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Per- and Polyfluoroalkyl Substances in Aqueous Samples: Performance Demonstration of EPA Method 1633 Using High Sensitivity Mass Spectrometry

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1. Introduction

Per- and poly-fluoroalkyl substances (PFAS) have presented unique analytical challenges around the globe while becoming one of the top emerging contaminants recently (on April 2024) regulated in drinking water by the US Environmental Protection Agency (EPA). The EPA finalized the 1633 method in January 2024 for solids, biosolids, tissue, and aqueous matrices, including wastewater, surface water, and groundwater. Forty target PFAS compounds are paired with 23 extracted internal standards (EIS) and 7 non-extracted internal standards (NIS) for quantitative analysis. This study demonstrates the developed method for LC-MS/MS analysis and its performance to fulfill selected quality control requirements outlined in EPA 1633 to accurately analyze PFAS in aqueous samples.

2. Methods

Five target PFAS standards and two IS mixtures were purchased from Wellington Laboratories (PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, PFAC-MXJ, MPFAC-HIF-IS, and MPFAC-HIF-ES). Calibration standards were made by serial dilution of the target stock standards using methanol containing 4% water, 1% ammonium hydroxide, and 0.625% acetic acid, starting at 10 times lower than calibration range listed in EPA 1633.

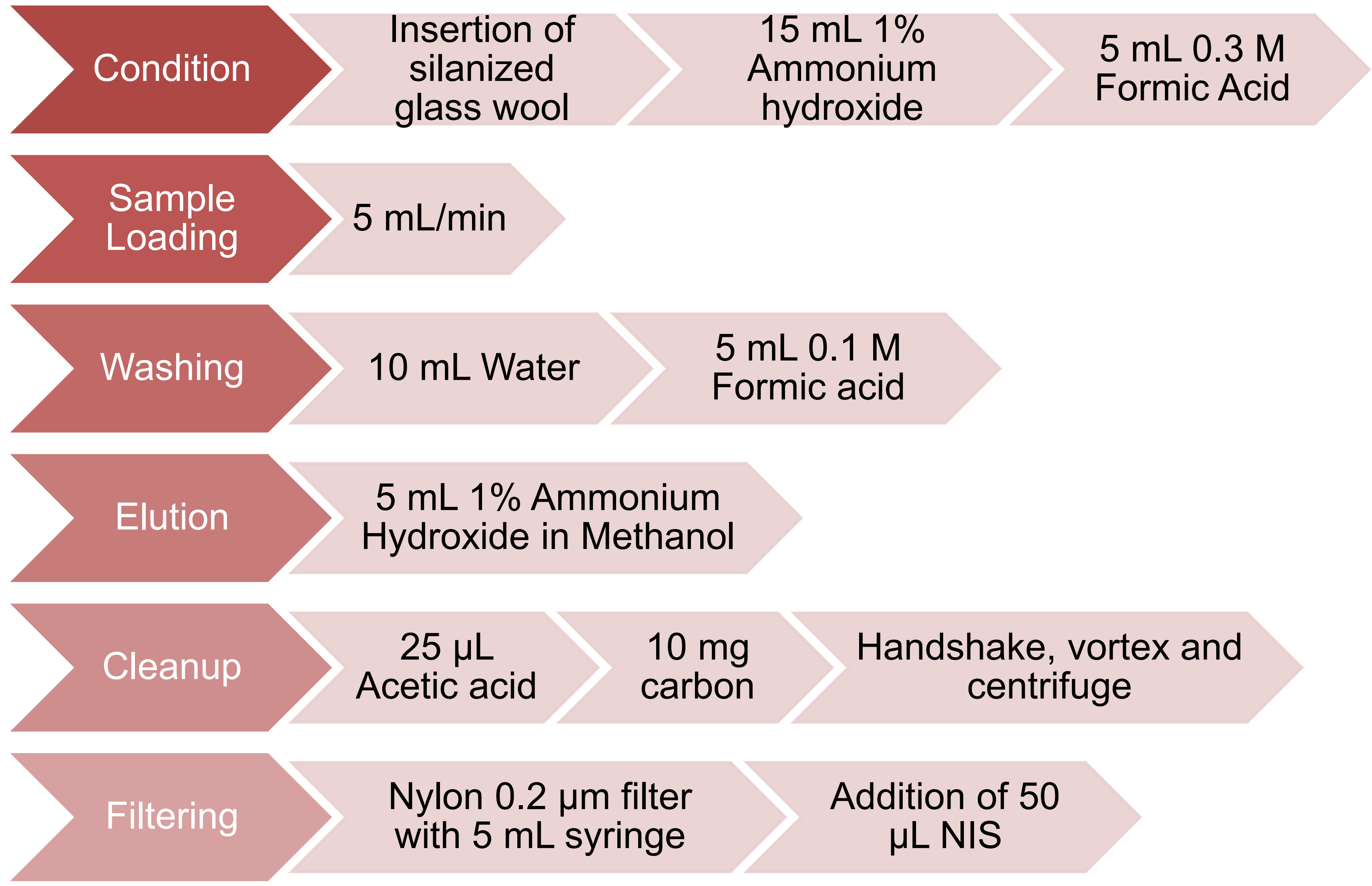
A Shimadzu LCMS-8060NX triple quadrupole mass spectrometer coupled with a Nexera HPLC system was used for LC-MS/MS analysis with the parameters listed in **Table 1**. This analysis utilized a Shim-pack Scepter C18-120 (3 µm, 2.1x100 mm) as the delay column to remove any background PFAS contamination, and a Shim-pack Scepter C18-120 (3 µm, 2.1x50 mm) as the analytical column.

Table 1. Summary of the LCMS method parameters

Parameter	Value
LCMS	Shimadzu LCMS-8060NX
Analytical Column	Shim-pack Scepter C18-120, 3.0 µm, 2.0 x 50mm
Delay Column	Shim-pack Scepter C18-120, 3.0 µm, 2.0 x 100mm
Injection Volume	10 µL
Pretreatment Mode	Co-Injection
Column Oven Temp.	40°C
Mobile Phase	A: 2 mM Ammonium Acetate in LCMS Grade Water
	B: LCMS Grade Acetonitrile
Flow Rate	0.4 mL/min
Run Time	14 minutes

2.1 Sample Preparation

A 500 mL volume of reagent water was spiked with 50 µL of EIS (800 µg/L 13C4-PFBA) and 200 µL of native compounds (2 µg/L PFBA). Method Blanks (MB) were also prepared and only spiked with EIS. To calculate the Method Detection Limits (MDL) of the target compounds, seven water samples spiked at concentrations 10x lower than EPA's limit of quantification (LOQ). Samples were extracted by solid-phase extraction (SPE) using Biotage EVOLUTE® EXPRESS WAX 150-mg/6-mL cartridges and following the procedure in **Figure 1**. After extraction, cleanup, and concentration of each sample, an aliquot was transferred to a 1 mL silanized amber glass vial and vortexed for LC-MS/MS analysis.



Condition → Insertion of silanized glass wool → 15 mL 1% Ammonium hydroxide → 5 mL 0.3 M Formic Acid

Sample Loading → 5 mL/min

Washing → 10 mL Water → 5 mL 0.1 M Formic acid

Elution → 5 mL 1% Ammonium Hydroxide in Methanol

Cleanup → 25 µL Acetic acid → 10 mg carbon → Handshake, vortex and centrifuge

Filtering → Nylon 0.2 µm filter with 5 mL syringe → Addition of 50 µL NIS

Figure. 1 Summary of sample extraction, cleanup, and concentration

2.2 LCMS Analysis

- Day 1 analysis included, a calibration curve, instrument blank, a calibration verification (CV), three method blanks, and three spiked water samples.
- Day 2 consisted of analyzing the instrument blank, CV, three MB, and three spiked water samples.
- This was repeated on Day 3 with the instrument blank, CV, two MB, and two spiked water samples.
- Before each LC-MS/MS batch, every vial was vortexed to resuspend PFAS compounds that may have adsorbed to the walls of their respective vials. This helps to improve relative standard error (RSE), as PFAS compounds are known to adsorb to the walls of sample vials.

3. Results

LC-MS/MS analysis for target PFAS and EIS compounds passed requirements outlined in EPA 1633 requiring a relative standard error (RSE) maximum of 20% for instrument linearity. This linear range varied for PFAS target compounds from 0.025 µg/L to 25 µg/L with a resulting RSE range from 9.8% for PFTrDA to 18.3% for PFHxA. EIS compounds had an RSE range from 0.71% for 13C$_5$-PFNA to 10.92% for 13C$_3$-HFPO-DA at concentration levels specified by the method. Guidelines from 40 CFR 136, Appendix B were followed to calculate an MDL value for the target PFAS compounds listed in EPA's 1633 method. The method detection limits for spiked samples (MDL_s) were computed by multiplying the standard deviation from the concentration of each compound by the appropriate t-value (99% confidence level). Per EPA 1633 criteria, method detection limits were also determined for method blanks (MDL_b) when an individual analyte has a numerical result in at least one sample. MDL_b was calculated the same way as the MDL_s with the addition of the average resulting concentration for each compound. Final MDL values are then chosen based on the greater value between the calculated MDL_s and MDL_b. 21 compounds including PFBA, 3:3 FTCA, PFHpA, and NMeFOSE had an MDL_b. Of all compounds with an MDL_b, only PFHpA had an MDL_b (0.23 ng/L) greater than its MDL_s (0.16 ng/L). Overall, the MDL ranged from 0.10 ng/L for PFEESA to 1.48 ng/L for 5:3 FTCA. **Figures 2 and 3** compare the calculated MDL values obtained with this workflow using the Shimadzu's LCMS-8060NX to the values report in EPA Method 1633.

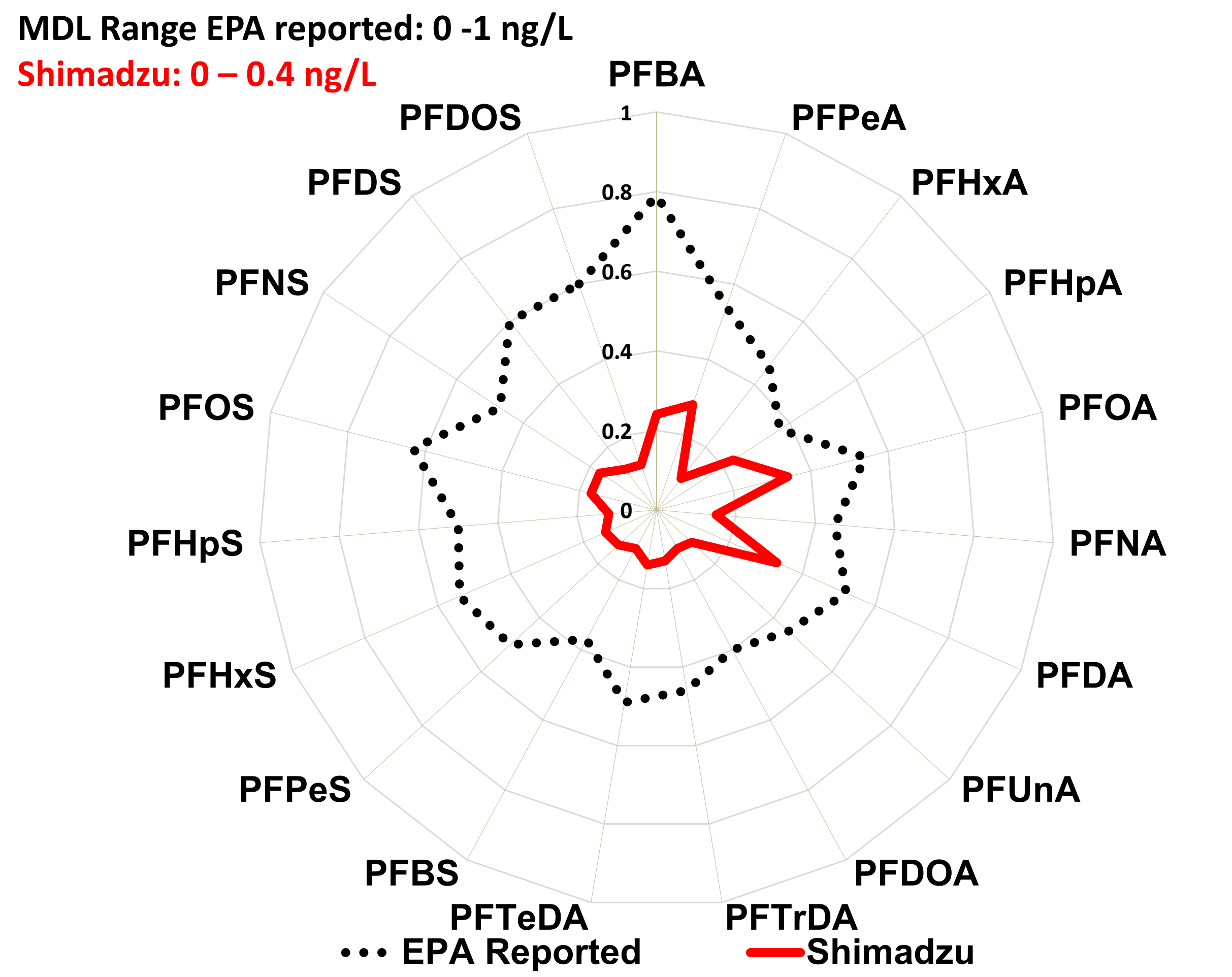


Figure 2. MDLs reported in EPA 1633 and obtained with Shimadzu's LCMS-8060NX of perfluoroalkyl carboxylic acids and sulfonic acids.

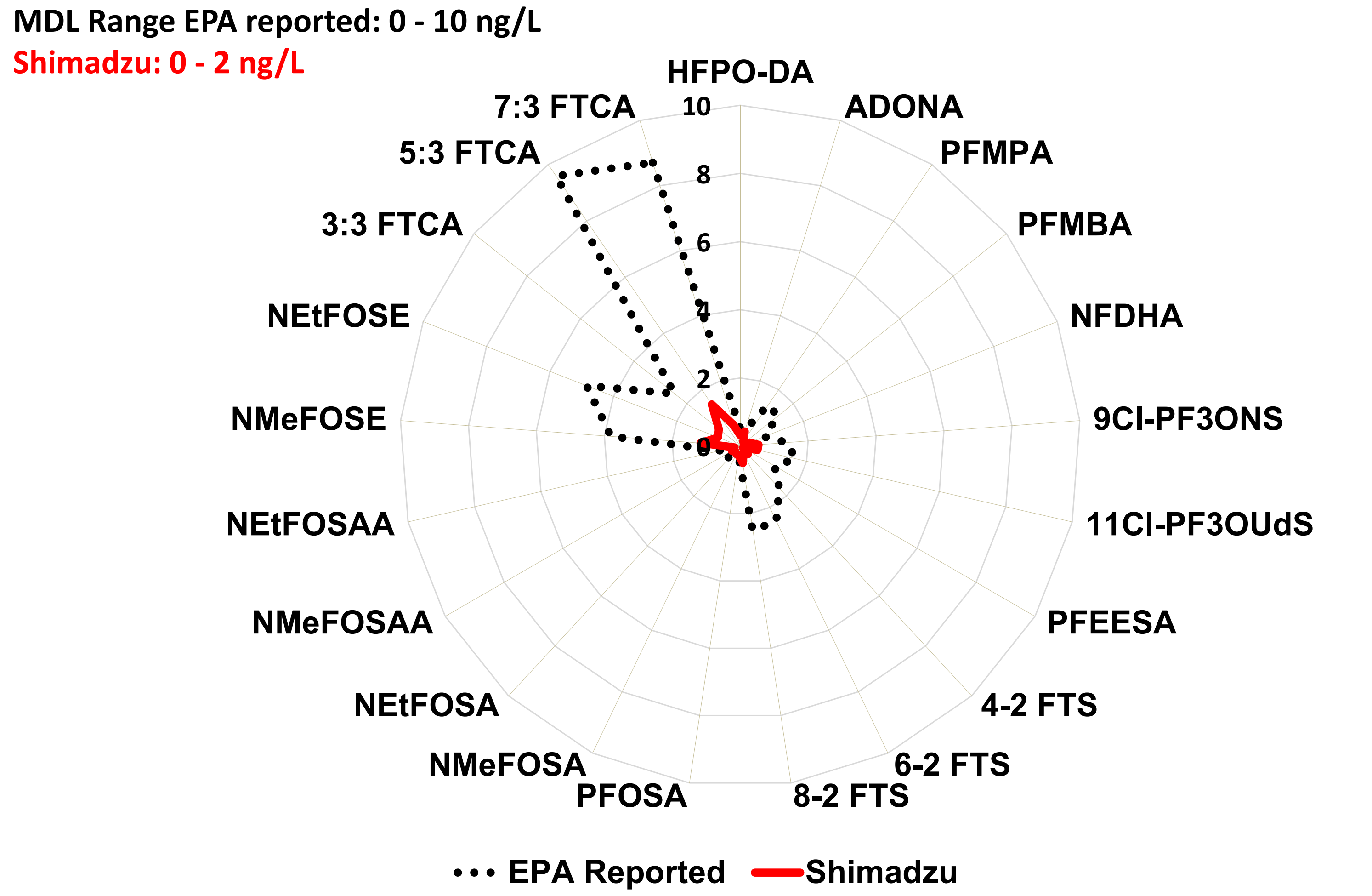


Figure 3. MDLs reported in EPA 1633 and obtained with Shimadzu's LCMS-8060NX of Per- and Polyfluoroether carboxylicacids, Ether sulfonic acids, Fluorotelomer sulfonic acids, Perfluorooctane sulfonamides, Perfluorooctane sulfonamidoacetic acids, Perfluorooctane sulfonamide ethanols, Fluorotelomer carboxylic acids.

4. Conclusions

- The Shimadzu LCMS-8060NX can detect 10x lower than EPA's LOQ in a neat standard matrix and extracted aqueous matrix.
- Excellent linearity was obtained with our method as observed by our low %RSDs and R^2 values.
- MDLs using the LCMS-8060NX were achieved that were up to 13.4x better than those reported by EPA method 1633.

Reference
(1) Method 1633* Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS
(2) (2) Appendix B to Part 136, Title 40 -- Definition and Procedure for the Determination of the Method Detection Limit—Revision 2

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