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

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1 Introduction

Growing environmental and health concerns about Per- and Polyfluoroalkyl Substances (PFAS) have led to stricter and more extensive regulations of these substances in drinking water, ground water, soil and food over the past decade. PFAS are man-made chemicals used in a wide variety of commercial products like nonstick cookware, food packaging, paints, clothing, fire retardants and surfactants since the 1940's. Due to their inert nature, PFAS are very persistent and have been found to accumulate throughout the environment. Originally considered biologically inactive, recent research has revealed their toxicity to humans and wildlife leading to stricter global regulations restricting their levels in food, water, air and soil. US EPA Method 537.1 is a widely used method for the determination of selected PFASs in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry. This poster will discuss the validation of EPA 537.1 on the QSight 220 LC/MS/MS and the many pitfalls of implementing this method. The QSight 220 demonstrates excellent sensitivity, precision and accuracy running EPA 537.1 that provides results for PFAS that lower than any current regulatory limits.

2 Equipment and Materials

All native analyte standards, surrogates and internal standards were purchased from Wellington Labs (Table 1) and solvents were acquired from Millipore Sigma. Solid Phase Extraction (SPE) cartridges (styrene-divinylbenzene, 6-mL, 500-mg) were obtained from Phenomenex. All SPE sample was performed on a 12-position vacuum manifold from PerkinElmer specially modified with linear low-density polyethylene (LLDPE) tubing to eliminate PFAS contamination.

Figure 1: PerkinElmer LX-50 UHPLC and QSight 220 MS/MS

Table 1: Names and acronyms of analytes, surrogates and internal standards used in EPA Method 537.1

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-d_3-perfluoro-1-octanesulfonamidoacetic acid	d3-NMeFOSAA

All LC/MS/MS experiments were conducted on the PerkiElmer LX-50 UHPLC system coupled to the Qsight 220 MS/MS detector (Figure 1). PerkinElmer Brownlee SPP columns were used for both the delay and analytical columns (Table 2). All instrument control, data acquisition and data processing were performed using Simplicity™ software.

3 Remediation of PFAS Background

PFAS compounds are ubiquitous in the environment and the laboratory since these materials are commonly used in many products including materials used to construct SPE apparatus and LC/MS/MS systems. Careful steps were taken to reduce or eliminate background levels of PFAS making possible the measurement of the PFAS target analytes at parts per trillion levels. The SPE system used in this study was modified to eliminate any parts constructed of polytetrafluoroethylene (PTFE) or PTFE copolymers. The PFAS background generated from the mobile phase solvents and UHPLC pump were remediated by the use of a delay column installed between the pump mixer and the autosampler valve (Figure 2). PFAS contamination arising from the LX-50 Autosampler was remediated by replacing all PTFE based tubing with PEEK Tubing.

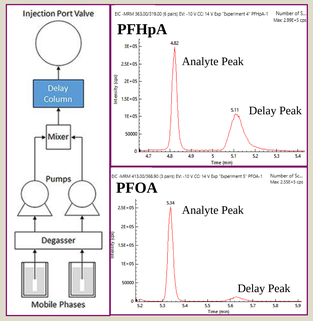

Figure 2: Schematic diagram of the position of the Delay Column installed between the pump and autosampler to capture any PFAS compounds coming from mobile phase solvents or pump materials (left). Two example MRM chromatograms demonstrating the separation of system background PFAS (Delay Peak) from analyte/standard PFAS (Analyte Peak).

4 Experimental

MS Method: MS/MS multiple reaction monitoring (MRM) experiments were developed for all analytes, surrogates and internal standards by syringe pump infusion directly into the QSight MS/MS electrospray ionization source (ESI). All MRMs were in negative ion ESI mode and two MRMs were established to monitor quantifier and qualifier ions for each analyte. The optimized MS source condition are shown in Table 2.

Table 2: Optimized QSight 220 ESI negative ionization source parameters.

MS Source Conditions	
Electrospray Voltage	-3500
Drying Gas	110
Nebulizer Gas	400
Source Temperature	350 °C
HSID Temperature	280 °C
Detection Mode	Time Managed MRM

Table 3: LX-50 UHPLC conditions and gradient program.

UHPLC Conditions	
Analytical Column	Brownlee™ SPP C18 Column, 75 x 4.6mm, 2.7mm, (PN: N9308415)
Delay Column	Brownlee™ SPP C18 Column, 50 x 3.0mm, 2.7mm (PN: N9308408)
Mobile Phase A	10 mM ammonium acetate in water
Mobile Phase B	Methanol
Flow Rate	0.8 mL/min
Column Temperature	40 °C
Injection Volume	10 µL

UHPLC Method: The UHPLC gradient method was developed and optimized to decrease the runtime, limit matrix interference and fully separate the branch chain isomers of PFHxS, PFOS, NMeFOSAA and NEtFOSAA, as required in EPA 537.1. The recommended HPLC method in EPA 537.1 has a 37-minute runtime which was reduced to 10 minutes in this study (Figure 3). The UHPLC conditions are summarized in Table 3.

Sample Preparation: All laboratory reagent blanks (LRB), laboratory fortified reagent blanks (LFB), field duplicate (FD) samples and laboratory fortified sample matrix (LFSM) samples were extracted, concentrated and reconstituted in strict adherence to the SPE method described in EPA Method 537.1.

Analyte	Peak #	RT (min)
PFBS	1	3.54
PFHxA	2	4.15
HFPO-DA	4	4.34
PFHpA	6	4.78
PFHxS	7	4.77
ADONA	8	4.84
PFOA	9	5.30
PFOS	11	5.73
PFNA	13	5.74
9CI-PF3ONS	14	5.93
PFDA	15	6.13
NMeFOSAA	17	6.31
PFUnA	19	6.45
NEtFOSAA	20	6.47
11CI-PF3OUdS	22	6.56
PFDoA	23	6.72
PFTrDA	24	6.96
PDTA	25	7.16
$^{13}C_2$-PFHxA SSrl1	3	4.15
$^{13}C_3$-HFPO-DA SSrl2	5	4.34
$^{13}C_4$-PFOA SSrl3	16	6.12
d_5-NEtFOSAA SSrl4	21	6.46
$^{13}C_8$-PFOA ISrl1	10	5.29
$^{13}C_4$-PFOS ISrl2	12	5.74
d_3-NMeFOSAA ISrl3	18	6.32

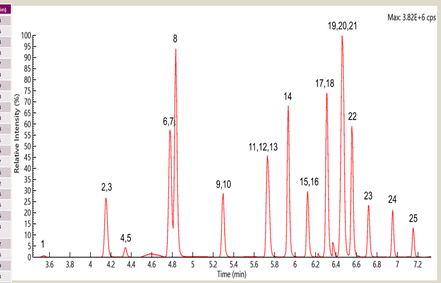

Figure 2: Total ion chromatogram of an 80 ng/L extracted LFB sample containing all method analytes, surrogates and internal standards.

5 Results and Discussion

Calibration standards were prepared at eight levels ranging from ~5 to 30,000 ng/L to evaluate the linear range and limits of detection (LOD) and quantitation of the instrument. Calibration standards were run in triplicate using internal standards for quantitation and fit using a non-weighted linear regression forcing the intercept through zero. All analytes and surrogates demonstrated excellent linearity with correlation coefficients (R^2) ≥0.996. **Table 4** shows the calibration ranges, R^2 values and instrument LOQs for analytes and surrogates and example calibration curves for PFOA and PFOS are shown in **Figure 3**.

Table 4: Calibration ranges,correlation coefficients and instrument LOQs for 8-point curves of analytes and surrogates.

Compound	Method Calibration Range (ng/L)[a]	R^2 [b]	Method LOQ (ng/L)[c]
PFBS	0.07 - 105.1	0.9994	0.027
PFHxA	0.02 - 118.8	0.9987	0.031
$^{13}C_2$-PFHxA	0.02 - 99.0	0.9989	0.004
$^{13}C_3$-HFPO-DA	0.27 - 99.0	0.9992	0.059
HFPO-DA	0.07 - 118.8	0.9985	0.089
PFHpA	0.02 - 118.8	0.9984	0.028
PFHxS	0.02 - 112.9	0.9998	0.005
ADONA	0.02 - 112.9	0.9990	0.003
PFOA	0.02 - 118.8	0.9998	0.034
PFOS	0.02 - 114.1	0.9974	0.012
PFNA	0.07 - 118.8	0.9993	0.034
9Cl-PF3ONS	0.02 - 111.1	0.9998	0.008
PFDA	0.32 - 118.8	0.9990	0.029
$^{13}C_4$-PFDA	0.02 - 99.0	0.9988	0.010
NMeFOSAA	0.02 - 118.8	0.9998	0.004
PFUnA	0.07 - 118.8	0.9968	0.047
NEtFOSAA	0.02 - 118.8	0.9968	0.003
d5-NEtFOSAA	0.07 - 396.0	0.9962	0.006
11Cl-PF3OUdS	0.02 - 112.3	0.9997	0.006
PFDoA	0.07 - 118.8	0.9963	0.027
PFTrDA	0.02 - 118.8	0.9959	0.021
PFTA	0.02 - 118.8	0.9967	0.057

a) Method calibration range is determined by multiplying the instrument calibration range by 1/250 to account for the SPE sample preparation/concentration.
b) R^2 values are the average of triplicate calibration curves.
c) Method LOQ was determined using the signal to noise ratio (S/N) of the peak from the lowest detectable calibration standard and extrapolating to the concentration at which the S/N = 10 then multiplying by 1/250 to account for the 250 to 1 sample concentration from the SPE extraction. LOQ is an estimate of sensitivity and cannot be used as method MRLs

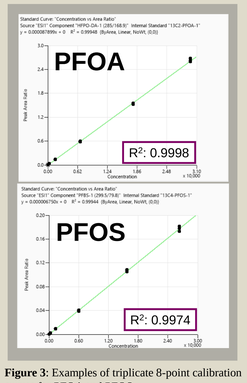

Figure 3: Examples of triplicate 8-point calibration curves for PFOA and PFOS.

Recovery studies were carried out by evaluating seven replicate LFBs per level at four fortification levels ranging from 0.3 – 80 ng/L. **Figure 4** summarizes the results of these experiments. These experiments verify that all recoveries were well within the limits of 70% to 130% recovery specified in the EPA Method 537.1. In fact, even the recoveries for LFBs fortified at 0.3 ng/L meet the requirements considering this fortification level is an order of magnitude below any state or federal mandates which require action limits of 7-10 ng/mL for specific contaminants like PFOA, PFOS and PFNA, confirming the validation of the methodology and instrumentation tested in this study.

Ten replicates LFBs at five fortification levels ranging from 0.2 – 80 ng/L were analyzed the were quantified to determine the Detection Limits (DL), Lowest Concentration Minimum Reporting Level (LCMRL) and Minimum Report Limit (MRL). The results are summarized in **Table 5** compared to the values reported in EPA 537.1. The LCMRLs and MRLs are lower than the benchmark levels reported in the EPA method demonstrating the suitability of this instrument and method.

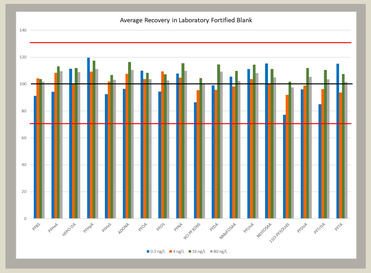

Figure 4: Plot of average recoveries of seven replicate LFBs for all method analytes at fortification concentrations of 0.3. 4. 16 & 80 ng/L. The black horizontal line represents 100% recovery and the red horizontal lines represent the 70% and 130% recovery limits required in EPA Method 537.1.

Analyte	Experimental DL (ng/L)[a]	EPA 537.1 DL (ng/L)[b]	Experimental LCMRL (ng/L)[a]	EPA 537.1 LCMRL (ng/L)[b]	Experimental MRL (ng/L)[a]
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

Table 5: Method detection limits (DL), 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.

Municipal tap waters (M1, M2 & M3) samples were collected and analyzed in three different locations in the Southeastern US. **Table 6** summarizes the results and demonstrates the method performance. Although, multiple analytes were detected above the MRLs at each location the reportable levels were below any current action limits.

Table 8. 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

6 Summary

This study demonstrates that the QSight 220 MS/MS system with the LX 50 UHPLC system is more than capable of analyzing drinking water samples for PFAS contamination according to EPA 537.1 for the analysis of 18 PFAS analytes. The validation studies prove the instrument meets sensitivity, linearity and repeatability requirements of the method and the manual SPE sample preparation exceeds the DL, LCMRL and MRL requirements of any current state and federal limits.