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APPLICATION NOTE 65499

Secondary validation study for EPA Method 537.1 using automated SPE followed by LC-Q Exactive Orbitrap MS

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Keywords

High-resolution accurate mass spectrometry, PFOS, PFOA, GenX, ADONA, PFASs, emerging contaminants, EPA 537.1, Orbitrap, AutoTrace

Goal

To demonstrate method performance for the per- and polyfluorinated alkyl substances (PFAS) analysis using Orbitrap™ high-resolution mass spectrometry as an alternative to conventional triple quadrupole instruments for determination of PFAS in drinking water matrices using EPA Method 537.1.

Introduction

Within the last decade, liquid chromatography-tandem mass spectrometry (LC-MS/MS) sensitivity has increased by at least a factor of ten and is therefore sensitive enough for quantitation of targeted compounds for validated methods. The ease of use for detecting polar compounds makes LC-MS/MS the technique of choice for analysis of compounds of emerging concern (CECs) in environmental samples. However, with the development of high-resolution accurate mass (HRAM) spectrometers, sensitivity rivals that of triple quadrupole MS instruments and, in addition, mass resolution provides the added benefits of accurate quantitation along with unknown screening capabilities. HRAM using Orbitrap technology combines the sensitivity of a triple quadrupole analyzer for quantitation with the confidence of full scan data for quantitative identification and confirmation similar to MS/MS instruments that participated in a method validation study.

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This application note highlights the Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer used as one of the outside laboratory validations for updating EPA Method 537 r1.1 - *Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS).*

EPA 537 Rev. 1.1, first published in 2009 to determine 14 different PFAS in drinking water, has been updated to EPA Method 537.1 and includes four more PFAS. These new PFAS that have been replacing PFOA and PFOS in manufacturing processes are GenX chemicals, specifically the hexafluoropropylene oxide dimer acid, as well as 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS), 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS), and 4,8-dioxa-3H-perfluorononanoic acid (ADONA). EPA Method 537.1 can be used by EPA's Regions and other government and commercial environmental laboratories to measure PFAS in finished drinking water.

Experimental

This application note describes the quantitation of selected PFAS reagent and drinking water using EPA Method 537.1. The list of PFAS included in this study is shown in Table 1.

Sample preparation

PFAS standard solutions

Target, internal, and surrogate PFAS standard mixtures were provided by the EPA. These were originally purchased from Wellington Laboratories for the four new compounds plus the isotopically labeled targeted compounds added to EPA Method 537.1. Legacy PFAS analytes were obtained from AccuStandard. A stock solution of 18 target PFAS compounds was prepared in methanol/water 96/4 (v/v) at a concentration of 2 µg/mL prior to shipment to the three outside laboratories involved in the secondary validation study. Calibration solutions, with concentrations of 0.1–40 ng/L (ppt), were prepared by serial dilutions of the stock solution in 96:4 (v/v) methanol/water and appropriate internal standards and surrogate were added according to the method.

Table 1. List of PFAS compounds included in this method

Analyte	Acronym	CASRN
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorohexanoic acid	PFHxA	307-24-4
Hexafluoropropylene oxide dimer acid	GenX	13252-13-6
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluoroheptanoic acid	PFHpA	375-85-9
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanoic acid	PFNA	375-95-1
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
Perfluorodecanoic acid	PFDA	335-76-2
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluoroundecanoic acid	PFUnA	2058-94-8
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTA	376-06-7
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9

Sample and extracted QC preparation

A 250 mL water sample was preserved with Trizma®, fortified with surrogate standards, and passed through a solid phase extraction (SPE) cartridge containing SDVB to extract the method analytes and surrogates using a semi-automated Thermo Scientific™ Dionex™ AutoTrace™ 280 Solid-Phase Extraction instrument. The compounds were eluted from the solid phase with a small amount of methanol. The extract was concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1 mL volume with 96%/4% (v/v) methanol/water after adding the internal standards.

Drinking water matrix for LFSM

Monrovia, California, tap water, a finished drinking water from a combined ground and surface water source, was collected and preserved according to EPA Method 537.1. This matrix served as the laboratory fortified sample matrix (LFSM).

LC-MS/MS analysis

Since the required limits of detection are in the low ng/L range, careful selection of reagents and consumables is necessary to ensure they are PFAS-free. The LC-MS/MS system, composed of a Thermo Scientific™ UltiMate™ 3000 UHPLC and a Q Exactive mass spectrometer equipped with a H-ESI II ionization probe, also included an isolator column installed after the LC pump and prior to the injection valve. The isolator column offsets background contaminants from the LC pump, degasser, and mobile phases.

LC conditions	
Analytical column:	Waters™ Atlantis™ dC18 2.1 x 150 mm column packed with 5.0 µm particles
Isolator column:	Thermo Scientific™ Hypersil™ C18, 5 µm, 2.1 x 50 mm (P/N 28105-052130)
Column temp.:	25 °C
Flow rate:	0.5 mL/min
Solvent A:	Water containing 20 mM ammonium acetate
Solvent B:	Methanol
Injection volume:	10 µL

LC gradient	
Time (min)	% Methanol
0	30
0.63	30
15	90
16.3	90
16.4	30
21	30

MS conditions

The H-ESI II source was used in the negative ionization mode and the optimized MS parameters were as follows: spray voltage at 2.5 kV; sheath gas at 60; auxiliary gas at 12; probe heater temperature at 437 °C, and capillary temperature at 269 °C.

Both EPA Method 537 Rev. 1.1 and Method 537.1 require MS/MS for the method analytes within specified retention time segments and a minimum of 10 scans across the chromatographic peak for adequate precision.

EPA Method 537.1 measures precursor and product ion transitions, termed Selected Reaction Monitoring (SRM). Similarly, the Q Exactive mass spectrometer performs MS/MS in Parallel Reaction Monitoring (PRM) mode. In PRM mode, a list of targeted precursor ions, retention times, and collision energies can be included in the method (Table 2). When detecting a targeted ion, the system isolates that precursor ion in the quadrupole and triggers the MS/MS, generating MS/MS spectra that can be used for both quantitation and qualitative identification. Both the quantitation and identification are performed taking into account product ions generated after the isolation of a specific precursor ion. This operating mode is similar to SRM (also called MRM) using a triple quadrupole instrument.

In PRM, the third quadrupole of a triple quadrupole instrument is substituted with the HRAM mass analyzer to permit the parallel detection of all target product ions in one concerted high-resolution mass analysis. Thus, instead of serially monitoring target transitions over several ion injections and low-resolution mass measurement periods as in SRM, PRM monitors all product ions of a mass-selected targeted compound in parallel with one ion injection and full mass range Orbitrap mass analysis (Figure 1).

Table 2. Monitored PRM transitions details and instrument parameter: S-lens is set at 50 for all compounds.

Compound	Retention Time (min)	Precursor (*m/z*)	Quant. Product (*m/z*)	Normalized Collision Energy (NCE)
PFBS	7.5	298.9430	79.9561	60
PFHxA	9.2	312.9728	268.9829	20
GenX	9.8	284.9779	168.9884	20
PFHpA	10.8	362.9696	318.9794	20
PFHxS	10.8	398.9366	79.9560	60
ADONA	10.9	376.9689	250.9761	35
PFOA	12.0	412.9664	368.9767	20
PFOS	12.9	498.9302	79.9560	60
PFNA	13.0	462.9632	418.9737	20
9Cl-PF3ONS	13.4	530.8956	350.9454	35
PFDA	13.8	512.9600	468.9703	20
NMeFOSAA	14.2	569.9673	418.9736	20
PFUnA	14.5	562.9568	168.9886	20
NEtFOSAA	14.5	583.9830	418.9738	20
11CL-PF3OUdS	14.8	630.8892	450.9390	35
PFDoA	15.1	612.9537	168.9883	20
PFTrDA	15.6	662.9504	168.9887	20
PFTA	16.1	712.9473	168.9886	20
13C2-PFDA	13.8	514.9667	469.9735	20
13C2-PFHxA	9.2	314.9795	269.9864	20
13C3-GenX	9.8	286.9849	168.9884	20
d5-NEtFOSAA	14.5	589.0143	418.9735	35
13C2-PFOA	12.0	414.9652	369.9800	20
13C4-PFOS	12.9	502.9436	79.9560	60
d3-NMeFOSAA	14.2	572.9861	418.9735	35

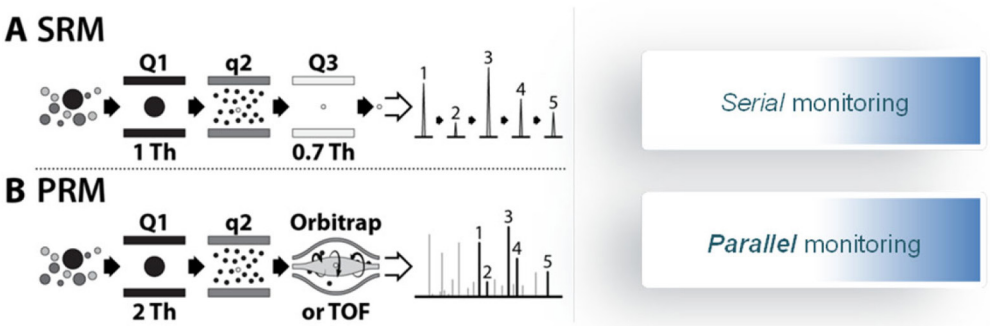

Figure 1. SRM and PRM

The number of scans across the chromatographic peak is dependent on the cycle time of the instrument and therefore on the set of conditions used (e.g. resolving power). These conditions can be optimized depending on the objectives of the analysis, in this case, accurate quantitation as well as unambiguous identification. The optimized conditions listed below produce >10 MS^2 scans using a resolution setting of 17,750 (full width at half maximum (FWHM)) at *m/z* 200.

Another important feature of the Q Exactive mass spectrometer is the ability to fill the C-trap in parallel to detection in the Orbitrap analyzer. This presents an enormous time savings so that more than 90% of the entire analysis time is spent on filling the C-trap, enhancing the sensitivity and selectivity. To make the most effective use of the duty cycle at 17,750 resolution setting, the Ion Transmission (IT) was set at 55 ms, and the Automatic Gain Control (AGC) at 2E5 for best sensitivity. With these settings, the EPA Method 537.1 requirement of >10 scans for all compounds was easily met. Figure 2 shows PFNA with >30 scans even though it is at the most overlapping scan window for the other nearby compounds.

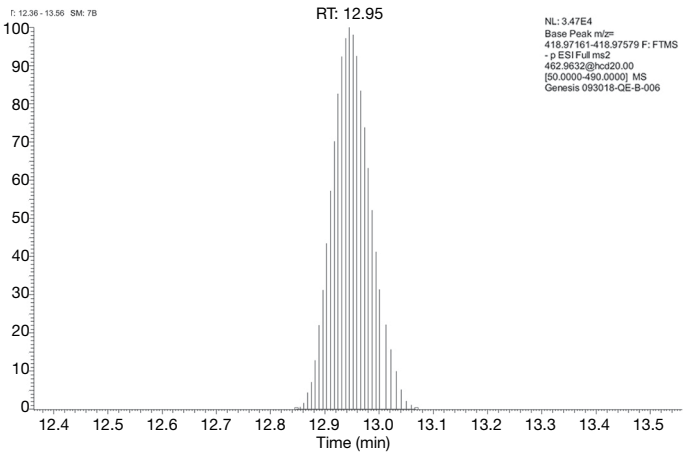

Figure 2. Greater than 30 scans for PFNA

Data processing

Thermo Scientific™ TraceFinder™ Chromatography Data System software, version 4.1 was used.

Secondary laboratory validation study requirement

Prior to publishing a new method such as EPA 537.1, laboratories involved in the inter-laboratory studies need to prove ruggedness of the new method by completing an initial demonstration of capability (IDC) and perform a lowest concentration minimum reporting limit (LCMRL) study for determination of Minimum Reporting Limit (MRL). The requirements are:

1. Demonstration of low background <1/3 of minimum reporting limit (MRL)

2. Demonstration of precision by analyzing four to seven extracted laboratory reagent waters (LFBs) near mid-level to obtain RSD of <20%

3. Demonstration of accuracy from 4–7 laboratory fortified blanks (LFBs) with recovery of 70–130%

4. Demonstration of precision and accuracy (P&A) for mid-level laboratory fortified sample matrix and laboratory fortified sample matrix duplicates (LFSM/LFSMD) with recovery of 70–130% and RSD of <30%

5. Determination of the LCMRL. The LCMRL is the lowest spiking concentration where the probability of spike recovery in the 50% to 150% range is at least 99%. It differs from MDL studies because it also accounts for accuracy beside precision. LCMRL procedures require, at a minimum, four replicates at each of seven fortification levels plus blanks to calculate MRL.

All the requirements listed above must be processed through the entire method from extraction to analysis.

Results and discussion

Linearity and sensitivity

Excellent linearity and quantitative accuracy were achieved over the range of 0.1 to 40 ng/L, with correlation coefficients greater than 0.995 for all transitions using unweighted linear regression and forced to zero. The respective residuals were less than 30% of the nominal values. Representative calibration curves for PFOS and PFOA are shown in Figure 3, with correlation coefficients of 0.9998 and 0.9998, respectively. Figure 4 also shows chromatograms of quantitation ions injected at 0.1 ng/L demonstrating the high sensitivity achieved with the Q Exactive mass spectrometer for the quantitation of PFAS at ultra-low levels (sub-ppt range) for four new compounds added to EPA Method 537.1.

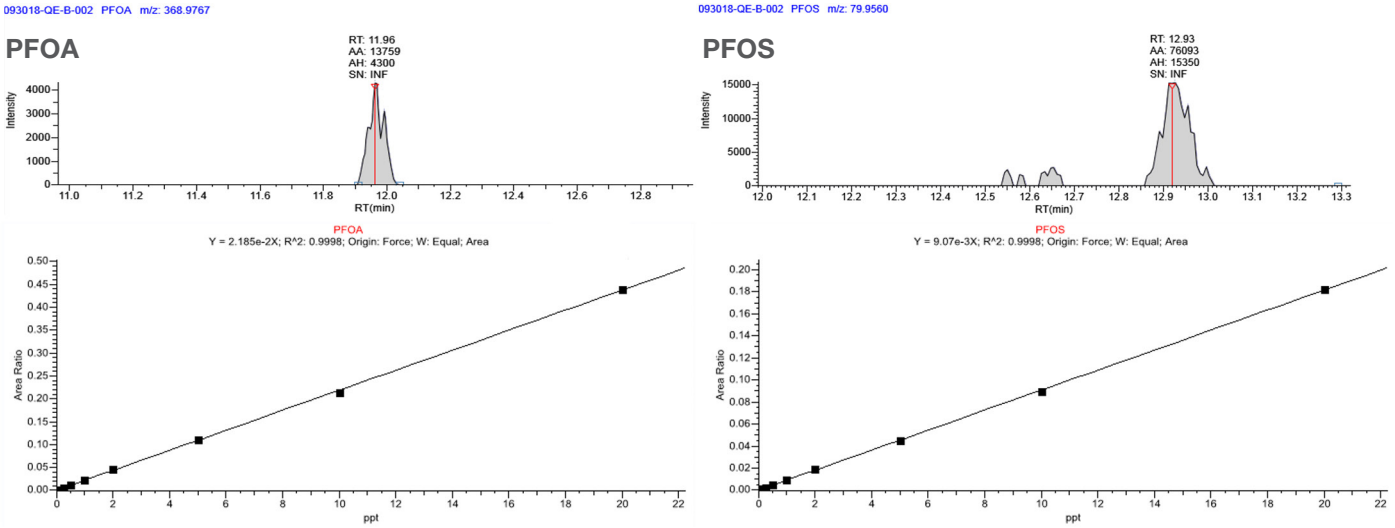

Figure 3. Calibration and chromatogram of 0.1 ppt the lowest calibration point used for this study for PFOA (left) and PFOS (right)

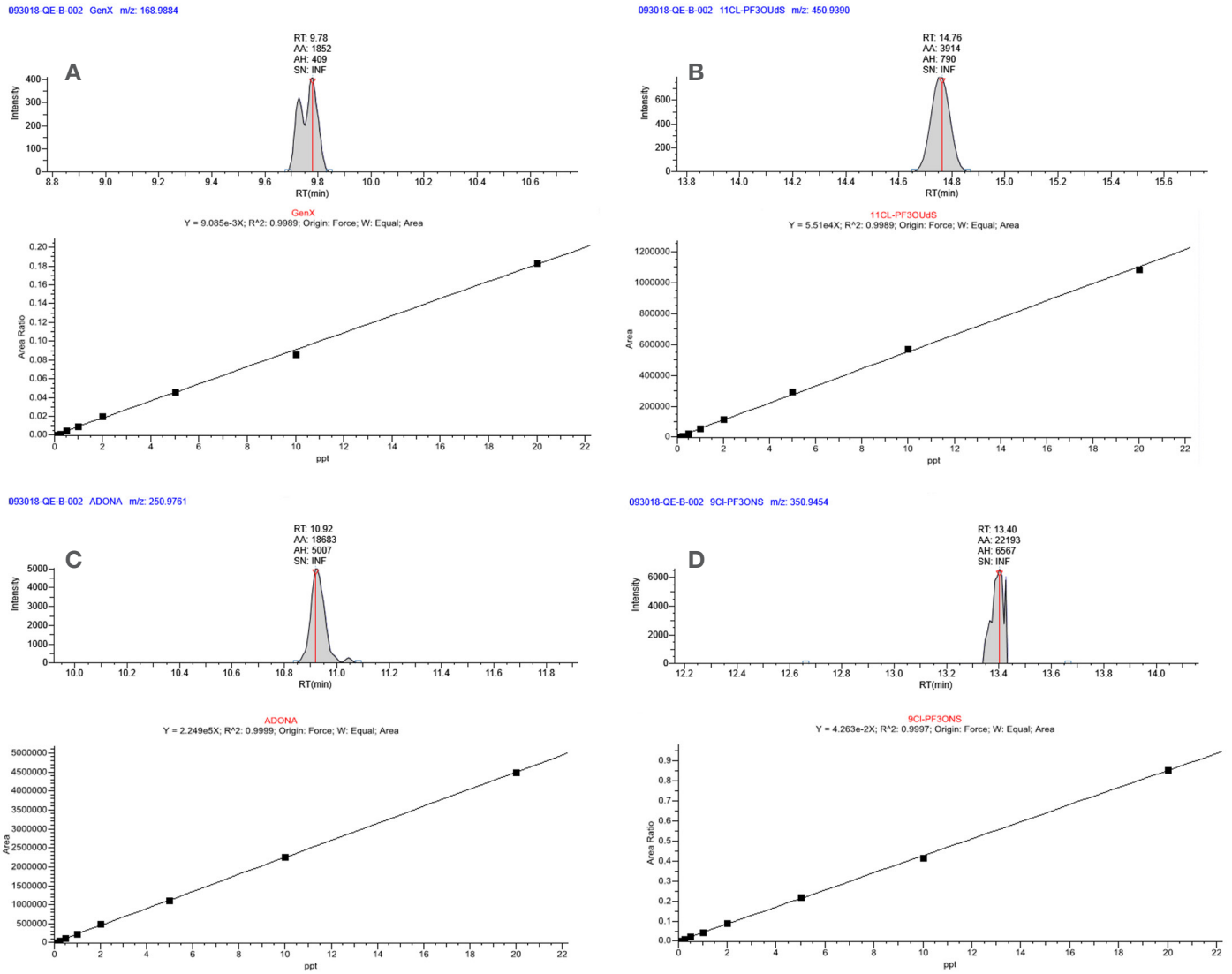

Figure 4. Calibration and 0.1 ppt level for (A) GenX, (B) 11CL-PF3OUdS, (C) ADONA, and (D) 9CI-PF3ONS. All correlation coefficients were >0.998.

Peak asymmetry

One of the method's requirements is to have a peak asymmetry factor (AF) of >0.8 and <1.5 for the first eluting peaks, PFBS and PFHxA, at mid-point calibration standard concentration as shown in Figure 5.

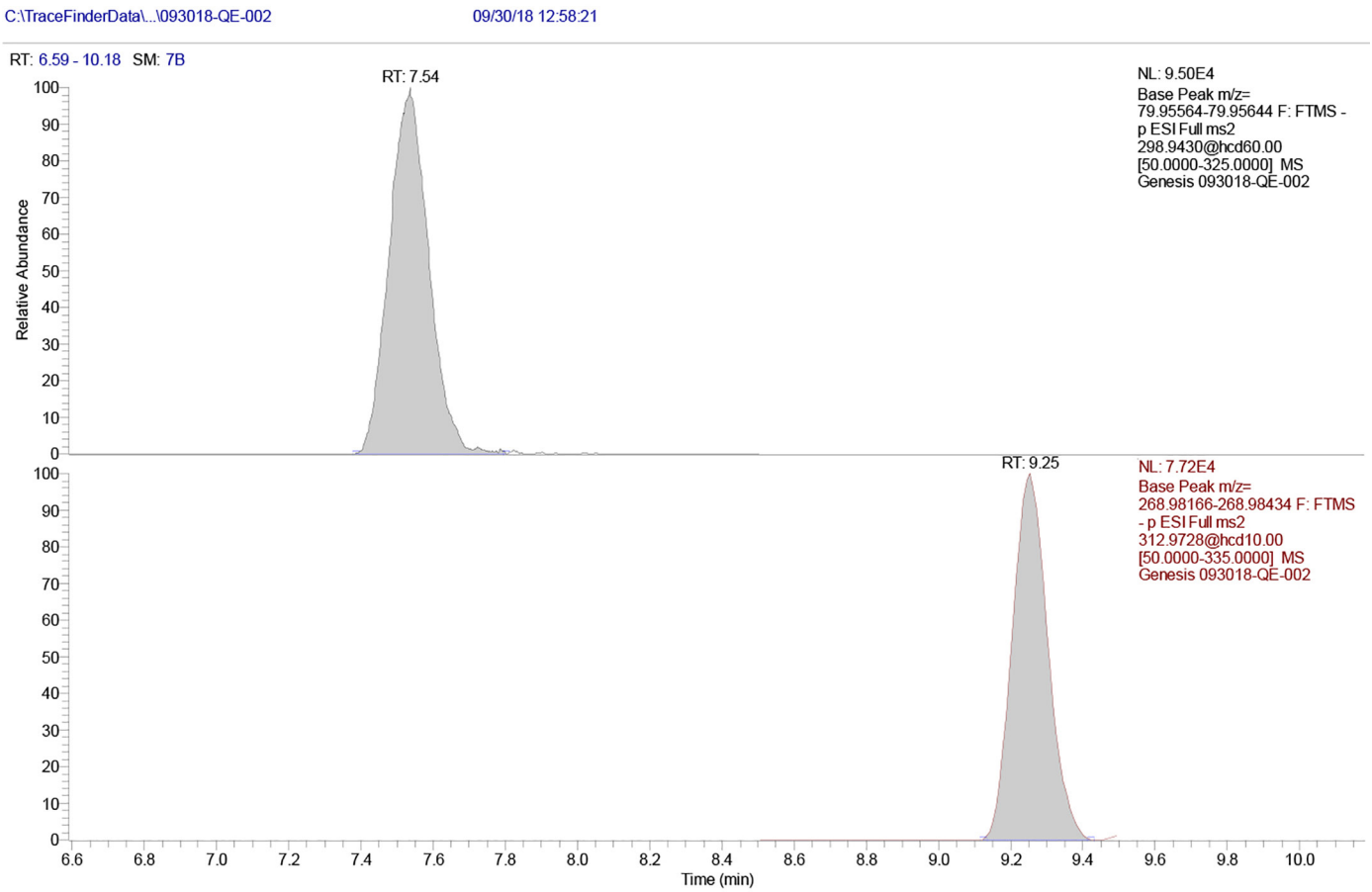

Figure 5. Asymmetry for PFBS (top) and PFHxA (bottom)

Initial demonstration of capability

1. Low system background was measured. All method blanks exhibited very low levels of contamination compared to the lowest calibration level at 0.1 ppt for all analytes (Table 3).

Table 3. Low system background in extracted method blanks. Levels shown below LCMRL calculated levels shown in Table 6 should be considered only as an estimate.

Extract	11CL-PF3OUdS (ng/L)	9Cl-PF3ONS (ng/L)	ADONA (ng/L)	GenX (ng/L)	NEtFOSAA (ng/L)	NMeFOSAA (ng/L)	PFBS (ng/L)	PFDA (ng/L)	PFDoA (ng/L)
Method blank -1	0	0.002	0.004	0	0	0	0	0	0.005
Method blank -2	0	0.003	0.074	0	0.035	0.009	0.001	0	0.01
Method blank -3	0	0.005	0.111	0	0	0.011	0.002	0	0.025
Method blank -4	0	0.007	0.129	0	0	0.013	0	0	0.04
Extract	**PFHpA (ng/L**	**PFHxA (ng/L**	**PFHxS (ng/L**	**PFNA (ng/L**	**PFOA (ng/L**	**PFOS (ng/L**	**PFTA (ng/L**	**PFTrDA (ng/L**	**PFUnA (ng/L**
Method blank -1	0.003	0.038	0	0	0.019	0.052	0.008	0	0.009
Method blank -2	0.005	0.039	0.001	0.007	0.024	0.055	0.013	0.009	0.011
Method blank -3	0	0.04	0	0	0.025	0.059	0.029	0.013	0.023
Method blank -4	0	0.054	0	0	0.031	0.069	0.036	0.016	0.059

2. The initial demonstration of precision and accuracy was met by analyzing seven LFBs extracted over three days spiked at 25 ng/L <20% RSD and ±30 difference achieved (Table 4).

Table 4. Data for precision and accuracy for six laboratory fortified blanks

Compound	Average Concentration (ng/L)	Theoretical Concentration (ng/L)	% Difference	% Recovery	Limit	% RSD
PFBS	26.719	25.000	6.87	107%	70-130%	4.76
PFHxA	26.166	25.000	4.66	105%	70–130%	3.59
GenX	25.459	25.000	1.83	102%	70–130%	4.61
PFHxS	25.739	25.000	2.95	103%	70–130%	2.13
PFHpA	24.744	25.000	-1.02	99%	70–130%	2.77
ADONA	22.629	25.000	-9.48	91%	70–130%	5.52
PFOA	28.394	25.000	13.57	114%	70–130%	3.24
PFOS	27.329	25.000	9.32	109%	70–130%	2.89
PFNA	26.596	25.000	6.39	106%	70–130%	4.60
9Cl-PF3ONS	25.982	25.000	3.93	104%	70–130%	5.49
PFDA	25.791	25.000	3.16	103%	70–130%	5.08
11CL-PF3OUdS	24.883	25.000	-0.47	100%	70–130%	5.49
NMeFOSAA	25.722	25.000	2.89	103%	70–130%	5.36
PFUnA	27.007	25.000	8.03	108%	70–130%	5.64
NEtFOSAA	25.534	25.000	2.14	102%	70–130%	6.66
PFDoA	26.028	25.000	4.11	104%	70–130%	5.36
PFTrDA	24.620	25.000	-1.52	98%	70–130%	5.13
PFTA	25.489	25.000	1.96	102%	70–130%	3.70

3. Monrovia, CA, tap water was spiked at 25 ng/L extracted over two batches in duplicates and analyzed. Results are shown in Table 5. The %RSD of less than 30% and recoveries of ±30% of spike amount were met.

Table 5. (part 1) Showing data for precision and accuracy for four laboratory fortified sample matrix

	Spike (ng/L)	LFSM	LFSM	LFSM	LFSM	Average	STDEV	%REC.	%RSD
PFBS	25	19.8	21.4	25.1	25.5	23.0	2.8	91.9%	12%
11CL-PF3OUdS	25	21.1	22.7	24.2	24.8	23.2	1.7	92.9%	7%
9Cl-PF3ONS	25	22.0	22.1	26.7	25.9	24.2	2.5	96.6%	10%
ADONA	25	18.2	19.8	19.4	19.6	19.2	0.7	77.0%	4%
GenX	25	22.0	23.0	24.4	24.4	23.4	1.2	93.8%	5%
NEtFOSAA	25	21.2	21.8	24.2	24.4	22.9	1.6	91.6%	7%
NMeFOSAA	25	20.6	22.4	24.3	26.0	23.3	2.3	93.3%	10%
PFDA	25	21.7	22.7	24.6	25.7	23.7	1.8	94.7%	8%
PFDoA	25	21.6	24.0	24.8	26.0	24.1	1.9	96.3%	8%
PFHpA	25	19.6	21.3	24.0	24.2	22.3	2.2	89.1%	10%
PFHxA	25	20.9	22.0	24.4	25.1	23.1	2	92.3%	9%
PFHxS	25	20.6	22.2	23.5	23.5	22.5	1.4	89.8%	6%
PFNA	25	23.5	23.5	25.9	26.2	24.8	1.5	99.2%	6%
PFOA	25	22.0	23.1	24.8	25.3	23.8	1.5	95.2%	6%
PFOS	25	22.5	24.0	25.6	26.2	24.6	1.7	98.3%	7%
PFTA	25	21.9	23.2	26.9	28.0	25.0	2.9	100.0%	12%
PFTrDA	25	21.3	23.1	25.5	25.4	23.8	2	95.3%	9%
PFUnA	25	22.2	24.8	26.1	26.5	24.9	1.9	99.5%	8%

Table 5. (part 2) Showing data for recovery of internal standards and surrogates used in laboratory fortified sample matrix

	Spike (ng/L)	LFSM	LFSM	LFSM	LFSM
Surrogate:					
13C2-PFHxA	40	111%	113%	113%	111%
13C3-GenX	40	100%	103%	106%	101%
d5-NEtFOSAA	160	115%	119%	113%	103%
13C2-PFDA	40	116%	116%	108%	111%
Internal standard:					
13C2-PFOA	10	114%	110%	101%	119%
13C4-PFOS	20	113%	112%	101%	119%
d3-NMeFOSAA	40	106%	100%	101%	113%

4. For the LCMRL calculation, four replicates at concentrations of 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 16 ng/L were extracted and analyzed. The LCMRL and DL were calculated using the LCMRL calculator from the EPA website: http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods_ogwdw.cfm. Table 6 shows the results. The Reported DLs are calculated using the same LCMRL calculator.

Table 6. Summary of LCMRL and calculated detection limit

Analyte	DL (ng/L)	LCMRL (ng/L)
PFBS	0.42	2.5
PFHxA	0.22	0.71
GenX	0.34	1.1
PFHpA	0.18	1.3
PFHxS	0.17	0.38
ADONA	0.15	0.25
PFOA	0.16	0.73
PFOS	0.11	0.5
PFNA	0.3	0.58

Analyte	DL (ng/L)	LCMRL (ng/L)
9Cl-PF3ONS	0.14	0.29
PFDA	0.26	0.34
NMeFOSAA	0.24	0.44
PFUnA	0.45	0.64
NEtFOSAA	0.21	0.34
11CL-PF3OUdS	0.33	0.43
PFDoA	0.78	2.5
PFTrDA	0.13	0.58
PFTA	0.1	0.56

Conclusions

The method referenced in this application note is rugged and reproducible and shows excellent quantitative performance of the Q Exactive Orbitrap mass spectrometer in PRM mode for EPA Method 537.1 with enhanced selectivity and specificity.

References

1. Winslow, S.D.; Pepich, B.V.; Martin, J.J.; Hallberg, G.R.; Munch, D.J.; Frebis, C.P.; Hedrick, E.J.; Krop, R.A. Statistical Procedures for Determination and Verification of Minimum Reporting Levels for Drinking water Methods. *Environ. Sci. Technol.* **2004,** *40,* 281–288.
2. J.A. Shoemaker and D.R. Tettenhorst, Office of Research and Development: Method EPA 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). https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=343042&simpleSearch=0

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