

Perfluoroalkyl Substances (PFAS) Testing Guide

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.



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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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 Perfluorooctane-sulfonic 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

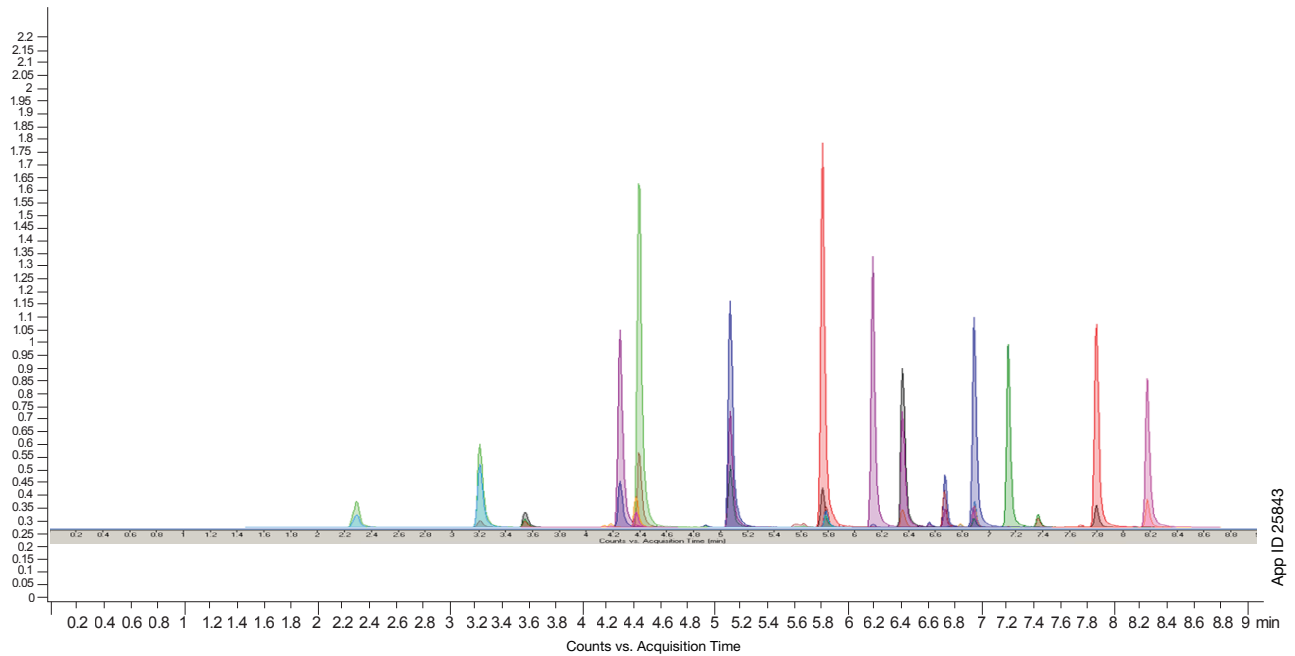
Data and Results

PFAS Target Analytes and UHPLC Retention Times

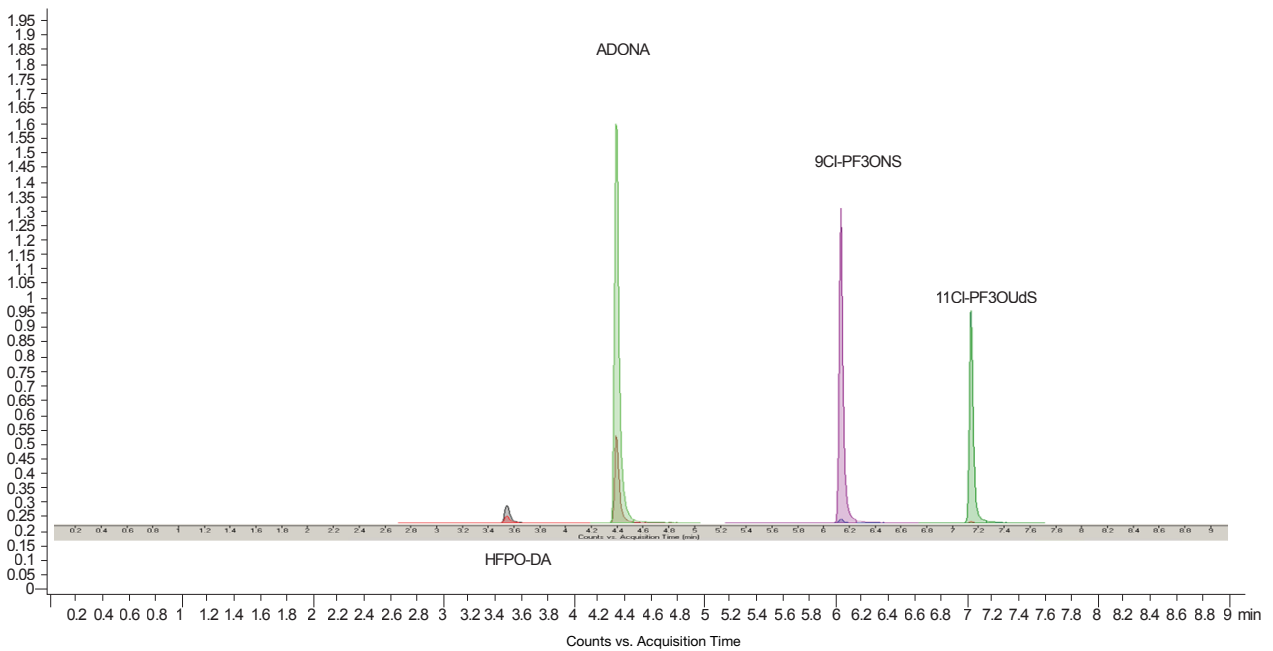
Analyte	RT (min)	Internal Standard
PFBS	2.29	13C4 -PFOS
PFHxA	3.20	13C2 -PFOA
HFPO-DA	3.55	13C2 -PFOA
PFHpA	4.24	13C2 -PFOA
PFHxS	4.39	13C4 -PFOS
ADONA	4.41	13C2 -PFOA
PFOA	5.08	13C2 -PFOA
PFOS	5.72	13C4 -PFOS
PFNA	5.77	13C2 -PFOA
9CI-PF3ONS	6.15	13C4 -PFOS
PFDA	6.35	13C2 -PFOA
NMeFOSAA	6.70	d3 -NMeFOSAA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

Full PFAS Target Analytes

PFAS Replacement Compounds



Short Chain ("Gen X") PFAS Analytes



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 500mg/6mL and Luna® Omega 1.6µm PS C18 for use in EPA Method 537.1. Luna Omega 1.6µm 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 1.6µm 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 70µg/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 1.6µm PS C18 for this essential environmental application.

References

1. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=348508&Lab=CESER&simpleSearch=0&showCriteria=2&searchAll=537.1&TIMSType=&dateBeginPublishedPresented=03%2F24%2F2018



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

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 C ₄ -PFOS
¹³ C ₆ -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 ₆ -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
9CI-PF3ONS	35	14.53	¹³ C ₆ -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
11CI-PF3OUdS	42	16.70	¹³ C ₆ -PFOS
PFDoA	44	17.13	¹³ C ₂ -PFDoA

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

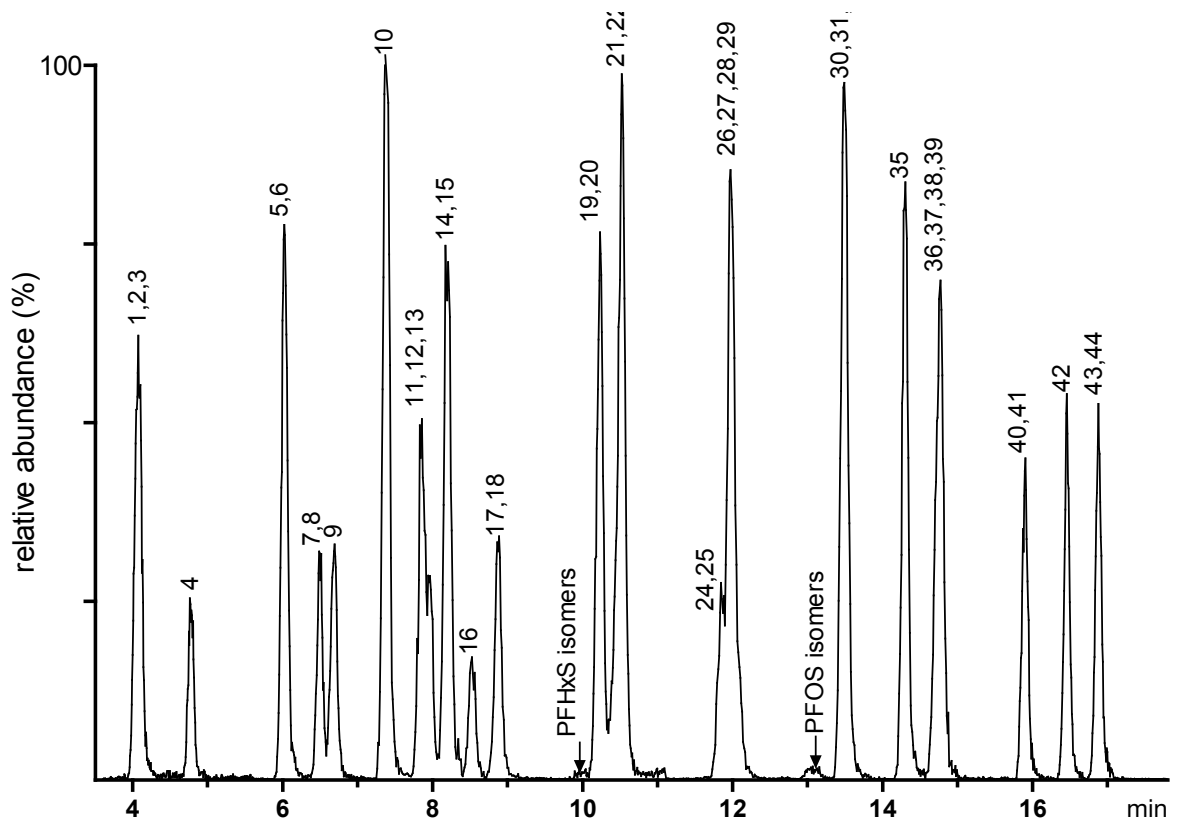
Table 6.
EPA 533 Precision and Accuracy Data from a Commercial Laboratory

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



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 Pierrri, 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>

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.

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 polyethylene 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% NH₄OH, 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.

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

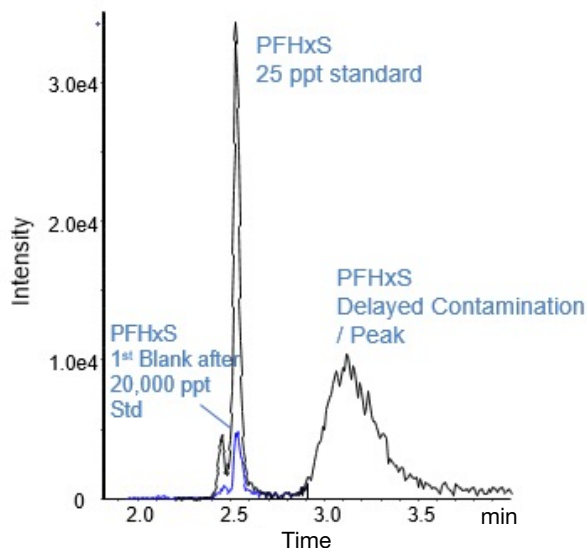
Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	-4500 V
Temperature (TEM)	600 °C
Nebulizer Gas (GS1)	50 psi
Heater Gas (GS2)	50 psi

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

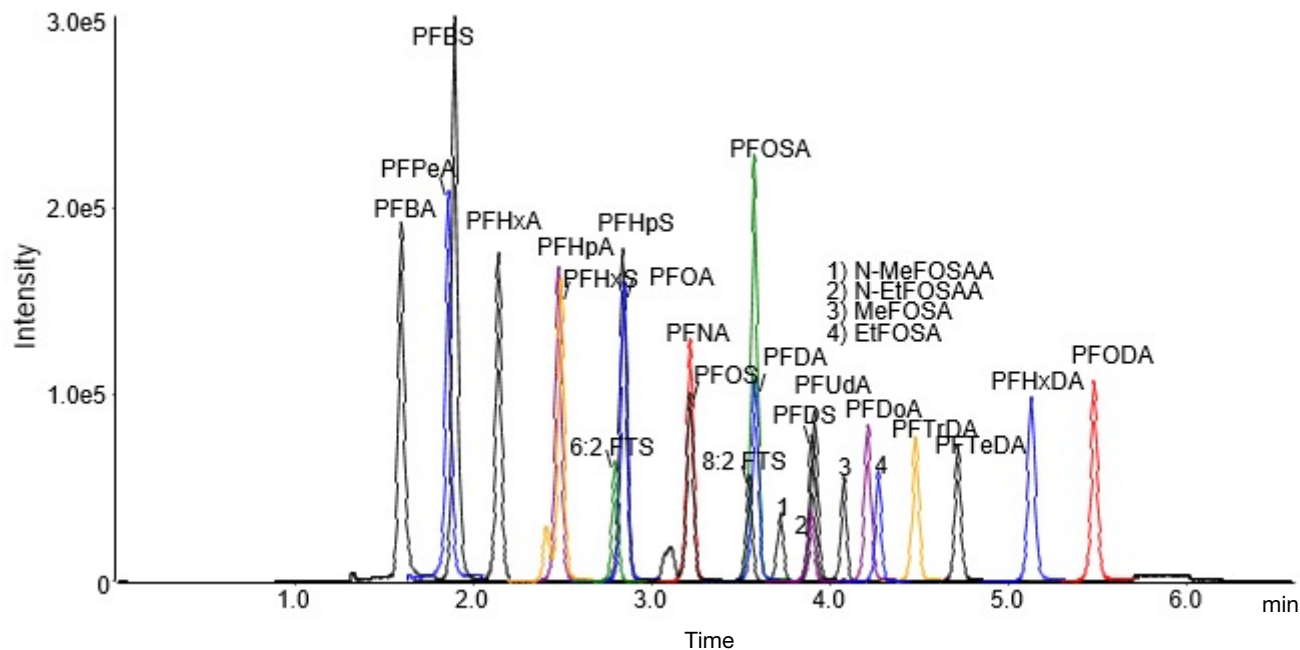
The Gemini® C18 column was selected for both methods based on its strong retention and predictable resolution of PFASs. All of the other columns tested exhibited breakthrough of the short chain acids in the column dead volume during optimization of the 950 µL injection method. The Luna® C18(2) column 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.

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



Overlaid Chromatograms of a 1 μ g/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.

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.

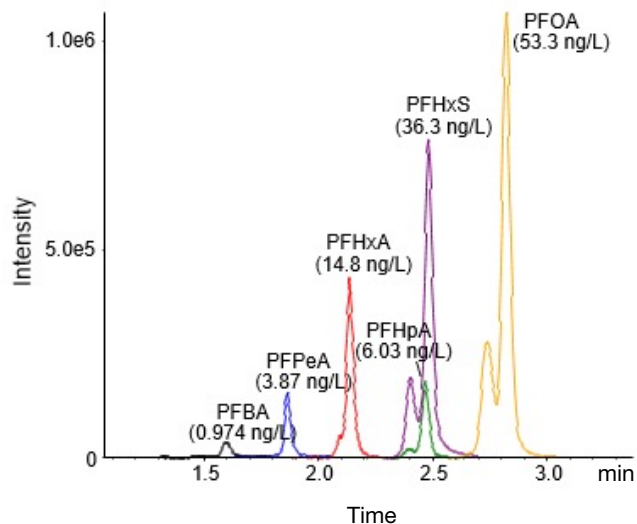


Table 5.
Calibration curves for method 1 and 2.

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
PFCA								
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%				
PFSA								
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%				

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.



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.

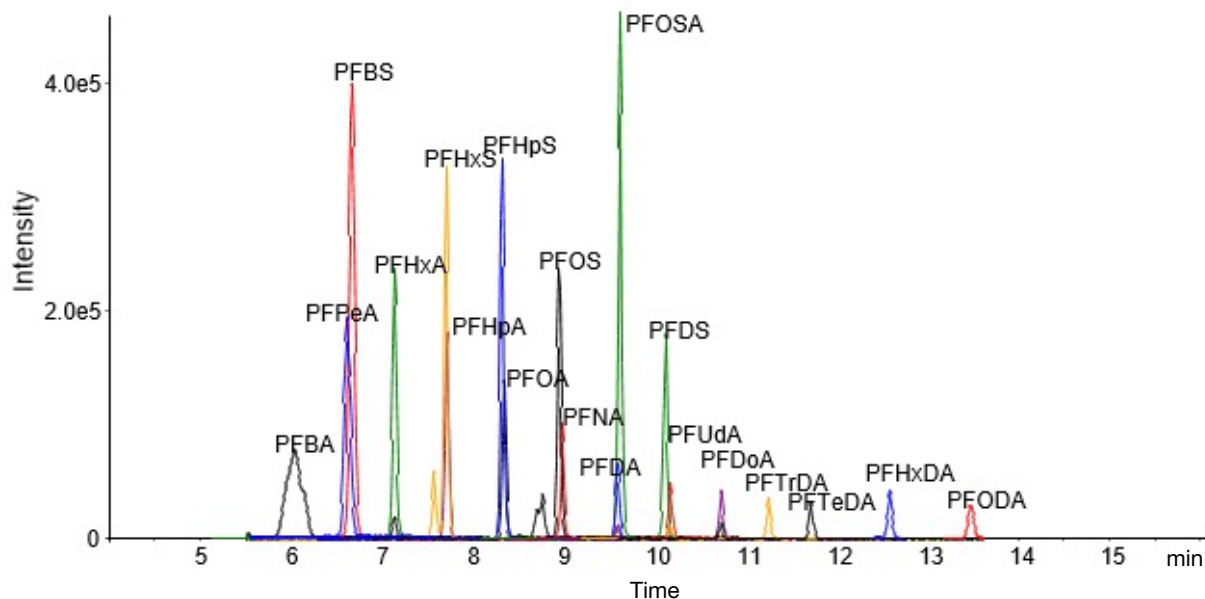
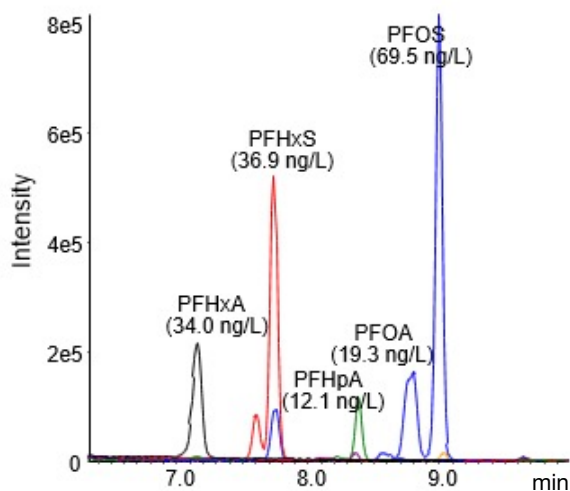


Figure 6
Overlaid MRM traces of PFASs detected in a groundwater sample with the calculated concentrations of each PFAS.

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 PFTTrDA (81%) and PFBS (84%). Due to limited availability of surrogate standards, PFBS was analyzed using 18O2 PFHxS as an internal standard, and PFTTrDA 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.

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
PFASs				
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

Applications

Acknowledgements

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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
<i>13C4_PFBa</i>	217	172	-25	-12
<i>13C5_PFPeA</i>	268	223	-20	-12
<i>13C2_PFHxA</i>	315	270	-25	-12
<i>13C4_PFHpA</i>	367	322	-25	-12
<i>13C2_PFOA</i>	415	370	-25	-14
<i>13C4_PFOA</i>	417	372	-25	-14
<i>13C5_PFNA</i>	468	423	-25	-14
<i>13C2_PFDA</i>	515	470	-25	-16
<i>13C2_PFUdA</i>	565	520	-25	-18
<i>13C2_PFDoA</i>	615	570	-25	-18
<i>13C2_PFTeDA</i>	715	670	-25	-22
<i>13C2_PFHxDA</i>	815	770	-25	-24
<i>18O2_PFHxS</i>	403	84	-60	-74
<i>13C4_PFOS</i>	503	80	-65	-108
<i>13C8_PFOSA</i>	506	78	-60	-85
<i>M2-6:2FTS</i>	429	409	-50	-32
<i>M2-8:2FTS</i>	529	509	-50	-40
<i>d3MeFOSA</i>	515	169	-75	-37
<i>d5EtFOSA</i>	531	169	-75	-37
<i>d3-MeFOSAA</i>	573	419	-40	-36
<i>d3-EtFOSAA</i>	589	419	-50	-36

Perfluoroalkyl Substances (PFAS) Testing Guide

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