Application Note

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EPA Method 533 for Analysis of Per/Polyfluoroalkyl Substances in Drinking Water Using Agilent 6470 Triple Quadrupole LC/MS

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Introduction

The United States EPA (USEPA) has developed Method 533 for the analysis of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water. The method addresses some of the challenging compounds in USEPA Methods 537 and 537.1 for C_4 and C_5 acids and sulfonates, while limiting chain length to C_{12} acids and adding some emerging PFAS into the method. The work shown here was used as a second-lab demonstration for evaluating EPA method 533.

PFAS are extracted from water using off-line solid phase extraction (SPE), followed by LC/MS/MS using triple quadrupole mass spectrometry. Twenty-five compounds were measured, including the C_4 to C_{12} acids, C_4 to C_8 sulfonates, fluorotelomers, and mono/poly perfluorinated ethers. Ten of the 14 compounds in Method 537 are included, plus the four additional compounds in Method 537.1. USEPA Method 533 includes isotope dilution analogs to assess recoveries through the sample preparation as well as internal standard addition for the native compounds. Branched and linear PFHxS and PFOS isomers are summed in the calibration and quantitation of these compounds, and are discussed more fully in the USEPA method.

There are several competing issues in PFAS analyses, complicating the development of a single comprehensive method. Trace PFAS amounts can originate from sample collection and preparation tools such as filters and pipettes, LC systems as background, and from mobile phase solvents. A delay column is used between the LC pump and autosampler to separate the background PFAS compounds from those in the sample, enabling proper quantitation. Details of this mechanism and setup are explained in other Agilent Application Notes. Another issue is that longer chain compounds require higher concentrations of methanol (MeOH) in the final extract to remain in solution (96% in USEPA 537.1), but this causes peak shape distortion for the smaller, early-eluting compounds. Lowering the concentration of methanol in the final extract to 80% helps improve the peak shape of the early eluters and increases retention but reduces long chain solubility and reduces quantitative accuracy and precision as they drop out of solution.

This Application Note provides results obtained in a second lab demonstration for USEPA Method 533, using an Agilent 6470 triple quadrupole LC/MS/MS.

MS instrument conditions

Parameter	Value
MS	Agilent 6470 Triple Quadrupole MS/MS with Agilent Jet Stream ESI source
Source Parameters	
Gas Temperature	230 °C
Gas Flow	4 L/min
Nebulizer	20 psi
Sheath Gas Temperature	375 °C
Sheath Gas Flow	12 L/min
Capillary Voltage (Neg)	2,500 V
Nozzle Voltage (Neg)	0 V

dMRM transitions

The method used dynamic multiple reaction monitoring (dMRM), and was run in electrospray negative mode using an Agilent 6470 LC/MS/MS.

All compound parameters including precursor ion, product ion, fragmentor voltages, and collision energies were optimized for each compound with Agilent Optimizer Software (Table 1). Refer to Application Note 5991-7951 for more details. The cell accelerator voltage was selected as 2 V for all compounds.

LC instrument conditions

Parameter	Value
LC	Agilent 1260 series Infinity binary pump, G1367E Infinity ALS, G1316A Infinity thermostated column compartment
Analytical Column	Agilent ZORBAX Eclipse Plus C18, 3 × 50 mm; 1.8 µm (p/n 959757-302)
Delay Column	Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm (p/n 835975-902)
Column Temperature	50 °C
Injection Volume	10 uL
Mobile Phase	A) 20 mM Ammonium acetate in water (LC grade) B) MeOH (LC grade)
Gradient Flow Rate	0.4 mL/min
Gradient	Time (min) %B 0.0 5 0.5 5 3.0 40 16.0 80 18.0 80 20.0 95
Stop Time	20.0 minutes
Post Time	6 minutes

Note: It is highly recommended to run at least one blank injection at the start of the worklist to remove any built-up contaminants from the system.

Table 1. Compound parameters.

Compound	Precursor Ion	Product Ion	Ret Time (min)	Fragmentor (V)	Collision Energy (V)
11Cl-PF3OUdS	630.9	450.9	17.67	165	32
11Cl-PF3OUdS	630.9	82.9	17.67	165	32
$^{13}C_2$-4:2FTS	329.0	309.0	9.18	125	20
$^{13}C_2$-6:2FTS	429.0	409.0	13.06	125	24
$^{13}C_2$-8:2FTS	529.0	509.0	15.96	170	28
$^{13}C_2$-PFDoA	614.9	570.0	18.09	79	5
$^{13}C_2$-PFOA	415.0	369.9	13.17	80	8
$^{13}C_2$-PFOA	415.0	168.9	13.17	80	20
$^{13}C_3$-HFPO-DA	287.0	184.9	10.03	160	20
$^{13}C_3$-HFPO-DA	287.0	168.9	10.03	160	4
$^{13}C_3$-PFBA	216.0	171.9	4.91	65	8
$^{13}C_3$-PFBS	302.0	80.0	7.71	100	45
$^{13}C_3$-PFHxS	402.0	80.0	11.58	100	45
$^{13}C_4$-PFBA	217.0	172.0	4.91	60	8
$^{13}C_4$-PFHpA	367.0	322.0	11.40	72	0
$^{13}C_4$-PFOS	502.9	98.9	14.73	180	48
$^{13}C_4$-PFOS	502.9	79.9	14.73	180	52
$^{13}C_5$-PFHxA	318.0	273.0	9.37	70	8
$^{13}C_5$-PFPeA	268.0	223.0	7.24	60	8
$^{13}C_6$-PFDA	519.0	474.0	15.99	81	4
$^{13}C_7$-PFUnA	570.0	525.0	17.12	73	5
$^{13}C_8$-PFOA	421.0	376.0	13.16	69	4
$^{13}C_8$-PFOS	507.0	80.0	14.72	100	50
$^{13}C_9$-PFNA	472.0	427.0	14.68	66	4
4:2FTS	327.0	306.9	9.18	125	20

Table 1. Compound parameters, continued.

Compound	Precursor Ion	Product Ion	Ret Time (min)	Fragmentor (V)	Collision Energy (V)
4:2FTS	327.0	80.9	9.18	125	36
6:2FTS	427.0	406.8	13.06	125	24
6:2FTS	427.0	80.9	13.06	125	40
8:2FTS	527.0	506.8	15.96	170	28
8:2FTS	527.0	80.9	15.96	170	40
9Cl-PF3ONS	530.9	350.9	15.52	145	28
9Cl-PF3ONS	530.9	83.0	15.52	145	32
ADONA	377.0	250.9	11.72	80	12
ADONA	377.0	85.0	11.72	80	36
HFPO-DA-CO2	285.0	184.9	10.03	155	16
HFPO-DA-CO2	285.0	168.9	10.03	155	4
NFDHA	295.0	201.0	9.10	75	5
NFDHA-CO2	251.0	84.9	11.71	130	20
PFBA	213.0	168.9	4.91	60	8
PFBS	298.9	98.9	7.71	100	29
PFBS	298.9	80.0	7.71	100	45
PFDA	513.0	469.0	15.99	81	4
PFDA	513.0	218.7	15.99	100	16
PFDoA	613.0	569.0	18.09	79	5
PFDoA	613.0	268.7	18.09	100	20
PFEESA	314.9	134.9	8.57	110	24
PFEESA	314.9	69.0	8.57	110	60
PFHpA	362.9	319.0	11.40	72	0
PFHpA	362.9	169.0	11.40	72	12
PFHpS	448.9	98.7	13.26	100	44
PFHpS	448.9	79.7	13.26	100	52
PFHxA	313.0	268.9	9.37	70	8
PFHxA	313.0	119.0	9.37	70	18
PFHxS	398.9	99.0	11.59	100	45
PFHxS	398.9	80.0	11.59	100	49
PFMBA	279.0	84.9	7.91	70	12
PFMPA	229.0	84.9	5.95	60	12
PFNA	463.0	419.0	14.69	66	4
PFNA	463.0	219.0	14.69	66	17
PFOA	413.0	369.0	13.17	69	4
PFOA	413.0	169.0	13.17	69	12
PFOS	498.9	99.0	14.73	100	50
PFOS	498.9	80.0	14.73	100	50
PFPeA	263.0	218.9	7.24	60	8
PFPeS	348.9	98.9	9.69	135	40
PFPeS	348.9	79.9	9.69	135	40
PFUnA	563.0	519.0	17.12	73	5
PFUnA	563.0	269.0	17.12	100	20

Preparation of calibration standards

The high calibrator was prepared by dilution of a 500 ng/mL analyte dilution standard containing all analytes of interest. 1:4 serial dilutions of the high calibrator were prepared in 80% MeOH for subsequent calibrators. Isotope dilution standard and internal standard were added before analysis.

Extraction

Water samples, 250 mL, were fortified with isotope dilution analogues, and were extracted using weak anion exchange cartridges according to EPA Method 533. Samples were eluted with an ammonium hydroxide/MeOH solution and concentrated to dryness before reconstituting with 80% MeOH. Internal standard was added before analysis.

Chromatography

The chromatograms shown in Figure 1 are from a 1.5 ng/mL (in vial) calibrator separated by compound group.

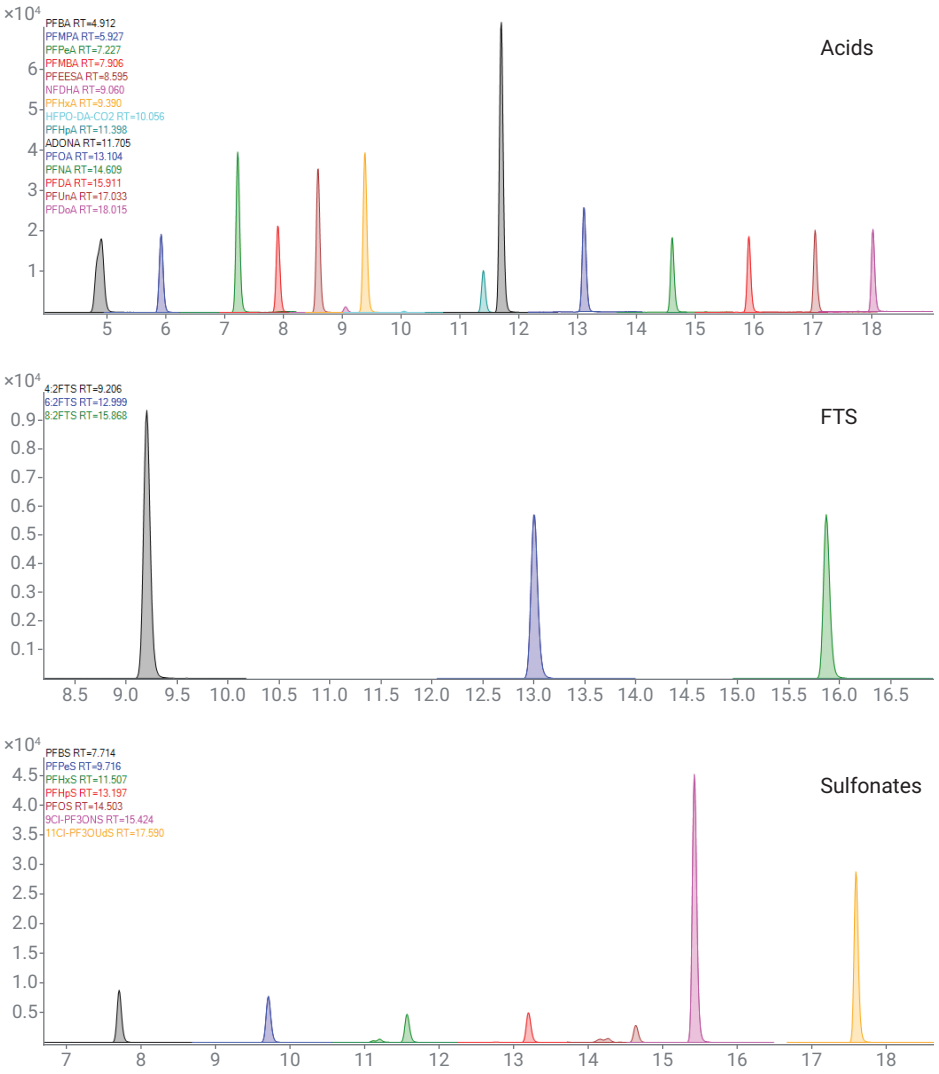

Figure 1. Chromatograms separated by compound group.

Performance: precision and accuracy

The precision and accuracy of the method was evaluated by fortifying five replicates of reagent and municipal-treated tap water samples at 48 ng/L.

Precision and accuracy were within EPA requirements. All PFAS compounds had recoveries between 87 and 103% in reagent and tap water, with a maximum of 5% RSD in reagent water and 12% RSD in tap water. Precision and accuracy were within EPA requirements as noted in USEPA method 533.

Table 2. Precision and accuracy of the method.

Compound	RT	Compound Group	Reagent Water Recovery	Reagent Water %RSD	Tap Water Recovery	Tap Water %RSD
PFBA	4.93	acid	98%	3%	97%	10%
PFMPA	5.95	acid	101%	2%	92%	10%
PFPeA	7.23	acid	98%	3%	94%	11%
PFMBA	7.91	acid	97%	3%	94%	11%
PFEESA	8.58	acid	99%	2%	96%	11%
NFDHA	9.04	acid	92%	5%	87%	11%
PFHxA	9.37	acid	97%	3%	93%	11%
HFPO-DA	10.03	acid	96%	5%	87%	11%
PFHpA	11.36	acid	99%	2%	94%	10%
ADONA	11.67	acid	103%	2%	96%	10%
PFOA	13.10	acid	97%	3%	94%	10%
PFNA	14.65	acid	99%	2%	94%	10%
PFDA	15.97	acid	98%	2%	94%	10%
PFUnA	17.10	acid	100%	3%	96%	11%
PFDoA	18.08	acid	99%	3%	96%	11%
4:2FTS	9.19	FTS	97%	2%	94%	11%
6:2FTS	13.00	FTS	99%	3%	96%	10%
8:2FTS	15.93	FTS	100%	3%	97%	11%
PFBS	7.70	sulfonate	98%	2%	94%	10%
PFPeS	9.69	sulfonate	97%	3%	95%	11%
PFHxS	11.54	sulfonate	99%	3%	96%	11%
PFHpS	13.20	sulfonate	102%	2%	97%	11%
PFOS	14.69	sulfonate	97%	3%	93%	11%
9Cl-PF3ONS	15.49	sulfonate	99%	2%	95%	11%
11Cl-PF3OUdS	17.66	sulfonate	98%	2%	92%	12%

Performance: low system background and LCMRL

Evidence of low system background is demonstrated by injecting a laboratory reagent blank (LRB) immediately following the high calibrator and evaluating the concentration of each analyte in the LRB. The LRB is an aliquot of reagent water fortified with the isotope dilution analogues and processed as a field sample. LRBs are used to determine if method analytes are introduced from the lab equipment, reagents, glassware, or extraction apparatus. Trace levels of a few PFAS were seen in the LRB, which could be due to contamination from sample preparation, etc. The instrument blanks that were 80% MeOH did not show any PFAS contamination, indicating that the LC/MS/MS was PFAS-free.

The lowest concentration minimum reporting limit (LCMRL) was determined by spiking progressively lower concentrations of the analytes in reagent water and extracting such that the recovery was still in the range of 50 to 150%.

Table 3. Low system background and LCMRL.

Compound	RT	Compound Group	Instrument Blank (ng/L)	LRB Concentration (ng/L)	LCMRL (ng/L)	EPA 533 LCMRL^
PFBA	4.93	acid	–	0.58	2.6	13
PFMPA	5.95	acid	–	0.01	0.087	3.8
PFPeA	7.23	acid	–	0.13	1.0	3.9
PFMBA	7.91	acid	–	0.01	0.12	3.7
PFEESA	8.58	acid	–	–	0.079	2.6
NFDHA	9.04	acid	–	–	0.16	16
PFHxA	9.37	acid	–	0.11	0.34	5.3
HFPO-DA	10.03	acid	–	–	1.8	3.7
PFHpA	11.36	acid	–	0.38	4.2* (1.3)	2.6
ADONA	11.67	acid	–	–	0.069	3.4
PFOA	13.10	acid	–	0.27	0.68	3.4
PFNA	14.65	acid	–	0.07	0.40	4.8
PFDA	15.97	acid	–	0.07	0.31	2.3
PFUnA	17.10	acid	–	0.05	0.31	2.7
PFDoA	18.08	acid	–	0.09	0.40	2.2
4:2FTS	9.19	FTS	–	0.04	0.17	4.7
6:2FTS	13.00	FTS	–	0.03	0.22	14
8:2FTS	15.93	FTS	–	0.03	0.18	9.1
PFBS	7.70	sulfonate	–	0.01	0.21	3.5
PFPeS	9.69	sulfonate	–	–	0.084	6.3
PFHxS	11.54	sulfonate	–	0.02	0.13	3.7
PFHpS	13.20	sulfonate	–	–	0.088	5.1
PFOS	14.69	sulfonate	–	0.15	0.47	4.4
9Cl-PF3ONS	15.49	sulfonate	–	–	0.11	1.4
11Cl-PF3OUdS	17.66	sulfonate	–	0.01	0.67	1.6

^ Single-lab LCMRLs determined during method development by EPA and listed in Table 7 of EPA method 533.

* One high outlier point was observed for PFHpA in one of the four spikes at 1.6 ng/L. All other compounds in those spikes had recoveries within the accuracy specifications. Because all compounds are spiked using a mixture containing all compounds, and LRB values were low in that batch, the high PFHpA result appears to be from the SPE used for that sample. Instrument sensitivity for PFHpA is much lower, as shown in Figure 2A. The LCMRL without the outlier is shown in the brackets.

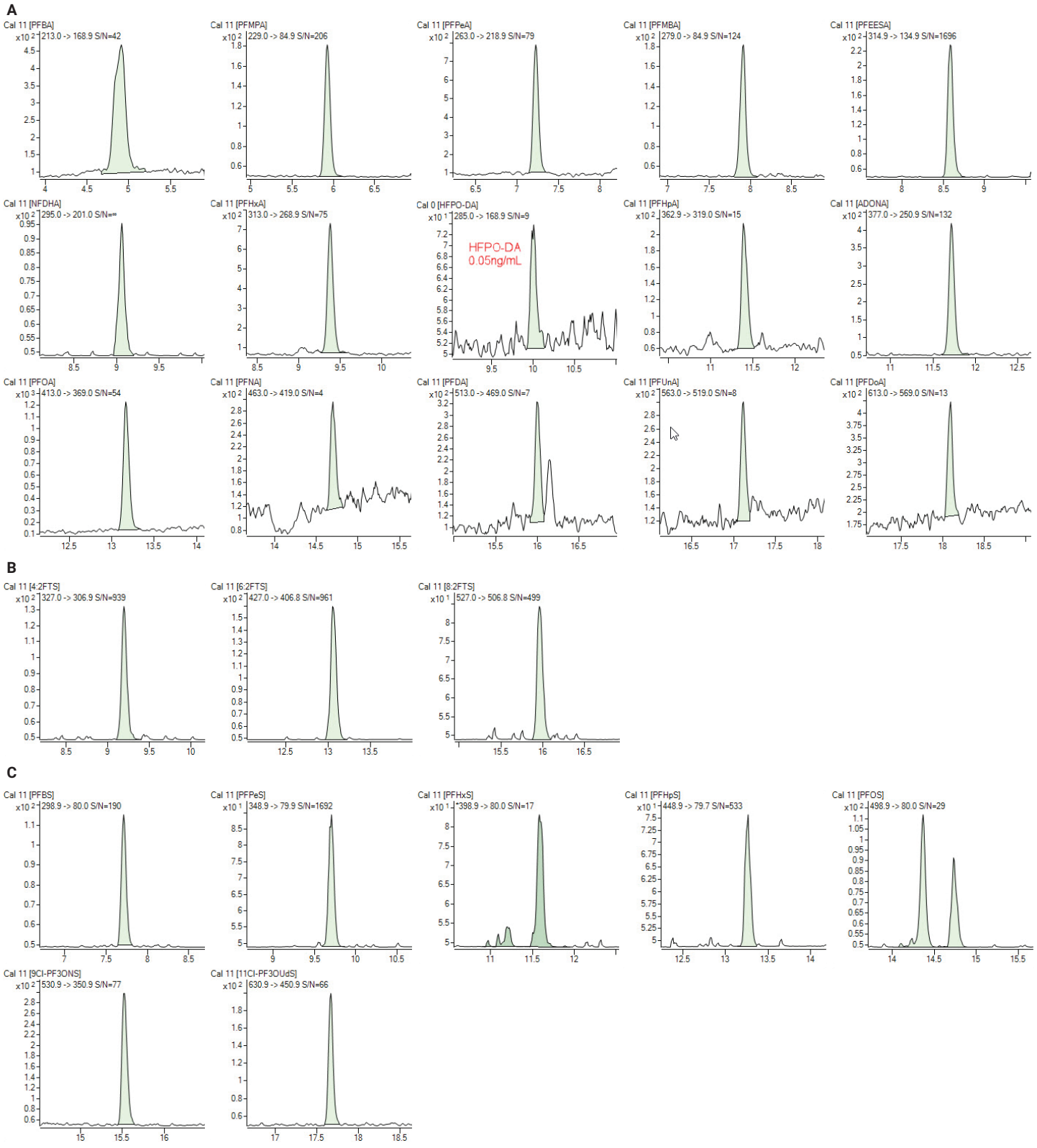

Figure 2. A, B, and C show chromatograms for acids, FTSs, and sulfonates, respectively, at the calibration concentration of 0.003 ng/mL in the vial (0.05 ng/mL for HFPO-DA).

Background chromatography

Double blanks do not contain internal standards (ISTDs), isotope dilution analogs (IDAs), or target compounds, nor do they get processed through sample extraction steps. They are simply solvent blanks to demonstrate background levels in the LC/MS/MS system. Blank++ injections are unextracted solvent blanks that contain both ISTDs and IDAs.

The chromatograms in Figure 3 are on the same scale, indicating the absence of PFAS in the LC/MS/MS system.

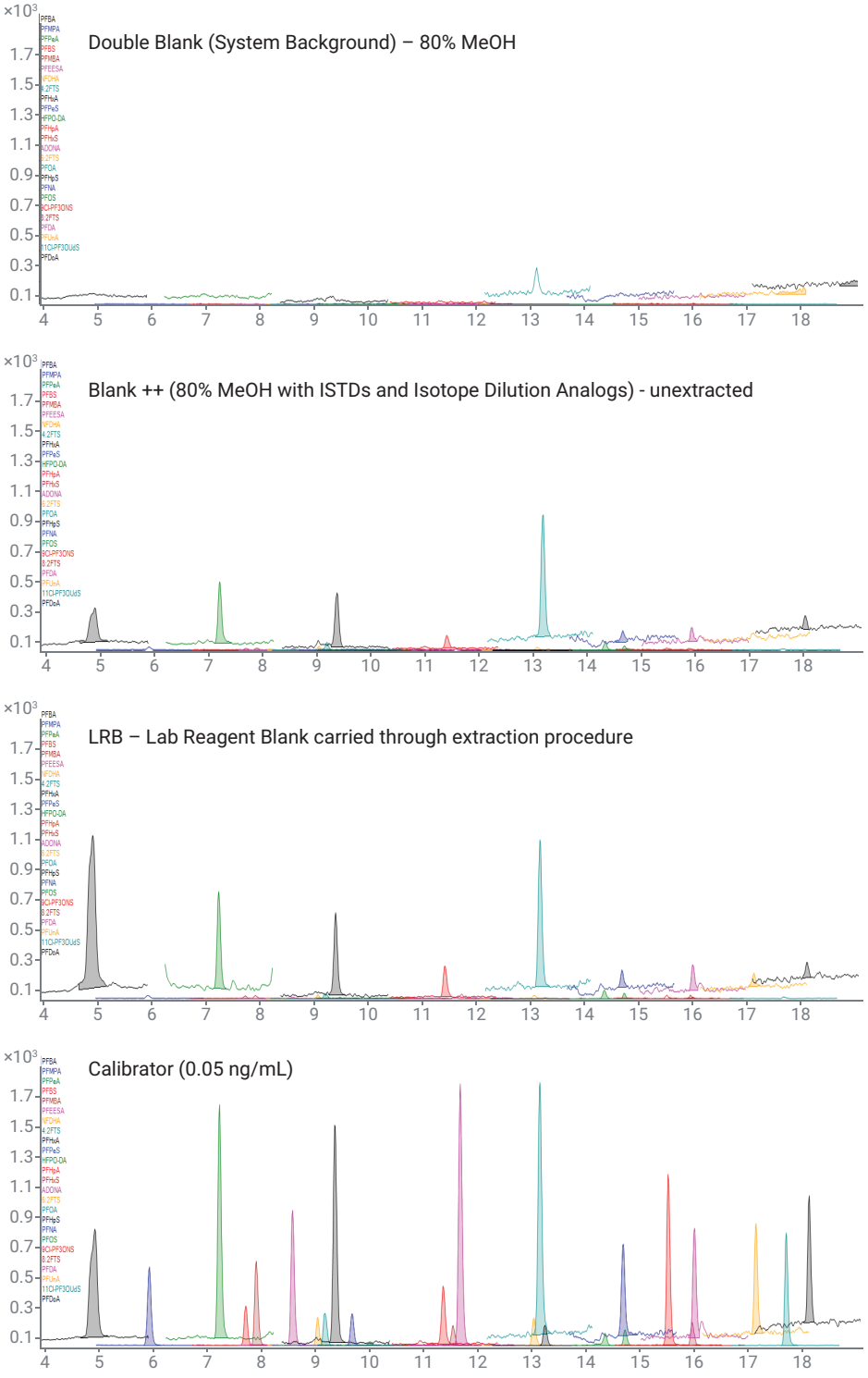

Figure 3. Background chromatography.

Conclusion

The EPA method allows flexibility in the choice of LC columns, LC conditions, and MS conditions. The Agilent ZORBAX Eclipse Plus C18 column used for this work provided baseline resolution for all compounds within each class, with minimal RT overlap among all compounds.

Precision and accuracy were within EPA requirements. Recoveries were between 87 and 103% for all PFAS compounds in reagent and tap water, with a maximum of 5% RSD in reagent water and 12% RSD in tap water. LCMRL values were all lower than EPA values listed in EPA 533 except for PFHpA, where determined LCMRL was slightly higher due to inclusion of an outlier in one of the four spikes that was attributed to the SPE cartridge for that sample. However, instrument sensitivity noted during testing was much higher than needed by the method. Of the 25 compounds in the method, 22 LCMRLs were determined to be at or below 1 ng/mL. The lowest LCMRL in the EPA method was 1.8 ng/L. This work has been used as a second-lab validation for the creation of EPA method 533, and data have been acknowledged in the official method.

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