

Enhanced performance for FOSAs and FOSEs analysis with an ultra-sensitive PFAS-multiclass method in environmental samples

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Introduction

With the increasing regulatory drivers and public pressure for managing PFAS in the environment, foods, and consumer and industrial products, laboratories are in need of efficient multiclass-PFAS methods that enable routine ultra-sensitive quantitation. As the classes of PFAS to be monitored expand, the instrument performance may be compromised for specific compounds as their ionization efficiency changes based on their physico-chemical properties. In this work we validated a LC-MS/MS method for a multiclass-PFAS panel including PFCAs, PFASs, FTSs, FOSAs, FOSAAs, FOSEs, FTCAS, PFESAs and PFECAs across matrices of environmental relevance (drinking water, wastewater, tissue...). In this presentation we will share the detailed results of the method validation, with emphasis on classes of PFASs that are historically worse performers in multiclass-PFAS methods, such as FOSAs and FOSEs.

Method

Two different methods were optimized in a LCMS 8065XE (Figure 1), equipped with a new ESI probe (StreamFocus) and a new collision cell (UFSweeper IV) for providing more sensitive and accurate results during PFAS analysis, and coupled with a coupled with a Shimadzu Nexera 40 series UHPLC. A delay column was installed between the mixer and autosampler to separate PFAS from the HPLC system and those in the sample. The two methods were developed according to the most commonly sample preparation approaches used for the analysis of PFAS in environmental samples: 1) co-solvation plus direct injection⁽¹⁾ (referred as “Direct Injection”), and 2) SPE or solvent extraction⁽²⁾ (referred as “Extraction Based”).

Difference in the LCMS methods were driven by the final composition of the samples to be analyzed and list of targeted compounds (Table 2).

Direct injection method

For simplicity, two identical columns were used as delay and analytical columns. The target list (29 compounds) was built as a combination of the targets from EPA 537.1 and EPA 533. The samples were prepared in H2O/MeOH (50/50, v/v) without time consuming pretreatment such as solid-phase extraction. Internal standards were added to the samples at concentrations from 10 ng/L to 40 ng/L (final concentration was compound dependent based on performance during the method optimization). The specific method conditions are listed in Table 1.

Extraction based method

In this method, the delay and analytical columns only differed on their particle size. The 40 PFAS targeted in EPA 1633A were analyzed, using 24 extracted internal standards (EIS), and 7 non-extracted internal standards (NIS). Calibration curves were generated by preparing methanol containing 4% water, 1% ammonium hydroxide, and 0.625% acetic acid. The stock standards were then diluted to yield concentration ranges of 2.5 to 62,400 ng/L for native PFAS targets, 1.0 to 20.0 µg/L for EIS, and 1.0 to 4.0 µg/L for NIS. 2.5 ng/L for native standards is equivalent to 0.00625 ng/g in tissue samples. Environmental samples were prepared according to EPA 1633. The LC and MS parameters are summarized in Table 1. Figure 2 shows the chromatographic separation of the 40 targeted PFAS in 10 min. With acetonitrile, the cholic acids eluted much earlier than both branched and linear PFOS. The retention time difference was 1.5 min, which exceeded the required one-minute separation from EPA 1633A.



Figure 1. LCMS-8065XE.

Table 2. Target compounds; compounds shaded in light green – from Direct injection method.

LC: Nexera X3	Direct Injection	Extraction based
Delay Column	Shimadzu Scepter C18-120, 2.1 × 100 mm, 3 µm	Shimadzu Scepter C18-120, 2.1 × 100 mm, 3 µm
Analytical Column	Shimadzu Scepter C18-120, 2.1 × 50 mm, 1.9 µm	Shimadzu Scepter C18-120, 2.1 × 50 mm, 1.9 µm
Flow rate	0.25 mL/min	0.3 mL/min
Mobile phase A	2mM Ammonium Acetate in Water	2mM Ammonium Acetate in Water
Mobile phase B	Methanol	Acetonitrile
Injection volume	20 µL	5 µL
Oven temperature	40 °C	35 °C
Measurement time	18 min	10 min

MS: LCMS-8065XE	Direct Injection	Extraction based
Nebulizing gas	1.1 L/min	1.1 L/min
Heating gas	15.0 L/min	15.0 L/min
Drying gas	5.0 L/min	5.0 L/min
Interface Temperature	200 °C	225 °C
DL Temperature		200°C
Heat Block Temperature	200 °C	250 °C
Interface voltage	-0.2 kV	-0.5 kV
Focus voltage		0 kV

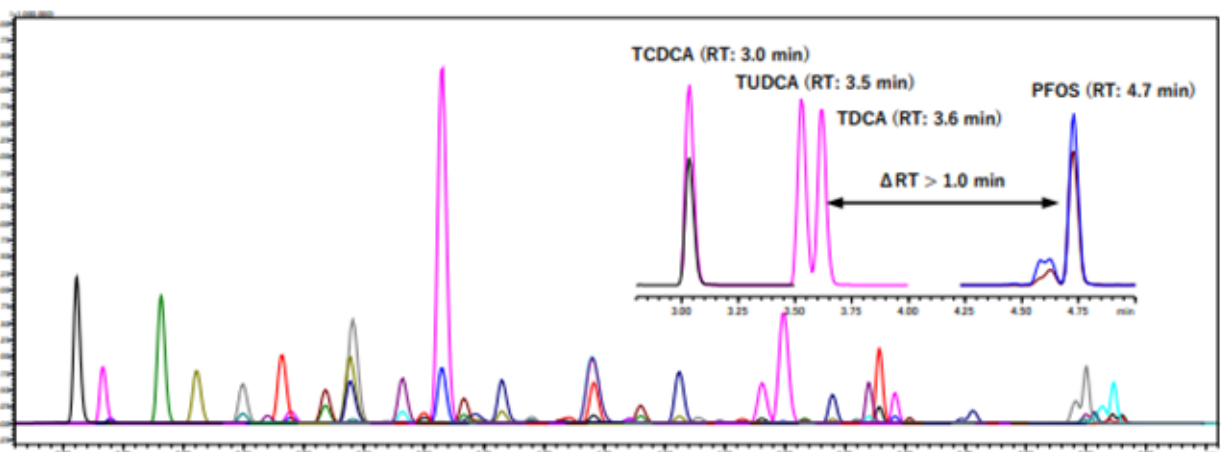


Figure 2. MS chromatogram of 40 PFAS and 3 cholic acids measured in 10 min.

Results

Calibration curves

In the *Direct Injection method*, calibration curves were created in the range of 1-100 ng/L. The calibration curves showed good linearity for all 29 target compounds with accuracy of each calibration point (7 standards) being within 80-120% for both PFOA and PFOS, and 70-130% for the other targets. In the *Extraction based method*, calibration curves (7 standards with concentrations ranging from 2.5 to 62400 ng/L, compound dependent) exhibited linear regression with R²>0.995, accuracies at each calibration points within 70-130%, and relative standard error (RSE) from 3% to 17%; these QA/QA parameters were within the acceptable ranges as listed in EPA 1633A.

Direct injection method – Focus on sensitivity

Seven replicates spiked at 1 ng/L were analyzed to calculate the Method Detection Limits (MDLs). As summarized in Figure 3, the MDLs are less than 1 ng/L without the need of sample preconcentration. These MDLs are suitable to support PFAS testing across the countries in the European Union and under the Water Framework Directive, where local limits are under development and the strictest being <1 ng/L. Figure 4 summarizes the results from the recovery and reproducibility (n=7) evaluation with drinking water samples spiked with 4 ng/L of each target; samples showed excellent recovery (80-120and reproducibility (<20%). PFBA and PFHxA were quantified in the analyzed samples.

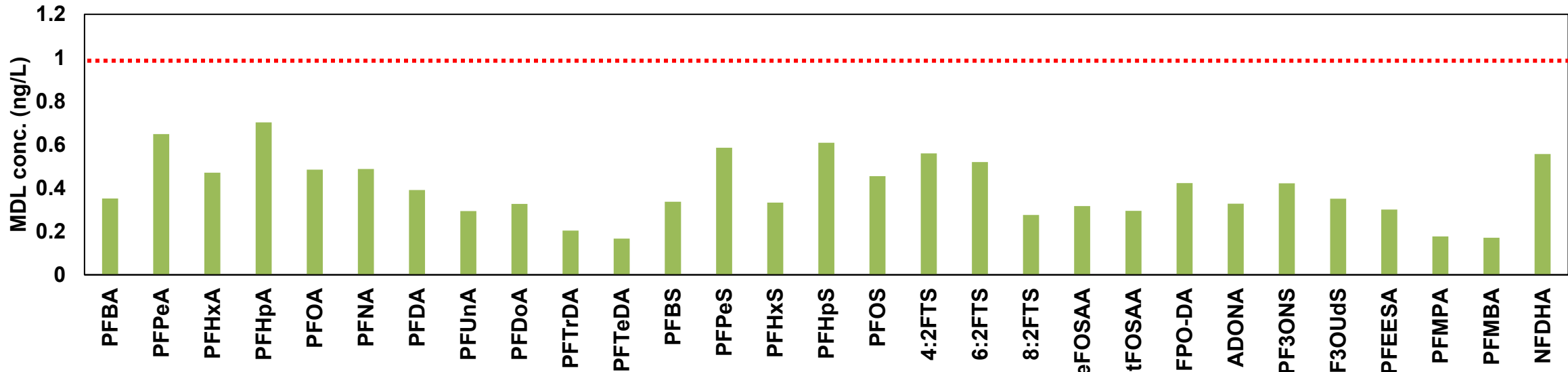


Figure 3. MDL (99% confidence interval) of the target compound

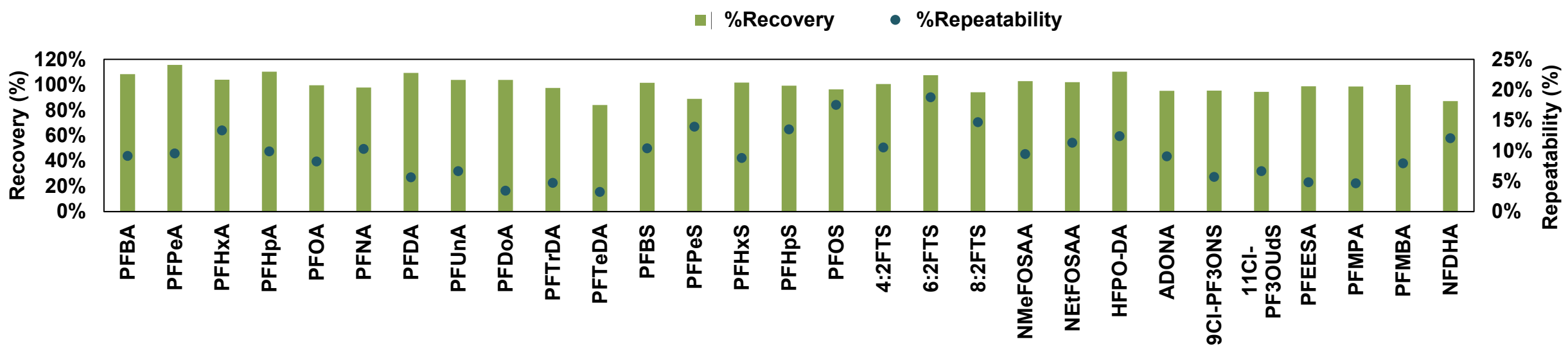


Figure 4. Recovery and reproducibility of drinking water spiked with 4 ng/L (n=7)

Extraction based method – Focus on robustness

The level 5 calibration standard (80x lower concentration than LOQ reported in EPA 1633) was used as the continuing calibration verification (CCV). This concentration was four times lower than the required LLOQ specified in EPA Method 1633A. CCVs were analyzed according to EPA's QA/QC requirements to monitor quantitative performance stability. Figure 5 shows the accuracy of representative compounds (PFBS, HFPO-DA, PFOA, PFHxS, PFNA, and PFOS) in the CCVs. A total of 930 injections of extracted tissue samples were performed. All CCVs (n=73), shown in Figure 5, demonstrated accuracies within 70-130%, noted with the red dotted line in the figure. These results highlight the excellent robustness of the LCMS-8065XE for analysis in complex sample matrices.

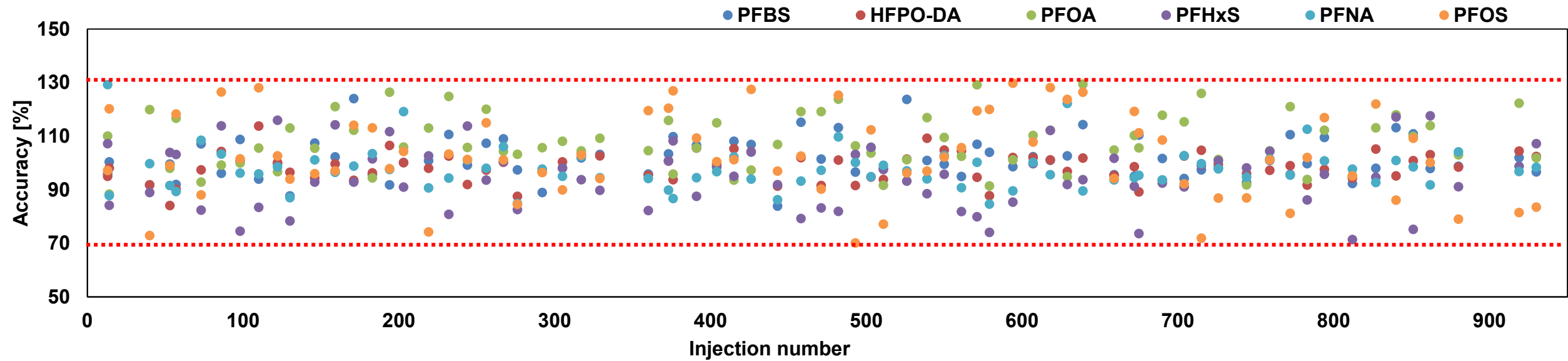


Figure 5. %Accuracy for selected compounds (PFBS, HFPO-DA, PFOA, PFHxS, PFNA and PFOS) in CCVs injected after 10 - 20 samples of extracted tissue blanks.

Challenging compounds in multi-class methods

In multi-class methods performance in terms of sensitivity is normally compromised and higher detection limits are accepted for “worst performers”; for PFAS compounds, these are normally FOSAs, FOSEs and others. LOQs for all 40 targets ranged from 0.003 ng/g to 0.625 ng/g. Figure 6 compares the LOQs reported in EPA 1633A (max and min) in tissues with those obtained in this work, demonstrating that new ESI source enables good sensitivity across PFAS classes.

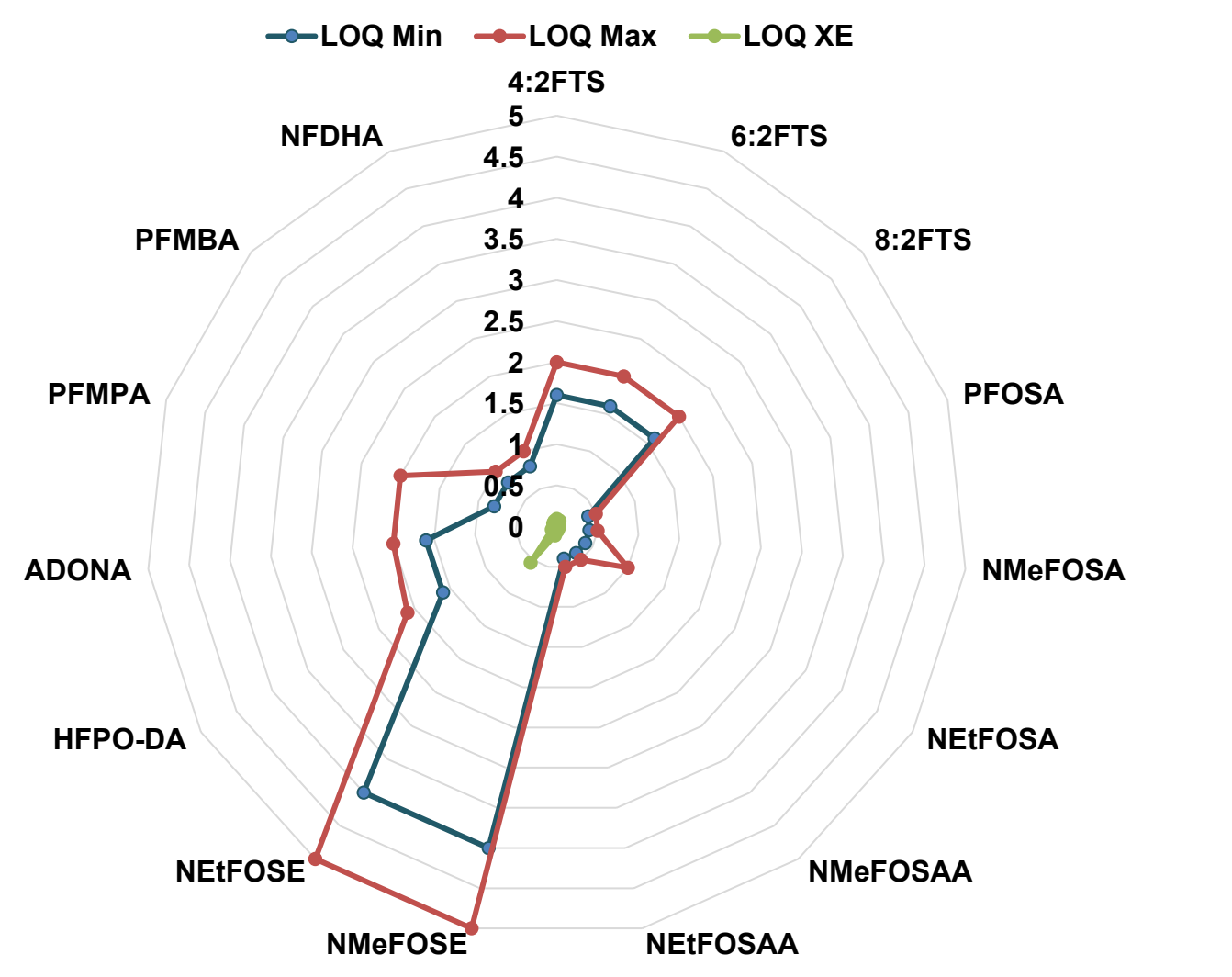


Figure 6. Comparison LOQs (ng/g) in tissue from EPA 1633: FTSs, FOSAs, FOSAAs, FOSEs, FTCAS, PFESAs and PFECAs

Conclusions

- A method for highly sensitive and accurate analysis of 29 PFAS down to less than 1 ng/L in drinking water was validated using the LCMS™-8065XE, in one cycle of 18 minutes by direct injection without sample preconcentration.
- A separate LCMS method for the analysis of 40 PFAS in extracted samples, following EPA 1633A as reference, was also validated. Concentrations up to 80 times lower than the LLOQ in EPA Method 1633A, using a neat standard solution were achieved with excellent linearity. High throughput and robust instrument performance was demonstrated by maintaining accurate quantification during extended operation (>900 injections) without maintenance or cleaning.
- The performance of a novel ESI source was demonstrated across multiple classes of PFAS in a single method.

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References

- An Ultra-high Sensitivity Analysis of 29 PFAS Compounds in Drinking Water by Direct Injection.
- Breakthrough Sensitivity and Robustness for PFAS Analysis for EPA Method 1633A.