Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water

Using automated solid phase extraction and LC-MS/MS for U.S. EPA Method 533

Authors: Xin Zhang, Changling Qiu, Rahmat Ullah, and Yan Liu

Thermo Fisher Scientific, Sunnyvale, CA

Keywords: Perfluorinated and polyfluorinated alkyl substances, PFAS, AutoTrace 280 PFAS, U.S. EPA Method 533, Acclaim 120 C18 column, Vanquish Flex UHPLC, TSQ Fortis triple quadrupole mass spectrometer

Goal

To demonstrate an efficient and reliable solid phase extraction method with a Thermo Scientific™ Dionex™ AutoTrace™ PFAS, an automated solid-phase extraction (SPE) system, for the determination of per- and poly-fluorinated compounds in drinking water per U.S. EPA Method 533

1. Introduction

Drinking water perfluorinated and polyfluorinated alkyl substances (collectively referred to as PFAS) occurrence studies have typically targeted perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), and as a result, these two are the most commonly detected compounds. This discussion focuses primarily on PFOS and PFOA. However, other compounds including PFBA, PFHxA, PFHpA, PFNA, PFUnDA, and PFHxS have also been detected in drinking water. In December 2019, the United States Environmental Protection Agency (U.S. EPA) took a key step in implementing the PFAS Action Plan by announcing a new validated method for testing PFAS in drinking water. This new validated test method complements other actions the agency is taking under the Action Plan to help communities address PFAS nationwide. It focuses on “short chain” PFAS, those PFAS with carbon chain lengths of four to twelve, and covers PFOS, PFOA, and other common PFAS that have been detected and reported. Currently most testing laboratories are

performing the sample extraction manually using a vacuum manifold, which is labor-intensive and time-consuming. In addition, the flow rate through the cartridge is difficult to control, which may yield low recoveries or cause false negatives. There is a high demand for automation of the SPE procedure. Here we discuss the development of an analytical method using an automated SPE system and LC-MS/MS for determination of twenty-five PFAS compounds following the guidelines provided by U.S. EPA Method 533. We have demonstrated that the automated SPE system provides reliable determination of PFAS in large-volume aqueous samples and saves time, solvent, and labor, while ensuring high reproducibility and productivity for analytical testing laboratories.

2. Experimental

2.1. Instruments

- Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS (P/N 22136-60101, PROD, AT280 PFAS, CARTRIDGE)
- Thermo Scientific™ Vanquish™ Flex UHPLC system
- Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer
- Organomation Associates™ OA-SYS™ heating system

2.2. Reagents, standards, and consumables

- Water, UHPLC-MS Grade, Fisher Scientific (P/N W81)
- Methanol, UHPLC-MS Grade, Fisher Scientific (P/N A458-1)
- Acetic acid, Optima™ LC/MS, Fisher Scientific (P/N A11310X1AMP)
- Ammonium acetate, Optima™ LC/MS, Fisher Scientific (P/N A11450)
- The native PFAS analyte primary dilution standard, 500 µg/L in MeOH/water (water<1%), Wellington Laboratories Inc. (EPA-533PAR), see Table 1 for analyte details
- Mass-labeled PFAS isotope dilution standards, 1000–4000 µg/L in MeOH/water (water <1%), 500–2000 µg/L in MeOH/water (water <1%), Wellington Laboratories Inc. (EPA-533ES), see Table 1 for compound details
- Mass-labeled PFAS isotope performance standards, 1000–3000 µg/L in MeOH/water (water <1%), Wellington Laboratories Inc. (P/N EPA-533IS), see Table 1 for compound details
- Polypropylene collection vials, Fisher Scientific (P/N 50-809-216)
- SPE Cartridges – 0.5 g, 6 mL Thermo Scientific™ Dionex™ SolEx™ WAX cartridges (P/N 088115)
- Thermo Scientific™ Vanquish™ system fitted with Thermo Scientific™ PFC-free kit (P/N 80100-62142)
- Thermo Scientific™ Acclaim™ 120 C18 column, 2.1 × 150 mm, 2.2 µm (P/N 071399)
- Thermo Scientific™ Hypersil™ BDS C18 column, 2.1 × 50 mm, 5 µm (P/N 28105-052130)

2.3. Method workflow

Figure 1 shows the workflow of the method that applies to the test blank, LCMRL, and the precision and accuracy test samples. Ammonium acetate (250 mg) was added to the 250 mL water samples as a preservation reagent to sequester free chlorine by forming chloramine. Different amounts of PFAS analyte primary dilution standards were spiked into the sample to achieve the desired concentration range (1–200 ng/L). Twenty microliters of the mass-labeled PFAS isotope dilution standard were added and vortexed into the above samples prior to WAX SPE extraction. The concentration of each target analyte is calculated using the isotope dilution technique. After WAX extraction with an automated SPE system, the extraction eluent was evaporated to dryness under nitrogen gas flow at 55–60 °C and reconstituted with 1 mL 80/20 MeOH/water (v/v). Ten microliters of mass-labeled PFAS isotope performance standard were added to the extraction eluent. After sufficient vortexing, the sample was transferred to a PFAS-free vial and was ready for LC-MS/MS analysis.

Table 1. Information for test analytes, dilution analogues, and internal standards

	Targeted analyte	Abbreviation	Isotope dilution standard	Isotope performance standard
1	Perfluorobutanoic acid	PFBA	$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
2	Perfluoro-3-methoxypropanoic acid	PFMPA	$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
3	Perfluoropentanoic acid	PFPeA	$^{13}C_5$-PFPeA	$^{13}C_3$-PFBA
4	Perfluorobutanesulfonic acid	PFBS	$^{13}C_3$-PFBS	$^{13}C_4$-PFOS
5	Perfluoro-4-methoxybutanoic acid	PFMBA	$^{13}C_5$-PFPeA	$^{13}C_3$-PFBA
6	Perfluoro(2-ethoxyethane) sulfonic acid	PFEESA	$^{13}C_3$-PFBS	$^{13}C_4$-PFOS
7	Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	$^{13}C_5$-PFHxA	$^{13}C_2$-PFOA
8	1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	$^{13}C_2$ 4:2 FTS	$^{13}C_4$-PFOS
9	Perfluorohexanoic acid	PFHxA	$^{13}C_5$-PFHxA	$^{13}C_2$-PFOA
10	Perfluoropentanesulfonic acid	PFPeS	$^{13}C_3$-PFHxS	$^{13}C_4$-PFOS
11	Hexafluoropropylene oxide dimer acid	HFPO-DA	$^{13}C_3$-HFPO-DA	$^{13}C_2$-PFOA
12	Perfluoroheptanoic acid	PFHpA	$^{13}C_4$-PFHpA	$^{13}C_2$-PFOA
13	Perfluorohexanesulfonic acid	PFHxS	$^{13}C_3$-PFHxS	$^{13}C_4$-PFOS
14	4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	$^{13}C_4$-PFHpA	$^{13}C_2$-PFOA
15	1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	$^{13}C_2$-6:2 FTS	$^{13}C_4$-PFOS
16	Perfluorooctanoic acid	PFOA	$^{13}C_8$-PFOA	$^{13}C_2$-PFOA
17	Perfluoroheptanesulfonic acid	PFHpS	$^{13}C_8$-PFOS	13C_4-PFOS
18	Perfluorononanoic acid	PFNA	$^{13}C_9$-PFNA	$^{13}C_2$-PFOA
19	Perfluorooctanesulfonic acid	PFOS	$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
20	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	^{9}Cl-PF_3ONS	$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
21	1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	$^{13}C_2$-8:2 FTS	$^{13}C_4$-PFOS
22	Perfluorodecanoic acid	PFDA	$^{13}C_6$-PFDA	$^{13}C_2$-PFOA
23	Perfluoroundecanoic acid	PFUnA	$^{13}C_7$-PFUnA	$^{13}C_2$-PFOA
24	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	^{11}Cl-PF_3OUdS	$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
25	Perfluorododecanoic acid	PFDoA	$^{13}C_2$-PFDoA	$^{13}C_2$-PFOA

Figure 1. U.S. EPA Method 533 procedure workflow using an automated SPE system

2.4. Sample preparation

Reagent water: DI water that does not contain any measurable quantities of method analytes or interfering compounds greater than 1/3 the MRL for each method analyte of interest is necessary for this analysis. For this work, water was further purified using a bench model Millipore™ water purification system (Millipore Corp, Billerica, MA, Model No. Milli-Q™ Gradient A10 or equivalent). This water is referred to as DI water in this document.

Standard calibration solution: The PFAS PDS was diluted with 80%/20% MeOH/DI water to produce standard solutions containing different concentration levels of each PFAS. The mass-labeled PFAS isotope dilution standard and mass-labeled PFAS performance dilution standard were added to each calibration standard at a constant concentration. The standard calibration solutions were used to quantify the samples (Table 2).

Table 2. Standard calibration solutions

Target PFAS conc. (µg/L)	Stock solution conc. (µg/L)	Volume of stock solution (µL)	80% MeOH (µL)	Isotope dilution standard (µL)	Isotope performance standard (µL)
100	2000	50	950	20	10
50	100	500	500	20	10
20	100	200	800	20	10
10	100	100	900	20	10
5	10	500	500	20	10
2	10	200	800	20	10
1	10	100	900	20	10
0.5	10	50	950	20	10
0.2	10	20	980	20	10
0.1	10	10	990	20	10

Lowest Concentration Minimum Reporting Level (LCMRL) and Method Detection Limits (MDL) solution: To determine LCMRL, seven replicates of fortified samples at different concentration levels (1, 2, 4, 6, 10, 14, 20, and 40 ng/L, preparation details are in Table 3) were processed through the entire method procedure (Figure 1). The LCMRLs were calculated according to the procedure in reference 1.

Table 3. Preparation of the fortified samples for the LCMRL test

Fortified conc. (ng/L)	DI water (mL) w/ ammonium acetate	Analyte stock conc. (µg/L)	Volume stock solution (µL)	Surrogate std. PDS (µL)
40	250	100	100	20
20	250	100	50	20
14	250	100	35	20
10	250	100	25	20
6	250	10	150	20
4	250	10	100	20
2	250	10	50	20
1	250	10	25	20

MDLs were determined by running seven replicate fortified samples at a concentration of 4 ng/L through the entire method procedure. The MDL was determined using the following equation

$$MDL = s \times t_{(n-1,\ 1-\alpha = 0.99)}$$

Where s = standard deviation of replicate analyses

$t_{(n-1,\ 1-\alpha = 0.99)}$ = Student's value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates

2.5. AutoTrace 280 PFAS sample extraction

The AutoTrace 280 PFAS system is constructed of inert material to ensure accurate and precise analysis for PFAS extraction. We modified the line function per the U. S. EPA Method 533 requirement. The solvent side lines were used just to condition and dry the cartridges. The sample side lines were used in sample load, rinse, and elution steps to maximize PFAS recoveries. Thus, both solvent and sample lines are flushed in the Sample Path Cleaning step. Figure 2 shows a general guideline for AutoTrace 280 sample extraction.

Figure 2. General guideline for AutoTrace 280 PFAS sample extraction

2.5.1. Create methods in the AutoTrace 280 PFAS SPE workstation software in the computer

The AutoTrace 280 PFAS extraction and clean-up methods for PFAS are specified below following U.S. Method EPA 533 guidelines and are divided into three parts (methods): Cartridge Conditioning and Sample Loading, Sample Elution, and Sample Path Cleaning. These methods are created and loaded into the AutoTrace 280 PFAS from the computer and run sequentially.

Solvents used for the three methods are listed below.

Solvent No.	Nomenclature
1	Solvent 1 (unused)
2	Water
3	Solvent 3 (unused)
4	MeOH (methanol)
5	0.1 M phosphate buffer (pH 7.0)

2.5.1.1. Method One: **Cartridge Conditioning and Sample Loading (Program this method in Solid Phase Extraction mode)**

No.	Method (Programmed)	User intervention/information
1	Process 6 samples using the following method steps.	N/A
2	Condition the cartridge with 10 mL of MeOH into solvent waste.	N/A
3	Condition the cartridge with 10 mL of phosphate buffer into aqueous waste.	N/A
4	Condition the cartridge with 3.0 mL of phosphate buffer into aqueous waste.	N/A
5	Condition the cartridge with 3 mL of water into aqueous waste.	N/A
6	Load 270.0 mL of sample onto the cartridge.	Sample bottle contains 250 mL of sample. The method is programmed to deliver 270 mL sample as it accounts for the delay volume in the system. Waste automatically goes to aqueous waste.
7	Pause and alert operator, resume when CONTinue is pressed.	Manually add 10 mL 1 g/L ammonium acetate into the sample bottle, swirl over the inner walls to rinse out any residual sample. Make sure the sample weights are at the bottom of the sample bottle submerged in liquid.
8	Load 25 mL of sample onto the cartridge.	The method is programmed to consider the delay volume.
9	Pause and alert operator, resume when CONTinue is pressed.	Manually add 1 mL MeOH into the sample bottle, swirl over the inner walls to rinse out any residual sample. Make sure the sample weights are at the bottom of the sample bottle submerged in liquid.
10	Load 16 mL of sample onto the cartridge.	The method is programmed to consider the delay volume and to pull all the aqueous phase from the tubes.
11	Dry cartridge with gas for 5 min.	N/A
12	End.	N/A

Set condition flow and loading flow at 5.0 mL/L; set air factor at 1.0.

2.5.1.2. Method Two: Sample Elution (Program this method in Solid Phase Elute mode)

No.	Method (Programmed)	User intervention/information
		This step must be performed before pressing CONTinue on the front panel. Manually add 5.0 mL MeOH with 2% ammonium hydroxide (v/v) into the sample bottle, swirl over the inner walls to rinse out any residual sample.
1	Process 6 samples using the following method steps.	N/A
2	Manually rinse sample container with 10.0 mL to collect.	First elution with 5 mL MeOH with 2% ammonium hydroxide (v/v). The method is programmed to consider the delay volume.
3	Pause and alert operator, resume when CONTinue is pressed.	Manually add 5.0 mL MeOH with 2% ammonium hydroxide (v/v) into the sample bottle, swirl over the inner walls to rinse out any residual sample.
4	Manually rinse sample container with 15.0 mL to collect.	Second elution with 5 mL MeOH with 2% ammonium hydroxide (v/v). The method is programmed to consider the delay volume and push out any residual MeOH.
5	End.	N/A

Set loading flow at 1.0 mL/L; set air factor at 1.0.
Do not detach the cartridges during Methods One and Two.

2.5.1.3. Method Three: Sample Path Cleaning (Program this method in Solid Phase Elute mode). Use six empty SPE cartridges in each cartridge holder and push down on the lever to engage to run the Sample Path Cleaning method.

No.	Method	User intervention/information
1	Process 6 samples using the following method steps.	Manually insert all the sampling lines into the methanol bottle. Insert the solvent lines into the assigned solvents.
2	Clean each sample path with 50.0 mL MeOH into solvent waste.	N/A
3	Collect 5.0 mL fraction into sample tube using MeOH.	Steps 3 and 6 are programmed to clean the solvent path. The fractions collected are discarded.
4	Pause and alert operator, resume when CONTinue is pressed.	Take out all the sample lines from methanol and manually insert all the sample lines into water.
5	Clean each sample path with 50.0 mL into aqueous waste.	N/A
6	Collect 5.0 mL fraction into sample tube using water.	Steps 3 and 6 are programmed to clean the solvent path. The fractions collected are discarded.
7	End.	N/A

Set condition and loading flow at 10 mL/min.

2.5.2. Load/Transfer method to AutoTrace 280 PFAS

Load/transfer all the extraction and cleanup methods to the AutoTrace 280 PFAS instrument. Perform extraction/clean-up from the instrument's front panel.

2.5.3. SPE extracting and eluting with the AutoTrace 280 PFAS

2.5.3.1. Preparing the AutoTrace 280 PFAS

- Turn on the system and gas supply, set the nitrogen gas gauge at 10 psi.
- Check that both solvent and aqueous waste containers are empty.

2.5.3.2. Cleaning the AutoTrace 280 PFAS

The cleaning protocol is performed prior to extraction to ensure the system is free of potential PFAS contamination in both the solvent lines and sample lines.

a) Insert the solvent lines into the assigned solvents, with line 2 in DI water, line 4 in MeOH, and line 5 in 10 mM pH 7 phosphate buffer (solvent side).

b) Place collection containers into each elution rack position.

c) Press "Load" multiple times to display Method 29 "Prime Solvents" (This method is built into the system). Press CONT once to select the method. Press CONT again to run the method. The method draws enough solvent from each port to prime the liquid lines. Repeat the procedure 3-4 times.

d) Place 6 empty SPE cartridges in each cartridge holder and push down on the lever to engage (green lights on).

e) Insert all the sample lines into the methanol solvent bottle (from sample side).

f) Load **"Method Three: Sample Path Cleaning"** method and run by selecting CONT

g) Follow the instrument display to proceed.

h) Run the Clean Sample Path method 1–2 times or until a desired background is achieved.

Note that whenever the system is idle for more than 24 h, a "Sample Path Cleaning" method with both methanol and water needs to be run to clean the lines and leave them filled with DI water.

2.5.3.3. SPE extracting and eluting with the AutoTrace 280 PFAS

a) Place collection vials into each elution rack position.

b) Place the SPE cartridge in each cartridge holder and engage the cartridge.

c) Place the sample lines into the sample bottles.

d) Load **"Method One: Cartridge Conditioning and Sample Loading"** and press CONT from front panel. This method will execute the following steps:

i. Condition the cartridge with methanol and water (Solvent lines)

ii. Load the sample (Sample lines)

iii. Rinse the sample bottle and cartridge with water (Sample lines)

iv. Dry with gas (Solvent lines)

e) Before loading Method Two, perform the methanol addition step into sample bottles as described in Method Two. Do not detach the cartridges during Methods One and Two.

f) Load **"Method Two: Sample Elution"** and press CONT from front panel. This method will execute the following steps:

v. Elute the sample with methanol (Sample lines).

vi. Collect the extract for the next step.

2.5.4. Extract evaporation, reconstitution, and transfer for LC-MS/MS analysis

a) Evaporate the extract to dryness with nitrogen flow in a heated water bath at ~55–60 °C, reconstitute to 1 mL with 80:20 (vol/vol) methanol/water, and vortex.

b) Transfer the final sample in a polypropylene autosampler vial for LC-MS/MS analysis.

2.6. LC-MS/MS conditions

LC system components, as well as the mobile phase constituents, may contain many of the analytes in this method. Thus, a Thermo Scientific™ PFC-free kit (P/N 80100-62142), which includes PFAS-safe tubing, fittings, solvent filter inlets, and sample vials, is strongly recommended. An isolator column, a Thermo Scientific™ Hypersil™ BDS C18, 2.1 × 50 mm column, was installed after the LC pump and prior to the injection valve to offset background contaminants from the LC pump, degasser, and mobile phases. To minimize the background PFAS peaks and to keep background levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, prior to daily use, flush the column with 100% methanol for at least 20 min before initiating a sequence. It may be necessary on some systems to flush other LC components such as wash syringes and sample needles before daily use.

2.6.1. LC conditions

Parameter	Value
Analytical column	Acclaim 120 C18, 2.1 × 150 mm, 2.2 µm
Isolator column	Hypersil BDS C18, 2.1 × 50 mm, 5 µm. This column was installed prior to the autosampler to remove any contaminants from the mobile phase.
Column temperature	45 °C
Flow rate	0.4 mL/min
Injection volume	5 µL
Autosampler temperature	6 °C
Mobile phase A	20 mM ammonium acetate
Mobile phase B	Methanol
Gradient	*Time (min)* / *%B*: -3 / 5 Equilibration; 0 / 5 Run; 0.5 / 5; 3 / 40; 14 / 85; 17 / 85; 18 / 5; 21 / 5

2.6.2. MS global parameters

Parameter	Value
Ion source type	H-ESI
Polarity	Negative
Negative ion	2,500 V
Sheath gas	50 arbitrary units
Aux gas	10 arbitrary units
Sweep gas	1 arbitrary units
Ion transfer tube temperature	275 °C
Vaporizer temperature	300 °C
Q1 resolution	0.7 FWHM*
Q3 resolution	1.2 FWHM*
CID gas	2 mTorr

*FWHM: Full width at half maximum

2.6.3. Optimized SRM transition parameters for the MS method

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	Tube lens (V)	Source fragmentation (V)
PFBA	212.979	168.97	7.78	77	0
$^{13}C_3$-PFBA	216	172	7.78	77	0
$^{13}C_4$-PFBA	217	172	7.78	77	0
PFMPA	229	85	9.6	76	0
PFPeA	262.976	219.042	7	82	0
$^{13}C_5$-PFPeA	267.993	222.99	7.27	82	0
PFMBA	279	85	11.32	41	0
HFPO-DA	285	169	5.25	64	32.5
$^{13}C_3$-HFPO-DA	287	169	5.25	64	32.5
NFDHA	295	201	5.25	77	0
PFBS	298.943	79.957	30.4	81	9.8
$^{13}C_3$-PFBS	301.953	79.96	30.4	81	9.8
PFHxA	312.973	268.97	10.23	60	5
PFEESA	315	135	26	120	5
$^{13}C_5$-PFHxA	317.9896	273	10	60	5
4:2 FTS	326.974	307.042	18.11	102	18
$^{13}C_2$-FTS 4:2	328.981	308.96	18	101	18
PFPeS	348.94	80.042	33.66	87	13
PFHpA Opt	362.97	319.042	10.23	64	5
$^{13}C_4$-PFHpA	366.983	321.98	10	64	5
ADONA	377	251	10	71	5
PFHxS	398.937	79.957	39	110	5
$^{13}C_3$-PFHxS	401.947	79.957	39	110	5
PFOA	412.966	369	10.23	74	5
$^{13}C_2$-PFOA	415	370	10.23	74	5
$^{13}C_8$-PFOA	421	376	10	74	5
6:2 FTS	426.968	406.988	21.45	118	5
$^{13}C_2$-FtS 6:2	428.975	408.96	21	118	5
PFHpS Opt	448.933	80.012	37.6	123	5
PFNA	462.963	418.97	10.23	79	5
$^{13}C_9$-PFNA	471.993	426.97	10	79	5
PFOS	499	80	47	130	26.1
$^{13}C_4$-PFOS	503	80	38.5	108	18
$^{13}C_8$-PFOS	506.957	79.957	42.66	107	18
PFDA Opt	512.96	469.042	10.23	84	5
$^{13}C_6$-PFDA	518.98	473.97	10	84	5
8:2 FTS	526.962	506.97	24	119	28
$^{13}C_2$-FtS 8:2	528.968	508.96	24	129	5
^{9}Cl-PF_3ONS	531	351	23.4	120	5
PFUNA Opt	562.957	518.97	10.23	93	5
$^{13}C_7$-PFUdA	570	525	10.23	93	5
PFDoA Opt	612.954	569	10.23	95	5
$^{13}C_2$-PFDoA	614.96	569.97	10	95	5
^{11}Cl-PF_3OUdS	631	451	26	120	5

2.7. Data collection software

Data were collected and processed using Thermo Scientific™ Chromeleon™ Data System (CDS) version 7.2.9. This method has three isotope performance standards that are used as reference compounds for the internal standard quantitation of the sixteen isotope dilution analogues. The sixteen isotope dilution analogues are used as reference compounds to quantitate the native analyte concentrations. The suggested isotope performance standards reference for each isotope dilution analogue and the suggested isotope dilution analogue references for the native analytes are listed in Table 1. In the Chromeleon CDS 7.2.9 software processing method, the three mass-labeled PFAS isotope performance standards were set as “external standards” and the sixteen mass-labeled PFAS isotope dilution standards were linked to targeted analytes as “internal standards” and calibrated by the external standards in “calibration of other components”.

3. Results and discussion

3.1. Demonstration of low system background

Due to the wide usage of PFAS, it is necessary to demonstrate low system background from consumables such as sample containers, tubing, SPE cartridges, and separation columns to instruments including the SPE auto extraction system, LC, and MS. A blank experiment was designed that consisted of reagent water going through the workflow. The final chromatogram shown in Figure 3 demonstrates the overall very low system background and qualifies PFAS system suitability.

To assess the carryover of the AutoTrace 280 PFAS system, a blank extraction was run after a 80 ng/L spiked water sample extraction with the AutoTrace 280 PFAS system. No significant carryover was observed in the blank with all tested analytes lower than 1/3 of the LCMRL.

3.2. Lowest concentration minimum reporting level (LCMRL)

The LCMRL is the lowest true concentration for which the future recovery is predicted to fall between 50 and 150% recovery with high confidence (99%). The experiment was designed and calculated according to U.S. EPA Method 533 guidelines with seven replicates at each concentration and a total of seven different concentrations. Table 4 shows the concentration detail of each tested analyte and effective LCMRLs ranging from 2 to 10 ng/L.

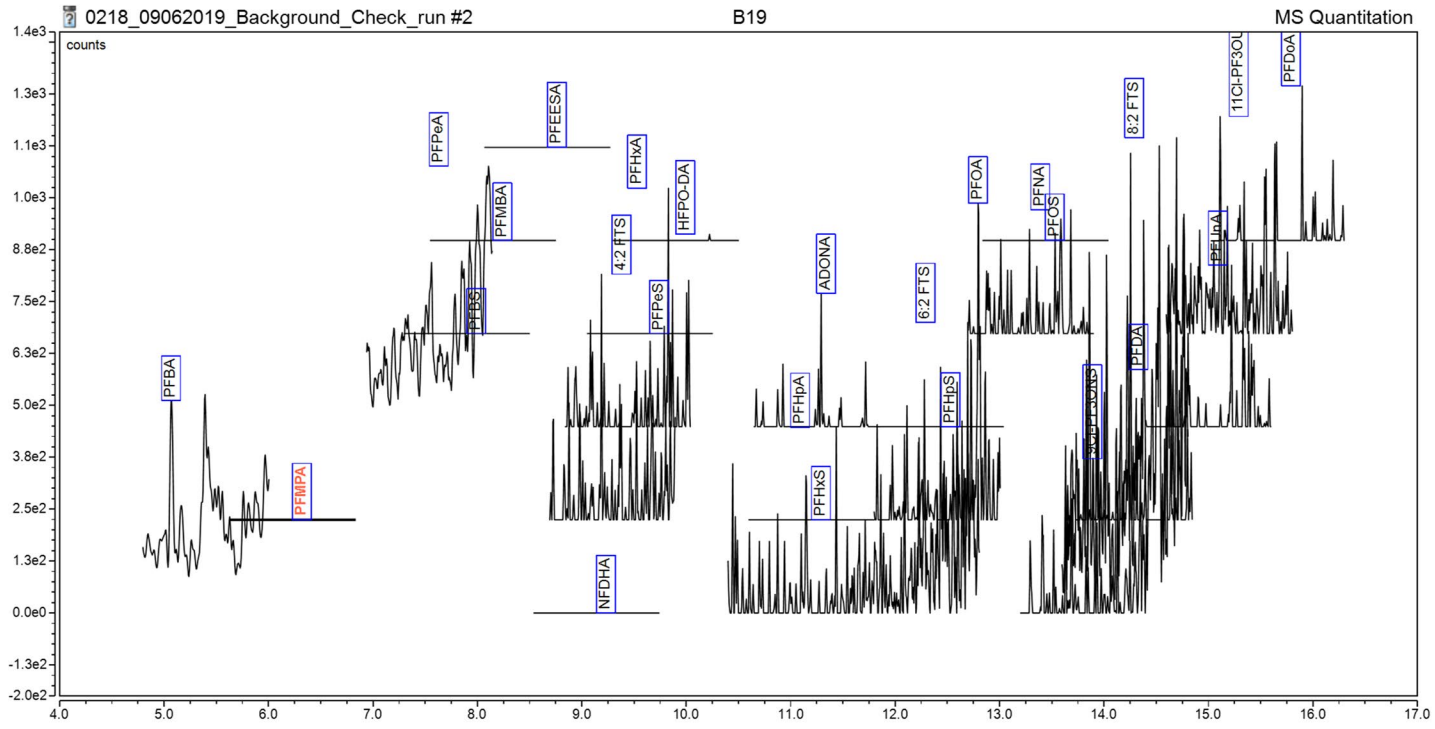

Figure 3. PFAS LC-MS/MS chromatogram for a method blank sample

Table 4. Lowest concentration minimum reporting levels

Number	Analytes	Fortification levels (ng/L)	LCMRL (ng/L)
1	PFBA	1.0, 2.0,4.0, 6.0, 10, 14, 20	8.6
2	PFMPA	1.0, 2.0,4.0, 6.0, 10, 14, 20	4.5
3	PFPeA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9
4	PFBS	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.2
5	PFMBA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9
6	PFEESA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.4
7	NFDHA	1.0, 2.0,4.0, 6.0, 10, 14, 20	5.7
8	4:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20	7.0
9	PFHxA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.2
10	PFPeS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.6
11	HFPO-DA	1.0, 2.0,4.0, 6.0, 10, 14, 20	7.4
12	PFHpA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.4
13	PFHxS	1.0, 2.0,4.0, 6.0, 10, 14, 20	6.5
14	ADONA	1.0, 2.0,4.0, 6.0, 10, 14, 20	1.6
15	6:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	5.7
16	PFOA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9
17	PFHpS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	5.8
18	PFNA	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.8
19	PFOS	1.0, 2.0,4.0, 6.0, 10, 14, 20	5.2
20	^{9}Cl-PF$_3$ONS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.9
21	8:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	9.5
22	PFDA	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.1
23	PFUnA	1.0, 2.0,4.0, 6.0, 10, 14, 20	4.1
24	^{11}Cl-PF$_3$OUdS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.4
25	PFDoA	1.0, 2.0,4.0, 6.0, 10, 14, 20,40	4.8

3.3. Calibration

During method development, multiple chromatographic peaks representing branched and linear isomers were observed for standards of PFHxS and PFOS. All the chromatographic peaks observed in the standard are integrated and the areas summed. Chromatographic peaks in all validation test samples are integrated in the same way as the calibration standard for analytes with quantitative standards containing the branched and linear isomers.

The targeted analytes were quantified by using the internal standard calibration technique. The internal standard technique calculates concentration based on the ratio of the peak area of the native analyte to that of the isotope dilution analogue. Calibration for all tested analytes from at least a 0.25–20 ng/L range are obtained, all with r^2 values of 0.99 or greater. For calibration levels that are less than or equal to the LOQ, the result for each analyte is within 50–150% of the true value. All other calibration points are within 70–130% of their true value. The detailed calibration range, coefficient of determination, and mean % deviation of all tested analytes are listed in Table 5.

Table 5. Calibration summary

Analytes	Cal range (ng/L)	r^2	Mean deviation
PFBA	0.25–50	1.000	6.7
PFMPA	0.25–50	0.994	13
PFPeA	0.5–50	1.000	4.7
PFBS	0.25–20	0.998	9.0
PFMBA	0.5–50	1.000	5.3
PFEESA	0.25–50	0.997	5.1
NFDHA	0.5–50	0.996	9.4
4:2 FTS	0.25–20	0.998	12
PFHxA	0.25–50	0.998	9.4
PFPeS	0.25–20	0.999	15
HFPO-DA	0.25–20	0.999	12
PFHpA	0.25–50	0.998	4.7
PFHxS	0.5–50	0.994	20
ADONA	0.25–50	0.999	6.4
6:2 FTS	0.25–20	1.000	13
PFOA	0.25–50	0.998	9.4
PFHpS	0.5–20	0.992	13
PFNA	0.25–50	0.998	7.2
PFOS	0.25–20	0.999	11
^{9}Cl-PF$_3$ONS	0.25–20	0.997	13
8:2 FTS	0.25–50	0.999	12
PFDA	0.25–50	0.994	7.7
PFUnA	0.25–50	0.999	4.8
^{11}Cl-PF$_3$OUdS	0.25–20	0.996	12
PFDoA	0.25–50	0.995	8.1

3.4. Demonstration of analyte precision and accuracy with reagent water

Two fortified concentration levels (10 ng/L and 80 ng/L) were analyzed to measure target analyte recovery and reproducibility (measured by RSD of the determined concentration). At each concentration level, six replicate fortified samples were preserved, prepared, extracted, evaporated and reconstituted, and analyzed by the method. The accuracy and precision results of the method in reagent water are presented in Table 6. At both 10.0 ng/L and 80.0 ng/L fortified concentration level, all recoveries were within the acceptable range of 70 to 130% according to U.S. EPA Method 533, ranging from 85.9 to 124%. The calculated RSDs were all less than 20%, suggesting good precision.

Table 6. Precision (RSD) and accuracy (%REC) for reagent water at different spiking levels

	Reagent water spike at 10 ng/L (Low)		Reagent water spike at 80 ng/L (High)	
Target Analyte	Mean %R	RSD	Mean %R	RSD
PFBA	119	4.5%	94.4	4.1%
PFMPA	106	9.2%	97.8	4.7%
PFPeA	100	5.8%	95.4	8.0%
PFBS	85.9	14%	96.2	9.5%
PFMBA	103	11%	96.3	5.1%
PFEESA	109	3.8%	91.9	4.5%
NFDHA	118	11%	104	2.3%
4:2 FTS	108	14%	95.8	2.4%
PFHxA	110	6.0%	96.0	4.2%
PFPeS	97.6	5.0%	109	7.2%
HFPO-DA	102	18%	100	14%
PFHpA	100	6.7%	90.5	6.2%
PFHxS	111	16%	96.5	8.4%
ADONA	104	1.5%	91.9	5.1%
6:2 FTS	99.0	3.0%	93.8	6.0%
PFOA	123	9.9%	99.9	4.0%
PFHpS	108	12%	105	10%
PFNA	105	3.2%	95.5	3.8%
PFOS	93.4	8.1%	101	8.4%
^{9}Cl-PF$_3$ONS	107	10%	94.3	6.5%
8:2 FTS	116	14%	107	4.4%
PFDA	106	4.6%	95.9	1.8%
PFUnA	107	9.2%	95.6	1.2%
^{11}Cl-PF^{3}OUdS	95.1	6.8%	78.9	9.9%
PFDoA	97.9	6.9%	91.4	2.4%

For quality control purposes, the percent recoveries of the isotope dilution analogues are calculated using the integrated peak areas of isotope performance standards, which are added to the final extract and function as traditional internal standards, exclusively applied to the isotope dilution analogues. The sixteen mass-labeled PFAS isotope dilution standards were monitored as well for the accuracy and precision under constant concentration (40 and 160 ng/L). As shown in Table 7, all recoveries ranged within 90 to 123% and RSDs were within 15% demonstrating good accuracy and precision.

Table 7. Precision and accuracy (P@A) in reagent water: isotope dilution analogue recovery data

	Fortification (ng/L)	REC (%) (P@A Low)	RSD (P@A Low)	REC (%) (P@A High)	RSD (P@A High)
$^{13}C_4$-PFBA	40	105	6.1	106	6.7
$^{13}C_5$-PFPeA	40	102	4.8	105	6.0
$^{13}C_3$-PFBS	40	106	2.6	117	3.9
$^{13}C_2$ 4:2 FTS	160	110	3.3	121	6.4
$^{13}C_5$-PFHxA	40	95.5	3.9	97.7	4.1
$^{13}C_3$-HFPO-DA	40	109	15	120	13
$^{13}C_4$-PFHpA	40	106	4.8	111	7.4
$^{13}C_3$-PFHxS	40	99.1	6.5	104	5.3
$^{13}C_2$-6:2 FTS	160	102	4.9	115	8.8
$^{13}C_8$-PFOA	40	101	6.7	106	8.4
$^{13}C_9$-PFNA	40	97.9	5.9	106	11
$^{13}C_8$-PFOS	40	92.3	11	105	13
$^{13}C_2$-8:2 FTS	160	97.6	8.3	103	11
$^{13}C_6$-PFDA	40	96.2	7.4	104	11
$^{13}C_7$-PFUnA	40	90.0	9.0	92.8	14
$^{13}C_2$-PFDoA	40	91.7	7.7	94.5	14

3.5. Precision and accuracy in finished drinking water

The mean percent recovery, corrected for the matrix contribution, and RSD for each tested analyte as well as tested concentration for unfortified drinking water sample are presented in Table 8. All corrected recoveries were within 85.6 to 120% and RSDs were within 15%. The average percent recoveries and RSDs of the isotope dilution analogues in finished drinking water samples are listed in Table 9. All the data indicate good precision and accuracy with the finished drinking water sample.

Table 8. Precision (RSD) and accuracy (%REC) for drinking water at different spiking levels

Target analyte	Avg ± St Dev (n=3)	Drinking water spike at 10 ng/L		Drinking water spike at 80 ng/L	
		Mean %Rec (n=6)	RSD	Mean % Rec (n=6)	RSD
PFBA	2.3 ± 0.4	94.8	6.1%	105.7	4.1%
PFMPA	ND	105	3.5%	110.8	6.5%
PFPeA	ND	107	7.9%	102.8	8.5%
PFBS	ND	91.3	18%	110.7	9.4%
PFMBA	ND	106	14%	114.0	3.7%
PFEESA	ND	110	8.3%	105.2	7.4%
NFDHA	ND	112	9.0%	117.2	4.1%
4:2 FTS	ND	106	6.4%	112.4	4.4%
PFHxA	ND	114	4.1%	108.5	5.4%
PFPeS	ND	103	15%	114.1	8.3%
HFPO-DA	ND	93.4	15%	120.5	3.6%
PFHpA	ND	104	6.2%	107.7	2.8%
PFHxS	ND	99.7	13%	107.2	5.2%
ADONA	ND	100	2.7%	108.9	3.5%
6:2 FTS	0.32 ± 0.29	94.0	4.7%	107.2	5.4%
PFOA	0.79 ± 0.09	110	4.2%	113.4	1.6%
PFHpS	ND	118	16%	119.6	6.7%
PFNA	ND	108	4.5%	112.8	3.1%
PFOS	ND	99.8	13%	117.1	5.7%
^{9}Cl-PF$_3$ONS	ND	97.7	5.6%	110.5	4.3%
8:2 FTS	ND	114	16%	124.1	6.8%
PFDA	ND	111	7.5%	111.0	3.0%
PFUnA	0.36 ± 0.06	104	3.3%	107.9	2.3%
^{11}Cl-PF^{3}OUdS	ND	85.6	7.5%	95.0	4.0%
PFDoA	0.55 ± 0.14	98.7	5.8%	101.9	2.2%

Table 9. Precision and accuracy (P@A) in drinking water: isotope dilution analogue recovery data

	Fortification (ng/L)	REC (%) (P@A Low)	RSD (P@A Low)	REC (%) (P@A High)	RSD (P@A High)
$^{13}C_4$-PFBA	40	108	3.7	110	6.9
$^{13}C_5$-PFPeA	40	104	4.1	112	5.2
$^{13}C_3$-PFBS	40	118	3.6	121	7.6
$^{13}C_2$ 4:2 FTS	160	123	3.2	128	5.1
$^{13}C_5$-PFHxA	40	98.4	2.6	103	7.3
$^{13}C_3$-HFPO-DA	40	117	9.8	123	8.4
$^{13}C_4$-PFHpA	40	110	3.4	112	5.4
$^{13}C_3$-PFHxS	40	113	6.8	118	7.4
$^{13}C_2$-6:2 FTS	160	117	2.3	123	6.0
$^{13}C_8$-PFOA	40	107	2.2	112	6.6
$^{13}C_9$-PFNA	40	105	3.7	107	3.8
$^{13}C_8$-PFOS	40	108	5.5	113	7.3
$^{13}C_2$-8:2 FTS	160	113	3.9	110	6.6
$^{13}C_6$-PFDA	40	104	5.8	108	5.3
$^{13}C_7$-PFUnA	40	102	10	101	4.2
$^{13}C_2$-PFDoA	40	101	10	101	6.6

4. Conclusions

We proposed and demonstrated a PFAS-inert automated SPE extraction LC-MS/MS system that can be used for the simultaneous extraction and determination of twenty-five PFAS in drinking water. The PFAS-inert material modified auto SPE system ensures inertness and prevents PFAS from leaching into sample during extraction; while at the same time delivering labor-and-time-efficient, consistent, and reliable performance. Full method validation has been successfully performed with both a reagent water and a finished drinking water sample. Acceptable low background, LCMRLs, calibration range, coefficient of determination and % deviation, precision, and accuracy for both tested analytes and isotope dilution analogue recoveries demonstrated the auto sample preparation system and UHPLC-MS/MS workflow as an efficient and reliable method to fulfill U. S. EPA Method 533 requirements.

thermo scientific

References

1. U.S. 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. https://www.epa.gov/dwanalyticalmethods/method-533-determination-and-polyfluoroalkyl-substances-drinking-water-isotope
2. Rahman, M.F.; Peldszus, S.; Anderson, W. B. Behaviour and fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in drinking water treatment: a review. *Water Research,* **2014,** *50,* 318–340.
3. Wilhelm, M.; Bergmann, S.; Dieter, H.H. Occurrence of perfluorinated compounds (PFCs) in drinking water of North Rhine-Westphalia, Germany and new approach to assess drinking water contamination by shorter-chained C4-C7 PFCs. *Int. J. Hyg. Environ. Health* **2010,** *213*(3), 224–232.
4. Ahrens, L.; Taniyasu, S.; Yeung, L.W.Y.; Yamashita, N.; Lam, P.K.S.; Ebinghaus, R. Distribution of polyfluoroalkyl compounds in water, suspended particulate matter and sediment from Tokyo Bay, Japan. *Chemosphere* **2010,** *79*(3), 266–272.
5. Ullah, S.; Alsberg, T.; Berger, U. Simultaneous determination of perfluoroalkyl phosphonates, carboxylates, and sulfonates in drinking water. *J. Chromatogr. A* **2011,** *1218*(37), 6388–6395.
6. U.S. EPA Method 533, EPA Press Office, Dec, 2019, https://www.epa.gov/newsreleases/epa-announces-new-method-test-additional-pfas-drinking-water
7. U.S. EPA Lowest concentration minimum reporting level. https://www.epa.gov/dwanalyticalmethods/lowest-concentration-minimum-reporting-level-lcmrl-calculator

Find out more at **thermofisher.com/autotrace**

ThermoFisher SCIENTIFIC