Application Note

Environmental

LC/MS/MS Determination of PFOS and PFOA in Water and Soil Matrices

Using an Agilent 1290 Infinity II LC with Ultivo Tandem Mass Spectrometry

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Abstract

This Application Note demonstrates the performance of the Agilent Ultivo triple quadrupole LC/MS combined with an Agilent 1290 Infinity II LC for the analysis of perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) in water and soil matrices based on a recent report[1]. Briefly, the environmental water samples were filtered, and the soil samples were extracted using methanol. The resulting samples were subjected to cleanup using weak anion exchange cartridges to enrich the target compounds and remove the interferants. The target compounds were then eluted at high pH, further evaporated under nitrogen, then redissolved in methanol for Ultivo LC/MS/MS analysis. The internal isotope dilution method was used for quantitation. Both PFOA and PFOS in solvent solution show an excellent linear relationship in the range of 0.5–200 µg/L, with linear regression coefficients reaching 0.997. The limits of detection (LODs) for PFOA and PFOS were at sub-ng/L levels in water, and at ng/kg levels in soil. The average spiking recoveries in pure water, river water, and wastewater at 2.5, 40, and 200 ng/L ranged from 88.4 to 98.8 %, and from 88.0 to 97.3 % for PFOA and PFOS, respectively, with all RSD values (n = 6) within 0.60–14 %. For spiked blank soil, field soil, and sediment matrices spiking at 0.50, 5.0, and 20 µg/kg, the recoveries for PFOA and PFOS were within 98.6–113 % and 96.8–111 %, respectively, with RSD for both compounds within 0.4–6.6 %. These results demonstrate that the method developed using the Ultivo LC/MS/MS is very accurate and reliable. This method also meets the criteria for routine monitoring of trace levels of PFOA and PFOS in a range of environmental water and soil matrices.

Introduction

The two primary perfluoroalkylated substances, PFOA and PFOS, are found in environmental settings including water, soil, sediment, silt, and biological matrices[2,3]. Studies on experimental animals and epidemiological exposures have demonstrated that PFOS and PFOA can have a harmful impact on human health including hepatotoxicity, developmental toxicity, possibly reproductive toxicity, and potential promotion of cancer[4]. The European Food Safety Authority (EFSA) released a tolerable daily intake (TDI) for PFOA and PFOS in 2008[4], and the US Environmental Protection Agency (EPA) issued a health advisory level for total PFOA and PFOS in drinking water in 2016[5]. China is the major country manufacturing and applying perfluoalkylated substances. In addition, more PFOA, PFOS, and related compounds have been reported in various environmental settings and food products in China in the past decade[6-8]. However, in China, no maximum allowable levels for PFOA and PFOS are regulated in either food or drinking water. Recently, China has issued a reference method for the determination of PFOA and PFOS in food of plant origin[9]. To ensure reliable monitoring of the residue status in the environment, it is essential to establish a robust method for the determination of PFOA and PFOS in various environmental matrices. This monitoring is beneficial for future environmental regulation.

To determinate the level of PFOA, PFOS, and other perfluoroalkylated substances in the matrix, solid-phase extraction (SPE) cleanup followed by liquid chromatography triple quadrupole tandem mass spectrometry (LC/MS/MS) analysis has been widely applied[2-3, 6-8]. The SPE cleanup cartridges used for perfluoroalkylated substances are primarily based on reversed-phase chromatography and weak anion exchange (WAX) mechanisms. These SPE cleanup cartridges can remove most sample matrices efficiently[6,8], and are beneficial for the subsequent accurate and robust measurement of the analytes using LC/MS/MS. This Application Note determined that WAX cartridge cleanup combined with the Ultivo LC/MS was a sensitive and reliable approach for the accurate determination of PFOA and PFOS in various environmental matrices.

Experimental

Materials and reagents

Stock standard solutions of PFOA, PFOS, $^{13}C_4$-PFOA, and $^{13}C_4$-PFOA were purchased from Wellington Laboratories, Canada, with the respective concentration of 50.00 µg/mL in methanol. Methanol, acetonitrile, acetic acid, ammonia acetate, and ammonia hydroxide (W% = 20 %) were HPLC grade, and purchased from Fisher Scientific (Fair Lawn, NJ). Milli-Q water was used throughout the experiment as pure water (resistance as 18 MΩ). All other reagents were analytical grade, and obtained from SinoChem (Beijing, China). WAX cartridges (150 mg/6 mL) were obtained from Agilent Technologies (Little Falls, DE).

Standard mixture calibration solution preparation

The highest concentration standard mixture calibration solution (200 ng/mL) containing each internal standard at 10 ng/mL was first prepared from the stock solutions. Then, 10 ng/mL of the internal standard mixture in methanol was prepared from the stock solution as dilution solvent. Other standard mixture calibration solutions (0.5, 1.0, 2.0, 5.0, 10, 20, 50, and 100 ng/mL) were diluted in series from the highest concentration standard mixture calibration solution using the dilution solvent containing 10 ng/mL of each internal standard prepared. The final concentration of internal standard in each calibration solution was 10 ng/mL.

Water and soil sample collection, shipping, and storage

Both surface water and wastewater were collected from a river and an industry waste exhaust in China. The soil sample collection, shipping, and storage followed the GB17378.3 and HJ/T 166 guidelines for soil sampling. All collected samples were stored in a polypropylene apparatus in the dark at 4 °C, were cleaned up within two weeks, and were analyzed within one month.

Sample cleanup and enrichment

The water sample (500 mL) was filtered through a quartz membrane. Then, 10 ng of $^{13}C_4$-PFOA and $^{13}C_4$-PFOA were added to the filtrate, which was vortexed for 30 seconds, before being allowed to sit at room temperature for 30 minutes. The WAX cleanup protocol followed the procedure in Reference 1, or the volume of solvents used in each step were reduced to make the method more environmentally friendly. This procedure is detailed as follows:

1. WAX cartridges were pre-activated using 0.5 % ammonia methanol solution (4 mL), methanol (4 mL), and water (4 mL) sequentially.
2. The water sample was then loaded onto the WAX cartridge at a flow rate of 3–5 mL/min.
3. After all the water sample had passed through the cartridge, water (5 mL) and acetic acid buffer (5 mL, pH 4.0) were used to wash the cartridge sequentially.
4. The cartridge was then dried under vacuum for one hour.
5. After drying, 3 mL of methanol was used to wash the cartridge, and the flowthrough solution was discarded.
6. Then, 0.5 % ammonia methanol solution (4 mL) was used to elute the target compounds from the cartridge; the eluate was collected in a 10-mL polypropylene test tube and dried under nitrogen at 40 °C to nearly dry.

7. Next, the residue was dissolved in 1 mL of methanol by vortexing thoroughly.
8. The resulting solution was further filtered using a 0.22-µm membrane, and transferred to a 2-mL polypropylene vial for LC/MS/MS analysis.

Soil/sediment sample extraction, cleanup, and enrichment

A 5.0 (±0.1) g amount of the dried sample was transferred into a 100-mL polypropylene tube. Then, 10 ng of each internal standard ($^{13}C_4$-PFOA and $^{13}C_4$-PFOA) were added to the sample, which was vortexed before being allowed to sit at room temperature for 30 minutes.

The sample was then subjected to extraction using methanol. The extraction protocol can follow the procedure in Reference 1. Alternatively, slightly changing the extraction conditions as follows can reduce the extraction time. Ten milliliters of methanol were added to the sample tube, and vortexed for homogenous mixing. The sample tube was then shaken for 20 minutes using a shaker at 37 °C, before centrifugation at 6,000 rpm for five minutes. The resulting supernatant solution was transferred to a 500-mL polypropylene beaker. The extraction procedure was repeated two times, and the resulting extracts were collected into the same beaker. Next, to make the concentration of methanol in the sample solution lower than 10 %, 300 mL of pure water were transferred to the beaker. The diluted sample extract was then subjected to further cleanup and enrichment using the WAX cartridge following the same procedure as for the water sample.

Spiking recovery test

Blank water, river water, and wastewater matrices were used to evaluate the recovery for water matrix. Blank soil, agricultural soil, and river sediment were used to test the recovery for soil matrices. For each matrix, 1 to 3 levels of PFOA and PFOS were spiked into the matrix with six replicates. Table 1 lists the level of PFOA and PFOS spiked in each matrix. In addition, 10 ng of the internal standard were added to the matrices. The spiked samples were vortexed at room temperature for 30 minutes before being subjected to sample preparation for water or soil matrix, respectively.

LC and MS conditions

LC/MS/MS analysis was conducted using a 1290 Infinity II LC coupled with the state-of-art Ultivo tandem quadrupole LC/MS. Table 2 shows the detailed LC and MS/MS conditions.

Table 1. The level of PFOA and PFOS spiked in each matrix.

Water matrix	L1 (ng/L)	L2 (ng/L)	L3 (ng/L)
Blank water	2.5	40	200
Surface water	2.5	40	–
Industrious wastewater	–	–	200
Soil matrix	L1 (µg/kg)	L2 (µg/kg)	L3 (µg/kg)
Blank soil	0.5	5	20
Soil	–	5	20
Sediment	–	5	20

Table 2. The detailed LC/MS/MS analysis conditions.

LC conditions	
Instrument	Agilent 1290 Infinity II LC with built-in degasser
Autosampler	Agilent 1290 Infinity II Autosampler with temperature control
Column temperature	1290 Infinity II thermostatted column compartment
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2,7 µm
Column temperature	35 °C
Mobile phase	2.0 mmol/L ammonium acetate solution B) Acetonitrile
Flow rate	0.30 mL/min
Injection volume	5.0 µL
Post time	3 minutes
Gradient elution profile	0–3 minutes: 30–65 % B; 3–4 minutes: 65 % B; 4–5 minutes: 65–100 % 5–8 minutes: 100 %
MS/MS conditions	
Instrument	Ultivo LC/TQ
Ionization mode	Negative
Drying gas temperature	325 °C
Drying gas flow rate	6 L/min
Nebulizer gas pressure	30 psi
Sheath gas temperature	350 °C
Sheath gas flow rate	11 L/min
Capillary voltage	2,500 V
Nozzle voltage	0 V
CAV	9 V

Results and discussion

Optimization of LC and MS/MS conditions

Initially, to find the correct precursor ions for detection of the two compounds, the standard solutions of PFOA and PFOS were subjected to a Q1 MS scan under negative ionization mode. The selected precursor ions were then subjected to product ion scanning. By optimizing the parameters for precursor transmission and fragmentation, transmissions of 413/369 and 499/99 were selected for quantitation of PFOA and PFOS, respectively. The other ion transmissions (413/169, 499/80) were selected for qualitative confirmation. Similarly, the transmissions for isotopic standards were also established, as shown in Table 3.

With the establishing MRM acquisition parameter, an InfinityLab Poroshell 120 EC-C18 column was selected for separating PFOA and PFOS using acetonitrile and water containing ammonium acetate as the mobile phase. Using a five-minute gradient elution, both PFOA and PFOS were baseline-separated, as shown in Figure 1. For a relatively clean water matrix, five minutes is sufficient for separation of the analytes. However, when analyzing complex matrices such as wastewater, soil, or sediment, the residue matrix in the sample after cleanup may interfere with the analysis and shorten the column life. Therefore, to ensure complete cleanup of the column after each analysis, the gradient profile was elevated to pure acetonitrile for three minutes right after both analytes eluted from the column. In addition, due to the prevalence of perfluoroalkylated substances, the background level from the LC system can be high. In this case, connecting a trapping column between the solvent mixture and the autosampler is suggested, to trap the residue[7] and eliminate the coelution interference, for accurate quantitation.

Table 3. MRM acquisition parameters for the detection of PFOA and PFOS.

Compound	Precursor (*m/z*)	Fragment ion (*m/z*)	Fragment voltage (V)	Collision energy (V)	Dwell time (ms)
PFOA	413	369*	80	1	30
		169	80	12	30
M4-PFOA	417	372*	80	1	30
		169	80	12	30
PFOS	499	99*	200	52	30
		80	200	68	30
M4-PFOS	503	99*	200	52	30
		80	62	68	30

*Quantification ion

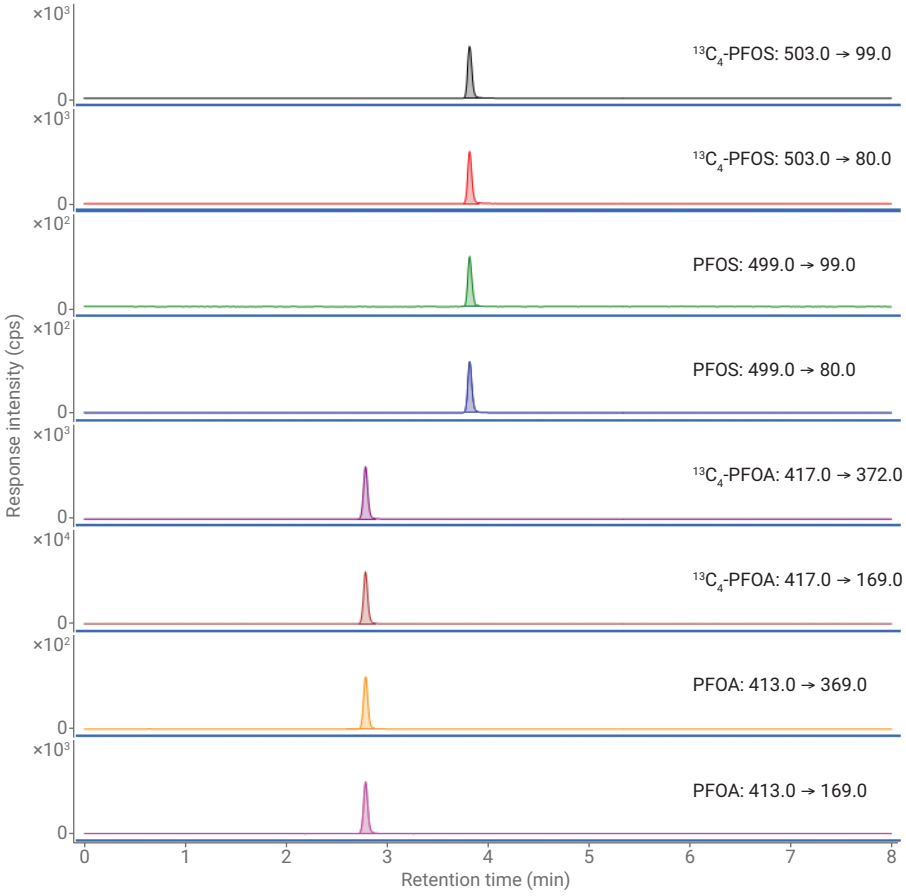

Figure 1. The typical MRM chromatograms for PFOA, PFOS, and their isotopically labeled analogs using an Ultivo LC/TQ.

Optimization for sample extraction and cleanup

Water samples were filtered before cleanup. However, for soil samples, an appropriate extraction procedure was needed to efficiently extract the target compounds. The extraction solvent, the modes of extraction, and the percentage of TOC in soil samples may affect the efficiency of the target compound extraction. At a fixed level of spiking of PFOA and PFOS in soil (5 µg/kg), the recoveries were compared while varying the extraction solvent, the modes of extraction, and the percentage of TOC in soil matrix. Varying the extraction mode using shaking or ultrasonication did not result in significant differences in recoveries between PFOA and PFOS. While varying the TOC percentage within the test TOC range of 0.81–2.4 %, did not result in significantly different recoveries for both PFOA and PFOS. However, when comparing different extraction solvents including water, methanol, and their combination (1:1, v/v) at neutral and at basic pH (0.1 % KOH), it was found that methanol is essential for the efficient simultaneous extraction of PFOA and PFOS. Both methanol/water (1:1. v/v) and methanol can extract PFOA and PFOS effectively. However, methanol can extract more interferences than methanol/water (1:1, v/v), which may explain the relative high deviation error in recovery.

The filtered water sample could be loaded directly onto a WAX cartridge for cleanup. The soil extracts were diluted so that the final methanol in the sample solution was below 10 % before being loaded onto the cartridge column. The WAX cartridge has the capacity to retain PFOA and PFOS strongly at pH 4.0. Therefore, after loading the sample onto the cartridge, the cartridge was subjected to washing using acetic acid (pH 4.0), then pure methanol. These washes do not elute out the target compounds, but do efficiently elute out the interfering compounds from soil extract. By changing the elution solvent of ammonia/methanol, the target compounds can be eluted out of the cartridge efficiently. The final optimized procedure is shown in the experimental section.

The calibration curves and sensitivity

The calibration solution was prepared from the standard mixture solution, with both PFOA and PFOS concentrations ranging from 0.5 to 200 µg/L, and each internal standard at a concentration of 10 µg/L. The ratios of the peak area of the target compound over the peak area of its internal standard were plotted against the ratios of the concentrations for the target and its internal standard in the solution. Figure 2 shows that the ratio of the peak areas correlates linearly with the ratio of the concentrations for both PFOA and PFOS, with regression coefficients as high as 0.997.

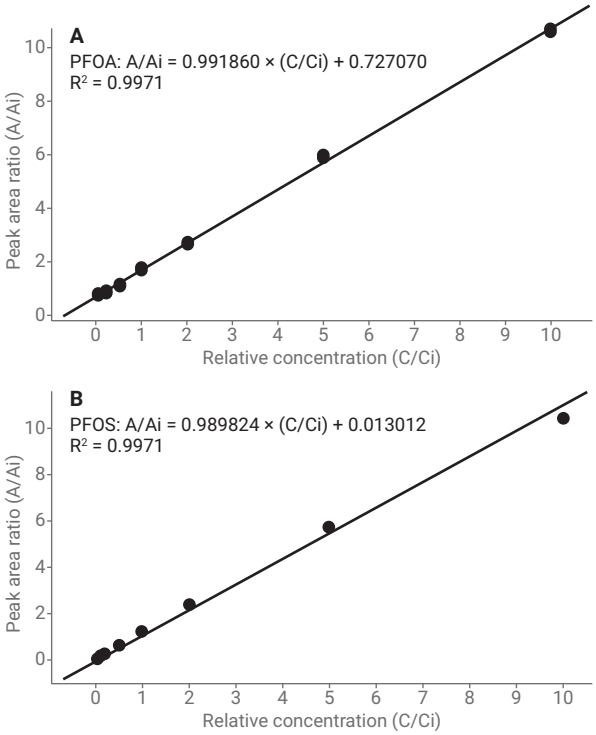

Figure 2. The calibration curve for PFOA and PFOS within the tested concentration range.

The sensitivity of the method was demonstrated at the lowest spiking level of PFOA and PFOS in pure water and blank soil. As shown in Figure 3, at a low 2.5 ng/L spiking level in water, the signal-to-noise ratio (S/N) for PFOA and PFOS was 1,193 and 120, respectively. These S/N ratios indicate that the LOD for both PFOA and PFOS in water can be at the sub-ng/L level or lower. At a 0.5 µg/kg spiking level in the blank soil, the S/N for PFOA and PFOS reached 1,382 and 226, respectively. This indicates that the LOD for both PFOA and PFOS can be at the ng/kg level or lower in blank soil. The results demonstrate that the developed method has the capacity to detect extremely low, trace amounts of PFOA and PFOS.

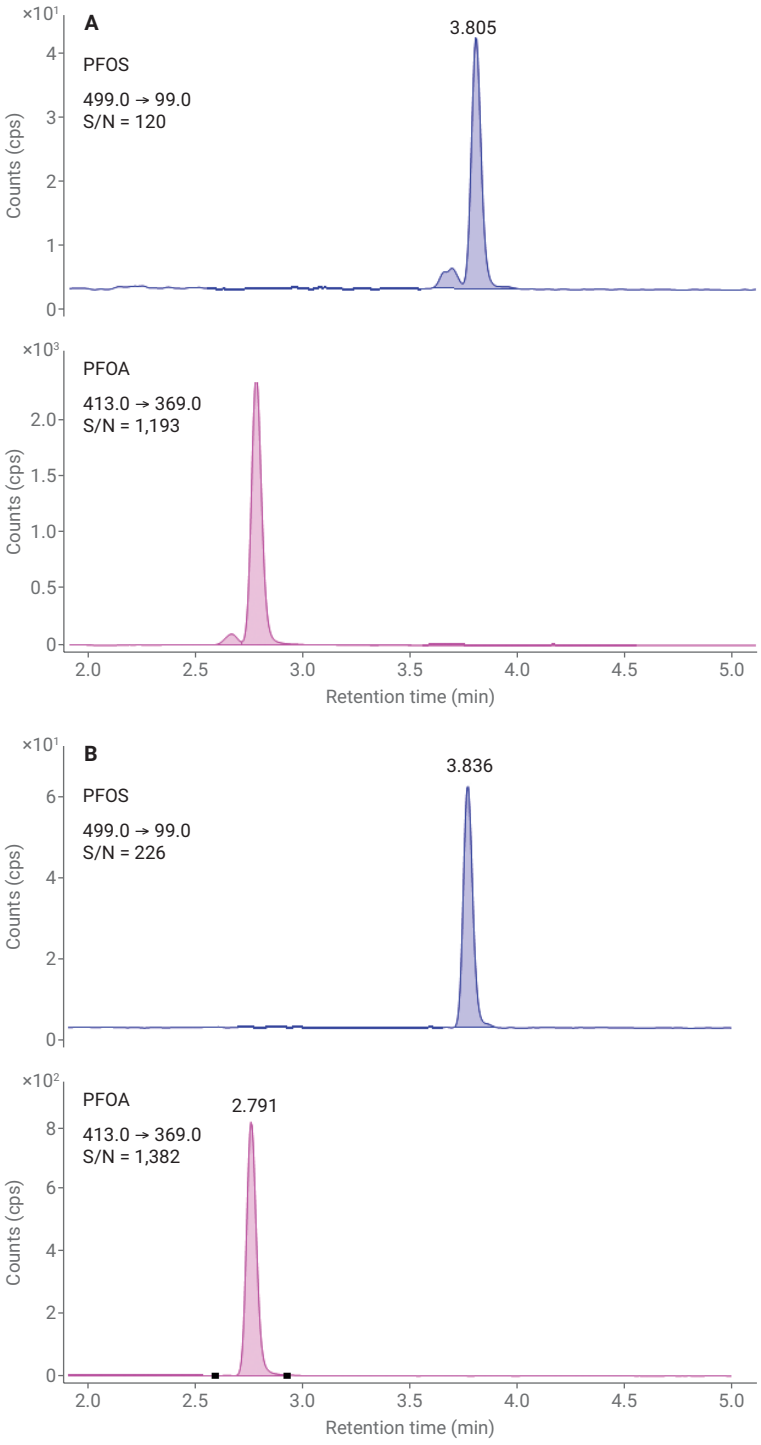

Figure 3. Chromatograms for PFOA and PFOS spiked at 2.5 ng/L in water (A) and 0.5 µg/kg in blank soil (B) respectively. Note: only a quantitative ion chromatogram was illustrated for each compound.

Method accuracy and precision

Spiking experiments were used to evaluate the accuracy and precision of the method. Pure water, river water, wastewater, blank soil (quartz sand), field soil, and sediment were selected as testing matrices. Figure 4 shows that at spiking levels of 2.5, 40, and 200 ng/L, the recoveries for PFOA are within 91.1–94.1 % with a relative standard deviation (RSD, n = 6) within 1.3–4.8 %. The recoveries for PFOS were within 88.0–93.8 %, with an RSD in the range of 0.8–5.3 %. For river water spiked at 2.5 and 40.0 ng/L, and wastewater spiked at 200 ng/L, the recoveries for both PFOA and PFOS were within 88.4–98.8 and 88.0–97.3 %, and the RSDs were within 2.2–13.8 and 0.9–4.1 %, respectively. For spiking in blank soil, field soil, and sediment at 0.5, 5.0, and 20.0 µg/kg, the recoveries for PFOA were within 98.5–112.8 % with RSDs within 0.6–5.7 %. The recoveries for PFOS were also within 96.8–111.1 % with RSDs within 0.4–6.6 %. The results demonstrate that the method can accurately and reliably determine trace levels of PFOA and PFOS in various environmental water and soil matrices.

Real sample analysis

The method was used to monitor the level of PFOA and PFOS in underground water, surface water, the surrounding soil, and sediment collected locally. The results suggested that both surface and underground water were contaminated by a very low level of PFOA and PFOS, ranging from several ng/L to several 10s of ng/L. For the surrounding soil and sediment, both PFOA and PFOS were not detected in most samples.

Conclusion

The 1290 Infinity II LC, coupled with the novel Ultivo quadrupole mass spectrometer, was applied to detect PFOA and PFOS in a range of environmental water and soil matrices. With WAX cartridge cleanup and enrichment, the LOD for both PFOA and PFOS in blank water can be as low as sub-ng/L. The LODs of PFOA and PFOS in blank soil can be at ng/kg levels. The isotopic dilution calibration demonstrates a good linear relationship within the test range of 0.5 to 200 µg/L, with regression coefficients as high as 0.997. The method is also accurate and precise, with spiking recoveries in all tested matrices ranging from 88 to 113 %, with RSDs within 0.6–13.8 %. It suggests that the method can reliably be applied for the routine measurement of trace PFOA and PFOS in environmental water and soil matrices.

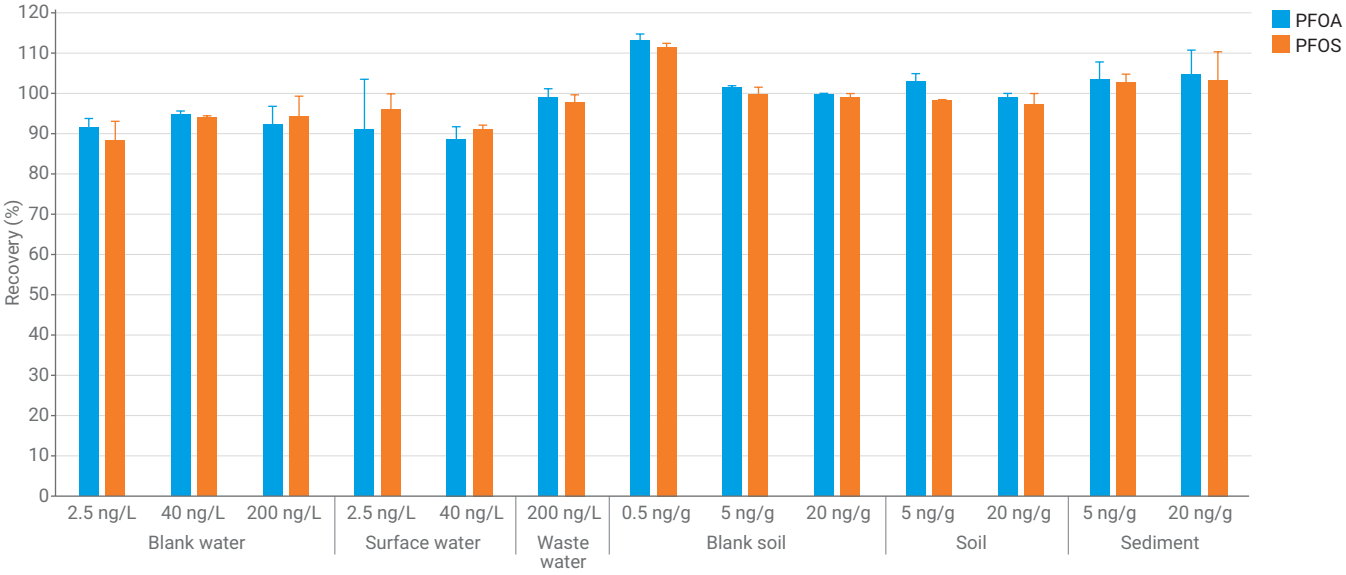

Figure 4. The recoveries of PFOA and PFOS at each spiked level in the tested water and soil matrices.

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