APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

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Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances in Drinking Water: Validation Studies of EPA Method 537.1 Using the QSight 220 UHPLC/MS/MS

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been used in a wide variety of industries around the world since the 1940s.[1,2] This includes equipment used to package and process foodstuffs, commercial household products like nonstick cookware and cleaning products, and industrial goods such as automotive lubricants and electronics, among numerous of other applications.[3-6] Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are the two most extensively produced and studied of these chemicals. Originally considered biologically inactive, more in-depth research has revealed their toxicity to humans and wildlife alike. Furthermore, many of these chemicals are incredibly stable in the environment and the human body, meaning they are resistant to breaking down and can accumulate over time.[7,8]

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Growing health concerns regarding PFAS and their prevalence in consumer goods and the environment indicates a critical need to simply and reliably execute existing and upcoming regulatory methods on commercially available instrumentation. The United States Environmental Protection Agency (EPA) recently updated their Method 537.1, which is the current standard method for analysis of PFAS in drinking water.[9] EPA Method 537.1 is utilized for the determination of selected PFAS in drinking water by solid phase extraction (SPE) and liquid chromatography/mass spectrometry (LC/MS/MS). Other published methods, including EPA Method 533 and provisional EPA Method 8327, may be utilized for analysis of PFAS in more diverse matrices and sample types. This application note will focus on the validation of EPA Method 537.1, as well as the development of an improved version of this methodology using the PerkinElmer QSight® LX50 ultra high-performance liquid chromatography (UHPLC) system coupled with the PerkinElmer QSight 220 triple quadrupole mass spectrometer. The results demonstrate that all the PFAS analytes listed in EPA Method 537.1 can be determined reliably by the QSight 220 LC/MS/MS system, with good recovery and precision at low limits of quantification (LOQs).

Experimental

Materials and Reagents

The mixed primary PFAS standards, surrogates and internal standards were obtained from Wellington Laboratories. The list of PFAS analytes, surrogates and internal standards are listed in Table 1. The LC/MS grade methanol (MeOH), LC/MS grade water (reagent water), ammonium acetate solution and Trizma® pre-set crystals were obtained from Sigma Aldrich.

The PerkinElmer SPE manifold system used for the extraction of all water samples was modified to allow for the extraction of large volume samples with the addition of linear low-density polyethylene tubing (LLDPE) obtained from Freelin-Wade, and SPE tube adaptors obtained from Sigma Aldrich. Styrenedivinyl-benzene (SDVB) SPE cartridges (0.5 g, 6-mL) were obtained from Phenomenex. The 250-mL high density polyethylene (HDPE) bottles used for preparation and extraction of all blanks, spiked blanks, field samples and QC samples were obtained from Sigma Aldrich. The nitrogen evaporation system with heated water bath used for the concentration of final extracts was obtained from Organomation Associates, Inc.

PerkinElmer low volume, 300-µL polyethylene (PE) vials were used in the HPLC autosampler, and the polyethylene vial caps were obtained from Restek. Polyethylene vials and caps are required to prevent adsorption of PFAS compounds on glass vials and to eliminate PFAS materials commonly used in HPLC vial septa.

Table 1: Target analytes, surrogates, internal standards and acronyms of PFAS compounds analyzed.

Native Analytes	Acronym
Potassium perfluoro-1-butanesulfonate	PFBS
Perfluoro-n-hexanoic acid	PFHxA
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA
Perfluoro-n-heptanoic acid	PFHpA
Sodium perfluoro-1-hexanesulfonate	PFHxS
Sodium dodecafluoro-3H-4,8-dioxanonanoate	ADONA
Perfluoro-n-octanoic acid	PFOA
Sodium perfluoro-1-octanesulfonic acid	PFOS
Perfluoro-n-nonanoic acid	PFNA
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS
Perfluoro-n-decanoic acid	PFDA
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA
Perfluoro-n-undecanoic acid	PFUnA
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS
Perfluoro-n-dodecanoic acid	PFDoA
Perfluoro-n-tridecanoic acid	PFTrDA
Perfluoro-n-tetradecanoic acid	PFTeDA
Surrogate Standards	**Acronym**
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	13C2-PFHxA
Tetrafluoro(heptafluoropropoxy)[$^{13}C_3$]propanoic acid	13C3-HFPO-DA
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	13C2-PFDA
N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	d5-NEtFOSAA
Internal Standards	**Acronym**
Perfluoro-n-[1,2-$^{13}C_2$]octanoic acid	13C2-PFOA
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	13C4-PFOS
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	d3-NMeFOSAA

Hardware/Software

A PerkinElmer QSight LX50 ultra high-performance liquid chromatography (UHPLC) system was used for the chromatographic separation of the analytes, with subsequent detection achieved with a PerkinElmer QSight 220 triple quadrupole mass spectrometer with a dual ionization source (ESI and APCI). The LX50 autosampler was modified by replacing all polytetrafluoroethylene (PTFE) based tubing with polyether ether ketone (PEEK) tubing to reduce or eliminate any contamination from PFAS compounds introduced by the PTFE tubing. In addition, a PEEK needle was installed in the autosampler. All instrument control, data acquisition, and data processing were performed using Simplicity™ 3Q software.

Method

LC Conditions and MS Parameters

The LC method and MS source parameters are shown in Table 2. A pair of C18 columns were used in this method. A delay column (Brownlee™ SPP C18 Column, 50 x 3.0 mm, 2.7 µm) was installed in-line between the LX50 pump and the autosampler to trap and delay possible interferent PFAS arising from the LC pump and solvent reservoirs. The analytical column (Brownlee™ SPP C18 Column, 75 x 4.6 mm, 2.7 µm) was used to separate the PFAS and any other interfering components. The LC gradient program was modified from the program recommended in EPA Method 537.1, as allowed in the method, to speed up the chromatographic analysis, as shown in Table 3.

For maximum sensitivity, the MS source parameters, which include the gas flows, temperature, and position settings, were optimized. The compound dependent parameters such as collision energies (CE), entrance voltages (EV), and the collision cell lens voltage (CCL2), were optimized for the target compounds as shown in Table 4.

Table 2: LC Method and MS Source Conditions.

LC Conditions	
Analytical Column	Brownlee™ SPP C18 Column, 75 x 4.6 mm, 2.7 µm, (PN: N9308415)
Delay Column	Brownlee™ SPP C18 Column, 50 x 3.0 mm, 2.7 µm, (PN: N9308408)
Mobile Phase A	10 mM ammonium acetate in water
Mobile Phase B	Methanol
Flow Rate	0.8 mL/min
Column Oven Temperature (°C)	40
Auto Sampler Temperature (°C)	15
Injection Volume	10
Needle Wash 1	25% acetonitrile in methanol
Needle Wash 2	50% water in methanol
MS Source Conditions	
Electrospray Voltage	-3500
Drying Gas	110
Nebulizer Gas	400
Source Temperature (°C)	350
HSID Temperature (°C)	280
Detection Mode	Time Managed MRM

Table 3: LC Gradient Program.

Step #	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
1	0.00	95	5
2	0.70	95	5
3	1.00	55	45
4	7.00	2	98
5	8.00	2	98
6	8.10	95	5
7	10.00	95	5

Table 4: Optimized MRM Parameters for the PFAS analytes, surrogates and internal standards continued...

Acronym	Precursor Ion	Product Ion	RT (min)	CE[a]	EV[b]	CCL2[c]	Quantifier/Qualifier
PFBS-1	299.5	79.8	3.53	59	-35	76	Quantifier
PFBS-2	299.5	98.8	3.53	38	-35	64	Qualifier
13C2-PFHxA-1	315.0	270.0	4.13	13	-10	48	Quantifier
13C2-PFHxA-2	315.0	119.0	4.13	32	-10	52	Qualifier
PFHxA-1	313.0	269.1	4.13	13	-10	52	Quantifier
PFHxA-2	313.0	118.9	4.13	31	-10	56	Qualifier
13C3-HFPO-DA-1	286.9	168.9	4.31	12	-5	44	Quantifier
13C3-HFPO-DA-2	286.9	184.9	4.31	28	-5	52	Qualifier
HFPO-DA-1	285.0	168.9	4.32	14	-5	40	Quantifier
HFPO-DA-2	285.0	184.9	4.32	28	-5	52	Qualifier
PFHpA-1	363.0	319.0	4.75	14	-10	56	Quantifier
PFHpA-2	363.0	169.0	4.75	24	-10	64	Qualifier
PFHxS-1	399.0	80.0	4.76	91	-45	120	Quantifier
PFHxS-2	399.0	99.0	4.76	46	-45	88	Qualifier
ADONA-1	377.0	251.1	4.81	17	-10	64	Quantifier
ADONA-2	377.0	84.9	4.81	64	-10	88	Qualifier
PFOA-1	413.0	368.9	5.27	14	-10	68	Quantifier
PFOA-2	413.0	168.9	5.27	25	-10	80	Qualifier

Table 4: Optimized MRM Parameters for the PFAS analytes, surrogates and internal standards.

Acronym	Precursor Ion	Product Ion	RT (min)	CE[a]	EV[b]	CCL2[c]	Quantifier/Qualifier
13C2-PFOA-1	415.0	370.0	5.27	15	-14	68	IS
PFNA-1	463.0	419.0	5.72	16	-10	76	Quantifier
PFNA-2	463.0	219.0	5.72	24	-10	76	Qualifier
13C4-PFOS-1	503.0	80.0	5.71	111	-69	124	IS
PFOS-1	499.1	79.9	5.71	100	-45	120	Quantifier
PFOS-2	499.1	98.9	5.71	55	-45	116	Qualifier
9Cl-PF3ONS-1	530.9	350.9	5.92	35	-30	112	Quantifier
9Cl-PF3ONS-2	530.9	83.0	5.92	35	-30	96	Qualifier
13C2-PFDA-1	515.0	469.9	6.11	16	-13	84	Quantifier
13C2-PFDA-2	515.0	219.0	6.11	24	-13	88	Qualifier
PFDA-1	513.0	468.9	6.11	16	-10	84	Quantifier
PFDA-2	513.0	219.0	6.11	25	-10	92	Qualifier
d3-NMeFOSAA-1	573.0	419.0	6.29	27	-25	104	IS
NMeFOSAA-1	570.0	419.0	6.29	27	-20	108	Quantifier
NMeFOSAA-2	570.0	482.9	6.29	20	-20	108	Qualifier
PFUnA-1	562.9	518.9	6.42	17	-10	96	Quantifier
PFUnA-2	562.9	269.0	6.42	26	-10	96	Qualifier
d5-NEtFOSAA-1	589.0	419.0	6.45	28	-20	112	Quantifier
d5-NEtFOSAA-2	589.0	531.0	6.45	27	-20	112	Qualifier
NEtFOSAA-1	584.0	418.9	6.45	27	-20	96	Quantifier
NEtFOSAA-2	584.0	482.9	6.45	20	-20	100	Qualifier
11Cl-PF3OUdS-1	630.9	450.9	6.55	36	-40	176	Quantifier
11Cl-PF3OUdS-2	630.9	199.0	6.55	32	-40	148	Qualifier
PFDoA-1	612.9	568.9	6.7	17	-10	104	Quantifier
PFDoA-2	612.9	319.0	6.7	27	-10	100	Qualifier
PFTrDA-1	662.9	618.9	6.94	18	-11	104	Quantifier
PFTrDA-2	662.9	368.9	6.94	28	-10	120	Qualifier
PFTA-1	712.9	668.8	7.14	17	-10	116	Quantifier
PFTA-2	712.9	368.9	7.14	29	-10	140	Qualifier

a. CE = Collision Cell Energy
b. EV = Entrance Voltage
c. CCL2 = Collision Cell Lens 2 voltage

Calibration Standards Preparation

The analyte stock standard solution and the surrogate primary dilution standard (SUR PDS) were combined and diluted with 96% MeOH/4% reagent water to prepare the primary dilution standard (PDS), per section 7.2.3.2 of EPA Method 537.1. The PDS was diluted in 96% MeOH to prepare eight calibration standards, as per Section 7.2.4 of EPA Method 537.1. Internal standards (IS) were added at a constant volume to each calibration standard. Analyte and surrogate concentrations in the calibration standards ranged from ~5 to 30,000 ng/L, except the d5-NEtFOSAA surrogate which ranged from ~20 to 100,000 ng/L. Calibration standards were transferred to low volume polyethylene vials and caps for UHPLC analysis. The broad range calibration standards were used to determine method linearity and instrument limits of detection (LOD), but a reduced range and number of calibrants at a higher minimum level can be utilized in general practice. The EPA method only requires a minimum five calibration levels.

Laboratory Reagent Blank and Laboratory Fortified Blank Preparation

All laboratory reagent blanks (LRB) and laboratory fortified blanks (LFB) were prepared in 250 mL polyethylene bottles by placing ~1.25 g of Trizma pre-set crystals into each bottle, and adding 250 mL of reagent water. A constant volume of SUR PDS was added to all LRBs and LFBs to monitor extraction efficiency based on surrogate recoveries. Analyte fortification solution was spiked into LFBs at varying amounts to evaluate and validate analyte recoveries, as well as determine the method detection limits

(DL), minimum reporting levels (MRL) and lowest concentration minimum reporting limits (LCMRL). All LRBs and LFBs were extracted and concentrated by the SPE sample preparation method, as defined and required in section 11 of EPA Method 537.1. Final extracts were spiked with a constant amount of internal standards prior to transferring an aliquot to PE vials with PE caps for analysis by LC/MS/MS. LRBs were analyzed daily on the LC/MS/MS system to ensure the adequate reduction or absence of PFAS interferences. LRBs were considered acceptable if the analyte concentrations were less than 1/3 the proposed MRL, in accordance with section 9.3.1 of EPA Method 537.1.

Field Samples, Field Reagent Blanks and Laboratory Fortified Sample Matrix

All field duplicate (FD) samples, laboratory fortified sample matrix samples (LFSM), and field reagent blanks (FRB) were collected in 250 mL polyethylene bottle containing a ~1.25 g of Trizma pre-set crystals, in accordance with section 8 of EPA Method 537.1. The FD and LFSM samples were collected at the source by opening the tap for 3 - 5 minutes and then collecting the sample from the flowing system. Field reagent blanks (FRB) were prepared by placing 250 mL of reagent water, plus Trizma, in the sample bottle in the laboratory. The FRB was then taken to the sampling site and transferred to a clean sample bottle. The purpose of the FRB was to ensure that no contamination was introduced by the sample collection process. All FDs, LFSMs and FRBs were stored at <10°C until extraction. A constant amount of SUR PDS was added to all FDs, LFSMs and FRBs prior to extraction. A constant amount of analyte fortification solution was added to all LFSMs prior to extraction. Final extracts were spiked with IS prior to transferring an aliquot to PE vials with PE caps for analysis by the LC/MS/MS system.

Solid Phase Extraction and Sample Concentration

A manual SPE vacuum manifold system was used for all extractions. The SPE system was equipped with LLDPE transfer lines, SPE tube adaptors and PTFE-free manifold valves to eliminate PFAS contamination introduced from the SPE system. Extractions were performed in strict accordance to the procedure defined in sections 11.3 - 11.5 in EPA Method 537.1, as required by the method. Styrenedivinyl-benzene (SDVB) SPE 6 mL tubes containing 0.5 g of sorbent were utilized. The SPE cartridges were conditioned with 15 mL of methanol followed by 18 mL of reagent water. Samples were introduced on the cartridges at a rate of 10 - 15 mL/min, followed by two 7.5 mL aliquots of reagent water used to rinse the bottles. PFAS analytes were eluted from the cartridges by rinsing the bottles with two 4 mL aliquots of methanol and then pulled through the extraction system. The methanol extracts were collected in 15 mL polyethylene tubes. The extracts were then evaporated to dryness under a gentle stream of nitrogen while heating in a water bath at 60°C. Samples were reconstituted with 1 mL of 96:4% (v/v) methanol/water, and the appropriate amount of IS was added. A small aliquot was transferred to a polyethylene vial for final LC/MS analysis.

Results and Discussion

Remediation of PFAS Background Contamination

One of the major challenges associated with trace analysis of PFAS is the contamination of blanks, samples and QC samples arising from the reagents, SPE apparatus, sample collection materials, volumetric ware, vials, the LC/MS system, and the lab environment. Many of these interferences can originate from the materials that are used in construction of volumetric ware, pipettes, syringes, tubing, and vials, as well as from PTFE parts in the LC/MS/MS system. In order to eliminate or reduce these interferences from the LC/MS/MS system, a delay column was placed between the mobile phase mixer in the pump and the sample valve in the autosampler to trap and delay any PFAS compounds arising from the pump and mobile phase solvents. By doing so, the PFAS chromatographic peaks in the sample are well separated from the incoming PFAS contaminant peaks from the pump system. The standard LX50 autosampler also contains PTFE tubing both internally and to the wash solution reservoirs that contribute to PFAS contamination. This contamination was remediated by replacing all PTFE tubing in the autosampler with PEEK tubing. All the materials used in this study were tested prior to running samples to check for PFAS contamination through the injection of blank samples. Through these experiments, it was confirmed that all the supplies used were free of PFAS contamination.

LC and MS/MS Methods

The QSight MS/MS MRM parameters were optimized for each analyte, surrogate and IS by direct infusion experiments using a syringe pump. Once precursor and product masses were determined, the entrance voltage (EV), collision cell energy (CE) and collision cell lens 2 voltage (CCL2) were optimized for each compound using the autotune feature in Simplicity 3Q. The optimized MRM parameters are shown in Table 4. MRM experiments were established for two precursor/product ion transitions for each analyte and surrogate to serve as quantifier and qualifier ions, as well as a single MRM transition for each IS. Once the retention times for each analyte were established, a time-managed MRM MS/MS method was used with optimized time windows and dwell times so that there were at least 10 scans across each analyte peak.

The LC gradient method was optimized to provide good separation of the analytes, minimize run time, and optimize peak symmetry. A high efficiency superficially porous particle (SPP) type column was chosen to provide narrow peaks and short run times. The original chromatographic method described in EPA 537.1 had a 37-minute runtime, while the method presented herein reduces the injection-to-injection run time to 10 minutes. The total ion chromatogram (TIC) is shown in Figure 1. In the initial demonstration of the LC method capability, the baseline separation of the branched vs. linear isomers was established for PHHxS, PFOS, NMeFOSAA and NEtFOSAA, as shown in Figure 2. In addition, the LC method meets the requirements for the initial demonstration of peak asymmetry factor described in section 9.2.5 of EPA 537.1. The peak asymmetry factors for the first two eluting peaks (PFBS and PFHxA) must fall between 0.8 and 1.5. The peak asymmetry factors for PFBS and PFHxA were 0.9 and 1.3, respectively.

Analyte	Peak #	RT (min)	IS# Ref
PFBS	1	3.54	2
PFHxA	2	4.15	1
HFPO-DA	4	4.34	1
PFHpA	6	4.78	1
PFHxS	7	4.77	2
ADONA	8	4.84	1
PFOA	9	5.30	1
PFOS	11	5.73	2
PFNA	13	5.74	1
9Cl-PF3ONS	14	5.93	2
PFDA	15	6.13	1
NMeFOSAA	17	6.31	3
PFUnA	19	6.45	1
NEtFOSAA	20	6.47	3
11Cl-PF3OUdS	22	6.56	2
PFDoA	23	6.72	1
PFTrDA	24	6.96	1
PDTA	25	7.16	1
$^{13}C_2$-PFHxA SS#1	3	4.15	1
$^{13}C_3$-HFPO-DA SS#2	5	4.34	1
$^{13}C_2$-PFDA SS#3	16	6.12	1
d_5-NEtFOSAA SS#4	21	6.46	3
$^{13}C_2$-PFOA IS#1	10	5.29	-
$^{13}C_4$-PFOS IS#2	12	5.74	-
d_3-NMeFOSAA IS#3	18	6.30	-

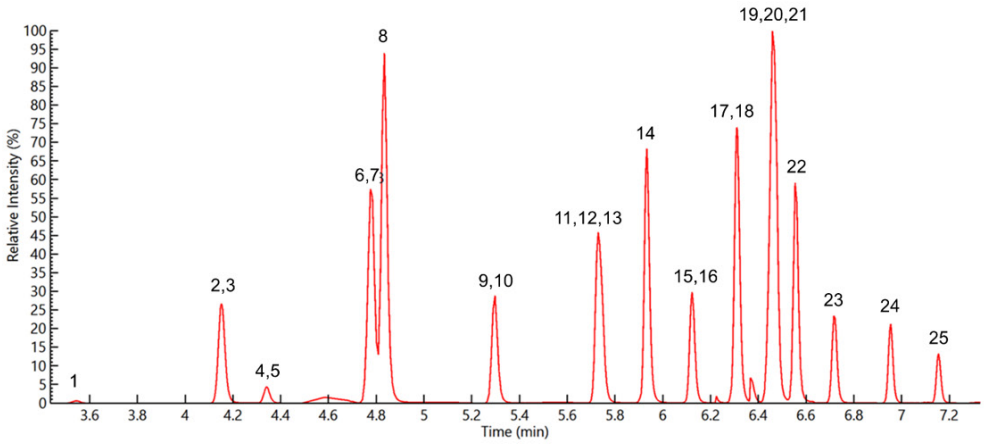

Figure 1: Total ion chromatogram of an 80 ng/L extracted LFB sample containing all method analytes, surrogates and internal standards.

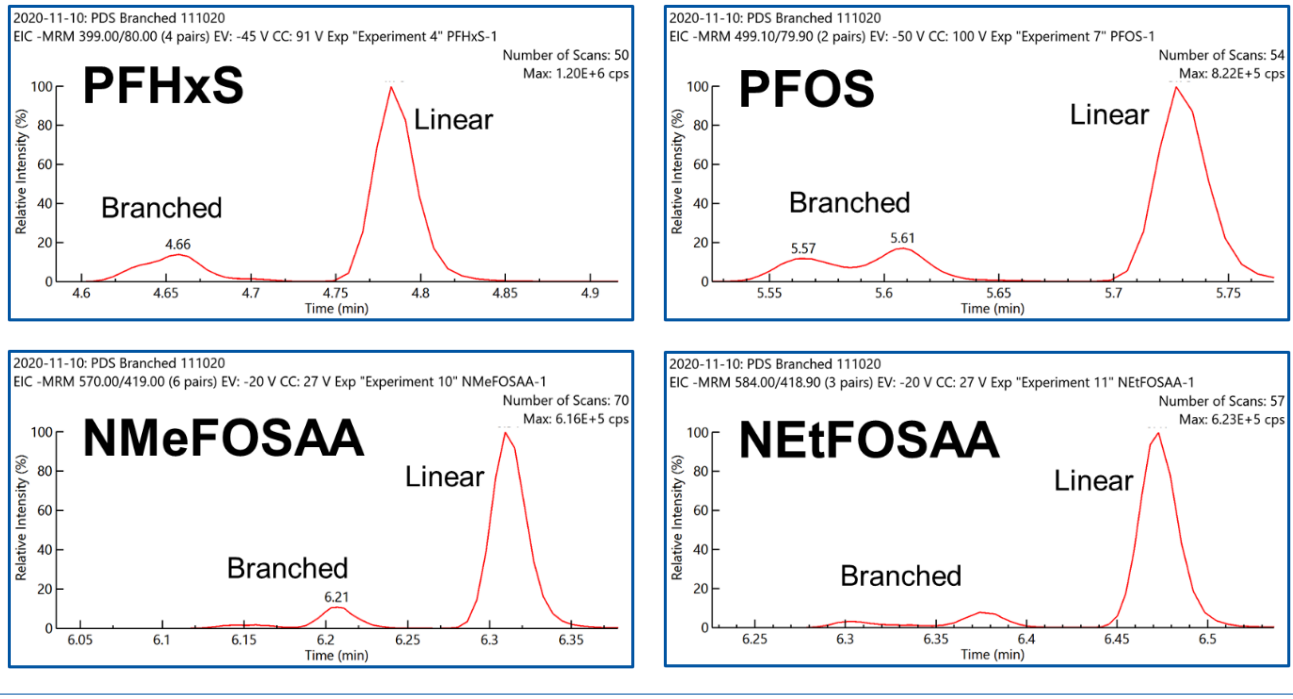

Figure 2: MRM chromatograms of PFHxS, PFOS, NMeFOSAA and NEtFOSAA showing the baseline separation of linear and branched chain isomers.

Linearity, Instrument Limits of Quantitation (LOQ) and Instrument Limits of Detection (LOD)

Calibration curves were used to assess linearity and to estimate the instrument limits of detection (LOD) and quantitation (LOQ) for all PFAS targets and surrogates. Eight-point calibration curves were constructed using a non-weighted linear regression with the intercept forced through zero in the concentration range of ~5 – 30,000 ng/L from three replicates at each level. Excellent linearity was achieved over the studied range of concentrations with correlation coefficient values (R^2) greater than 0.99 for all the analytes and surrogates, as shown in Table 5. Figure 3 shows representative calibration curves for triplicate injections of analytes PFOA, PFOS, HFPO-DA and 9Cl-PF3ONS.

The instrument limits of detection (LOD) and quantitation (LOQ) for each target analyte were determined at the lowest detectable standard on the calibration curve (ng/L) extrapolated to give a signal-to-noise ratio (S/N) of 3 for LOD and an extrapolated S/N equal to 10 for the LOQ. Table 6 is a summary of the instrument and method LODs and LOQs

Table 5: Instrument and Method Calibration Ranges and Linearity (R^2) for eight-point calibration curves of all EPA Method 537.1 analytes and surrogates.

Compound	Instrument Calibration Range (ng/L)[a]	Method Calibration Range (ng/L)[b]	R^2 [c]
PFBS	16.4 - 26287	0.07 - 105.1	0.9994
PFHxA	5.5 - 29703	0.02 - 118.8	0.9987
13C2-PFHxA	4.6 - 24752	0.02 - 99.0	0.9989
13C3-HFPO-DA	67.5 - 24752	0.27 - 99.0	0.9992
HFPO-DA	18.5 - 29703	0.07 - 118.8	0.9985
PFHpA	5.5 - 29703	0.02 - 118.8	0.9984
PFHxS	5.2 - 28218	0.02 - 112.9	0.9998
ADONA	5.2 - 28218	0.02 - 112.9	0.9990
PFOA	5.5 - 29703	0.02 - 118.8	0.9998
PFOS	5.3 - 28515	0.02 - 114.1	0.9974
PFNA	18.5 - 29703	0.07 - 118.8	0.9993
9Cl-PF3ONS	5.1 - 27772	0.02 - 111.1	0.9998
PFDA	81.0 - 29703	0.32 - 118.8	0.9990
13C2-PFDA	4.6 - 24752	0.02 - 99.0	0.9988
NMeFOSAA	5.5 - 29703	0.02 - 118.8	0.9998
PFUnA	18.5 - 29703	0.07 - 118.8	0.9968
NEtFOSAA	5.5 - 29703	0.02 - 118.8	0.9968
d5-NEtFOSAA	18.3 - 99010	0.07 - 396.0	0.9962
11Cl-PF3OUdS	5.2 - 28069	0.02 - 112.3	0.9997
PFDoA	18.5 - 29703	0.07 - 118.8	0.9963
PFTrDA	5.5 - 29703	0.02 - 118.8	0.9959
PFTA	5.5 - 29703	0.02 - 118.8	0.9967

a. Instrument calibration range is the actual concentration range of calibration standards used to determine calibration curves.
b. Method calibration range is determined by multiplying the instrument calibration range by 1/250 to account for the SPE sample preparation/concentration.
c. R^2 values are the average of triplicate calibration curves.

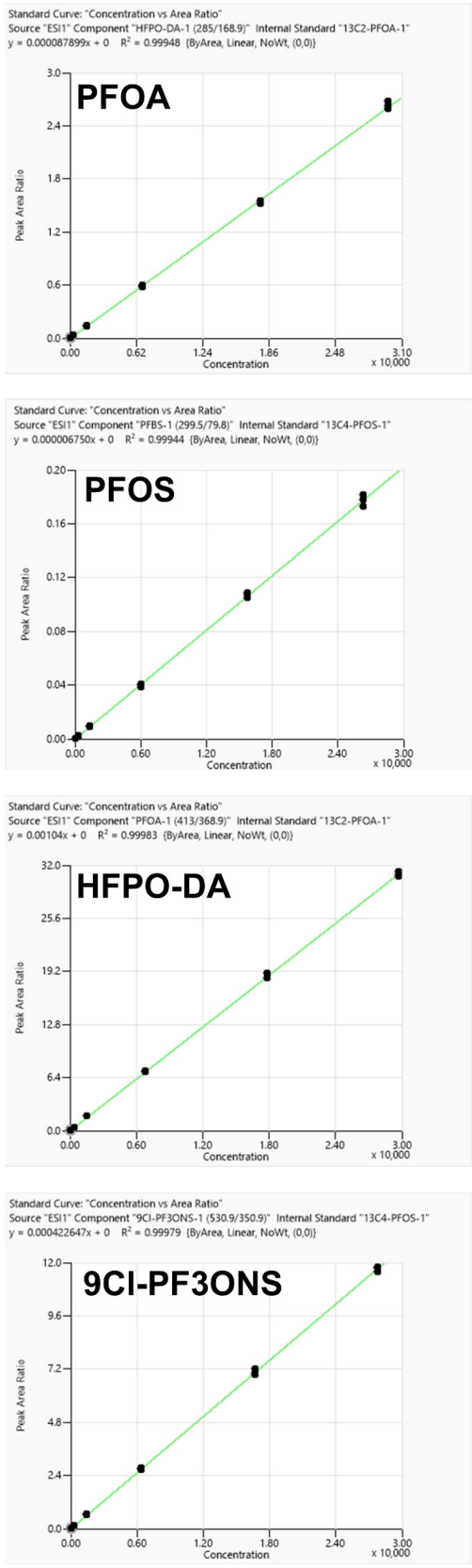

Figure 3: Triplicate injection calibration curves for representative analytes PFOA, PFOS, HFPO-DA and 9Cl-PF3ONS.

Table 6: Instrument and method limits of detection (LOD) and limits of quantitation (LOQ) for all target analytes in EPA Method 537.1.

Analyte	Instrument (ng/L)[a]		Method (ng/L)[b]	
	LOD	LOQ	LOD	LOQ
PFBS	2.00	6.68	0.008	0.027
PFHxA	2.31	7.70	0.009	0.031
HFPO-DA	6.70	22.35	0.027	0.089
PFHpA	2.10	6.99	0.008	0.028
PFHxS	0.38	1.28	0.002	0.005
ADONA	0.24	0.79	0.001	0.003
PFOA	2.57	8.56	0.010	0.034
PFOS	0.92	3.07	0.004	0.012
PFNA	2.52	8.40	0.010	0.034
9Cl-PF3ONS	0.60	2.00	0.002	0.008
PFDA	2.17	7.24	0.009	0.029
NMeFOSAA	0.29	0.96	0.001	0.004
PFUnA	3.50	11.67	0.014	0.047
NEtFOSAA	0.25	0.85	0.001	0.003
11Cl-PF3OUdS	0.44	1.48	0.002	0.006
PFDoA	2.02	6.73	0.008	0.027
PFTrDA	1.55	5.16	0.006	0.021
PFTA	4.29	14.30	0.017	0.057

a. Instrument LOD/LOQ was determined using the signal-to-noise ratio (S/N) of the peak from the lowest detectable calibration standard (5-18 ng/L) and extrapolating to the concentration at which the S/N = 3 or 10 for LOD or LOQ, respectively. This is an estimate to demonstrate expected LOD/LOQ and can vary from lab to lab.

b. Method LOD/LOQ is calculated by multiplying the Instrument LOD/LOQ by 1/250 to account for the 250 to 1 sample concentration from the SPE extraction. LOD/LOQ cannot be used as MRLs but provide an estimate of instrument sensitivity.

Table 7: PFAS analyte and surrogate recovery data for LRBs of reagent water spiked at 0.3, 4, 16 and 80 ng/L. Seven replicate samples were extracted at each fortification level.

Analyte	0.3 ng/L		4 ng/L		16 ng/L		80 ng/L	
	Average % Recovery	%RSD	Average % Recovery	%RSD	Average % Recovery	%RSD	Average % Recovery	%RSD
PFBS	91	14	104	10	104	3	102	4
PFHxA	94	6	108	12	113	4	110	6
HFPO-DA	111	17	100	13	112	5	109	6
PFHpA	119	13	109	13	117	5	111	7
PFHxS	92	5	102	11	107	3	103	4
ADONA	96	4	108	12	116	4	111	6
PFOA	110	5	104	11	108	3	104	4
PFOS	94	4	109	12	107	2	103	4
PFNA	108	14	105	13	116	4	110	7
9Cl-PF3ONS	86	21	95	10	104	3	100	4
PFDA	99	12	96	10	115	5	109	7
NMeFOSAA	106	9	98	11	110	5	102	4
PFUnA	111	9	104	11	114	4	108	7
NEtFOSAA	115	8	100	10	111	2	105	4
11Cl-PF3OUdS	77	10	92	7	102	2	98	3
PFDoA	96	10	99	10	112	4	105	7
PFTrDA	85	25	96	9	110	4	104	6
PFTA	115	44	94	8	108	5	102	6
Surrogates								
13C2-PFHxA	96	9	106	8	113	5	113	4
13C3-HFPO-DA	91	7	102	4	109	5	109	5
13C2-PFDA	84	9	105	3	115	5	115	5
d5-NEtFOSAA	89	4	106	4	113	3	113	3

Determination of Method DLs, MRLs and LCMRLs.

The method detection limits (DL), minimum reporting levels (MRL) and lowest concentration minimum reporting limits (LCMRL) were determined as described in EPA Method 537.1. Ten replicate reagent water samples were fortified (LFB) with method analytes at four different concentrations, representing the proposed MRL (0.3 ng/L), as well as low (4 ng/L), mid (16 ng/L) and high (80 ng/L) concentrations to evaluate method recoveries. A constant volume of SUR PDS was also added to each LFB, as described in section 7.2.2.2 of EPA Method 537.1. Each of these LFBs were then carried through the full sample preparation method including SPE, evaporation, reconstitution and IS addition. Aliquots of each LFB replicate were then transferred to polypropylene vials and analyzed on the LC/MS system to determine analyte and surrogate recoveries. The recoveries of all analytes at all fortification levels fell well within the required 70-130% recoveries, as shown in Table 6. Most of the RSDs for the ten replicates fortified at 0.3 ng/L were ≤ 25%, with the exception of PFTA. The recovery RSDs for 4 ng/L, 16 ng/L and 80 ng/L recoveries were < 13%, < 5% and < 7%, respectively. The 0.3 ng/L recovery RSD levels were expectedly higher than those for the low, mid and high fortification levels, but still demonstrate excellent method performance at a level well below any state or federal regulatory limits for PFAS compounds in drinking water.

The method DLs, MRLs and LCMRLs were calculated and validated using the ten replicate LFBs fortified at five levels ranging from 0.2 to 80 ng/mL using the statistical analysis methods described in EPA Method 537.1. Table 7 summarizes the statistical analysis and determinations of DLs, MRLs and LCMRLs in this study.

The method detection limits are not a specific requirement of EPA Method 537.1, but may be required by other regulatory bodies for compliance monitoring. The DLs are the minimum concentrations of analytes that can be measured, identified, and determined with a 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision, and accurate quantitation is not expected at this level.[10] The detection limits in this study were determined from ten replicate LFBs fortified at ~1.6 ng/L and calculated as described in section 9.8.2 of EPA 537.1.

The single laboratory LCMRLs are the lowest concentration for which future recoveries are expected, with 99% confidence, to be between 50 and 150% recovery. This value is not required to be determined by EPA, but provides good guidance on the expected method performance on a particular instrument in a specific laboratory. The LCMRLs were determined in this study to demonstrate method and instrument performance. To determine the LCMRLs, ten replicate LFBs at five fortification levels were carried through the full sample preparation method including SPE, evaporation, reconstitution and IS addition. Aliquots of the final samples were transferred to polypropylene vials and analyzed by LC/MS/MS to determine analyte concentrations. The concentrations were then analyzed using the LCMRL calculator[11] provided by EPA, using the statistical procedures described by Winslow, et. al, 2004.[12] The LCMRLs in this study are generally consistent with those reported, or are below those reported in EPA 537.1, demonstrating that this instrument is well suited for the analysis of PFAS compounds in drinking water using EPA Method 537.1.

The MRLs were determined by fortifying, extracting and analyzing seven replicate LFBs at proposed MRL concentrations ranging from 0.2 - 4 ng/L. Calculations were then performed for the mean and the standard deviation to determine the half range for prediction interval of results (HR_{PIR}). It was then confirmed that the upper and lower limits for the predicted interval for results (PIR) met the upper and lower recovery limits described in section 9.2.6 of EPA 537.1. The upper PIR recovery limit must be ≤ 150% and the lower PIR recovery limit must be ≥ 50%. The experimentally determined MRLs from this study are summarized in the last column of Table 7. These values are provided to reflect MRL values one can expect when performing EPA 537.1 using the QSight 220 LC/MS/MS system. The MRLs demonstrated here are well below any state or federal action limits for regulated PFAS contaminants in drinking water.

Table 8: Method detection limits (DL) and lowest concentration minimum reporting limits (LCMRL) and minimum reporting levels (MRL) determined experimentally on the QSight LC/MS/MS system and compared to reference values report in EPA Method 537.1 rev 2.0.

Analyte	Experimental DL (ng/L)[a]	EPA 537.1 DL (ng/L)[b]	Experimental LCMRL (ng/L)[c]	EPA 537.1 LCMRL (ng/L)[d]	Experimental MRL (ng/L)[e]
PFBS	1.1	6.3	0.72	1.8	1.4
PFHxA	1.5	1.7	0.93	1.0	0.30
HFPO-DA	1.5	4.3	0.57	1.9	1.6
PFHpA	1.6	0.63	0.10	0.71	1.6
PFHxS	1.2	2.4	0.60	1.4	0.29
ADONA	1.4	0.55	ND	0.88	0.28
PFOA	1.3	0.82	0.34	0.53	0.30
PFOS	1.4	2.7	1.0	1.1	0.29
PFNA	1.6	0.83	0.50	0.70	1.6
9Cl-PF3ONS	1.1	1.8	0.68	1.4	1.5
PFDA	1.1	3.3	0.40	1.6	0.30
NMeFOSAA	1.2	4.3	0.22	2.4	0.30
PFUnA	1.3	5.2	0.30	1.6	1.6
NEtFOSAA	1.2	4.8	0.73	2.8	1.6
11Cl-PF3OUdS	0.66	1.5	0.39	1.5	0.28
PFDoA	1.2	1.3	0.19	1.2	0.30
PFTrDA	1.0	0.53	0.82	0.72	4.0
PFTA	0.86	1.2	1.5	1.1	4.0

a. Experimental DL was determined from ten LFB replicates fortified at ~4.0 ng/L measured over three days and calculated according to section 9.2.8 in EPA Method 537.1 rev 2.0

b. Reference DL values from EPA Method 537.1 rev 2.0 (Table 5) determined from seven LFB replicates fortified at 4.0 ng/L measured over three days and calculated according to section 9.2.8

c. Experimental LCMRLs were determined from ten replicates each at five fortification levels ranging from ~0.2 – 80 ng/L using the EPA LCMRL Calculator.[11]

d. Reference LCMRL values from EPA Method 537.1 rev 2.0 (Table 5).

e. Experimental MRLs were determined from seven LFBs fortified at concentrations ranging from ~0.2 to 4.0 ng/L according to section 9.2.6 of EPA Method 537.1 rev 2.0 using the Half Range prediction interval method with confirmed upper and lower Prediction Interval Results (PIR) ≤150% and ≥50%, respectively.

Field Sample Analysis

Field samples of tap water were collected from three different municipalities in the Southeast US, and are designated M1, M2 and M3. Public drinking water in all three locations are sourced from groundwater. Four field samples and one FRB were collected at each location. Prior to extraction, all samples were spiked with a constant amount of SUR PDS and two field samples were fortified with method analytes at a concentration of ~8.0 ng/L, resulting in two FD samples, two LFSM samples and one FRB from each sampling location. All samples from a single location were then carried through SPE extraction, evaporation and reconstitution. The reconstituted samples were then spiked with IS, and an aliquot was transferred to a polypropylene vial for LC/MS/MS analysis.

The FRBs were evaluated to confirm that there was no contamination from sampling, and that all analytes were either not present or at <1/3 of the MRL concentrations, as required by EPA Method 537.1, indicating that the field sampling process was free of contamination.

Table 9 summarizes the results for all samples. All samples contained PFOA levels above the MRL of the method, but still below any state or federal regulatory action limit. The samples collected in locations M1 and M2 contained PFBS, PFHxA, PFHxS and PFDA above the method MRLs, and the samples from M2 also contained PFOS above the MRL. All other analytes were either not detected or below the MRLs, as indicated by <MRL in the table. The LFSM % recoveries were all within the method requirements of ≥70% and ≤130%. The RPD values for the LFSM are a measure of the percent difference between the two replicates, and are required to be ≤ 30%. All analytes are well below the RPD requirement. All calculations were performed according to the method definitions. Although a few PFAS analytes were detected in these samples, all levels were below existing federal and state health advisory and action limits indicating that these water sample were below any current PFAS standards.

Average FD and LFSM surrogate recoveries are summarized in Table 9. The values reported in the table are the average of duplicate samples for each sampling location. The recoveries all fall within the ≥70% and ≤130% requirements and verify the efficiency of the sample preparation.

Table 9: Average analyte field duplicate (FD) sample concentrations, average laboratory fortified sample matrix (LFSM) recoveries and LFSM relative percent difference (RPD) data for duplicate (2x) FDs and LFSMs from each sampling location.

Analyte	Average FD Conc (ng/L)			Average LFSM % Recovery[a]			LFSM RPD[b]		
	M1	M2	M3	M1	M2	M3	M1	M2	M3
PFBS	2.0	14.9	<MRL	120	100	119	5.6	16.0	1.4
PFHxA	1.8	2.0	<MRL	101	95	120	2.1	2.8	6.0
HFPO-DA	<MRL	<MRL	<MRL	116	90	108	4.1	18.0	1.1
PFHpA	<MRL	<MRL	<MRL	103	88	99	2.3	1.2	0.4
PFHxS	0.32	0.56	<MRL	89	75	81	5.3	0.4	0.0
ADONA	<MRL	<MRL	<MRL	114	107	111	2.5	6.8	1.3
PFOA	1.1	1.9	0.39	88	78	88	3.6	8.9	7.2
PFOS	<MRL	2.0	<MRL	129	111	126	0.1	7.0	2.9
PFNA	<MRL	<MRL	<MRL	90	82	92	9.1	12.8	0.1
9Cl-PF3ONS	<MRL	<MRL	<MRL	118	97	115	6.2	0.2	2.2
PFDA	0.35	0.37	<MRL	82	128	121	2.1	3.3	1.0
NMeFOSAA	<MRL	<MRL	<MRL	96	85	94	1.7	6.5	0.7
PFUnA	<MRL	<MRL	<MRL	75	120	139	0.2	1.4	5.0
NEtFOSAA	<MRL	<MRL	<MRL	98	84	97	6.3	6.6	0.3
11Cl-PF3OUdS	<MRL	<MRL	<MRL	57	86	100	9.0	2.3	4.3
PFDoA	<MRL	<MRL	<MRL	124	118	129	0.2	2.1	0.3
PFTrDA	<MRL	<MRL	<MRL	120	106	113	2.4	0.7	9.2
PFTA	<MRL	<MRL	<MRL	94	83	92	6.9	1.6	19.3

a. LFSM percent recovery calculated according to section 9.3.6.2 of EPA Method 537.1.

b. Relative percent difference (RPD) for duplicate LFSMs calculated according to section 9.3.7.3 of EPA Method 537.1.

Table 10: Percent recoveries for surrogates in field duplicates (FD) and laboratory fortified sample matrix (LFSM) samples. Surrogate recoveries are required to be ≥70% and ≤130% according to EPA Method 537.1. Values shown are the average of duplicate (2x) FDs and LFSMs.

Surrogates	Average FD %Recovery			Average LFSM %Recovery		
	M1	M2	M3	M1	M2	M3
$^{13}C_2$-PFHxA	104	112	127	100	106	117
$^{13}C_3$-HFPO-DA	106	93	103	104	94	97
$^{13}C_2$-PFDA	76	81	79	81	76	73
d5-NEtFOSAA	110	106	106	111	106	102

Conclusion

This application note reports the validation of an LC/MS/MS method for the determination of PFAS analytes and mass-labelled surrogates in drinking water listed in the US EPA Method 537.1 using the PerkinElmer QSight LX50 ultra high-performance liquid chromatography (UHPLC) system, coupled with the PerkinElmer QSight 220 triple quadrupole mass spectrometer. These validation studies demonstrate that excellent linearity was achieved for all PFAS analytes and surrogates, with the R^2 values ≥0.996. The instrument LODs and LOQs verify that the QSight 220 has ample sensitivity required to quantify the PFAS analytes listed in US EPA Method 537.1. Instrument modifications and the incorporation of a delay column are required to eliminate and reduce background PFAS contaminants, and have been verified to be effective by the analysis of blanks.

An improved chromatographic method has been developed to decrease LC/MS/MS runtimes to 10 minutes, as compared to the method described in EPA Method 537.1 with a runtime of 37 minutes; a 73% decrease in LC/MS/MS runtime. The chromatographic method was established to meet peak symmetry requirements and the baseline separation of linear and branched chain isomers of selected analytes. MRM experiments were optimized for all analytes, surrogates and internal standards on the QSight 220 tandem quadrupole mass spectrometer, including quantifier and qualifier MRMs for all analytes and surrogates. A time-managed MRM mass spectrometer method has been optimized to maximize dwell time for improved sensitivity, while maintaining more than 10 data points across each chromatographic peak. Recoveries for LFBs fortified at the very low concentration of 0.3 ng/mL ranged from 77% to 119% while recoveries for LFBs fortified at 4, 16 and 80 ng/mL ranged from 92% to 117%. EPA Method 537.1 requires that recoveries fall within 70-130% so the recoveries in this study are well within these requirements demonstrating the excellent performance of the sample preparation procedure. In addition, the experimentally determined LCMRLs are at or even well below those reported in the method further supporting the excellent method performance. The SPE extraction in this study was carried out on a manual SPE manifold system that was modified to eliminate any components constructed of PTFE to minimize

or eliminate PFAS contamination. Method MRLs could be improved by incorporating an automated or robotic SPE extraction system and these systems will be evaluated in future studies.

Municipal drinking water samples from three sampling sites were quantified with validated recoveries and repeatability within the method requirements. Surrogate standard recoveries in field samples validated the effectiveness of the sample preparation method. Overall, this validation study shows that the LX50 UHPLC system coupled to the QSight 220 tandem quadrupole mass spectrometer (LC/MS/MS) is an excellent system for the application of EPA Method 537.1 with ample sensitivity to measure all analytes.

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