Analysis of PFAS at low ppt levels in drinking water via EPA method 533

Using the SCIEX Triple Quad 4500 system

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Per- and polyfluorinated alkyl substances (PFAS) are a group of manmade chemicals that have been used for decades in a large host of applications. A recent review focused on 1,400 unique PFAS species[1] although current OECD databases have upwards of 4,500 unique PFAS compounds.[2] The resilience of some PFAS to degradation and their potential for biological harm have spurred health and environmental concerns amongst regulatory agencies. The CDC's national biomonitoring program has found evidence of widespread presence of certain PFAS residues in human serum[3]. One possible route of human exposure is from PFAS contaminated water.[4]

PFAS are known to enter the water supply and bioaccumulate in watershed ecosystems. This can be a health issue for humans who directly consume contaminated water. The US EPA published the fifth unregulated contaminant monitoring rule (UCMR5)[5], which proposes maximum residue limits (MRL) for PFAS residues in EPA method 533. Representative chemical structures are shown in Figure 1.

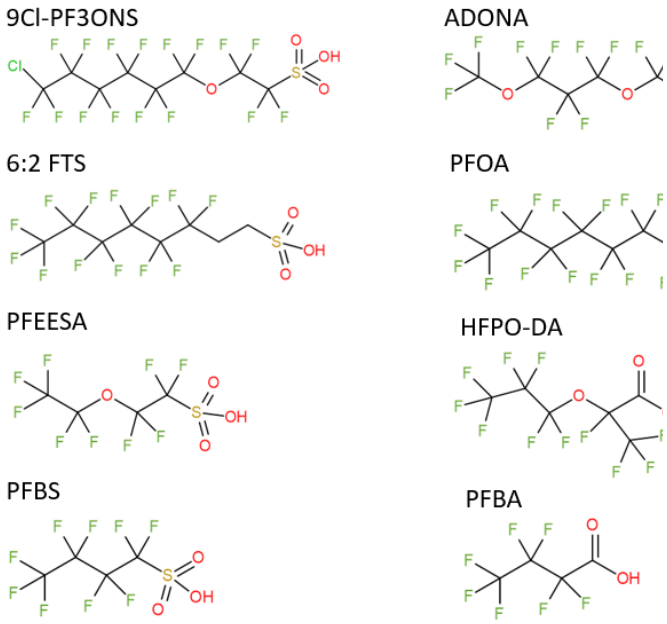

Figure 1. Representative structural diversity among EPA method 533 analytes. Compounds can have either sulfonate (left) or carboxylate head groups (right). Structure variability can also include per-/polyfluorinated species, the presence or absence of ether linkage(s) and alkyl chain length with or without branching.

This technical note presents data collected in accordance with EPA method 533 requirements for the initial demonstration of capability (IDC). Sample preparation was done using Phenomenex Strata-X-AW Weak Anion solid-phase extraction (SPE) and data acquisition was performed using the SCIEX Triple Quad 4500 system coupled to an ExionLC AC system.

Key method features

- MRLs ≤ 3 ng/L reported for all analytes, meeting UCMR5 requirements for EPA method 533 compounds
- Sensitive and robust performance meets all EPA method 533 performance criteria
- SCIEX OS software simplifies data review and report generation using custom calculations and flagging rules
- Specialized LC setup ensures low systemic contamination

Methods

Sample preparation: Sample preparation followed EPA method 533.[6] Briefly, 250 mL of water was fortified with analytical and isotopic standards (Wellington Laboratories). Water samples were passed through a conditioned anion exchange SPE column (Phenomenex Strata-X-AW Weak Anion 500 mg/6 mL). After rinsing with ammonium acetate the column bound residues were eluted with methanol containing 2% ammonium hydroxide, dried down under nitrogen gas, and reconstituted in 1 mL of 80:20 methanol/water containing isotopic performance standards.

Four real-world samples were collected from New England tap water and natural rivers, lakes, and ponds and then extracted following the EPA method 533 procedures. The isotopic dilution standards and isotopic performance standards were spiked as prescribed.

Care was taken to clean all sample preparation components with LC-MS grade methanol followed by MilliQ water to minimize contamination. This included sample containers, SPE connections, mobile phase bottles, and metal gas lines used for the dry down process.

Chromatography: HPLC separation was performed with the ExionLC AC system using a 12-minute gradient. The Phenomenex Gemini C18 (100 x 3.0 mm, 3.0 µm, 00D-4439-Y0) was used as the analytical column. The delay column used was a Phenomenex Luna C18 (30 x 3.0 mm, 00A-4252-Y0). The delay column was plumbed before the injection port to allow for separation of PFAS residues originating from the LC system and/or mobile phases from the analytical peak (Figure 2). Very low systemic contamination was observed for all analytes (1/3 of the MRL). The injection volume was 5 µL with a column temperature of 40°C using a flow rate of 0.6 mL/min. Mobile phases consisted of 10 mM ammonium acetate and methanol with 10 mM ammonium acetate. Figure 3 displays representative chromatographic separations that were achieved with the system.

Mass spectrometry: A Turbo V ion source was used with electrospray ionization in negative ion mode. Acquisition was performed using Analyst software 1.7.2. The Scheduled MRM algorithm was used to optimize duty cycle to give greater than 12 scans across the chromatographic peaks.

Source conditions were optimized to give the highest ionization efficiency with respect to flow rate and mobile phase composition. Compound specific DP, CE, and CXP values were used to give maximum sensitivity and selectivity for all transitions and values were similar to previous PFAS SCIEX tech notes[7].

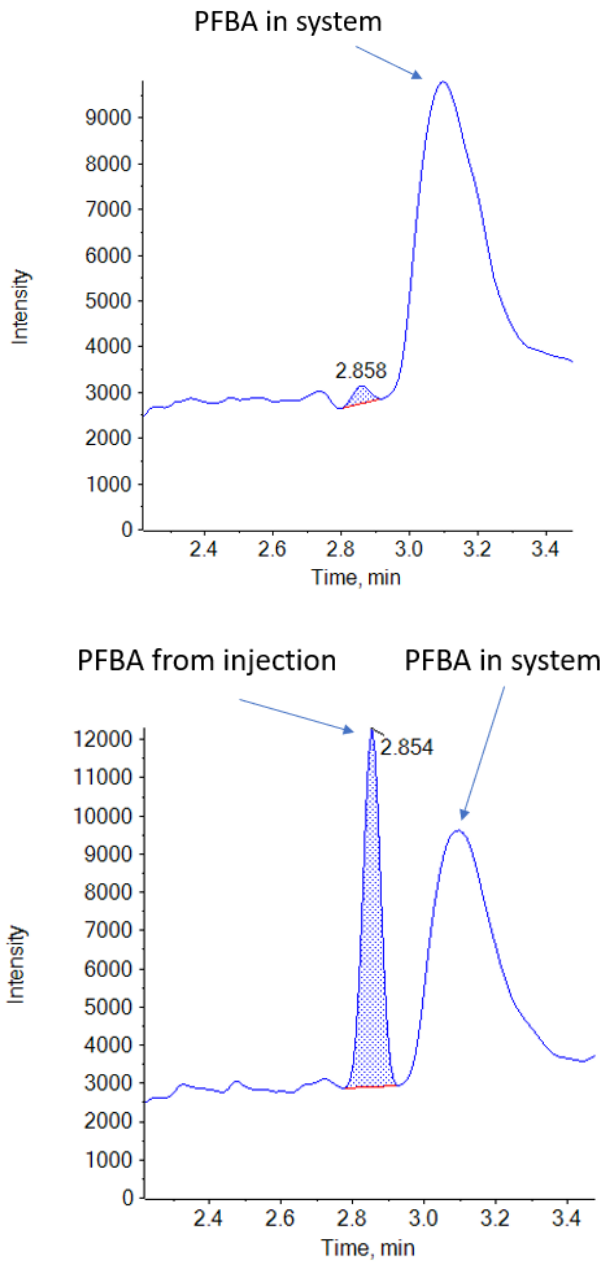

Figure 2. Effect of delay column in the event of systemic contamination. PFBA exhibited low systemic contamination. A solvent blank showed a delayed, relatively broad PFBA peak (top). A 0.1 ng/mL injection, corresponding to 1/5 of the MRL, showed injected PFBA at 2.85 minutes with the delayed peak corresponding to systemic contamination (bottom).

Data processing: Data were processed using SCIEX OS software 2.1. The processing method was built to assign the appropriate isotopic dilution standard to the appropriate quantification transition for each PFAS analyte. This is accomplished in the software during construction of the processing method and allows for straightforward, internal standard corrected quantification.

Calibration curves were constructed between 0.5 – 15 ng/mL (corresponding to 2 – 60 ng/L in sample) and were forced through zero per method requirements. Linearity was > 0.98 for all analytes. The statistics pane in SCIEX OS software allows for easy evaluation of the accuracy and precision of each calibration curve. This feature facilitated rapid assessments of method performance criteria specific to MRL calculations.

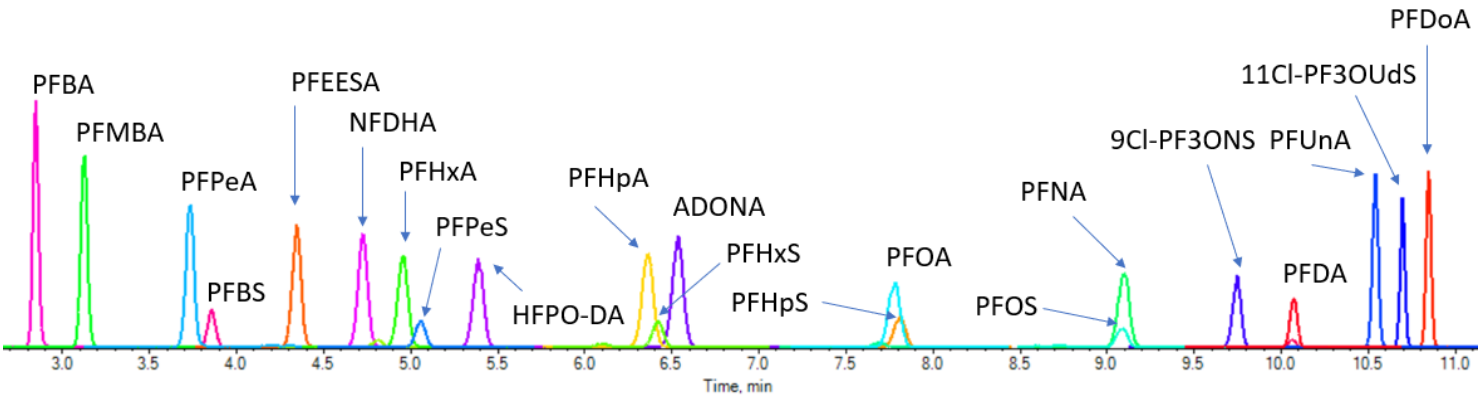

Figure 3. TIC chromatogram of 15 ng/mL solvent standard showing chromatographic separation of EPA method 533 PFAS analytes. Representative target residues are displayed here as extracted from their corresponding MRM in the mass spectrometry method.

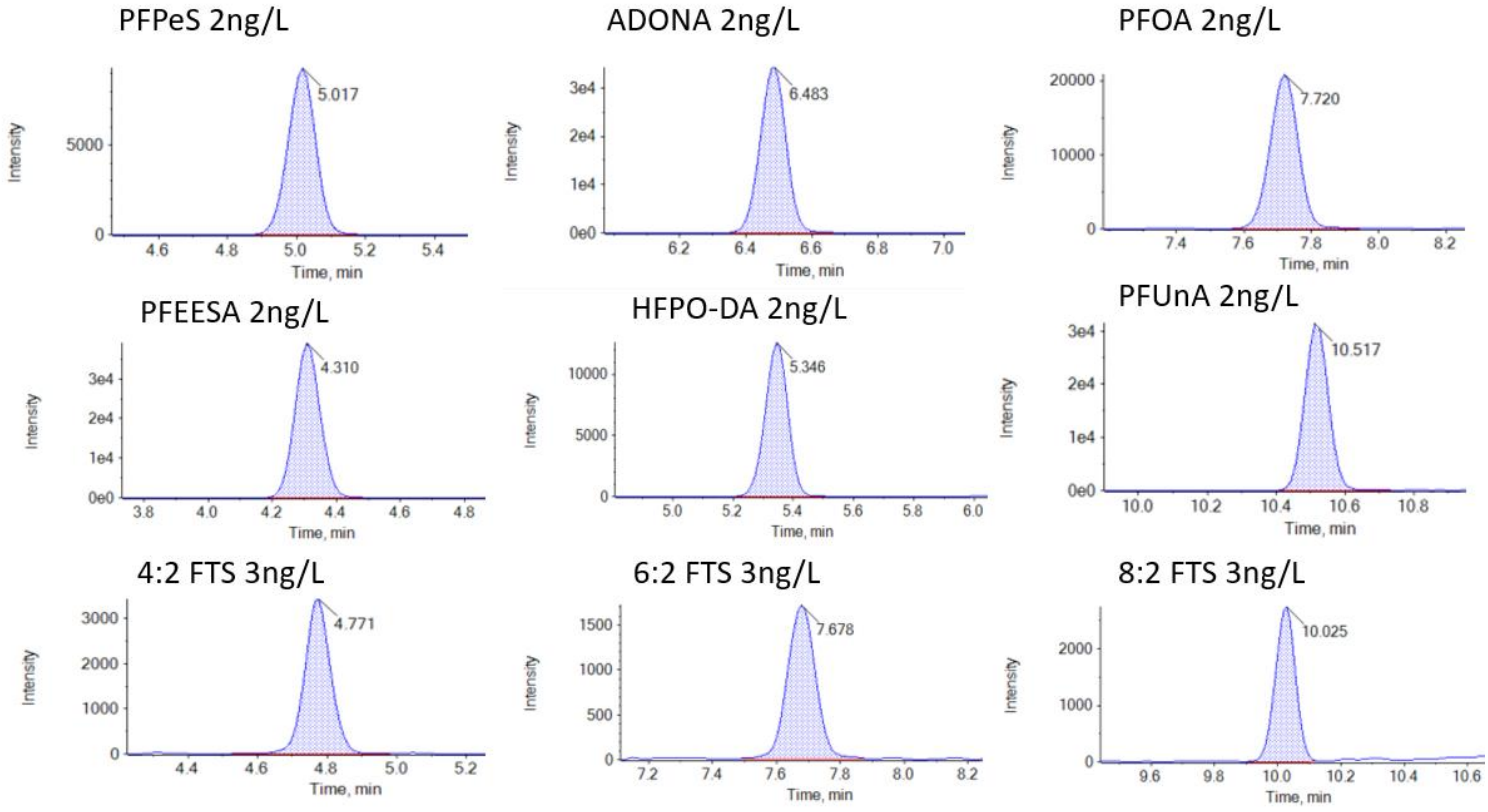

Figure 4. Representative compound chromatograms at the verified MRL values. All compounds in the method were found to have MRL values of 2 ng/L except for 4:2 FTS, 6:2 FTS, and 8:2 FTS, which had verified MRL values of 3 ng/L.

Method chromatography, precision, and accuracy

Excellent peak shape and analyte separation were achieved using the 12 min gradient (Figure 3). Target MRLs for IDC requirements included accuracy and precision characteristics. Requirements were for seven replicate spiked samples to display accuracies of 70%-130% and precision ≤ 20% CV. All compounds in the study were in acceptable ranges for their verified MRL values (Table 1). Most compounds displayed accuracy in the 80%-110% range or better. Precision values are listed for each compound alongside the compound MRL value in Table 1.

MRL determinations

For an MRL to be considered verified, the seven replicate extracts at the target MRL fortification must pass statistical rigor as described in EPA method 533. The standard deviation from the seven replicates is multiplied by t-value (3.963) to give the Half Range for the Prediction Interval of Results (HRPIR). Then the Upper and Lower Prediction Interval of Results (UPIR and LPIR) are calculated as follows:

$$Upper\ PIR\ Limit = \frac{Mean\ + HRPIR}{Fortified\ Concentration} \times 100$$

$$Lower\ PIR\ Limit = \frac{Mean - HRPIR}{Fortified\ Concentration} \times 100$$

The MRL is verified for a given concentration when 50% ≤ PIR ≤ 150%. MRLs verified here met or exceeded UCMR5 reporting criteria (Table 1). Figure 4 illustrates representative analyte chromatograms at their found MRL values.

Representative sample data

Several tap water and natural water sources were collected from New England and subjected to the extraction method. All tap water samples showed low level ng/L PFOS and PFOA (Table 2). The river water sample had higher concentrations compared to treated tap water. However, PFAS findings were, in all cases, below the EPA drinking water recommendation for summed PFOA and PFOS (70 ng/L).[8] Chromatograms of a representative water sample finding vs. the analytical standard are shown in Figure 5.

Table 1. Accuracy and precision of method. Precision CV measurements were based on the area response of the quantifier transition obtained from 7 replicates of water fortified at 6 ng/L. Mean % area values are based on 7 replicates of water fortified at 4 ng/L. Verified MRLs were established using EPA method 533 criteria. All residues passed data quality requirements in section 9 of EPA method 533.

Compound	Verified MRL (ng/L)	CV (%)	Mean accuracy (%)	UCMR5 MRL (ng/L)
11Cl-PF3OUdS	*2*	*6.6*	*85.2*	*5*
9Cl-PF3ONS	*2*	*7.4*	*96.8*	*2*
ADONA	*2*	*3.5*	*94.9*	*3*
HFPO-DA	*2*	*5.4*	*94.5*	*5*
NFDHA	*2*	*4.3*	*91.1*	*20*
PFBA	*2*	*7.2*	*109.8*	*5*
PFBS	*2*	*6.6*	*97.9*	*3*
8:2 FTS	*3*	*9.5*	*117.0*	*5*
PFDA	*2*	*4.5*	*96.2*	*3*
PFDoA	*2*	*6.1*	*102.6*	*3*
PFEESA	*2*	*7.3*	*102.6*	*3*
PFHpS	*2*	*7.5*	*98.8*	*3*
PFHpA	*2*	*7.5*	*104.4*	*3*
4:2 FTS	*3*	*6.5*	*122.2*	*3*
PFHxS	*2*	*7.7*	*99.9*	*3*
PFHxA	*2*	*4.2*	*98.4*	*3*
PFMPA	*2*	*3.4*	*100.4*	*3*
PFNA	*2*	*4.2*	*93.3*	*4*
6:2 FTS	*3*	*5.1*	*112.9*	*5*
PFOS	*2*	*8.9*	*98.8*	*4*
PFOA	*2*	*8.0*	*100.0*	*4*
PFPeA	*2*	*4.2*	*101.4*	*3*
PFPeS	*2*	*7.1*	*98.5*	*4*
PFUnA	*2*	*4.1*	*100.2*	*2*

Table 2. PFAS water concentrations from tap water and river water samples collected from New England. Concentrations were below the current EPA guidelines of 70 ng/L.

Compound	Tap water 1 (ng/L)	Tap water 2 (ng/L)	Tap water 3 (ng/L)	River water (ng/L)
^{11}Cl-PF$_3$OUdS	*ND*	*ND*	*ND*	*ND*
^{9}Cl-PF$_3$ONS	*ND*	*ND*	*ND*	*ND*
ADONA	*ND*	*ND*	*ND*	*ND*
HFPO-DA	*ND*	*ND*	*ND*	*ND*
NFDHA	*ND*	*ND*	*ND*	*ND*
PFBA	*ND*	*ND*	*ND*	*ND*
PFBS	*ND*	*ND*	*ND*	*ND*
8:2 FTS	*ND*	*ND*	*ND*	*ND*
PFDA	*ND*	*ND*	*ND*	*ND*
PFDoA	*ND*	*ND*	*ND*	*ND*
PFEESA	*ND*	*ND*	*ND*	*ND*
PFHpS	*ND*	*ND*	*ND*	*ND*
PFHpA	*ND*	*ND*	*ND*	*ND*
4:2 FTS	*ND*	*ND*	*ND*	*ND*
PFHxS	*ND*	*ND*	*ND*	*4.6*
PFHxA	*ND*	*ND*	*ND*	*5.1*
PFMPA	*ND*	*ND*	*ND*	*ND*
PFNA	*ND*	*ND*	*ND*	*4.5*
6:2 FTS	*ND*	*ND*	*ND*	*ND*
PFOS	*ND*	*2.0*	*ND*	*15.9*
PFOA	*3.3*	*3.8*	*3.3*	*7.1*
PFPeA	*ND*	*ND*	*ND*	*7.6*
PFPeS	*ND*	*ND*	*ND*	*ND*
PFUnA	*ND*	*ND*	*ND*	*ND*

ND means compounds were below the detection limits of the assay.

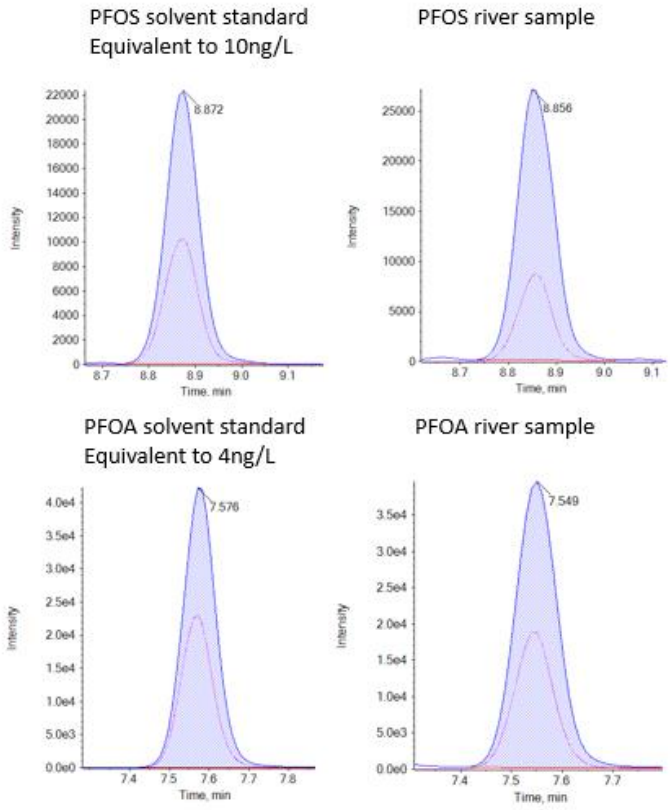

Figure 5. PFOS and PFOA findings in a representative river sample. Analyte transitions (blue) and their respective internal standards (pink) are shown for PFOS and PFOA. Solvent standards are shown (left) alongside actual findings in the sample (right).

Conclusions

- The SCIEX Triple Quad 4500 system coupled to an ExionLC AC system can achieve, and in many cases surpass, target MRLs for PFAS residues specified in EPA method 533 and UCMR5
- Simple upgrades to the LC system can limit contamination and separate background contaminants from residues in the analytical injection
- The method was able to successfully test for PFAS contamination in New England water samples

References

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