High Performance Liquid Chromatograph Mass Spectrometer    LCMS-8060RX

# Application News

# Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water in Accordance with EPA Method 537.1

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## User Benefits

◆ Based on EPA Method 537.1, 18 PFAS in drinking water can be accurately analyzed at concentrations equivalent to EPA's Final MCLs.

◆ PFAS analysis can be done immediately by using the LC/MS/MS Method Package for PFAS in Drinking Water, minimizing the effort required to set up an analytical system.

## ■ Introduction

Per- and polyfluoroalkyl substances (PFAS) are organofluorine compounds that are used in a wide range of consumer products and other applications due to their water repellency, heat resistance, chemical resistance, and other beneficial properties. However, their extremely high chemical stability prevents them from breaking down easily. Consequently, due to their persistence in the environment and possible toxicity to biological organisms, some PFAS have been designated as being subject to the Stockholm Convention on Persistent Organic Pollutants (POPs Convention), which restricts their manufacture and use. In recent years, there have been efforts to strengthen PFAS restrictions and determine their actual levels, resulting in a need to standardize the methods used to analyze them.

In April 2024, the U.S. Environmental Protection Agency (EPA) announced the following final maximum contaminant levels (Final MCLs) for certain PFAS in drinking water,[1] 4 ng/L PFOA, 4 ng/L PFOS, 10 ng/L PFHxS, 10 ng/L PFNA, and 10 ng/L HFPO-DA.

This article describes the results from using the LC/MS/MS Method Package for PFAS in Drinking Water to simultaneously analyze 18 PFAS target compounds in drinking water in accordance with EPA Method 537.1,[2] which was published by the EPA in 2018.

## ■ Analyte List

The compounds targeted for measurement included 18 types of target compounds specified in EPA Method 537.1, 3 types of internal standard (IS) substances, and 4 types of surrogate compounds. The measured compounds are listed in Table 1.

## ■ Sample Preparation

Samples were pretreated in accordance with EPA Method 537.1.

10 μL of a standard surrogate mixture solution and 1.25 g of Trizma were added to water samples and extracted using a solid phase column containing styrenedivinylbenzene (SDVB) polymeric sorbent phase. A GL Sciences AL898 Aqua Loader unit with an aspiration delivery kit for PFAS extraction was used to pass the sample water through the solid phase column. After fully drying the eluate solution eluted from the column, it was dissolved in 1 mL of 96 % methanol solution, and an internal standard was added for LC/MS/MS analysis. The pretreatment process flow is shown in Fig. 1.

## ■ Analytical Conditions

HPLC and MS analytical conditions are indicated in Table 2.

Samples were measured using the LC/MS/MS Method Package for PFAS in Drinking Water. It includes ready-to-use methods that are compliant with EPA methods 533 and 537.1 and the corresponding precautions. This article describes using the method to comply with EPA Method 537.1.

Table 1 Analyte List

| Acronym | Compound |
|---|---|
| **Analytes** | |
| HFPO-DA | Hexafluoropropylene oxide dimer acid |
| NEtFOSAA | N-ethyl perfluorooctanesulfonamidoacetic acid |
| NMeFOSAA | N-methyl perfluorooctanesulfonamidoacetic acid |
| PFBS | Perfluorobutanesulfonic acid |
| PFDA | Perfluorodecanoic acid |
| PFDoA | Perfluorododecanoic acid |
| PFHpA | Perfluoroheptanoic acid |
| PFHxS | Perfluorohexanesulfonic acid |
| PFHxA | Perfluorohexanoic acid |
| PFNA | Perfluorononanoic acid |
| PFOS | Perfluorooctanesulfonic acid |
| PFOA | Perfluorooctanoic acid |
| PFTA | Perfluorotetradecanoic acid |
| PFTrDA | Perfluorotridecanoic acid |
| PFUnA | Perfluoroundecanoic acid |
| 11Cl-PF3OUdS | 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid |
| 9Cl-PF3ONS | 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid |
| ADONA | 4,8-dioxa-3H-perfluorononanoic acid |
| **Internal Standards** | |
| $^{13}C_2$-PFOA | Perfluoro-[1,2-$^{13}C_2$]octanoic acid |
| $^{13}C_4$-PFOS | Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate |
| d$_3$-NMeFOSAA | N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid |
| **Surrogates** | |
| $^{13}C_2$-PFHxA | Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid |
| $^{13}C_2$-PFDA | Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid |
| d$_5$-NEtFOSAA | N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid |
| $^{13}C_3$-HFPO-DA | Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid |
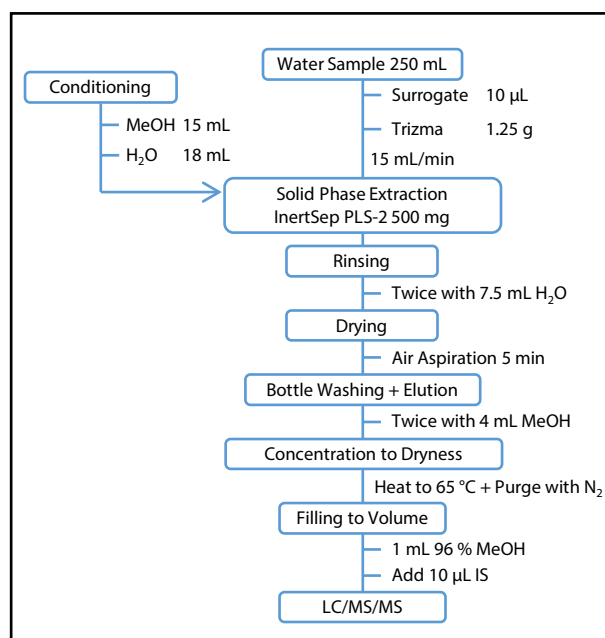


Fig. 1 Pretreatment Process Flow

Table 2 Analytical Conditions

| HPLC (Nexera™-X3) | | MS (LCMS-8060RX) | |
|---|---|---|---|
| Analytical Column: | Shim-pack Velox™ SP-C18 (50 mm × 2.1 mm I.D., 2.7 μm, P/N:227-32003-02) | Ionization: | ESI (Negative mode) |
| Solvent Delay Column: | Shim-pack™ GIST C18 (50 mm × 3.0 mm I.D., 5 μm, P/N 227-30015-03) | Mode: | MRM |
| | | Nebulizing Gas: | 3 L/min |
| Mobile Phase A: | 5 mM Ammonium Acetate in reagent water | Drying Gas Flow: | 5 L/min |
| Mobile Phase B: | Methanol | Heating Gas Flow: | 15 L/min |
| Gradient Program: | B 5 % – 40 % (1 min) – 95 % (8 min) – 100 % (8.1-13.0 min) – 5 % (13.1-18.0 min) | DL Temp.: | 150 °C |
| | | Block Heater Temp.: | 250 °C |
| Flowrate: | 0.25 mL/min | Interface Temp.: | 100 °C |
| | | Probe Position: | +1.5 mm |
| Column Temp.: | 45 °C | | |
| Injection Volume: | 2 μL | | |
| Run Time: | 18 min | | |

## ■ Measuring Standard Solutions

For standard solutions, a standard mixture solution of 18 PFAS (Cat. No. EPA-537APDS, Wellington Laboratories), a standard surrogate solution (Cat. No. EPA-537SS-R1, Wellington Laboratories), and an internal standard solution (Cat. No. EPA-537IS, Wellington Laboratories) were diluted with 96 % methanol to prepare solutions with 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, and 25 μg/L concentrations. The quantity of the internal standard and surrogate substances added conformed to EPA Method 537.1. The successive five injections were made per concentration.

MRM chromatograms for the lowest calibration curve concentrations (0.05 μg/L in solution) of the 18 target PFAS are shown in Fig. 2. Good chromatograms were confirmed for each component.

The coefficient of correlation (R) and the area repeatability at the lowest calibration curve concentration (0.05 μg/L) are listed in Table 3. The values show that good linearity was obtained with coefficient of correlation (R) > 0.997 for all compounds. They also confirm good repeatability at the lowest calibration curve concentration (0.05 μg/L) with area %RSD < 11 % (Table 3).

Fig. 3 shows the accuracy of concentrations at each calibration point. It shows that those are within ±30 % of the specified concentration. Calibration curve examples for PFOA and PFOS are shown in Fig. 4.

Table 3 Correlation Coefficient of Calibration Curve and Area Repeatability at Calibration Curve Lowest Concentration

| Compound | Retention Time (min) | Correlation Coefficient (R) | 0.05 μg/L Standard Solution Area %RSD (%, n = 5) |
|---|---|---|---|
| PFBS | 3.595 | 0.9975 | 1.4 |
| PFHxA | 4.108 | 0.9986 | 4.3 |
| HFPO-DA | 4.301 | 0.9986 | 5.8 |
| PFHpA | 4.777 | 0.9984 | 2.0 |
| PFHxS | 4.837 | 0.9973 | 9.6 |
| ADONA | 4.874 | 0.9983 | 3.0 |
| PFOA | 5.382 | 0.9986 | 2.3 |
| PFOS | 5.932 | 0.9984 | 3.9 |
| PFNA | 5.918 | 0.9987 | 7.6 |
| 9Cl-PF3ONS | 6.224 | 0.9981 | 4.6 |
| PFDA | 6.395 | 0.9988 | 5.5 |
| NMeFOSAA | 6.626 | 0.9990 | 3.3 |
| PFUnA | 6.813 | 0.9986 | 5.0 |
| NEtFOSAA | 6.844 | 0.9984 | 10.7 |
| 11Cl-PF3OUdS | 7.023 | 0.9977 | 8.1 |
| PFDoA | 7.177 | 0.9987 | 5.7 |
| PFTrDA | 7.503 | 0.9987 | 3.8 |
| PFTA | 7.795 | 0.9985 | 3.3 |
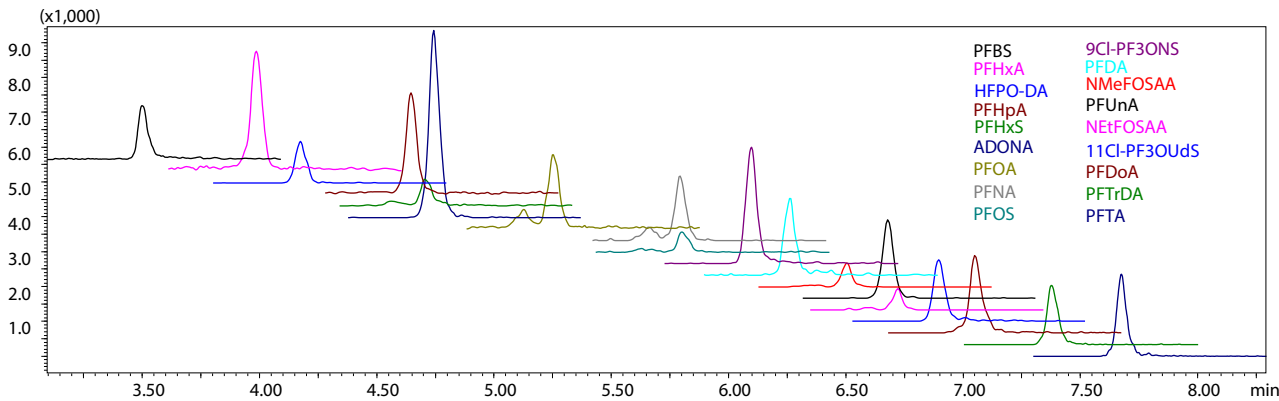


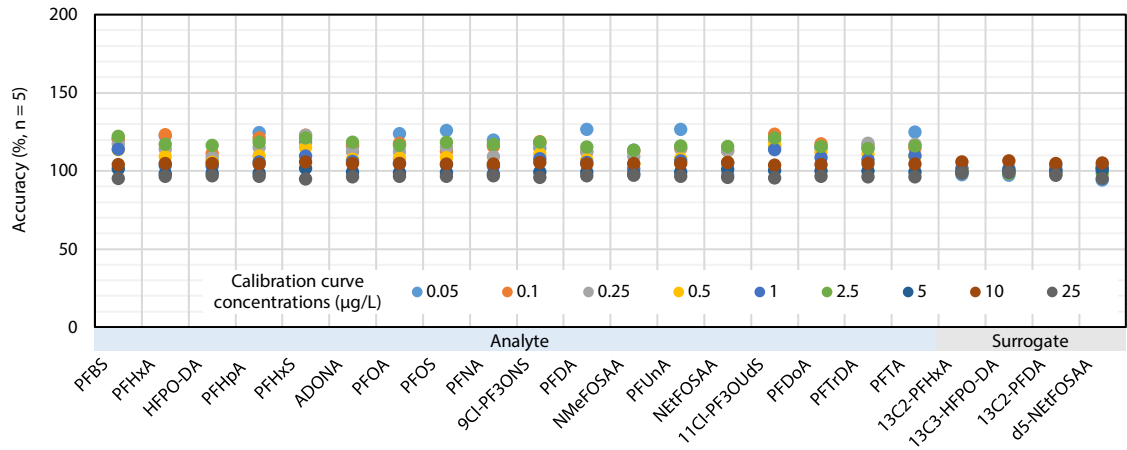Fig. 2 MRM Chromatograms at Lowest Calibration Curve Concentration (0.05 μg/L)



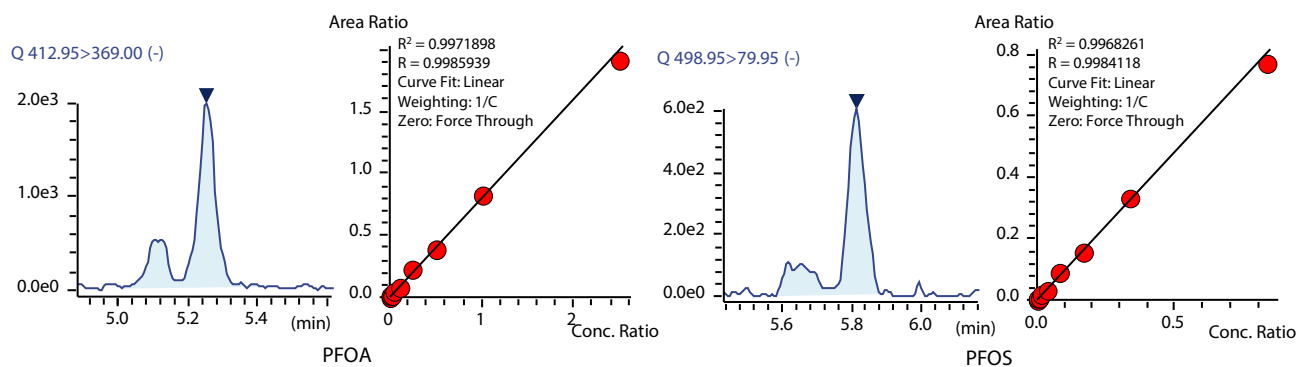Fig. 3 Accuracy of Each Calibration Curve Concentration

Fig. 4 MRM Chromatogram (0.05 µg/L in Solution) and Calibration Curve for PFOA (Left) and PFOS (Right)

## ■ Spike-and-Recovery Tests Using Ultrapure Water

Seven samples each were prepared by spiking with "Low" (1 ng/L in water, 0.25 µg/L in solution) or "High" (4 ng/L in water, 1 µg/L in solution) concentrations to ultrapure water, and pretreated according to the procedure shown in Fig. 1. The spike recovery rates and concentration repeatability from spike-and-recovery tests using ultrapure water are shown in Fig. 5.

Good recovery rates between 93 and 109 % were obtained for all compounds in low- and high-concentration spiked samples, and good repeatability results were also obtained, with concentration %RSD (n = 7) < 10 %.

## ■ Spike-and-Recovery Tests Using Drinking Water

Spike-and-recovery tests were performed using drinking water samples. Two drinking water samples spiked with 4 ng/L in water (1 µg/L in solution) of target compounds were prepared according to the process shown in Fig. 1 to obtain a Laboratory Fortified Sample Matrix (LFSM) and a Laboratory Fortified Sample Matrix Duplicate (LFSMD). Variability was evaluated based on relative percent difference (RPD) calculated from the above results. For the RPD calculation method, refer to EPA Method 537.1.

The recovery rates and RPD for the LFSM and LFSMD solutions are indicated in Fig. 6. It shows good results with recovery rates within the 84 to 101 % range. For %RSD < 6 %, good results were also obtained from the drinking water spike-and-recovery tests.
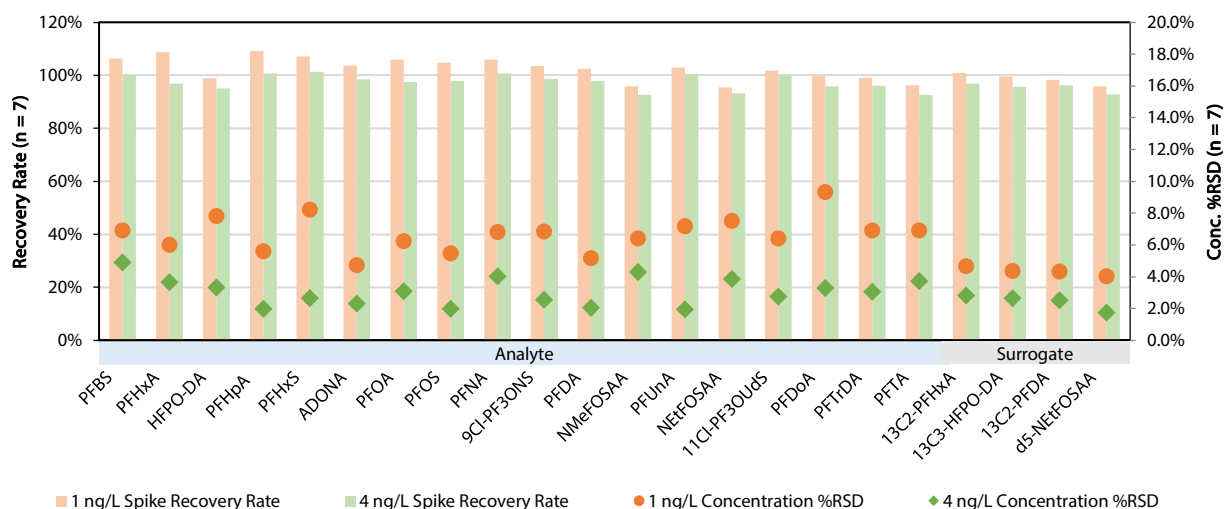


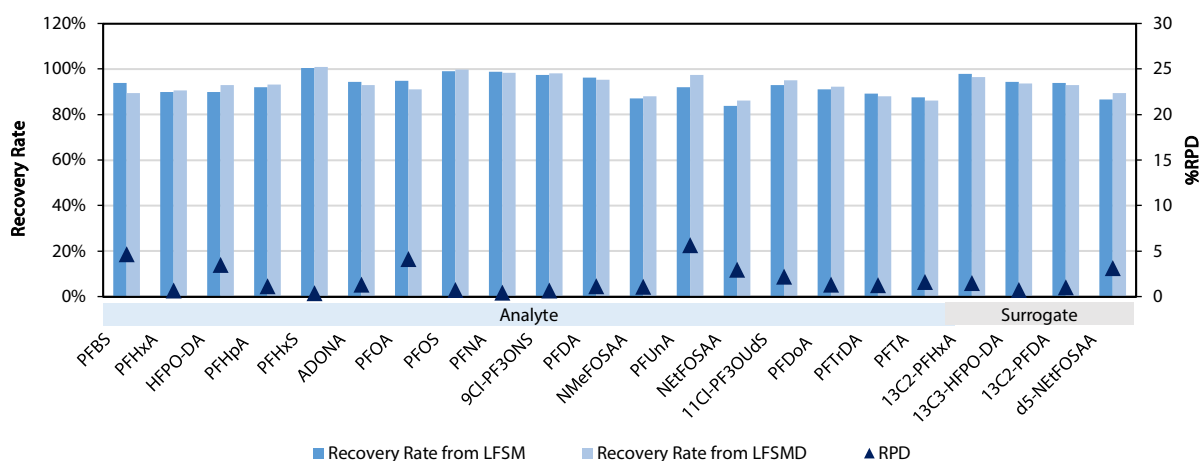Fig. 5 Spike-and-Recovery Test Results Using Ultrapure Water



Fig. 6 Spike-and-Recovery Test Results Using Drinking Water

## ■ Conclusion

By using an LCMS-8060RX liquid chromatograph mass spectrometer and the LC/MS/MS Method Package for PFAS in Drinking Water, 18 PFAS in drinking water were measured in accordance with EPA Method 537.1.

The above set up was able to measure concentration levels that were one-tenth those published by the EPA as Final MCLs (4 ng/L PFOA, 4 ng/L PFOS, 10 ng/L PFHxS, 10 ng/L PFNA, and 10 ng/L HFPO-DA).

Based on spike-and-recovery test results using ultrapure water, good recovery rates and repeatability results were obtained when spiking the ultrapure water with the equivalent of 1/4 the Final MCLs (1 ng/L in sample water). Similarly, good recovery rates and repeatability results were obtained when spiking drinking water samples with the Final MCLs (4 ng/L in sample water). These results confirm that PFAS compounds can be simultaneously analyzed in accordance with EPA Method 537.1 with good accuracy.

In addition, the LC/MS/MS Method Package for PFAS in Drinking Water makes it easy to set up an analytical system, and it enables accurate analysis.

Reference Documents

1) Per- and Polyfluoroalkyl Substances (PFAS)
   Final PFAS National Primary Drinking Water Regulation
   https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas
2) USEPA. Method 537.1, Detections of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS). Version 2.0, March 2020
   https://www.epa.gov/water-research/epa-drinking-water-research-methods

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Triple Quadrupole LC-MS/MS

> LC/MS/MS Method Package for PFAS in Drinking …

> Shim-pack Velox LC Columns
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