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Targeted Quantitation of Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in Water Using the Agilent 6470 Triple Quadrupole LC/MS System

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Abstract

The presence of per- and polyfluoroalkyl substances (PFAS) in drinking water is of emerging concern globally due to their widespread usage, environmental persistence, and bio-accumulative tendency.[1] Therefore, it is important that analytical methods are accurate and reliable to facilitate the screening and quantitation of multiple PFAS in water matrices. A comprehensive workflow using solid phase extraction and LC/MS/MS was developed for the analysis of more than 100 native and isotopically labeled PFAS in water, with the intention of accelerating and simplifying routine laboratory testing. Compound separation was achieved on an Agilent 1290 Infinity II LC System equipped with a PFC-Free HPLC Conversion Kit and analyzed with the Agilent 6470 Triple Quadrupole LC/MS. The MRM transitions and optimized MS parameters of the analytes were easily and quickly exported from the Agilent PFAS MRM Database for LC/TQ to build the acquisition method. A solid phase extraction (SPE) protocol using an Agilent weak anion exchange cartridge was developed for the extraction of the analytes from the water matrices. Method detection limits were determined using ultrapure water samples and ranged from 0.14 to 14 ng/L for 60 PFAS. The interbatch precision and recovery for these 60 analytes in drinking water were within the acceptable limits of 2.2 to 16.7% RSD and 76 to 119%, respectively. The interbatch precision for 60 PFAS in surface water ranged from 1.6 to 19.9% RSD with recovery of 72 to 120%. This confirmed the method applicability for a routine and more comprehensive analysis to allow an expanded scope of PFAS testing in these two water matrices.

The method described in this application note is available as an electronic eMethod: *PFAS in Drinking and Surface Water by LC/TQ (G5285AA)*. The eMethod includes a comprehensive step-by-step workflow guide, ready to run acquisition and quantitation methods, and detailed ordering information to facilitate implementation of new PFAS analysis workflow.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals widely used in consumer products and industrial processes due to their unique and desirable chemical properties. Because of their widespread usage, environmental persistence and bio-accumulative nature, legacy PFAS are ubiquitous in the environment and new fluorochemicals are being found in the environment frequently.[1] With increasing evidence of environmental and human health impact associated with PFAS, public awareness of these chemicals is high and environmental groups have been pressing for the removal of these contaminants from drinking water and water supplies.[2] Currently, there are various standard methods such as the USA EPA Methods 537.1 and 533 for drinking water, and USA EPA Method 8327, ASTM 7979, and ISO methods for nonpotable waters. Typically, these methods require analysis of up to 30 compounds but due to rapidly evolving regulatory initiatives across various regions and countries, they are expected to change over time with the introduction of more target analytes. This makes it challenging for laboratories to stay current with their PFAS analysis and requires frequent method protocol updates.

Due to its high sensitivity and specificity, triple quadrupole LC/MS (LC/MS/MS) is the most widely used technique for PFAS analysis and quantitation. SPE is the most common sample cleanup approach for extracting PFAS from water matrices, as demonstrated in several standard methods, including the US EPA drinking water methods and ISO 21675. In this application note, a comprehensive method was developed for the accurate and reliable analysis of more than

100 native and isotopically labeled PFAS in drinking water and surface water using an Agilent 1290 Infinity II LC interfaced with a 6470 Triple Quadrupole LC/MS. To simplify implementation and reduce method development requirements, the eMethod includes a comprehensive guide for sample extraction, the chromatographic separation, and MS detection including electronic methods, as well as details about targeted quantitation and data processing.

Experimental

Reagents and standards

Native and isotopically labeled PFAS analytical standards were purchased as individual stock solutions, solution mixes, or powdered standards from Wellington Laboratories Inc. (Guelph, ON, Canada) and Toronto Research Chemicals (Toronto, ON, Canada). LC/MS grade methanol, ammonium acetate (LC/MS grade), glacial acetic acid, and ammonium hydroxide (28% ammonia in water, ≥99.99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-Propanol was purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q water system.

Calibration standards

The stock solutions and solution mixes of the native PFAS were combined to prepare a calibration mix in methanol such that the concentrations are 250 to 2,500 ng/mL per PFAS. A surrogate mix was prepared in methanol by combining stock solutions and solution mixes of mainly isotopically labeled PFAS at 250 to 2,000 ng/mL. An isotope performance standard mix consisting of three isotopically labeled PFAS at 500 or 1,500 ng/mL, was prepared in methanol containing 1 mM sodium hydroxide.

The calibration mix was diluted with 80/20 methanol/water to prepare calibration standards with concentrations ranging from 0.04 to 100 ng/mL for PFPAs, MeFBSA, MeFOSE, and EtFOSE; 0.1 to 250 ng/mL for n:2 FTCAs; and 0.01 to 25 ng/mL for all other PFAS. The surrogate mix was added at a constant amount to each calibration standard so the final concentration of each surrogate is 20 ng/mL for $^2H_7$-MeFOSE, $^2H_9$-EtFOSE, and Cl-PFOPA; 40 ng/mL for $^{13}C_2$-n:2 FTCAs; and 5 ng/mL for all other labeled PFAS. The isotope performance standard mix was also added at a constant amount to each calibration standard so that the final concentration of each labeled PFAS is 5 or 15 ng/mL.

Some of the standards such as the PFSAs, DONA, diPAPs, PFESAs, FTSAs, PFPiAs, and diSAmPAP were purchased in their salt forms. Therefore, the concentrations of these analytes are reported as free acids (anions).

Method detection limit

The method detection limits (MDLs) were calculated based on the procedure described in 40 CFR Part 136 Appendix Revision 2.[3] Briefly, seven replicates of 250 mL of ultrapure water were spiked with a PFAS spike mix solution containing native PFAS at 1 to 25 ng/L. Surrogate mix was added to these samples and they were extracted using the SPE protocol described in the next section and analyzed over three separate days. MDL was calculated using the formula below:[3]

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

where

s = standard deviation of the replicate spiked sample analyses

$t_{(n-1,\ 1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates

Sample preparation

Each of the 250 mL of water sample was collected in polypropylene bottles and its pH was adjusted to approximately three by adding 2.5 mL of glacial acetic acid. After this, the sample was spiked with the surrogate mix at concentrations of 20, 80, and 160 ng/L to match the expected concentrations of 5, 20, and 40 ng/mL in the final extract. To prepare matrix spike samples, an appropriate amount of native PFAS spike mix solution was added at two concentration levels by referencing the approach used in EPA Method 533,[4] namely low spike at 5 to 50 ng/L and high spike at 20 to 200 ng/L. Unspiked matrix samples (matrix blank) were prepared by omitting the addition of PFAS spike mix solution. SPE was performed using Agilent SampliQ Weak Anion Exchange (WAX), 6 mL, 150 mg cartridges (part number 5982-3667), which were conditioned with 4 mL of 0.1% ammonia in methanol, 4 mL of methanol, 4 mL of water, and 3 mL of 1% acetic acid in water. The water samples were loaded onto the cartridges under vacuum at approximately 2 to 3 mL/min. The cartridges were washed with 4 mL of 25 mM acetate buffer (pH 4) followed by 4 mL of water, and dried under high vacuum for 10 minutes. The analytes were eluted from the cartridges using 4 mL of methanol followed by 4 mL of 0.1% ammonia in methanol. The eluates were concentrated to 0.5 mL under a gentle stream of nitrogen gas in a water bath at 30 to 35 °C. The isotope performance standard mix was added to the concentrated extract and its volume was adjusted with methanol and water to 1 mL to obtain a methanol to water ratio of 80/20. This results in a sample concentration factor of 250-fold. The sample preparation is summarized in Figure 1.

Instrumentation

Chromatographic separation was achieved using an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column (part number 959758-902) installed on an Agilent 1290 Infinity II UHPLC system consisting of the following modules:

– Agilent 1290 Infinity II High Speed Pump (G7120A)

– Agilent 1290 Infinity II Multisampler with Multiwash Option (G7167B)

– Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)

A 12-minute gradient elution was performed with 5 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B) at 0.4 mL/min with a total run time of approximately 18 minutes (injection to injection). To minimize background PFAS contamination, the Agilent PFC-Free HPLC Conversion Kit
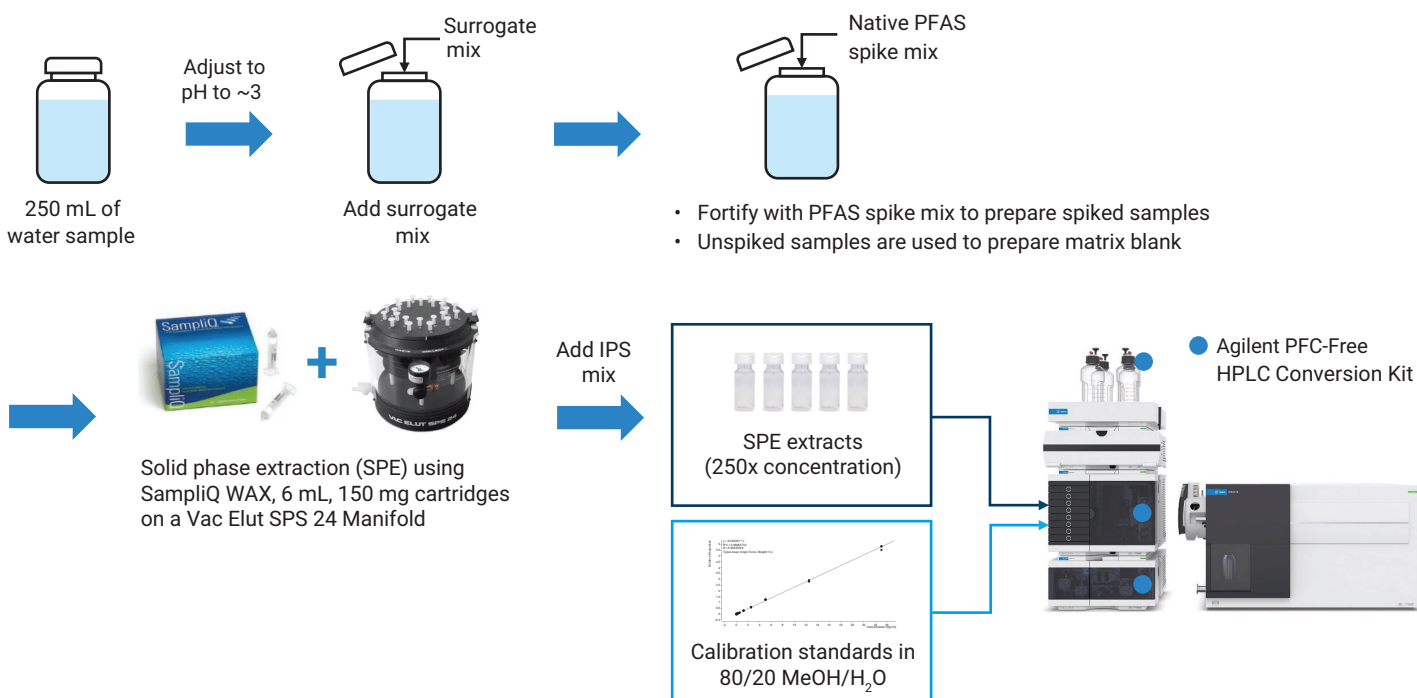

Figure 1. Flowchart of solid phase extraction protocol using Agilent SampliQ WAX cartridges.

(part number 5004-0006) was installed on the UHPLC system. This easy-to-install conversion kit includes substitutes for all critical LC system parts containing organic fluorine compounds and a newly developed PFC delay column (part number 5062-8100) for delaying potential per- or polyfluorochemicals impurities from the mobile phases (Figure 1).

Dynamic MRM (dMRM) analysis was performed using a 6470 LC/TQ with an Agilent Jet Stream (AJS) ion source operated in negative ionization mode. The LC/TQ autotune was performed in unit mode with report m/z <100 mode enabled. Data acquisition and processing were performed using Agilent MassHunter Data LC/MS Acquisition software version 10.1 and Quantitative Analysis software version 10.2, respectively.

Results and discussion

Agilent PFAS MRM database for triple quadrupole LC/MS

The Agilent PFAS MRM Database (part number G1736AA) is a curated database that allows for the customization of MRM sub-methods based on a target list of interest or standard methods, and includes:

– Intrinsic properties and identifiers such as compound name, molecular formula, and CAS number.

– Optimized MRM parameter settings for the acquisition of 72 native and 36 isotopically labeled analytes from 14 PFAS groups for all current Agilent LC/TQ models (Figure 2A). These analytes include those that are listed in regulations such as the European

Drinking Water Directive; standard methods from the EPA , ASTM, and ISO; and emerging PFAS compounds.

– Retention time information derived from an optimized chromatographic method (Figure 2B).

In this study, the MRM transitions and optimized MS parameters of all 108 analytes were exported from this database using the MassHunter LC/MS Data Acquisition software to create the acquisition method. The method was set up for the analysis of 71 native PFAS analytes, 33 labeled PFAS, and one native PFAS, which were used as surrogates for isotope dilution or internal standard quantitation of the native PFAS. Three labeled PFAS were used as internal standards for calculating the surrogate recoveries.

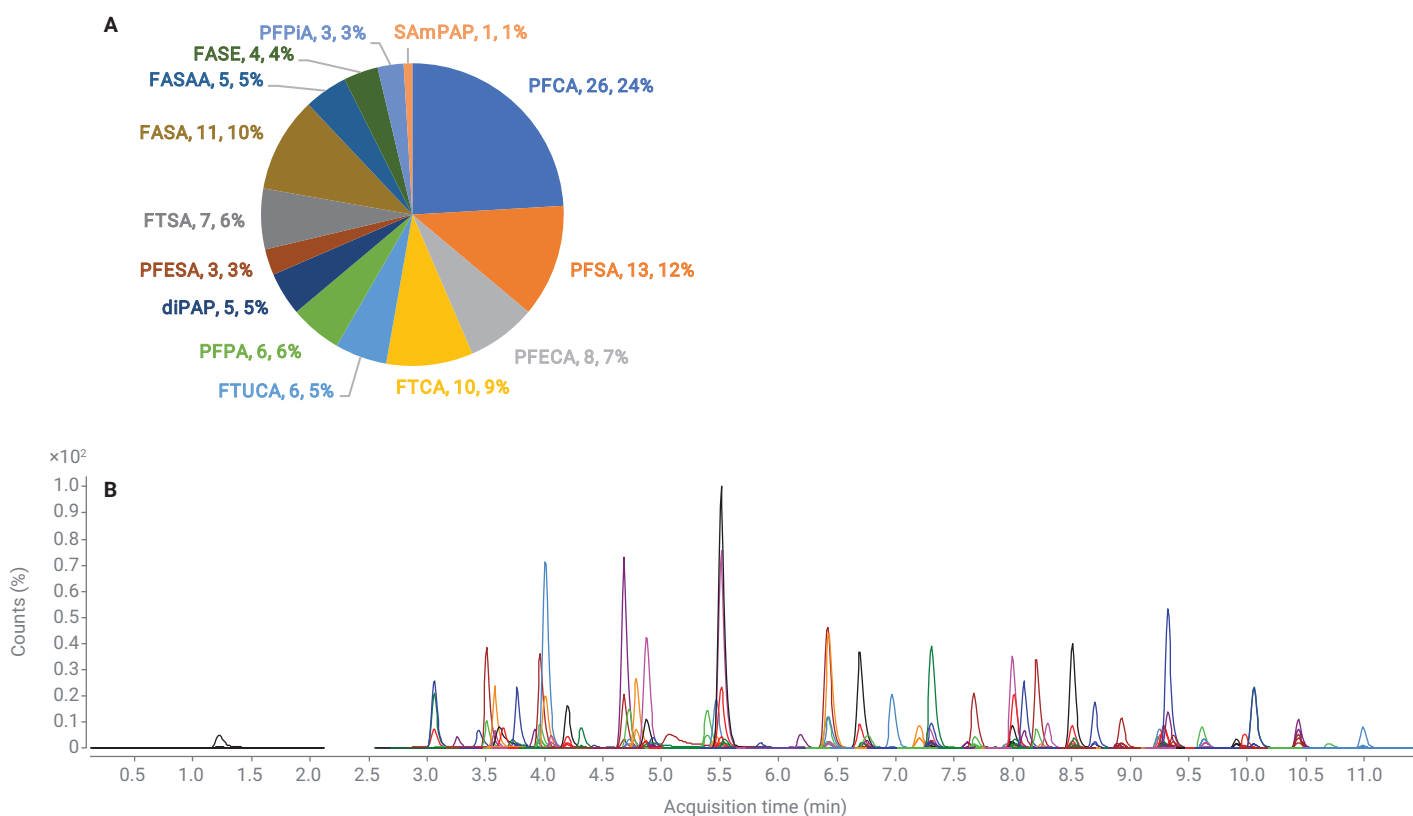**Figure 2.** (A) Classification of the PFAS compounds in the database (denoted by group, number of PFAS and % of total PFAS). (B) MRM chromatogram of 108 native and isotopically labeled PFAS in the SPE extract of a drinking water sample spiked at 5 to 50 ng/L. The symmetric sharp peaks for the majority of the analytes demonstrated the efficient chromatographic separation of the analytes within the retention time window.

Analytical range and accuracy

For each PFAS except FTSAs, the calibration curve was generated using linear regression by forcing it through the origin with 1/x weighting. For FTSAs, quadratic regression was used. All 71 analytes demonstrated a wide analytical range of at least three orders of magnitude with good linear or quadratic fit of $R^2 \geq 0.99$ (Figure 3 and Table 1). For all analytes, the accuracies of the calibration standards included in the calibration curves were within the typical acceptable limits of 70 to 130% with precision of ≤20% RSD.

Background interference

In this study, the use of the Agilent PFC-Free HPLC Conversion Kit effectively reduced the background PFAS contamination as the routine analyses of instrument blank (gradient program with no injection) and solvent blanks (80/20 methanol/water) had no detectable PFAS peaks. In addition, evidence of low system background is demonstrated by injecting a laboratory reagent blank (LRB) immediately after the highest calibration standard. The LRB is prepared from 250 mL of ultrapure water spiked with surrogate mix and processed using the same SPE protocol as the matrix blank samples. Trace levels of a few PFAS were seen in the LRB, but their concentrations were all below MDLs. Thus, demonstrating that there was minimal contamination from lab equipment, reagents, glassware, or extraction apparatus.
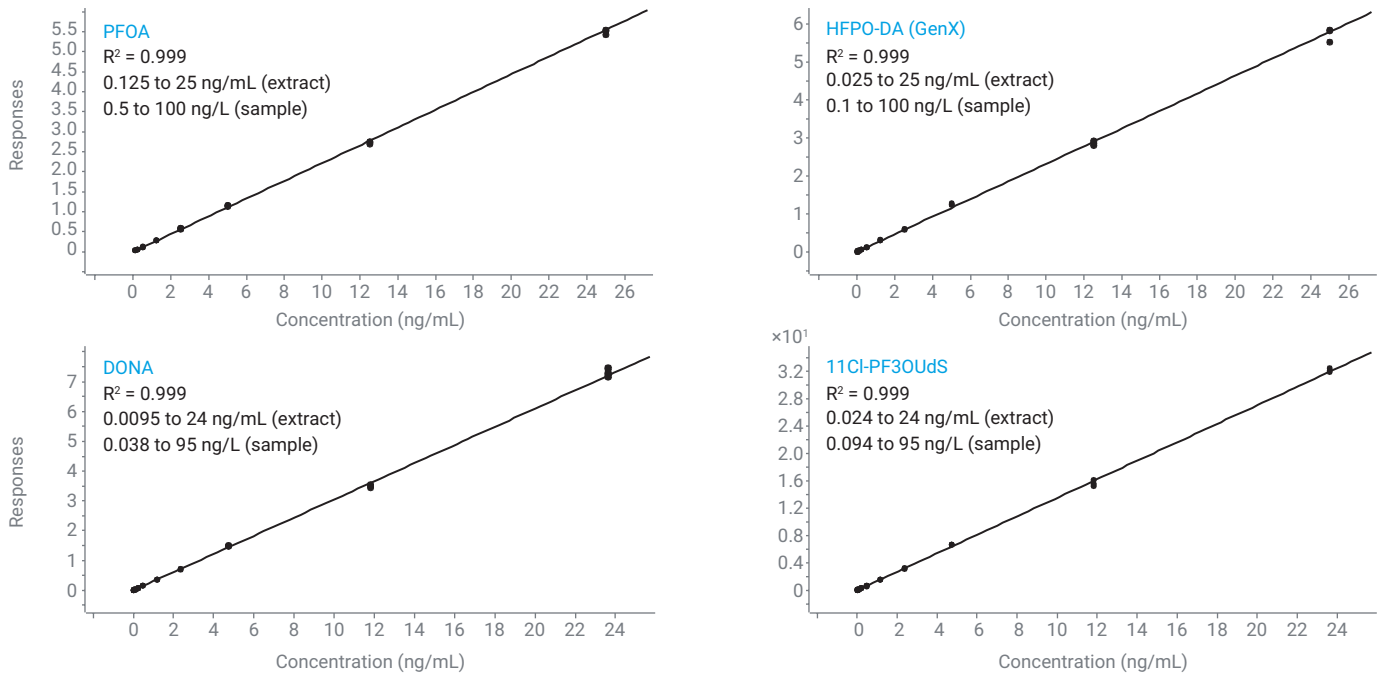

Figure 3. Linear calibration curves for four of the PFAS (3 injections per calibration level).

Table 1. Summary of the method performance results including MDLs, calibration analytical range, method precision, and method recovery. The method precision and recovery were based on results from interbatch analyses of low spike drinking water and low spike surface water samples (8 replicates per matrix).

No.	Compound	PFAS Group	CAS number	Surrogate	MDL (ng/L)	Calibration analytical range (ng/L)	Low Spike Drinking Water		Low Spike Surface Water	
							Precision (%RSD)	Recovery (%)	Precision (%RSD)	Recovery (%)
1	PFBA	PFCA	375-22-4	$^{13}C_4$-PFBA	0.24	0.2 to 100	7.5	102	8.6	103
2	PFPeA	PFCA	2706-90-3	$^{13}C_5$-PFPeA	0.24	0.5 to 100	4.8	98	7.9	100
3	PFHxA	PFCA	307-24-4	$^{13}C_5$-PFHxA	0.35	1 to 100	3.3	103	8.5	104
4	PFHpA	PFCA	375-85-9	$^{13}C_4$-PFHpA	0.30	0.2 to 100	3.2	102	8.9	107
5	PFOA	PFCA	335-67-1	$^{13}C_8$-PFOA	0.38	0.5 to 100	3.9	97	6.4	106
6	PFNA	PFCA	375-95-1	$^{13}C_9$-PFNA	0.29	0.2 to 100	4.7	104	8.7	105
7	PFDA	PFCA	335-76-2	$^{13}C_6$-PFDA	0.28	0.1 to 100	5.2	104	8.3	107
8	PFUnDA	PFCA	2058-94-8	$^{13}C_7$-PFUnDA	0.30	0.1 to 100	8.1	102	9.9	108
9	PFDoDA	PFCA	307-55-1	$^{13}C_2$-PFDoDA	0.32	0.2 to 100	5.0	104	7.0	107
10	PFTrDA	PFCA	72629-94-8	$^{13}C_2$-PFDoDA	0.26	0.2 to 100	8.2	95	9.1	94
11	PFTDA	PFCA	376-06-7	$^{13}C_2$-PFTDA	0.28	0.2 to 100	4.5	104	11.5	105
12	PFHxDA	PFCA	67905-19-5	$^{13}C_2$-PFHxDA	2.1	0.5 to 100	4.2	98	8.2	102
13	PFODA	PFCA	16517-11-6	$^{13}C_2$-PFHxDA	N.D.	0.1 to 100	35.1	14	53.1	73
14	PFBS	PFSA	375-73-5	$^{13}C_3$-PFBS	0.21	0.088 to 89	4.7	106	11.4	111
15	PFPeS	PFSA	2706-91-4	$^{13}C_3$-PFHxS	0.35	0.094 to 94	5.3	96	8.0	99
16	PFHxS	PFSA	355-46-4	$^{13}C_3$-PFHxS	0.27	0.18 to 91	5.1	103	7.1	106
17	PFHpS	PFSA	375-92-8	$^{13}C_8$-PFOS	0.52	0.095 to 95	8.2	97	6.4	97
18	PFOS	PFSA	1763-23-1	$^{13}C_8$-PFOS	0.27	0.19 to 93	6.9	100	5.7	100
19	PFNS	PFSA	68259-12-1	$^{13}C_8$-PFOS	0.50	0.096 to 96	7.9	100	4.0	99
20	PFDS	PFSA	335-77-3	$^{13}C_8$-PFOS	0.54	0.19 to 96	10.0	95	3.9	92
21	PFDoS	PFSA	79780-39-5	$^{13}C_8$-PFOS	0.50	0.48 to 97	14.7	77	17.4	66
22	4-PFecHS	PFSA	646-83-3	$^{13}C_8$-PFOS	0.40	0.037 to 92	7.4	90	7.9	89
23	HFPO-DA	PFECA	13252-13-6	$^{13}C_3$-HFPO-DA	0.24	0.1 to 100	7.4	103	7.7	103
24	HFPO-TA	PFECA	13252-14-7	$^{13}C_9$-PFNA	0.26	0.095 to 95	4.2	86	3.6	84
25	DONA	PFECA	919005-14-4	$^{13}C_4$-PFHpA	0.23	0.038 to 95	6.4	101	8.9	102
26	PFMPA	PFECA	377-73-1	$^{13}C_4$-PFBA	0.22	0.1 to 100	9.8	97	10.4	104
27	NFDHA	PFECA	151772-58-6	$^{13}C_5$-PFHxA	0.25	0.1 to 100	5.4	95	8.7	100
28	PFMBA	PFECA	863090-89-5	$^{13}C_5$-PFPeA	0.19	0.1 to 100	3.3	115	5.3	110
29	P5MeODIOXOAc	PFECA	1190931-41-9	$^{13}C_3$-HFPO-DA	0.27	0.5 to 100	6.3	107	9.4	114
30	6:2 FTCA	FTCA	53826-12-3	$^{13}C_2$-6:2 FTCA	3.2	5 to 1000	8.6	116	14.4	113
31	8:2 FTCA	FTCA	27854-31-5	$^{13}C_2$-8:2 FTCA	5.8	10 to 1000	9.9	104	15.8	100
32	10:2 FTCA	FTCA	53826-13-4	$^{13}C_2$-10:2 FTCA	14	10 to 1000	12.5	106	19.9	115
33	3:3 FTCA	FTCA	356-02-5	$^{13}C_5$-PFPeA	0.60	0.5 to 100	10.4	100	9.9	91
34	5:3 FTCA	FTCA	914637-49-3	$^{13}C_2$-6:2 FTUCA	0.45	0.2 to 100	3.6	86	4.1	97
35	7:3 FTCA	FTCA	812-70-4	$^{13}C_2$-8:2 FTUCA	0.39	0.2 to 100	4.7	80	4.2	100
36	8:3 FTCA	FTCA	34598-33-9	$^{13}C_6$-PFDA	N.D.	0.2 to 50	8.1	55	6.6	73
37	6:2 FTUCA	FTUCA	70887-88-6	$^{13}C_2$-6:2 FTUCA	0.23	0.2 to 100	6.1	110	3.6	111
38	8:2 FTUCA	FTUCA	70887-84-2	$^{13}C_2$-8:2 FTUCA	0.19	0.2 to 100	8.2	110	3.0	111
39	10:2 FTUCA	FTUCA	70887-94-4	$^{13}C_2$-10:2 FTUCA	0.24	0.2 to 100	9.7	111	4.1	109
40	PFBPA	PFPA	52299-24-8	Cl-PFOPA	N.D.	0.8 to 400	43.6	108	10.9	68
41	PFHxPA	PFPA	40143-76-8	Cl-PFOPA	N.D.	2 to 400	27.3	221	9.6	177
42	PFOPA	PFPA	40143-78-0	Cl-PFOPA	N.D.	2 to 400	7.1	134	9.4	148
43	PFDPA	PFPA	52299-26-0	Cl-PFOPA	N.D.	4 to 400	13.7	54	12.9	65

No.	Compound	PFAS Group	CAS number	Surrogate	MDL (ng/L)	Calibration analytical range (ng/L)	Low Spike Drinking Water		Low Spike Surface Water	
							Precision (%RSD)	Recovery (%)	Precision (%RSD)	Recovery (%)
44	Cl-PFHxPA	PFPA	N/A	Cl-PFOPA	N.D.	2 to 400	15.6	151	9.2	112
45	6:2 diPAP	diPAP	57677-95-9	$(^{13}C_2)_2$-6:2 diPAP	0.38	0.19 to 97	4.7	103	6.0	103
46	6:2/8:2 diPAP	diPAP	943913-15-3	$(^{13}C_2)_2$-6:2 diPAP	N.D.	0.2 to 98	27.1	73	7.3	56
47	8:2 diPAP	diPAP	678-41-1	$(^{13}C_2)_2$-8:2 diPAP	0.67	0.2 to 98	5.9	110	8.0	110
48	PFEESA	PFESA	113507-82-7	$^{13}C_3$-PFBS	0.15	0.089 to 89	2.9	102	7.2	105
49	9Cl-PF3ONS	PFESA	756426-58-1	$^{13}C_8$-PFOS	0.26	0.094 to 94	7.3	94	7.2	95
50	11Cl-PF3OUdS	PFESA	763051-92-9	$^{13}C_8$-PFOS	0.25	0.094 to 95	9.9	87	9.6	67
51	4:2 FTSA	FTSA	757124-72-4	$^{13}C_2$-4:2 FTSA	0.24	0.19 to 93	3.9	105	4.7	108
52	6:2 FTSA	FTSA	27619-97-2	$^{13}C_2$-6:2 FTSA	0.23	0.19 to 95	3.7	101	6.0	107
53	8:2 FTSA	FTSA	39108-34-4	$^{13}C_2$-8:2 FTSA	0.28	0.19 to 96	4.8	103	4.3	107
54	10:2 FTSA	FTSA	120226-60-0	$^{13}C_2$-8:2 FTSA	0.54	0.19 to 96	8.4	85	12.0	87
55	FBSA	FASA	30334-69-1	$^{13}C_3$-PFHxS	0.39	0.2 to 100	11.2	90	3.8	94
56	FHxSA	FASA	41997-13-1	$^{13}C_8$-PFOS	0.38	0.1 to 100	13.1	90	5.6	92
57	PFOSA	FASA	754-91-6	$^{13}C_8$-PFOSA	0.14	0.1 to 100	8.4	111	3.8	110
58	FDSA	FASA	N/A	$^{13}C_8$-PFOSA	0.21	0.2 to 100	9.8	95	7.4	86
59	MeFBSA	FASA	68298-12-4	$^{13}C_8$-PFOSA	0.69	0.8 to 400	5.1	91	7.6	88
60	MeFHxSA	FASA	68259-15-4	$^{13}C_8$-PFOSA	0.31	0.45 to 90	10.7	76	9.6	74
61	N-MeFOSA	FASA	31506-32-8	$^2H_3$-N-MeFOSA	1.0	0.2 to 100	6.5	104	5.3	113
62	N-EtFOSA	FASA	4151-50-2	$^2H_5$-N-EtFOSA	1.0	0.5 to 100	6.5	106	5.7	106
63	FOSAA	FASAA	2806-24-8	$^2H_3$-N-MeFOSAA	N.D.	0.2 to 100	19.4	48	17.5	72
64	N-MeFOSAA	FASAA	2355-31-9	$^2H_3$-N-MeFOSAA	0.28	0.2 to 100	4.7	102	8.7	103
65	N-EtFOSAA	FASAA	2991-50-6	$^2H_5$-N-EtFOSAA	0.20	0.2 to 100	6.1	95	5.2	96
66	MeFOSE	FASE	24448-09-7	$^2H7$-MeFOSE	0.97	0.8 to 400	7.3	110	3.8	116
67	EtFOSE	FASE	1691-99-2	$^2H_9$-EtFOSE	0.50	0.8 to 400	7.4	114	7.0	114
68	6:6 PFPi	PFPiA	40143-77-9	$^{13}C_2$-PFDoDA	0.26	0.19 to 97	7.0	81	8.7	79
69	6:8 PFPi	PFPiA	610800-34-5	$(^{13}C_2)2$-6:2 diPAP	0.51	0.49 to 97	16.7	87	8.1	48
70	8:8 PFPi	PFPiA	40143-79-1	$(^{13}C_2)2$-6:2 diPAP	N.D.	0.2 to 98	55.8	60	16.9	40
71	diSAmPAP	SAmPAP	2965-52-8	$(^{13}C_2)2$-8:2 diPAP	N.D.	0.2 to 98	51.9	44	42.8	38

N.D.: Not determined

Method sensitivity

The method sensitivity was assessed by calculating MDLs. For 60 out of 71 analytes, the MDLs ranged from 0.14 to 14 ng/L within a single analytical method (Figure 4 and Table 1). The precision and recoveries for these 60 analytes were 3.7 to 19.3% RSD and 70 to 121% , respectively. Due to low recoveries, MDLs were not determined for 11 analytes, namely 8:3 FTCA; 6:2/8:2 diPAP, 8:8 PFPi, PFODA, the five PFPAs, FOSAA, and diSAmPAP. Some of these analytes such as 8:3 FTCA, 6:2/8:2 diPAP and 8:8 PFPi can still be analyzed in the method if recoveries between 46 to 59% are acceptable to the user. The five PFPAs, PFODA, FOSAA, and diSAmPAP had low recoveries with poor precision (<20% and >47% RSD, respectively) probably because they were not completely eluted from the SPE cartridges. Nevertheless, the method demonstrated good sensitivity for most PFAS with 51 of them having MDLs ≤0.60 ng/L. Notably for PFOS, its MDL is 0.27 ng/L (Figure 4B), which is below the European Union Water Framework Directive annual average environmental quality standard (AA-EQS) limit value of 0.65 ng/L established for PFOS and its derivatives in inland surface waters.[5] Some analytes require low source temperature (e.g. HFPO-DA) while others perform better with higher temperatures (e.g. PFSAs). However, the method demonstrated good overall sensitivity despite compromising on source parameters for some analytes.
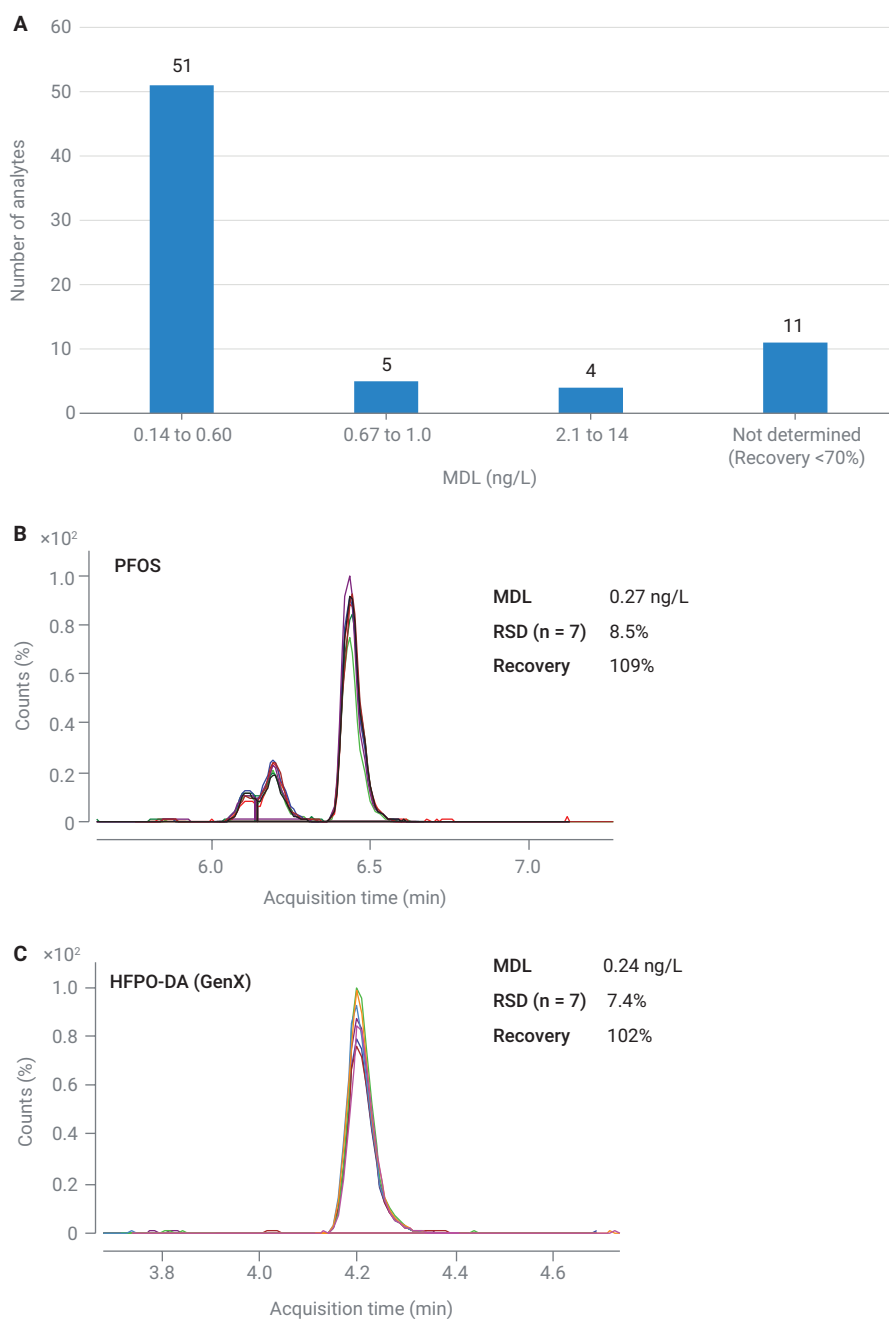
Figure 4. Distribution of the MDLs for the 71 PFAS in ultrapure water (A). Overlay of the MRM chromatograms of seven replicate analyses of spiked ultrapure water samples fortified with 1 ng/L of PFOS (B) and HFPO-DA (C).

Interbatch method precision and recovery

Method precision and recovery were assessed using spiked drinking water and surface water matrices. The measured concentration of each analyte in a spiked matrix sample was corrected by subtracting its native level present in the unspiked matrix sample. For each water matrix, interbatch method precision was determined from the percent relative standard deviation (%RSD) of the corrected concentrations from eight and six replicate extractions of low spike (5 to 50 ng/L) and high spike (20 to 200 ng/L) water samples, respectively, from two batches separately prepared by two analysts and analyzed using two different units of 6470 LC/TQs across two days to mimic real lab conditions with multiple operators or shifts. Using the same

set of replicate data, the mean percent recovery was calculated for determining interbatch method recovery. Typically, the acceptable precision and recovery limits are ≤20% RSD and 70 to 130%[4], respectively.

For low spike drinking water samples, 60 out of 71 analytes had interbatch precision ranging from 2.9 to 16.7% RSD and recoveries between 76 to 116%, which were well within the acceptable limits (Table 1). The same 60 analytes had precision ranging from 2.2 to 11.7% RSD and recoveries between 79 to 119% in high spike drinking water samples, thus demonstrating that there is minimal saturation of the SPE sorbent at high spike concentrations. For low spike surface water samples, 60 out of 71 analytes had interbatch precision ranging from 3.0 to 19.9% RSD and recoveries between 72 to 116% (Table 1).

The same 60 analytes had precision ranging from 1.6 to 16.5% RSD and recoveries between 73 to 120% in high spike surface water samples. Out of these 60 analytes in surface water, 57 are identical to those in drinking water which met the acceptable limits. For both matrices, the recoveries of a few analytes were less than 70% or more than 130% but their precisions were within 17% RSD, demonstrating consistency within each technical preparation and thus confirming the repeatability of analyte recovery using this method.

Among 71 analytes, results of more than 80% of targets were within 20% RSD and 70 to 130% recovery limits. These results confirm the precision of method performance across different experimental conditions for the two water matrices.
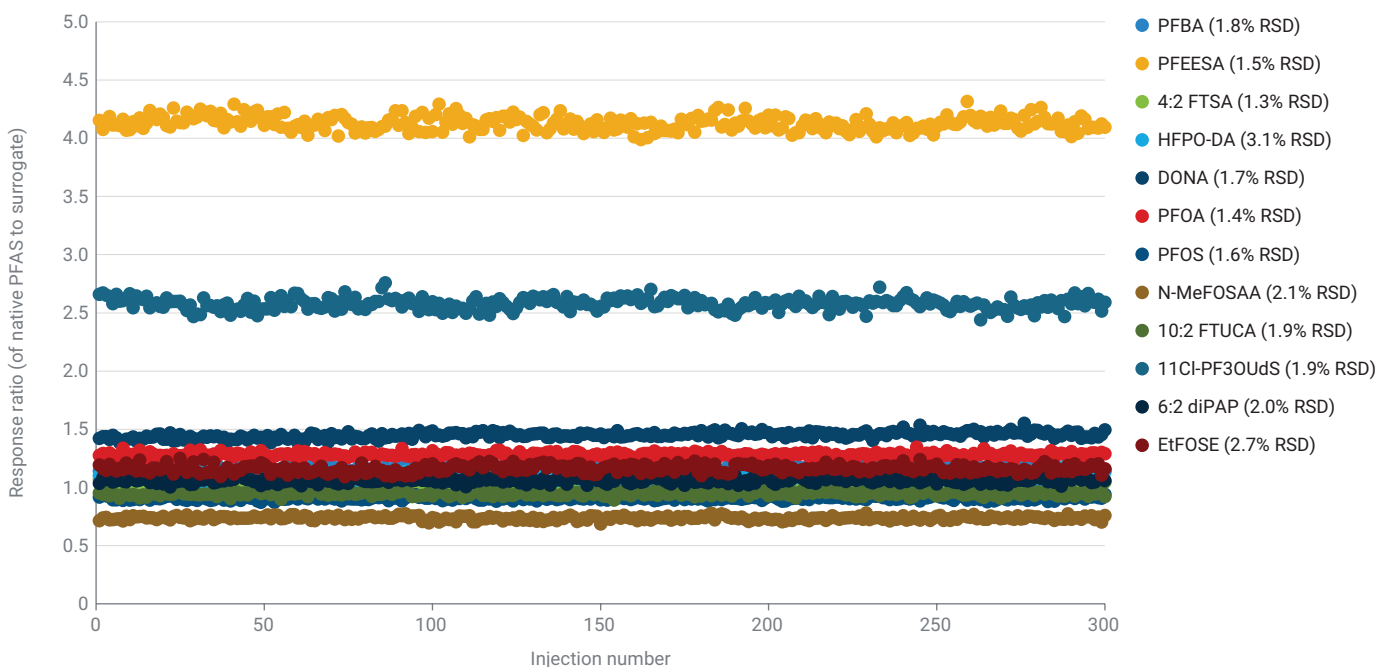

Figure 5. Response ratio of the 12 representative PFAS, sorted in ascending RT, over 93 hours of continuous injections of high spike surface water sample. The response ratio reproducibility (% RSD) for each of the PFAS is shown in the parentheses in the legend on the right.

Legend:
- PFBA (1.8% RSD)
- PFEESA (1.5% RSD)
- 4:2 FTSA (1.3% RSD)
- HFPO-DA (3.1% RSD)
- DONA (1.7% RSD)
- PFOA (1.4% RSD)
- PFOS (1.6% RSD)
- N-MeFOSAA (2.1% RSD)
- 10:2 FTUCA (1.9% RSD)
- 11Cl-PF3OUdS (1.9% RSD)
- 6:2 diPAP (2.0% RSD)
- EtFOSE (2.7% RSD)

Method robustness

Method robustness was assessed by analyzing 300 continuous injections of high spike surface water samples (20 to 200 ng/L) across a continuous batch spanning 93 hours on the unattended instrument. Twelve analytes were selected to represent nine different PFAS groups, namely PFCA, PFSA, PFECA, FTUCA, FTSA, diPAP, PFESA, FASAA, and FASE (Figure 5). The retention times (RT) of these compounds ranged from 3.05 to 9.63 minutes and spanned evenly across the elution window. As shown in Figure 5, a good response ratio reproducibility RSD of ≤3.1% and RT RSD of ≤0.10% were observed for all 12 analytes over 300 injections. The method robustness, calculated from almost four days of continuous data acquisition, confirmed the sustainable performance of the LC/MS/MS method for day-to-day operations without need for frequent maintenance.

Conclusion

A method for the targeted quantitation of 71 PFAS from 14 different PFAS groups, including all PFAS listed in current EPA, ASTM, and ISO methods, using a single LC/MS/MS and SPE method was successfully developed and applied to drinking water and surface water matrices. The use of the Agilent PFAS MRM Database in this study facilitated the quick creation of the LC/MS/MS acquisition method for 108 native and labeled PFAS for a more comprehensive, targeted PFAS analysis. The method uses SPE with SampliQ WAX cartridges which provided selective and reproducible extraction for effective sample cleanup and concentration of PFAS in water matrices. The 18-minute LC method using ZORBAX RRHD Eclipse Plus C18 column demonstrated good chromatographic and even RT distribution for all analytes.

The method performance was verified based on calibration curve analytical range and accuracy, method sensitivity (MDL), method precision, and method recovery. The method demonstrated good sensitivity where most analytes had MDLs at low to sub-ng/L concentrations. Method precision and recovery were verified from two batch analysis of two different water matrices and demonstrated the applicability of the quantitative analytical method for at least 60 PFAS in drinking water and surface water matrices.

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