Analysis of PFAS in drinking water with EPA method 537.1 and the SCIEX QTRAP 4500 system

Achieving 537.1 method requirements in a robust 10-minute method

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In the United States, EPA method 537.1 describes the sample preparation, reporting guidelines, and quality control for the analysis of a suite of 14 per- and polyfluorinated substances (PFAS) in drinking water. The EPA 537.1 method guidelines provide some flexibility in the liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Within these guidelines, optimization of certain aspects of the method, such as column chemistry, chromatography, mobile phases, gradient profile, and MS/MS transitions, was performed. Sample preservation and preparation guidelines published in EPA 537.1 are prescriptive and were therefore closely followed.

Table 1. PFAS in EPA method 537.1. Names, abbreviations, and method detection limits (MDLs) for 14 PFAS compounds included in EPA Method 537.1 along with the reporting limits (MRLs) published by the UCMR3 guidelines 6 of the PFAS compounds. MDLs and MRLs shown as ng/L (ppt) here.

Compound	Abbreviation	Method detection limit (ng/mL)	UCRM3 reporting limit (ng/mL)
Perfluorohexane carboxylate	*PFHxA*	*0.09*	-
Perfluoroheptane carboxylate	*PFHpA*	*0.1*	*10*
Perfluorooctane carboxylate	*PFOA*	*0.1*	*20*
Perfluorononane carboxylate	*PFNA*	*0.09*	*20*
Perfluorodecane carboxylate	*PFDA*	*0.1*	-
Perfluoroundecane carboxylate	*PFUnDA*	*0.1*	-
Perfluorododecane carboxylate	*PFDoA*	*0.1*	-
Perfluorotridecane carboxylate	*PFTrDA*	*0.2*	-
Perfluorotetradecane carboxylate	*PFTeDA*	*0.2*	-
Perfluorobutane sulfonate	*PFBS*	*0.1*	*90*
Perfluorohexane sulfonate	*PFHxS*	*0.08*	*30*
Perfluorooctane sulfonate	*PFOS*	*0.1*	*40*
n-ethyl perfluorooctane sulfonamidoacetic acid	*n-EtFOSAA*	*0.1*	-
n-methyl perfluorooctane sulfonamidoacetic acid	*n-MeFOSAA*	*0.09*	-

Key features of PFAS analysis on the SCIEX QTRAP 4500 system

- Robust and reproducible results with qualifying accuracy and precision for calculated concentrations, asymmetry factor, and linearity
- Total sample runtime takes only 8-10 min, depending on autosampler settings and system dead volume
- Sensitive MDLs of 0.08-0.2 ng/L for the entire suite of 14 PFAS compounds

Methods

Sample preparation: Sample preservation and preparation were performed according to the guidelines in EPA Method 537.1. Briefly, 1 g of Trizma was added to 250 mL polypropylene bottles. Bottles were pre-weighed to calculate the mass of sample collected. Surrogate standards were added to the sample container to achieve a final concentration of 2 ng/L in the 250 mL water sample.

The water samples were extracted using the following procedure with Phenomenex Strata-XL solid phase extraction cartridges (6 mL, 500 mg):

1. Condition SPE tubes with 15 mL of methanol followed by 18 mL of water
2. Add sample to tubes at a flow rate of approximately 10-15 mL per minute.
3. Rinse tubes with 7.5 mL of water and repeat
4. Dry tubes under vacuum for 5 minutes
5. Rinse sample bottle with 4 mL of methanol and transfer methanol to SPE tube while collecting eluent and repeat
6. Evaporate sample to dryness under nitrogen at 40-60°C
7. Reconstitute sample in 1 mL of 96% methanol 4% water containing 1 ng/L of internal standards
8. Transfer a 0.25 mL aliquot to a polypropylene vial and archive the remaining volume

Chromatography: An Agilent 1200 binary pump was modified by replacing all clear fluoroethylene polymer (FEP) tubing with 1/8 in or 1/16 inch PEEK tubing. A delay column (Phenomenex Luna C18(2), 5µm, 30x2mm) was inserted between the gradient mixing chamber and the autosampler valve to retain contaminants from the eluents or pumps for an extra 1-2 min compared with target analytes eluting from the analytical column.

An Agilent 1200 autosampler injected 10 ul of each sample onto the analytical column (Phenomenex Gemini C18, 3µm, 50x2mm), which was heated to 40°C. Gradient separation was performed at a flow rate of 0.6 mL/min using the gradient shown in Table 2.

Mass spectrometry: Samples were ionized using negative mode electrospray at the source conditions shown in Table 3 and the Q1/Q3 masses, declustering potentials, and collision energies shown in Table 5. Calibration was performed using an 8-point curve at concentrations of 50, 100, 200, 500, 1000, 2000, 5000, and 10000 ng/L and the concentrations of surrogates and internal standards was 1,000 ng/L in all final sample extracts, standards, method blanks, and quality control samples.

Table 2. Gradient program.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	*95*	*5*
0.1	*45*	*55*
4.5	*1*	*99*
8	*1*	*99*
8.5	*95*	*5*

Mobile phase A - 20 mM ammonium acetate
Mobile phase B – methanol

Data processing: Quantitation was performed using MultiQuant software 3.0.2 using 1.0 point Gaussian smoothing and 1/x weighted linear regression forced through the origin (as required by EPA 537.1). A concentration factor of 250 was applied to samples as a result of the concentration of 250 mL to the final 1 mL extract.

Table 3. Source conditions.

Parameter	Value
CAD	*9*
GS1	*30*
GS2	*40*
CUR	*60*
IS Voltage	*-4500*
TEM	*450*

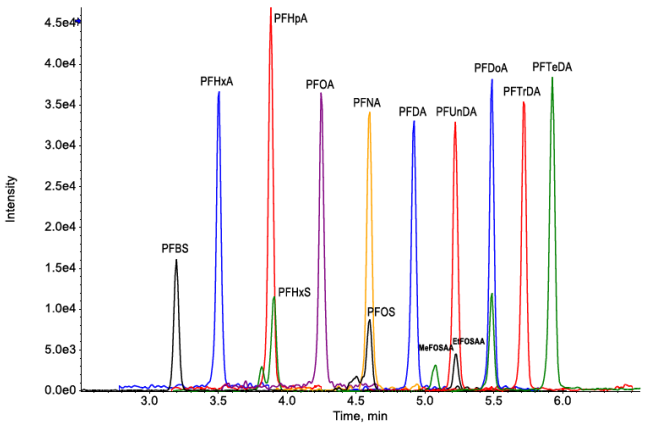

Figure 1. Chromatographic separation of PFAS standard mix. PFAS standard mix at 50 ng/L (ppt) containing all 14 compounds in EPA 537.1 eluting within 6 minutes for an 10 minute total run time with a 10 uL injection.

Calibration curve results

The initial calibration curve results achieved the following guidelines prescribed in EPA method 537.1:

1. Linearity (r2>0.99) (as shown in Figure 2)
2. Accuracy (+/- 30% for each calibrator)
3. Precision (RSD <20% of 4 replicates of a fortified blank)
4. Asymmetry factor (>0.8 and <1.5 for the first 2 peaks in the chromatogram as shown in Figure 3)
5. Surrogate recovery +/- 30% of expected response
6. Laboratory reagent blanks (LFBs) and field reagent blanks (FRBs) quantitated at <1/3 of the MRL.

To calculate method detection limits, 9 water samples were spiked with approximately 0.2 ng/L of each of the 14 PFAS compounds and analyzed following the full analytical protocol. The calculated MDLs shown Table 1 were calculated according to EPA 537.1 using the mean and standard deviation of the replicated spiked samples. The MDLs for all 14 compounds was below 0.2 ng/L, which highlights the excellent sensitivity of the method.

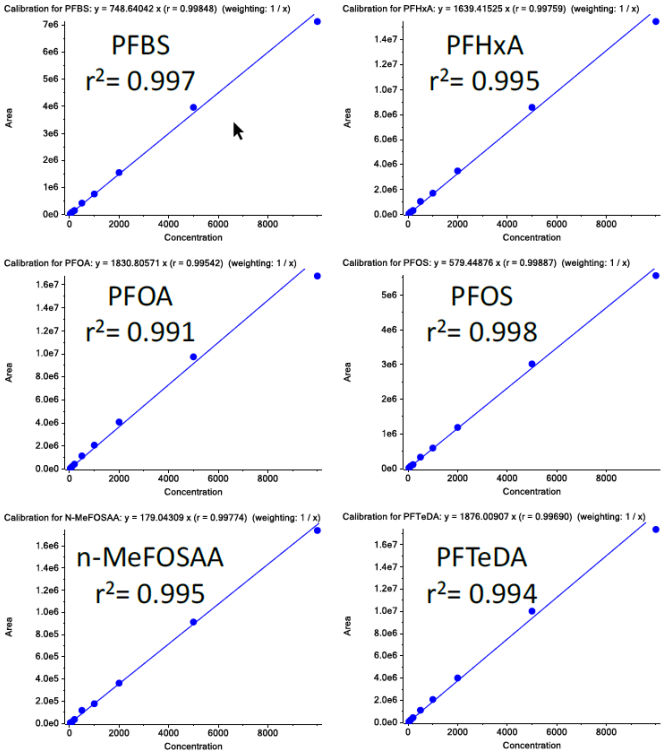

Figure 2. Calibration curves. Linearity of 6 of the PFASs analyzed out of the suite of 14 showing r2>0.99 with a linear fit forced through the origin and 1/x concentration weighting. The other 8 PFAS compounds also showed r2>0.99.

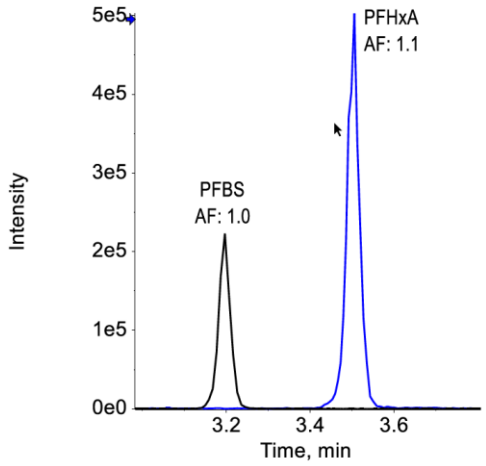

Figure 3. Asymmetry factor (AF). The AB is calculated for the first 2 eluting peaks, PFBS and PFHxA, at a mid-point standard concentration of 500 ng/L.

After 8 days of analyzing samples to calculate the MDL and other samples, the continuing calibrations still met the requirements of +/- 30% of expected calculated concentration for all 14 analytes, as shown in Table 4.

Table 4: Calibration curve statistics. Accuracy of a 50 ng/L calibration standard injected immediately after the initial calibration curve and 6 and 8 days after the calibration curve.

Days after calibration	0	6	8	0	6	8
Compound	Calc. conc. (ng/L)			Accuracy (%)		
PFBS	*44.8*	*54.6*	*57.7*	*90*	*109*	*115*
PFHxA	*54.8*	*52.2*	*52.6*	*110*	*104*	*105*
PFHpA	*55.1*	*50.4*	*58.0*	*110*	*101*	*116*
PFHxS	*47.1*	*53.9*	*49.3*	*94*	*108*	*99*
PFOA	*52.2*	*58.9*	*53.7*	*104*	*118*	*107*
PFNA	*55.1*	*50.9*	*51.3*	*110*	*102*	*103*
PFOS	*47.0*	*45.5*	*48.7*	*94*	*91*	*97*
PFDA	*52.7*	*53.5*	*52.3*	*105*	*107*	*105*
PFUdA	*52.9*	*48.5*	*52.9*	*106*	*97*	*106*
PFDoA	*54.5*	*56.9*	*55.0*	*109*	*114*	*110*
PFTrDA	*52.0*	*54.8*	*51.5*	*104*	*110*	*103*
PFTeDA	*51.6*	*51.0*	*53.0*	*103*	*102*	*106*
n-EtFOSAA	*55.7*	*53.9*	*57.2*	*111*	*108*	*114*
n-MeFOSAA	*56.7*	*61.6*	*58.2*	*113*	*123*	*116*

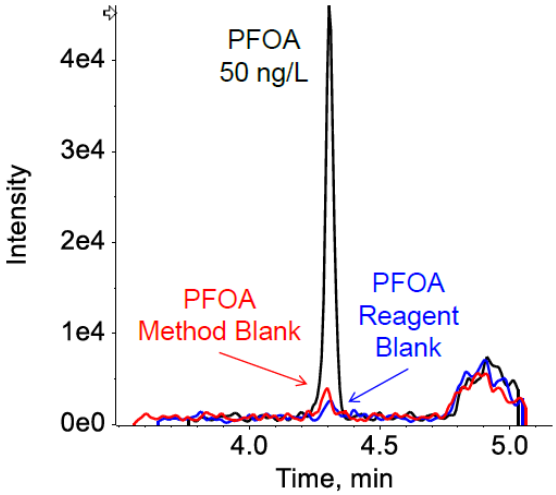

Figure 4: Blank samples. Blank chromatograms showing extremely low contamination in blank samples compared with the 50 ng/L calibration standard. The small peak at 4.9 min is the PFOA contamination eluting from the delay column, which originates from the HPLC pumps and eluent.

Method validation

Blank samples showed very low responses and were always below the requirement of <1/3 of the MRL. Figure 4 shows the response of a method blank (red), which was a 250 mL water sample taken through the entire sample preparation protocol, and a reagent blank, which was 96% methanol and 4% water prepared in an autosampler vial. A small peak at 4.9 min in the chromatogram in Figure 4 shows presence of PFOA contamination presumably in the HPLC pumps or eluents and demonstrates the adequate separation from the quantitated analyte peak as a result of the delay column installment.

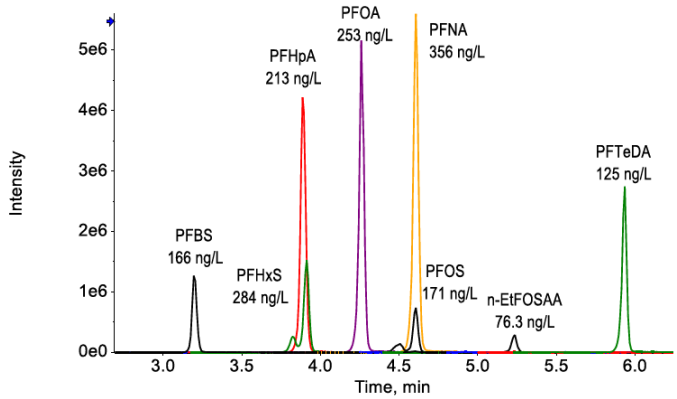

Figure 5. Proficiency testing. Proficiency testing samples were run to establish method performance met EPA requirements.

Proficiency testing (PT) samples were obtained from ERA and were analyzed along with the MDL replicates. The PT samples were diluted 10:1 with water and analyzed according to the procedures described in this note. The results of the 8 analytes present in PT CAT: 960 were all within +/- 18% of the assigned concentration of the PT study (Figure 5).

The HPLC method and MS/MS analysis for this validated 537 method were both fully compatible with an extended list of 25 analytes. However, the EPA method 537 sample preparation protocol relies on reverse phase, hydrophobic interactions for solid phase extraction (SPE) retention and, therefore, fails to adequately retain shorter chain PFCAs, including perfluorobutane carboxylate and perfluoropentane carboxylate. Modifying this sample preparation method by replacing Strata-XL with Strata-XL-AW (weak anion exchange) solid phase extraction tubes and altering the solvents used during extraction allows a longer, extended list to be analyzed using the same method.

Conclusions

Robust and reproducible results with quantitative accuracy and precision for calculated concentrations, asymmetry factor, and linearity were achieved in a single ten-minute LC-MS/MS acquisition on the SCIEX QTRAP 4500 system. Following stringent protocols for sample preparation and preservation defined by the EPA 537.1 method, method optimization steps were taken to continue to improve PFAS analysis workflow and quantitative performance.

- Background contamination was minimized through LC system adjustments (replacing FEP tubing and installing delay column).
- Analysis of shorter-chain PFCAs was improved through selection of weak anion exchange sorbent for sample SPE.
- Sensitive MDLs of 0.08-0.2 ng/L for the entire suite of 14 PFAS compounds were achieved, all of which meet or exceed the requirements of the US EPA's UCMR3 list for drinking water, and method robustness was demonstrated by sustained accuracy of measured concentration in a QC sample over eight days without need for re-injection of calibration standards.

Table 5. MS method information. MRM transitions, instrument voltage parameters, and retention times.

Compound	Q1	Q3	RT (min)	DP	CE
PFBS	298.9	80	3.2	-20	-56
PFBS_2	298.9	99	3.2	-20	-46
13C3_PFBS	302	80	3.2	-20	-56
PFHxA	313	269	3.5	-10	-14
PFHxA_2	313	119	3.5	-10	-25
13C2_PFHxA	315	270	3.5	-10	-14
13C5_PFHxA	318	273	3.5	-10	-14
PFHpA	363	319	3.9	-10	-14
PFHpA_2	363	169	3.9	-10	-25
13C4_PFHpA	367	322	3.9	-10	-14
PFHxS	399	80	3.9	-20	-74
PFHxS_2	399	99	3.9	-20	-60
13C3_PFHxS	402	80	3.9	-20	-74
PFOA	413	369	4.3	-10	-14
PFOA_2	413	169	4.3	-10	-26
13C2_PFOA	415	370	4.3	-10	-14
13C8_PFOA	421	376	4.3	-10	-14
PFNA	463	419	4.7	-10	-16
PFNA_2	463	169	4.7	-10	-26
13C9_PFNA	472	427	4.7	-10	-16
PFOS	499	80	4.7	-20	-95
PFOS_2	499	99	4.7	-20	-87
13C4_PFOS	503	80	4.7	-20	-95
13C8_PFOS	507	80	4.7	-20	-95

Table 5. MS method information cont'. MRM transitions, instrument voltage parameters, and retention times.

Compound	Q1	Q3	RT (min)	DP	CE
PFDA	513	469	5	-10	-17
PFDA_2	513	169	5	-10	-27
13C2_PFDA	515	470	5	-10	-17
13C6_PFDA	519	474	5	-10	-16
PFUdA	563	519	5.3	-10	-18
PFUdA_2	563	469	5.3	-10	-28
13C7_PFUdA	570	525	5.3	-10	-18
N-MeFOSAA	570	419	5.2	-50	-28
N-MeFOSAA_2	570	483	5.2	-50	-22
d3-MeFOSAA	573	419	5.2	-50	-28
N-EtFOSAA	584	419	5.3	-50	-28
N-EtFOSAA_2	584	526	5.3	-50	-28
d5-EtFOSAA	589	419	5.3	-50	-28
PFDoA	613	569	5.6	-10	-18

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