Reducing the effects of system contamination in PFAS analysis

Using the ExionLC 2.0 system

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Per-- and polyfluoroalkyl substances (PFAS) are human made chemicals, developed to have resistance to grease, oil, water and heat. With these properties, PFAS are used in a wide range of consumer and industrial products, including stain and water-resistant fabrics and carpeting, cleaning products, paints, and firefighting foams. Limited use of certain PFAS has FDA approval to be used in cookware, food packaging and food processing equipment.

The strength of the C-F bond in PFAS makes natural degradation extremely difficult. The widespread use of PFAS and their ability to remain intact has caused increasing levels of environmental contamination over time from both past and current uses. This alarming accumulation rate has led to increased study of the potential environmental and health effects of PFAS. The resistance to degradation, however, also makes them a challenge to analyze because they are prevalent contaminants in analytical instrumentation. With guidance for PFAS concentrations continuingly decreasing the limits, there is an increasing need to manage the background levels in the analytical instrumentation used. This would allow an accurate measurement of PFAS amounts in environmental samples.

Here, the ExionLC 2.0 system with the optional wash system was investigated for its flexibility in minimizing background contamination of PFAS for LC-MS/MS analysis when analyzing PFAS containing samples. A SCIEX Triple Quad 7500 system

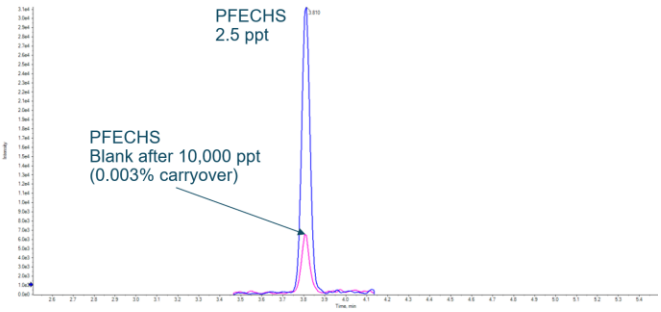

Figure 1. Carryover analysis. Blank extracted ion chromatogram for PFECHS showing extremely low contamination in blank samples (pink trace) analyzed immediately following the injection of the highest calibration standard of 10,000 ppt, overlaid with the LLOQ standard 2.5 ppt (blue trace).

coupled with an ExionLC 2.0 system was chosen as the highly sensitive test platform to investigate meeting a <0.05% carryover performance requirement after 1 ppb standard and a blank contamination <2.5 ppt for 52 PFAS compounds.

Key features of the ExionLC 2.0 system

- Flexibility in wash solvent selection and flow rate options for extended needle wash capabilities to minimize carryover, which reduces false positive rates and the need for repeat extractions and re-injections
- Ability to customize the system and optimize the cleanup of all autosampler solvents to reduce system contamination to a minimum, allowing accurate determination of PFAS even at low levels
- Accurate and precise quantification results with linear coefficient of determination performance (r^2) > 0.99, precision <10% coefficient of variation and asymmetry factors between 0.8 and 1.5
- Sensitive minimum reporting limits as low as 2.5 ppt with a calibration range 2.5-10,000 ppt (3.6 orders of magnitude LDR).

Methods

Materials: All experiments were performed utilizing the U.S. EPA Method 537 and 533 analyte primary dilution standard solution/mixtures (Wellington Laboratories Cat.#). Verex glass and polypropylene autosampler vials with Verex polytetrafluoroethylene/silicon caps were purchased from Phenomenex.

Sample preparation: Standards were prepared to cover the calibration range 2.5-10,000 ppt.

Chromatography: LC separation was achieved using the SCIEX ExionLC 2.0 system and a Phenomenex Gemini C18 column (3 µm, 3.0 x 50 mm, P/N 00B-4439-Y0). A Phenomenex Gemini C18 5 µm, 4.6 x 30 mm column (P/N 00A-4435-E0) was installed after the 50 µL mixer and utilized as a delay column to separate the binary pump solvent PFAS contamination peak from the analytical peak.[1] A 9.5 minute gradient of water and methanol with 10mM ammonium acetate buffer was used, with a flow rate of 0.6 mL/min and column temperature of 40 ℃.

The SCIEX ExionLC 2.0 system autosampler was used with a configuration consisting of a 250 µL syringe, 250 µL buffer tubing, 100 µL sample loop and 15 µL needle tubing. To optimize sample consumption and minimize the injection cycle time, the injection mode used was the microliter pick-up plus mode and the injection volume was set to 10.0 µL utilizing two 35.0 µL transport segments (water containing 10mM ammonium acetate). The syringe speed was set to low and speed factor to 0.8. All polymer tubing was replaced with PEEK, including between solvent bottles and solvent selector valve (with 1/8" outside diameter, or O.D. and an 0.08" internal diameter, or I.D.), solvent selector valve to degasser (1/8" O.D. x 0.08" I.D.), degasser to pump heads (1/8" O.D. x 0.08" I.D) and buffer tubing in the autosampler (1/16" O.D. x 0.03" I.D.).

Autosampler wash system: The wash system was used to deliver various volumes of multiple solvents of differing compositions at varying flow rates to provide the flexibility and capability to manage carryover. The wash program solvents, flow rates and volumes used are shown in Figure 2. All polymer tubing was replaced with PEEK (1/16 in x 0.03 in). A Phenomenex Gemini C18 5 µm, 4.6 x 30 mm column (P/N 00A-4435-E0) was used as a delay column to separate the autosampler wash and transport solvent PFAS contamination peaks from the analytical peak.

Mass Spectrometry: Mass spectrometry was performed using the SCIEX 7500 system, using electrospray ionization (ESI) in negative mode. One MRM transition was monitored for each analyte. The Scheduled MRM algorithm was used to monitor

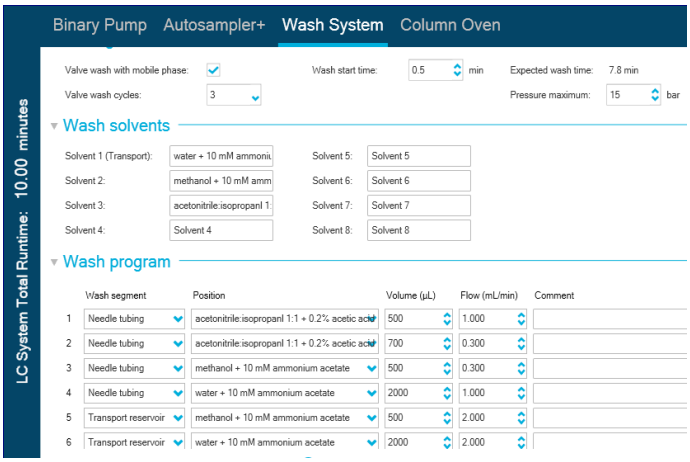

Figure 2. Flexible configuration of autosampler wash station. Up to eight different solvents can be used to generate the most efficient wash program. Here, three different solvents were used in the various steps: acetonitrile/isopropanol (1:1, v/v) with 0.1% acetic acid, methanol with 10mM ammonium acetate wash, and water with 10mM ammonium acetate.

compounds only during their expected retention time window to maximize both cycle time and dwell time. All the peaks in the method contained >12 points across the peak.

Data acquisition was performed using SCIEX OS Software 2.1.6 with Components for the ExionLC 2.0 system.

Data processing: Processing of MS data was performed using SCIEX OS Software 2.1.6 in which calibration curves, precision and accuracy statistics were generated and assessed.

Managing PFAS carryover using the autosampler wash system

The wash system on the ExionLC 2.0 system has the flexibility to wash inside the autosampler tubing as well as the capability to perform an aggressive wash of the outside of the sample needle, using up to 8 different solvents at different flow rates (Figure 3). There is also the option to perform an autosampler valve rinse, using up to 3 autosampler injector valve toggles, and wash with gradient pump solvents prior to performing the wash program with wash system solvents. The wash procedure can be programed to start at any time after the injection, so the valve wash can be performed during the high organic portion of the gradient. A valve rinse is not performed during the wash program so there is no danger of introducing differing solvent compositions to the gradient and affecting chromatographic separation. To optimize sample consumption as well as minimize the injection cycle time, the injection mode used was the microliter pickup plus mode. A transport solvent segment is used in this injection mode to sandwich the sample and ensure delivery of all the sample onto the column. The use of the wash system, which is required for PFAS analysis on the ExionLC 2.0

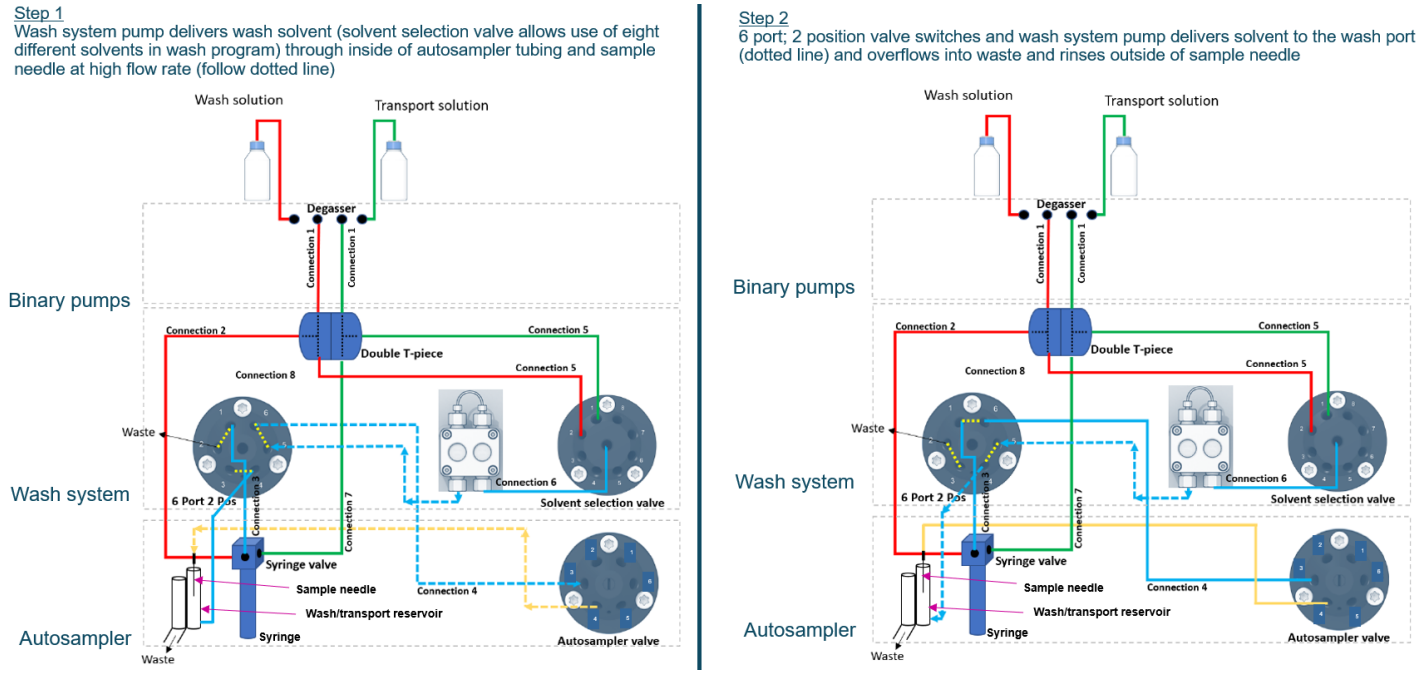

Figure 3. Two step sequential wash sequence of the wash system. Step 1 describes the washing inside the sample needle. Step 2 describes the washing of the outside of the sample needle. Up to 8 solvents can be selected and used with different flow rates in the wash program for maximum flexibility and efficiency.

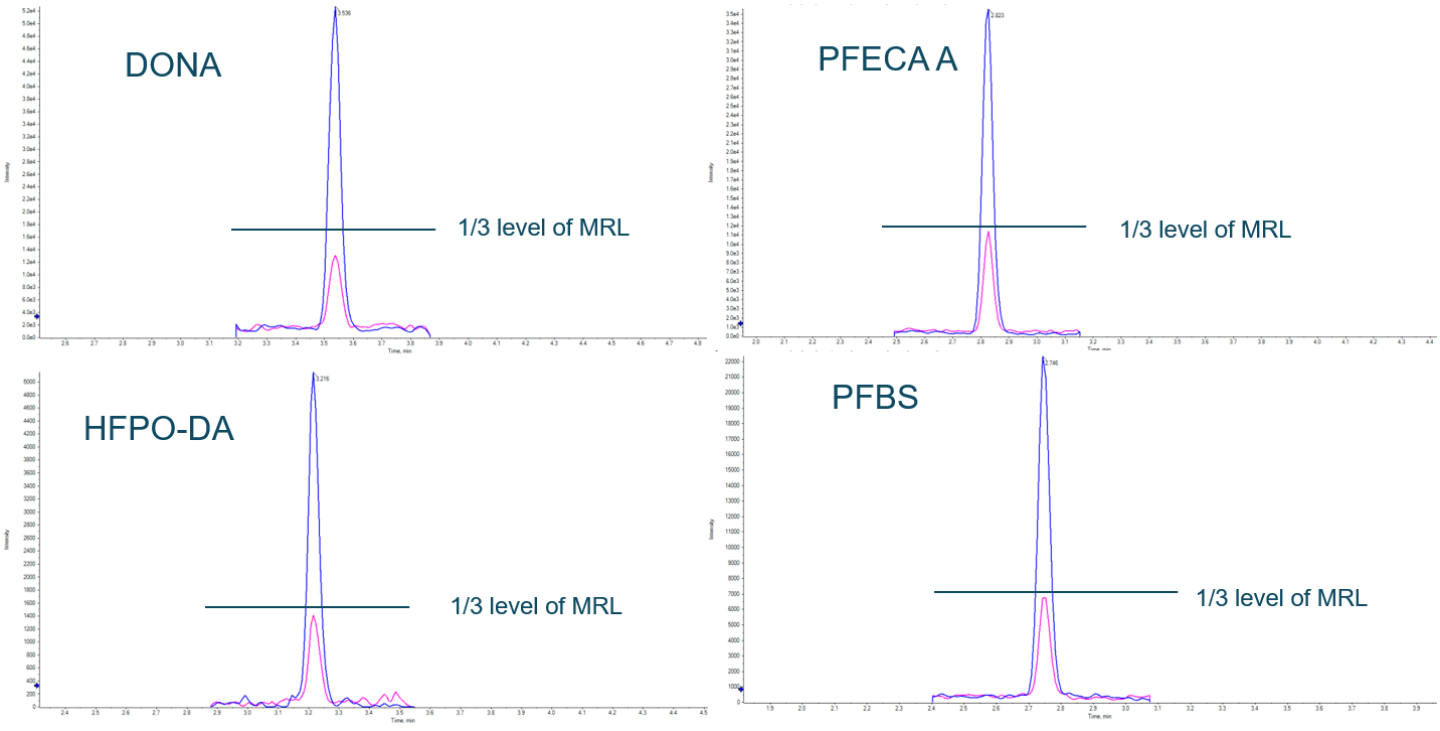

Figure 4. Blank samples. Blank extracted ion chromatograms for select PFAS compounds showing extremely low contamination in blank samples (pink trace). These were analyzed immediately following the injection of the highest calibration standard of 10,000 ppt, and are overlaid with the LLOQ standard 2.5 ppt (blue trace). Requirements specify the blank must be less than 1/3 the level of the MRL.

system allows inclusion of an additional delay column prior to the autosampler valve to separate any PFAS contamination present in the autosampler solvents from the PFAS analytes in the samples and standards.

Evaluating carryover

Blank samples showed very low responses and were below the requirement of <1/3 of the maximum residue limit (MRL). Figure 4 shows carryover peaks for select PFAS compounds in a blank (pink trace) analyzed immediately following the injection of the highest calibration standard of 10,000 ppt, overlaid with the LLOQ standard 2.5 ppt (blue trace). The integrated areas of the first blank after the highest concentration sample (10,000 ppt) were less than 32% of the lowest calibrator for all compounds. For example, the area of the first blank analyzed after the 10,000 ppt calibration standard was 19% of the area of the 2.5 ppt standard for PFECHS. The area of all the carryover peaks was lower than 0.01% of the highest standard peak area (10,000 ppt).

The wash system programing allows selection of the volume of wash solvent to use as well as the flow rate. This, in combination with the ability to use a delay column to clean the autosampler solvents, allowed the management of carryover for the ExionLC 2.0 system to meet the <0.05% carryover performance requirement. This was shown after a 10, 000 ppt standard was run, by a blank contamination of <2.5 ppt for 52 PFAS compounds. Example carryover performance for selected PFAS compounds is shown in Table 1.

Table 1. Carryover analysis.

Compound	% Carryover (after 10,000 ppt standard)
PFHxA	*0.003*
PFOA	*0.001*
PFBA	*0.013*
DONA	*not detected*
PFOS	*0.007*
PFBS	*0.007*
HFPO DA	*0.005*
PFNA	*0.006*
PFECHS	*0.003*
PFHxS	*not detected*
PFDoA	*0.003*
PFECA A	*0.009*

Carryover – area of first blank peak analyzed after the 10,000 ppt calibration standard as a % of the area of the 10, 000 ppt standard peak

Method performance

The chromatographic separation of 52 PFAS compounds is shown in Figure 5. Very good separation was achieved, with narrow peak widths and very good peak symmetry, which is important when performing quantification.

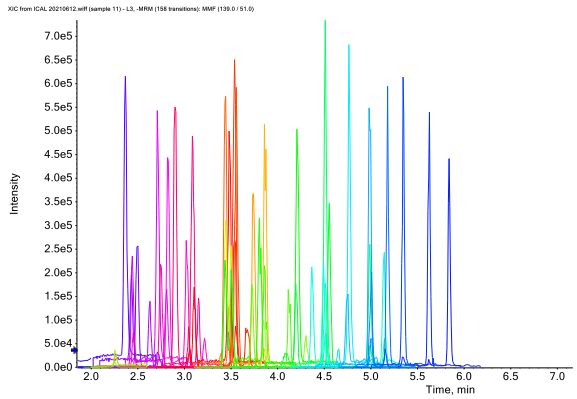

Figure 5. Chromatographic separation. Extracted ion chromatogram showing the PFAS elution profile of the 25 ppt standard.

Peak symmetry was measured using the asymmetry factor—the distance from the center line of the peak to the back slope, divided by the distance from the center of the peak to the front slope, at 10% of the maximum peak—which is typically around 1.0 and is expected to be >0.8 and <1.5. Figure 6 shows the extracted ion chromatogram of selected compounds at 2.5 ppt. For the compounds shown in this figure, the average asymmetry factor was 1.01. Across the concentration range 2.5 to 1000 ppt, the average asymmetry factor was 1.21 for PFBS, 1.03 for PFECA A, 1.17 for HFPO DA, 1.05 for PFHxS, 0.94 for DONA, 1.02 for PFECHS and 1.14 for PFOS. The separation and asymmetry factor for two early eluting peaks are shown on Figure 7.

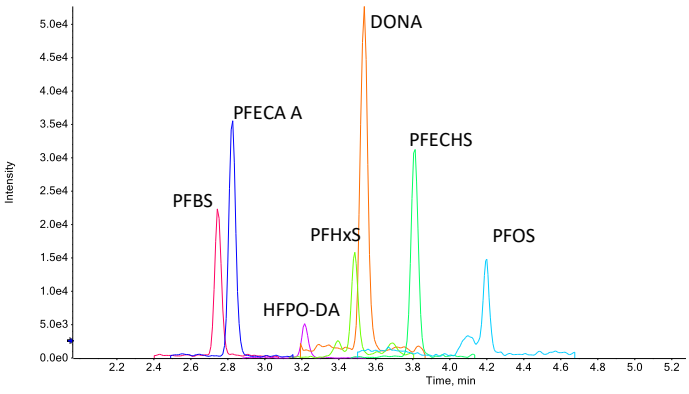

Figure 6. Signal at LLOQ. Extracted ion chromatogram of select PFAS from the 2.5 ppt standard. Very good peak symmetry was observed.

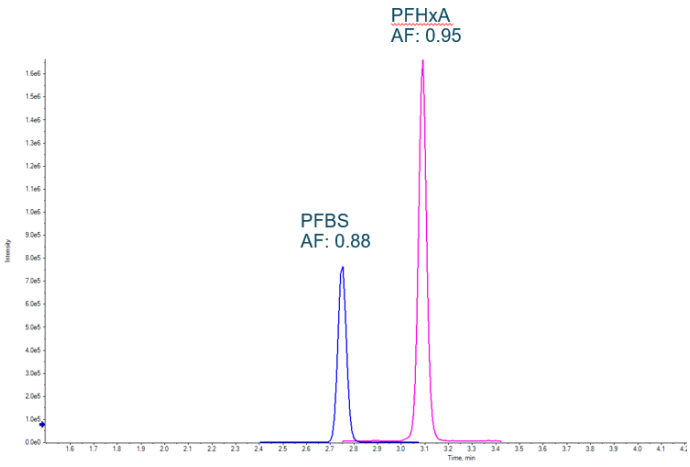

Figure 7. Asymmetry factor (AF). The AF is calculated for early eluting peaks, PFBS and PFHxA at a mid-point standard concentration of 100 ppt.

The 7-point calibration curve exhibited good accuracy within +/- 10% of the expected values for all points (Table 2) and R^2 coefficients of >0.990, as shown in Figure 9. The area reproducibility was also computed from 7 replicate injections of 2.5 ppt, then 7 replicate injections of 25 ppt standard. Typical variance results are shown using selected compounds in Table 3. Further excellent area reproducibility is demonstrated in the metric plot of internal standard area reproducibility over 30 injections (Figure 8). The mean internal standard area was calculated, and all collected data points fell within ±10% mean

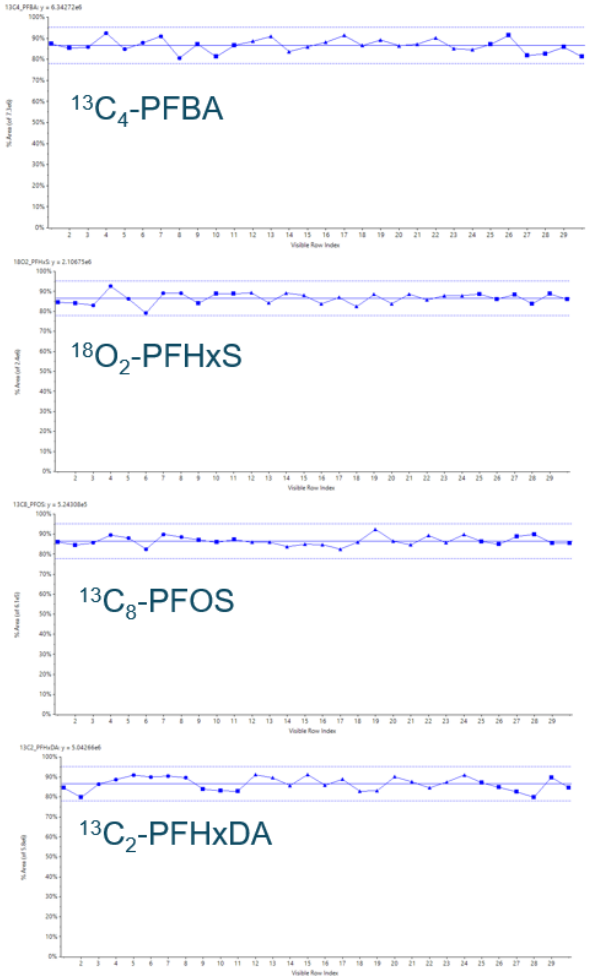

Figure 8. Reproducibility of data. Metric plot of internal standard area reproducibility over 30 injections. Mean regression with ±10% mean regression deviation (dashed lines).

Table 2. Calibration curve statistics. % Accuracy of calibration curve standards across the concentration range interrogated.

Concentration (ppt)	PFHxA	PFOA	PFBA	DONA	PFOS	PFBS	HFPO DA	PFNA	PFECHS	PFHxS	PFDoA	PFECA A
2.50	89.8	97.5	99.5	79.4	99.0	104.9	97.1	108.0	107.7	81.2	80.6	99.4
5.00	94.1	88.9	92.9	101.1	97.8	102.2	94.6	88.2	101.7	90.6	99.3	104.5
25.00	108.2	106.0	103.6	114.1	104.2	98.6	98.8	107.7	96.4	104.5	118.7	99.0
100.00	105.6	109.0	101.9	111.4	101.7	98.2	104.3	91.3	92.7	122.6	106.0	100.0
250.00	101.4	97.4	103.4	93.8	99.1	98.3	107.1	99.1	92.3	102.2	94.7	97.1
500.00	103.7	103.0	99.6	100.2	96.6	94.2	100.4	109.6	113.1	103.4	101.0	98.6
1000.00	97.1	98.2	99.1	116.9	101.7	103.5	97.7	96.2	96.2	95.5	99.8	101.4

Table 3. QC peak area precision. The % coefficient of variation (n=7) for two concentration samples are shown.

Concentration (ppt)	PFHxA	PFOA	PFBA	DONA	PFOS	PFBS	HFPO DA	PFNA	PFECHS	PFHxS	PFDoA	PFECA A
2.5	5.3	9.0	*4.0*	*4.3*	*6.7*	*4.9*	*6.8*	*6.2*	*4.1*	*7.8*	*7.8*	*3.5*
25	4.7	*1.0*	*3.6*	*2.5*	*1.7*	*4.8*	*4.3*	*2.9*	*5.6*	*5.0*	*3.4*	*3.3*

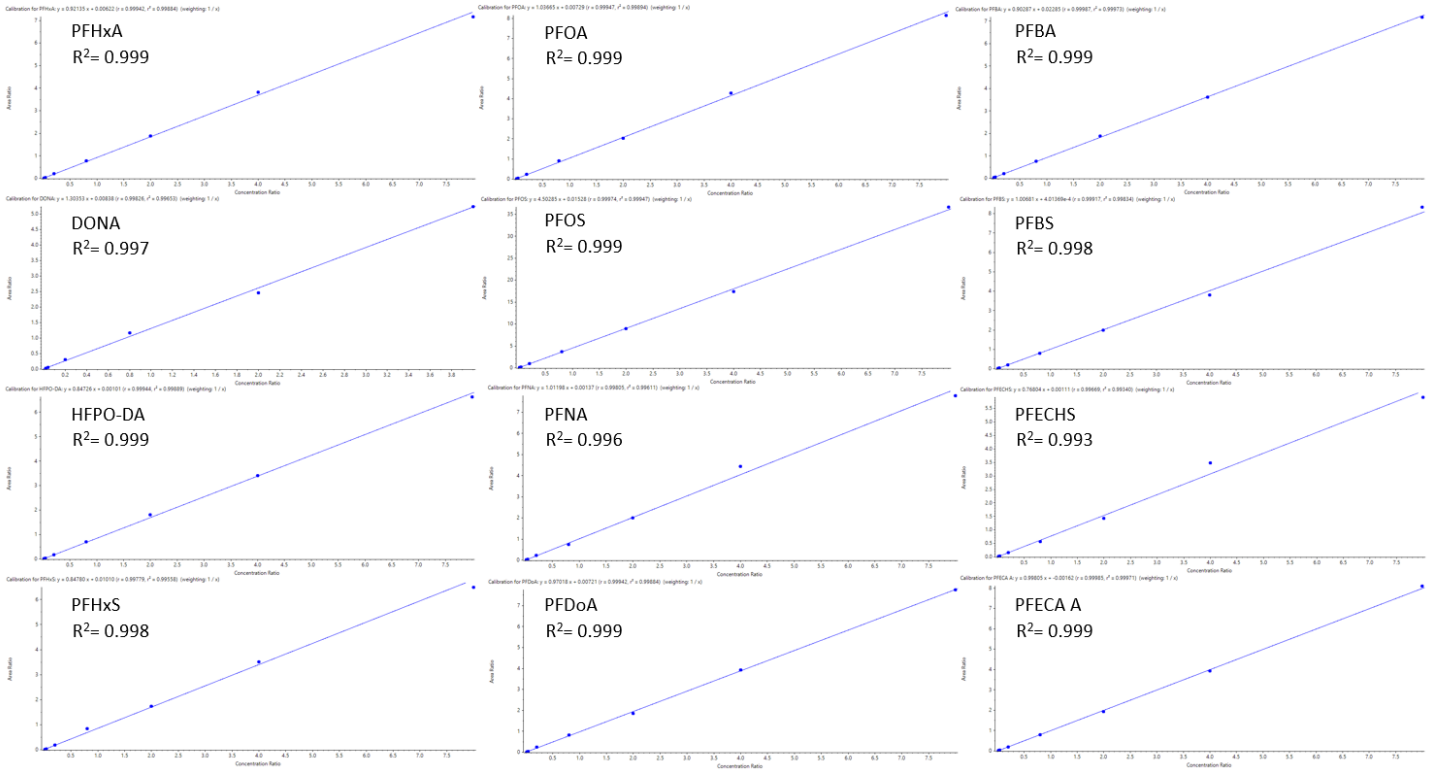

Figure 9. Calibration curves for selected PFAS compounds. Concentration lines were generated from the PFAS mixtures over a concentration range from 2.5 ppt to 1000 ppt. Excellent linearity was observed with r^2 values better than 0.99.

regression deviation (dashed lines). Area %CV for $^{13}C_4$-PFBA, $^{18}O_2$-PFHxS, $^{13}C_8$-PFOS and $^{13}C_2$-PFHxDA were 3.7, 3.2, 2.7 and 4.0% respectively.

Retention time reproducibility

Retention time stability is critical when using narrow retention time windows in combination with time-scheduled MRM acquisition, to ensure the peaks remain within the detection windows. Stability of the LC system over time is critical to ensure consistent retention times are delivered across large sample batches. This reduces the time spent re-adjusting methods to accommodate retention time drifts and minimizes any lost data due to peaks shifting out of target windows.

As shown in Figure 10, the retention time precision of each of the analytes across a range of retention times is less than 0.1% CV, with a mean of 0.05 % CV for the 52 compounds. For most compounds tested, the maximum retention time difference over 20 injections was <1 second.

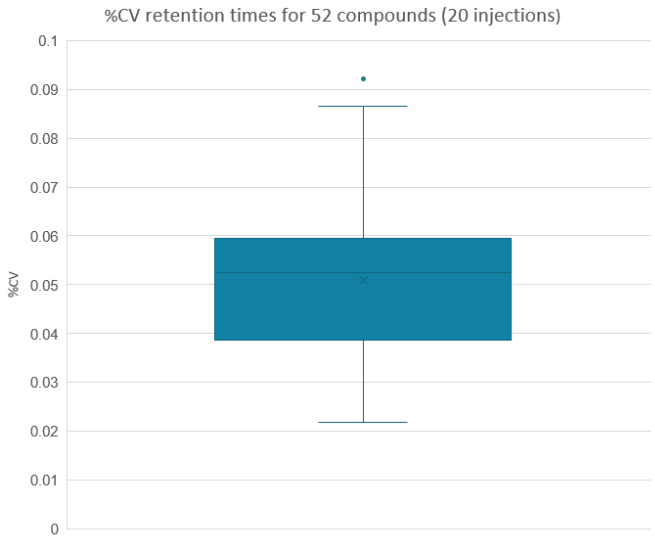

Figure 10. Retention time precision. Box and whisker plot showing the spread of the retention time %CV from 20 injections.

Conclusions

Robust and reproducible results and linearity, with quantitative accuracy and precision for calculated PFAS concentrations, were achieved in a single ten-minute LC-MS/MS acquisition on the SCIEX Triple Quad™ 7500 system. This was enabled by a high-quality separation on the ExionLC 2.0 system, as demonstrated by the retention time precision and peak shape quality.

An important aspect of the accurate measurement of PFAS amounts in environmental samples is the management of the background PFAS levels from the analytical instrumentation.

This was easily implemented on the SCIEX ExionLC 2.0 system by using the optional wash system and additional delay columns, which minimize carryover, reducing false positive rates and the need for repeat extractions and re-injections.

- The installation of a delay column after the LC pumps to separate the PFAS contamination coming from the analytical solvents in addition to the installation of an autosampler delay column that can be used to separate the autosampler solvent PFAS contamination peaks from the analytical peaks
- Flexibility in wash solvent selection and flow rate options for extended needle wash capabilities
- Installation of PEEK tubing throughout system

The replacement of FEP tubing and installation of delay columns, in combination with the use of the wash system, minimized background contamination to allow sensitive MDLs for the entire suite of 52 PFAS compounds studied in this work.

References

1. Quantitation of PFASs in water samples using LC-MS/MS large-volume direct injection and solid phase extraction. SCIEX technical note, RUO-MKT-02-4707-A.

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