

Analysis and Quantitation of Polyfluorinated Alkyl Substances (PFAS) in EPA Method 537.1 Using High Resolution Accurate Mass Spectrometry

Brahm Prakash, Christopher Gilles, Ruth Marfil-Vega, William Lipps
Shimadzu Scientific Instruments, Inc., Columbia, Maryland

1. Overview

Compounds listed in EPA Method 537.1¹ are quantitated using a high-resolution accurate mass QTOF. Quantitation limits are comparable to a triple quadrupole, and the high-resolution capability of a QTOF allow for identification of PFAS not listed in EPA Method 537.1

2. Introduction

EPA Method 537 was expanded to EPA Method 537.1 in November 2018 to include four new per-/polyfluoroalkyl substances (PFAS) compounds. While EPA Method 537.1 focuses on well-known PFAS, such as PFOA, PFOS, and GenX², there are thousands of unknown PFAS that can potentially contaminate drinking water. Analysis of unknown contaminants requires high resolution and accurate mass capabilities in order to positively identify the molecular formula. This poster demonstrates that the quantitation of all PFAS outlined in EPA Method 537.1 can be performed on a quadrupole time-of-flight mass spectrometer (QTOF) at low parts per trillion concentrations in environmental drinking water samples. Quantitation limits on the QTOF are compared to a triple quadrupole mass spectrometer (QQQ). Additionally, the workflow to tentatively identify untargeted PFAS is also included.

3. Methods

ESI source conditions were optimized on a QQQ and applied to the QTOF. MRM transitions were determined on a QQQ, and accurate mass precursor/product ions were determined on a QTOF. The chromatographic parameters are based on the chromatographic method used in EPA Method 537.1. A Shim-pack XR-ODS 50 x 3.0 mm column was used as a delay column, and a Shim-pack™ Velox 150mm x 2.1mm x 2.7µm column was used as the analytical column. Quantitation was performed using MRM on the QQQ and high-resolution MRM on the QTOF. Details are included in Tables 1 and 2.

Table 1. LC System and Parameters

LC System	Nexera-X2 UHPLC System
Analytical Column	Shim-pack™ Velox , 150mm x 2.1mm x 2.7µm, Part No. 227-320094-04
Solvent	Shim-pack XR-ODS
Delay Column	50mm x 2mm x 2.2µm, Part No. 228-41605-93
Column Temp.	40 °C
Inj Volume	5 µL
Mobile Phase	A: 20 mM Ammonium Acetate B: Methanol
Flow Rate	0.25 mL/min
Run Time	35 minutes

Table 2. LCMS Acquisition Parameters

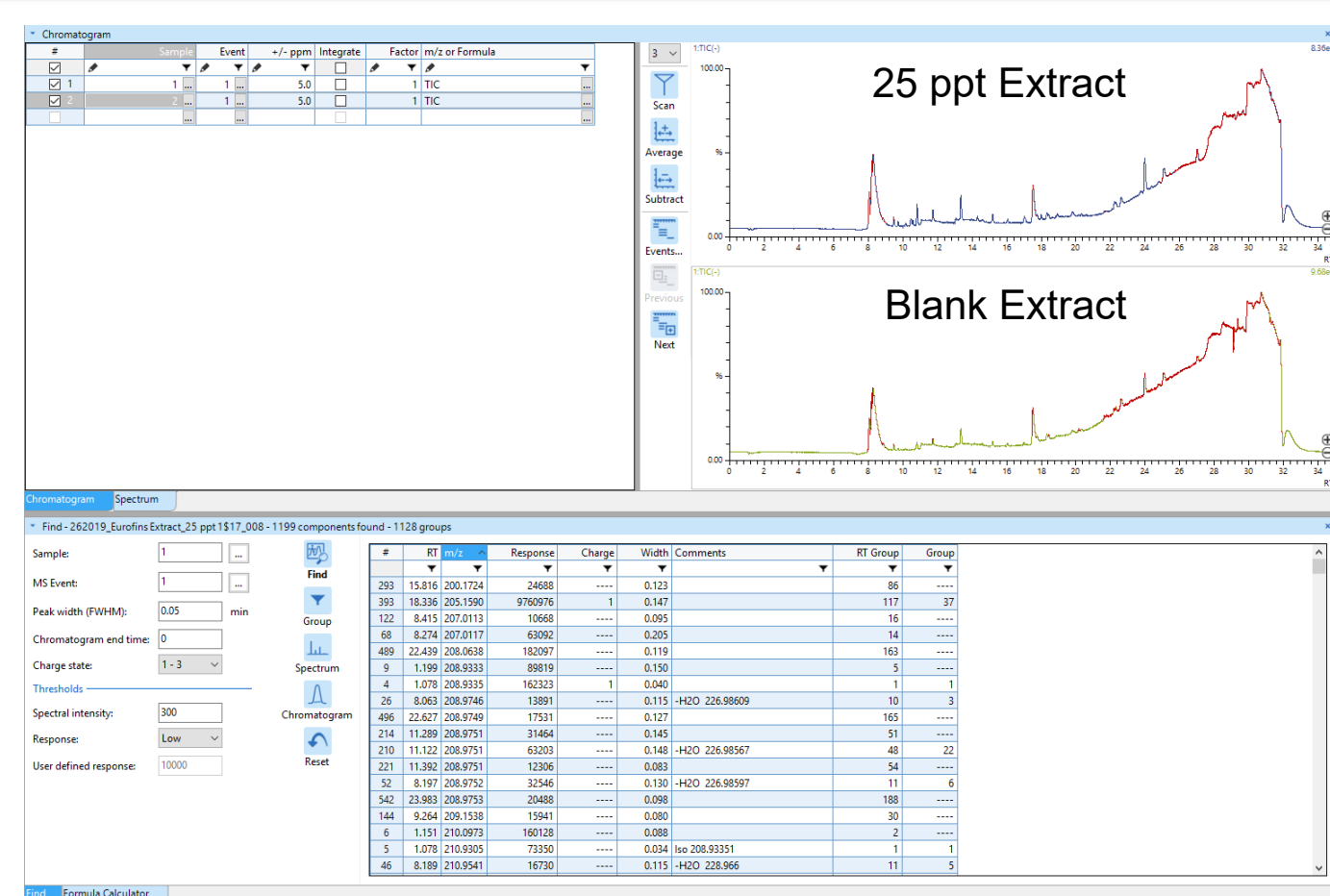
MS Instrument	LCMS-8045 and LCMS-9030
Interface	Electrospray Ionization (ESI) Negative mode
Interface Temp.	100 °C
Desolvation Line Temp.	100 °C
Heat Block Temp.	200 °C
Heating Gas Flow	15 L/min
Drying Gas Flow	5 L/min
Nebulizing Gas Flow	3 L/min
Total MRMs	48

4. Results

4-1. Qualitative Analysis

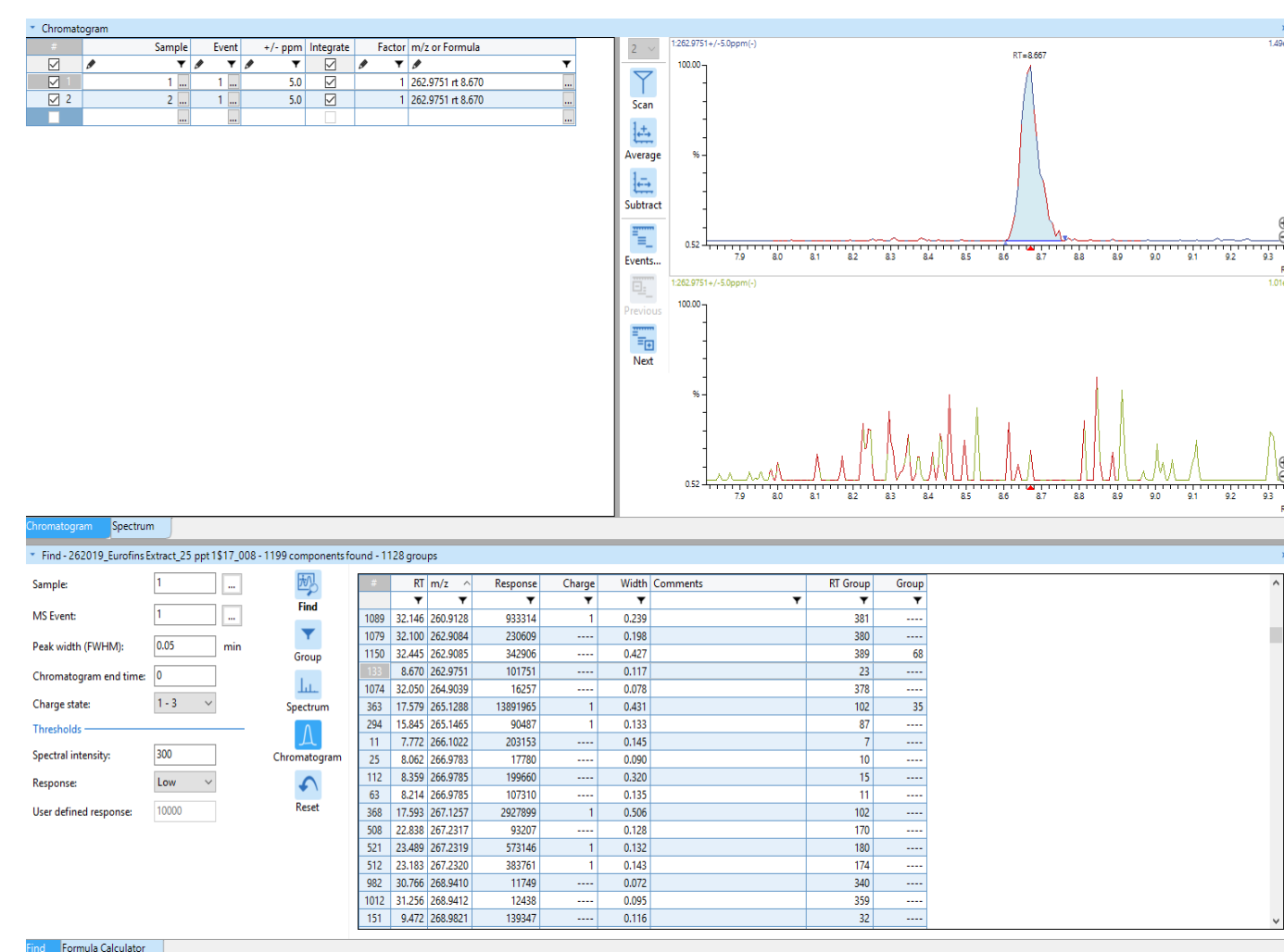
The overall workflow for identifying unknown PFAS in environmental samples can be broken down into four steps. One representative ion was chosen to outline the workflow for tentatively identifying an unknown compound using Insight Explore.

Step 1: Load the .lcm and .lcl files into Insight Explore, then use Find to identify features of interest.



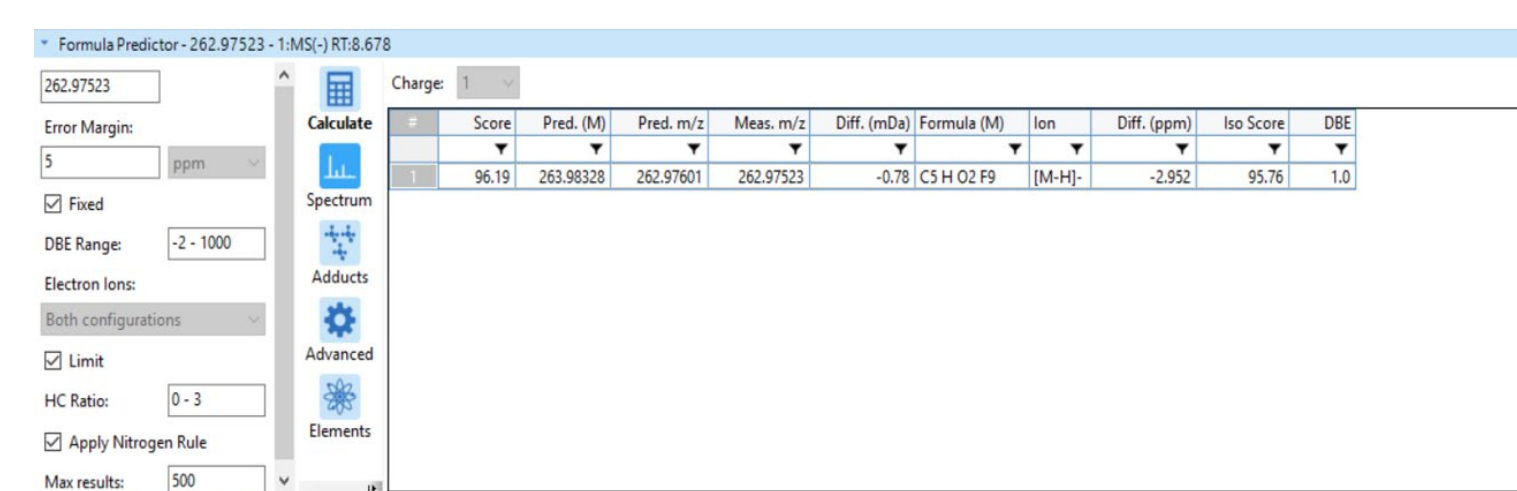
Step 2: Find the feature compounds that are present in the sample but not in the blank.

An unknown compound with the accurate mass of 262.9751 m/z was found at retention time 8.670 min. This compound appeared in an extract, however it is not present in the blank extract.



Step 3: Identify the possible formula using Formula Predictor included in Insight Explore using accurate mass and isotopic pattern.

Using the *Formula Predictor* function, Insight Explore predicted the most likely formula for 262.9751 m/z to be C₅H₉O₂F₉.



Step 4: Confirm the formula by comparing MS or MS/MS spectrum with a Database.

The predicted molecular weight of 263.98323 and the predicted molecular formula of C₅H₉O₂F₉ was used to search DSSTox. A total of 10 candidates were found in the database, with four candidates being adduct ions of Perfluoropentanoic acid. Further information can be acquired using MS/MS scans using 262.9751 as a precursor ion. Comparing m/z, formula, structures from DSSTox and MS/MS data, the potential structures were narrowed down to four potential candidates including PFPeA.

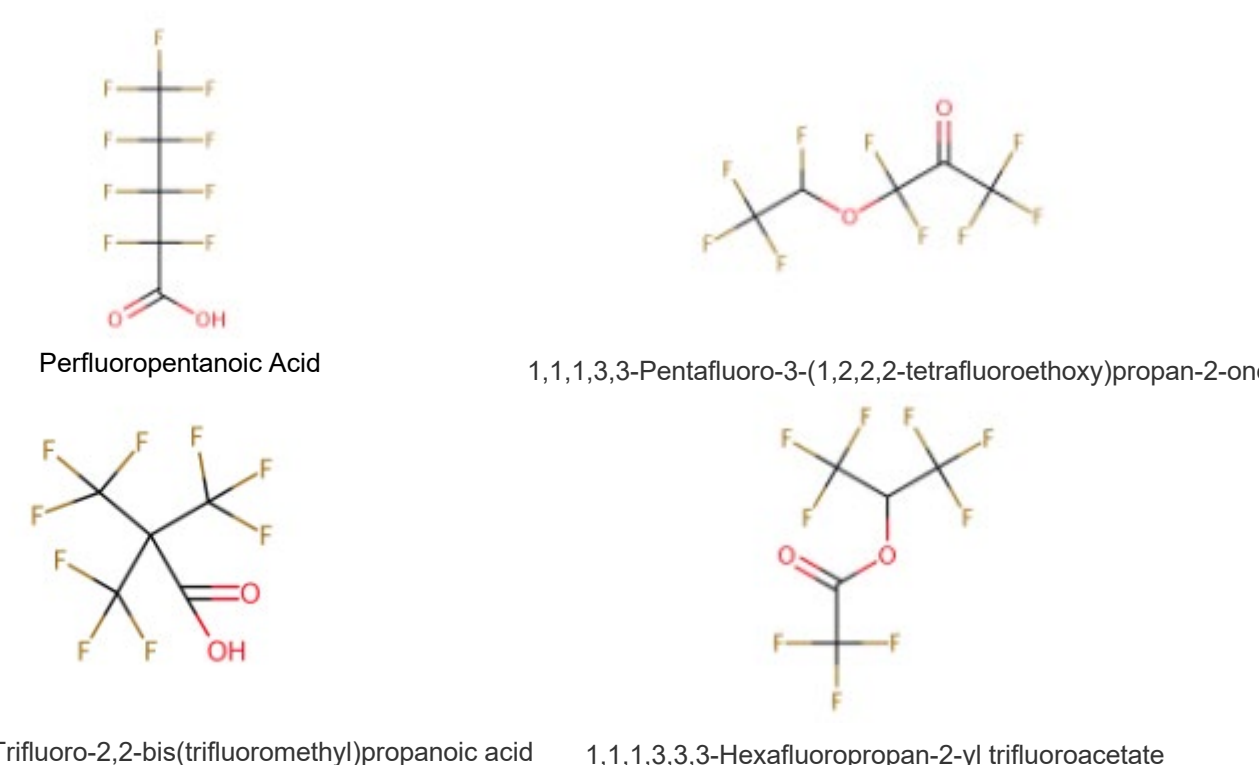


Figure 1. Potential structures

4-2. Quantitative Analysis

The quantitative capabilities of a QTOF for PFAS analysis were compared to a triple quadrupole mass spectrometer traditionally used for EPA Method 537.1. Since a TOF mass analyzer does not operate in the same fashion as a quadrupole mass analyzer, a MRM width of 20 ppm was used for all product ions. All calibration curves showed a r² value greater than 0.99 as required by EPA Method 537.1. Figure 2 compares the chromatograms at a sample concentration of 5 ppt for all new targets in EPA Method 537.1. In order to assess TOF stability, a precision and accuracy study was conducted at 25 ppt. Table 3 compares replicated 25 ppt injections for the QTOF against a triple quadrupole. Figure 3 compares the LOQs from QTOF and QQQ, with the results for the majority of compounds being less than 3 ng/L.

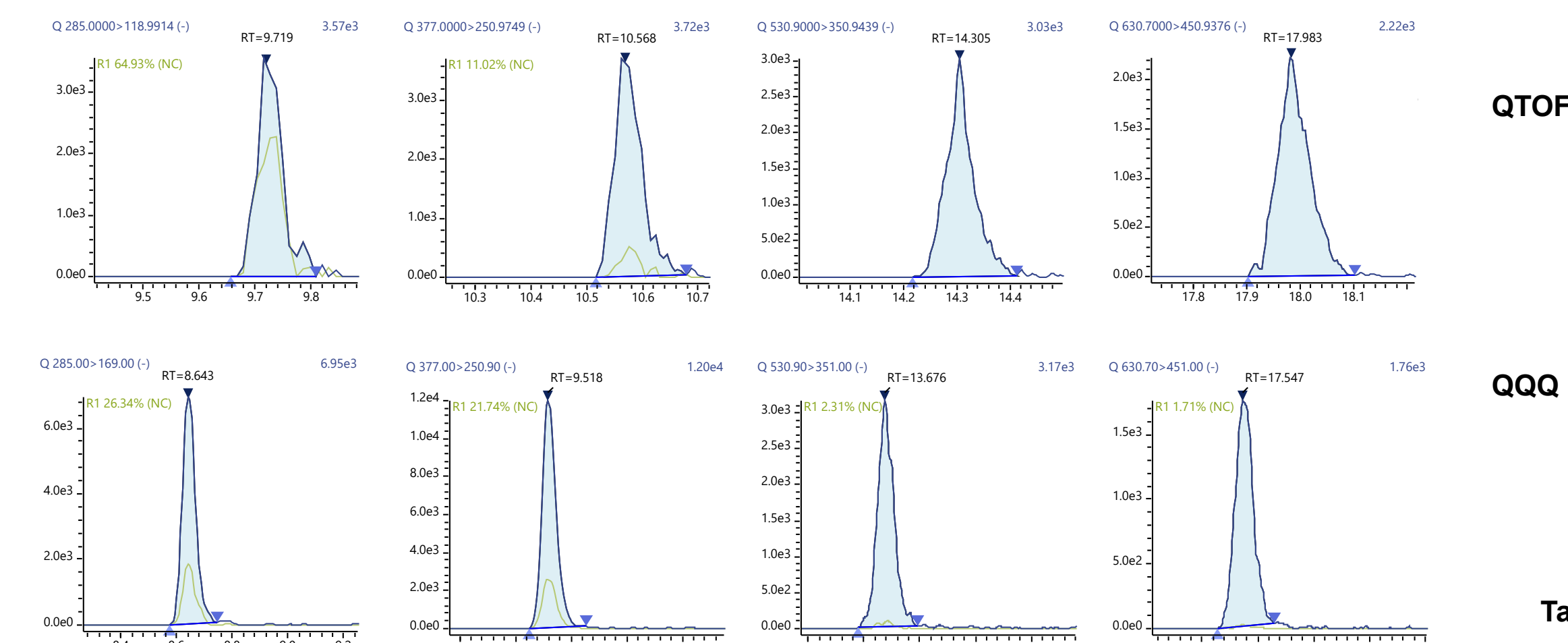


Figure 2. QTOF (top) Vs QQQ (bottom) Chromatograms at 5ppt of GenX, ADONA, 9CI-PF3ONS and 11CI-PF3OUds

Table 3. Summary P&A from QTOF (left) and QQQ (right).

QTOF 25 ppt P&A	True Value	Avg	Avg.%REC	%RSD	Std Dev
PFBS	22.2	23.7	107	7.6	1.80
PFHxA	25	29	116	4.8	1.39
HFPO-DA	62.5	80.4	129	7.3	5.89
PFHpA	25	27.6	110	7.8	2.16
PFHxS	22.8	25.9	113	14.4	3.73
ADONA	25	46.2	185	6.1	2.80
PFOA	25	28.7	115	5.8	1.65
PFOS	23.1	21.7	94	23.1	5.00
PFNA	25	28.2	113	6.3	1.78
9CI-PF3ONS	23.2	28	121	6	1.68
PFDA	25	25	100	6.3	1.56
N-MeFOSAA	25	29.2	117	11.3	3.31
N-EtFOSAA	25	25.9	104	23.6	6.11
PFUnA	25	24.4	98	7.7	1.88
11CI-PF3OUds	23.5	44.5	189	6.6	2.94
PFDoA	25	22.1	88	6.5	1.43
PFTriA	25	22.8	91	6.3	1.44
PFTreA	25	22.8	91	6.8	1.55

QQQ 25 ppt P&A	True Value	Avg	Avg.%REC	%RSD	Std Dev
PFBS	22.2	25.6	115	2.5	0.629
PFHxA	25	25.6	103	3.9	1.01
HFPO-DA	62.5	67.2	108	3.7	2.46
PFHpA	25	26.4	106	3.6	0.954
PFHxS	32.8	26.4	80	4.1	1.09
ADONA	25	39.7	159	3.2	1.29
PFOA	25	25.1	100	2.5	0.619
PFNA	23.1	25.8	112	3.1	0.797
PFOS	25	25.7	103	3.6	0.936
9CI-PF3ONS	23.2	27.7	119	2.9	0.809
PFDA	25	23.4	94	2.9	0.686
N-MeFOSAA	25	31.4	126	5.6	1.75
N-EtFOSAA	25	34.8	139	5.5	1.90
PFUnA	25	24.1	96	3.6	0.875
11CI-PF3OUds	23.5	46	196	4	1.85
PFDoA	25	23.5	94	2.8	0.665
PFTriA	25	24.4	98	3.8	0.916
PFTreA	25	25.2	101	2.9	0.726

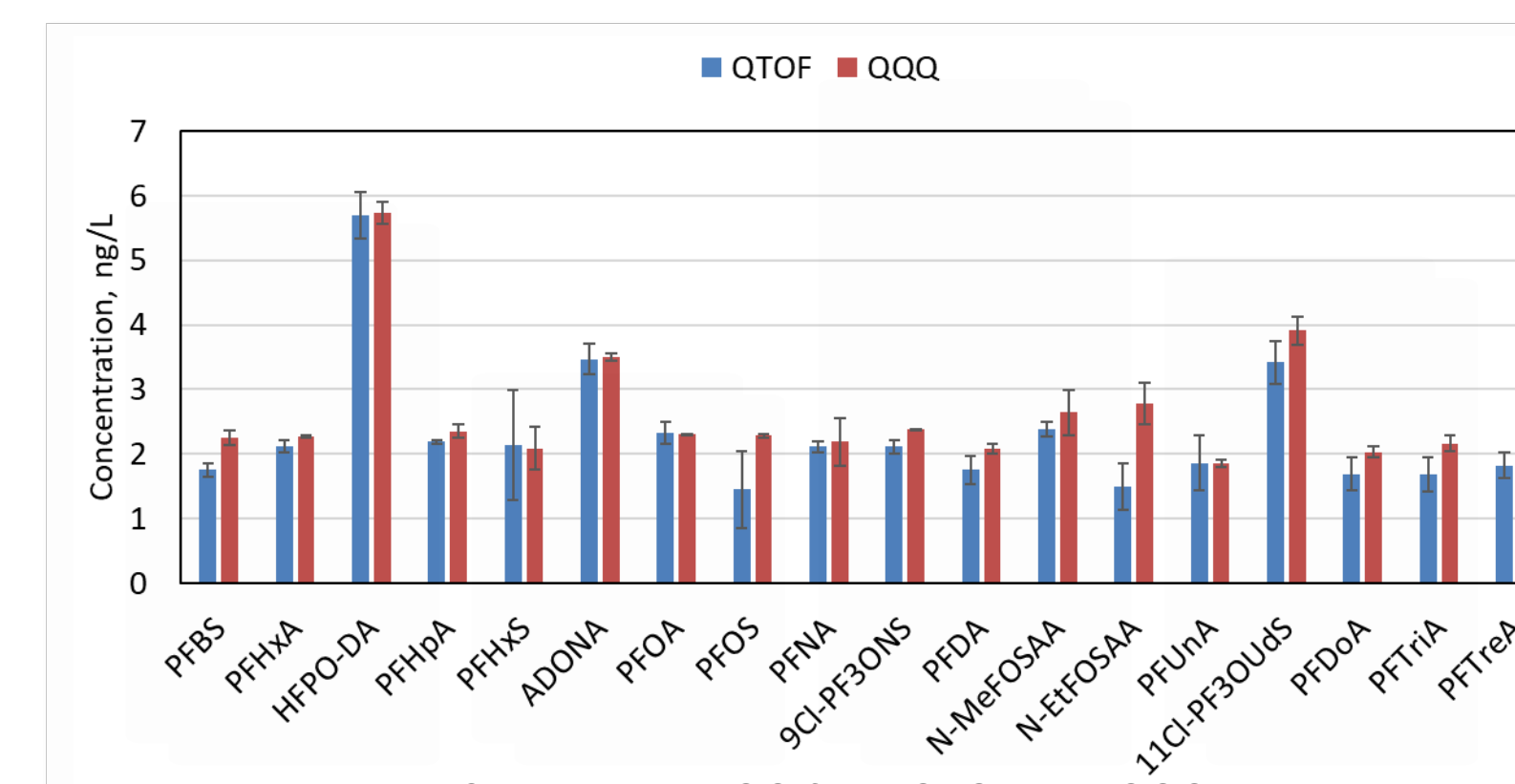


Figure 3. Comparison LOQ from QTOF and QQQ.

5. Conclusions

Comparable quantitative results can be obtained by using either a LCMS QQQ or a LCMS QTOF, with the QTOF having the additional ability to simultaneously screen for potential unknown PFAS contaminants with a streamlined workflow using LabSolutions Insight Explore. EPA Method 537.1 allows for additional compounds to be added to the method, as long as the QC requirements are met. Simultaneous Qualitative and Quantitative Analysis allows for laboratories to constantly screen for PFAS not specifically in EPA Method 537.1, while quantifying known PFAS contaminants.

6. References

- EPA Method 537 rev 1.1, 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).
- EPA Method 537.1, Determination of selected per- and polyfluorinated alkyl substances in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS), Version 1.0. (U.S. Environmental Protection Agency, Washington, D.C., Nov.2018).