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Analysis of Per- and Poly-fluoroalkylated Substances (PFAS) Specified in EPA Method 1633 Using Triple Quadrupole LC-MS/MS

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1. Overview

A triple quadrupole mass spectrometer was used to quantify per- and poly-fluoroalkylated substances (PFAS) to meet the requirements set by the EPA 1633 draft method.

2. Introduction

The growing importance of per and poly-fluoroalkylated substances (PFAS) as a global public health threat is driving regulatory action. The EPA has developed methods for the measurement of PFAS in various matrices, such as Draft Method 1633 (EPA1633), which describes the analysis of forty PFAS in wastewater, solids, biosolids, and tissue samples.

If wastewater becomes regulated, treatment utilities will be required to adopt methods such as EPA1633 which have been validated for analysis of PFAS in complex wastewater matrices. EPA1633 is currently in the third draft, which lists low detection and reporting limits for wastewater that may result in EPA establishing permit limits that will challenge existing water systems. Laboratories will need to measure at these low method reporting limits, and perhaps even lower to provide utilities a higher confidence in the results at these concentrations.

3. Method

The instrument was calibrated according to the method using the Calibration Standards listed in **Table 2** (Wellington PFAC) and verified to meet the performance criteria of EPA1633. The forty PFAS listed in EPA1633 were chromatographically separated on a Shim-pack Scepter C18 column (50 x 2.1 mm, 3 μm) by gradient elution using 2 mM ammonium acetate in water and acetonitrile (no additives) as the mobile phases at a flow rate of 0.4 mL/min (**Figure 1**). A Shimadzu GIST C18 column (50 × 3 mm, 5 μm) was used as a delay column to reduce the system PFAS interferences. Multiple reaction monitoring (MRM) analysis was performed on a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer with neat standards of the forty PFAS listed in EPAM1633 ranging from 0.3 ppt to 625 ppb. LabSolutions and LabSolutions Insight software were used to obtain the data and perform data analyses.

Table 1. Summary of LCMS method parameters

LCMS-8060NX	Parameters	LC	Parameters
Ion source	ESI Ion Focus	Column	Shimadzu Scepter C18, 2.1 × 50 mm, 3 μm
Nebulizing gas	3.0 L/min	Flow rate	0.4 mL/min
Heating gas	15.0 L/min	Mobile phase A	2mM Ammonium Acetate in Water
Drying gas	5.0 L/min	Mobile phase B	Acetonitrile
Interface Temperature	190 °C	Injection volume	1 μL
DL Temperature	200°C	Column oven temperature	40 °C
Heat Block Temperature	300°C	Diluent	Methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid

The signal-to-noise ratio (SNR) for CS1 was well above 10 for all analytes, easily meeting the method's sensitivity requirements. To determine how much lower in concentration each of the instruments can detect, we serially diluted and analyzed CS1. PFAS were detected at or below the limits of quantitation (LOQ) established in EPA1633 in neat standards These data are shown in **Table 2**.

Silanized glass vials and silicone/polyethylene polymer caps were used to hold PFAS standards which significantly reduced PFAS interferences compared to other materials. Shimadzu LabSolutions Insight LCMS software was used to quickly process the data and determine that the sensitivity would meet the performance criteria of EPA1633 (e.g., confirm the relative standard error and relative standard deviation were less than 20%).
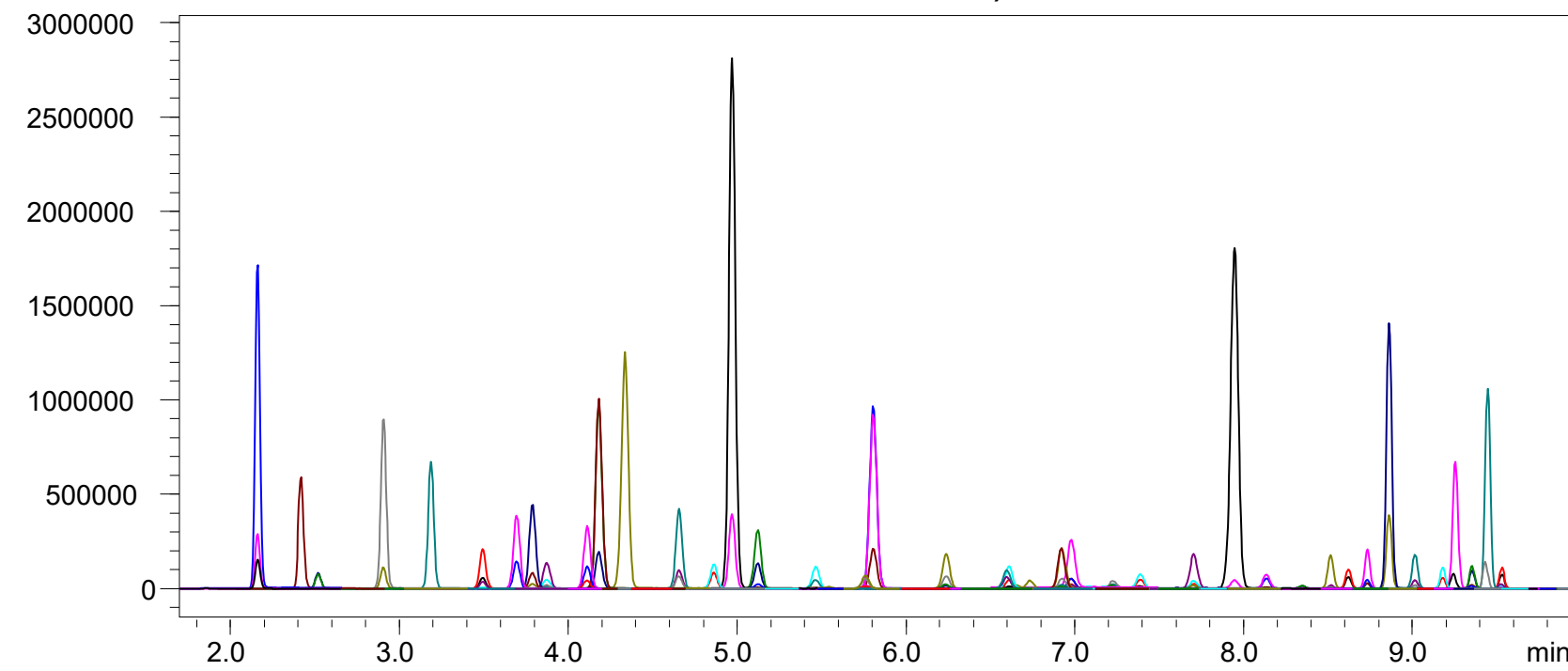

Figure 1. Chromatograms of 1633 PFAS analytes with non- and extracted internal standards
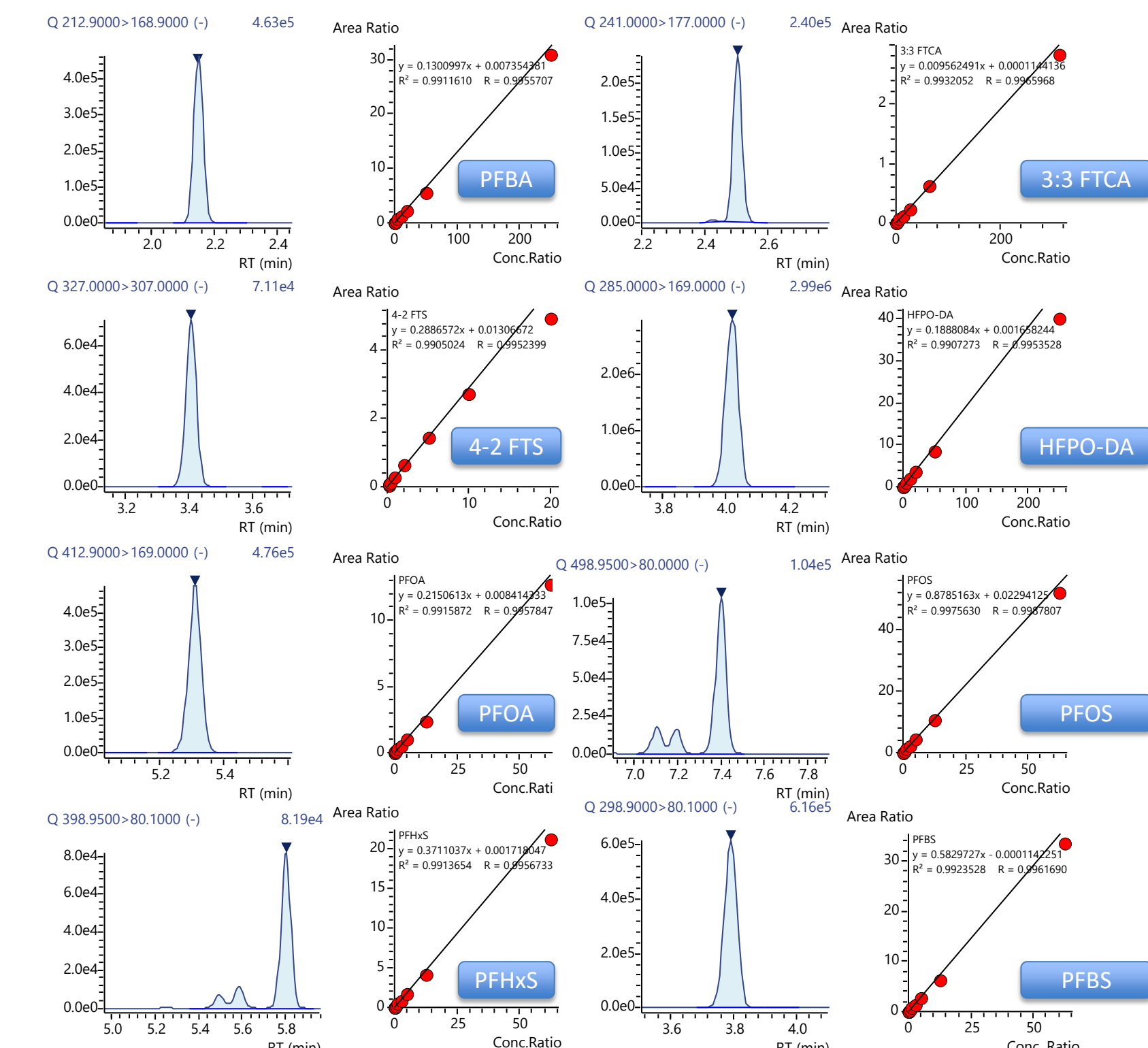

Figure 2. Examples of PFAS chromatograms and respective calibration curves

4. Results

LabSolutions Insight software was used to efficiently review and analyze the data (see **Figure 3**).The signal-to-noise ratio for all PFAS at LOQ concentrations were above 10. The calibration range has the linearly of 0.99 or greater with %RSE of <20%. The IDL was calculated using 10 replicates of spiked ultrapure water samples. The theoretical MDL was calculated based on an assumption of 100% extraction efficiency and a concentration factor of 100X; these conditions may vary with laboratory environment, sample matrix, and analyst extraction proficiency. The Shimadzu LCMS-8060NX was not only able to meet EPA 1633 draft but was able to detect much lower concentrations.

Table 2. Summary of LCMS acquisition parameters and performance for EPAM1633 standards.

| Acronym	Precursor m/Z	Ref.(1) m/z	Ref.(2) m/z	Ret. Time	%RSE	LOQ (ng/mL)	EPA LOQ (ng/mL)	IDL (ng/mL) n=10	Theoretical MDL (ng/L)	EPA Pooled MDL (ng/L)
PFBA	212.98	168.90	N/A	2.16	11	0.0250	0.80	0.004	0.041	0.800
PFMPA	228.97	85.00	N/A	2.42	10	0.0125	0.40	0.053	0.526	0.540
3:3 FTCA	241.01	177.00	117.00	2.53	11	0.1248	1.00	0.053	0.526	2.540
PFPeA	262.98	219.00	N/A	2.91	9	0.0125	0.40	0.003	0.028	0.530
PFMBA	278.97	85.00	N/A	3.19	10	0.0250	0.40	0.003	0.025	0.530
4:2 FTS	326.97	307.00	80.90	3.50	10	0.1000	0.80	0.027	0.267	1.740
NFDHA	294.97	201.15	85.00	3.70	11	0.0063	0.40	0.005	0.049	1.920
PFHxA	312.97	269.00	119.10	3.80	11	0.0125	0.20	0.004	0.037	0.480
PFBS	298.94	80.10	99.10	3.88	12	0.0125	0.20	0.009	0.092	0.370
HFPO-DA	328.97	169.00	118.90	4.12	12	0.0125	0.80	0.007	0.067	1.540
5:3 FTCA	341.00	237.10	217.10	4.20	12	0.0780	5.00	0.055	0.552	9.920
PFEESA	314.94	134.85	N/A	4.34	10	0.0063	0.40	0.001	0.007	0.790
PFHpA	362.97	319.00	169.00	4.67	10	0.0125	0.20	0.002	0.023	0.390
PFPeS	348.94	80.00	99.00	4.87	14	0.0125	0.20	0.010	0.098	0.530
ADONA	376.97	250.90	84.80	4.98	12	0.0063	0.80	0.002	0.019	1.470
6:2 FTS	426.97	407.00	81.00	5.14	9	0.0250	0.80	0.013	0.132	2.520
PFHxS	398.94	80.10	99.10	5.47	11	0.0125	0.20	0.008	0.079	0.560
PFOA	412.97	369.00	169.00	5.48	11	0.0250	0.20	0.008	0.076	0.550
7:3 FTCA	441.00	317.00	337.00	5.83	11	0.0790	5.00	0.066	0.657	9.140
PFNA	462.96	418.90	219.00	6.25	11	0.0125	0.20	0.004	0.036	0.460
PFHpS	448.93	80.00	99.00	6.61	11	0.0125	0.20	0.010	0.095	0.870
8:2 FTS	526.96	506.95	81.05	6.63	12	0.1000	0.80	0.036	0.358	2.580
NMeFOSAA	569.97	483.00	419.00	6.95	15	0.0250	0.20	0.019	0.186	1.040
PFDA	512.96	469.00	269.05	7.00	16	0.0250	0.20	0.038	0.379	0.530
NEtFOSAA	583.98	418.95	482.95	7.25	19	0.0500	0.20	0.050	0.501	0.800
PFOS	498.93	80.00	99.05	7.40	10	0.0250	0.20	0.015	0.147	0.640
PFUnA	564.97	518.95	269.00	7.72	14	0.0250	0.20	0.007	0.069	0.440
9Cl-PF3ONS	530.90	351.00	83.10	7.96	10	0.0063	0.80	0.002	0.021	1.420
PFNS	548.93	80.05	99.00	8.15	11	0.0125	0.20	0.009	0.092	0.490
PFDoA	612.95	569.00	433.10	8.36	11	0.0125	0.20	0.007	0.074	0.370
PFOSA	497.95	78.05	168.90	8.53	15	0.0031	0.20	0.004	0.040	0.320
PFDS	598.92	80.05	98.95	8.63	11	0.0125	0.20	0.005	0.052	0.900
PFTrDA	662.95	619.00	168.95	8.74	10	0.0125	0.20	0.003	0.032	0.460
11Cl-PF3OUdS	630.89	451.00	452.95	8.87	12	0.0063	0.80	0.004	0.041	1.780
PFTeDA	712.95	668.90	169.00	9.02	11	0.0125	0.20	0.004	0.041	0.510
PFDoS	698.92	80.00	98.95	9.19	13	0.0125	0.20	0.006	0.060	0.640
NMeFOSE	555.99	58.95	N/A	9.26	12	0.0156	2.00	0.010	0.101	3.930
NMeFOSA	511.96	169.05	219.10	9.36	19	0.0250	0.20	0.032	0.317	0.410
NEtFOSE	570.00	59.05	N/A	9.45	10	0.0156	2.00	0.006	0.063	5.130
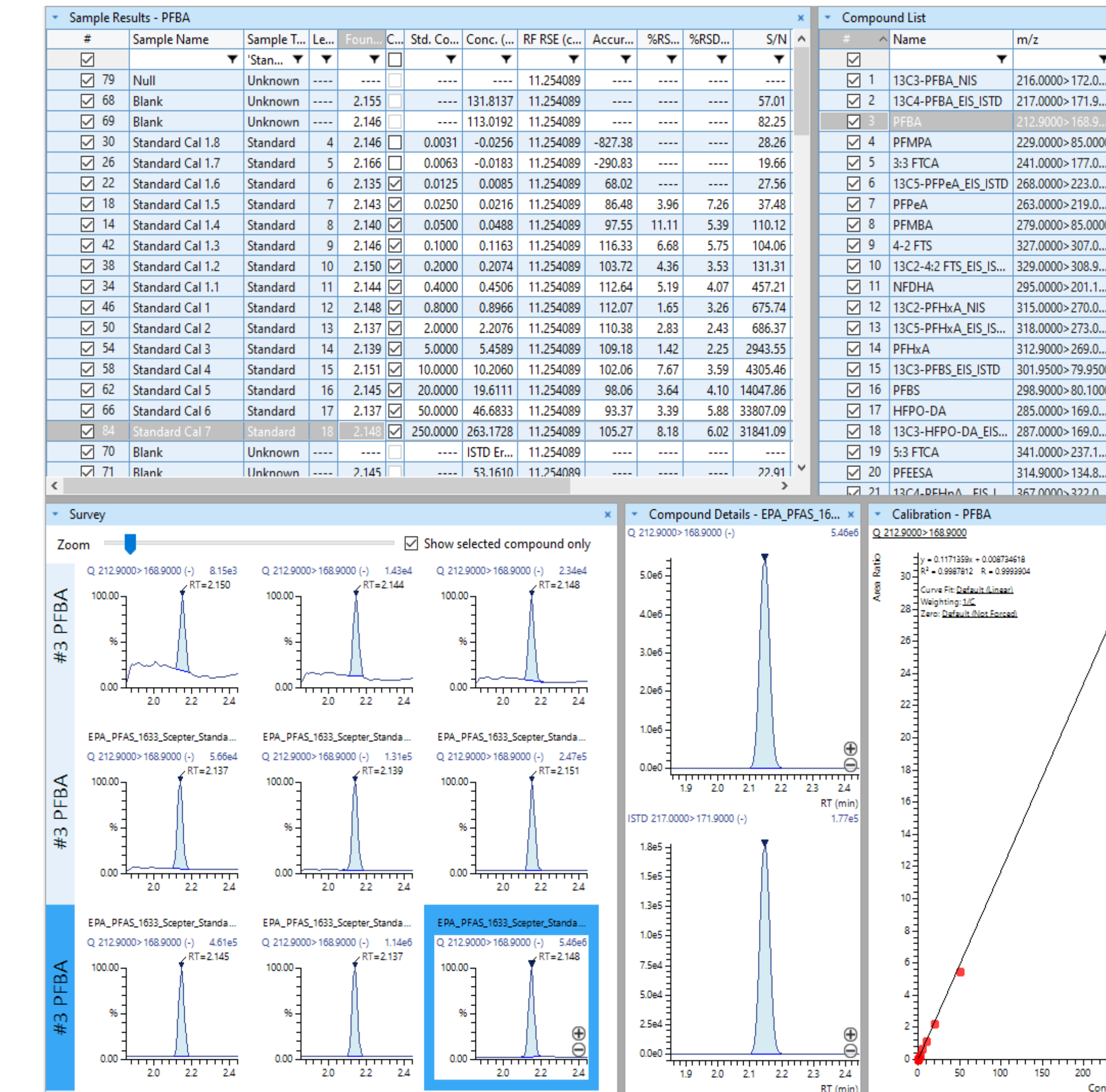
Figure 3. Screenshot of EPAM1633 data in LabSolutions Insight software.

5. Conclusions

The Shimadzu LCMS-8060NX was easily able to meet the LOQ requirements set by the EPA 1633 3rd draft method and Shimadzu LabSolutions Insight was used to efficiently process the acquired data. This processing software calculated %RSD, SNR, and %RSE as required by EPA 1633.

Reference

United States Environmental Protection Agency, Office of Water. 3rd Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC/MS/MS, 2022. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P101687F.txt (accessed June 1, 2023)