APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances by EPA Method 8327 Using the QSight 220 UHPLC/MS/MS

Introduction

Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals that have been widely used over the past 60 years in commercial and industrial products such as fire-fighting foams, water proofing treatments in clothing and furniture, household items, nonstick cookware, and paper.[1-3] PFAS are carbon-chain based compounds of different chain lengths where at least one, or all, of the hydrogen atoms are replaced with fluorine atoms.[2] Owing to the strong covalent C-F bonds, PFAS are thermally stable, chemically stable, and resistant to degradation. As a result of their unique physicochemical properties and potentially bioaccumulative capabilities, PFAS have been identified as persistent organic pollutants and considered contaminants of concern to human health and wildlife.[4,5] Recently, the effluent of domestic and/or industrial wastewater treatment plants (WWTPs) has been recognized as one of the main contributors to PFAS found in natural waters.[6]

The United States Environmental Protection Agency (US EPA) recently validated SW-846 Method 8327 for the analysis of PFAS in four non-potable aqueous matrices (reagent water, groundwater, surface water, and wastewater effluent) using external standard calibration and liquid chromatography/tandem mass spectrometry (LC/MS/MS).[7]

In this application note, we discuss the development of a fast and robust method for the analysis of all analytes listed in EPA Method 8327 (see Table 1) using a 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 PFAS analytes listed in EPA Method 8327 can be determined reliably by the QSight 220 LC/MS/MS system, with good recovery and precision at low limits of quantification (LLOQs) in reagent water, wastewater, downstream, and upstream surface water samples.

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Experimental

Materials and Reagents

Mixed primary PFAS standards and surrogates were obtained from Wellington Laboratories (Guelph, Ontario). The LC/MS grade methanol (MeOH) and acetic acid were obtained from Sigma Aldrich, and the LC/MS grade water and ammonium acetate were obtained from Fisher Scientific. The syringe filters used were Phenex™-Regenerated Cellulose (RC) 0.2 µm, 26 mm filters. The LC autosampler vials used were amber glass vials from PerkinElmer, with polyethylene septum-less caps. The treated wastewater sample, as well as the upstream and downstream surface water samples, were obtained from the Grand River outflow of the Galt wastewater treatment plant in Cambridge, ON, Canada. The reagent water sample was sourced from Fisher Scientific. The PFAS analytes and surrogates are listed in Table 1.

Hardware and 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). All instrument control, data acquisition, and data processing were performed using PerkinElmer Simplicity™ 3Q Software.

Method Parameters

The LC method and MS source parameters are shown in Table 2. Two C18 columns were used in this study, one was a delay column (Brownlee, SPP C18, 50 x 4.6 mm, 2.7 µm) to separate and delay possible interferent PFAS compounds coming from the LC system, and the other was an analytical column (Brownlee, SPP C18, 100 x 3.0 mm, 2.7µm) used to separate the PFAS and any other interfering components. The LC gradient program is shown in Table 3.

Table 1. Target analytes, surrogates, CAS # and acronyms of PFAS analyzed.

Compounds	Acronym	CAS #	Surrogate	Acronym
Carboxylic Acids				
Perfluoro-n-Butanoic Acid	PFBA	375-22-4	Perfluoro-n-[$^{13}C_4$]Butanoic Acid	MPFBA
Perlfuoro-n-Pentanoic Acid	PFPeA	2706-90-3	Perfluoro-n-[$^{13}C_5$]Pentanoic acid	M5PFPeA
Perfluoro-n-Hexanoic Acid	PFHxA	307-24-4	Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]Hexanoic Acid	M5PFHxA
Perfluo-n-Heptanoic Acid	PFHpA	375-85-9	Perfluoro-n-[1,2,3,4-$^{13}C_4$]Heptanoic Acid	M4PFHpA
Perfluoroctanoic Acid	PFOA	335-67-1	Perfluoro-n-[$^{13}C_8$]Octanoic Acid	M8PFOA
Perfluoro-n-Nonanoic Acid	PFNA	375-95-1	Perfluoro-n-[$^{13}C_9$]Nonanoic Acid	M9PFNA
Perfluoro-n-Decanoic Acid	PFDA	335-76-2	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]Decanoic Acid	M6PFDA
Perfluoro-n-Undecanoic Acid	PFUnDA	2058-94-8	Perfluo-n-[1,2,3,4,5,6,7-$^{13}C_7$]Undecanoic Acid	M7PFUdA
Perfluoro-n-Dodecanoic Acid	PFDoDA	307-55-1	Perfluo-n-[1,2-$^{13}C_2$]Dodecanoic Acid	MPFDoA
Perfluoro-n-Tetradecanoic Acid	PFTeDA	376-06-7	Perfluoro-n-[1,2-$^{13}C_2$]Tetradecanoic Acid	M2PFTeDA
Perfluoro-n-Tridecanoic Acid	PFTriA	72629-94-8	-	-
Sulfonic Acids				
Potassium Perfluoro-1-Butanesulfonate	PFBS	375-73-5	Sodium Perfluoro-1-[2,3,4-$^{13}C_3$]Butanesulfonate	M3PFBS
Potassium Perfluorohexanesulfonate	PFHxS	355-46-4	Sodium Perfluoro-1-[1,2,3-$^{13}C_2$] Hexanesulfonate	M3PFHxS
Perfluorooctyl Sulfonic Acid	PFOS	1763-23-1	Sodium Perfluoro-1-[$^{13}C_8$]Octanesulfonate	M8PFOS
Sodium 1H, 1H, 2H, 2H-Perfluoro-1-Hexanesulfonate	4:2 FTS	757124-72-4	Sodium 1H, 1H, 2H, 2H-Perfluoro-1-[1,2-$^{13}C_2$]Hexanesulfonate	M2-4:2 FTS
Sodium 1H, 1H, 2H, 2H-Perfluorooctane Sulfonic Acid	6:2 FTS	27619-97-2	Sodium 1H, 1H, 2H, 2H-Perfluoro-1-[1,2-$^{13}C_2$]Octanesulfonate	M2-6:2 FTS2
Sodium 1H, 1H, 2H, 2H-Perfluorodecane Sulfonic Acid	8:2 FTS	39108-34-4	Sodium 1H, 1H, 2H, 2H-Perfluoro-1-[1,2-$^{13}C_2$] Decanesulfonate	M2-8:2 FTS
Sodium Perfluoro-1-Pentanesulfonate	PFPeS	2706-91-4	-	-
Perfluoheptane Sulfonic Acid	PFHpS	375-92-8	-	-
Sodium Perfluoro-n-Nonanesulfonate	PFNS	68259-12-1	-	-
Sodium Perfluo-1-Decanesulfonate	PFDS	335-77-3	-	-
Sulfonamides and Sulfonamidoacetic Acids				
N-methylperfluoro-1-Octanesulfoniamidoacetic Acid	N-MeFOSAA	2355-31-9	N-Methyl-d3-Perfluoro-1-Octanesulfonamidoacetic Acid	d3-N-MeFOSAA
N-ethylperfluoro-1-Octanesulfonamidoacetic Acid	N-EtFOSAA	2991-50-6	-	-
Perfluoro-1-Octanesulfonamide	FOSA	754-91-6	-	-

Table 2. LC Method and MS Source Conditions.

LC Conditions	
Analytical Column	PerkinElmer Brownlee SPP C18, 100 x 3.0 mm, 2.7µ m (PN: N9308410)
Delay Column	PerkinElmer Brownlee SPP C18, 50 x 4.6 mm, 2.7 µm (PN: N9308414)
Mobile Phase A	2.5 mM Ammonium Acetate in Water
Mobile Phase B	2.5 mM Ammonium Acetate in Methanol
Flow Rate	0.5 mL/min
Column Oven Temperature	40 °C
Auto Sampler Temperature	10 °C
Injection Volume	25 µL
Needle Wash 1	50:50 Methanol:Water
Needle Wash 2	95:5 Methanol:Water
MS Source Conditions	
Electrospray Voltage	-3000 V
Drying Gas	200
Nebulizer Gas	200
Source Temperature	350 °C
HSID Temperature	275 °C
Detection Mode	Time managed MRM

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 energy (CE), entrance voltage (EV), and lens voltage (CCL2) were optimized for the target compounds as shown in Table 4.

Table 3. LC Gradient Program.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	95	5
1.00	95	5
1.50	55	4
7.00	2	98
8.00	2	98
8.10	95	5
12.00	95	5

Table 4. Optimized MRM Parameters for the PFASs compounds and surrogates.

Analytes	Precursor Ion	Product Ion	Retention Time (min)	CE	EV	CCL2	Quantifier/ Qualifier
PFBA1	213.1	169.1	3.6	13	-9	36	Quantifier
MPFBA	217	172	3.6	14	-4	40	Quantifier
PFPeA1	263.1	219	4.33	12	-8	84	Quantifier
M5PFPeA	268	223	4.33	12	-12	45	Quantifier
PFBS1	299.1	80	4.45	63	-35	84	Quantifier
PFBS2	299.1	99	4.45	45	-19	84	Qualifier
M3PFBS	302	80	4.45	67	-28	80	Quantifier
4:2 FTS1	327	81	4.96	38	-4	65	Quantifier
4:2 FTS2	327	307	4.96	21	-2	65	Qualifier
M2-4:2 FTS	329	81	4.96	53	-36	60	Quantifier
PFHxA1	313.3	269.2	5.01	13	-10	50	Quantifier
PFHxA2	313.3	119	5.01	31	-6	50	Qualifier
M5PFHxA	318	273	5.01	12	-4	52	Quantifier
PFPeS1	349	80	5.07	73	-6	100	Quantifier
PFPeS2	349	99	5.07	47	-34	65	Qualifier
PFHpA1	363.1	319.1	5.61	14	-6	75	Quantifier
PFHpA2	363.1	169.1	5.61	23	-14	60	Qualifier
M4PFHpA	367	322	5.61	12	-10	60	Quantifier
PFHxS1	399.1	79.9	5.62	77	-22	85	Quantifier
PFHxS2	399.1	99	5.62	53	-20	80	Qualifier
M3PFHxS	402	80	5.62	84	-8	100	Quantifier
6:2 FTS1	427	81	6.09	65	-8	80	Quantifier
6:2 FTS2	427	407	6.09	29	-28	115	Qualifier
M2-6:2 FTS	429	409	6.09	28	-16	124	Quantifier
PFOA1	413.2	369.1	6.11	14	-2	65	Quantifier
PFOA2	413.2	168.9	6.11	24	-2	75	Qualifier
M8PFOA	421	376	6.11	15	-4	84	Quantifier

Table 4. Optimized MRM Parameters for the PFASs compounds and surrogates (continued)

Analytes	Precursor Ion	Product Ion	Retention Time (min)	CE	EV	CCL2	Quantifier/ Qualifier
PFHpS1	449	80	6.11	86	-18	90	Quantifier
PFHpS2	449	99	6.11	52	0	80	Qualifier
PFOS1	499.1	79.8	6.52	78	0	110	Quantifier
PFOS2	499.1	99	6.52	49	-10	85	Qualifier
M8PFOS	507	80	6.52	107	-22	140	Quantifier
PFNA1	463.1	419.1	6.54	15	-20	75	Quantifier
PFNA2	463.1	218.9	6.54	24	-12	85	Qualifier
M9PFNA	472	427	6.54	15	-14	76	Quantifier
PFNS1	549	80	6.88	96	-24	110	Quantifier
PFNS2	549	99	6.88	62	-34	130	Qualifier
PFDA1	513.1	469.1	6.9	15	-4	90	Quantifier
PFDA2	513.1	219	6.9	24	-6	80	Qualifier
M6PFDA	519	474	6.9	15	0	88	Quantifier
8:2 FTS1	527	81.1	6.9	73	-40	115	Quantifier
8:2 FTS2	527	507.1	6.9	35	-28	95	Qualifier
M2-8:2 FTS	529	81	6.9	34	-53	108	Quantifier
N-MeFOSAA1	570.2	419.2	7.07	27	-2	90	Quantifier
N-MeFOSAA2	570.2	483.2	7.07	20	-26	100	Qualifier
d3-N-MeFOSAA	573	419	7.07	26	-26	116	Quantifier
PFDS1	599.1	80.1	7.18	138	-2	175	Quantifier
PFDS2	599.1	98.9	7.18	62	-14	150	Qualifier
PFUnDA1	563.2	519.1	7.2	16	-2	100	Quantifier
PFUnDA2	563.2	169.1	7.2	28	-22	115	Qualifier
M7PFUdA	570	525	7.2	15	-16	88	Quantifier
N-EtFOSAA1	584.1	419.2	7.22	27	-22	125	Quantifier
N-EtFOSAA2	584.1	169	7.22	22	-8	85	Qualifier
FOSA1	498	78	7.31	92	-40	110	Quantifier
PFDoDA1	613.1	569.1	7.48	17	-9	100	Quantifier
PFDoDA2	613.1	169.1	7.48	35	-11	96	Qualifier
MPFDoA	615	570	7.48	17	-14	104	Quantifier
PFTriA1	663.1	619.1	7.71	16	-1	108	Quantifier
PFTriA2	663.1	169.1	7.71	38	-1	124	Qualifier
PFTeDA1	713.1	669.1	7.9	17	-4	120	Quantifier
PFTeDA2	713.1	169.1	7.9	44	-3	120	Qualifier
M2PFTeDA	715	670	7.9	17	-14	108	Quantifier

Standard and Sample Preparation

Standard Preparation

The primary standard solution was diluted to prepare working standards as per Section 7.4 of EPA Method 8327, with 95/5 acetonitrile:water as the diluent. The working standard solutions were then diluted with 50/50 methanol:water, with 0.1% acetic acid to make a nine-point calibration ranging from 5 to 200 ng/L. Filtration was not performed on the calibration standards.

Water Sample Preparation

The water samples were prepared as described in EPA Method 8327. Briefly, a water sample was prepared by extraction with methanol containing 0.1% acetic acid in 50/50 v/v ratio. The sample was then vortexed and filtered through a 0.2 µm syringe filter directly into an autosampler vial for LC/MS/MS analysis.

Quality Control Sample Preparation

To obtain reliable sample results, it is critical to avoid any contamination of the samples during sample collection, sample preparation and sample analysis.

To ensure the absence of PFAS in samples, a Laboratory Reagent Blank (LRB) was prepared on each day of analysis to test for possible contamination from reagents, glassware, and materials used for the sample preparation process. The LRB sample was prepared by the same procedures as the water sample preparation described above, using reagent water (LC/MS grade water) as sample. The values of LRB should be close to zero or at least less than the LLOQ of the method. Otherwise, an investigation on the source of contamination must be carried out.

To study possible analyte loss or contamination during sample preparations, a Laboratory Fortified Blank (LFB) sample (or method blank as described in the EPA Method) was prepared per day or per work shift. An LFB sample was prepared by following the same water sample preparation procedures described above, using a reagent water spiked with a known amount of analyte (surrogates in this study) solution. One of the advantages of using surrogates in the LFB is that they are not naturally found in any water samples and can be used to further evaluate PFAS contamination during sample preparation processes. However, the isotope purity of the surrogates must be high (close to 100%) and any parent PFAS residuals (if any) in the surrogates must not be detectable, or at least below the LLOQ. Therefore, monitoring an LFB sample prepared with surrogates and PFAS-free reagent water during the method validation can examine both the recovery of the method for surrogates, and the isotope purity of the surrogates.

To evaluate sample matrix effects and analyte recovery from real water sample matrices, Laboratory Fortified Matrix (LFM) samples were prepared per day or per work shift. An LFM sample was prepared by following the same water sample preparation procedures as described above, using a real water sample spiked with a known amount of analyte. The percent recovery is calculated by comparing the difference of the spiked (LFM sample) and non-spiked water sample results and the expected (spiked) value. During method validation, three LFM samples were prepared using an upstream and a downstream surface water sample, as well as a wastewater sample. Each matrix was spiked with 160 ng/mL of surrogates, and then the three LFM samples were prepared by following the same water sample preparation procedures described above. It is recommended to inject a middle level calibration standard after analyzing a batch of 10 water samples to check instrument performance.

Results and Discussion

Linearity and Lower Limits of Quantitation (LLOQ)

Calibration curves were used to assess linearity and limits of quantification for all PFAS targets and surrogates. Nine-point calibration curves were created from three replicate injections at each concentration level using a 1/x weighted linear regression, without forcing through zero in the concentration range of 5 – 200 ng/L. Excellent linearity was achieved over the studied range of concentration, with correlation coefficient values (R^2) greater than 0.99 for all analytes and surrogates, as shown in Table 5. Figure 1 shows representative calibration curves for analytes (PFBA, PFPeA, and PFHxS) and surrogates (M6PFDA, M5PFPeA, and M2PFTeDA). The values for %RSD for each target compound at 5 ng/L are also listed in Table 5.

The lower limit of quantitation (LLOQ) for each target analyte was determined as the lower point on the calibration curve (ng/L in this work) verified to give a signal-to-noise ratio (S/N) that is greater than three, with accuracy in the range of 50-150%, and %RSD ≤ 20%. Figure 2 shows an overlay of the TICs for the three injections performed at 5 ng/L. All compounds presented a S/N greater than three and % RSDs ≤ 20%.

Figure 3 illustrates an overlay of the extracted ion chromatograms (EICs) of the 24 PFAS analytes spiked into a reagent water sample.

Table 5. R^2, % RSD, and S/N for the lowest calibration standard (LLOQ) 5 ng/L for target analytes and surrogates in EPA Method 8327.

Analyte	R^2	%RSD	S/N Ratio
PFBA	0.9989	10	12
MPFBA	0.9997	3	168
PFPeA	0.9990	15	10
M5PFPeA	0.9991	14	93
PFBS	0.9995	3	77
M3PFBS	0.9994	0	215
4:2 FTS	0.9986	5	42
M2-4:2 FTS	0.9975	15	40
PFHxA	0.9964	4	28
M5PFHxA	0.9986	9	63
PFPeS	0.9980	6	76
PFHpA	0.9990	4	21
M4PFHpA	0.9990	8	123
PFHxS	0.9993	8	12
M3PFHxS	0.9988	7	102
6:2 FTS	0.9974	20	48
M2-6:2 FTS	0.9971	17	30
PFOA	0.9978	14	15
M8PFOA	0.9989	6	64
PFHpS	0.9985	15	82
PFOS	0.9988	8	14
M8PFOS	0.9986	9	87

Analyte	R^2	%RSD	S/N Ratio
PFNA	0.9981	7	9
M9PFNA	0.9989	16	66
PFNS	0.9982	18	88
PFDA	0.9971	8	8
M6PFDA	0.9983	5	47
8:2 FTS	0.9982	6	19
M2-8:2 FTS	0.9962	14	10
N-MeFOSAA	0.9959	17	13
d3-N-MeFOSAA	0.9959	16	11
PFDS	0.9980	10	47
PFUnDA	0.9975	12	5
M7PFUdA	0.9984	19	22
N-EtFOSAA	0.9969	17	7
d5-N-EtFOSAA	0.9967	20	11
FOSA	0.9990	5	269
PFDoDA	0.9979	14	13
MPFDoA	0.9977	4	17
PFTriA	0.9962	18	4
PFTeDA	0.9961	9	4
M2PFTeDA	0.9963	13	16

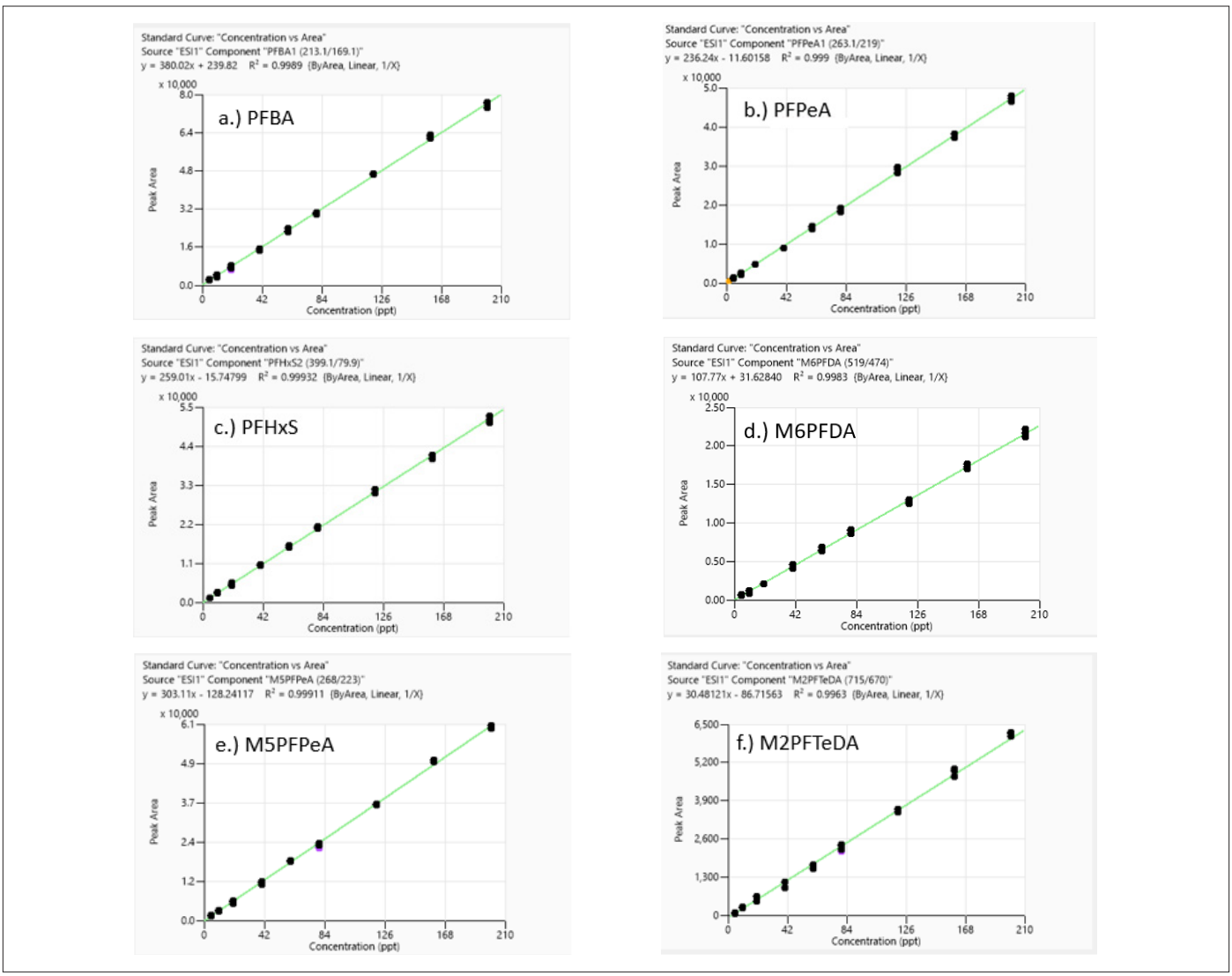

Figure 1. Calibration curves for representative analytes (PFBA, PFPeA, PFHxS) and surrogates (M6PFDA, M5PFPeA, M2PFTeDA) in triplicates.

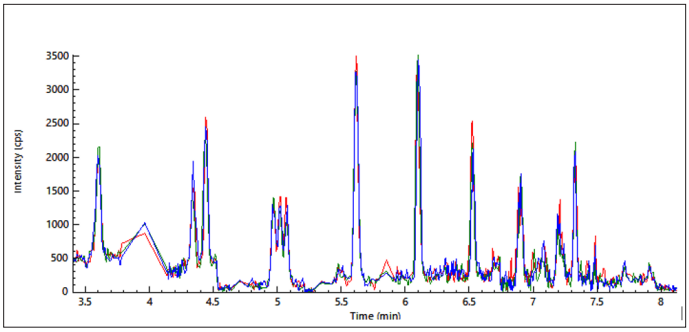

Figure 2. TIC overlay of all PFAS in EPA Method 8327 for all three injections at the lowest level calibrator, 5 ng/L.

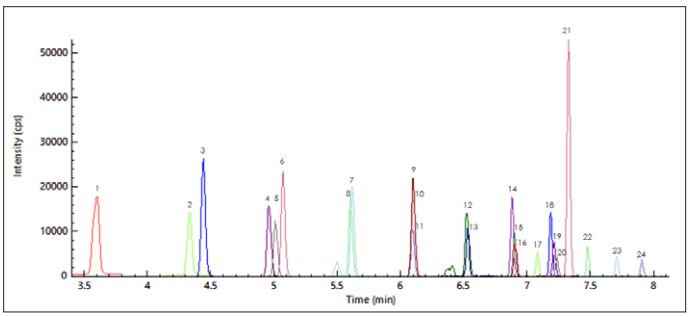

Figure 3. Overlay of extracted ion chromatograms for the 24 PFAS analytes at 160 ppt.

Contamination, Carryover Effect, and Sample Matrix Effects

One of the major challenges associated with trace analysis of PFAS is the possibility of contamination from the materials used in both the preparation and analysis of the samples, i.e., glassware, pipettes, tubing, or PTFE-coated portions of the LC system, to name a few. A prime example, and one of the most common sources of this contamination, is the presence of fluoropolymers in various laboratory consumables. As per EPA Method 8327, blanks are acceptable from a quality standpoint as long as the concentration of target analytes in the blank are less than one half of the lowest limit of quantitation (LLOQ).

To eliminate or reduce these interferences from the LC/MS/MS system, a delay column was placed between the mobile phase mixer and the autosampler injection valve. By doing so, the PFAS compounds in the sample are well separated from the PFAS contaminants from the mobile phase solvent lines. For an example, please refer to Figure 1 of our previous application notes.[8-9] Furthermore, all materials used in this study were tested prior to running the samples to check for PFAS contamination through the injection of blank samples. An LRB was prepared and injected each time a new material was introduced, whether it be from the introduction of new autosampler vials and caps, to the introduction of new pipette tips used in sample preparation, or to the opening of a new bottle of solvents. No PFAS contamination was found in any of the LRB samples, thus confirming that all supplies used were free of PFAS contamination. During method validation, the LFB samples were prepared and the recoveries were determined. Good recoveries of the surrogates (93% - 103%) were obtained from the LFB samples (as shown in Table 6), indicating no analyte loss or contamination during sample preparations.

During the sequence, a blank was also injected in between the injections for standards and samples to monitor any carryover effects. As shown in Figure 4, no carryover was observed in either the LRB or LFB samples. The figure also demonstrates the absence of PFAS contamination from the instrument and materials used during analysis.

Sample matrix effects (MEs) are one of the main challenges in LC/MS/MS method development and validation. In this study, sample MEs were evaluated by comparing the responses obtained from surrogates prepared in different water sample matrices to those obtained from surrogates prepared in LC/MS grade water, or by evaluating the recoveries of surrogates spiked in various water sample matrices (LFM samples) using the external calibration method. As shown in Table 7, all surrogates studied show good recoveries from the water sample matrices, demonstrating that sample MEs are not significant. These results are in line with other studies in the literatures on drinking water and surface water analysis, in that the sample matrix effects are less than 20% and external calibration method can be applied for quantification without significant error.[8-11]

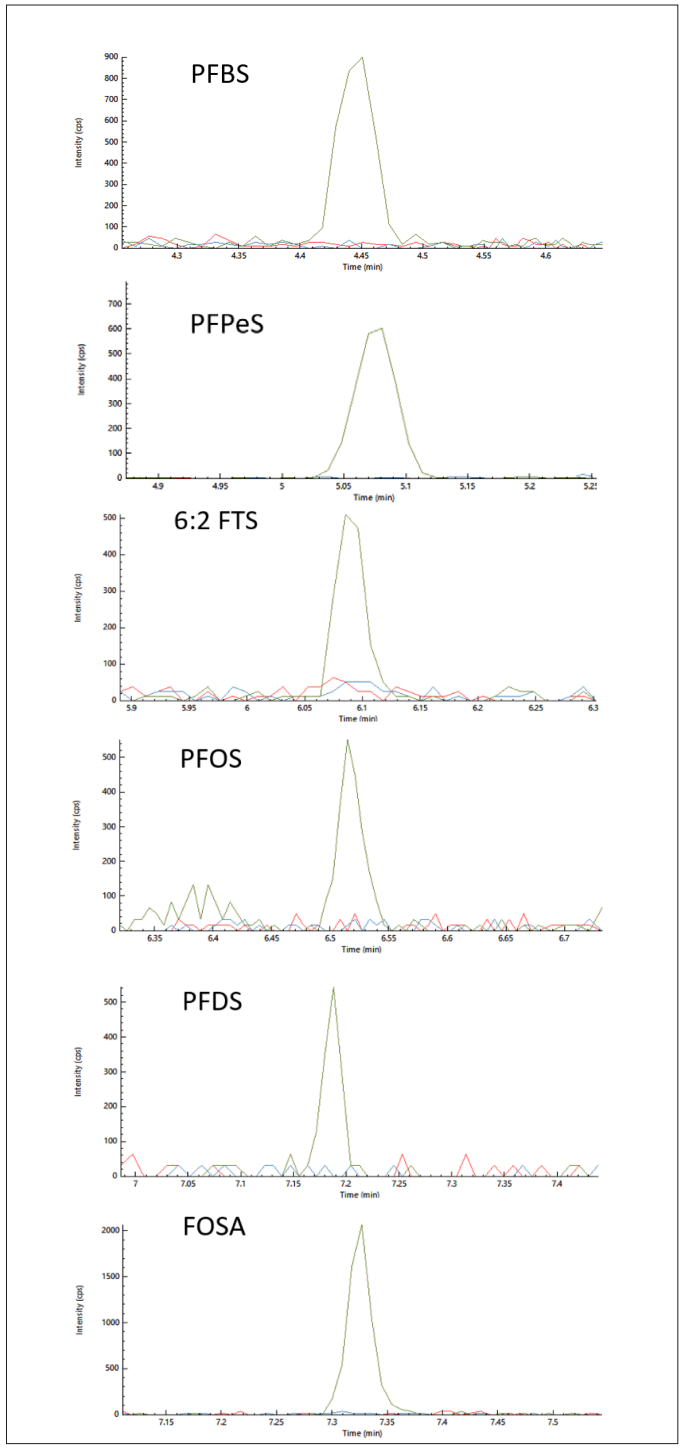

Figure 4. Overlays of MRM chromatograms of analytes in LRB (in red), LFB (in blue) and a standard (5 ng/mL in green).

Method Precision and Accuracy

Method precision was assessed based on replicate analyses of spiked water samples (n = 5). The precision was then calculated based on the coefficient of variation (RSD%) of the collected data. Method accuracy assesses how close the experimental value is to the expected value. Method accuracy was evaluated by the recovery of a known amount of analyte spiked into a sample. As shown in Table 7, the recovery for the PFAS surrogates spiked in wastewater and surface water samples were within 82 - 104%, with RSD ≤ 10%, demonstrating good accuracy and precision of the method. Similarly, as shown in Table 8, the recoveries of analytes and surrogates were within 70 - 130% from the spiked reagent water samples, with RSD less than 20%.

Figure 5 shows TIC overlays of the reagent water spiked with analytes at a) 40 ng/L, b) 80 ng/L, and c) 160 ng/L. Figure 6 shows the TIC overlays of blank water samples (wastewater, downstream surface water, and upstream surface water) and the spiked water samples.

Table 6. Average concentration and % recovery results from Laboratory Fortified Blank spiked with 160 ng/L PFAS surrogates.

Analyte	Laboratory Fortified Blank (LFB)	
	Average Concentration(ng/L)	Average Recovery(%)
MPFBA	153.07	96
M5PFPeA	149.80	94
M3PFBS	149.31	93
M2-4:2 FTS	152.86	96
M5PFHxA	159.08	99
M4PFHpA	149.87	94
M3PFHxS	151.46	95
M2-6:2 FTS	154.38	96
M8PFOA	153.39	96
M8PFOS	153.65	96
M9PFNA	148.94	93
M6PFDA	156.74	98
M2-8:2 FTS	165.53	103
d3-N-MeFOSAA	161.30	101
M7PFUdA	161.22	101
d5-N-EtFOSAA	152.43	95
MPFDoA	161.09	101
M2PFTeDA	162.41	102

Table 7. PFAS surrogate average concentration, % RSD, and % recovery results from wastewater, downstream surface water, upstream surface water samples spiked with 160 ng/L.

	Wastewater 160 ng/L			Downstream Surface Water 160 ng/L			Upstream Surface Water 160 ng/L		
Analyte	Average Concentration (ng/L)	%RSD	% Average Recovery	Average Concentration (ng/L)	%RSD	% Average Recovery	Average Concentration (ng/L)	%RSD	% Average Recovery
MPFBA	155.24	0	97	154.74	3	97	151.18	2	94
M5PFPeA	154.35	2	96	152.68	2	95	151.26	2	95
M3PFBS	152.02	3	95	155.83	2	97	150.10	2	94
M2-4:2 FTS	161.66	4	101	164.29	6	103	161.59	5	101
M5PFHxA	166.43	3	104	155.96	8	97	147.78	8	92
M4PFHpA	151.75	4	95	147.16	3	92	142.78	4	89
M3PFHxS	150.83	5	94	151.42	4	95	152.16	3	95
M2-6:2 FTS	154.60	7	97	148.48	2	93	147.82	2	92
M8PFOA	158.37	3	99	150.61	3	94	144.67	3	90
M8PFOS	152.85	3	96	153.23	2	96	152.65	3	95
M9PFNA	158.92	3	99	148.72	4	93	140.38	4	88
M6PFDA	148.20	3	93	147.51	5	92	145.52	8	91
M2-8:2 FTS	158.33	6	99	151.90	6	95	153.40	5	96
d3-N-MeFOSAA	164.51	10	103	157.85	8	99	161.48	7	101
M7PFUdA	160.20	8	100	154.82	8	97	142.13	8	89
d5-N-EtFOSAA	131.97	10	82	140.66	10	88	150.12	7	94
MPFDoA	150.35	5	94	146.64	4	92	147.61	5	92
M2PFTeDA	157.42	9	98	163.30	8	102	149.84	8	94

Table 8. PFAS analyte and surrogate average concentration, % RSD, and % recovery results from Reagent Water Samples spiked with 40 ng/L, 80 ng/L, and 160 ng/L.

	40 ng/L			80 ng/L			160 ng/L		
Analyte		160 ng/L	% Average Recovery	Average Concentration (ng/L)	%RSD	% Average Recovery	Average Concentration (ng/L)	%RSD	% Average Recovery
PFBA	35.18	6	88	72.24	1	90	140.62	8	88
MPFBA	38.25	3	96	73.18	3	91	150.08	2	94
PFPeA	40.51	6	101	74.89	5	94	144.27	5	90
M5PFPeA	37.83	3	95	71.66	4	90	147.85	2	92
PFBS	39.04	9	98	73.65	3	92	140.77	5	88
M3PFBS	37.04	5	93	72.31	4	90	147.67	2	92
4:2 FTS	41.26	7	103	80.21	9	100	155.22	4	97
M2-4:2 FTS	39.07	8	98	75.24	10	94	157.07	5	98
PFHxA	37.32	10	93	74.71	7	93	145.58	7	91
M5PFHxA	37.58	10	94	73.14	10	91	149.76	5	94
PFPeS	37.86	5	95	75.27	10	94	140.83	8	88
PFHpA	41.81	8	105	77.44	4	97	143.01	9	89
M4PFHpA	36.71	6	92	70.22	6	88	146.67	3	92
PFHxS	38.43	6	96	71.20	3	89	137.04	9	86
M3PFHxS	36.36	5	91	71.33	4	89	142.35	4	89
6:2 FTS	38.40	8	96	74.38	6	93	152.85	10	96
M2-6:2 FTS	40.51	2	101	72.16	3	90	148.23	3	93
PFOA	37.89	5	95	74.60	6	93	140.22	10	88
M8PFOA	38.54	5	96	71.69	4	90	150.10	3	94
PFHpS	38.89	4	97	72.19	3	90	139.53	5	87
PFOS	39.17	2	98	72.52	5	91	140.30	9	88
M8PFOS	37.83	3	95	72.14	4	90	143.04	2	89
PFNA	38.48	10	96	75.88	7	95	141.53	8	88
M9PFNA	37.39	8	93	70.62	3	88	142.86	4	89
PFNS	37.71	4	94	72.66	5	91	134.60	7	84
PFDA	35.24	8	88	71.27	7	89	129.32	5	81
M6PFDA	35.54	4	89	66.04	9	82	139.08	3	87
8:2 FTS	38.42	7	96	74.09	10	93	132.21	5	83
M2-8:2 FTS	36.31	8	91	75.03	5	94	146.40	5	92
N-MeFOSAA	41.92	11	105	86.86	8	109	151.33	3	95
d3-N-MeFOSAA	37.06	10	93	66.10	9	83	151.33	8	95
PFDS	36.15	10	90	70.18	10	88	132.29	6	83
PFUnDA	41.66	8	104	79.54	8	99	139.53	9	87
M7PFUdA	38.30	11	96	65.59	2	82	148.31	6	93
N-EtFOSAA	34.85	9	87	80.84	9	101	138.02	10	86
d5-N-EtFOSAA	32.92	10	82	70.79	10	88	140.35	10	88
FOSA	37.79	7	94	72.69	3	91	135.63	6	85
PFDoDA	35.40	6	89	69.91	10	87	125.01	6	78
MPFDoA	34.12	2	85	65.97	7	82	132.95	7	83
PFTriA	39.91	10	100	74.50	9	93	128.37	8	80
PFTeDA	34.72	9	87	78.55	7	98	122.65	8	77
M2PFTeDA	36.91	4	92	61.74	9	77	114.81	9	72

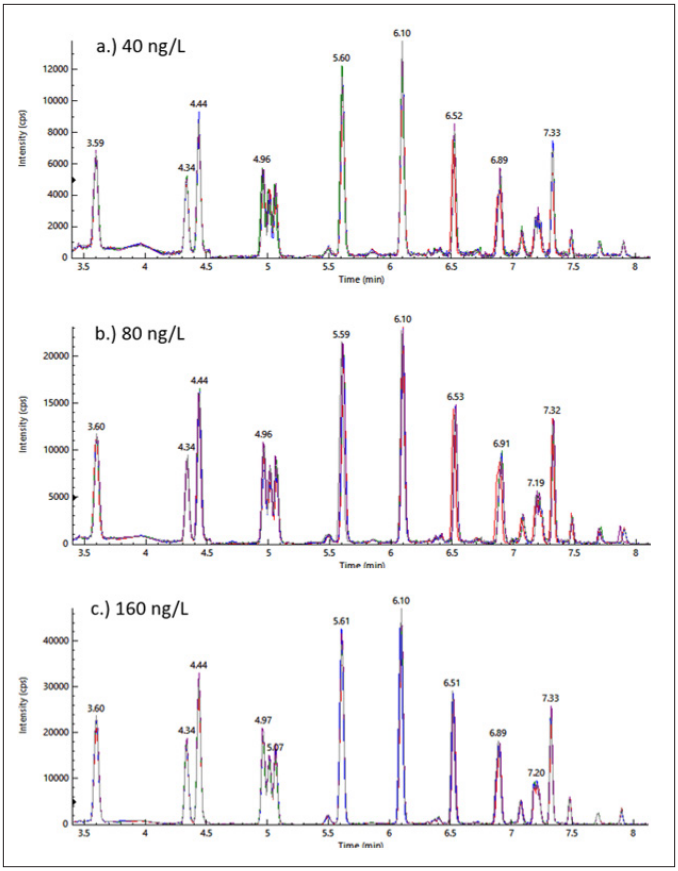

Figure 5. TIC overlay of all replicates of Reagent Water spiked at 40 ng/L, 80 ng/L, and 160 ng/L.

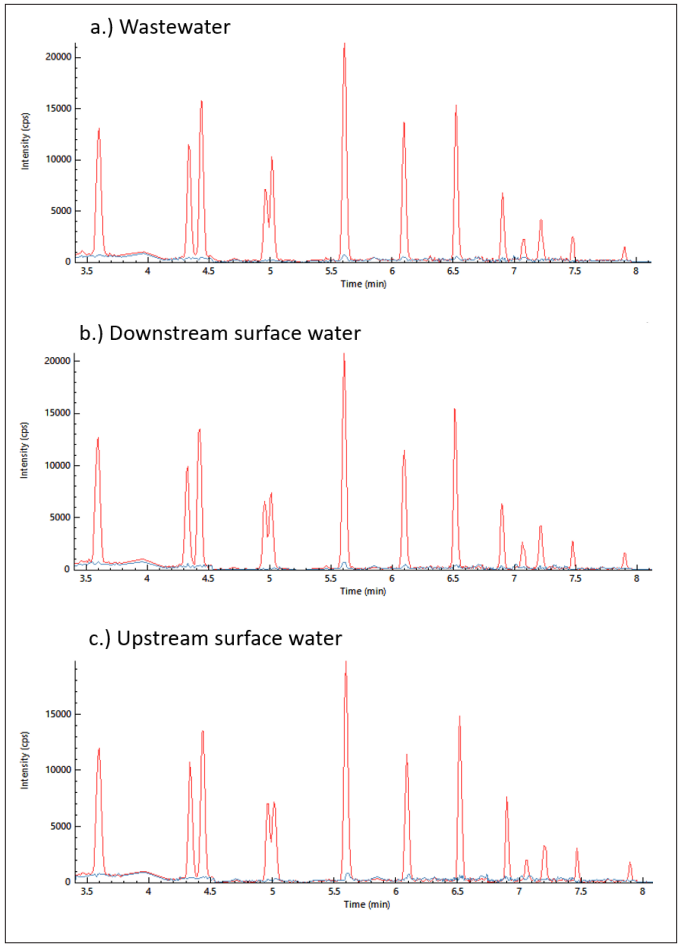

Figure 6. TIC overlay of blank water sample (in blue) and the spiked water samples (in red): a. Wastewater, b. Downstream surface water, and c. Upstream surface water.

Water Sample Analysis

The validated method was applied to the analysis of PFAS in four water samples. Among the 24 PFAS analytes, five were found in the wastewater, downstream surface water, and upstream surface water samples, with the results presented in Table 9. Some are below the LLOQ of the method, but they could still be detected. The identity of the analytes in the samples were confirmed by comparing the analyte retention time and ion ratios of the qualifier ions against the quantifier ions in the samples with those in the reference standards. This is illustrated in Figure 7, where the ion ratio of the qualifier ions against quantifier ions and the retention time in wastewater sample for PFHpA is consistent with the reference standards sample, therefore confirming the existences of the analytes in the water samples. No PFAS analytes were detected in the reagent water sample.

Table 9. The Measured PFAS Results from the Tested Wastewater, Downstream Surface Water, and Upstream Surface Water Samples in ng/L.

Analyte	Wastewater (ng/L)	Downstream Surface Water (ng/L)	Upstream Surface Water (ng/L)
PFPeA	15.30	6.55	3.47
PFBS	5.90	3.13	3.44
PFHpA	12.94	3.94	3.79
PFHxS	15.10	15.79	15.18
PFOA	2.88	0.95	0.87

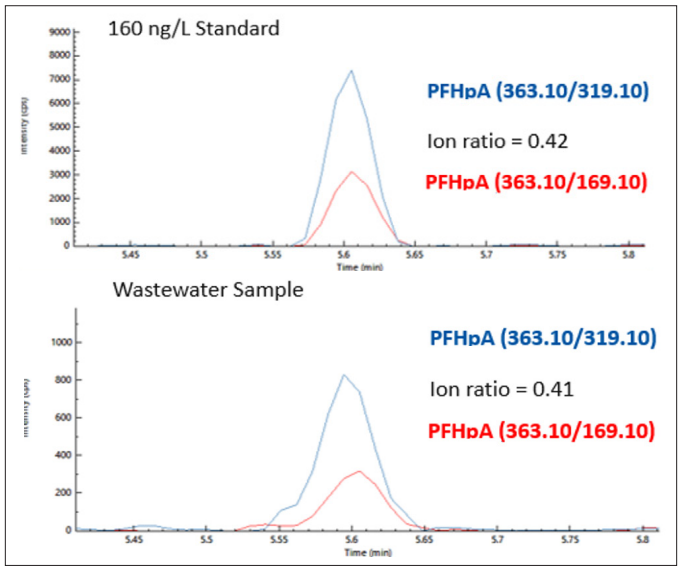

Figure 7. Chromatograms of PFHpA in 160 ng/L standard and a wastewater sample.

Conclusion

This application note reports an LC/MS/MS method for the determination of PFAS analytes and stable isotope-labelled surrogates listed in the US EPA Method 8327 using a PerkinElmer QSight 220 mass spectrometer. QSight 220 mass spectrometer. Excellent linearity was achieved for all PFAS analytes and surrogates with the R^2 values greater than 0.996. The calculated LLOQs were well below the suggested LLOQ values in Method 8327. The method was applied to the analysis of four different types of water samples: reagent water, wastewater, downstream wastewater, and upstream wastewater. The recoveries attained were between 72-109% for the reagent water samples spiked with PFAS analytes and surrogates. The recoveries of PFAS from wastewater, downstream surface water, and upstream surface water samples spiked with PFAS surrogates were between 82-104%. The calculated % RSDs were ≤ 15% for all the studied water samples. Therefore, the method can be used for PFAS analysis to achieve reliable and accurate results and meet or exceed requirements set by the EPA Method 8327.

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