

Simple and rapid quantification of per- and polyfluoroalkyl substances (PFAS) in seawater

Using the SCIEX 7500 system

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In this technical note, a method is presented for quantifying per- and polyfluoroalkyl compounds (PFAS) in seawater at the low ng/L range using a simple sample preparation approach, with no solid phase extraction (SPE). The sensitivity of the SCIEX 7500 system¹ allowed for the ultra-trace level quantification of PFAS in un-spiked seawater samples using only direct injection analysis.

With seafood consumption identified as a major pathway for human exposure to PFAS, and PFAS regulations continuously tightening as concerns about exposure rise, the ability to monitor PFAS levels in seawater has become critical. The ocean has been referred to as a “terminal sink” for PFAS, with perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (PFHxA) and perfluorooctanoic acid (PFOA) reported as being abundant in surface and subsurface seawater.² While recent advancements suggest that petrochemicals released into the sea could be one source of PFAS in seawater,³ the distribution and abundance of PFAS in seawater is still poorly understood.²

Previous analytical methods from SCIEX have enabled the detection of low ng/L levels for various PFAS compounds in drinking water and surface water to help meet European Union regulatory requirements.^{4,5,6} Seawater is a very difficult matrix to analyze due to the high salt levels which typically results in poor quantification due to high matrix effects. Here, we provide a robust and sensitive method for quantifying PFAS in seawater using external standards and a standard addition workflow.



Key features of PFAS analysis using the SCIEX 7500 system

- Simple, reproducible and robust sample preparation was used, with no SPE needed
- Good chromatographic peak-to-peak separation was achieved with an HPLC run time of 15 min (Figure 2)
- Excellent sensitivity was achieved with limit of quantification (LOQ) values at sub-ng/L levels, as shown in Figure 1
- The average accuracy (%) for PFAS standards in both solvent and spiked seawater samples was within acceptable criteria (70%–130%), and the area %CV <15% against an external calibration curve
- An external calibration curve in solvent and a standard addition workflow were easily implemented using SCIEX OS software to further confirm the concentration of PFAS compounds detected in un-spiked seawater samples

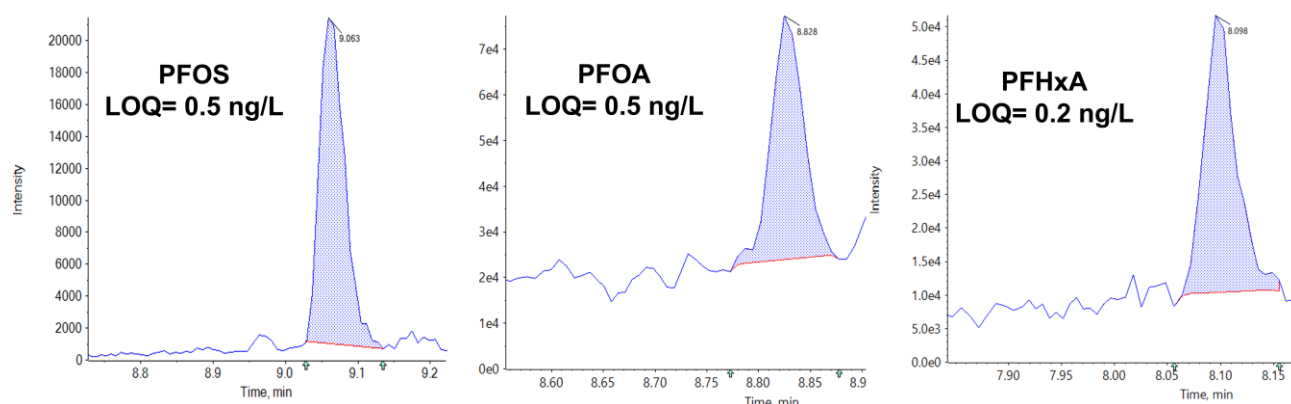


Figure 1. Extracted ion chromatograms (XICs) for PFAS compounds reported to be abundant in surface and subsurface seawater at their respective LOQs.² LOQ levels of 0.5 ng/L were achieved for PFOS and PFOA, and an LOQ level of 0.2 ng/L was achieved for PFHxA. This figure demonstrates the sensitivity and low-level quantification achieved on the SCIEX 7500 system. LOQ levels were determined by average accuracy ($\pm 30\%$), area %CV (<10%) and r^2 (>0.99).

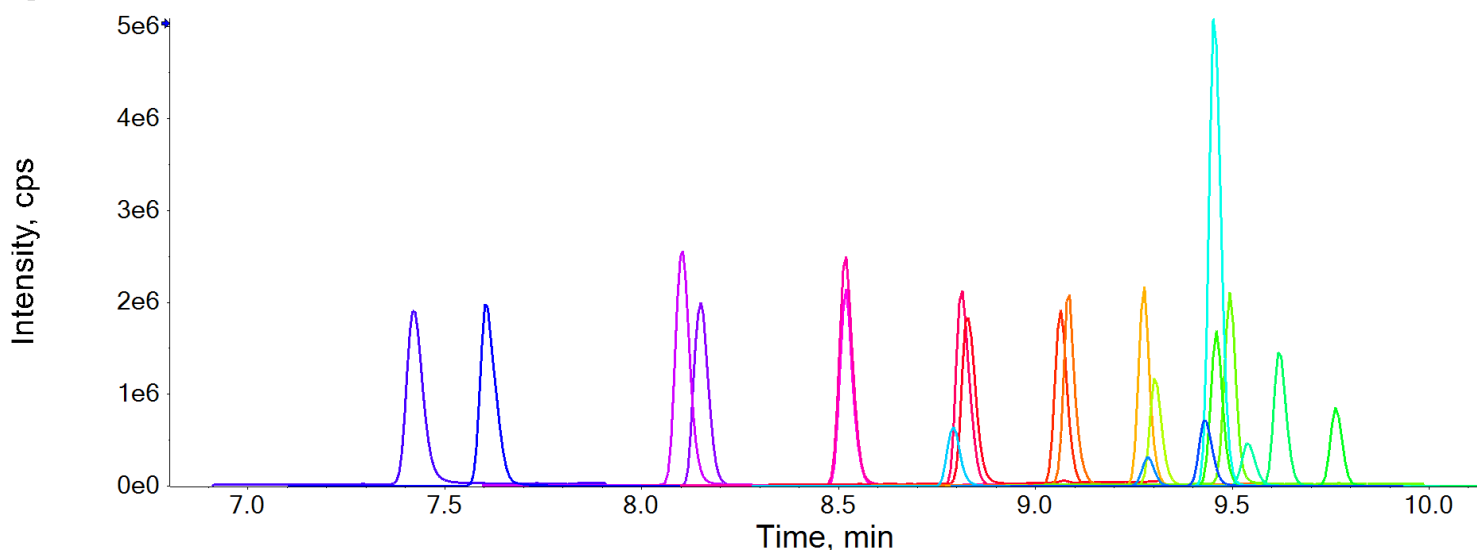


Figure 2. XICs of all PFAS compounds analyzed at 50 ng/L. Good chromatographic peak-to-peak separation was achieved.

Methods

Standard preparation: Mixed standards were prepared in a mixture of LC-MS water at a ratio of 2.5 mL LC-MS water to 2 mL 50:50 (v/v) acetonitrile/methanol + 0.22% formic acid.

Sample preparation: 2.5 mL of filtered seawater samples (collected from the Irish sea and filtered once with a 0.2 μm , 25 mm diameter Phenomenex regenerated cellulose (RC) syringe filter PN: AF0-8459) were added to 2 mL 50:50 (v/v) acetonitrile/methanol + 0.22% formic acid solution prior to analysis.

Chromatography: Chromatographic separation was performed using a Phenomenex Luna Omega PS C18, 100 \AA , 100 mm x 2.1 mm, 3 μm (PN: 00D-4758-AN) column and a Phenomenex Gemini C18, 110 \AA , 100 mm x 2.0 mm, 3 μm (PN: 00D-4439-B0) delay column. The injection volume was 50 μL and a flow rate of 0.4 mL/min was used. Mobile phase A was 20mM ammonium acetate in water and mobile phase B was methanol. Gradient conditions are shown in Table 1.

Table 1. Chromatographic gradient program.

Time (min)	% A conc	% B conc
0.0	90	10
1.5	90	10
8.0	1.0	99
12.0	1.0	99
12.5	90	10
15.0	90	10

Mass spectrometry: The analysis was performed using a SCIEX 7500 system operated with electrospray ionization in negative ion mode. The optimized source and gas parameters were similar to those in a previously published SCIEX technical note.⁶

Data processing: Processing was performed using SCIEX OS software 3.0. The peak-to-peak algorithm was used for signal-to-noise (S/N) determination.

Ultra-trace level sensitivity of PFAS standards in solvent

Table 2 highlights the LOQ values that were achieved for the PFAS compounds tested with this method in solvent (n=3). The criteria for LOQ determination were based on an average accuracy (%) of 70%–130% and an area %CV of <15%. The lowest current limit for PFAS compounds set by the European Parliament and Council of the European Union for drinking water is 100 ng/L. Table 2 illustrates the excellent levels of sensitivity achieved using this method.

In addition, the linearity of each PFAS compound was evaluated (Table 2) with all r^2 values >0.99 using a 1/x weighting. Figure 3 shows the calibration curve for PFOS and the respective r^2 value.

Table 2. LOQ for each PFAS compound analyzed in diluent. Three commonly reported PFAS compounds in seawater are highlighted in bold (PFOS, PFHxA and PFOA). The peak-to-peak S/N algorithm was used, and S/N is included to show each peak is quantifiable with S/N >10.

Compound name	Chemical formula	LOQ (ng/L)	Peak-to-peak S/N
PFOS	C8HF17O3S	0.50	17.0
PFHxA	C6HF11O2	0.20	23.3
PFOA	C8HF15O2	0.50	15.6
PFNA	C9HF17O2	0.20	15.7
6:2 FTS	C8H5F13SO3	0.50	35.1
8:2 FTS	C10H5F17SO3	0.50	25.6
N-EtFOSSA	C12H8F17NO4S	0.20	33.4
N-MeFOSSA	C11H6F17NO4S	0.50	15.5
FOSA	C8H2F17NO2S	0.50	41.0
PFUdS	C11HF23O3S	0.20	18.8
PFDS	C10HF21O3S	0.20	20.1
PFBS	C4HF9O3S	0.10	41.1
PFPeS	C5HF11O3S	0.10	35.1
PFPeA	C5HF9O2	0.20	21.2
PFHpA	C7HF13O2	0.20	25.8
PFHxS	C6HF13O3S	0.20	14.3
PFHpS	C7HF15O3S	0.20	17.3
PFDA	C10HF19O2	0.50	26.2
PFNS	C9HF19O3S	0.20	20.1
PFDoS	C12HF25O3S	0.50	18.8
PFUdA	C11HF21O2	0.50	14.8

Precision and accuracy in 10 ng/L standard

Precision and accuracy were further assessed through multiple injections of a 10 ng/L standard (n=6). Table 3 shows that each PFAS compound was within the acceptable criteria with an average accuracy (%) of $\pm 30\%$ and an area %CV of <15%. Specifically, for the 3 PFAS commonly detected in seawater (PFOS, PFHxA, PFOA) the average accuracy ranged from 93.4-99.9% and the area %CV ranged from 2.8-7.5%.

Calibration for L-PFOS 1: $y = 1.10862e5 x + 3...0.99729$, $r^2 = 0.99458$ (weighting: $1/x^2$)

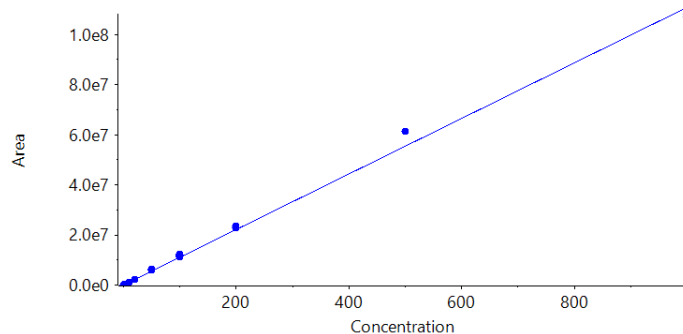


Figure 3. Calibration curve for PFOS. The linear range is 0.2–1,000 ng/L and $r^2 > 0.99$ (weighting $1/x$).

Table 3. The area %CV and average accuracy (%) across six standard injections at 10 ng/L. For each PFAS compound in diluent analyzed, the area %CV values are $\leq 15\%$ and average accuracy (%) values are 70%–130%. Three commonly reported PFAS compounds in seawater are highlighted in bold (PFOS, PFHxA and PFOA).

Compound name	Area %CV	Average accuracy (%)
PFOS	6.7	99.9
PFHxA	2.8	95.3
PFOA	7.5	93.4
PFNA	14	98.5
6:2 FTS	4.8	108
8:2 FTS	6.2	98.5
N-EtFOSSA	4.4	107.8
N-MeFOSSA	6.7	101.4
FOSA	7.6	125
PFUdS	6.0	98.3
PFDS	5.2	97.6
PFBS	4.2	101
PFPeS	7.2	96.9
PFPeA	2.3	104
PFHpA	3.8	101
PFHxS	4.6	103
PFHpS	5.0	106
PFDA	13	100
PFNS	3.2	89.5
PFDoS	6.3	90.9
PFUdA	12.2	91.6

Spiked seawater samples

Un-spiked and spiked seawater samples (10 ng/L) were injected to assess accuracy. Table 4 provides a summary of the calculated concentration of PFAS compounds in un-spiked seawater injected once (correction has been applied based on the average area of detected PFAS compounds in un-spiked seawater samples) when compared to the external standard calibration curve. Figure 6 highlights the XICs of three PFAS compounds, including the blank, un-spiked and spiked seawater samples.

Table 4. The concentration (conc) of PFAS compounds in the un-spiked sample (ng/L) and the accuracy of PFAS compounds analyzed with a 10 ng/L spike. As shown in the table, accuracy (%) values for a 10 ng/L spiked sample were 86.4%–124.8%.

Compound name	Un-spiked conc (ng/L)	Spiked conc: 10 ng/L	Accuracy (%): 10 ng/L spiked
PFOS	<LOQ	10.4	99.6
PFHxA	0.54	10.1	103.9
PFOA	1.91	12.5	100.5
PFNA	<LOQ	9.23	88.5
6:2 FTS	<LOQ	10.8	108
8:2 FTS	ND	10.1	106
N-EtFOSSA	ND	10.2	102
N-MeFOSSA	ND	12.1	112
FOSA	ND	12.5	125
PFUdS	ND	8.64	86.4
PFDS	ND	9.22	92.2
PFBS	0.61	11.6	109
PFPeS	ND	10.1	101
PFPeA	0.58	11.2	102
PFHpA	0.54	10.9	93.4
PFHxS	<LOQ	10.9	109
PFHpS	ND	11.5	115
PFDA	ND	11.0	110
PFNS	ND	9.53	95.3
PFUdA	ND	8.67	86.7

* ND = not detected, <LOQ = below the limit of quantification

* Blank correction has been applied based on the average area of detected PFAS compounds in un-spiked seawater samples for average accuracy (%) and concentration for compounds spiked at 10 ng/L

The average accuracy and precision were assessed for four of the most commonly reported PFAS compounds spiked at 10 ng/L in order to gain an average accuracy and %CV of area. Table 5 shows the calculated concentration of PFOS, PFHxA, PFOA and perfluorononanoic acid (PFNA) compounds in un-spiked seawater when compared to the external standard calibration curve, area %CV and average accuracy (%) for three injections at 10 ng/L.

Average accuracy was within acceptable criteria (70%–130%) and area %CV was $\leq 4.3\%$ for spiked samples. From Table 4 and 5, we see comparable levels of PFOS, PFOA, PFHxA and PFNA in un-spiked seawater, which demonstrates the sensitivity, robustness, and reproducibility of this method.

Table 5. The average concentration (conc) of PFOS, PFHxA, PFOA and PFNA in the un-spiked sample (ng/L), area %CV (n=3) and average accuracy (n=3) analyzed at a 10 ng/L spike. As shown in the table, all area %CV is ≤ 4.26 and average accuracy (%) is 116%–127%.

Compound name	Mean un-spiked conc (ng/L)	Mean spiked conc: 10 ng/L	Mean spiked conc (corrected): 10 ng/L	%CV of area: 10 ng/L spiked	Mean accuracy (%): 10 ng/L spiked
PFOS	<LOQ	12.7	12.4	3.2	123
PFHxA	0.85	13.4	12.7	3.9	127
PFOA	2.08	14.1	11.6	4.3	116
PFNA	<LOQ	12.6	12.1	0.94	121

*<LOQ= less than the limit of quantification

* Correction has been applied based on the average area of detected PFAS compounds in un-spiked seawater samples for average accuracy (%) and concentration for compounds spiked at 10 ng/L

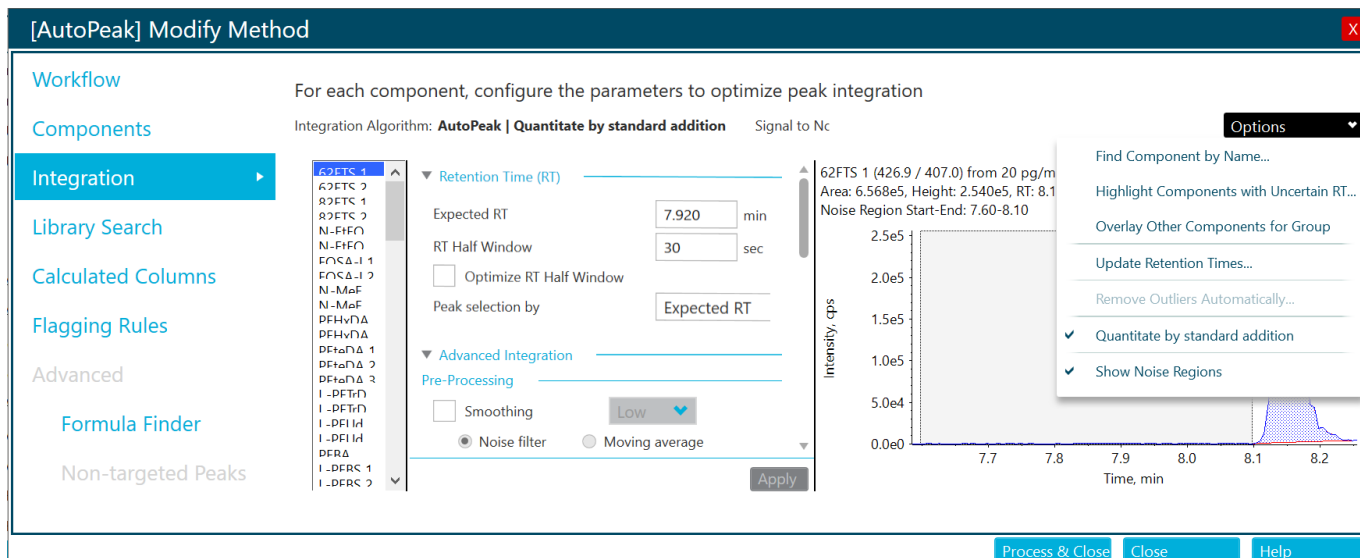


Figure 5. The “Quantitate by standard addition” function can be easily implemented in the process method. The function can be enabled under Integration > Options.

Standard addition in SCIEX OS improves quantification confidence

Standard addition is an analytical technique that improves quantification accuracy in samples with high matrix effects, for example complex environmental samples with high backgrounds. Standard addition is beneficial when surrogate standards are not readily available.⁷ In this study, an external standard calibration curve in solvent and a standard addition workflow was assessed to determine the suitability of an external standard calibration curve for seawater. See Figure 4, which highlights the standard addition calibration curve for PFNA.

Table 6 compares PFAS compounds detected in un-spiked seawater on two different SCIEX 7500 systems when using the standard addition function in SCIEX OS software. The concentration of PFAS compounds detected in un-spiked seawater is comparable on both instruments when using standard addition. The average concentration of PFAS compounds in un-spiked seawater (Table 6) is also comparable to the concentrations calculated against the external standard calibration curve shown in Table 4, highlighting the robustness of this analytical method. Therefore, for this application, standard addition is viable when quantifying PFAS in the low ng/L range in seawater.

Table 6. The concentration of PFAS compounds detected in un-spiked seawater using the standard addition function in SCIEX OS software. The data below were acquired on two different SCIEX 7500 systems.

Compound name	Instrument 1: Average concentration of un-spiked standard addition (ng/L)	Instrument 2: Average concentration of un-spiked standard addition (ng/L)
PFOS	<LOQ	<LOQ
PFHxA	0.74	0.94
PFOA	2.34	2.06
PFNA	<LOQ	<LOQ
6:2 FTS	0.35	0.46
PFHpA	1.10	1.22
PFHxS	<LOQ	<LOQ

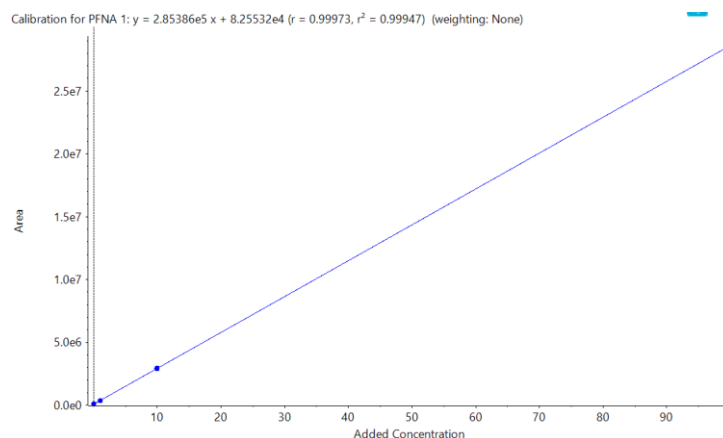


Figure 4. Standard addition calibration curve in seawater for PFNA. The embedded standard addition function in SCIEX OS software was used. Spiked concentrations were 1, 10 and 100 ng/L. An r^2 value of 0.99973 was obtained

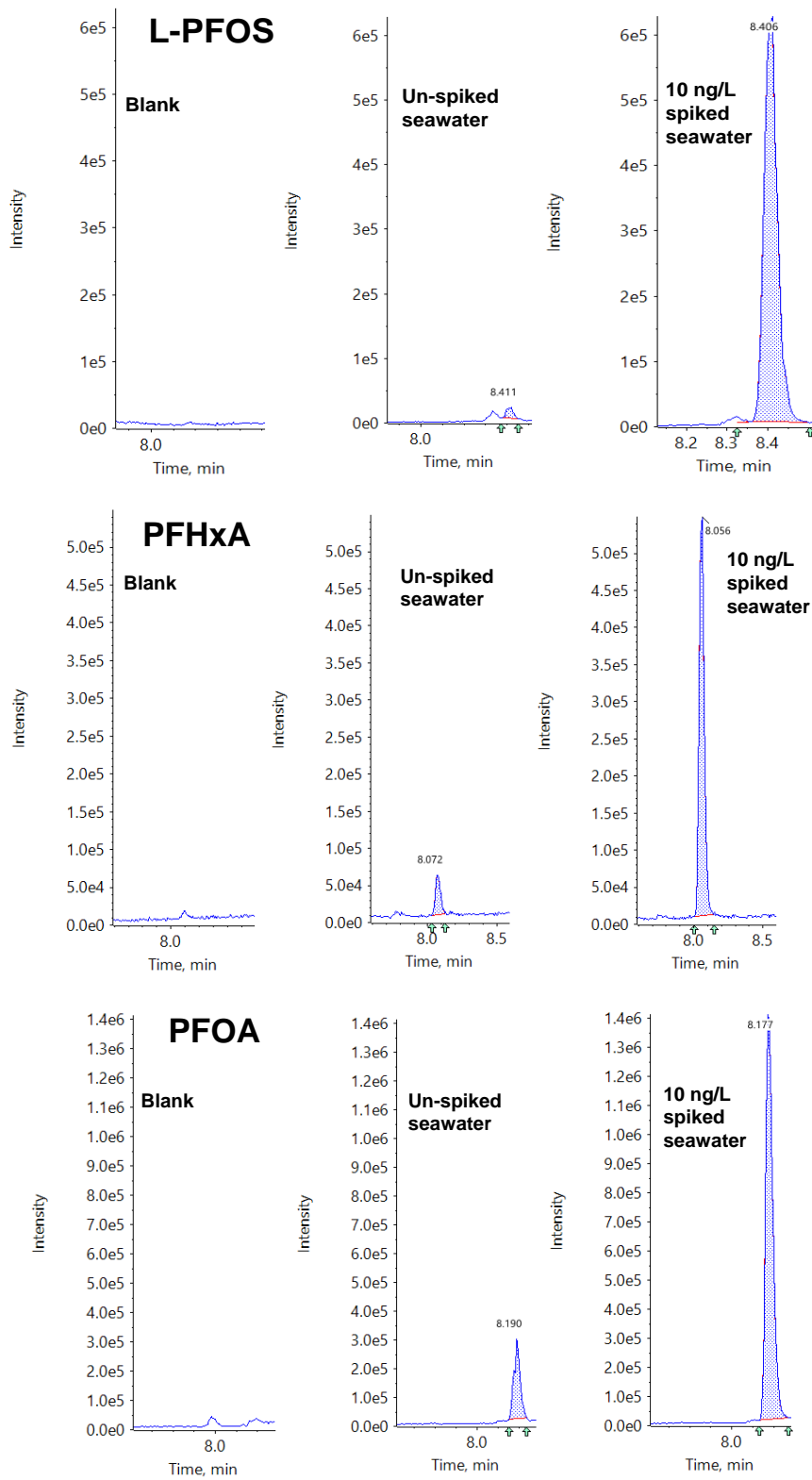


Figure 6. XICs of PFOS, PFHxA and PFOA. From left to right: blank, un-spiked seawater sample and 10 ng/L spiked seawater sample.

Conclusions

- It is possible to achieve high-level sensitivity when analyzing PFAS compounds in the low ng/L range using the SCIEX 7500 system
- For PFAS standards in solvent, the area %CV was <15% and average accuracy (%) was 89.5%–125.5%, which were all within acceptable criteria (<15% for %CV and 70%–130% for average accuracy)
- For PFAS compounds (PFOS, PFHxA, PFOA and PFNA), excellent precision, accuracy and linearity were achieved in seawater samples, confirming the robustness and reproducibility of the method
- Both an external standard calibration curve and a standard addition workflow can be used for quantification of PFAS in seawater samples
- All PFAS compounds detected in the un-spiked seawater samples were at low ng/L levels

References

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