# Analysis of Per- and Polyfluoroalkyl Substances (PFAS) Specified in EPA M533 Using LCMS Triple Quadrupole

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## Novel Aspect

The quantitation of short chain per- and polyfluoroalkyl substances (PFAS) in drinking water[1] by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS).

## Introduction

Recently, EPA announced a new method for testing short chain per- and polyfluoroalkyl substances (PFAS) in drinking water. Structures for select short chain and long chain PFAS[2] are included in Figure 1. Method 533[3] measures PFAS by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS). The lowest concentration minimum reporting levels (LCMRLs) for the method analytes range from 1.4 to 16 ng/L. Shimadzu Scientific Instruments was one of eight laboratories that participated in providing EPA with outside laboratory validation data along with a review of the method draft. This poster includes Shimadzu Scientific Instruments data from the validation study.

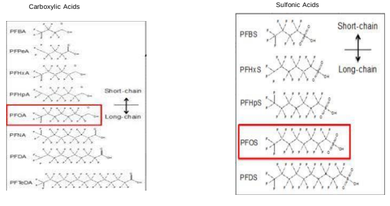



**Figure 1.** Structure of Long and Short Chain PFAS compounds.

## SPE Method

Solid Phase Extraction (SPE) with a WAX sorbent (500 mg) was used for the extraction, as outlined in EPA method 533 (section 6.8.1). Each cartridge was cleaned and conditioned first, following EPA 533 (section 11.4.1). A vacuum manifold with a high-volume sampling kit outfitted with large bore PEEK tubing was used to reduce potential contamination.

All sample bottles were rinsed with the elution solvent prior to use. Each water sample (250 mL) was adjusted to pH 6-8 and fortified with PFAS analyte and isotope dilution analogues, mixed, and loaded onto the conditioned cartridge. Compounds were eluted at a high pH from the solid phase with two 5 mL aliquots of methanol containing 2% ammonium hydroxide (v/v) and evaporated to dryness using nitrogen. Extracted samples were reconstituted to a final volume of 1 mL in 80:20 methanol:$H_2O$ with internal standards added.

Extraction for Precision & Accuracy study was performed by fortifying five replicates of reagent water and tap water samples at 10 ng/L. For LCMRL calculations (results not shown in this poster) samples were extracted at eight concentration levels ranging from 0.2 ppt and 14 ppt. Four replicates were prepared at each concentration level and a minimum of four laboratory reagent blanks (LRB) were also included in the extraction batches.

**Figure 2**. LCMS-8045 triple quadrupole mass spectrometer

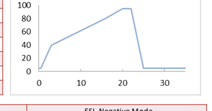
## Instrumental Method

The analysis of 25 PFAS compounds, with16 isotope dilution analogues and 3 post extraction internal standards was performed using a UHPLC system coupled with a triple quadrupole mass spectrometer. MRM transitions were optimized using Flow Injection Analysis for all compounds[4]. Source parameters were optimized to reduce fragmentation and increase sensitivity. Fluorotelomer acids, observed as [M-H]- and [M-HF-H]- can result in an ion with the same m/z as the unsaturated fluorotelomer acid. Even under optimized chromatography, these compounds have near identical retention times. The lower ESI heater temperature reduces HF loss and minimizes false identification of fluorotelomer acids. The chromatographic parameters are based on the chromatographic method used in EPA Method 533. A Shim-pack XR-ODS 50 x 3.0 mm column was used as a delay column, and a Phenomenex Gemini™ C18, 2.0 mm ID × 50 mm, 3.0 µm particle size column was used as the analytical column. Quantitation was performed using MRM on tandem mass spectrometer (LC-MS/MS). LCMS system and instrumental conditions are included in Table 1 and MRM transitions are included in Table 2.

**Table 1**. LCMS Method conditions

| | |
|---|---|
| **LCMS Instrument** | Shimadzu LCMS-8045 |
| **Analytical Column** | Gemini 3µm C18 110A LC Column 50 x 2mm |
| **Solvent Delay Column** | Shim-pack XR-ODS 2.2-micron, 3.0 x50mm |
| **Injection Volume** | 10 µL |
| **LC Flow Rate** | 0.25 mL/min |
| **Mobile Phase A** | 20 mM Ammonium Acetate in LCMS-grade Water |
| **Mobile Phase B** | Methanol |
| **Run / Acquisition Cycle Time** | 35 minutes (all 44 PFAS compounds are eluted in 20 minutes) |

| | Time (min) | % MPA | %MPB |
|---|---|---|---|
| **Gradient Conditions** | 0 | 95 | 5 |
| | 3 | 60 | 40 |
| | 16 | 20 | 80 |
| | 20 | 5 | 95 |
| | 22 | 5 | 95 |
| | 25 | 95 | 5 |




| | |
|---|---|
| **Interface** | ESI, Negative Mode |
| **Interface Temperature** | 100 °C |
| **Desolvation Line Temperature** | 160 °C |
| **Heat Block Temperature** | 200 °C |
| **Heating Gas Flow** | 15 L/min |
| **Drying Gas Flow** | 5 L/min |
| **Nebulizing Gas Flow** | 3 L/min |
| **Total MRMs** | 66 |
| **Minimum Dwell Time** | 19 msec |
| **Maximum Dwell Time** | 124 msec |

## Calibration

Standards available from Wellington Laboratories were used for these studies (EPA method analyte stock 2 mL volume in methanol at 1 ug/L, Internal standard in methanol Wellington Catalog No. 533-IS and Isotope Dilution Analogue PDS in Methanol Wellington Catalog No. 533-ES). These standards were then diluted to working standards as outlined in Section 7.17.5 of EPA Method 533 using 20% water in methanol as diluent to match the extract solvent composition. The working standards were used to create a calibration curve ranging from 1 ng/L to 1000 ng/L for NFDHA, and from 0.1 ng/L to 100 ng/L for all other analytes. During this study Initial Calibration curve was ran 5 consecutive days. Figure 3 shows aggregate calibration curve for PFMPA and PFPeA and Figure 4 shows aggregate calibration curve for PFDA and example MRL 0.1 ng/L chromatogram. The chromatogram shown in Figure 5 is from a level 7, 6 ng/L calibrator. Figure 6 shows a clean instrument blank (80:20 MeOH:$H_2O$), indicating that the system is free from PFAS contamination as no PFAS was detected.

**Table 2.** Target and labelled PFAS m/z, retention times, and correlation coefficients from the aggregate curve (Days 1-5)

| ID# | Compound | MRL invial (ng/mL) | MRL in sample(ng/L) | Type | ISTD Group# | m/z | RT | Collision | R2 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M3PFBA | ---- | ---- | ISTD | 3 | 216.00>172.00 | 5 | 10 | ---- |
| 2 | MPFBA | ---- | ---- | Surrogate | 1 | 217.00>172.00 | 5 | 10 | ---- |
| 3 | PFBA | 0.05 | 0.2 | Target | 1 | 212.90>168.90 | 5.18 | 10 | 0.9945 |
| 4 | PFMPA | 0.025 | 0.1 | Target | 1 | 229.00>85.00 | 6.2 | 10 | 0.9947 |
| 5 | PFPeA | 0.05 | 0.2 | Target | 1 | 263.00>219.00 | 7.95 | 8 | 0.9947 |
| 6 | M5PFPeA | ---- | ---- | Surrogate | 1 | 268.00>223.00 | 7.94 | 8 | ---- |
| 7 | M3PFBS | ---- | ---- | Surrogate | 1 | 302.00>80.00 | 8.54 | 34 | ---- |
| 8 | PFBS | 0.1 | 4 | Target | 2 | 298.90>80.10 | 8.55 | 30 | 0.9949 |
| 9 | PFMBA | 0.025 | 0.1 | Target | 1 | 279.00>85.00 | 8.72 | 20 | 0.994 |
| 10 | PFEESA | 0.025 | 0.1 | Target | 1 | 314.90>134.85 | 9.54 | 25 | 0.9958 |
| 11 | NFDHA | 5 | 20 | Target | 1 | 295.00>201.15 | 10.08 | 8 | 0.9982 |
| 12 | M2-4-2 FTS | ---- | ---- | Surrogate | 2 | 329.00>309.00 | 10.22 | 20 | ---- |
| 13 | 4-2 FTS | 1 | 4 | Target | 2 | 327.00>307.00 | 10.2 | 18 | 0.9938 |
| 14 | PFHxA | 0.05 | 0.2 | Target | 1 | 312.90>269.00 | 10.48 | 8 | 0.9947 |
| 15 | PFPeS | 0.1 | 0.4 | Target | 2 | 349.00>80.00 | 10.82 | 9 | 0.9949 |
| 16 | HFPO-DA | 0.025 | 0.1 | Target | 1 | 285.00>169.00 | 11.21 | 42 | 0.9953 |
| 17 | 13C-HFPO-DA | ---- | ---- | Surrogate | 1 | 287.00>169.20 | 11.21 | 8 | ---- |
| 18 | PFHpA | 0.025 | 0.1 | Target | 1 | 362.90>319.00 | 12.57 | 9 | 0.9942 |
| 19 | M4PFHpA | ---- | ---- | Surrogate | 1 | 367.00>322.00 | 12.57 | 10 | ---- |
| 20 | M3PFHxS | ---- | ---- | Surrogate | 2 | 402.00>80.00 | 12.75 | 9 | ---- |
| 21 | PFHxS | 0.1 | 0.4 | Target | 2 | 398.90>80.10 | 12.08 | 49 | 0.9965 |
| 22 | ADONA | 0.025 | 0.1 | Target | 1 | 377.00>250.90 | 12.8 | 43 | 0.9948 |
| 23 | 6-2 FTS | 0.5 | 2 | Target | 2 | 427.00>407.00 | 14.12 | 11 | 0.9955 |
| 24 | M2-6-2 FTS | ---- | ---- | Surrogate | 2 | 429.00>409.00 | 14.14 | 22 | ---- |
| 25 | M8PFOA | ---- | ---- | Surrogate | 1 | 421.00>376.00 | 14.27 | 23 | ---- |
| 26 | PFOA | 0.1 | 0.4 | Target | 1 | 412.90>369.00 | 14.25 | 10 | 0.9944 |
| 27 | M2PFOA | ---- | ---- | ISTD | 1 | 415.00>370.00 | 14.28 | 10 | ---- |
| 28 | PFHpS | 0.1 | 0.4 | Target | 2 | 449.00>80.00 | 14.33 | 10 | 0.9952 |
| 29 | PFNA | 0.05 | 0.2 | Target | 1 | 462.90>418.90 | 15.76 | 51 | 0.9942 |
| 30 | M8PFOS | ---- | ---- | Surrogate | 3 | 507.00>80.00 | 15.75 | 12 | ---- |
| 31 | M9PFNA | ---- | ---- | Surrogate | 1 | 472.00>427.00 | 15.73 | 11 | ---- |
| 32 | PFOS | 0.05 | 0.2 | Target | 2 | 498.90>80.10 | 15.23 | 45 | 0.9952 |
| 33 | M4PFOS | ---- | ---- | ISTD | 2 | 503.00>80.00 | 15.76 | 45 | ---- |
| 34 | 9Cl-PF3ONS | 0.025 | 0.1 | Target | 1 | 530.90>351.00 | 16.5 | 54 | 0.9954 |
| 35 | 8-2 FTS | 1 | 4 | Target | 2 | 527.00>507.00 | 16.97 | 27 | 0.997 |
| 36 | M2-8-2 FTS | ---- | ---- | Surrogate | 2 | 529.00>509.00 | 16.98 | 26 | ---- |
| 37 | PFDA | 0.025 | 0.1 | Target | 1 | 512.90>468.90 | 17.04 | 26 | 0.9952 |
| 38 | MPFHxA | ---- | ---- | Surrogate | 1 | 318.00>273.00 | 10.48 | 12 | ---- |
| 39 | PFUnA | 0.025 | 0.1 | Target | 1 | 562.90>519.00 | 18.14 | 11 | 0.9948 |
| 40 | M7PFUnA | ---- | ---- | Surrogate | 3 | 570.00>525.00 | 18.11 | 12 | ---- |
| 41 | 11Cl-PF3OUdS | 0.025 | 0.1 | Target | 1 | 630.70>451.00 | 18.63 | 12 | 0.9953 |
| 42 | PFDoA | 0.025 | 0.1 | Target | 1 | 612.90>568.90 | 19.06 | 30 | 0.9951 |
| 43 | M2PFDoA | ---- | ---- | Surrogate | 3 | 615.00>570.00 | 19.06 | 10 | ---- |
| 44 | MPFDA | ---- | ---- | Surrogate | 1 | 519.00>474.10 | 17.04 | 12 | ---- |

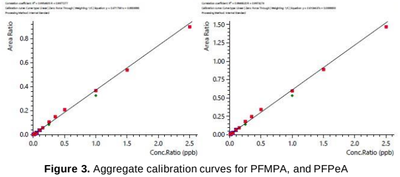



**Figure 3.** Aggregate calibration curves for PFMPA, and PFPeA

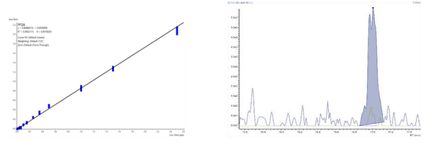


**Figure 4**. Aggregate calibration curve for PFDA and example MRL 0.1 ng/L chromatogram

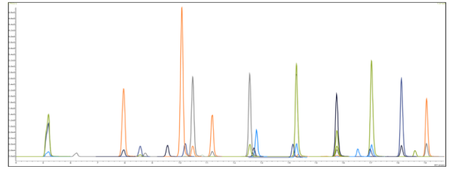


**Figure 5.** TIC of all 44 compounds at Level 7, 6 ng/L.

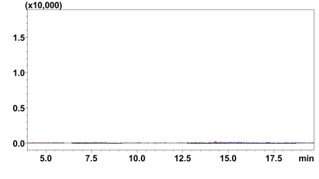



**Figure 6.** Blank: 80:20 MeOH:H2O.

## Results

*Method development for PFAS*

The use of a Phenomenex Gemini™ C18, 2.0 mm ID × 50 mm, 3.0 µm particle size analytical column and a Shim-Pack XR-ODS 50 x 3.0 mm column as a delay column provided a good chromatographic separation for all compounds including branched and linear isomers. Calibration curves for PFAS analytes were prepared in the range of 0.025 – 25 ng/mL, representing pre-SPE sample concentrations of 0.1 – 100 ng/L (except for NFDHA which was analyzed from 0.25 – 250 ng/L). All calibration curves (aggregate curve and 5 individual curves analyzed I 5 consecutive days) demonstrated $r^2$ values greater than 0.99. All RSD results for the aggregate curve were less than 20%. All MRL level accuracies were between 50 – 150%. Accuracies at the MRL for each day (against the aggregate curve), and %RSDs are shown in Figure 7. Precision and accuracy studies in reagent water (RW) and tap water (TW) were performed at 10 ng/L and recoveries of majority of analytes were within 70-130% with %RSDs below 20% for all method analytes. The P & A study results were within EPA method 533 requirements; the data is included in Figure 8.

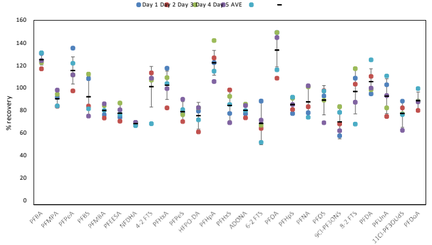



**Figure 7.** %recovery (individual injections from five consecutive days and average) at MRL.

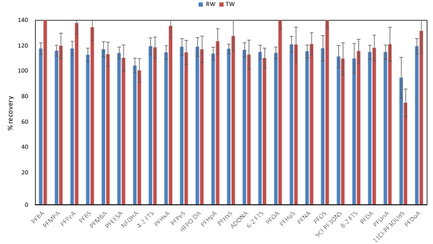



**Figure 8**. Precision and accuracy results/

## Conclusions

This study showed good chromatographic separation for all compounds listed in the method using the delay and analytical columns recommended by EPA. Recoveries for most target compounds and precision and accuracy data for all target analytes in reagent water and tap water were within EPA requirements of 70 -130%, with %RSD below 20% for all method analytes. This data was generated as part of the EPA method 533 second laboratory validation organized by EPA. Shimadzu participated in this validation, as acknowledged in the final method.

## References

(1) EPA Method 537 rev1.1, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (U.S. Environmental Protection Agency, Washington, D.C., Sept. 2009).
(2) Evoqua Water Technologies, Webinar, March 6, 2018
(3) EPA method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution anion exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry(U.S. Environmental Protection Agency, Washington, D.C., December 2019).
(4) Shimadzu Application News No. C184, "Analysis of PFAS Specified in EPA Method 537 and Beyond Using Shimadzu UFMS", 2019

(*)Brahm Prakash is not currently affiliated with Shimadzu Scientific Instruments