APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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A Simple and Sensitive Method for Rapid Determination of PFOA and PFOS in Water Samples by Direct Injection UHPLC/MS/MS

Introduction

Per- and polyfluoroalkyl substances (PFASs), or Per- and polyfluorinated compounds (PFCs), represent a group of anthropogenic chemicals that have been produced and widely used in industrial applications and consumer products since the 1950s. The unique physical and chemical characteristics of these compounds (highly stable and resistant to degradation), along with their ubiquitous use, have led to the accumulation of PFAS in the environment, with growing concern of human exposure to these chemicals.[1-3] Among PFASs, perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been the most prevalent in the environment, and have thus attracted the most attention. Exposure to these chemicals in the United States and Europe is mainly from legacy use of PFAS containing products which are persistent in the environment. PFOA and PFOS have been found around the world in different water resources, including drinking, surface, ground and waste water.[1-6] High concentrations of PFASs were reported in water near crash and fire training military bases.[7] As PFAS production shifted from Western to Asian countries, such as China, increased amounts of PFOS and PFOA were detected in water samples collected from rivers and coastal drain outlets around the Bohai Sea, China.[8]

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PFOS and PFOA have been included in many advisory guidelines. For example, the United States Environmental Protection Agency (U.S. EPA) issued a health advisory of 70 parts per trillion (ppt) for PFOA and PFOS in drinking water.[9] The latest European Commission adopted proposal for PFASs are 100 ppt for an individual PFAS compound, and 500 ppt for total exposure to PFASs.[10]

The development of an efficient strategy for identification and quantification of PFASs is essential for risk assessment. The most widely used analytical method for PFAS monitoring is LC/MS/MS owing to its high sensitivity, selectivity and robustness. Multiple methods have been developed for the analysis of PFAS in environmental matrices, including:

- **United States Environmental Protection Agency (U.S. EPA):** In 2009, EPA published Method 537 for the determination of 14 PFASs in drinking water, and updated the method to EPA 537.1 in 2018 to include 4 new compounds.[11-12] Currently, EPA is working on Method 8327, designed to measure a group of 24 PFAS compounds in ground, surface, and waste water samples using LC/MS/MS with external calibration.[13]
- **International Organization for Standardization (ISO):** Developed in 2009, ISO 25101 is utilized for the determination of the PFOS and PFOA in unfiltered samples of drinking, ground and surface water (fresh water and sea water) by coupling solid phase extraction (SPE) with LC/MS/MS.[14]
- **The American Society for Testing and Materials (ASTM):** Two methods, ASTM D7979-17 for environmental waters,[15] and ASTM D7968-17a for soil,[16] were developed by ASTM for the determination of PFAS using multiple reaction monitoring (MRM) mass spectrometry.

For the determination of low levels of PFAS, it is necessary to utilize either a highly sensitive mass spectrometer, or a sample preparation technique that includes a concentration step. Coupling solid phase extraction (SPE) with LC/MS/MS has been one of the most popular approaches to PFAS analysis in aqueous samples, and has been employed in EPA Method 537 and 537.1, as well as ISO 25101. Recently, with the advancement and availability of highly sensitive mass spectrometers, a trend towards developing a high throughput analytical method for monitoring PFASs by direct injection without SPE has been appreciated, as shown in the ASTM methods[15-16] and various publications.[4-5,17] The direct injection approach can achieve higher levels of sample throughput, and reduce potential analyte loss and contamination caused by SPE sample preparation, as demonstrated by this study using PerkinElmer's QSight® 420 mass spectrometer, coupled with UHPLC for the determination of trace amount of PFOA and PFOS in drinking and surface water samples.

Experimental

Hardware/Software

Chromatographic separation was conducted utilizing a PerkinElmer QSight LX50 ultra-high-performance liquid chromatography (UHPLC) system, and detection was achieved using a PerkinElmer QSight 420 triple quadrupole mass spectrometer with a dual ionization source (ESI and APCI). All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q Software.

Method

Standard and Sample Preparation

Primary PFOS and PFOA standards were obtained from Wellington Laboratories (Guelph, Ontario). LC/MS grade methanol (MeOH) and water were obtained from Fisher Scientific. A mixed standard stock solution was prepared in methanol by dilution of the primary standard solutions. The mixed standard stock solution was diluted with methanol and water 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 surface water samples from Lake Ontario, Canada. Water samples were analyzed directly without any pretreatment.

LC Conditions and MS Parameters

The LC method and MS source parameters are shown in Table 1. Two C18 columns (Bownlee, SPP C18, 50 x 3 mm, 2.7 µm) were used in this study: one was used as a delay column to separate possible interferent perfluorinated components (PFCs) coming from the LC system; another was used as the analytical column to separate PFOA and PFOS, as well as any interfering components. The applied LC gradient program is shown in Table 2. MS source parameters, including gas flows, temperature and position settings, were optimized for maximum sensitivity. Compound-dependent parameters, such as collision energies (CE), entrance voltages (EV), and lens voltages (CCL2), were optimized for PFOA and PFOS and are shown in Table 3. During method development, the retention times for PFOA and PFOS were determined, and then the potential interfering PFC components from the LC system and mobile phases were identified and separated from analyte peaks using a delay column as shown in Figure 1. 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 PFOA and PFOS.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
Analytical Column	Bownlee, SPP C18, 50 x 3 mm, 2.7 µm (PN: N9308408)
Delay Column	Bownlee, SPP C18, 50 x 3 mm, 2.7 µm (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 °C
Auto Sampler Temperature	15 °C
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 °C
HSID Temperature	280 °C
Detection Mode	Time managed MRM

Table 2. LC Gradient Program.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	95	5
1.3	95	5
1.6	55	45
4.5	30	70
6.0	15	85
6.1	2	98
7.1	2	98
7.2	95	5
10.0	95	5

Table 3. Optimized MRMs and Compound-dependent Parameters for PFOA and PFOS.

Compound Name	Q1 Mass	Q2 Mass	RT	CE	EV	CCL2
PFOA-1	413.2	169.1	4.58	25	-14	124
PFOA-2	413.2	369.1	4.58	14	-14	124
PFOA-3	413.2	218.9	4.58	22	-14	125
PFOS-1	499.1	80.0	5.09	90	-63	179
PFOS-2	499.1	99.0	5.09	60	-56	161

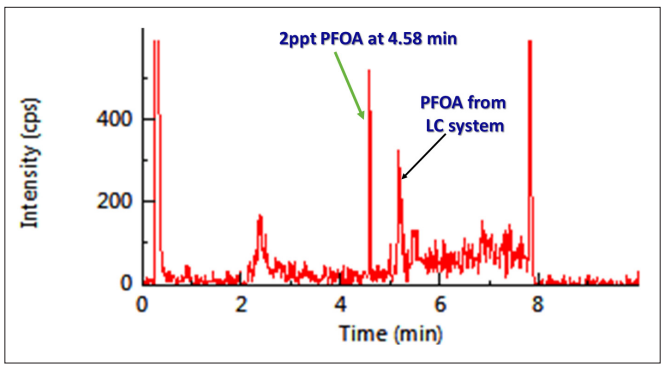

Figure 1. Chromatogram of PFOA in a 2 ng/L(ppt) standard solution and the delayed/isolated PFOA peak coming from LC system contamination.

Quality Control Sample Preparation

Avoiding contamination during sample collection, preparation and analysis are all crucial for reliable sample analysis. The following quality control measures were taken:

- **Laboratory Reagent Blank (LRB):** To test possible interference or contamination from reagents and glassware, and from the sample preparation processes, a Laboratory Reagent Blank (LRB) was prepared per day, or per each work shift. The values of the LRB should be close to zero, or at least less than the LOQ (limit of quantification) of the method. Otherwise, an investigation of the source of contamination must be carried out. An LRB sample was prepared by following the same procedures as those followed for normal sample preparation, using LC/MS grade water as the sample matrix.
- **Laboratory Fortified Blank (LFB):** To study possible analyte loss or contamination during sample preparations, a Laboratory Fortified Blank (LFB) sample was prepared per day, or per work shift. An LFB sample is prepared by following the same water sample preparation procedures, using LC/MS grade water spiked with a known amount of analyte solution. During method validation, LFB samples were prepared by spiking the analyte in three different concentration levels as shown in Table 4, and three replicates of the LFB samples at each level were prepared on three separate days.
- **Laboratory Fortified Matrix (LFM):** To evaluate sample matrix effects and analyte recovery from the real water sample matrix, a Laboratory Fortified Matrix sample (LFM) was prepared per day or per work shift. An LFM sample can be prepared by following the same water sample preparation procedures, using a real water sample spiked with a known amount of analyte. During method validation, the LFM samples were prepared using a river water sample matrix and three different concentration levels of analyte were spiked to the sample matrix.

The percent recovery for quality control samples is calculated by comparing the difference of the spiked (LFM sample) and non-spiked water sample results and the expected (spiked) value.

Table 4. The Spiked PFOA and PFOS amounts in QC Samples and the Recovery Results.

Sample ID	Spiked Amount (ng/L)		Recovered (ng/L)		Recovery (%)	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
LRB	0	0	0	0		
LFB1	1	1	0.91	0.87	91	87
LFB2	10	10	10.4	11.2	104	112
LFB3	100	100	98.6	103.7	99	104
LFM1	1	1	1.01	0.94	101	94
LFM2	10	10	9.24	10.5	92	105
LFM3	100	100	107.4	113.3	107	113

Results and Discussion

Contamination, Sample Matrix Effects and Carryover Effect

System contamination can be the main concern for LC/MS/MS analysis of PFASs. The contamination could be caused by mobile phases, containers, tubing, filters, fittings, pumps and pump seals used in the LC system. To remedy this contamination issue, a delay column as described earlier in the experimental section was inserted between the mixing valve of the pump and the autosampler to trap PFASs from the LC system. As shown in Figure 1, the PFOA analyte peak in a standard can be well separated from the PFOA system contamination peak by the delay column. Internal standard solutions could be another source of contamination, as identified in our previous study.[17] Such contamination can lead to errors in quantification, especially at low concentration levels. Thus, internal standards were not used in this study.

Sample matrix effects (MEs) are one of the main challenges in LC/MS/MS method development and validation, especially for complex sample matrices. In this study, sample MEs were evaluated by comparing the slopes of calibration curves obtained from standards prepared in a river water sample matrix, to slopes obtained from standards prepared in LC/MS grade water. The sample 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. The results show that this river water sample matrix has a signal enhancement effect for PFOA (ME = 11%), while for PFOS, a signal suppression effect was observed (ME = -3%). These results are in line with other literature studies on drinking and surface water analysis, in that the sample matrix effects are less than 20%, and an external calibration method can be applied for quantification without significant error,[4-5] as the studied drinking and surface water matrices are relatively clean as compared to industrial waste water.

The carryover effect was investigated by injecting the highest concentration calibration standard (2000 ng/L in this case) followed by a blank water (LC/MS grade water) injection. The results show that the carryover effect is minimum, or at least less than the LOQ of the method.

Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Method linearity was studied by external calibration method. As shown in Figure 2, good linearity was obtained from 0.5 to 2000 ng/L (ppt) for both PFOA and PFOS, with regression coefficients (R^2) greater than 0.998. To measure the lower level of analytes more accurately, calibration curves were also built at a low concentration range from 0.5 to 100 ng/L (ppt), and again, good linearity (R^2 ≥0.997) was achieved as shown in Figure 3. The limit of detection (LOD) and limit of quantification (LOQ) were estimated based on the signal to noise ratio (S/N ≥ 3 for LOD, and S/N ≥ 10 for LOQ) of the analyte's quantifier ion. As shown in Figure 4, the signal to noise ratios for both PFOA and PFOS are greater than 40 (without smoothing the chromatograms) in a river water sample spiked with 1 ng/L (ppt) of analytes. Based on these S/N results, the estimated LODs are ≤ 0.2 ng/L (ppt) for both PFOA and PFOS, and the estimated LOQs are ≤ 0.5 ng/L (ppt) for both PFOA and PFOS.

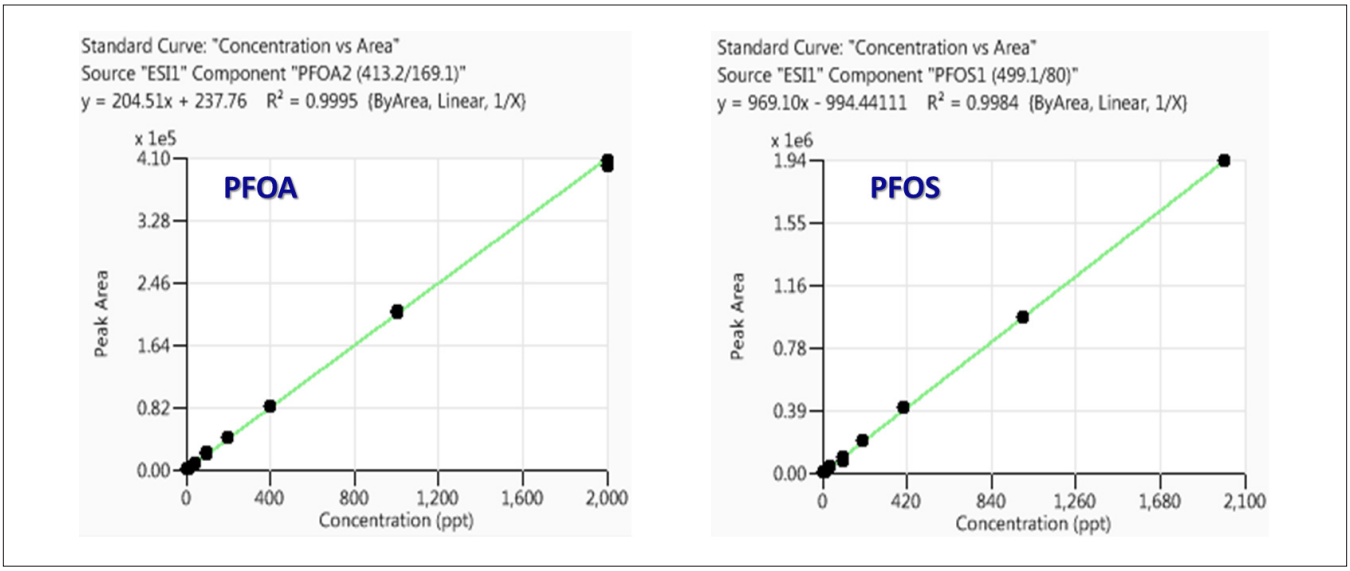

Figure 2. Calibration curves for PFOA and PFOS with concentrations from 0.5 to 2000 ng/L (ppt).

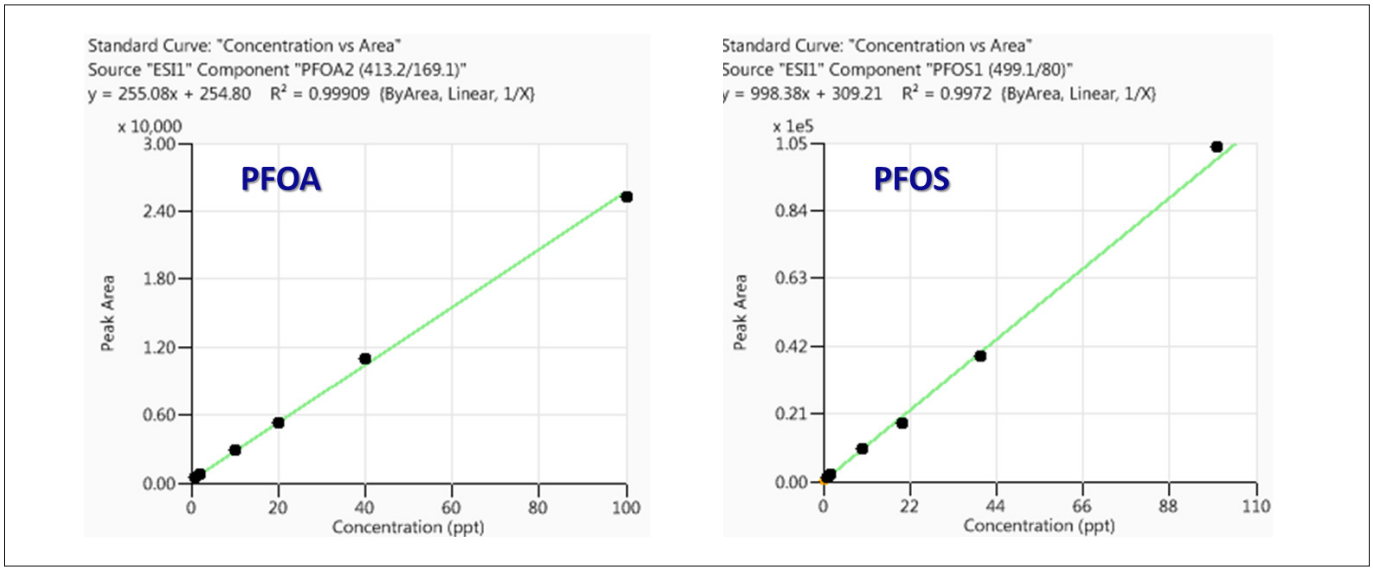

Figure 3. Calibration curves for PFOA and PFOS at low concentrations from 0.5 to 100 ng/L (ppt).

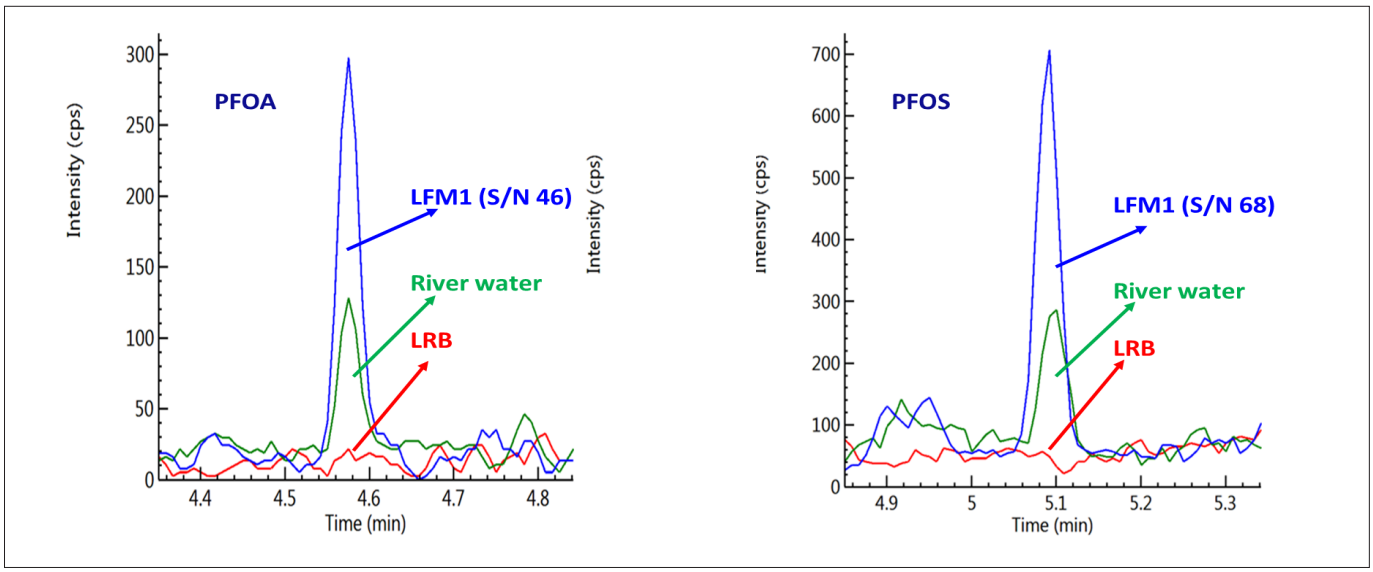

Figure 4. Chromatograms of LRB sample (in red color, prepared using LC/MS grade water), River water sample (in green color) and LFM1 sample (in blue color, prepared using the river water sample spiked with 1 ng/L of analytes).

Method Validation

As shown in Table 4 and Figure 4, no interference or contamination from reagents or glassware was observed in this study, as demonstrated by the LRB sample results. Good recoveries were obtained for LFB samples, indicating no analyte loss or contamination during sample preparations. The method's selectivity and analyte confirmation from samples were evaluated by comparing the analyte retention time and mass spectra information between the reference standard and tested samples. Per the regulatory guidance on analytical method validation,[18] at least two MS/MS transition ion pairs were used in the method, and the product ion ratios (qualifier vs. quantifier) were within the 20% tolerance windows of the expected ratio (1.25 for PFOA and 0.33 for PFOS, obtained from their reference standards).

Method precision was assessed based on replicate analyses of a middle level standard (7 replicates), and a spiked river water sample (10 replicates), on three days. The precision was then calculated based on the coefficient of variation (RSD%) of the collected data:

- Within-day RSDs:
  - Middle Level Standard (at 10 ng/L): 3.1% for PFOA, and 2.7% for PFOS
  - River water sample spiked with 1 ng/L of analytes: 8.3% for PFOA, and 9.1% for PFOS
- Inter-day RSDs:
  - Middle Level Standard (at 10 ng/L): 4.9% for PFOA, and 3.3% for PFOS
  - River water sample spiked with 1 ng/L of analytes: 8.7% for PFOA, and 9.6% for PFOS

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 to a sample (LFM samples). As shown in Table 4, the recoveries of analytes from the spiked samples were between 87% and 113%, demonstrating good accuracy of the method. Figure 4 shows the overlapped chromatograms of analytes in LRB, river water and the spiked river water (LFM1) samples.

Sample Analysis

The developed LC/MS/MS method was applied for the analysis of PFOA and PFOS in 12 water samples including drinking, rain, river and lake water samples. As shown in Table 5, very low amounts of PFOA and PFOS were detected in the river, lake and some tap water samples, although their amounts are much lower than any of the drinking water health advisory limits. The results demonstrated the superior sensitivity of the QSight 420 LC/MS/MS system. The chromatograms of PFOA and PFOS for a local river water sample (S6) are shown in Figure 5. Although the chromatographic baseline of the qualifier ion pair (413.2/369.1) for PFOA is slightly higher compared to those of reference standards, the ion ratios of the qualifier ion against the quantifier ion in the sample for both PFOA and PFOS (as shown in Figure 5) are very consistent with those obtained from reference standards (1.25 for PFOA and 0.33 for PFOS), positively confirming the identity and existence of the analytes in the sample. No PFOA or PFOS were found in a commercially available bottled drinking water sample (S1), or the LC/MS grade water (S12) used in this study.

Conclusions

A simple, rapid, sensitive and cost-effective LC/MS/MS method has been developed and validated for the analysis of PFOA and PFOS 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. In addition to its high sensitivity, the method showed a wide linear dynamic range (0.5 to 2000 ppt), and eliminated the SPE sample preparation procedures, and therefore not only reduced costs and saved time for sample analysis, but also prevented potential contamination from SPE sample preparation steps. The method has been applied for real water sample analysis with good precision and accuracy.

Table 5. PFOA and PFOS results from tested water samples in ng/L (ppt).

Analyte	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
PFOA	< LOD	0.8	1.4	1.8	< LOQ	4.7	2.3	2.4	1.8	0.5	1.1	< LOD
PFOS	< LOD	< LOQ	1.1	1.6	2.5	4.4	1.7	1.6	1.9	< LOD	< LOD	< LOD

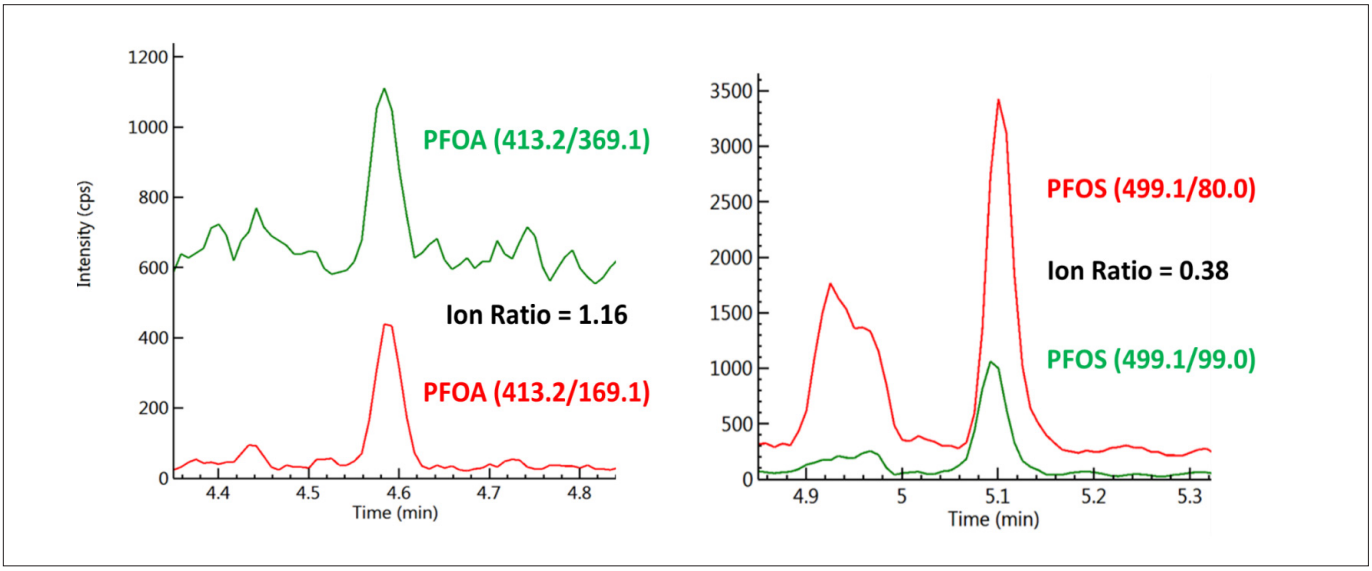

Figure 5. Chromatograms of PFOA and PFOS obtained from sample S6 (Red - quantifier ion pair; and green, qualifier ion pair).

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