorinated Alkyl Substances (PFAS) are well known environmental contaminants that have a newly recognized potential to taint certain food products through agricultural consumption via environmental transport from contaminated industrial sites [1]. The analysis of PFAS in food products requires more extensive analytical preparation techniques, compared to PFAS testing of simple matrices such as drinking water, in order to reduce the impact of sample matrix interferences on the subsequent instrumental analysis. An example is provided of a PFAS method applicable to milk, butter, cheese and fish.

The Prequel
Per- and Polyfluorinated Alkyl Substances (PFAS) are an extensive family of synthetic, fluorochemicals with a unique set of physical and chemical properties. These properties have resulted in their widespread commercial use over the past 50 years in diverse applications ranging from fire fighting foams to stain resistant carpet to grease-proof pizza boxes. However, these same unique physical and chemical properties also have been found to bear serious environmental consequences: widespread dispersion ability, extreme environmental persistence and a high degree of bioaccumulation [2]. Although PFAS do not exhibit acute toxic properties, researchers have found that PFAS can demonstrate a large number of subtle, chronic health effects, primarily affecting the endochrine and reproductive systems. Consequently, health experts have long been concerned that low-level, cumulative exposure to PFAS over an extended period of time could have serious health consequences [3]. Therefore, chronic lifetime PFAS exposure pathways - such as through food or drinking water – are of particular concern to regulators and are receiving enhanced scrutiny.

Initial Concerns
In the US, the initial US Food and Drug Administration (FDA) concern about PFAS centered about the contamination of food products through contact with PFAS containing food packaging (and to a lesser extent with food processing equipment). The classic examples are those PFAS coated pizza boxes, fast-food hamburger wrappers and microwave popcorn bags that have done such a marvelous job of keeping grease off our clothes. That problem was summarily solved in late 2016 when FDA removed the approval for the use of PFAS in food packaging [4].

Likewise, the primary US Environmental Protection Agency (EPA) focus has been on drinking water as a primary source of lifetime PFAS exposure. EPA is continuing to conduct extensive nationwide testing for PFAS in drinking water under the Unregulated Contaminant Monitoring Rule (UCMR) program [5]. These efforts will very likely result in specific regulatory limits for the allowable concentration of certain PFAS in drinking water.

Concurrently, other government agencies, such as the US Department of Defense (DOD) have been extensively studying the widespread environmental contamination of military facilities owing to the extensive historical use of PFAS firefighting foams, principally at air bases [6].

Convergence
Initially, these three individual trains of concern seemed to be running on separate tracks. It was only more recently that they were seen to be converging toward a much larger, more complex problem requiring multimedia, multi agency examination and the use of more sophisticated analytical tools. The simplified pathway model shown in **Figure 1** illustrates the general scope of the problem. By the end of 2019, the FDA was fully on board with concerns about PFAS entering the general food supply through environmental sources, potentially leading to the contamination of dairy products, bottled water, seafood and other consumables [7].

Analytical Implications
This expanded concept of the PFAS problem is clearly a major step forward, but it has presented some analytical challenges. Much of the official PFAS methodology developed over the past decade has been focused on the analysis of drinking water and aimed at a very limited list of analytes. With little challenge from matrix interference, easily surmountable chromatography issues and straight forward mass spectrometry, these official drinking-water-only methods proved to be inadequate when applied to the analysis of PFAS in soil, sediment, sludge and wastewater. When applied to the analysis of foods - with a myriad of complex matrices, they are quite ineffective, resulting in a surge in PFAS analytical method development centered about complex matrices, with food testing occupying a prominent position. The following section features one such application as an illustration of the approaches now being pursued in pursuit of the expanded PFAS challenge.

Analysis of PFAS in Dairy Products, Eggs, and Fish by LC-MS/MS

Method Introduction
The following work was performed through a collaboration between Weck Laboratories, Inc., City of Industry, CA, USA and Phenomenex, Inc.,Torrance, CA, USA, for the development of new sample preparation and analysis procedures for determining low levels of PFAS in food products. This particular application was directed at achieving sub-ppb sensitivity for 23 PFAS analytes in dairy products (milk, butter and cheese), eggs and fish as representative of difficult to analyze fatty matrices. The following discussion is a synopsis of the full work [8].

Sample Preparation
One gram of homogenized sample was spiked with internal standards and surrogates and an analyte mix of 23 PFAS compounds (**Table 1**) at the 1 ng/g level, followed by the addition of 10 mL acetonitrile and 10 mL water. Four replicates of each matrix (milk, eggs, butter, cheese and fish) were prepared. The samples were processed by a modified QuEChERs procedure using a commercial kit (Phenomenex roQ™ Extraction Kit). An aliquot (500 µL) of the cleaned acetonitrile phase was transferred to an LC vial for analysis. **Figure 2** displays an extraction blank and the five sample types following sample preparation.

7. New Concerns about PFAS in Food (continued)

Optional Solid Phase Extraction

A dispersive SPE cleanup was used to achieve a 10-fold lower level of quantitation. Four replicate samples of the egg matrix were spiked with the PFAS analyte mix at the 0.1 ng/g level and processed by the QuEChERs procedure. Following extraction, 500 µL of the acetonitrile phase was diluted with 15 mL of water and loaded onto a preconditioned, weak-ion-exchange SPE tube (Phenomenex Strata®-X-AW 200 mg). The analytes of interest were then eluted with 4 mL of 0.3 % NH_4OH-acetonitrile.The eluate was evaporated to dryness, reconstituted with 500 µL of acetonitrile and transferred to an LC autosampler vial for analysis.

Optional Solid Phase Extraction

A dispersive SPE cleanup was used to achieve a 10-fold lower level of quantitation. Four replicate samples of the egg matrix were spiked with the PFAS analyte mix at the 0.1 ng/g level and processed by the QuEChERs procedure. Following extraction, 500 µL of the acetonitrile phase was diluted with 15 mL of water and loaded onto a preconditioned, weak-ion-exchange SPE tube (Phenomenex Strata-X-AW 200 mg). The analytes of interest were then eluted with 4 mL of 0.3 % NH_4OH-acetonitrile.The eluate was evaporated to dryness, reconstituted with 500 µL of acetonitrile and transferred to an LC autosampler vial for analysis.

LC-MS/MS Analysis

The chromatography was performed on an Agilent® 1290 UHPLC system. The LC column employed was a Phenomenex Luna® Omega 1.6 µL PS C18 operating at 40 degrees Celsius with a flow rate of 0.55 mL/min and an injection volume of 20 µL. The mass spectrometer used was an Agilent 6460 QQQ. Various LC-MS/MS conditions were explored and an ammonium acetate/acetonitrile gradient (**Table 2**) proved to be optimum, resulting in a run time of approximately 4 minutes.

Results and Discussion

System calibration showed a linear dynamic response from 0.05 ppb – 1000 ppb with a lower limit of quantization of 0.05 ppb as shown in **Figure 3** and a calibration chromatogram at the 0.05 ppb level is shown in **Figure 4**. Recovery data for the five matrix types is summarized in **Figures 5**–**9**. Four replicates of each matrix were spiked at the 1 ng/g level and prepared for analysis as described above (but were not subjected to the solid phase extraction process). **Figure 10** presents the recovery data for four replicates of the egg matrix spiked at 0.1 ng/g and prepared as described above, but with the addition of the solid phase extraction step to increase method sensitivity.

The recovery data show good recovery for all five matrices spiked at the 1 ng/g level, with most analytes falling into the 80 % - 120 % recovery range. Precision is generally somewhat poorer for the higher fat dairy products than for the lower fat matrices. The recoveries on tuna fish are particularly good, considering the complexity of the matrix. In comparing the analyte recoveries from eggs at the 1 ng/g and 0.1 ng/g levels (**Figure 9** and **Figure 10**), both show comparable recoveries although, as expected, the higher spike level shows greater precision. Overall, the data suggest that the method has sufficient accuracy and precision to potentially be used to assess environmental PFAS contamination of food products. Clearly, this is preliminary data and further development and multi-laboratory validation would be required to demonstrate such a purpose. However, the data clearly show that current sample preparation techniques, coupled with the power of advanced chromatography and triple-quad mass spectrometry represent a suitable workflow.

The Sequel

The earlier discussion showed the use of current analytical technology to address the challenge of environmental PFAS contamination of the food supply. However, care should be taken since experience with analytical chemistry teaches us that we will inevitably be facing further analytical challenges from the realm of the “unknown-unknowns”.

In PFAS analysis, we are currently discussing a target analyte list of 20, 30 or 40 compounds? However, the number of compounds in the PFAS universe has been estimated at 5000 - and even as high as 8,000 - which doesn’t include potential degradation products. Toxicity is largely a function of the unique chemical and configurational state of a molecule that controls the biochemical interaction with the organism. So, there is much more analytical work to identify the most important PFAS compounds from a toxicity perspective.

Excellent work is being done with accurate mass and advanced data analysis to give us a broader understanding of the chemical complexity of the PFAS universe. However, given the complexity and extent of the problem of environmental PFAS contamination, it is clear that a lot of hard work has yet to be done.

Acknowledgements

The contribution of Dr. Agustin Pierri and his team at Weck Laboratories, City of Industry, California, USA is gratefully acknowledged.

WECK LABORATORIES, INC.

7. New Concerns about PFAS in Food (continued)

Figure 1.
Pathway Model for Environmental Transmission of PFAS to Food and Consumer

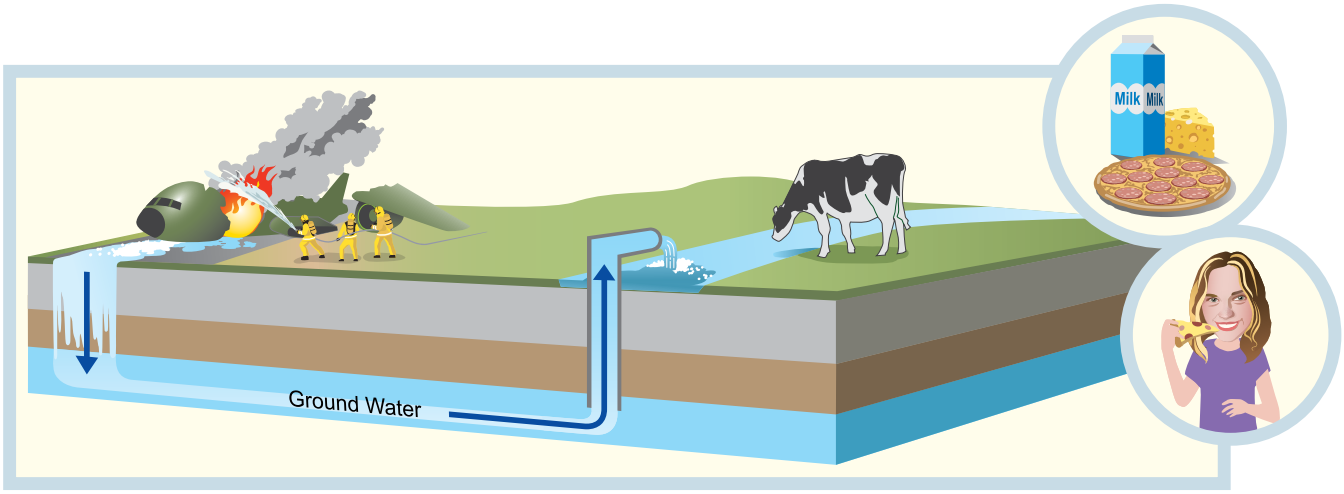
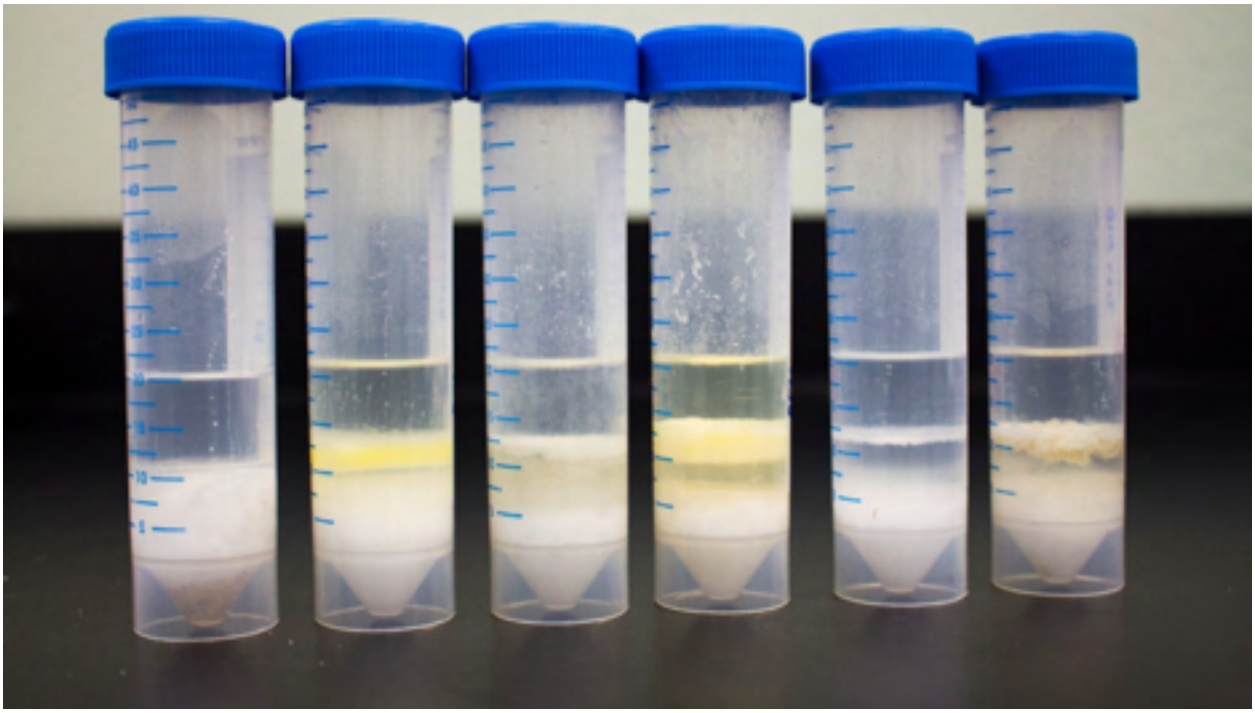


Figure 2.
Samples after QuEChERs Cleanup:
From Left to Right: Blank, Butter, Cheese, Egg, Milk and Fish



7. New Concerns about PFAS in Food (continued)

Applications

Figure 3.
System Calibration Dynamic Range (0.05 – 1000 ppb)

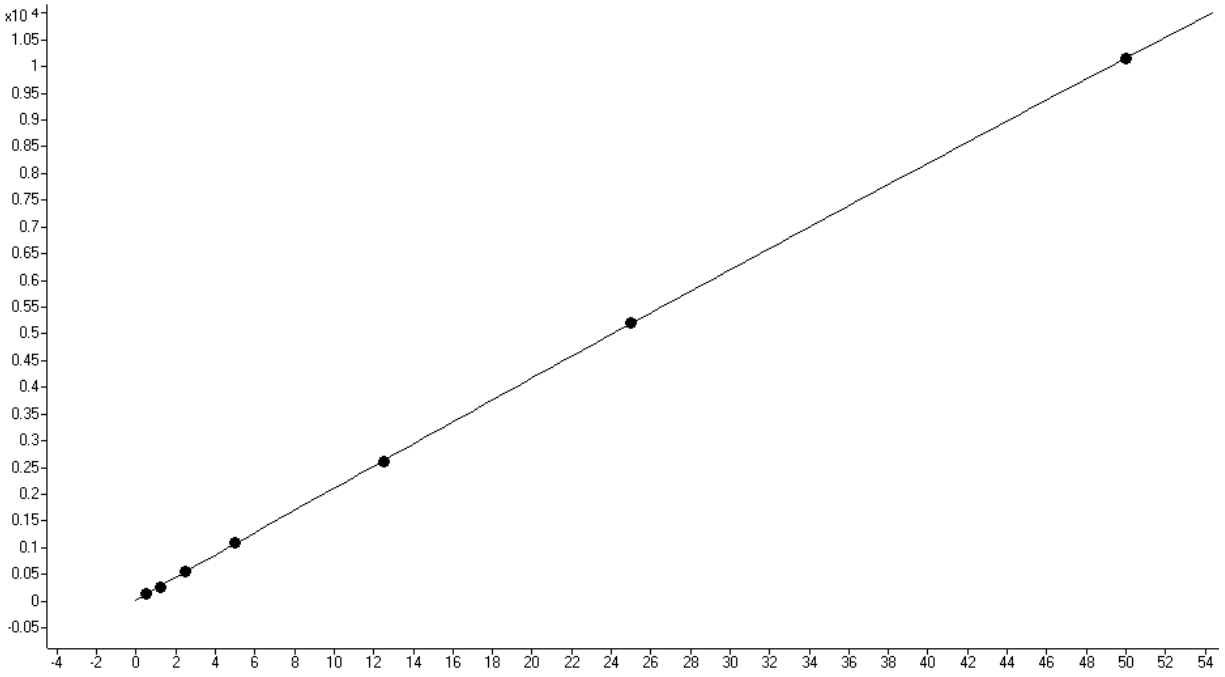
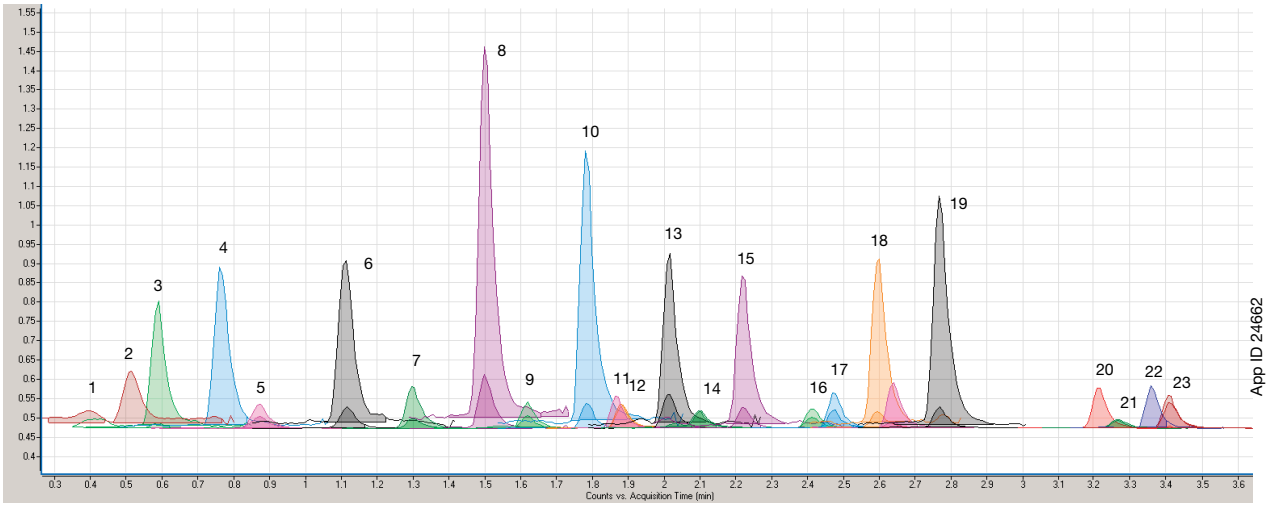


Figure 4.
Chromatogram of 0.05 ppb Lower Limit of Quantization Standard



7. New Concerns about PFAS in Food (continued)

Figure 5.
Milk Recoveries (QuEChERs: 1 ng/g, n=4)

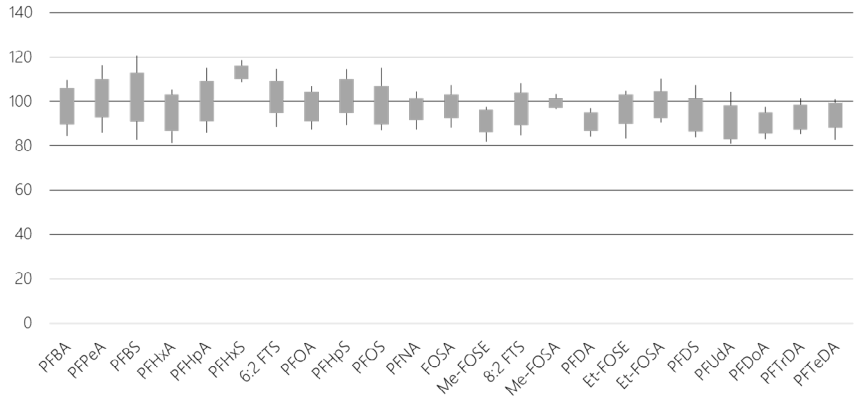


Figure 6.
Butter Recoveries (QuEChERs: 1 ng/g, n=4)

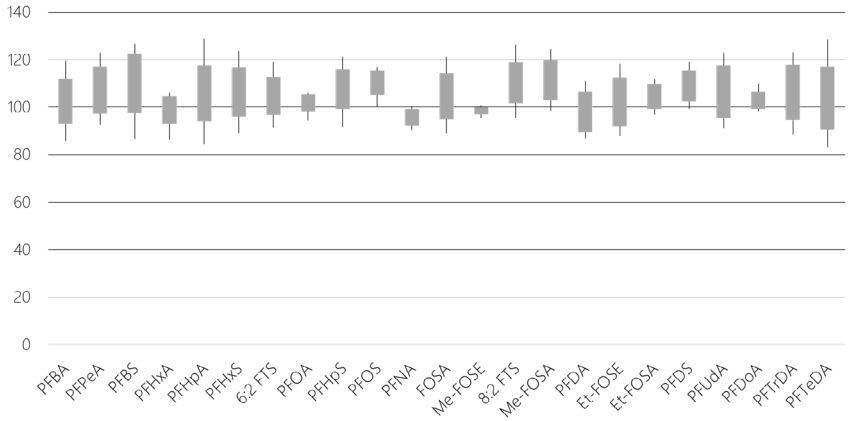
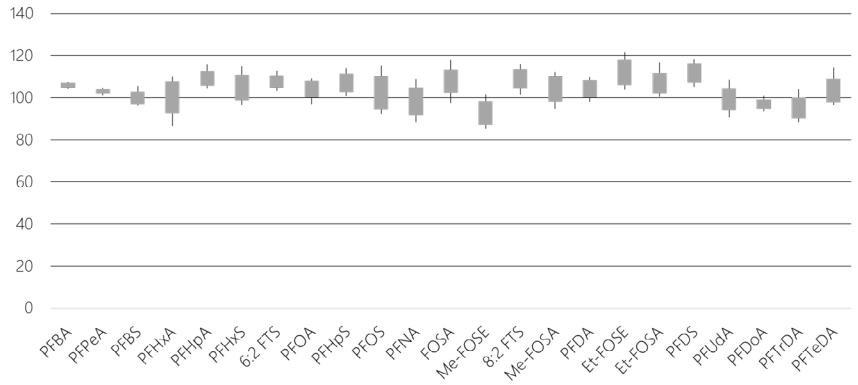


Figure 7.
Tuna Recoveries (QuEChERs: 1 ng/g, n=4)



7. New Concerns about PFAS in Food (continued)

Figure 8.
Cheese Recoveries (QuEChERs:1 ng/g, n=4)

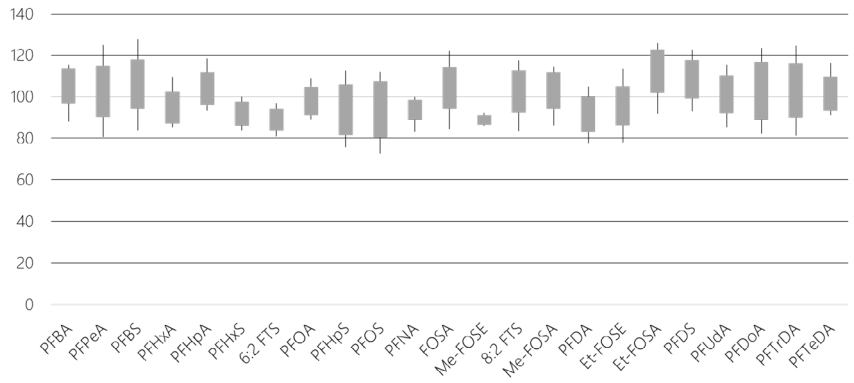


Figure 9.
Egg Recoveries (QuEChERs: 1 ng/g, n=4)

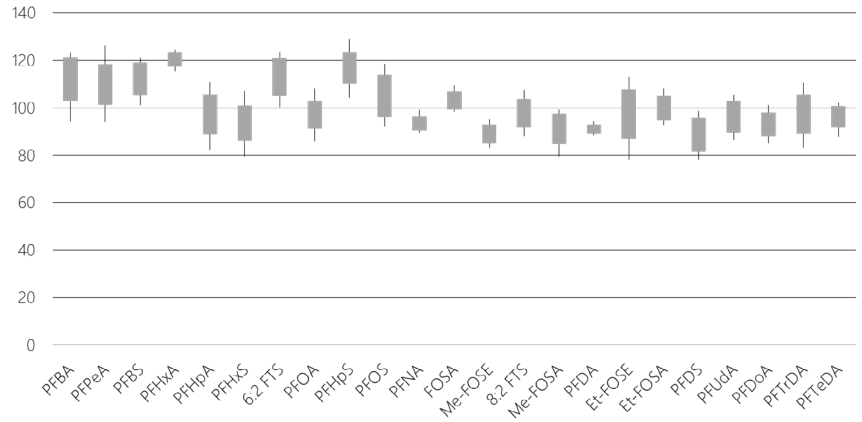
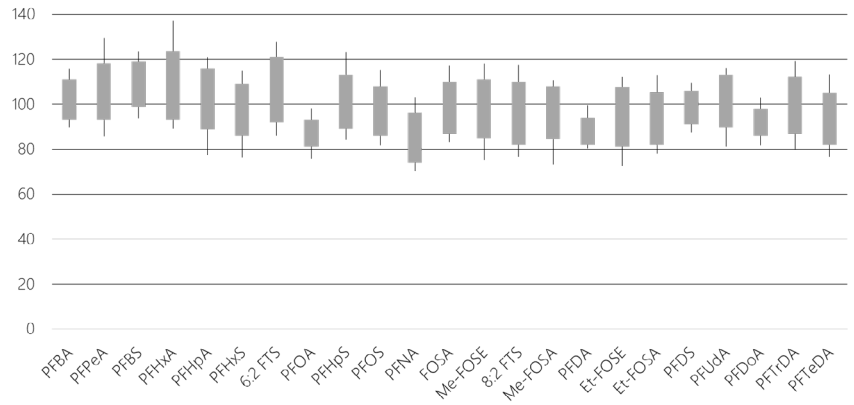


Figure 10.
Egg Recoveries (QuEChERs + SPE: 0.1 ng/g, n=4)



7. New Concerns about PFAS in Food (continued)

Table 1.
PFAS Analyte List

Analytes:		
1. PFBA	9. PFHpS	17. Et-FOSE
2. PFPeA	10. PFOS	18. Et-FOSA
3. PFBS	11. PFNA	19. PFDS
4. PFHxA	12. FOSA	20. PFDS
5. PFHpA	13. Me-FOSE	21. PFDoA
6. PFHxS	14. 8:2 FTS	22. PFTrDA
7. 6:2 FTS	15. Me-FOSA	23. PFTeDA
8. PFOA	16. PFDA	

Table 2.
LC-MS/MS Conditions

Column: Luna® Omega 1.6 µm PS C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4752-AN
Mobile Phase: A: 5 mM Ammonium Acetate in Water
B: Acetonitrile
Gradient:

Time (min)	% B
0	40
0.5	40
3	90
3.1	100
4	100

Flow Rate: 0.55 mL/min
Injection: 20 µL
Temperature: 40 °C
UHPLC System: Agilent® 1290
Detection: Agilent 6460 QQQ
Analytes:

1. PFBA	9. PFHpS	17. Et-FOSE
2. PFPeA	10. PFOS	18. Et-FOSA
3. PFBS	11. PFNA	19. PFDS
4. PFHxA	12. FOSA	20. PFUdA
5. PFHpA	13. Me-FOSE	21. PFDoA
6. PFHxS	14. 8:2 FTS	22. PFTrDA
7. 6:2 FTS	15. Me-FOSA	23. PFTeDA
8. PFOA	16. PFDA	

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8. PFAS in Food Contact Materials

Identification and Quantification of PFAS in Food Contact Materials using MRM^{HR} Workflow on X500R QTOF System

Wang Sha, Liu Bingjie, Cheng Haiyan, Li Lijun, Jin Wenhai
SCIEX, China

Introduction

In comparison to other surfactants, perfluorinated alkyl substances (PFAS) have stable physiochemical structures with hydrophobic and oleophobic properties. They are widely used in industrial and consumer products like plastic packaging materials for food and as coating in non-stick pans. Due to their chemical stability and low reactivity, PFAS are highly resistant to degradation even in living organisms and can therefore be accumulated in the food chain. Human exposure to PFAS residues has been implicated in incidences of cancer, obesity, endocrine system disruption and other adverse health effects. [1]

With the rapid growth in the food delivery industry in China (and globally) in the past two years, one-time-use plastic packaging materials are widely used by merchants due to their low cost and high durability [2]. One-time-use plastic has become a source of public concern and environmental pollution. Given the tremendous persistence of PFAS in the environment and the adverse effect on human health, monitoring of PFAS residue has gained traction in China and elsewhere.

In China, the level of PFOS and PFOA in food contact materials and products is regulated according to the latest National Food Safety Standard (GB 31604.35-2016). The detection limit is set at 1.0 ng/g while the quantification limit is set at 2.0 ng/g. In 2006, the European Union (EU) has set a regulation that the level of PFOS in finished products should not exceed 0.005 % of the product mass.

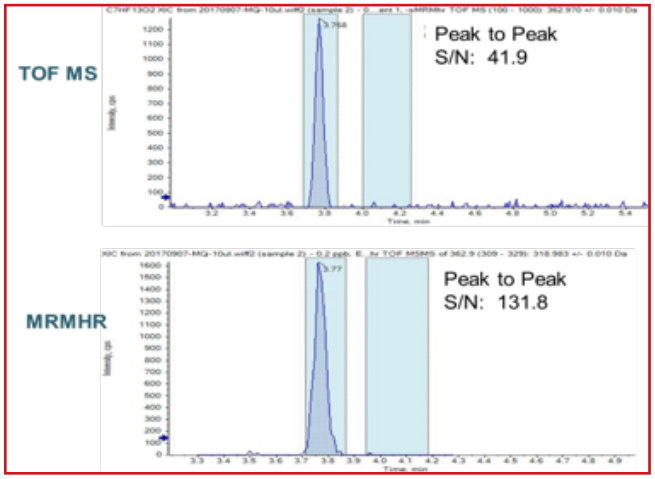


Figure 1.
Signal-to-Noise Comparison of PFHpA using TOF-MS and MRMHR Data Using a Post Spiked 0.2 ppb Matrix Blank

Monitoring the transition and the high resolution fragment ion results in greater specificity and reduced baseline, so signal-to-noise demonstrates marked improvement and method sensitivity is maximized.

The X500R QTOF system has the industry's fastest scanning speed, allowing for the implementation of the unique MRM^{HR} acquisition mode to provide excellent quantitative performance using high-resolution MS/MS data. This approach to quantitation with LC-QTOF-MS/MS minimizes matrix interferences and the patented Turbo V™ ion source with curtain gas interface, twin sprayer technology and built-in automatic calibration system help to improve and maintain instrument robustness and maintain high mass accuracy results. The high resolution MS/MS spectra can also be used for qualitative analysis by calculating the ion ratio for confirmation, thus reducing false positives by taking advantage of the data acquired on the LC-QTOF platform.

Key Workflow Advantages

- PFAS quantitation using an easily established method and minimal method development
- 10-minute run time using a Phenomenex Kinetex® C18 column demonstrates separation of PFAS targets
- MRM^{HR} workflow using MS/MS for selectivity vs high resolution TOF MS mode provides improved signal-to-noise
- QTOF technology can be utilized for quantitative analysis of PFAS suite without compromising method performance (excellent sensitivity, linearity demonstrated)

8. PFAS in Food Contact Materials (continued)

Methods

Sample Preparation

The food packaging material to be tested is cut into small pieces. For coating sample, scrape it with a small knife. The sample preparation procedure was adapted from National Standard of China (document number GB 31604.35-2016) which is implemented on 19 April 2017 (**Figure 2**).

A total of eight samples were collected as test samples which include disposable meal box, plastic bag, beverage bottle, coating of non-stick pan, etc. Packaging materials in the collected samples were mainly polyethylene, polystyrene and polytetrafluoroethylene.

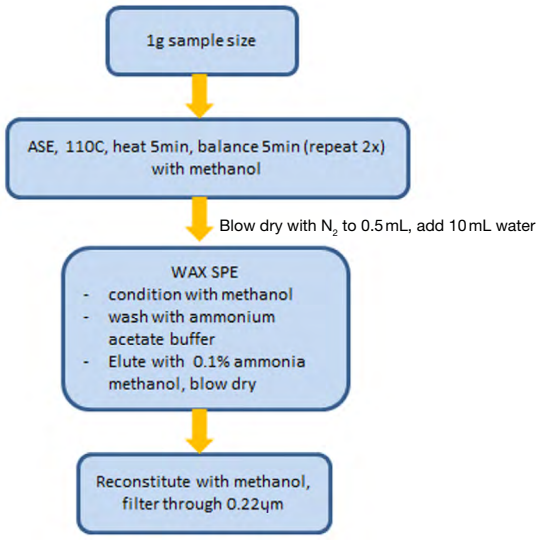


Figure 2.
Extraction and Clean-up Process Flow Diagram

Chromatography

Using the SCIEX® ExionLC™ AD System with a Phenomenex Kinetex®, 2.6 µm C18, 100 X 2.0 mm, compounds were separated using a gradient elution with mobile phase A of 5 mM NH_4AC in water and mobile phase B of 5 mM NH_4AC in methanol (flow rate of 0.3 mL/min, column temperature 40 °C).

Mass Spectrometry

The SCIEX X500R QTOF System was used to analyse the compounds operating in negative ion polarity using the Scheduled MRM^{HR} acquisition mode (**Table 1**). Source conditions were as follows: CUR of 30psi; CAD of 7; IS of -4500V; Temp 500 °C; GS1 of 50psi; GS2 of 55psi.

Data Processing

All data was processed with SCIEX OS Software.

Table 1.
Scheduled MRM^{HR} Method Setup in SCIEX OS
Unique RTs can be defined for each transition for each analyte.

Mass Table ● Apply fragment ion mass ○ Apply TOF start/stop mass ☑ Apply scan schedule

	Compound ID	Group name	Precursor ion (Da)	Fragment ion (Da)	Accumulation time (sec)	Declustering potential (V)	Collision energy (V)	Retention time (min)
1	PFBA	PFBA	212.90	168.9000	0.0600	-80	-35	2.55
2	PFPeA 1	PFPeA	262.90	218.9000	0.0600	-80	-35	3.22
3	PFPeA 2	PFPeA	262.90	69.0000	0.0600	-80	-35	3.22
4	PFBS 1	PFBS	298.90	80.0000	0.0600	-80	-35	3.29
5	PFBS 2	PFBS	298.90	99.0000	0.0600	-80	-35	3.29
6	PFHxA 1	PFHxA	312.90	268.9000	0.0600	-80	-35	3.55
7	PFHxA 2	PFHxA	312.90	119.0000	0.0600	-80	-35	3.55
8	PFHxS 1	PFHxS	362.90	318.9000	0.0600	-80	-35	3.79
9	PFHxS 2	PFHxS	362.90	168.9000	0.0600	-80	-35	3.79

Establishing the Scheduled MRM^{HR} Quantitative Method

The SCIEX OS software is fully automated with a user-friendly interface, greatly reducing the time to establish the acquisition method. The MRM parameters can be set up easily in two different ways. For compounds which are in MS/MS spectral library, fragment ions can be imported easily from the library to build the MRM^{HR} method list. Up to 5 fragment ions can be imported at the same time using a single click. For compounds not found in the spectral library, spectra can be added easily to the library using TOF MS-IDA-MS/MS data acquired for standards of the desired targets.

MRM parameters like retention time, declustering potential (DP) and collision energy (CE) from an existing triple quadrupole method are fully transferrable.

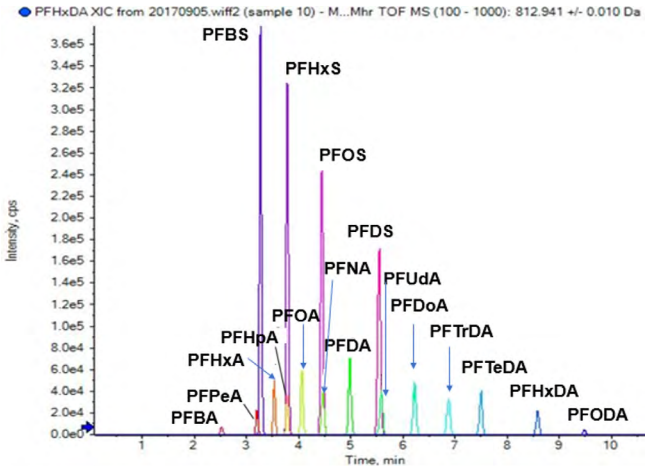


Figure 3.
TOF MS Extracted Ion Chromatogram of 17 PFAS
Good separation was achieved for most of the 17 PFAS compounds analysed.

8. PFAS in Food Contact Materials (continued)

MRM^{HR} Quantitation of PFAS

Chromatogram of 17 PFAS utilizing extracted precursor ion data from TOF-MS scan are shown (**Figure 3**).

High Selectivity Data

Comparing 0.2 ppb post spiked in matrix blank, PFHpA show higher selectivity in MRM^{HR} mode as compared to TOF-MS mode for quantification (**Figure 1**). Monitoring the high resolution fragment ion from the full scan MS/MS data collected provides greater specificity and reduced baseline, so signal-to-noise demonstrates marked improvement and method sensitivity is maximized.

Linearity and Accuracy

The 17 monitored PFAS demonstrate good linearity and accuracy (**Figure 4**) with the correlation coefficients above 0.99. Accuracy values are within the permissible deviation range for LOD and LOQ according to the national standards.

Ion Ratio Calculations

Ion ratios can be easily calculated using the SCIEX® OS software. Ion ratio confirmation can be visually displayed in the chromatogram and result table. Depending on the requirement, the confirmation tolerance can be defined using either constant tolerance or variable tolerance as shown in **Figure 5**.

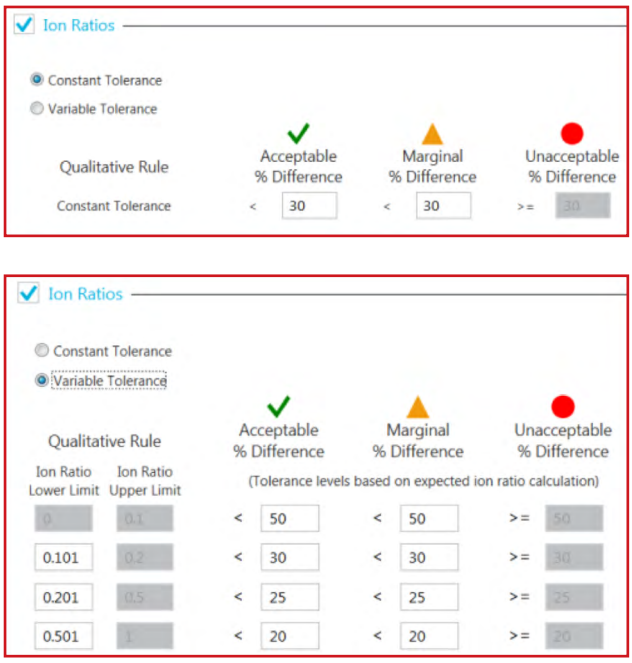


Figure 5.
Setting up Tolerance for Ion Ratios Confirmation

Constant tolerance (same percent difference from measured standard ion ratio) or variable tolerance (varying percent difference dependant on concentration level) can be utilized when determining whether an unknown same meets the criteria for qualitative analyte identification by ion ratio confirmation. Different levels of percent difference can be defined by the user to be flagged as within “Acceptable,” “Marginal,” or “Unacceptable.”

Detection of PFAS in Food Contact Materials

SCIEX OS software combines both qualitative and quantitative results in one single interface (**Figure 6**). The result table show the retention time, concentration, peak area, ion ratio confirmation and the mass error of 0.9 ppm for a sample tested positive with PFOA.

Among the eight samples, eight types of PFAS were detected as shown in **Table 2**. Two out of eight samples have levels which exceeded regulated level of 1 ng/g by national standard. Most of the detected PFAS are the acid derivatives of PFOA and primarily found in non-stick pan coating and disposable meal boxes. The number of actual samples collected in this test is rather small; hence statistically it does not imply that all related products are unsafe for consumers.

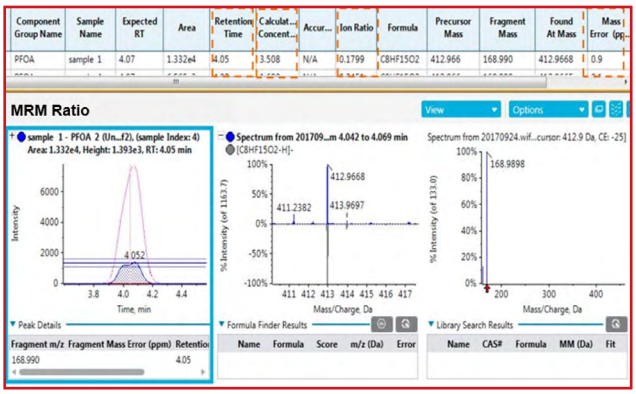


Figure 6.
PFOA Results in Actual Sample

8. PFAS in Food Contact Materials (continued)

Summary

The SCIEX® X500R QTOF system and SCIEX OS software brings powerful performance capabilities for routine testing of PFAS. The unique MRM^{HR} quantification method enables high selectivity even in real sample with matrix interference. This improves the detection and quantification of PFAS which can meet the EU regulation and national standards in China.

Although the concentration of PFAS in most of the test samples falls below the regulated level, the detection rate of perfluorinated alkyl substances is relatively high indicating that the quality of food contact/packaging materials may pose potential risks to consumer's health.

References

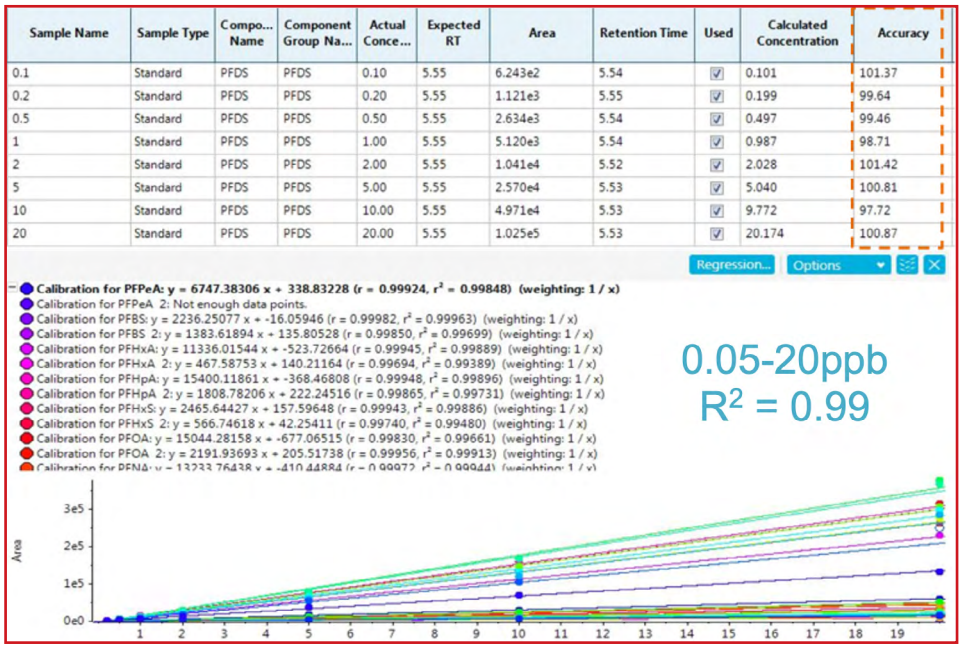
1. Xu R, Tan H, Yang H B, et al. (2014) Food and Fermentation Industries, 40(10): 205.
2. Kannan K, Tao L, Sinclair E, et al. (2005) Arch Environ Con Tox, 48: 559.
3. GB 31604.35-2016, National Food Safety Standard - Food contact materials and products - Determination of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Table 2.
PFAS Content in Different Food Contact Samples

	Detected Amount (ng/g)							
	PFHxA	PFHpA	PFOA	PFDA	PFuDA	PFDoA	PFTrDA	PFTeDA
Meal box 1	0.14	0.16	3.15	-	-	-	-	-
Meal box 2	-	-	3.12	-	-	-	-	-
Plastic bag 1	-	-	-	-	-	-	-	-
Plastic bag 2	-	-	-	-	-	-	-	-
Drink bottle 1	-	-	-	-	-	-	-	-
Drink bottle 2	-	-	-	-	-	-	-	-
Non-stick pan 1	-	-	-	0.11	0.15	0.13	0.15	-
Non-stick pan 2	-	-	-	-	-	-	-	0.17

- Falls below the detection level of this method.

Figure 4.
Calibration Curve of 17 PFAS with Acceptable Accuracy and Linear Response



New Analytical Frontiers

The number of PFAS compounds found on current analyte lists represents less than 1 % of the potential environmental contaminants that could be contributed by this large class of compounds. However, researchers continue to identify additional PFAS compounds with potential human health and environmental impacts, thereby increasing the scope of the problem. Consequently, it is inevitable that PFAS analyte lists will continue to grow, and, future analytical challenges - sample preparation, chromatograpy and mass spectrometry - will become more complex and difficult to overcome. Therefore, we close this PFAS Guide with two visionary technical notes which propose new analytical approaches that will help meet the evolving PFAS challenge.

9. pH-Variable LC Mobile Phase Gradient

PFAS Analysis Based Upon a pH-Variable LC Mobile Phase Gradient

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Introduction

Polyfluoroalkyl substances (PFAS) have been an environmental concern ever since the 1970s when initial reports of potential adverse health effects first came to light. While the analysis of PFAS compounds has been ongoing for some time in academia, they are a fairly recent addition to the suite of analyses commonly performed by commercial environmental laboratories. The only official methods for the analysis of PFAS in drinking water are EPA 537/537.1 and EPA 533 and there are currently no official methods for the analysis of PFAS in complex environmental matrices such as Wastewater, Sediment, and Soil. Although ASTM has released methods for the analysis of PFAS in complex matrices (ASTM D7979 and D7968), they have not gained widespread use within the environmental testing community. As PFAS analyte lists continue to expand and matrices become ever more complex, we anticipate the need for a scalable analytical framework that will enable the development of analytical methods for a wider range of PFAS compounds and matrices. In this Technical Note we present such a framework, based upon the use of a variable pH mobile phase gradient, which could facilitate the expansion of PFAS analyte lists beyond those in common use today.

Method Limitations

Most PFAS methods in use today employ an ammonium acetate (NH_4OAc) mobile phase at a pH of 7 and with a concentration between 2 and 20 mM. Although EPA methods 537.1 and 533 both specify 20 mM NH_4OAc, EPA's method flexibility criteria allow for the use of alternative mobile phases (1, 2). This allowance is useful in pursuing potentially better eluent systems and allowing the analyst to run various PFAS methods on the same instrument using the same column and similar mobile phase. The benefit of changing the eluent system is the ability to change analyte selectivity and potentially analyte resolution. Selectivity differences can also be useful when trying to discriminate analytes from matrix interferences. However, the drawback to changing eluent systems is that it takes time and can create other issues associated with differing mobile phase composition.

Recently introduced regulations in California (3) have significantly expanded the PFAS target analyte list to include compounds such as PFBA, PFMBA, PFHxDA and PFOcDA, which have very large differences in hydrophobicity. This presents a significant analytical challenge because PFHxDA (C16) and PFOcDA (C18) are very hydrophobic with limited solubility in water. The predicted solubility of PFOA (C8) and PFOcDA (C18) are 480,000 and 0.00047 ng/L respectively, using the WS-KOWIN from the USEPA EPISuite Software (4).

In addition, chromatographic analysis of PFBA in an extract that is > 90 % organic results in poor peak shape for this early eluting compound. Most methods that can successfully analyze for PFBA are either direct injection (100 % water), a 1:1 water-methanol dilution or have at least 20 % water in the extract (EPA 533). Some methods (ASTM D7979, D7968 and EPA 8327) add acetic acid to the extract to help improve the peak shape of PFBA. However, this results in poorer chromatographic performance for the longer chain PFHxDA (C16) and PFOcDA (C18).

A New Strategy

In recognition of these limitations, we have pursued a new chromatographic strategy using a 100 % organic system (for long chain PFAS solubility) and variable mobile phase pH to provide good chromatography for PFBA and other early eluting PFAS compounds. By staying within the confines of the NH_4OAc mobile phase composition but employing pH as a variable, one can realize the potential advantages mobile phase variation allowed by EPA while avoiding the primary disadvantages. This approach could be useful in overcoming the difficulty of expanding the analyte lists of the existing PFAS methods to incorporate both the hydrophilic shorter chain compounds and the extremely hydrophobic longer chain compounds.

Technical Approach

This work specifically focused on a secondary chemical characteristic of most PFAS compounds: the hydrophilic or polar functional head of the molecule which are either carboxylic or sulfonic acids which can be charged or neutral, depending on the pH of the eluent. Chromatographers can take advantage of secondary interactions by employing a mobile phase in which a pH gradient is performed, i.e. changing the pH of the mobile phase over time. Mobile phase pH becomes important when analytes contain acidic, basic or both functional groups. The mobile phase pH determines the charge state (protonation state) of the analyte and thereby influences its interactions with the mobile and stationary phase. This technique allows for more control of the ionic interactions between the PFAS analytes within a column's stationary phase and the mobile phase. This is analogous to the WAX SPE technique used in EPA method 533, wherein the ion exchange mechanism allows for stronger interaction with the shorter-chain PFAS compounds than does the styrenedivinylbenzene (SVDB) SPE sorbent used in method 537.1 which operates primarily in a reversed phase mode. Shorter chain PFAS compounds have a lower degree of binding ability due to their shorter chain length and thus often pass through, owing to binding mechanisms that rely exclusively or primarily on a reversed phase interaction.

9. pH-Variable LC Mobile Phase Gradient (continued)

In this new technique, the mobile phase at the beginning of the run has a low pH (~ pH 3.9) and changes over time to a higher pH (~ pH 9.3). This protonates or deprotonates the functional heads of the various PFAS compounds over time, depending upon the pKa of the functional group. This correspondingly changes the elution profile for the separation, in terms of both relative and absolute retention times. In principle, the protonation of short-chain, anionic PFAS will lead to greater retention, while the deprotonation of the later-eluting, long-chain PFAS may lead to less retention, thereby compressing the chromatogram. This will lead to less suppression from non-retained interferences, and shorter run times, allowing greater sample throughput. Separating interferences from early eluting analytes is particularly important when there is only one sensitive MRM transition available, as in the case of PFBA and PFPeA. It is reasonable to think that these orthogonal retention mechanisms (hydrophobicity vs. ionizability or pKa) could offer greater opportunity to resolve complex PFAS mixtures. This Technical Note provides an illustration of the potential power of this approach.

Experimental Conditions

Instrumentation and Consumables. All PFAS analyses were performed on an Agilent® 1100 HPLC with a Thermo Scientific® TSQ Vantage triple quadrupole mass spectrometer. All samples were prepared using a Phenomenex Strata®-X-AW 200 mg 33 µm in a 6cc format (pn: 8B-S038-FCH). The LC column employed was a Phenomenex Kinetex® C18 EVO 5 µm 100 x 2.1 mm (pn: 00B-4633-AN).

Reagent Preparation. Eluents: (1A) Ammonium Acetate (NH_4OAc) was prepared at 20 mM by dissolving 1.54 g NH_4OAc into 1.0 L of water. LC-MS methanol (MeOH) was used for (1B). Acetic acid (HOAc) was prepared at 20 mM by diluting 1.22 mL of glacial acetic acid into 1.0 L of water (2A). Basic methanol was prepared by diluting 1.46 mL of concentrated. Ammonium Hydroxide (NH_4OH) into 1.0 L of LC-MS methanol. Reference materials were purchased from Wellington Labs (Guelph, Canada) and diluted into LC-MS methanol for analysis.

Mass Spectrometer Operating Conditions: The capillary and vaporizer temperature were 250 ˚C and 300 ˚C respectively. The sheath and aux gas were held at 40 arb and 50 arb respectively. The ESI voltages for positive and negative mode were +3.0/-2.5add spacekV. See Appendix 1 for MS/MS Parameters.

LC Operating Conditions: A moderate organic gradient profile was used in both analyses being compared. The only difference between the two LC systems was the pH modifiers that were used in the aqueous and organic eluents. To illustrate the effect of improved peak shape and selectivity differences solely due to the pH modifiers, the times used to change from aqueous to high organic were identical.

Results and Discussion

Although it is difficult to determine the actual pH in any eluent system especially in the presence of methanol and a particular stationary phase, this was estimated in an offline experiment. In order to ascertain the pH change as 20 mM HOAc mixes with the 25 mM NH_4OH, the pH was measured offline for different mixture ratios of this binary system. The measured pH values are shown in **Table 3**. Based on this data, it is estimated that the gradient pH elution profile has a pH no wider than 3.9 and 9.3 from start to finish respectively.

Table 1.
LC Conditions (neutral, pH=7)

	20 mM NH_4OAc	MeOH
Time	% A	% B
0.00	95	5
1.20	55	45
3.60	35	65
11.00	10	90
13.00	10	90
13.01	95	5
17.00	95	5

Table 2.
LC Conditions (gradient pH)

	20 mM HOAc	25 mM NH_4OH in MeOH
Time	% A	% B
0.00	95	5
1.20	55	
3.60	35	65
11.00	10	
13.00	10	90
13.01	95	5
17.00	95	5

Table 3.
Measured pH of a Binary Mixture of Eluents

20 mM HOAc	25 mM NH_4OH in MeOH	
% A	% B	Actual pH
100	0	3.62
95	5	3.86
90	10	4.17
80	20	4.55
70	30	5.14
60	40	5.77
50	50	6.45
40	60	7.13
35	65	8.15
30	70	8.52
20	80	8.98
10	90	9.33
0	99.5	10.25

9. pH-Variable LC Mobile Phase Gradient (continued)

One of the first notable improvements using the new gradient pH upon injecting an extract containing PFAS in 100 % methanol is that the peak shape for PFBA is drastically improved due to shifting the equilibrium of unprotonated PFBA to a protonated form. Protonated PFBA will interact with the nonpolar stationary phase much more than the mobile phase causing increased retention and a better focused peak. This is illustrated in **Figures 1** and **2**; PFBA (light blue). Under the commonly used eluent system of 20 mM NH_4OAc, PFBA and PFMPA exhibit severe fronting in 100 % methanol (required for PFODA solubility). However, using the gradient pH profile, these peaks are focused much better on the column.

Additionally, the latest eluters (PFTrA, PFTeDA, PFHxDA and PFODA) not only elute early, but the peak height is noticeably higher. The increase in height would improve detection limit with a greater s/n. This indicates that NH_4OH, which increases in concentration as the organic (methanol) gradient progresses, is affecting analyte retention by shifting their equilibrium to a deprotonated anion since the anions favor interactions with the mobile phase and the neutral analyte favors interaction with the stationary phase. In fact, the NH_4OH must be present in slightly higher molar concentration than the HOAc in order to move the pH into the slightly basic range.

Figure 1.
Chromatogram of 48 PFAS using 20 mM NH_4OAc (pH=7)

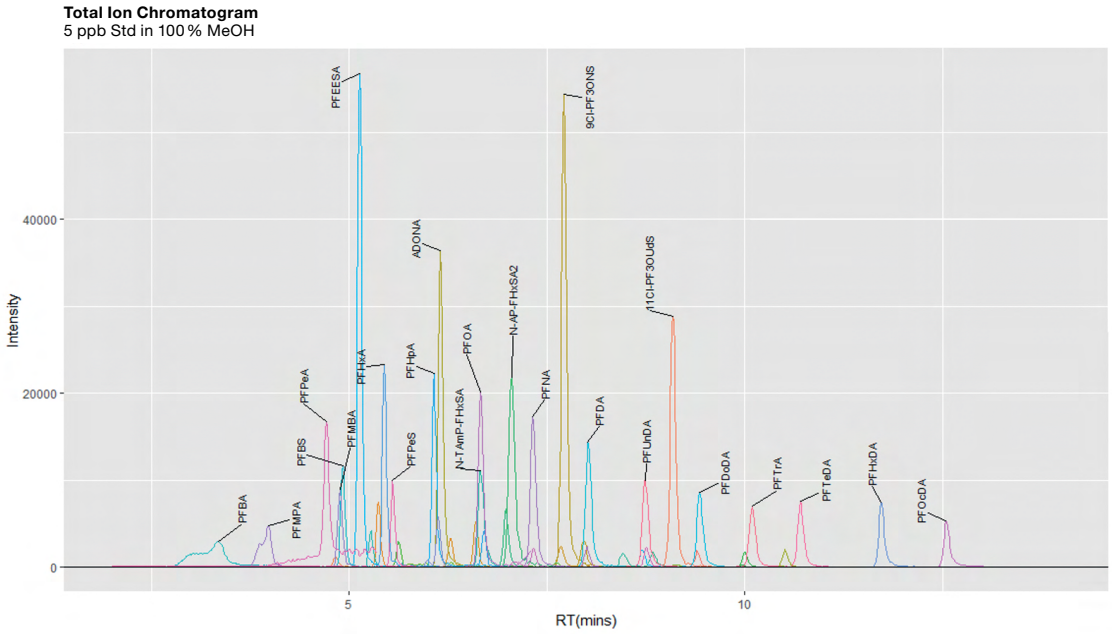
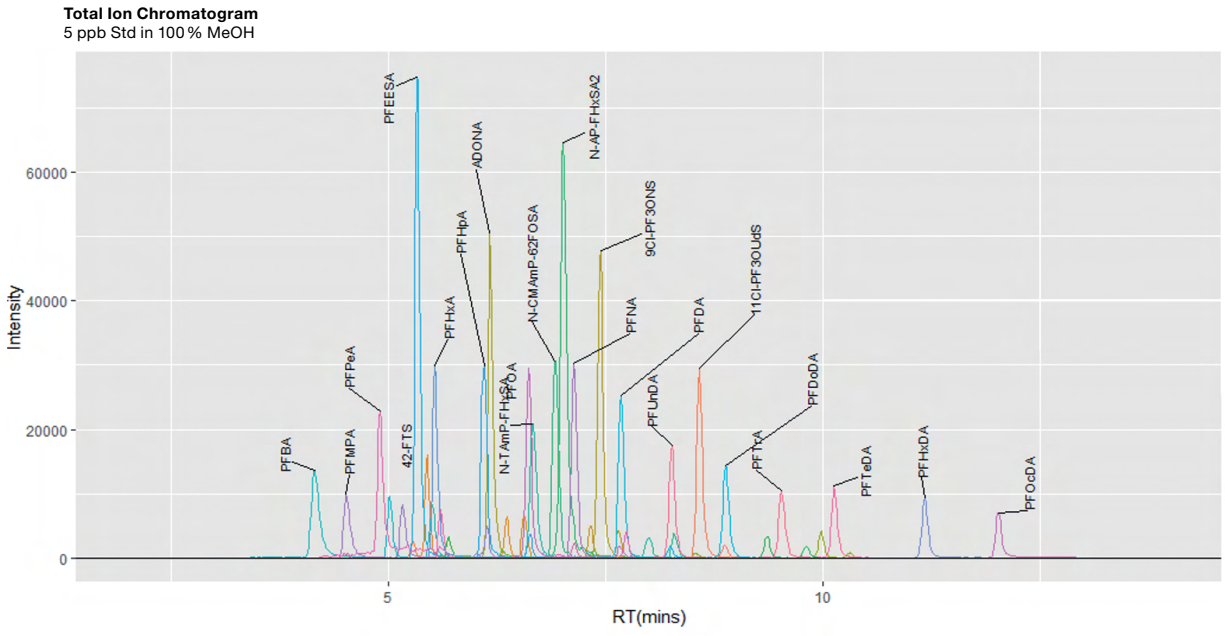


Figure 2.
Chromatogram of 48 PFAS using 20 mM HOAc and 25 mM NH_4OH (varied pH from 3.9 to 9.3)



9. pH-Variable LC Mobile Phase Gradient (continued)

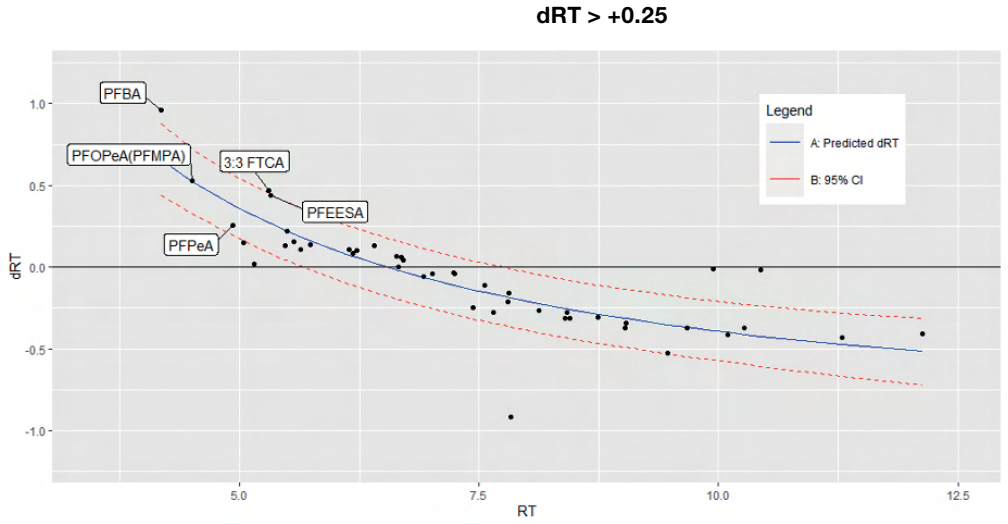
The selectivity of these two mobile phase systems was further investigated to see how they affect different PFAS compounds varying in chain length.

Upon close examination of the ΔRT data there were certain analytes (e.g PFOSA) that indicated possible differences in selectivity. In order to evaluate significant selectivity differences between the two eluent systems that were not obvious, a statistical approach was used. This is necessary because not every slight change in RT or resolution may be significant. First, a least squares regression was performed on the ΔRT as a function of RT of the new method. The equation that was used to model the change in the two systems is listed in equation (1) where a, b, c are the coefficients for the intercept, linear term, and inverse term respectively:

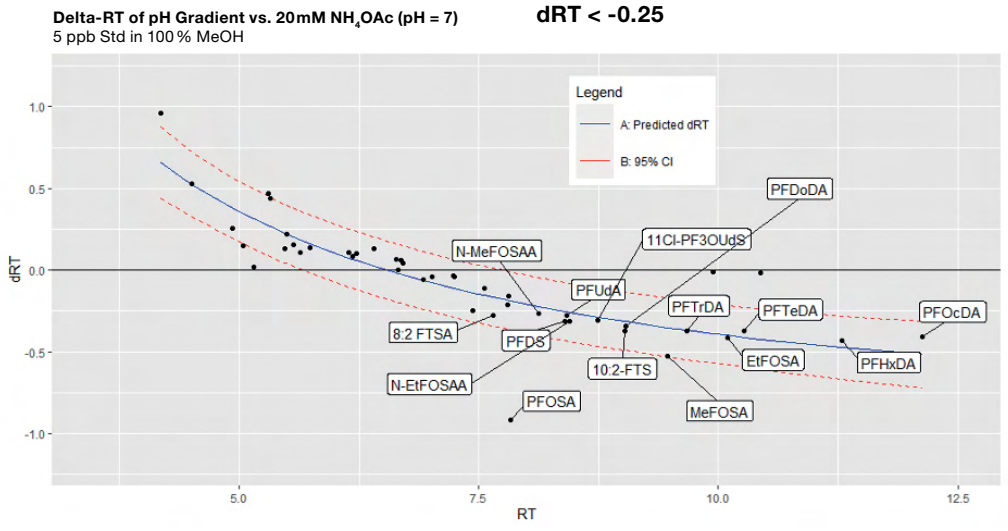
$$\Delta RT = a + b \cdot RT + c \cdot \frac{1}{RT} \qquad (1)$$

To validate the regression model and the prediction interval of significance at 95 %, a Global Validation of Linear Models Assumptions (GVLMA) was used (5) The plots in **Figure 3** highlight the most important aspects of the advantages of this new system. These are increased retention for early eluters (3a), decreased elution for late eluters (3b), and significant selectivity differences (3c). To evaluate significant differences, the x-axis shows the retention time (RT) for the new mobile phase and the y-axis shows the ΔRT relative to the neutral ammonium acetate mobile phase.

Figure 3.
Notable Mobile Phase Elution Changes
a) PFAS Analytes with Increased Retention



b) PFAS Analytes with Decreased Retention

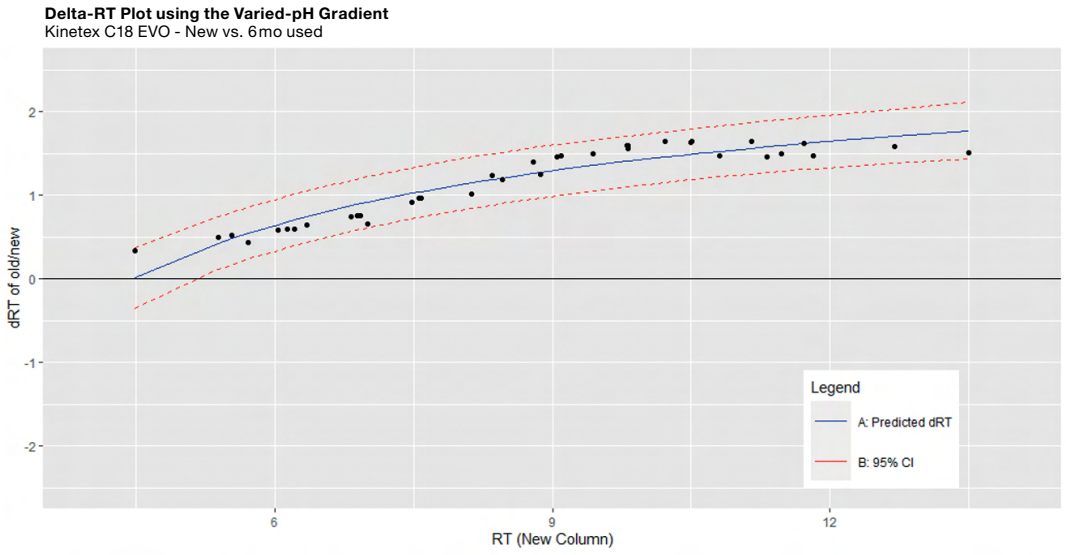


9. pH-Variable LC Mobile Phase Gradient (continued)

It is also worth noting that this new eluent system also has an effect on sensitivity for certain compounds. Specifically, N-TAmP-FHxSA, N-CMAmP-62FOSA, and N-AP-FHxSA2 (which are detected in ESI+) had an increase in response more than 2x in the new pH gradient eluent system (**Figure 1-2**).

Lastly, the robustness of the stationary phase was examined by evaluating a "well used" LC column versus a brand new column. The "well used" column had been used to analyze thousands of samples over approximately six months. This included drinking water extracts as well as non-potable aqueous and soil extracts. The Kinetex® EVO C18 showed reasonable robustness and, although some retention is lost over time, there was no significant ($P<0.05$) selectivity difference observed. Again, the GVLMA cross-validation was used (**Figure 4**) to detect significant elution order changes (ie: all analytes had statistically the same elution order) although "absolute" elution order was different in some cases.

Figure 4.
Retention Difference of New vs Used column Under Varied pH conditions



Conclusion

The objective of using a pH gradient mobile phase for PFAS analysis is that it allows the analyst to widen the scope of analyte chemistry to properly chromatograph short-chain and long-chain PFAS in 100 % organic extracts as well as change the selectivity of the method. This holds true for any analyte panel outside the scope of method EPA 537.1 and EPA 533, in that the absolute and relative retention of some analytes are different than when using a standard organic gradient with ammonium acetate (NH_4OAc).

Additionally, this solution may provide the ability to move certain peaks away from interferences and high ion suppression zones at the beginning of the chromatographic run. It may also allow for the inclusion of other PFAS analytes with a minimal redevelopment and optimization. The pH gradient method shows excellent robustness and reproducibility, with stable PFAS analyte retention times, even when using different columns, systems, and analysts. The changes in retention times (both absolute and relative) offer another tool for more complex PFAS mixtures - either those with more PFAS analytes or from working with dirtier matrices.

Moving forward, this promising mobile phase gradient approach could be combined with work investigating alternative HPLC stationary phases to determine optimal conditions for PFAS panels that are much broader in scope and chemistry. In principle, this approach should allow the separation of an even wider class of PFAS including non-volatile short-chain PFAS. Preliminary data suggest that the use of Formic acid (ie: 25 mM HOFo) instead of 25 mM HOAc can drop the pH slightly lower; closer to pH = 3. This has the benefit of increased retention for TFA, TFMS, and PFPrA in extracts that are 100 % methanol.

9. pH-Variable LC Mobile Phase Gradient (continued)

Appendix 1.
Instrumental Conditions for MS/MS Analysis and RT Data

						Retention Time Data	
Analyte	Precursor	Product	CE	S-Lens	Polarity	Gradient pH	Constant pH=7
PFBA	213	169	9	35	-	4.18	3.22
PFMPA	229	85	12	35	-	4.51	3.98
PFPeA	263	219	9	38	-	4.93	4.68
3:3-FTCA	241	177	8	37	-	5.30	4.83
PFEESA	315	135	23	90	-	5.32	4.88
PFBS	299	80	36	100	-	5.04	4.89
PFMBA	279	85	12	40	-	5.15	5.13
NFHDA	295	201	10	33	-	5.50	5.28
4:2-FTS	327	307	20	110	-	5.48	5.35
PFHxA	313	269	9	47	-	5.57	5.42
PFPeS	349	80	41	100	-	5.64	5.53
HFPO-DA	285	169	8	37	-	5.74	5.60
PFHpA	363	319	9	56	-	6.15	6.04
PFHxS	399	80	44	120	-	6.18	6.10
ADONA	377	251	11	60	-	6.22	6.12
5:3-FTCA	341	237	13	57	-	6.41	6.28
6:2-FTS	427	407	22	130	-	6.64	6.57
PFOA	413	369	9	62	-	6.69	6.63
N-TAmP-FHxSA	499.1	60	37	140	+	6.66	6.66
PFHpS	449	80	46	110	-	6.71	6.66
N-CMamP-6:2FOSA	571.1	440	31	140	+	6.92	6.98
N-AP-FHxSA	485.1	85	34	130	+	7.01	7.05
PFNA	463	419	10	65	-	7.24	7.27
PFOS	499	80	46	105	-	7.25	7.29
9Cl-PF3ONS	530.9	351	28	120	-	7.56	7.67
7:3FTCA	441	337	11	70	-	7.44	7.68
8:2-FTS	527	507	27	130	-	7.65	7.93
PFDA	513	469	10	75	-	7.81	7.97
PFNS	549	80	48	130	-	7.80	8.01
N-MeFOSAA	570	419	20	120	-	8.13	8.40
PFUnDA	563	519	10	85	-	8.42	8.69
PFDS	599	80	49	110	-	8.40	8.71
PFOSA	498	78	34	110	-	7.84	8.75
N-EtFOSAA	584	419	20	120	-	8.45	8.76
11Cl-PF3OUdS	630.9	451	30	120	-	8.74	9.05
PFDoDA	613	569	12	92	-	9.04	9.38
10:2-FTS	627	607	31	150	-	9.03	9.40
MeFOSE	616	59	15	90	-	9.95	9.96
MeFOSA	512	169	30	110	-	9.48	10.00
PFTrA	663	619	12	101	-	9.67	10.05
EtFOSE	630	59	15	91	-	10.45	10.46
EtFOSA	526	169	30	120	-	10.10	10.51
PFTeDA	713	669	12	108	-	10.27	10.64
PFHxDA	813	769	12	120	-	11.29	11.72
PFOcDA	913	869	13	140	-	12.13	12.54

References

1) U.S. Environmental Protection Agency, Method 537.1—Determination of Selected Per- And Polyfluorinated Alkyl Substances In Drinking Water By Solid Phase Extraction And Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). November 2018.

2) U.S. Environmental Protection Agency, Method 533— Determination of Per- And Polyfluoroalkyl Substances In Drinking Water By Isotope Dilution Anion Exchange Solid Phase Extraction And Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). November 2019.

3) Analytical Reporting Limits for PFAS compliance with DoD Table B-15 of QSM, Version 5.1 or later (updated 07/22/20) https://www.waterboards.ca.gov/pfas/docs/reporting_limits_dod_qsm_v5_1_or_later_july_22_2020.pdf

4) U.S. Environmental Protection Agency, Estimation Program Interface Suite (EPISuite) Software, v4.11, 2000-2017. https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411

5) Peña, Edsel A., and Elizabeth H. Slate. "Global Validation of Linear Model Assumptions." Journal of the American Statistical Association, vol. 101, no. 473, 2006, pp. 341–54, doi:10.1198/016214505000000637.

10. Column Chemistry Considerations

Column Chemistry Considerations for Full Coverage of PFAS Analyte Ranges

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Introduction

Per- and polyfluorinated alkyl substances (PFAS) are man-made chemicals, that have been widely used since the 1940s. They have been employed in a large variety of consumer products, such as nonstick cookware, food containers, stain and water repellent fabrics, polishes, waxes, paints, and cleaning products and are now widely distributed in the global environment. A significant source of PFAS environmental contamination has been the widespread use of PFAS-containing aqueous firefighting foams (AFFF), which are known to migrate into groundwaters at airports and military bases. Further environmental exposure to PFAS comes from industrial production facilities (e.g. chrome plating, electronics, manufacturing, or oil recovery). Living organisms, including plants, animals, and humans, can accumulate PFAS compounds in their tissue, which can build up over time and impact their health.[1-3] A total of 9,252 PFAS are listed in EPA's most recent list of PFAS substances. [4] However, only a handful of these, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been widely monitored in the environment or have been thoroughly studied for their toxicological effects.

Common Chromatographic Approaches

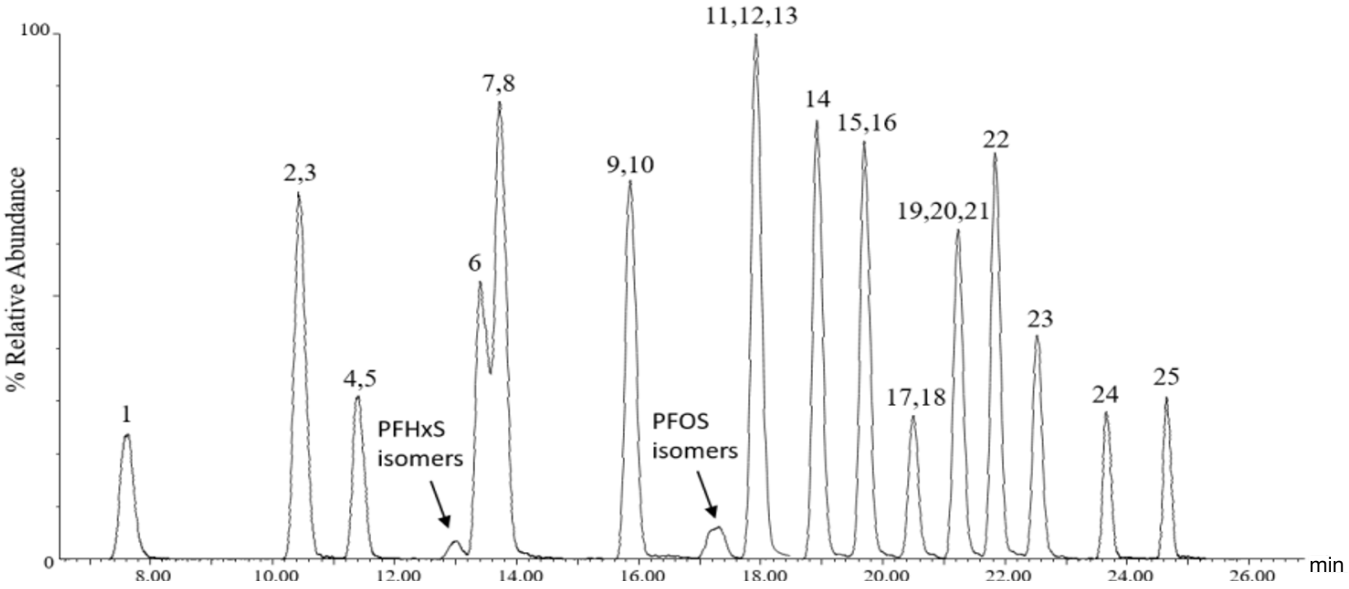
PFAS compounds are typically determined by LC-MS/MS and LC-HRMS instrumentation. The use of mass spectrometry detection has played a significant role in the quantitation of specific compounds where standards are available. Where standards are not available, the use of time of flight (TOF) and Orbitrap™ MS detectors are used to semi-quantify unknown PFAS compounds. The chromatographic separation of PFAS compounds in currently validated methods typically involves a reversed phase mechanism using a C18 or Phenyl column in an acidic-methanol eluent. For example, EPA method 537.1 uses a C18 column (5 µm, 2.1 x 150 mm C18) and EPA Method 533 was validated using a C18 Phenomenex Gemini® column (3 µm, 2 x 50 mm). Conversely, ASTM D7979 and EPA 8327 were validated using a Phenyl-Hexyl column (1.7 µm, 2.1 × 100 mm), ISO 21675 used a C18 column (5 µm, 2 × 50 mm) and the Department of Agriculture CLG-PFAS 2.01 method used a C8 column, Phenomenex Luna® C8(2) (3 µm, 2 x 50 mm).

PFAS Chromatographic Challenges

While these methods are generally adequate for a limited list of analytes, the large number of potential PFAS analytes that could potentially be present in a sample will inevitably challenge simple chromatographic separation approaches. This phenomenon was seen early in the development of the EPA drinking water methods. EPA 537.1 when validated, identified several overlapping peaks which can be seen in **Figure 1** as demonstrated by peaks, 2,3; 4,5; 7,8; 9,10; 11,12,13; 15,16; 17, 18; 19, 20, 21.

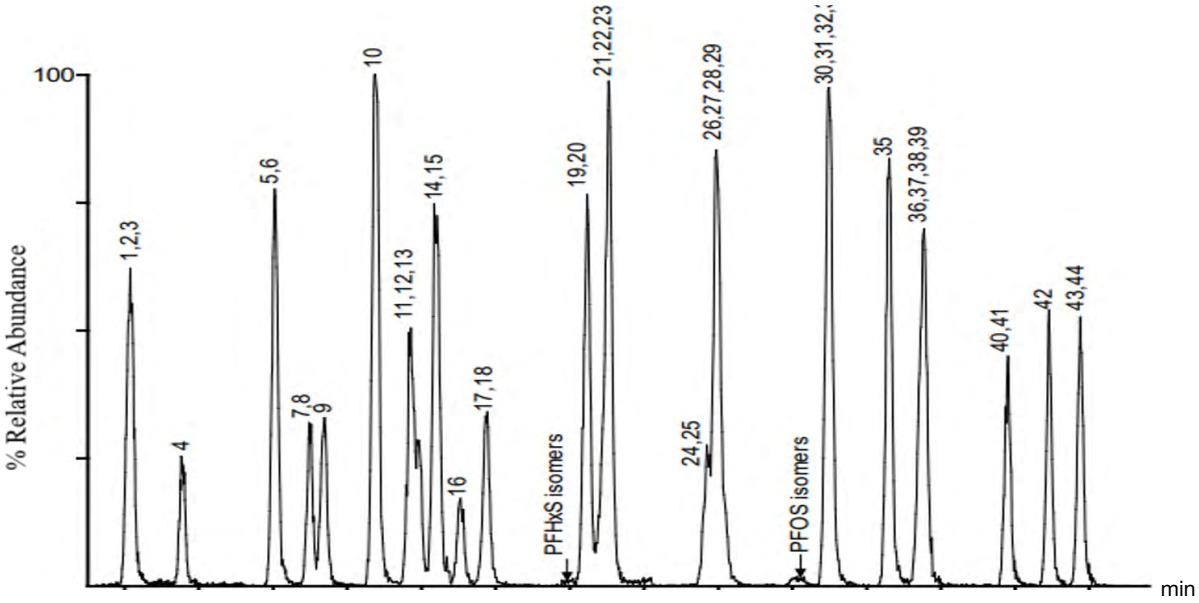
10. Column Chemistry Considerations (continued)

Figure 1.
Example chromatogram for reagent water fortified with method 537.1 analytes at 80 ng/L



Likewise, when EPA 533 was developed and validated with an expanded list of PFAS compounds, it also shows several overlapping peaks, as seen in **Figure 2**.

Figure 2.
Example chromatogram for reagent water fortified with method 533 analytes at 80 ng/L



10. Column Chemistry Considerations (continued)

Whereas many of these overlapping peaks can be successfully resolved by the mass analyzer, the potential presence of isobaric homologues and unresolved matrix interferences point to the continuing need for good chromatographic separation to assure reliable identification and quantitation. Although the problem may be manageable for today's small analyte lists, the challenge will inevitably grow as new PFAS compounds are added for investigational or regulatory purposes.

Looking to the Future

Current PFAS methods primarily rely upon C18 solid phase chemistry and simple methanol-ammonium acetate mobile phase gradients. These methods do not make full use of all the tools in the chromatographer's toolbox, nor need they, given today's limited analyte lists. However, this simple situation will inevitably change and there will be a need to develop more sophisticated chromatographic methods to tease out the subtle chemical and structural differences between closely related PFAS compounds. Chief among these will be the application of novel stationary phases and mobile phases to exploit the different interactions between closely related PFAS molecules. This Tech Note was designed to provide a vision of the potential power of such new chromatographic approaches.

Scope

In this Tech Note we will present data for a select list of PFAS compounds (**Table 1**) that were selected to reflect some of the chemical diversity of the PFAS universe. This color-coded grouping will be used to illustrate the differences in chromatographic retention time and elution order between various stationary phases including C8, C18, Phenyl-Hexyl, Biphenyl and F5 which can have significantly different sorptive properties. We will also examine how differing mobile phase polarity (e.g., methanol vs. acetonitrile) influences chromatographic performance for these various phases. Ideally, this information can be used to enhance chromatographic resolution as the list of PFAS compounds continues to increase. The goal is to provide insights that will allow method developers to identify useful separation strategies.

Table 1.

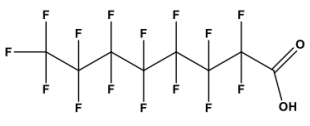
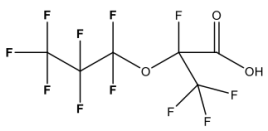
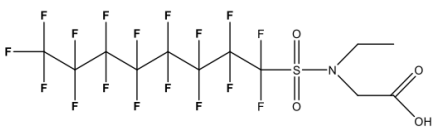
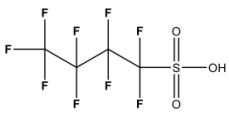
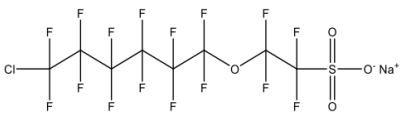
Chemical Name	Abbreviation
Perfluoroalkyl carboxylic acids (PFCAs)	
Perfluorohexanoic acid	PFHxA
Perfluoroheptanoic acid	PFHpA
perfluoro-n-octanoic acid	PFOA
perfluoro-n-nonanoic acid	PFNA
perfluoro-n-decanoic acid	PFDA
perfluoro-n-undecanoic acid	PFUdA
perfluoro-n-dodecanoic acid	PFDoA
perfluoro-n-tridecanoic acid	PFTrDA
perfluoro-n-tetradecanoic acid	PFTeDA
Perfluoroalkyl ether carboxylic acids (PFECA)	
hexafluoropropylene oxide-dimer acid	HFPO-DA
dodecafluoro-3H-4,8-diosanonanoate	NaDONA
Perfluorooctane sulfonamides and derivatives	
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA
Perfluorinated sulfonic acids (PFSAs)	
Perfluorobutanesulfonic acid	L-PFBS
perfluoro-1-hexanesulfonate	L-PFHxS
perfluoro-1-octanesulfonate	L-PFOS
Chlorinated polyfluoroalkyl ether sulfonic acids (Cl-PFESAs)	
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS
11-chloroeicosalfluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS

Method Variables

PFAS Chemistries

There are established, validated methods set forth by the EPA and ISO for chromatographic separation of PFAS compounds using specific types of columns and packing materials. Unfortunately, not all PFAS compounds can be separated with sufficient accuracy using these methods because of the different types of functional groups that are on different PFAS compounds. In the select list that was used, there are 5 categories of PFAS compounds as shown in **Figure 3**, with an example of each. Owing to the variety of functional groups that can potentially be found on PFAS compounds, there are a variety of HPLC column chemistries that could aid enhanced separation.

Figure 3.

Perfluoroalkyl carboxylic acids (PFCAs)	Perfluoroalkyl ether carboxylic acids (PFECAs)	Perfluoro sulfomamide carborylic acids (PFSACAs)
 Perfluoro-n-octanoic acid	 Hexafluoropropylene oxide-dimcer acid	 N-ethylperfluoro-1-octanesulfonamidoacetic acid
Perfluorinated sulfonic acids	**Chlorinated polyfluoroalkyl ether sulfoninic acids**	
 Perfluorobutanesulfonic acid	 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	

10. Column Chemistry Considerations (continued)

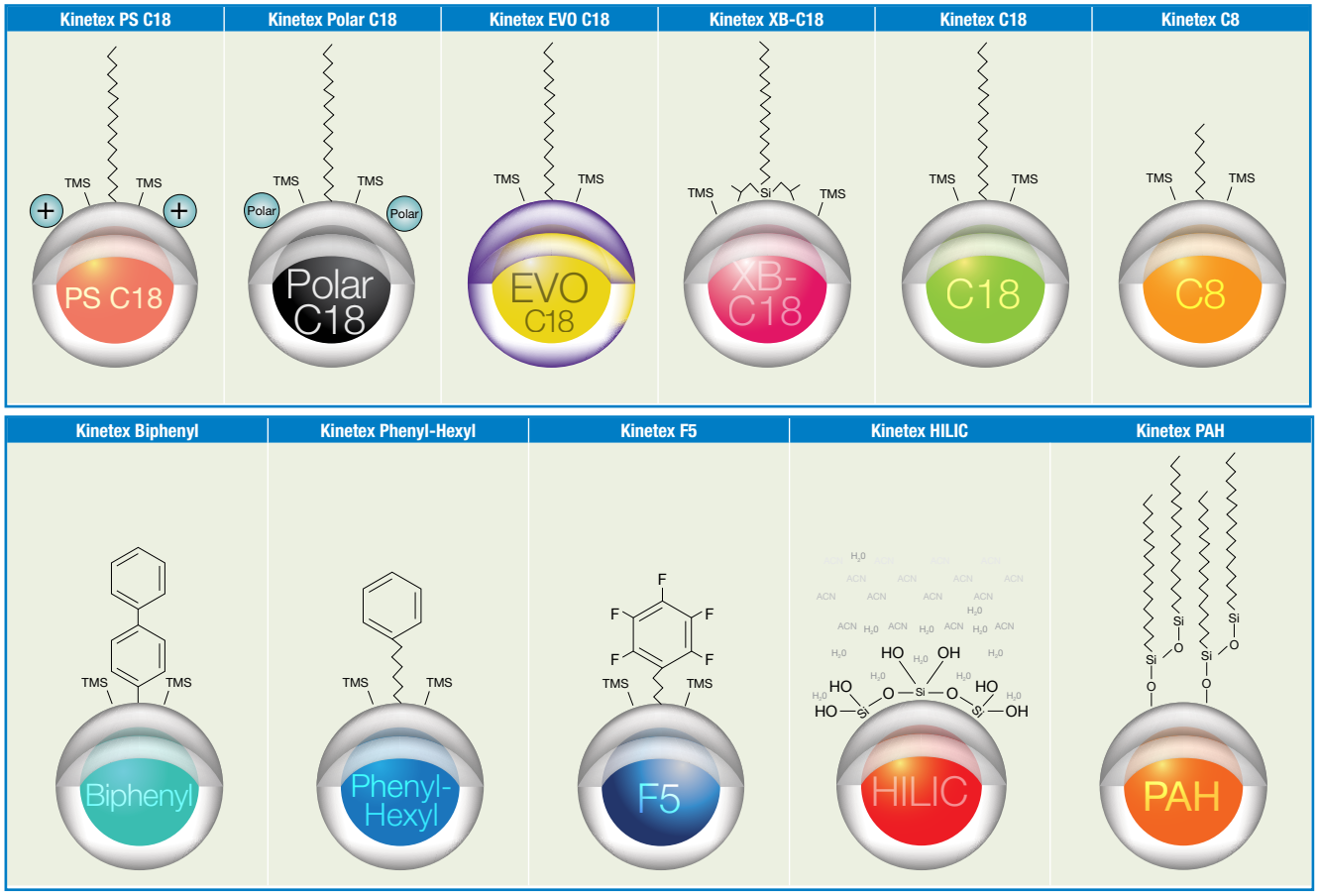
Solid Phase Chemistries

A representation of the different solid phase chemistries that are available in Phenomenex HPLC columns that could be used in PFAS separations is presented in **Figure 4**. This wide variety of ligand chemistries – combined with differences in porosity and other morphological variations – was developed to offer a wide range of variables for method development.

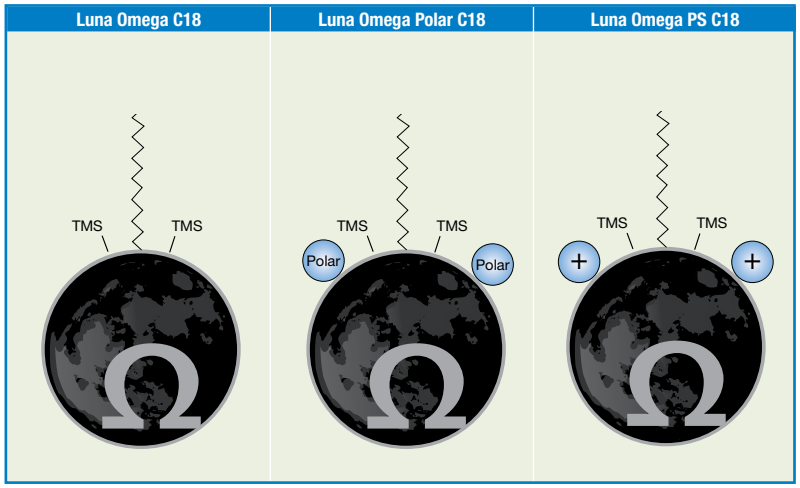
Different combinations of these variables serve to enhance the separation of polar compounds, increase surface areas, add pH stability, decrease system backpressures, etc. These, and additional column properties, provide chromatographers with a high degree of flexibility with which to tackle challenging separations.

Figure 4.
Available column chemistries appropriate for PFAS compound separation

Kinetex® Core-Shell 1.3, 1.7, 2.6, and 5 µm



Luna® Omega Fully Porous 1.6, 3, and 5 µm



10. Column Chemistry Considerations (continued)

Mobile Phase Chemistries

However, in addition to column selection, chromatographers can also make changes in mobile phase polarity to further enhance selectivity. For example, EPA method 533 was altered in several ways to enhance separation of the selected PFAS compounds used in the present study. In the first elution regime, the percentage of methanol was increased at run initiation and then further increased to a higher percentage than had been previously used in the published method. This decreased the overall run time but kept the percentage increase of methanol roughly the same. This elution regime will be referred to later in this Tech Note as "533 Similar" (**Table 2**).

In the second elution regime, acetonitrile was added to the mobile phase at a ratio of 80:20 methanol:acetonitrile to increase mobile phase polarity (but with all other factors remaining the same as in the "533 Similar" elution regime). This second elution regime will be referred to as "533 Acetonitrile Altered" (**Table 3**). The results from these two elution regimes will be addressed separately. Clearly, there are many other potential mobile phase variations that could be investigated. However, the two variations presented here will suffice to demonstrate the power of mobile phase polarity combined with solid phase chemistry variation to effect PFAS chromatographic behavior.

Table 2.

EPA 533 - As Published		
Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	95	5
0.5	95	5
3	60	40
16	20	80
18	20	80
20	5	95
22	5	95
25	95	5
35	95	5

40 → 80 in 13 min 3.08 % per min

533 Similar		
Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45

40 → 90 in 15 min 3.0 % per min

Table 3.

533 Similar		
Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45

533 Acetonitrile Altered		
Time (min)	% 20 mM Ammonium Acetate	% 80-20 Methanol Acetonitrile
Initial	55	45
15	10	90
21	10	90
21.5	55	45

Results and Discussion

For ease of comparison, all chromatographic data will be presented in tabular format with the chromatography columns on the left, the PFAS compounds across the top, and the specific analyte retention times under the PFAS compounds. The highlighted boxes identify two compounds that have overlapping retention times ($\Delta RT \leq 5$ seconds) and the arrows at the bottom indicate when two compounds have changed elution order. The different PFAS compound classes are represented by the colors referenced in **Table 1**. This representation is a more insightful way to present the data because overlaying or stacking individual chromatograms makes it very difficult to compare results across columns. The two mobile phase chemistry regimes identified above will now be discussed separately.

10. Column Chemistry Considerations (continued)

1. EPA 533 Similar

In order to determine how the selected PFAS compounds would elute and separate, seven different chromatography columns with different solid phase chemistries were examined. **Figure 5** displays columns that have C18-functionality or PAH-functionality. The PFAS elution order was generally consistent for most of the C18 phases, although specific elution times varied. The Kinetex® PAH column demonstrated two compound functional pairs with a reverse elution order: NaDONA (a perfluoroether carboxylic acid) vis-á-vis L-PFHxS (a perfluronated sulfonic acid) and PFUdA (a perfluoroalkyl carboxylic acid) vis-á-vis N-EtFOSSA (a perfluorooctane sulfonamide). In addition, there were slight differences in overlapping peaks amongst the various C18 phases, whereas the Kinetex PAH phase had only one overlapping pair. When compared to two C8 phases (**Figure 6**), the elution order was similar to the C18 phases, and the retention times were similar, but there were fewer overlapping peak pairs (one pair vs. 3 pairs).

However, the C8 phases also demonstrated two compound functionality pairs with a reverse order elution from the C18 phases: L-PFOS (a perfluoronated sulfonic acid) vis-á-vis PFNA (a perfluroalkyl carboxylic acid) and (again) PFUdA vis-á-vis N-EtFOSSA, presumably is response to the lower hydrophobicity of the C8 phase functionality. Interestingly, both C8 phases and the PAH phase had fewer overlapping peaks compared to the C18 phases, but in different parts of the elution order spectrum. This likely represents the greater contribution of pi-electron interaction with the PAH phase in contrast with more consistent hydrophobic interaction characteristic of the C18 phases. These variations are subtle rather than dramatic, but they offer insights into interactions between solid phase chemistry and PFAS compound class that could be useful for better separating adjacent compound pairs or shifting analytes away from mass spectral interferences.

Figure 5.
C18 and PAH summary

		L-PFBS	PFHxA	HFPO-DA	PFHpA	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini®	C18	5.28	7.37	8.23	9.53	10.02	10.09	11.41	13.12	13.12	13.58	14.28	15.03	15.35	15.36	16.05	16.34	17.23	18.11
Luna®Omega	Polar C18	4.15	6.18	7.10	8.31	8.38	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.04	14.05	14.38	14.59	15.47	16.29
Luna Omega	Ps C18	4.29	6.34	7.21	8.46	8.55	9.05	10.35	12.06	12.03	12.53	13.24	14.01	14.31	14.36	14.58	15.23	16.08	16.44
Kinetex	C18	3.36	6.38	7.38	9.44	10.04	10.07	12.03	13.48	13.52	14.4	15.1	15.41	16.17	16.15	16.5	17.15	18.06	18.48
Kinetex	XB-C18	3.27	5.30	6.18	7.56	8.10	8.13	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.02	14.31	14.56	15.47	16.31
Kinetex	Polar C18	3.05	4.59	5.49	7.18	7.33	7.37	9.16	10.54	10.57	11.52	12.17	12.51	13.25	13.27	14.03	14.25	15.18	16.05

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	PAH	1.24	2.07	2.60	3.99	4.27	4.31	5.81	7.59	7.76	8.61	9.16	9.73	10.32	10.52	11.14	11.69	12.75	13.71

Elution Order Shifts from C18

Elution Order Shifts from C18
Overlaps are Eliminated

Figure 6.
C8 summary

		L-PFBS	PFHxA	HFPO-DA	PFHpA	L-PFHxS	NaDONA	PFOA	L-PFOS	PFNA	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Luna	C8	5.41	7.51	8.38	9.51	10.04	10.15	11.33	12.47	12.49	13.28	13.53	14.12	14.40	14.46	15.13	15.33	16.14	16.48
Kinetex	C8	5.30	7.54	8.47	10.14	10.26	10.33	11.57	13.18	13.19	14.03	14.26	14.45	15.15	15.24	15.56	16.14	16.56	17.33

Elution Order Shifts from C18

10. Column Chemistry Considerations (continued)

Finally, additional differences are seen when comparing Kinetex® Biphenyl, Phenyl-Hexyl, and F5 columns. These phases were designed with different chemistries having varying polarities to provide better selectivity for aromatic compounds. However, these polarity differences and greater pi-electron interactability also come into play with the different PFAS chemistries, as evidenced by the various reverse order elution pairs from the C18 phases.

The elution order in the Kinetex Biphenyl and Phenyl-Hexyl columns are consistent, but markedly different from the Kinetex F5 column. The Biphenyl and F5 phases showed only one set of overlapping peaks, but the Phenyl-Hexyl column had 3 sets of overlapping peaks. Interestingly, the compound classes that overlapped were different between the Phenyl-Hexyl and Biphenyl columns (**Figure 7**).

Figure 7.
Phenyl Stationary Phase Summary

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA	9-Cl-PF3ONS	N-MeFOSAA	PFUdA	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	Biphenyl	1.47	2.06	2.19	3.67	4.00	4.43	5.53	7.02	7.49	8.64	8.21	9.23	9.24	9.88	10.16	10.56	10.96	11.67
Kinetex	Phenyl-Hexyl	2.53	3.89	4.57	5.97	6.39	6.42	7.69	9.10	9.35	10.30	10.37	11.13	11.33	11.69	12.21	12.31	13.01	13.67

Elution Order Shifts from C18

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	F5	3.37	5.12	5.84	7.49	7.85	7.98	9.45	11.10	11.35	11.88	12.47	13.37	13.63	13.84	14.09	14.61	15.47	16.18

Elution Order Shifts from C18

2. EPA 533 Acetonitrile Altered

Acetonitrile is a highly polar molecule and is often added to the mobile phase to alter how analytes interact with the solid phase. The previously discussed experimental sequence was repeated using a 80:20 methanol:acetonitrile mobile phase with the same PFAS compounds and HPLC columns. The C18 columns all still had a consistent elution order as compared to 533 Similar but displayed earlier retention times (**Figure 8**). However, compared to 533 Similar, the conditions of 533 Acetonitrile Altered resulted in a much larger number of retention time elution order shifts.

The addition of acetonitrile to the mobile phase increased the number of overlapping peaks for the Gemini® C18, Luna® Omega Polar C18, and Kinetex Polar C18 columns, but it conversely decreased the number of overlapping peaks for the Luna Omega PS-C18 and Kinetex C18 columns. In the Kinetex PAH column, the methanol:acetonitrile mobile phase also significantly changed the elution order as compared to methanol-only mobile phase, but with some differences in the effected compounds (**Figure 8**). However, with Kinetex PAH there were also more overlapping peaks, resulting in compromised separation for early eluters.

Figure 8.
C18 and PAH Summary

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini	C18	2.42	2.55	2.97	3.88	4.45	5.55	5.47	7.25	8.89	9.08	9.10	9.82	10.24	10.43	11.85	12.96	13.15	14.40
Luna Omega	Polar C18	1.83	1.97	2.25	2.92	3.43	4.31	4.51	5.75	7.14	7.35	7.35	7.98	8.44	8.45	9.69	10.84	10.88	12.01
Luna omega	Ps C18	1.87	1.99	2.34	3.12	3.66	4.69	4.87	6.29	7.74	7.90	8.02	8.71	8.91	9.10	10.38	11.46	11.71	12.91
Kinetex	C18	1.42	1.47	1.69	2.30	2.79	4.12	4.31	6.40	8.28	8.45	8.54	9.28	9.62	9.90	11.36	12.51	12.72	14.00
Kinetex	Polar C18	1.32	1.39	1.58	1.99	2.38	3.13	3.29	4.45	5.90	6.09	6.16	6.80	7.21	7.29	8.53	9.62	9.65	10.76

Elution Order Shifts from "EPA 533 Similar"

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	PFUdA	9-Cl-PF3ONS	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	XB-C18	1.37	1.42	1.63	2.23	2.69	3.68	3.76	5.27	6.80	6.87	7.26	7.94	7.96	8.20	9.52	10.57	10.82	12.08

Elution Order Shifts from "EPA 533 Similar"

10. Column Chemistry Considerations (continued)

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFNA	PFNA	L-PFOS	PFDA	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex®	PAH	0.76	0.76	0.83	0.95	1.05	1.33	1.34	2.19	3.44	3.49	3.78	4.30	4.31	4.78	6.10	6.90	7.47	8.82

Elution Order Shifts from "EPA 533 Similar"

The methanol:acetonitrile mobile phase also resulted in more overlapping pairs and changes in elution order with the C8 columns (**Figure 11**). The elution order was consistent between the two C8 columns using this method, but there were many shifts in elution order compared to the methanol-only eluent. Finally, the methanol:acetonitrile method and the methanol-only method showed similar but not identical elution orders in the Kinetex Biphenyl and Phenyl-Hexyl Columns. The elution order with Kinetex F5 was less comparable with Kinetex Biphenyl and Phenyl-Hexyl columns with the acetonitrile altered eluent than previously seen with the methanol-only eluent. However, with the acetonitrile altered eluent, Kinetex F5 was more similar in elution order to the C18 columns than to the phenyl stationary phases.

The 533 Acetonitrile Altered method also showed increased overlapping peaks in all phenyl and F5 stationary phases (**Figure 12**), although the shorter run times may have contributed significantly to these increases. All things considered, the methanol:acetonitrile data demonstrate that mobile phase polarity (in conjunction with stationary phase chemistry) has a great deal of influence over the sorption behavior of the different classes of PFAS compounds and could be a powerful tool with which to influence chromatographic behavior.

Figure 9.
C8 Summary

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Luna®	C8	2.05	2.15	2.34	3.22	3.55	4.51	5.07	6.22	7.48	7.53	8.04	8.28	9.05	9.09	10.25	11.33	11.36	12.42
Kinetex	C8	2.14	2.22	2.41	3.30	4.10	5.19	5.34	7.01	8.32	8.35	8.47	9.11	9.50	9.53	11.06	12.17	12.14	13.17

Elution Order Shifts from "EPA 533 Similar"

Figure 10.
Phenyl Stationary Phase Summary

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini®	C18	2.42	2.55	2.97	3.88	4.45	5.55	5.47	7.25	8.89	9.08	9.10	9.82	10.24	10.43	11.85	12.96	13.15	14.40

Benchmark: C 18 "EPA 533 Acetonitrile Altered"

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	9-Cl-PF3ONS	N-EtFOSAA	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	F5	1.27	1.32	1.45	1.93	2.29	3.12	3.26	4.55	5.92	6.05	6.54	6.84	7.03	7.22	8.44	9.24	9.63	10.76

Elution Order Shifts from C18 Acetonitrile Altered

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	PFUdA	9-Cl-PF3ONS	PFDoA	PFTrDA	11Cl-PF3OUdS	PFTeDA
Kinetex	Phenyl-Hexyl	1.05	1.14	1.18	1.39	1.60	1.99	2.25	2.96	4.09	4.33	4.47	4.82	5.16	5.46	6.17	7.09	7.45	7.95
Kinetex	Biphenyl	0.85	0.90	0.90	0.99	1.07	1.20	1.32	1.55	2.11	2.32	2.37	2.68	2.81	3.23	3.59	4.31	4.83	5.05

Elution Order Shifts from C18 Acetonitrile Altered

10. Column Chemistry Considerations (continued)

Conclusions

The HPLC methodology in EPA methods 537, 537.1 and 533 are all based upon a C18 stationary phase and a methanol-water mobile phase. In this study we have shown that the use of alternative stationary phases of varying surface chemistry and eluents of varying polarity can significantly alter the sorption-elution characteristics of different classes of PFAS compounds. This orthogonal approach to PFAS HPLC chromatography should serve as a fruitful avenue for future method development. As analyte lists increase in size and complexity, a variety of HPLC column chemistries and eluent compositions will be needed to accommodate the wide range of PFAS related compounds that might be encountered such as polar acids, non-polar acids, esters, amides, sulfonamides, and telomere length, all of which can be complicated with branched vs. linear isomers.

The work presented here is merely illustrative and should be considered a starting point for column chemistry and mobile phase considerations for PFAS HPLC methodology. Even though the demonstration sample contained a nice mix of PFAS compounds with varied functional groups, there are certainly many more compounds in the 9000-strong (and growing) PFAS inventory that will challenge LC-MS methodology. National and state PFAS analyte panels are constantly being updated and expanded. There is increasing emphasis on identifying and quantifying PFAS related isomers, unique functional groups and degradation products across a wide range of sample matrices. With regulated detection and quantitation limits being driven lower and lower, sensitivity is a significant issue. The choice of HPLC column chemistry will play a significant role in successfully meeting all these future challenges.

References

1. National Institute of Environmental Health Sciences. NIEHS Perfluorinate Chemicals (PFCs) fact sheet, 2012. https://www.atsdr.cdc.gov/sites/pease/documents/perflourinated_chemicals_508.pdf (accessed Dec 1, 2019).
2. United States Environmental Protection Agency. Per- and Polyfluoroalkyl Substances (PFAS). https://www.epa.gov/pfas (accessed Dec 1, 2019).
3. Agency for Toxic Substances and Disease Registry (ATSDR). An Overview of Perfluoroalkyl and Polyfluoroalkyl Substances and Interim Guidance for Clinicians Responding to Patient Exposure Concerns, 2019. https://www.atsdr.cdc.gov/pfas/docs/ATSDR_PFAS_ClinicalGuidance_12202019.pdf (accessed Apr 6, 2020).
4. PFAS List of PFAS Substances (Version 2). CompTox Chemicals Dashboard | PFASMASTER Chemicals (epa.gov) Accessed April 7, June 9, 2001.

Product Guide

Table 1.
Phenomenex PFAS Products Referenced or Applicable in Official Methods

Regulatory Method	Product	Part Number
USEPA 537.1: Determination of Selected Per-and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS) (5)	**Strata® SDB-L 500 mg/6 mL**	8B-S014-HCH
	Gemini® 3 µm C18, 50 x 3 mm or	00B-4439-B0
	Luna® Omega 1.6 µm PS C18 100 x 2.1 mm	00D-4752-AN
USEPA Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution, Anion Exchange Solid Phase Extraction and LC-MS/MS. (1)	**Strata-X-AW 500 mg/6 mL**	8B-S038-HCH
	Gemini 3 µm C18 50 x 2 mm or	00B-4439-B0
	Luna Omega 1.6 µm PS C18 100 x 2.1 mm	00D-4752-AN
US Food and Drug Administration: Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances(PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). (2)	**Strata-XL-AW 200 mg/3 mL**	8B-S051-FBJ
US Department of Agriculture: Screening, Determiation and Confirmation of PFAS by UPLC-MS-MS (3)	**Luna C8(2) 3 µm 50 x 2 mm**	00B-4248-B0
US Department of Defense: Quality Systems Manual (QSM) for Environmental Laboratories (4)	**Strata PFAS (WAX/GCB)** 200 mg/50 mg/6 mL, 30/box 500 mg/50 mg/6 mL, 30/box	 CSO-9207 CSO-9208
	Gemini 3 µm C18 50 x 2 mm	00B-4439-B0

References

1. Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) | Science Inventory | US EPA
2. Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry | Methods Approved to Analyze Drinking Water Samples to Ensure Compliance with Regulations | US EPA
3. Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances in Food using Liquid Chromatography-Tandem Mass Spectrometry (fda.gov)
4. Screening, Determination and Confirmation of PFAS by UPLC-MS-MS (usda.gov)
5. https://denix.osd.mil/edqw/documents/manuals/qsm-version-5-3-final/

Table 2.
Recommended HPLC Products for Routine PFAS Analysis

Description and Function	Product	Part Number
Analytical Column (UHPLC)	**Kinetex® 5 µm EVO C18 100 x 2.1 mm**	00D-4633-AN
	Luna Omega C18 1.6 µm 50 x 2.1	00B-4752-AN
	Gemini 3 µm C18 50 x 3 mm	00B-4439-Y0
Analytical Column	**Gemini 3 µm C18 50 x 3 mm**	00B-4439-Y0
Analytical Column (> 100 µL injection)	**Gemini 3 µm C18 100 x 3 mm**	00D-4439-Y0
Analytical Column (improved. lmwt acids)	**Luna Omega 3 µm PS C18 50 x 3 mm**	00B-4758-Y0
Delay Column	**Kinetex 5 µm EVO C18, 50 x 2.1 mm**	00B-4633-AN 00A-4252-Y0
SecurityGuard	**Luna Omega PS C18** **4 x 3.0/10 pack for ID: 3.2-8.0 mm** **4 x 2.0/10 pack for ID: 2.0-3.0 mm**	 AJ0-7606 AJ0-7605

Product Guide (continued)

Table 3.
Recommended SPE Products

Description and Function	Product	Part Number
SPE Cartridge for EPA 537.1	**Strata® SDB-L 500 mg/6 mL, 30/box**	8B-S014-HCH
SPE Cartridge for EPA 533	**Strata-X-AW 33um Polymeric Weak Anion, 500 mg/6 mL, 30/box**	8B-S038-HCH
SPE Cartridge (Rev. Phase, High Perf.)	**Strata-XL 500 mg/6 mL, 30/box**	8B-S043-HCH
SPE Stacked Cartridge (DOD QSM 5.3)	**Strata PFAS (WAX/GCB) 200 mg/50 mg/6 mL, 30/box**	CS0-9207
SPE Stacked Cartridge (DOD QSM 5.3)	**Strata PFAS (WAX/GCB) 500 mg/50 mg/6 mL, 30/box**	CS0-9208
SPE Cartridge (WAX for DOD QSM 5.3)	**Strata-XL-AW 500 mg/6 mL, 30/box**	8B-S051-HCH
GCB** Cartridge (GCB for DOD QSM 5.3)	**Strata GCB 250 mg/6 mL, 30/box**	8B-S528-FCH
SPE Cartridge (WAX* for FDA Method)	**Strata-XL-AW 100 µm 200 mg/3 mL, 50/box**	8B-S051-FBJ

(*WAX = Weak Anion Exchange)
(**GCB = Graphitized Carbon Black)

Table 4.
Recommended QuEChERs Products

Description and Function	Product	Part Number
QuEChERs Extraction (Soil/Sediment)	**roQ QuEChERs Extraction Kit**	KS0-8911
QuEChERs dSPE (Soil/Sediment)	**roQ QuEChERs dSPE Kit, 15 mL**	KS0-9516
QuEChERs Extraction (Dairy/Eggs/Fish)	**roQ QuEChERs Extraction Kit**	KS0-8910
QuEChERs dSPE (Dairy/Eggs/Fish)	**roQ QuEChERs dSPE Kit**	KS0-9511

Table 5.
Recommended Accessories

Description and Function	Product	Part Number
SPE Sample Reservoir	**75 mL Sample Reservoir**	H0-7005
Large Volume SPE	**Adaptor Cap for 12,20, 60 mL SPE Tubes**	AH0-7379
Autosampler Vials	**Polypropylene, 300 µm + PE Starburst Cap**	AR0-9995-12-C
Polypropylene Vials	**Vial 9 mm Screw Thd PP 2 mL, 1000 Pk**	AR0-89C7-13
Vial Caps	**Cap 9 mm Solid Top Black Unlined**	8B-S528-FCH
PEEK Capillary Tubing	**Capillary Tubing Kit, Various Sizes**	AT0-1964
PEEK Tubing Cutter	**Cutter for PEEK Capillary Tubing**	AT0-1110

roQ™ An Easier QuEChERS Solution

roQ™ Extraction Kits

Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Ordering Information

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Kits		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	KS0-8911*
EN 15662 Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KS0-8909*
Original Non-Buffered Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	KS0-8910
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	KS0-8912

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KS0-8911-NC and KS0-8909-NC

roQ dSPE Kits

dSPE kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Ordering Information

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg $MgSO_4$, 25 mg PSA, 25 mg C18E	100/pk	KS0-9504
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	100/pk	KS0-9505
150 mg $MgSO_4$, 25 mg PSA, 7.5 mg GCB	100/pk	KS0-9506
150 mg $MgSO_4$, 25 mg PSA	100/pk	KS0-9503
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18E, 50 mg GCB	100/pk	KS0-9514
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18E	100/pk	KS0-9512
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	100/pk	KS0-9513
150 mg $MgSO_4$, 50 mg PSA	100/pk	KS0-9511
15 mL dSPE Kits		
900 mg $MgSO_4$, 150 mg PSA, 150 mg C18E	100/pk	KS0-9508
900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB	100/pk	KS0-9509
900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB	100/pk	KS0-9510
900 mg $MgSO_4$, 150 mg PSA	100/pk	KS0-9507
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18E, 400 mg GCB	100/pk	KS0-9518
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18E	100/pk	KS0-9516
1200 mg $MgSO_4$, 400 mg PSA, 400 mg GCB	100/pk	KS0-9517
1200 mg $MgSO_4$, 400 mg PSA	100/pk	KS0-9515

roQ Extraction Salt Packets

Salt packets only. Centrifuge tubes not included.

Ordering Information

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Packets		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	AH0-9043
EN 15662 Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	AH0-9041
Original Non-Buffered Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	AH0-9042
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	AH0-9044

Bulk roQ QuEChERS Sorbents

Ordering Information

Phase	10 g	100 g
C18-E	—	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	—	04G-4610

Strata®-X Polymeric SPE

Strata-X

Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S100-TAK**	1 mL (100/box)
	30 mg	8B-S100-TBJ	3 mL (50/box)
	60 mg	8B-S100-UBJ**	3 mL (50/box)
	100 mg	8B-S100-EBJ	3 mL (50/box)
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
	500 mg	8B-S100-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S100-HDG	12 mL (20/box)
	1 g	8B-S100-JDG	12 mL (20/box)
	1 g	8B-S100-JEG	20 mL (20/box)
	2 g	8B-S100-KEG	20 mL (20/box)
	5 g	8B-S100-LFF	60 mL (16/box)
Teflon® Tube			
	200 mg	8B-S100-FBJ-T	3 mL (50/box)
	200 mg	8B-S100-FDG-T	12 mL (20/box)

On-line Extraction Cartridge

Description	Part Number	Unit/Box
Strata-X on-line extraction cartridge, 20 x 2.0 mm	00M-S033-B0-CB	ea
Cartridge holder, 20 mm	CH0-5845	ea

**Tab-less tubes available. Contact Phenomenex for details.

Strata-XL

Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S043-TAK	1 mL (100/box)
	60 mg	8B-S043-UBJ	3 mL (50/box)
	100 mg	8B-S043-EBJ	3 mL (50/box)
	200 mg	8B-S043-FBJ	3 mL (50/box)
	200 mg	8B-S043-FCH	6 mL (30/box)
	500 mg	8B-S043-HCH	6 mL (30/box)
Giga Tube			
	2 g	8B-S043-KDG	12 mL (20/box)
	2 g	8B-S043-KEG	20 mL (20/box)
	5 g	8B-S043-LEG	20 mL (20/box)
	5 g	8B-S043-LFF	60 mL (16/box)
	10 g	8B-S043-MFF	60 mL (16/box)
	30 mg	8E-S043-TGB	2 Plates/Box

* To control flow rate with Strata-XL, use a stopcock (AH0-6048) when processing samples with a vacuum manifold.

Strata Solid Phase Extraction (SPE)

PFAS (WAX/GCB)

Consists of a stacked single cartridge solution filled with polymeric WAX (200 mg) and GCB sorbents (50 mg) that functions to meet the DOD guidelines for PFAS testing. It is ideal for complex biota matrices and reduces the need for multiple extraction tubes.

Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	200 mg / 50 mg	CS0-9207	6 mL (30/box)

Gemini® pH Flexible LC Columns

Ordering Information

3 µm Microbore, Minibore and MidBore™ Columns (mm)										SecurityGuard™ Cartridges (mm)
Phases	50 x 1.0	20 x 2.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* /10pk
C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596

for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)						SecurityGuard™ Cartridges (mm)
Phases	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* /10pk
C18	00A-4439-E0	00B-4439-E0	00D-4439-E0	00F-4439-E0	00G-4439-E0	AJ0-7597

for ID: 3.2-8.0 mm

Kinetex® Core-Shell LC Columns

Ordering Information

2.6 µm Micro LC Columns (mm)						
Phases	30 x 0.3	50 x 0.3	100 x 0.3	150 x 0.3	50 x 0.5	150 x 0.5
EVO C18	—	00B-4725-AC	—	00F-4725-AC	00B-4725-AF	—

2.6 µm MercuryMS™ LC-MS Cartridges (mm)		
Phases	20 x 2.0	20 x 4.0
Biphenyl	00M-4622-B0-CE	00M-4622-D0-CE

MercuryMS Cartridge Holders		
Part No.	Description	Unit
CH0-7188	Direct-Connect Cartridge Holder, 20 mm	ea
CH0-5845	Standard Cartridge Holder, 20 mm	ea

2.6 µm Minibore Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298

for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297

for 3.0 mm ID

2010 R&D 100 Award Recipient

Ordering Information

5 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	00A-4633-AN	00B-4633-AN	00D-4633-AN	00F-4633-AN	AJ0-9298

for 2.1 mm ID

5 µm MidBore™ Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
EVO C18	00A-4633-Y0	00B-4633-Y0	00D-4633-Y0	00F-4633-Y0	AJ0-9297

for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296

for 4.6 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
***SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-9281
*PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223

Luna® One of The World's Leading LC Columns

Luna C18

Ordering Information

5 µm MidBore and Analytical Columns (mm)								SecurityGuard™ Cartridges (mm)	
Phases	30 x 3.0	50 x 3.0	150 x 3.0	250 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	4 x 2.0*	4 x 3.0*
								/10pk	/10pk
C18(2)	00A-4252-Y0	00B-4252-Y0	00F-4252-Y0	00G-4252-Y0	00A-4252-E0	00B-4252-E0	00C-4252-E0	AJ0-4286	AJ0-4287
								for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Analytical and Semi-Prep Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	100 x 4.6	150 x 4.6	250 x 4.6	250 x 10	4 x 3.0*	10 x 10‡
					/10pk	/3pk
C18(2)	00D-4252-E0	00F-4252-E0	00G-4252-E0	00G-4252-N0	AJ0-4287	AJ0-7221
					for ID: 3.2-8.0 mm	9-16 mm

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282
‡SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-9281

Luna Omega PS C18 and Luna C18

Ordering Information

1.6 µm Microbore Columns (mm)			
Phases	50 x 1.0	100 x 1.0	150 x 1.0
PS C18	—	00D-4752-A0	—
C18	00B-4742-A0	00D-4742-A0	00F-4742-A0

1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502
					for 2.1 mm ID

Perfluoroalkyl Substances **(PFAS)** Testing Guide

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