Rapid and Sensitive Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances in Water by Direct Injection with QSight 420 UHPLC-MS/MS

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1 Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are synthetic compounds, widely used in industrials and consumer products. There is growing health concerns to these chemicals due to their highly toxic persistent properties. The widely used method for PFASs analysis is LC-MS/MS due to its high sensitivity and selectivity. Coupling SPE with LC-MS/MS is a popular approach and has been employed in EPA methods. Recently, a high throughput approach for monitoring PFASs has been appreciated using direct injection without SPE, which not only achieves high sample throughput, reduces time and cost, but also minimizes analyte loss and contamination from SPE processes, as demonstrated by this study using QSight 420 mass spectrometer coupled with UHPLC for trace PFASs analysis in drinking and surface water samples.

2 Experimental Conditions

Hardware and Software

Chromatographic separation of PFASs from potential interfering components was conducted by a PerkinElmer QSight LX50™ ultra-high-performance liquid chromatography (UHPLC) system and determination of PFASs was achieved using a PerkinElmer QSight® 420 triple quadrupole mass detector with a dual ionization source. All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q Software.

Method Parameters

LC Method and MS Source Conditions

The LC method and MS source parameters are shown in **Table 1** and the LC gradient program is shown in **Table 2**. Two C18 columns (Brownlee, SPP C18, 50 x 3mm, 2.7µm) were used in this study: one was used as a delay column to separate possible interferent PFASs coming from the LC system; another was used as analytical column to separate PFASs as well as any interfering components. The multiple reaction monitoring mode (MRM) transitions of PFAS and their optimized parameters are shown in **Table 3**. During method development, the retention times for PFASs were determined, then the potential interfering components from LC system and mobile phases were identified and separated from analyte peaks using a delay column. As shown in **Figure 1**, analyte peaks were well separated from the system contamination peaks by the delay column. Finally, the MS acquisition method was generated using Simplicity software in the time-managed-MRM module with the retention times and corresponding retention time windows for all PFASs.

Table 1: LC method and MS source conditions

LC Conditions	
Analytical Column	Brownlee, SPP C18, 50 x 3mm, 2.7µm (PN: N9308408)
Delay Column	Brownlee, SPP C18, 50 x 3mm, 2.7µm (PN: N9308408)
Mobile Phase A	5 mM ammonium acetate in water
Mobile Phase B	LC/MS grade methanol
Mobile Phase Gradient	See Table 2
Flow Rate	0.8 mL/min
Column Oven Temperature	30 ºC
Auto Sampler Temperature	15 ºC
Injection Volume	50 µL
Needle wash 1	50% methanol in water
Needle wash 2	95% methanol in water
MS Source Conditions	
ESI Voltage (Negative)	-2500 V
Drying Gas	110
Nebulizer Gas	400
Source Temperature	350 ºC
HSID Temperature	280 ºC
Detection mode	Time managed MRM

Table 2: LC Gradient Program

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	95	5
1.00	95	5
1.50	55	45
7.00	2	98
8.00	2	98
8.10	95	5
12.00	95	5

Standards, Solvents and Sample Preparation

Primary PFASs standards were obtained from Wellington Laboratories (Guelph, Ontario). LC-MS grade methanol (MeOH) and water were obtained from Fisher Scientific. A mixed intermediate standard solution was prepared in methanol by dilution of the primary standard solutions. The mixed intermediate standard solution was diluted with 50% methanol to make calibration standards ranging from 0.5 to 2000 ng/L (ppt). A variety of drinking water and surface water samples were analyzed in this study: bottled drinking water purchased from a local store; tap water obtained from two different cities in Ontario (Toronto and Kitchener); rain water collected from Kitchener, Ontario; river water samples from Japan and Ontario, Canada; and lake water samples from Lake Ontario, Canada. The water samples were analyzed directly after extraction with methanol and then analyzed without further pretreatment to minimize potential contamination.

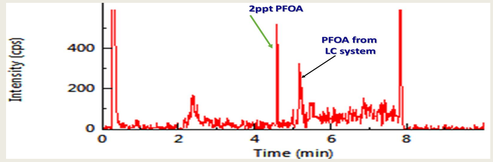

Figure 1: ***Chromatogram of PFOA in a 2 ng/L(ppt) standard solution and the delayed/isolated PFOA peak coming from LC system contamination.***

Table 3: Optimized mass dependent parameters for PFAS

Compound Name	Acronym	Q1 (amu)	Q2 (amu)	RT (min)	CE	EV	CCL2
Perfluorobutanoate	PFBA1	213.1	169.1	2.02	12	-14	124
	PFBA2	213.1	69.1	2.02	90	-14	124
Perfluoropentanoate	PFPeA1	262.9	218.9	2.63	12	-11	96
	PFPeA2	262.9	69.1	2.63	65	-12	88
Perfluorobutylsulfonate	PFBS1	299.1	98.9	2.74	42	-21	104
	PFBS2	299.1	80.1	2.74	50	-36	100
Perfluorohexanoate	PFHxA1	313.3	269.2	3.16	12	-10	112
	PFHxA2	313.3	119.0	3.16	32	-10	112
Perfluoroheptanoate	PFHpA1	363.1	319.1	3.71	13	-14	115
	PFHpA2	363.1	169.1	3.71	25	-14	115
Perfluorohexylsulfonate	PFHxS1	399.1	99.0	3.77	48	-20	128
	PFHxS2	399.1	79.9	3.75	59	-20	128
Perfluorooctanoate	PFOA1	413.2	369.1	4.22	14	-14	124
	PFOA2	413.2	168.9	4.22	25	-14	124
Perfluorononanoate	PFNA1	463.1	419.1	4.67	15	-12	168
	PFNA2	463.1	219.1	4.67	24	-12	164
Perfluorooctanesulfonate	PFOS1	499.1	80.0	4.68	90	-63	179
	PFOS2	499.1	99.0	4.68	60	-56	161
Perfluorodecanoate	PFDA1	513.1	469.1	5.57	16	-14	170
	PFDA2	513.1	219.1	5.57	25	-14	170
Perfluorodecylsulfonate	PFDS1	599.1	80.1	5.92	110	-14	230
	PFDS2	599.1	99.0	5.92	57	-14	240
Perfluoroundecanoate	PFUnDA1	563.2	519.1	5.93	16	-14	185
	PFUnDA2	563.2	219.1	5.93	27	-14	185
Perfluorododecanoate	PFDoDA1	613.1	569.1	6.24	15	-14	200
	PFDoDA2	613.1	169.1	6.24	45	-14	200
Perfluorotridecanoate	PFTriDA1	663.1	169.1	6.51	41	-14	220
	PFTriDA2	663.1	619.1	6.51	16	-14	220
Perfluorotetradecanoate	PFTeDA1	713.1	169.1	6.74	46	-14	240
	PFTeDA2	713.1	219.1	6.74	35	-14	240
Perfluorohexadecanoate	PFHxDA1	813.1	169.1	7.12	49	-20	240
	PFHxDA2	813.1	219.1	7.12	39	-20	240
Perfluorooctadecanoate	PFODA1	913.1	169.1	7.41	52	-14	245
	PFODA2	913.1	219.1	7.41	35	-14	245

Quality Control Sample Preparation

To test possible interference or contamination from reagents and glassware and from the sample preparation processes, a Laboratory Reagent Blank (LRB) was prepared per each work shift. The values of LRB should be close to zero or at least less than LOQ of the method. Otherwise, an investigation on the source of contamination must be carried out. The LRB sample was prepared by following the same procedures as for a real water sample preparation. To study possible analyte loss or contamination during sample preparations, a Laboratory Fortified Blank (LFB) was prepared per work shift. The LFB sample was prepared by following the same water sample preparation procedures spiked with a known amount of analyte solution. During method validation, LFB samples were prepared by spiking the analyte at three concentration levels (10, 100 and 1000 ng/L), respectively. To evaluate sample matrix effects and analyte recovery from real water sample matrix, a Laboratory Fortified Matrix sample (LFM) was prepared per work shift. The LFM sample was prepared by following the same water sample preparation procedures 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, the LFM samples were prepared using a river water sample matrix spiked at three concentration levels (10, 100, and 1000 ng/L), respectively.

3 Results and Discussion

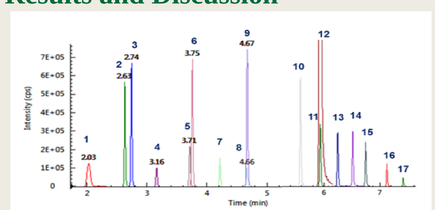

Figure 2: ***MRM chromatograms of the 17 PFASs***

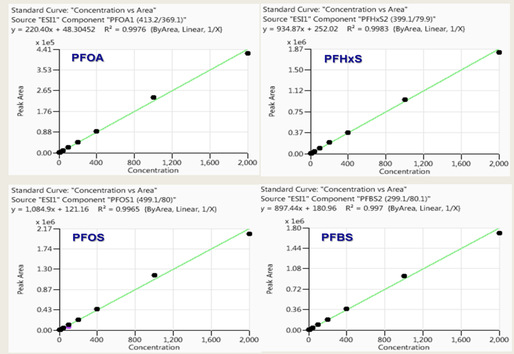

Figure 3: ***Example calibration curves with concentrations up to 2000 ng/L (ppt)***

Linearity, Limit of Quantification, QC Sample Results and Analyte Recovery

As shown in **Table 4** and **Figure 3**, good linearity was obtained for each analyte from low ng/L up to 2000 ng/L with regression coefficients (R^2) greater than 0.99 by external calibration method. The linear calibration ranges for all analytes are much wider than the suggested ranges (10 - 400ng/L) in the latest U.S. EPA method 8324. The limit of quantification (LOQ) of the method was estimated based on the signal to noise ratio (S/N ≥ 10) of analyte's quantifier ion. As shown in **Table 4**, the estimated LOQs ranged from 0.5 ng/L for PFOS to 40 ng/L for PFHxDA, which are all lower than the Lower Limit of Quantification (LLOQ) suggested in the U.S. EPA method 8324.

For QC sample analysis, after isolating the LC system contaminates by a delay column, no other interference or contamination from reagents and glassware was observed as demonstrated by the LRB sample results (LRB < LOQ). Good recoveries (close to 100%) were obtained for LFB samples, indicating no analyte loss or contamination during sample preparations (LRB and LFB data were not shown, but available upon request). Analyte recoveries from the spiked river water samples (LFM samples) are between 70.2 to 119% as shown in **Table 4**, demonstrated good accuracy of the method.

Sample Matrix Effects and Carryover

In this study, sample matrix effects were evaluated by comparing the slopes of calibration curves obtained from standards prepared in a river water sample matrix/methanol (1:1 by v/v) to slopes obtained from standards prepared in LC-MS grade water/methanol (1:1 by v/v). Sample matrix effects ME (%) for each analyte was calculated by the percentage difference between the slopes. When the percentage difference is positive, there is a signal enhancement effect, whereas a negative value indicates signal suppression effect. For example, the river water sample matrix had a signal enhancement effect for PFOA (ME = 11%), while for PFUnDA, a signal suppression effect was observed (ME = -15%). The results obtained in this study 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, because the studied drinking water and surface water matrices are relatively clean compared to industrial waste water.

The carryover effect was investigated by injecting a highest concentration calibration standard (2000 ng/L in this case) followed by a blank injection. The results showed that the carryover effect was less than the LOQ of the method.

Table 4: The Method's LOQ, Linear Range and Recovery Results.

Analyte	LOQ (ng/L)	Linear Range (ng/L)	Linearity (R^2)	Recovery (%) (Spiked 10ng/L)	Recovery (%) (Spiked 100ng/L)	Recovery (%) (Spiked 1000ng/L)
PFBA	4	4 - 2000	0.998	119	115	99.3
PFPeA	4	4 - 2000	0.995	101	117	100
PFBS	1	1 - 2000	0.995	108	102	101
PFHxA	4	4 - 2000	0.997	105	104	113
PFHpA	4	4 - 2000	0.999	116	111	104
PFHxS	1	1 - 2000	0.997	108	103	99.1
PFOA	1	1 - 2000	0.999	90.0	102	101
PFNA	10	10 - 2000	0.998	72.0	98.0	93.2
PFOS	0.5	0.5 - 2000	0.999	97.0	98.0	99.8
PFDA	10	10 - 2000	0.996	99.2	88.9	104
PFUnDA	10	10 - 2000	0.997	82.1	93.9	107
PFDoDA	10	10 - 2000	0.998	77.5	79.3	101
PFDS	1	1 - 2000	0.998	98.2	81.4	102
PFTriDA	20	20 - 2000	0.998	-	72.3	111
PFTeDA	10	10 - 2000	0.996	70.2	76.6	94.1
PFHxDA	40	40 - 2000	0.990	-	93.1	87.4
PFODA	4	4 - 2000	0.996	78.3	82.5	99.8

4 Sample Results

The developed LC-MS/MS method was applied for the analysis of PFASs in 15 water samples including drinking water, rain water, river water and lake water samples.

As shown in **Table 5**, among the seventeen PFAS compounds, six of them were found from river water, lake water and some tap water samples, although their amounts are much lower than any of the drinking water health advisory limits.

The identity of the analytes in these samples was confirmed by comparing the analyte retention time and the ion ratios of the qualifier ion against quantifier ion in the samples with those in the reference standards. For an example, as illustrated in **Figure 4**, the ion ratios of the qualifier ions against quantifier ions in a local river water sample (S6) for PFBS, PFHxS, PFOS and PFOA are consistent with those obtained from their reference standards, positively confirmed the existence of these analytes in the water sample. These results demonstrated the superior sensitivity and selectivity of the QSight 420 LC/MS/MS system for analysis of PFASs in water.

Table 5: The Measured PFASs Results from the Tested Water Samples in ng/L (ppt).

Analyte	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
PFBS	NQ	NQ	NQ	1.0	4.6	1.0	0.9	NQ	0.7	NQ	0.6	0.6	1.3	0.8
PFHxA	NQ	NQ	NQ	NQ	5.2	NQ	2.8	NQ	3.2	NQ	2.0	NQ	2.0	2.4
PFHpA	NQ	NQ	NQ	NQ	2.9	NQ	1.7	NQ	2.0	NQ	NQ	NQ	NQ	NQ
PFHxS	NQ	NQ	0.7	NQ	6.2	0.8	0.6	NQ	0.7	NQ	0.5	NQ	NQ	NQ
PFOA	0.8	1.4	1.8	NQ	4.7	0.9	2.3	NQ	2.4	1.3	1.8	1.0	NQ	1.4
PFOS	NQ	1.1	1.6	NQ	4.4	1.0	1.7	0.8	1.6	0.7	1.9	1.9	NQ	NQ

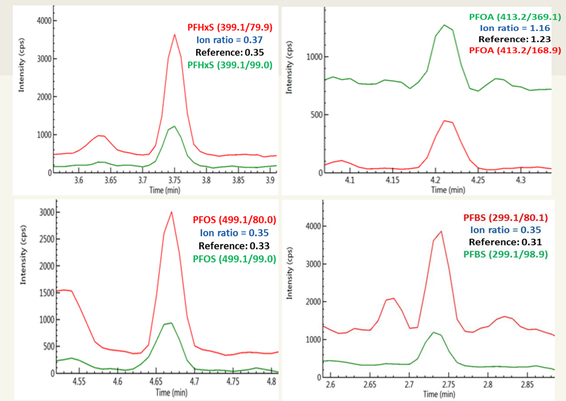

Figure 4: ***Chromatograms of, PFHxS, PFOA, PFOS and PFBS obtained from water sample S6 (Red, quantifier ion pair; and green, qualifier ion pair).***

5 Summary

A simple, rapid, sensitive and cost-effective LC-MS/MS method has been developed and validated for the analysis of 17 PFASs in drinking and surface water samples at sub to low ng/L (ppt) levels by coupling a LX-50 UHPLC system to a QSight 420 triple quadrupole mass spectrometer.

The method has been applied for real water sample analysis with good accuracy and high sensitivity, and it showed a wide linear dynamic range and eliminated the SPE sample preparation procedures. Therefore not only reduced the cost and saved time for sample analysis, but also prevented potential contamination from SPE sample preparation steps.

References:

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