# EPA Method 1633:

## Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

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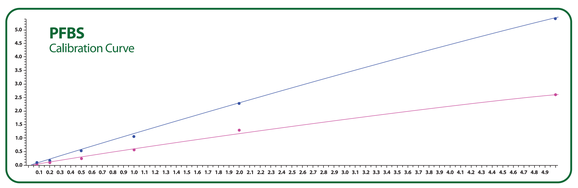
## INTRODUCTION

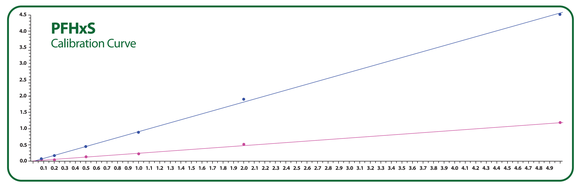
The list of Per- and polyfluoroalkyl substances (PFASs) is continually expanding such that the EPA released a new draft method 1633 [1] for monitoring these compounds in aqueous, solid, and tissue samples. These analytes are very resistant to degradation and must be identified and quantified in various media.

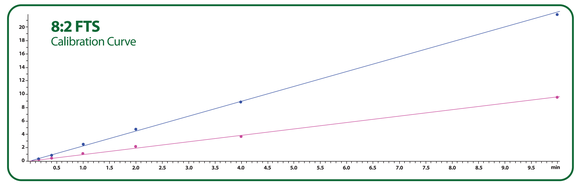
The diversity of these analytes, which stretches from very hydrophobic long chain molecules to very short chain polar analytes, makes it challenging to extract them from a sample using a single technique. Clean extracts along with excellent recoveries were successfully achieved using UCT Enviro-Clean® WAX SPE columns.

## LC-MS/MS PARAMETERS

| | | | |
|---|---|---|---|
| **HPLC system** | SCIEX Exion LC | **Detector** | ABSciex Qtrap 6500+ |
| **Delay column** | **Selectra® C18 50 x 4.6 mm x 5µm (p/n: SLC-1850ID46-5UM)** | | |
| **HPLC column** | **SelectraCore® C18 50 x 2.1mm x 2.7µm (p/n: SCS27-C18521)** | | |
| **Guard column** | **SelectraCore® 5 x 2.1mm x 2.7µm (p/n: SCS27-C18GDC21)** | | |
| **Guard column holder** | **Selectra® (p/n: SLGRDHLDR)** | | |
| **Oven temperature** | 40°C | **Flow rate** | 0.4 mL/Min |
| **Injection Volume** | 2 µL | | |
| **Mobile Phase** | **Bottle A:** 20mM Ammonium acetate in water / **Bottle B:** Acetonitrile | | |
| **Gradient ( B Conc. )** | 0 min (2%), 0-7.5 min (100%), 7.5-8.5 min (100%), 8.5-8.51 min (2%), 8.51-11.5 min (2%) | | |




References:
[1] 2nd Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid,Biosolids, and Tissue Samples by LC-MS/MS. URL: https://www.epa.gov/system/files/documents/2022-07/2nd%20Draft%20of%20Method%201633%20June%202022%20508-compliant.pdf

**Disclosure**: The speaker, author, moderator, planning member and/or presenter/s do have financial relationships with UCT, Inc. , as defined in the AACC policy on potential bias or conflict of interest. The specific product/s : Enviro-Clean® WAX156-P, Enviro-Clean® Graphitized Carbon, Selectracore® HPLC columns, Selectracore® Guard Columns, Selectra® Delay Column and Selectra® Guard Column Holder will be mentioned and/or discussed.



Questions / Comments: methods@unitedchem.com

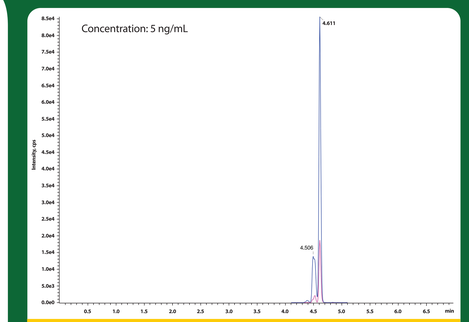



Figure 1: Linear and Branched Isomer Separation (PFOS)

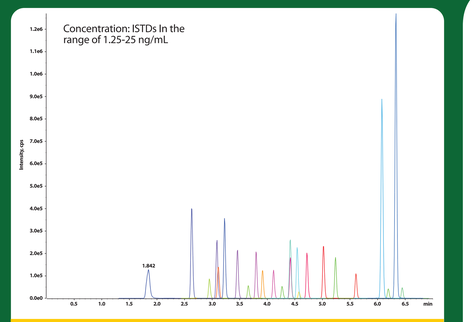



Figure 2: 1633 Extracted Internal Standards

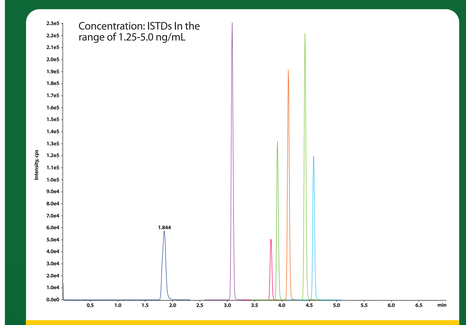



Figure 3: 1633 Non-Extracted Internal Standards

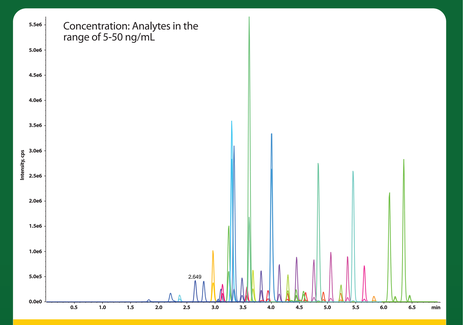



Figure 4: 1633 Targeted Analytes

## SPE METHOD

### System Set-up

a) Pack clean silanized glass wool to half the height of the WAX SPE cartridge barrel.
b) Set up the vacuum manifold with one WAX SPE cartridge plus a reservoir and reservoir adaptor for each cartridge for each sample.

### SPE Conditioning

a) Rinse SPE cartridge (ECWAX156-P) with15 mL of 1% methanolic ammonium hydroxide.
b) Rinse the cartridge with 10 mL of 0.3M formic acid, not allowing the water to drop below the top edge of the packing.
c) Close the valve and add 2–3 mL of 0.3M formic acid to the cartridge reservoir and the remaining volume with reagent water.

Note: Do not allow cartridge packing material to go dry during any of the conditioning steps.

### Sample Extraction/Drying

a) Pour the sample into the reservoir.
b) Adjust the vacuum so that the flow rate is approximately 5 mL/min.
c) Rinse the walls of the reservoir with 5 mL reagent water twice.
d) Rinse the walls of the reservoir with 5 mL of 1:1 0.1M formic acid/methanol.
e) Dry the cartridge under a high vacuum (15-20 in.Hg) for 5 minutes.

### Elution

a) Insert a collection rack containing 15 mL polypropylene collection tubes into the extraction manifold. DO NOT add NIS to these collection tubes.
b) Rinse the inside of the sample bottle with 5 mL of freshly prepared 1% methanolic ammonium hydroxide.

Note: Due to the volatility of ammonium hydroxide, it is highly recommended to use freshly prepared elution solvent. Rinsing the sides of the container is important for obtaining good recovery of the long-chain hydrophobic PFAS.

c) Transfer the rinse to the SPE reservoir, washing the walls of the reservoir. Use a low vacuum setting to let the solvent drip in a dropwise fashion.
d) Add 25 µL of concentrated acetic acid to each sample eluted in the collection tubes and vortex.
e) Add 10 mg of carbon (EUCARB00X) to each sample and hand-shake occasionally for no more than 5 minutes.
f) Vortex and centrifuge at 2800 rpm for 10 minutes.
g) Add NIS solution to a clean collection tube.
h) Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant the sample supernatant into the syringe barrel. Replace the plunger and filter the entire extract into the new collection tube containing the NIS.
i) Vortex and transfer a portion of the extract into a 1-mL polypropylene microvial for LC-MS/MS analysis.

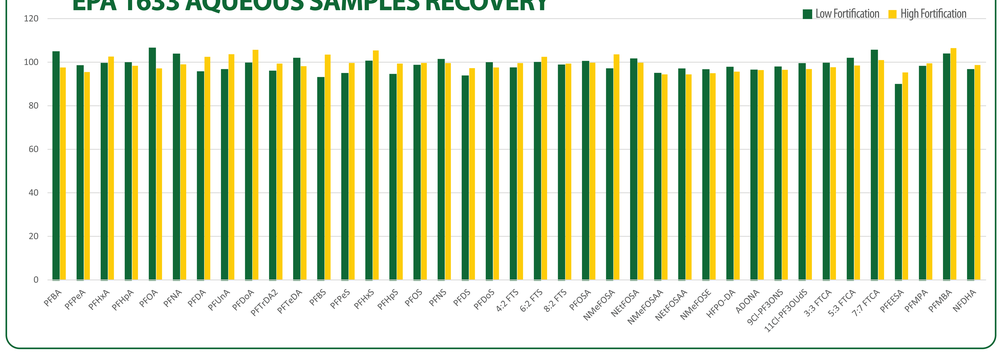
## SPE RESULTS IN AQUEOUS SAMPLES

(n=5)

| Analyte | Low Fortification | | | High Fortification | | |
|---|---|---|---|---|---|---|
| | Conc. (ng/mL)) | Rec. (%) | RSD (%) | Conc. (ng/mL)) | Rec. (%) | RSD (%) |
| PFBA | 5.0 | 104.6 | 0.9 | 25 | 97.5 | 1.1 |
| PFPeA | 2.5 | 98.2 | 1.0 | 12.5 | 95.4 | 3.7 |
| PFHxA | 1.25 | 99.3 | 0.7 | 6.25 | 102.5 | 1.1 |
| PFHpA | 1.25 | 99.6 | 0.6 | 6.25 | 98.3 | 2.0 |
| PFOA | 1.25 | 106.3 | 2.1 | 6.25 | 97.1 | 2.6 |
| PFNA | 1.25 | 103.5 | 1.3 | 6.25 | 98.9 | 1.2 |
| PFDA | 1.25 | 95.4 | 0.9 | 6.25 | 102.4 | 1.4 |
| PFUnA | 1.25 | 96.4 | 0.6 | 6.25 | 103.6 | 1.7 |
| PFDoA | 1.25 | 99.4 | 0.7 | 6.25 | 105.6 | 1.0 |
| PFTrDA2 | 1.25 | 95.7 | 2.6 | 6.25 | 99.3 | 4.3 |
| PFTeDA | 1.25 | 101.6 | 0.6 | 6.25 | 98.1 | 0.9 |
| PFBS | 1.25 | 92.8 | 0.8 | 6.25 | 103.4 | 1.8 |
| PFPeS | 1.25 | 94.6 | 0.7 | 6.25 | 99.6 | 1.9 |
| PFHxS | 1.25 | 100.3 | 1.3 | 6.25 | 105.3 | 5.5 |
| PFHpS | 1.25 | 94.2 | 1.2 | 6.25 | 99.3 | 5.9 |
| PFOS | 1.25 | 98.4 | 1.0 | 6.25 | 99.6 | 4.7 |
| PFNS | 1.25 | 101.1 | 0.9 | 6.25 | 99.5 | 1.4 |
| PFDS | 1.25 | 93.5 | 0.8 | 6.25 | 97.2 | 1.4 |
| PFDoS | 1.25 | 99.6 | 1.0 | 6.25 | 97.5 | 0.9 |
| 4:2 FTS | 5.0 | 97.2 | 0.9 | 25 | 99.5 | 0.8 |

| Analyte | Low Fortification | | | High Fortification | | |
|---|---|---|---|---|---|---|
| | Conc. (ng/mL)) | Rec. (%) | RSD (%) | Conc. (ng/mL)) | Rec. (%) | RSD (%) |
| 6:2 FTS | 5.0 | 99.7 | 0.9 | 25 | 102.4 | 4.1 |
| 8:2 FTS | 5.0 | 98.5 | 1.3 | 25 | 99.3 | 3.0 |
| PFOSA | 1.25 | 100.2 | 1.0 | 6.25 | 99.7 | 1.8 |
| NMeFOSA | 1.25 | 96.8 | 1.3 | 6.25 | 103.5 | 4.5 |
| NEtFOSA | 1.25 | 101.3 | 1.9 | 6.25 | 99.7 | 2.7 |
| NMeFOSAA | 1.25 | 94.7 | 1.5 | 6.25 | 94.3 | 2.9 |
| NEtFOSAA | 1.25 | 96.7 | 1.1 | 6.25 | 94.3 | 1.7 |
| NMeFOSE | 12.5 | 96.4 | 1.7 | 62.5 | 94.9 | 1.7 |
| NEtFOSE | 12.5 | 98.6 | 2.8 | 62.5 | 97.4 | 0.7 |
| HFPO-DA | 5.0 | 97.5 | 4.2 | 25 | 95.6 | 3.9 |
| ADONA | 5.0 | 96.2 | 0.8 | 25 | 96.3 | 2.7 |
| 9Cl-PF3ONS | 5.0 | 97.6 | 2.9 | 25 | 96.4 | 1.2 |
| 11Cl-PF3OUdS | 5.0 | 99.1 | 1.5 | 25 | 96.8 | 0.3 |
| 3:3 FTCA | 6.25 | 99.3 | 2.6 | 31.2 | 97.6 | 4.2 |
| 5:3 FTCA | 31.3 | 101.6 | 0.8 | 156 | 98.4 | 0.6 |
| 7:7 FTCA | 31.3 | 105.3 | 1.0 | 156 | 100.9 | 0.3 |
| PFEESA | 2.5 | 89.6 | 0.8 | 12.5 | 95.2 | 0.9 |
| PFMPA | 2.5 | 97.9 | 0.8 | 12.5 | 99.4 | 1.4 |
| PFMBA | 2.5 | 103.6 | 0.8 | 12.5 | 106.4 | 1.4 |
| NFDHA | 2.5 | 96.4 | 0.6 | 12.5 | 98.6 | 0.9 |

## EPA 1633 AQUEOUS SAMPLES RECOVERY




## CONCLUSION

This poster outlines the analysis of PFAS in aqueous samples according to EPA 1633 second draft method utilizing UCT's Enviro-Clean® polymeric weak-anion exchange (WAX) SPE cartridges (ECWAX156-P). In addition, the use of EUCARB00X helped produce a cleaner extract. LC-MS/MS analysis was carried out using a SelectraCore® C18 HPLC analytical column (SCS27-C18521), while a short (5cm) C18 delay column (SLC-1850ID46-5UM) was used to reduce potential PFAS contamination from the HPLC system. The chromatography was optimized to obtain a maximum resolution with minimum co-elution of isomers, including the critical linear and branched isomers of PFHxS and PFOS. For quantitation, a six-point calibration was performed, and all compounds were found to be linear with R2 values > 0.99. The extraction method was evaluated by spiking reagent water samples with PFAS at 5 ng/L and 25 ng/L. Recoveries of all analytes were within a range of 80-120% and RSD values <10%. Due to high background contamination that may arise from the solvent lines in the LCMS system, the instrument was plumbed with only Polyethylene lines to eliminate any interferences.

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