APPLICATION NOTE 73346

Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water using automated solid-phase extraction and LC-MS/MS

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1. Goal

To demonstrate an efficient and reliable solid-phase extraction method with the Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS Solid-Phase Extraction instrument for the determination of per- and poly-fluorinated compounds in drinking water per U.S. EPA Method 537.1

2. Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a group of man-made chemicals including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and GenX chemicals that have been manufactured and used in a variety of industries globally.[1,2] These compounds have a wide range of commercial product applications including industrial polymers, stain repellents, surfactants, waterproofing products, packaging, and aqueous film forming foams used for firefighting. PFAS are highly soluble in water, chemically stable, persistent in the environment, and can accumulate in the human body over time, leading to adverse human health effects.[3] PFOA and PFOS are no longer manufactured in the U.S. due to their persistence and potential human health risks.

In November 2018, the United States Environmental Protection Agency (U.S. EPA) published Method 537.1 "Determination of selected per- and polyfluorinated alkyl substances in drinking water by solid phase extraction and LC/MS/MS".[4] The method uses an offline solid-phase extraction (SPE) with liquid chromatography tandem mass spectrometry (LC-MS/MS) to extract, enrich, and determine 18 PFAS in drinking water. Currently most testing laboratories perform the sample extraction manually using a vacuum manifold, which is labor-intensive, time-consuming, and the flow rate through the cartridge is difficult to control. There is a high demand for automation of the SPE procedure.

In this application note, we discuss the development of an analytical method using an automated SPE system, AutoTrace 280 PFAS, and LC-MS/MS for determination of eighteen PFAS following the guidelines provided by U.S. EPA Method 537.1. We have demonstrated that the AutoTrace 280 PFAS instrument provides reliable automated SPE for determination of PFAS in large-volume (20 mL–4 L) aqueous samples.

3. Experimental

3.1. Instruments

- Thermo Scientific Dionex AutoTrace 280 PFAS System (P/N 22136-60101)
- Thermo Scientific™ Vanquish™ Flex Duo UHPLC system, fitted with Thermo Scientific™ PFC free kit (P/N 80100-62142), including:
  - System Base (P/N VF-S02-A)
  - Dual Pump F (P/N VF-P32-A)
  - Dual Split Sampler FT (P/N VF-A40-A)
  - Column Compartment H (P/N VH-C10-A)
- Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer (P/N TSQ02-10003)
- Organomation Associates™ 12 Position N-EVAP Nitrogen Evaporator (P/N 11155)

3.2. Reagents, standards, and consumables

- Water, UHPLC-MS Grade, Fisher Scientific (P/N W81)
- Methanol (MeOH), UHPLC-MS Grade, Fisher Scientific (P/N A458-1)
- Trizma™ Pre-Set Crystals, (Bioperformance certified), Fisher Scientific (P/N NC0829165)
- Acetic acid, Optima™ LC/MS, Fisher Scientific (P/N A11310X1AMP)
- Ammonium acetate, Optima LC/MS, Fisher Scientific (P/N A11450)
- Analyte Primary Dilution Standard (branched/linear mix), 2000 µg/L in MeOH/water (water<1%), Wellington Laboratories Inc. (P/N EPA-537PDS-R1), see Table 1 for analyte details
- Surrogate Primary Dilution Standard (SUR PDS), 1000–4000 µg/L in MeOH/water (water<1%), Wellington Laboratories Inc. (P/N EPA-537SS-R1), see Table 1 for compound details
- Internal Standard Primary Dilution Standard (IS PDS), 1000–4000 µg/L in MeOH/water (water<1%), Wellington Laboratories Inc. (P/N EPA-537IS), see Table 1 for compound details
- Polypropylene collection vials, Fisher Scientific (P/N 50-809-216). Polypropylene vials are used instead of glass vial due to adherence of PFAS compounds to the glass surface.
- SPE Cartridges – 0.5 g, 6 mL SPE cartridges containing styrenedivinyl-benzene (SDVB) polymer compliant with U.S. EPA Method 537.1
- Thermo Scientific™ Accucore™ RP-MS column, 2.1 × 100 mm, 2.6 µm (P/N 17626-102130)
- Thermo Scientific™ Hypersil™ BDS C18 column, 2.1 × 50 mm, 5 µm (P/N 28105-052130)

3.3. Method workflow

Figure 1 shows the workflow of the method that applies to the test blank, LCMRL, and the precision and accuracy test samples. Trizma (1.25 g) was added to the 250 mL water samples as a preservation reagent to remove free chlorine. Ten microliters of the SUR PDS were added prior to SPE extraction. After extraction with the AutoTrace 280 PFAS system, the extraction eluent was evaporated to dryness under nitrogen gas flow at 55–60 °C and reconstituted with 1 mL 96%/4% MeOH/water. Ten microliters of IS PDS were then 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.

3.4. Sample preparation

Reagent water - 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. For this work, water was obtained from a bench model Millipore water purification system (Millipore Corp, Billerica, MA, Model No. Milli-QR Gradient A10 or equivalent). This water is referred to as deionized water (DI water) in this document.

Table 1. Information for test analytes, surrogates, and internal standards

Analytes	Acronym	Chemical Abstract Services Registry Number (CASRN)
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6b
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9c
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1d
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4e
Surrogates	**Acronym**	
Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid	$^{13}C_2$-PFHxA	
Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid	$^{13}C_2$-PFDA	
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-NEtFOSAA	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	
Internal standards	**Acronym**	
Perfluoro-[1,2-$^{13}C_2$] octanoic acid	$^{13}C_2$-PFOA	
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	$^{13}C_4$-PFOS	
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-NMeFOSAA	

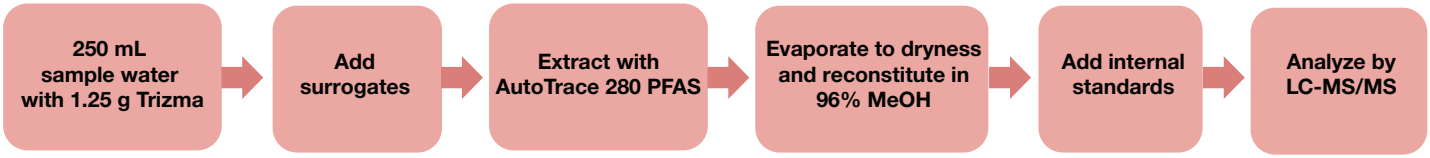

Figure 1. U.S. EPA Method 537.1 procedure workflow

Standard calibration solution - The PFAS PDS was diluted with 96%/4% MeOH/DI water to produce standard solutions containing different concentration levels of each PFAS. The IS PDS and SUR PDS were added to each calibration standard at a constant concentration. The standard calibration solutions were used to quantify all the samples (Table 2).

Table 2. Standard calibration solutions

Target PFAS conc. (µg/L)	Stock solution conc. (µg/L)	Volume of stock solution (µL)	96% MeOH (µL)	Surrogate standard PDS (µL)	Internal standard PDS (µL)
100	2000	50	950	10	10
50	100	500	500	10	10
20	100	200	800	10	10
10	100	100	900	10	10
5	10	500	500	10	10
2	10	200	800	10	10
1	10	100	900	10	10
0.5	10	50	950	10	10
0.2	10	20	980	10	10
0.1	10	10	990	10	10

Lowest Concentration Minimum Reporting Level (LCMRL) and Method Detection Limits (MDL) solution - To determine LCMRL, seven replicates of fortified samples prepared at different concentration levels (0.2, 0.4, 0.8, 2.0, 4.0, 8.0, and 32 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.

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

3.5. AutoTrace 280 PFAS sample extraction

The AutoTrace 280 PFAS system was modified to reduce Teflon™ components and replace with alternative inert materials. Historically, the solvent side lines of the AutoTrace 280 PFAS system were used for the condition, dry, and elute functions and the sample side lines were used for sample loading and rinsing. We modified the line function per the U. S. EPA Method 537.1 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 elute to maximize PFAS recoveries. Thus, both solvent and sample lines need to be flushed in the sample path cleaning step. Figure 2 shows a general guideline for AutoTrace 280 PFAS sample extraction.

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

Fortified conc. (ng/L)	DI water with Trizma (mL)	Analyte stock conc. (µg/L)	Volume stock solution (µL)	Surrogate standard. PDS (µL)
32	250	100	80	10
8	250	100	20	10
4	250	100	10	10
2	250	10	50	10
0.8	250	10	20	10
0.4	250	10	10	10
0.2	250	10	5	10

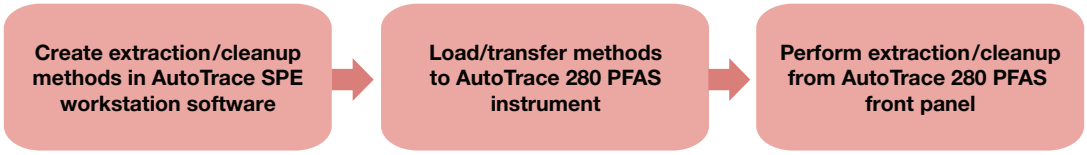

Figure 2. General guideline for AutoTrace 280 PFAS sample extraction

3.5.1. Create methods in the AutoTrace 280 PFAS SPE workstation software

The AutoTrace 280 PFAS extraction and cleanup methods for PFAS are specified below following U.S. Method EPA 537.1 guidelines and are divided into three parts (methods), cartridge conditioning and sample loading, sample elution, and sample path cleaning. These methods are loaded into the AutoTrace 280 PFAS instrument from the software provided with the system and run sequentially.

Solvents used for the three methods are listed below.

Solvent No.	Nomenclature
1	Solvent 1
2	Water
3	Solvent 3
4	MeOH (methanol)
5	Solvent 5

Method One: Cartridge Conditioning and Sample Loading (program this method in solid-phase extraction mode)

No.	Method (programmed)	User intervention/information
1	Process six samples using the following method steps	
2	Condition cartridge with 7.5 mL of MeOH into solvent waste	
3	Condition cartridge with 7.5 mL of MeOH into solvent waste	
4	Condition cartridge with 9.0 mL of water into aqueous waste	
5	Condition cartridge with 9.0 mL of water into aqueous waste	
6	Load 270.0 mL of sample onto cartridge	Sample bottle actually 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	Add 7.5 mL reagent water into 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 into sample.
8	Load 17.5 mL of sample onto cartridge	The method is programmed to consider the delay volume
9	Pause and Alert operator, resume when CONTinue is pressed	Add 7.5 mL water into 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 into sample.
10	Load 21.5 mL of sample onto 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 10.0 minutes	
12	End	

Step	Flow rate (mL/min)
Cond flow	10.0
Load flow	10.0
Rinse flow	10.0
Elute Flow	1.0
Cond air push	15.0
Rinse air push	20.0
Elute air push	5.0

SPE parameters	
Push delay	5 s
Air factor	1.0
Autowash vol.	1.0 mL

Instrument parameters	
Max elution vol.	20.0 mL
Exhaust fan on	Yes
Beeper on	Yes

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. Add 4.0 mL methanol into 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 into that 4 mL methanol.
1	Process six samples using the following method steps	
2	Manually rinse sample container with 14.0 mL to collect	First, elute with 4.0 mL MeOH. The method is programmed to consider the delay volume.
3	Pause and alert operator, resume when CONTinue is pressed	Add 4.0 mL methanol into 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 into that 4 mL methanol.
4	Manually rinse sample container with 18.0 mL to collect	Second, elute with 4.0 mL MeOH. The method is programmed to consider the delay volume and push out any residual methanol.
5	End	

Step	Flow rate (mL/min)
Cond flow	1.0
Load flow	1.0
Rinse flow	1.0
Elute flow	1.0
Cond air push	15.0
Rinse air push	20.0
Elute air push	5.0

SPE parameters	
Push delay	5 s
Air factor	1.0
Autowash vol.	1.0 mL

Instrument parameters	
Max elution vol.	20.0 mL
Exhaust fan on	Yes
Beeper on	Yes

*Do not detach the cartridges during methods one and two

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 (programmed)	User intervention/information
1	Process six samples using the following method steps	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 into solvent waste	
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 insert all the sample lines into water.
5	Clean each sample path with 50.0 mL into aqueous waste	
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	

Step	Flow rate (mL/min)
Cond flow	10.0
Load flow	10.0
Rinse flow	10.0
Elute flow	5.0
Cond air push	15.0
Rinse air push	20.0
Elute air push	5.0

SPE parameters	
Push delay	5 s
Air factor	1.0
Autowash vol.	1.0 mL

Instrument parameters	
Max elution vol.	20.0 mL
Exhaust fan on	Yes
Beeper on	Yes

3.5.2. Load/transfer method to AutoTrace 280 PFAS instrument

Load/transfer all the extraction and cleanup methods to the AutoTrace 280 PFAS instrument.

3.5.3. Perform extraction/cleanup from AutoTrace 280 PFAS front panel

3.5.3.1. Preparing the AutoTrace 280 PFAS instrument

- Turn on the gas supply
- Check that both solvent and aqueous waste containers are empty

3.5.3.2. Cleaning the AutoTrace 280 PFAS instrument

The cleaning protocol is performed prior to extraction to ensure the system is free of potential PFAS contamination for both solvent lines and sample lines when the system is idle for more than 24 h or the first time it is used.

a) Insert the solvent lines into the assigned solvents, with two solvent lines in methanol and three lines in DI water (from solvent side).

b) Place collection containers into each elution rack position.

c) Press “Load” multiple times to display Method 29 “Prime Solvents”. 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 six empty SPE cartridges in each cartridge holder and push down on the lever to engage.

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 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, run a Clean Sample Path method with both methanol and water to clean the lines and leave them filled with DI water.

3.5.3.3. Extracting and eluting with the AutoTrace 280 PFAS instrument

a) Place collection vials into each elution rack position.

b) Place an 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:

   i. Elute the sample with methanol (sample lines).

   ii. Collect the extract for the next step.

3.5.3.4. Cleaning the AutoTrace 280 PFAS instrument

a) Place six empty SPE cartridges in each cartridge holder and push down on the lever to engage.

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

c) Load “Method Three: Sample Path Cleaning” method and run by selecting CONT.

d) Follow the instrument display to proceed.

e) Run the Clean Sample Path method.

3.6. 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 96:4 (vol/vol) methanol/water, vortex.

b) Add internal standards to the sample.

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

3.7. LC-MS/MS analysis

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-free tubing, fittings, solvent filter inlets, and sample vials is strongly recommended. An isolator column, a Hypersil BDS C18, 2.1 x 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.

3.7.1. LC conditions

Parameter	Value
Analytical column	Accucore RP-MS, 2.1 × 100 mm, 2.6 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 temp.	45 °C
Flow rate	0.5 mL/min
Injection volume	5 µL
Autosampler temp.	6 °C
Solvent A	Water containing 0.1% acetic acid
Solvent B	Methanol containing 0.1% acetic acid
Solvent C	20 mM ammonium acetate in water
Gradient	Time (min) %B %C 0 30 5 1 30 5 14 95 5 17 95 5 18 30 5 21 30 5

3.7.2. MS global parameters

Parameter	Value
Ion source type	H-ESI
Polarity	Negative
Negative ion	2500 V
Sheath gas	50 arbitrary units
Aux gas	10 arbitrary units
Sweep gas	1 arbitrary units
Ion transfer tube temp.	325 °C
Vaporizer temp.	300 °C
Q1 resolution (FWHM*)	0.7
Q3 resolution (FWHM*)	1.2
CID gas	2 mTorr

*FWHM: Full width at half maximum

3.7.3. Optimized SRM transition parameters

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	Tube lens (V)	Source fragmentation (V)
PFBS	298.943	79.957	34	90	5
PFHxA	312.973	268.97	10.23	60	5
$^{13}C_2$-PFHxA	315	269.97	7.19	50	0
HFPO-DA	285.012	169.167	5.25	64	32.6
^{13}C-HFPO-DA	286.95	168.946	5.8	72	37.5
PFHpA	362.97	319.042	10.23	64	5
ADONA	376.97	251	10	71	5
PFHxS	398.937	79.957	39	110	5
PFOA	412.966	369.042	10.23	74	0
$^{13}C_2$-PFOA	414.962	370.03	7.78	65	0
PFNA	462.963	418.97	10.23	79	5
PFOS	498.93	79.957	47	130	26.1
$^{13}C_4$-PFOS	502.95	79.957	38.45	108	26.1
9Cl-PF3ONS	531.03	351	23.41	120	5
PFDA	512.96	469.042	10.23	84	0
$^{13}C_2$-PFDA	514.95	470.042	8.37	75	0
NMeFOSAA	569.967	418.97	18.42	116	5
d_3-NMeFOSAA	572.986	418.97	18	116	5
PFUnA	562.957	518.97	10.23	93	5
NEtFOSAA	583.983	418.97	18.34	117	5
d_5-NEtFOSAA	589.014	418.97	18	117	5
11Cl-PF3OUdS	630.958	450.833	26	120	5
PFDoA	612.954	569	10.23	95	5
PFTrDA	662.95	619.042	10.23	101	5
PFTA	712.947	668.971	10.23	105	5

4. Results and discussion

4.1. LC-MS/MS chromatograms

Figure 3 shows the chromatograms of 4 µg/L PFAS standards. The peak identification information along with the peak asymmetry factors, retention times, and internal standards are listed in Table 4. All the analytes are detected in 15 min and peak asymmetry factors are within 0.8–1.2, meeting the U.S. EPA Method 537.1 requirement.

4.2. Demonstration of low system background

A low system background needs to be demonstrated before running the samples. This is to ensure that no potential background contaminants interfere with the identification or quantitation of method analytes. The minimum reporting level (MRL) of U.S. EPA Method 537.1 for the 18 PFAS is 0.53–6.3 ng/L. The interference from solvents, reagents, containers, and SPE instrument needs to be maintained below 1/3 of the MRL value. Interference can come from contaminants of similar properties and also from the analytes that are present in many common laboratory supplies and SPE devices. The EPA method emphasizes that care must be taken with automated SPE systems to ensure that PFAS safe material used in these systems does not contribute to unacceptable analyte concentrations in the blank test.

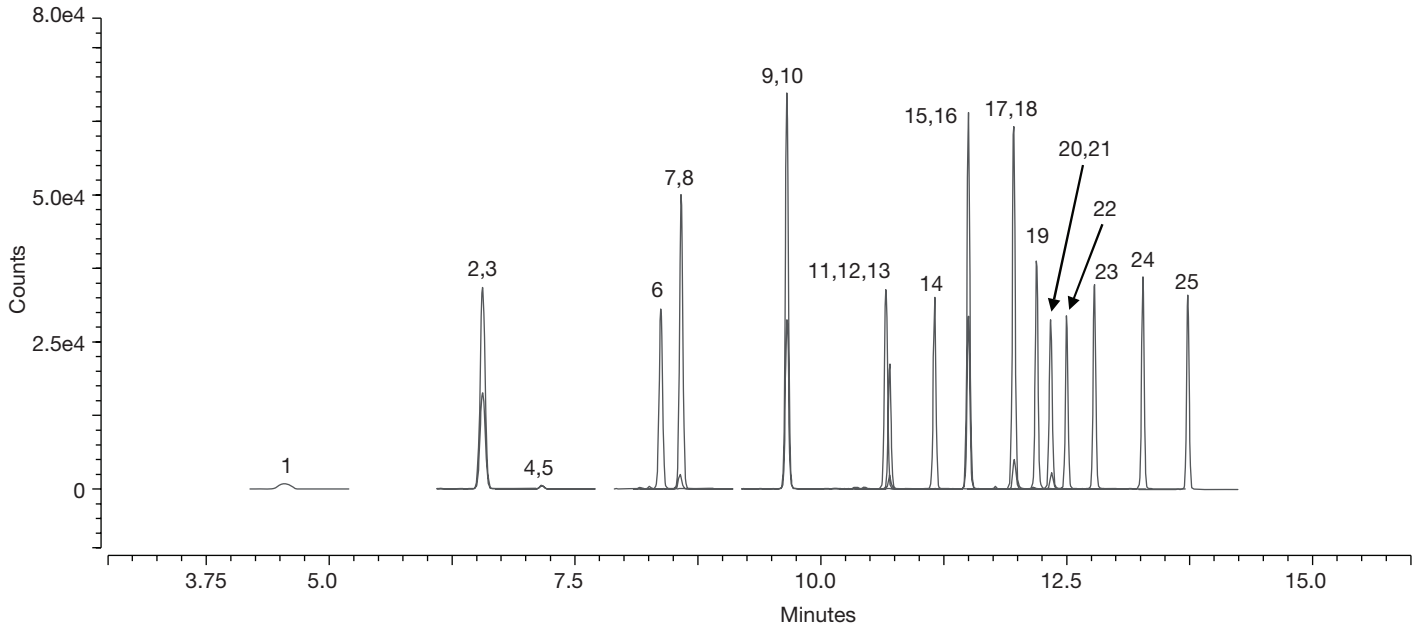

Figure 3. LC-MS/MS chromatograms of PFAS at 4 µg/L standard solution

Table 4. Retention time, asymmetry factor, and internal standards for method PFAS

Peak No.	Peak Name	Retention Time (min)	Asymmetry Factor	IS # ref
1	PFBS	4.56	1.09	$^{13}C_4$-PFOS
2	PFHxA	6.56	1.01	$^{13}C_2$-PFOA
3	$^{13}C_2$-PFHxA	6.56	0.96	$^{13}C_2$-PFOA
4	HFPO-DA	7.16	0.84	$^{13}C_2$-PFOA
5	^{13}C-HFPO-DA	7.16	0.84	$^{13}C_2$-PFOA
6	PFHpA	8.37	1.01	$^{13}C_2$-PFOA
7	ADONA	8.57	1.12	$^{13}C_4$-PFOS
8	PFHxS	8.58	0.95	$^{13}C_2$-PFOA
9	PFOA	9.65	1.06	$^{13}C_2$-PFOA
10	$^{13}C_2$-PFOA	9.66	0.98	--
11	PFNA	10.66	0.99	$^{13}C_2$-PFOA
12	PFOS	10.70	1.03	$^{13}C_4$-PFOS
13	$^{13}C_4$-PFOS	10.70	1.04	--
14	9Cl-PF3ONS	11.16	1.16	$^{13}C_4$-PFOS
15	PFDA	11.50	1.03	$^{13}C_2$-PFOA
16	$^{13}C_2$-PFDA	11.50	0.95	$^{13}C_2$-PFOA
17	NMeFOSAA	11.96	1.08	--
18	d_3-NMeFOSAA	11.97	1.05	d_3-NMeFOSAA
19	PFUnA	12.19	1.00	$^{13}C_2$-PFOA
20	NEtFOSAA	12.34	0.93	$^{13}C_2$-PFOA
21	d_5-NEtFOSAA	12.35	1.10	d_3-NMeFOSAA
22	11Cl-PF3OUdS	12.50	1.05	$^{13}C_4$-PFOS
23	PFDoA	12.78	1.07	$^{13}C_2$-PFOA
24	PFTrDA	13.27	1.01	$^{13}C_2$-PFOA
25	PFTA	13.70	0.94	$^{13}C_2$-PFOA

The AutoTrace 280 PFAS system was modified to reduce Teflon components and replace them with alternative inert materials. The LC solvent lines were modified similarly, and an isolate column was installed prior to the injection to minimize the PFAS contamination. The Sample Path Cleaning method with methanol and water should be run after each sample in the extraction process. The Sample Path Cleaning method with methanol and water should be run whenever the system has been idle for more than 24 h. The Sample Path Cleaning method can be run a second time if needed to achieve a low background.

4.3. Calibration and quantification

For the calibration curves, nine concentrations (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 50, and 100 µg/L) of standards were prepared and run. Calibration curves were created by plotting concentrations versus peak area ratios of analyte to internal standard. A linear regression or quadratic calibration curve was processed for each of the analytes with forced through zero setting as specified in U.S. EPA Method 537.1. Good fitting with the chosen model was obtained over the calibration range for all the method analytes. Figure 4 shows three typical calibration curves representing early, middle and late eluting PFAS.

4.4. The LCMRL and MDL

Lowest concentration minimum reporting level (LCMRL) is the lowest true concentration for which the future recovery is predicted to fall between 50% and 150% recovery with high confidence (99%). Detection limit (DL) is the minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. The calculated LCMRLs and DLs for each method analyte are presented in Table 5. The calculated LCMRLs ranged from 0.20 to 3.5 ng/L and the MDLs ranged from 0.30 to 2.5 ng/L.

Table 5. Calculated lowest concentration minimum reporting level and method detection limit results

Analyte	AutoTrace LCMRL (ng/L)[a]	AutoTrace DL (ng/L)[b]
PFBS	0.30	0.59
PFHxA	0.63	0.44
HFPO-DA	2.2	1.8
PFHpA	0.38	0.42
PFHxS	0.68	0.49
ADONA	0.20	0.30
PFOA	0.59	0.41
PFNA	0.23	0.38
PFOS	0.89	1.2
9Cl-PF3ONS	1.1	0.77
PFDA	0.72	0.75
PFUnA	1.2	0.79
NMeFOSAA	1.5	1.1
11Cl-PF3OUdS	2.1	0.62
NEtFOSAA	3.5	2.5
PFDoA	1.6	0.99
PFTrA	2.6	0.71
PFTA	2.5	0.86

[a]LCMRL were calculated according to the procedure in reference 1
[b]Detection limits were determined by analyzing seven replicates according to "Sample preparation" section, MDL= s × $t_{(n-1,\ 1-\alpha\ =\ 0.99)}$

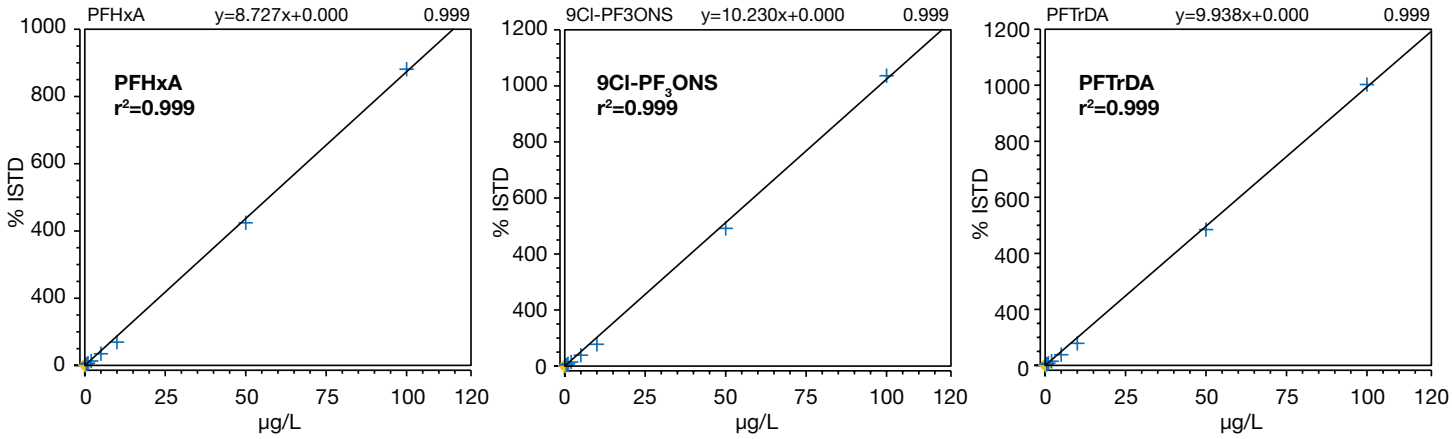

Figure 4. Typical calibration curves for PFAS

4.5. Method precision and accuracy

Precision and accuracy were evaluated to determine the method's extraction efficiency for PFAS determinations in drinking water samples. Two fortified concentration levels (16 ng/L and 80 ng/L) were analyzed to measure recovery and evaluate accuracy. At each concentration level, six replicate fortified samples were preserved, prepared, extracted, evaporated and reconstituted, and analyzed by the method.

The precision and accuracy results of the method are presented in Table 6. At both 16.0 ng/L and 80.0 ng/L fortified concentration levels, all recoveries were within the acceptable range of 70–130% according to U.S. EPA Method 537.1, ranging from 84.1% to 123%. The calculated relative standard deviations (RSD) were all less than 10%, suggesting good precision.

5. Conclusions

This application note reports a method that can be used for the extraction and determination of 18 PFAS in drinking water with a PFAS-safe AutoTrace 280 extraction system and LC-MS/MS. The modified AutoTrace 280 extraction system ensures inertness and prevents PFAS from leaching into sample during extraction, while at same time delivering consistent and reliable performance. Both sample path cleaning in SPE and separation method precaution for the LC system maintained a low system background, meeting the EPA method requirement. The calculated LCMRLs ranged from 0.20 to 3.5 ng/L and the MDLs ranged from 0.30 to 2.5 ng/L, which were below or comparable to those values reported in U.S. EPA Method 537.1. At both 16.0 ng/L and 80.0 ng/L fortified concentration levels, all the recoveries were within the acceptable range of 70–130%. The calculated RSDs were all less than 10%, suggesting good precision. Thermo Scientific LC-MS/MS with the automatic extraction AutoTrace 280 PFAS system demonstrated an efficient, reliable, and sensitive method to fulfill the requirements of U.S. EPA Method 537.1.

Table 6. Precision and accuracy (n=6) of PFAS in fortified drinking water

Analyte	Fortified conc. (ng/L)	Mean recovery (%)	RSD (%)	Fortified conc. (ng/L)	Mean recovery (%)	RSD (%)
PFBS	16.0	107	3.3	80.0	98.3	3.6
PFHxA	16.0	108	2.3	80.0	106	2.6
HFPO-DA	16.0	84.1	7.5	80.0	88.6	6.3
PFHpA	16.0	113	2.7	80.0	117	1.3
PFHxS	16.0	120	3.4	80.0	123	2.1
ADONA	16.0	117	2.5	80.0	121	1.1
PFOA	16.0	113	2.5	80.0	119	1.6
PFNA	16.0	114	2.9	80.0	118	2.1
PFOS	16.0	113	4.5	80.0	117	2.9
9Cl-PF3ONS	16.0	96.1	4.1	80.0	103	2.6
PFDA	16.0	105	3.2	80.0	111	2.1
PFUnA	16.0	96.8	5.0	80.0	103	3.1
NMeFOSAA	16.0	103	5.2	80.0	110	5.2
11Cl-PF3OUdS	16.0	88.5	5.5	80.0	97.1	4.8
NEtFOSAA	16.0	100	9.9	80.0	104	2.3
PFDoA	16.0	89.8	4.4	80.0	97.3	3.4
PFTrA	16.0	89.6	3.8	80.0	95.8	3.7
PFTA	16.0	89.0	4.8	80.0	98.1	3.3

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6. Acknowledgements

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