DETERMINATION OF PER- AND POLYFLUORINATED ALKYL SUBSTANCES (PFAS) IN DRINKING WATER USING AUTOMATED SOLID-PHASE EXTRACTION AND LC-MS/MS

GOAL

TO DEMONSTRATE AN EFFICIENT AND RELIABLE SOLID-PHASE EXTRACTION METHOD WITH THE THERMO SCIENTIFIC™ DIONEX™ AUTOTRACE™ 280 INSTRUMENT FOR THE DETERMINATION OF PER- AND POLY-FLUORINATED COMPOUNDS IN DRINKING WATER PER U.S. EPA METHOD 537.1.

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 United States 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.

This article will discuss the development of an analytical method using an automated SPE system, AutoTrace 280, and LC-MS/MS for the determination of 18 PFAS following the guidelines provided by U.S. EPA Method 537.1.

The method discussed in this article demonstrated that the AutoTrace 280 system provides reliable automated SPE for determination of PFAS in large-volume (20 mL–4 L) aqueous samples.

Experimental

Instruments

- Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS System
- Thermo Scientific™ Vanquish™ Flex Duo UHPLC system, fitted with Thermo Scientific™ PFC free kit

- Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer
- Organomation Associates™ 12 Position N-EVAP Nitrogen Evaporator

* For information on reagents, standards and consumables, please refer to reference 5.

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 Surrogate Primary Dilution Standard (SUR PDS) were added prior to SPE extraction. After extraction with the AutoTrace 280 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 Internal Standard Primary Dilution Standard (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.

Sample preparation

Reagent water - Water that does not contain any measurable quantities of method analytes or interfering compounds greater than 1/3 the minimum reporting level (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 article.

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 1).

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 2) 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.

AutoTrace 280 sample extraction

The AutoTrace 280 system was modified to reduce Teflon™ components and replace with alternative inert materials. Historically, the solvent side lines of the AutoTrace 280 system were used for the condition, dry, and elute functions and the sample side lines were used for sample loading and rinsing. The line function per the U. S. EPA Method 537.1 requirement was modified in the method discussed. 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 sample extraction.

Create methods in the AutoTrace 280 SPE workstation software

The AutoTrace 280 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 instrument from the software provided with the system and run sequentially.

* For information on the three methods and solvent used, please refer to reference 5.

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 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

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

Table 1. 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

Table 2. Preparation of the fortified samples for the LCMRL test

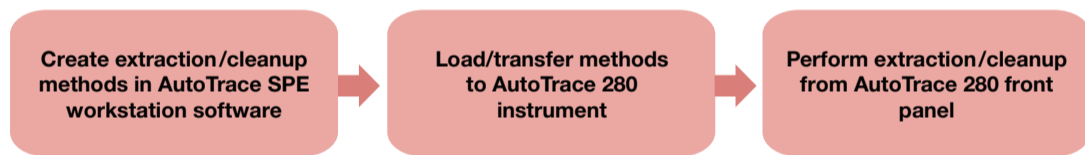| Fortified conc. (ng/L)	DI water with Trizma (mL)	Analyte stock conc. (µg/L)	Volume stock solution (µL)	Surrogate standard. PDS (µL)
32	250	100	80	10
8	250	100	20	10
4	250	100	10	10
2	250	10	50	10
0.8	250	10	20	10
0.4	250	10	10	10
0.2	250	10	5	10

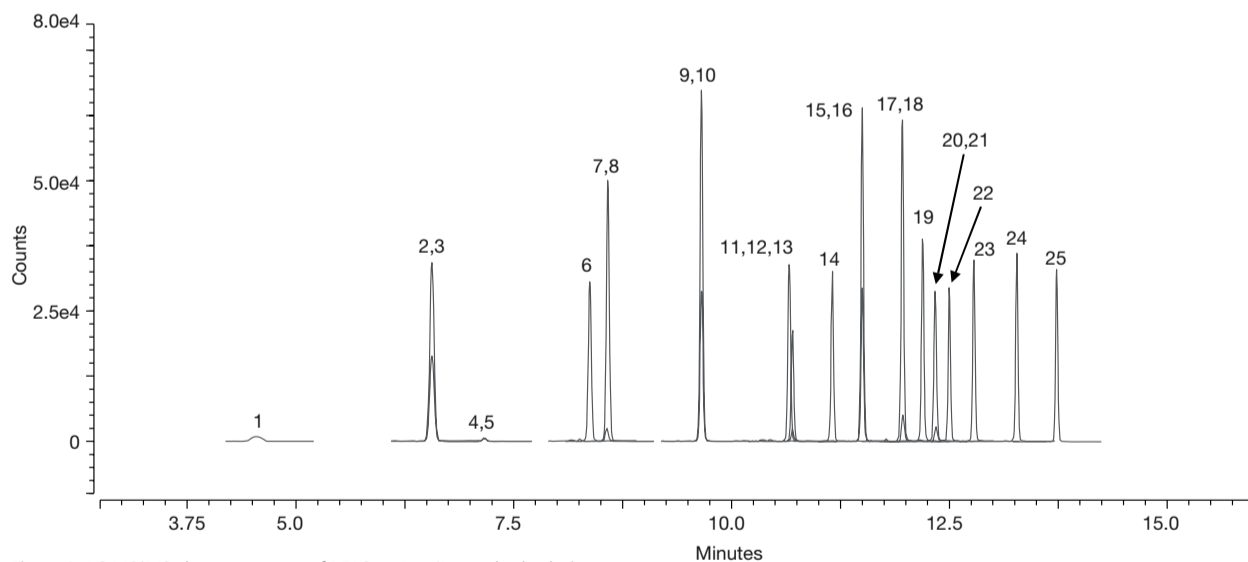Figure 2. General guideline for AutoTrace 280 sample extraction

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

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.

For information on LC conditions, please refer to reference 5.

Results and discussion

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 3. All the analytes are detected in 15 minutes and peak asymmetry factors are within 0.8– 1.2, meeting the U.S. EPA Method 537.1 requirement.

Demonstration of low system background

To ensure that no potential background contaminants interfere with the identification or quantitation of method analytes, a low system background needs to be demonstrated before running the samples. 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.

The AutoTrace 280 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.

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.

The LCMRL and MDL
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 4. The calculated LCMRLs ranged from 0.20 to 3.5 ng/L and the MDLs ranged from 0.30 to 2.5 ng/L.

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 (16.0 ng/L and 80.0 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 5. 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.

Conclusions
The results demonstrated that the method described 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 system demonstrated an efficient, reliable, and sensitive method to fulfill the requirements of U.S. EPA Method 537.1.

Table 3. Retention time, asymmetry factor, and internal standards for method PFAS

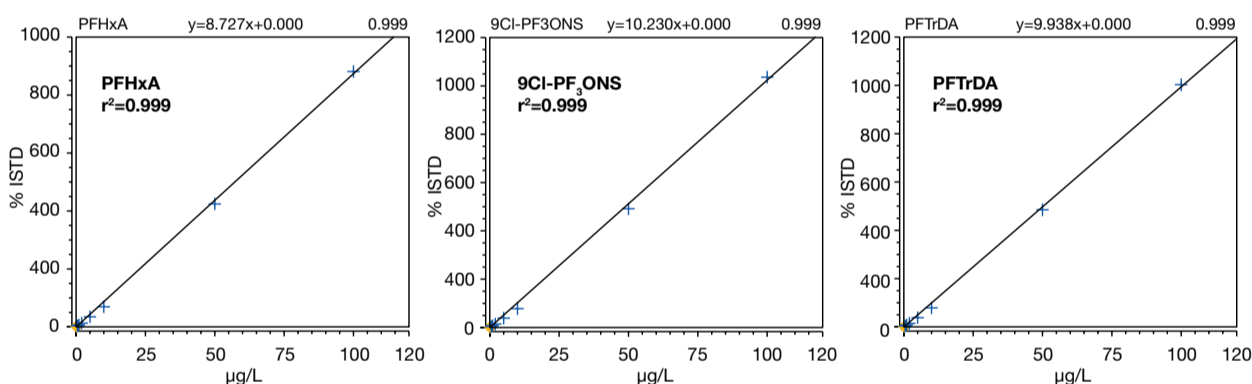| Peak No.	Peak Name	Retention Time (min)	Asymmetry Factor	IS # ref
1	PFBS	4.56	1.09	$^{13}C_4$-PFOS
2	PFHxA	6.56	1.01	$^{13}C_2$-PFOA
3	$^{13}C_2$-PFHxA	6.56	0.96	$^{13}C_2$-PFOA
4	HFPO-DA	7.16	0.84	$^{13}C_2$-PFOA
5	^{13}C-HFPO-DA	7.16	0.84	$^{13}C_2$-PFOA
6	PFHpA	8.37	1.01	$^{13}C_2$-PFOA
7	ADONA	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

Figure 4. Typical calibration curves for PFAS

Table 5. 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

Table 4. 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

References

1. U.S. EPA. Basic Information about Per- and Polyfluoroalkyl Substances (PFAS).

2. National Institute of Health, National Institute of Environmental Health Sciences, Polyfluoroalkyl Substances (PFAS).

3. U.S. EPA. Drinking Water Health Advisories for PFOA and PFOS.

4. U.S. EPA. Method 537.1, Detections of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Version 1.0, November 2018

5. Thermo Fisher Scientific, Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water using automated solid-phase extraction and LC-MS/MS, AN 73346-EN 0220S

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