

# Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

## ASMS 2019

Gerard Byrne II<sup>1</sup>, Brahm Prakash<sup>1</sup>, Evelyn Wang<sup>1</sup>,  
Christopher Gilles<sup>1</sup>, William Lipps<sup>2</sup>

<sup>1</sup>Shimadzu Scientific Instruments, Inc., Columbia,  
Maryland;

<sup>2</sup>Eurofins Eaton Analytical, 750 Royal Oaks Drive,  
Monrovia, CA

# Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

## Overview

A highly sensitive LCMS method with increased detection confidence using a QTOF with an accurate mass library was developed

## Introduction

Per-and-polyfluorinated alkyl substances (PFAS) are a diverse group of compounds used to manufacture many industrial and household products, ranging from carpeting and upholstery to fire-fighting foam. Current methods for PFAS analysis typically use triple quadrupole mass spectrometers due to the highly sensitive nature of these

instruments. However, PFAS are also analyzed in highly complex matrices, and as a result there is a risk for false positives and false negatives. One way to reduce the risk of false positives and false negatives is by using a high resolution accurate mass quadrupole time-of-flight (QTOF) mass spectrometer.

## Methods

A newly developed QTOF mass spectrometer was used to analyze PFAS in sample extracts. ESI source conditions were optimized on a QTOF. LC parameters were optimized so that baseline separation was achieved for all compounds in a given mixture. Library spectra of authentic compounds were acquired by injecting neat standards and

chromatographically separating all compounds in a given mixture. The library spectra was validated using Formula Predictor Software and comparing to the DSSTox database provided by the EPA. The built-in Library Spectra Matching function helped reduce false positives in compounds with non-specific transitions

Table 1. LC System and Parameters

LC System	: Nexera-X2 UHPLC System
Analytical Column	: Restek Raptor C-18, 150mm x 2.1mm x 2.7µm, Part No. 9304A62
Solvent Delay Column	: Shim-pack XR-ODS 50mm x 2mm x 2.2µm, Part No. 228-41605-93
Column Temp.	: 40 °C
Injection Volume	: 5 µL <sup>#</sup>
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-9030
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

# Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

## Results

### Mass Accuracy Stability

High resolution accurate mass spectrometers typically require frequent calibration, or an internal mass calibration may be needed to achieve the desired results. In order to assess how often the instrument requires calibration, a stability study was performed using an external mass

calibration. A MS scan range of 100-800 m/z was used with Q1 and the collision cell acting as ion guides. Table 1 outlines the results of this study. All data shown was acquired using the initial calibration, and no mass correction was performed.

Table 3. Measured mass of PFAS in EPA Method 537.1 over three days using the initial mass calibration

Compound	Monoisotopic Mass	Day 1		Day 2		Day 3	
		Measured Mass	Mass error	Measured Mass	Mass error	Measured Mass	Mass error
PFBS	298.9429	298.9424	-1.67	298.9427	-0.67	298.9428	-0.33
PFHxA	312.9722	312.9719	-0.96	312.9725	0.96	312.9723	0.32
HFPO-DA	284.9773	284.977	-1.05	284.9775	0.70	284.9775	0.70
PFHpA	362.969	362.969	0.00	362.9696	1.65	362.9694	1.10
PFHxS	398.9366	398.9361	-1.25	398.9369	0.75	398.9366	0.00
ADONA	376.9683	376.9678	-1.33	376.9686	0.80	376.9689	1.59
PFOA	412.9658	412.9656	-0.48	412.9658	0.00	412.9661	0.73
PFOS	498.9302	498.9299	-0.60	498.9305	0.60	498.9301	-0.20
PFNA	462.9626	462.9625	-0.22	462.963	0.86	462.9631	1.08
9CI-PF3ONS	530.895	530.8953	0.57	530.8962	2.26	530.8961	2.07
PFDA	512.9595	512.9597	0.39	512.9602	1.36	512.9602	1.36
N-MeFOSAA	569.9667	569.9669	0.35	569.9673	1.05	569.9675	1.40
N-EtFOSAA	583.9829	583.9824	-0.86	583.9826	-0.51	583.983	0.17
PFUnA	562.9563	562.9563	0.00	562.9572	1.60	562.9569	1.07
11CI-PF3OUdS	630.8886	630.8891	0.79	630.8897	1.74	630.8895	1.43
PFDoA	612.9531	612.9532	0.16	612.9544	2.12	612.9539	1.31
PFTriA	662.9504	662.9495	-1.36	662.9511	1.06	662.9508	0.60
PFTreA	712.9472	712.9467	-0.70	712.9484	1.68	712.9478	0.84

### Quantitation

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. Accuracies were within the acceptance criteria outlined in

EPA Method 537.1. Levels 2 through 10 were within 70-130%, and Level 1 was between 50 and 150% for all compounds. Figure 1 shows a representative TIC for the LLOQ calibrator. Figure 2 shows the chromatograms for the four new compounds listed in EPA Method 537.1.

# Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

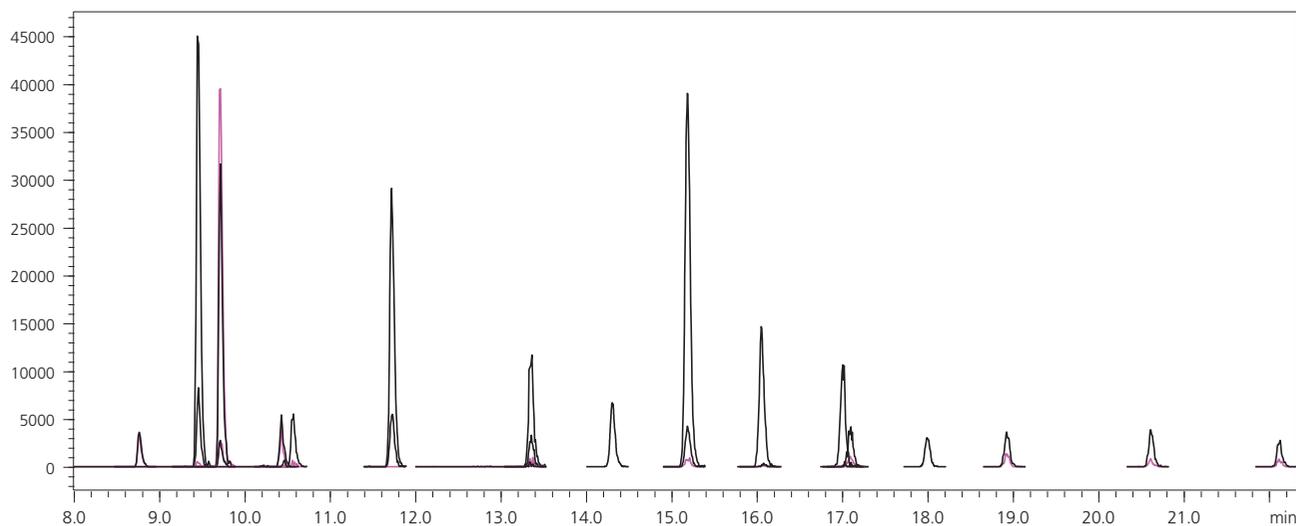


Figure 1. Representative TIC of all PFAS listed in EPA Method 537.1 at 5ppt sample concentration

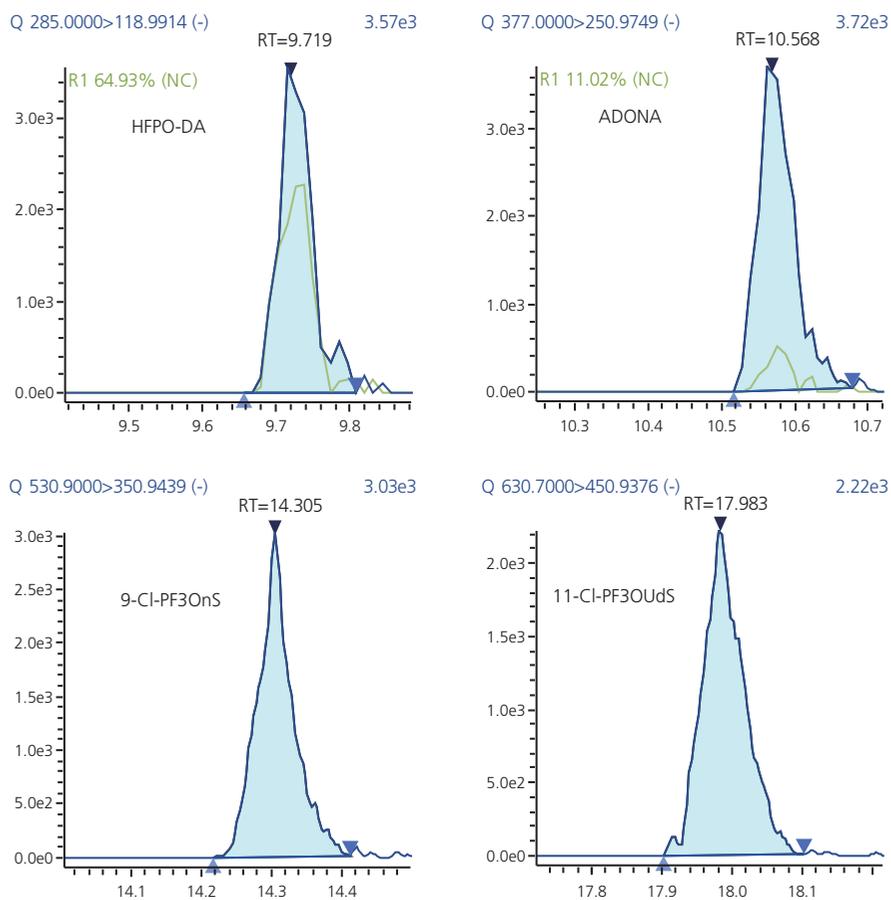


Figure 2. Representative chromatograms for all new targets in EPA Method 537.1

# Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

Table 4. LLOQ repeatability study at 5 ppt sample concentration

QTOF LOQ 2 ng/L	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
9CI-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
11CI-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

Table 5. Precision and accuracy study performed at 25ppt sample concentration

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	26.37	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
9CI-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
11CI-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

# Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

## Library Searching

MS scan data was acquired to measure the accurate masses for all precursor ions. The MS scan data was compared against data in the DSSTox and CompTox databases provided and maintained by the EPA. MS/MS spectral libraries were created separately to further reduce false positives. MS/MS spectral libraries also allow for

compounds with branched isomers to be distinguished from the linear isomers. All compounds in the MS and MS/MS spectral libraries were created using a chromatographic method based on EPA Method 537.1. Information on the retention times can be included to aid in compound identification.

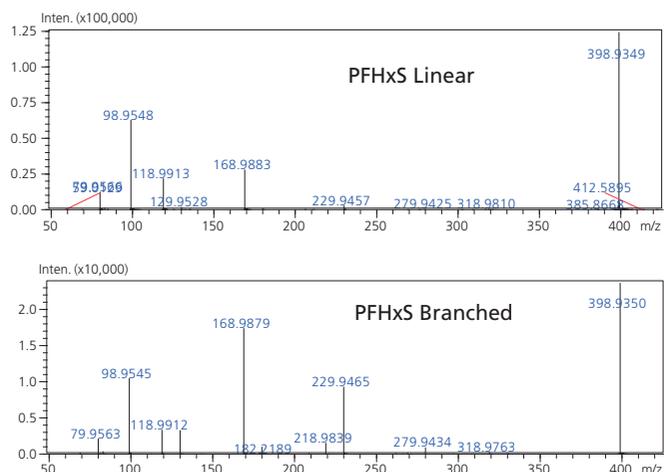


Figure 3. MS Spectra of linear and branched isomers of PFHxS at collision energy of 30 V

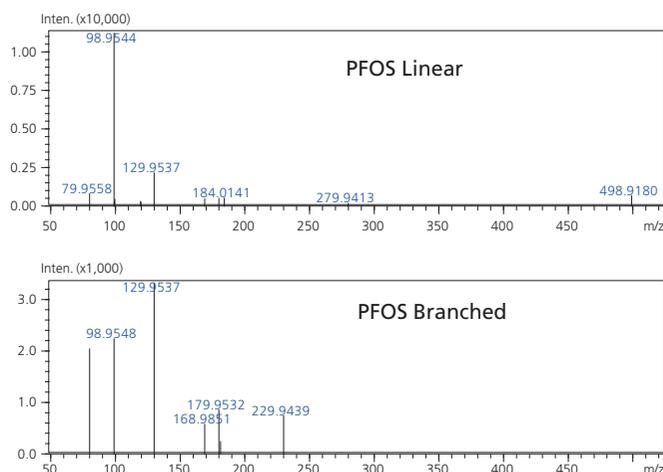


Figure 4. MS Spectra of linear and branched isomers of PFOS at collision energy of 45 V

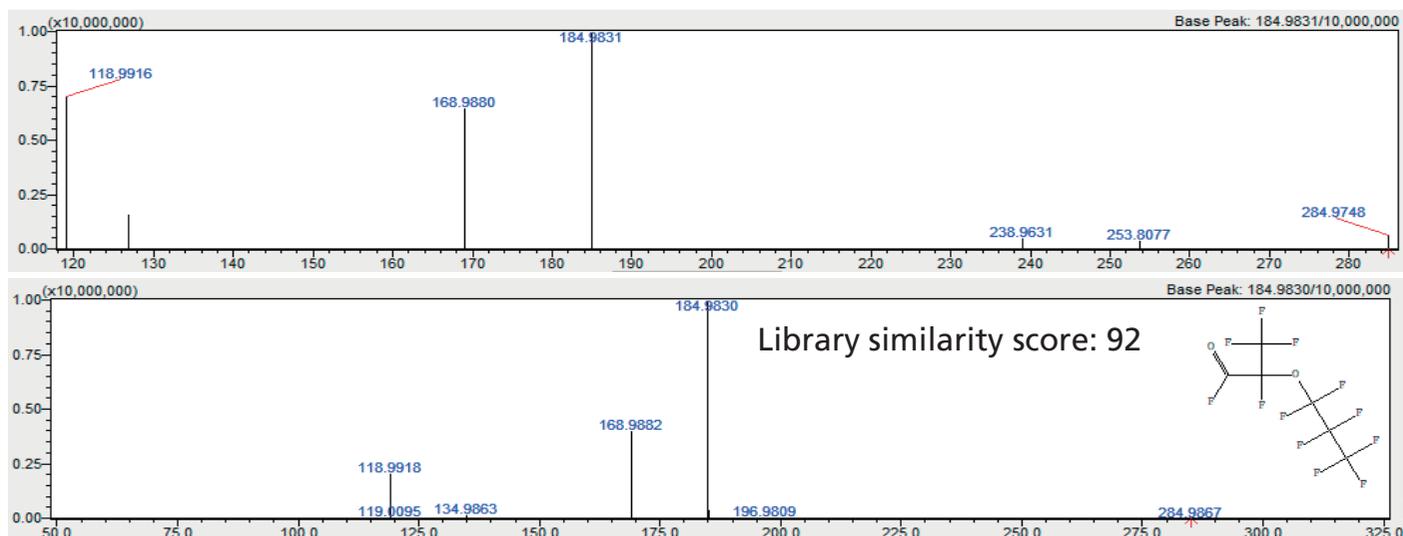


Figure 5. MS Spectra for HFPO-DA found in 25 ppt extracts compared against Library MS Spectra

## Sensitive Perfluoroalkyl Substance (PFAS) Screening Using High Resolution Accurate Mass Spectral Library

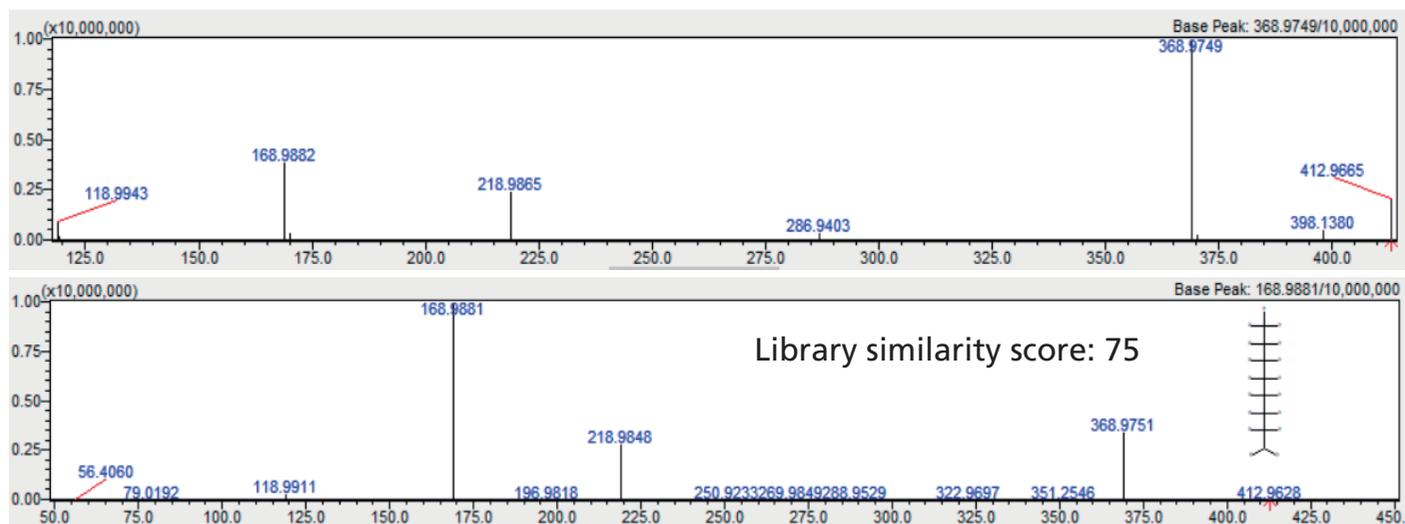


Figure 6. MS Spectra for PFOA found in 25 ppt extracts compared against Library MS Spectra

## Conclusions

A high resolution accurate mass spectral library was developed using a QTOF mass spectrometer. Mass accuracy is stable, with no mass correction or frequent tuning needed. Quantitative capabilities are comparable

to a triple quadrupole, while also allowing the use of Library spectra matching to reduce false positives in complex matrices.

For Research Use Only. Not for use in diagnostic procedures. This presentation may contain references to products that are not available in your country. All rights reserved. Information subject to change without notice.