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

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1. Overview

Compounds listed in EPA Method 537.1[1] 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 recently 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 GenX[2], 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).

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.

Table 2. 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 Delay Column	:	Shim-pack XR-ODS 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 3. LCMS Acquisition Parameters

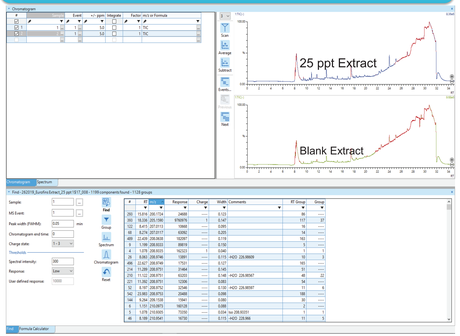
MS Instrument	:	**LCMS-8045**
Interface	:	Electrospray Ionization (ESI)
Interface Temp.	:	100 °C
Desolvation Line Temp.	:	100 °C
Heat Block Temp.	:	200 °C
Heating Gas Flow	:	15 L/min
Drying Gas Flow	:	5 L/min
Nebulizing Gas Flow	:	3 L/min
Total MRMs	:	48

4. Results

4-1. Qualitative Analysis

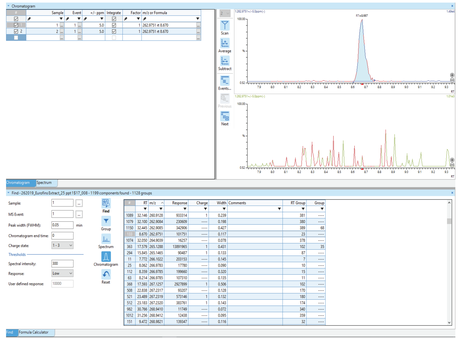
The overall workflow for identifying unknown PFAS in environmental samples can be broken down into four steps outlined on the following pages. One representative ion was chosen to outline the workflow for tentatively identifying an unknown compound using Insight Explore

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



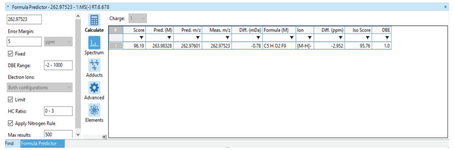
Step 2
Find the feature compounds that are present in the sample but not in the blank.

An unknown compound with the accurate mass of 262.9751 *m/z* was found at retention time 8.670 min using ESI negative mode. This compound appeared in a 25 ppt extract dated 1/17, however it is not present in the blank extract.



Step 3
Identifying 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 $C_2HO_2F_6$. Based on the information obtained from Insight Explore and comparing to the DSSTox database maintained by the USA EPA, the potential structures were narrowed down to three potential candidates including PFPeA.

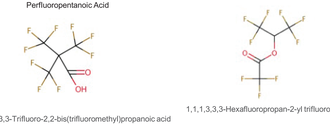


Step 4
Confirming the formula by comparing MS or MS/MS spectrum with a Database.

The predicted molecular weight of 263.98323 and the predicted molecular formula of C5HO2F9 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.

Perfluoropentanoic Acid

1,1,1,3,3-Pentafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-one



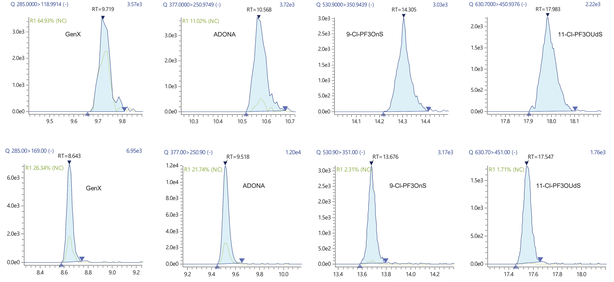
3,3,3-Trifluoro-2,2-bis(trifluoromethyl)propanoic acid

1,1,1,3,3,3-Hexafluoropropan-2-yl trifluoroacetate

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 TOF mass analyzers do not operate in the same fashion as a quadrupole mass analyzer, a MRM Integrating range of 20 ppm was used for all product ions. All calibration curves showed a r^2 value greater than 0.99 as required by EPA Method 537.1. Figure 1 compares the chromatograms at a sample concentration of 5 ppt for all new targets in EPA Method 537.1. Table 4 compares replicate 5ppt Injs for the QTOF against a triple quadrupole. In order to assess TOF stability, a precision and accuracy study was conducted at 25 ppt. Table 5 compares these results against a triple quad.

Figure 1.
QTOF Vs QQQ Chromatograms at 5ppt.



Tabl1 5

QTOF 25 ppt P&A	True Value	Inj 1	Inj 2	Inj 3	Inj 4	Inj 5	Inj 6	Inj 7	Inj 8	Avg	Avg.%REC	%RSD	Std Dev
PFBS	22.2	25.08	22.31	23.43	27.62	23.13	22.80	22.53	22.69	23.7	107	7.6	1.80416
PFHxA	25	29.31	26.73	29.77	31.29	29.35	27.60	29.34	28.65	29.0	116	4.8	1.38519
HFPO-DA	62.5	84.95	74.32	83.62	90.74	76.10	73.58	78.86	81.33	80.4	129	7.3	5.89465
PFHpA	25	30.66	25.74	26.61	31.22	24.87	25.65	26.88	27.50	27.6	110	7.8	2.16336
PFHxS	22.8	26.58	17.62	29.09	27.93	28.65	27.77	24.68	24.71	25.9	113	14.4	3.72591
ADONA	25	50.03	43.51	46.16	49.75	44.66	42.23	45.62	47.60	46.2	185	6.1	2.80041
PFOA	25	29.26	25.60	29.08	30.47	29.06	27.06	28.97	30.46	28.7	115	5.8	1.65379
PFOS	23.1	13.83	16.49	26.52	26.09	18.62	20.59	26.66	24.49	21.7	94	23.1	4.99594
PFNA	25	28.71	25.19	26.63	27.97	28.96	27.73	29.72	30.91	28.2	113	6.3	1.78411
9Cl-PF3ONS	23.2	29.58	26.07	28.35	30.69	27.30	25.64	28.40	28.25	28.0	121	6.0	1.68409
PFDA	25	26.91	21.79	24.95	26.01	24.87	23.96	25.33	25.94	25.0	100	6.3	1.56208
N-MeFOSAA	25	29.06	25.84	32.58	28.29	23.18	31.03	31.94	31.66	29.2	117	11.3	3.31232
N-EtFOSAA	25	33.44	20.11	24.78	34.05	21.12	25.33	18.12	30.33	25.9	104	23.6	6.11145
PFUnA	25	26.16	22.01	25.08	25.97	23.04	21.58	26.01	25.23	24.4	98	7.7	1.88443
11Cl-PF3OUdS	23.5	48.33	41.80	43.80	47.30	43.24	39.56	45.81	46.05	44.5	189	6.6	2.93683
PFDoA	25	23.07	21.07	21.55	23.02	21.26	19.65	23.89	23.24	22.1	88	6.5	1.43221
PFTriA	25	24.39	22.11	22.53	23.80	21.17	20.44	23.93	23.79	22.8	91	6.3	1.44296
PFTreA	25	25.40	22.34	23.11	23.22	20.27	21.33	23.75	22.65	22.8	91	6.8	1.54577

QQQ P&A	True Value	Inj 1	Inj 2	Inj 3	Inj 4	Inj 5	Inj 6	Inj 7	Inj 8	Avg	Avg.%RE	%RSD	Std Dev
PFBS	22.2	24.45	26.59	25.49	25.76	25.33	25.90	25.49	26.12	25.6	115	2.5	0.62923
PFHxA	25	24.76	27.72	25.82	25.45	24.86	24.81	25.38	26.40	25.6	103	3.9	1.00900
HFPO-DA	62.5	65.03	71.08	66.73	66.09	66.17	64.30	67.80	70.57	67.2	108	3.7	2.45917
PFHpA	25	25.48	28.53	26.06	26.74	25.95	25.98	25.79	26.54	26.4	106	3.6	0.95392
PFHxS	32.8	25.26	27.43	26.09	27.26	26.16	27.89	24.70	26.38	26.4	80	4.1	1.09444
ADONA	25	38.26	41.51	38.99	39.60	39.45	38.10	40.40	41.32	39.7	159	3.2	1.28787
PFOA	25	24.02	25.99	24.89	25.50	25.08	24.84	24.78	25.70	25.1	100	2.5	0.61854
PFNA	23.1	24.36	27.15	26.17	25.73	25.57	25.59	25.74	26.36	25.8	112	3.1	0.79690
PFOS	25	25.42	26.05	25.55	26.59	26.17	23.58	25.82	26.32	25.7	103	3.6	0.93568
9Cl-PF3ONS	23.2	26.88	28.81	28.18	28.12	27.52	26.34	27.47	28.28	27.7	119	2.9	0.80942
PFDA	25	22.49	24.45	23.68	23.71	22.86	22.81	23.19	24.10	23.4	94	2.9	0.68554
N-MeFOSAA	25	29.30	32.51	31.50	32.24	29.67	29.72	32.26	34.30	31.4	126	5.6	1.74514
N-EtFOSAA	25	32.93	36.76	36.41	36.58	33.77	31.62	34.84	35.86	34.8	139	5.5	1.90454
PFUnA	25	23.45	25.16	24.22	24.20	23.83	22.68	23.62	25.32	24.1	96	3.6	0.87477
11Cl-PF3OUdS	23.5	43.83	47.97	47.73	47.48	45.75	43.40	44.48	47.10	46.0	196	4.0	1.85480
PFDoA	25	22.53	24.56	24.15	23.55	23.29	22.78	23.30	23.56	23.5	94	2.8	0.66533
PFTriA	25	23.05	25.79	25.38	24.72	24.33	23.47	24.00	24.51	24.4	98	3.8	0.91626
PFTreA	25	23.61	26.10	25.47	25.33	25.28	25.33	25.10	25.69	25.2	101	2.9	0.72595

Table 4.

QTOF LOQ ng/L 2	True Value	Inj 1	Inj 2	Inj 3	Avg. Conc ng/L	Avg. %REc	%RSD	Std Dev
PFBS	1.8	1.8648	1.6588	1.716	1.75	97	6.1	0.10634
PFHxA	2	2.0164	2.2196	2.1124	2.12	106	4.8	0.10165
HFPO-DA	5	5.5528	6.104	5.4312	5.70	114	6.3	0.35853
PFHpA	2	2.166	2.184	2.2284	2.19	110	1.5	0.03212
PFHxS	1.8	1.7756	3.104	1.5168	2.13	118	39.9	0.85155
ADONA	2	3.7372	3.2864	3.3984	3.47	174	6.8	0.23472
PFOA	2	2.1672	2.4972	2.3256	2.33	117	7.1	0.16504
PFOS	1.9	1.5552	1.9996	0.8077	1.45	77	41.4	0.60234
PFNA	2	2.0328	2.108	2.1904	2.11	106	3.7	0.07883
9Cl-PF3ONS	1.9	2.0424	2.0464	2.2296	2.11	111	5.1	0.10694
PFDA	2	1.9144	1.51	1.8148	1.75	87	12.1	0.21070
N-MeFOSAA	2	2.2564	2.4268	2.4716	2.38	119	4.8	0.11354
N-EtFOSAA	2	1.9216	1.2868	1.3	1.50	75	24.1	0.36275
PFUnA	2	2.0916	1.3648	2.1324	1.86	93	23.2	0.43188
11Cl-PF3OUdS	1.9	3.616	3.0344	3.6044	3.42	180	9.7	0.33249
PFDoA	2	1.978	1.498	1.5932	1.69	84	15.0	0.25414
PFTriA	2	1.7364	1.3856	1.9176	1.68	84	16.1	0.27047
PFTreA	2	1.918	1.5872	1.9424	1.82	91	10.9	0.19841

QQQ LOQ	True Value	Inj 1	Inj 2	Inj 3	Avg. Conc ng/L	Avg %Rec	%RSD	Std Dev
PFBS	1.8	2.2468	2.364	2.1416	2.25	125.0	4.9	0.11125
PFHxA	2	2.2584	2.2908	2.2588	2.27	113.5	0.8	0.01859
HFPO-DA	5	5.7972	5.5472	5.8652	5.74	114.7	2.9	0.16746
PFHpA	2	2.468	2.2772	2.3	2.35	117.4	4.4	0.10420
PFHxS	1.8	2.4696	1.924	1.8828	2.09	116.2	15.7	0.32754
ADONA	2	3.4836	3.4636	3.5704	3.51	175.3	1.6	0.05678
PFOA	2	2.3168	2.3028	2.2864	2.30	115.1	0.7	0.01522
PFNA	1.9	2.2864	2.246	2.2932	2.28	119.7	1.1	0.02552
PFOS	2	2.6048	2.0668	1.904	2.19	109.6	16.7	0.36676
9Cl-PF3ONS	1.9	2.3688	2.3928	2.3812	2.38	125.3	0.5	0.01200
PFDA	2	2.02	2.0504	2.1616	2.08	103.9	3.6	0.07454
N-MeFOSA A	2	2.3484	2.5488	3.0312	2.64	132.1	13.3	0.35097
N-EtFOSAA	2	2.6572	2.5368	3.1528	2.78	139.1	11.7	0.32649
PFUnA	2	1.9112	1.7916	1.8608	1.85	92.7	3.2	0.06005
11Cl-PF3OUdS	1.9	4.0976	3.6736	3.9628	3.91	205.9	5.5	0.21663
PFDoA	2	2.0976	2.0704	1.9364	2.03	101.7	4.2	0.08630
PFTriA	2	2.292	2.1128	2.0636	2.16	107.8	5.6	0.12021
PFTreA	2	2.2848	2.1684	2.0508	2.17	108.4	5.4	0.11700

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

1. 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).
2. 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).