Analysis of Per/Polyfluoroalkyl Substances in Water Using an Agilent 6470 Triple Quadrupole LC/MS

Application Note

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Introduction

The analysis of per/polyfluoroalkyl substances (PFASs) or perfluorinated compounds (PFCs), in particular the perfluorinated alkyl acids (PFAAs), is currently a hot topic in water analysis. The unique chemical properties of these compounds make them components used in a variety of applications such as nonstick cookware, fire resistant clothing, fire-fighting foams, and others. However, these compounds are considered toxic, persistent, and bioaccumulative in wildlife and the environment. Consequently, the United States Environmental Protection Agency (USEPA) has recently issued drinking water health advisories for two PFASs, perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) at 70 ng/L (combined). Several states such as New Jersey, New York, and North Carolina already have public health guideline values varying from 20–400 ng/L for several PFAS including PFOA, PFOS, perfluorohexanoic acid (PFHxA), and perfluorononanoic acid (PFNA) in water.

USEPA Method 537 highlights a method for the analysis of 14 PFASs in drinking water with solid phase extraction (SPE) and LC/MS/MS. However, several other classes of PFASs are also currently in use and need to be monitored in the environment.

This application note describes the analysis of 30 PFASs in eight different classes, including all 14 PFASs in EPA Method 537, using a single analytical method on an Agilent 6470 triple quadrupole LC/MS/MS.

Agilent Technologies

Materials and Conditions

EPA Method 537 currently analyzes for 14 compounds, and is in use by many labs. This application expands coverage to 30 compounds, and includes additional chemical classes.

The 16 compounds highlighted in green are newer PFASs that are potentially of concern and has thus resulted in interest for environmental monitoring.

These compounds include eight different PFAS classes: perfluoroalkyl acids (acid), fluorooctane sulfonamide (FOSA), fluorooctance sulfonamide acetic acid (FOSAA), fluorotelomer alcohol-ethanoic acid (FTA-e), fluorotelomer alcohol-propanoic acid (FTA-p), fluorotelomer sulfonate (FTS), fluorotelomer unstaturated acid (FTUA), and perfluoroalkyl sulfonate (sulfonates).

Target compounds

Compound	Acronym	Class	Fluorinated C chain	EPA 537 or Additional
Perfluorobutanoate	PFBA	Acid	C4	Additional
Perfluoropentanoate	PFPeA	Acid	C5	Additional
Perfluorohexanoate	PFHxA	Acid	C6	537
Perfluoroheptanoate	PFHpA	Acid	C7	537
Perfluorooctanoate	PFOA	Acid	C8	537
Perfluorononanoate	PFNA	Acid	C9	537
Perfluorodecanoate	PFDA	Acid	C10	537
Perfluoroundecanoate	PFUdA	Acid	C11	537
Perfluorododecanoate	PFDoA	Acid	C12	537
Perfluorotridecanoate	PFTrDA	Acid	C13	537
Perfluorotetradecanoate	PFTeDA	Acid	C14	537
Perfluorooctanesulfonamide	FOSA	FOSA	C8	Additional
N-Ethyl-N-((heptadecafluorooctyl)sulfonyl)glycine	N-EtFOSAA	FOSAA	C8	537
N-((Heptadecafluorooctyl)sulfonyl)-N-methylglycine	N-MeFOSAA	FOSAA	C8	537
2-Perfluorohexyl ethanoic acid	FHEA	FTA-e	C6	Additional
2-Perfluorooctyl ethanoic acid	FOEA	FTA-e	C8	Additional
2-Perfluorodecyl ethanoic acid	FDEA	FTA-e	C10	Additional
3-Perfluoroheptyl propanoic acid (FHpPA)	PFHpPA	FTA-p	C7	Additional
4:2 Fluorotelomer sulfonate	4-2 FTS	FTS	C4	Additional
6:2 Fluorotelomer sulfonate	6-2 FTS	FTS	C6	Additional
8:2 Fluorotelomer sulfonate	8-2 FTS	FTS	C8	Additional
2H-Perfluoro-2-octanoic acid (FHUEA)	6-2 FTUA	FTUA	C6	Additional
2H-Perfluoro-2-decanoic acid (FOUEA)	8-2 FTUA	FTUA	C8	Additional
Perfluorobutylsulfonate	PFBS	Sulfonate	C4	537
Perfluoropentylsulfonate	PFPeS	Sulfonate	C5	Additional
Perfluorohexylsulfonate	PFHxS	Sulfonate	C6	537
Perfluoroheptylsulfonate	PFHpS	Sulfonate	C7	Additional
Perfluorooctylsulfonate	PFOS	Sulfonate	C8	537
Perfluorononylsulfonate	PFNS	Sulfonate	C9	Additional
Perfluorodecylsulfonate	PFDS	Sulfonate	C10	Additional

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, 3.0 × 50 mm; 1.8 µm (p/n 959757-302)
Delay column	Agilent Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm (p/n 959943-902)
Column temperature	50 °C
Injection volume	5 uL
Mobile phase	A) 5 mM Ammonium acetate in water (LC grade) B) 5 mM Ammonium acetate in 95 % MeOH (LC grade)
Gradient flow rate	0.4 mL/min
Gradient	Time (min) %B 0.0 10 0.5 10 2.0 30 14.0 95 14.5 100
Stop time	16.5 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.

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	15 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Capillary voltage (Neg)	2,500 V
Nozzle voltage (Neg)	0 V
Acquisition	
Cycle time	500 ms
Total MRMs	72
Max concurrent MRMs	22
Min/Max dwell	21 ms/500 ms

Parameters were optimized with Agilent Source Optimizer software. All data were processed with Agilent MassHunter Quantitative software (B.07.01).

d-MRM Transitions

The method used dynamic multiple reaction monitoring (dMRM), and was run in electrospray negative mode using an Agilent 6470 LC/MS/MS (Table 1).

All compound parameters including precursor ion, product ion, fragmentor voltages, and collision energies were optimized for each compound with Agilent Optimizer Software. The cell accelerator voltage was selected as 2 for all compounds.

Preparation of calibration standards

The 50 µg/mL stock solutions of 12 individual compounds, as well as some mixes (PFAC-MXB and FTA-MXA) at 2 µg/mL were supplied by Wellington Labs.

- The 2 µg/mL mix of 12 individual PFASs was prepared by mixing 40 µL of each 50 µg/mL stock and 520 µL of ACN.
- The 100 ng/mL mix of 27 PFASAs, PFSAs, FTUAs, FTSs, and FTA-p was prepared by mixing 50 µL of each 2-µg/mL solution: PFAS-MXA (from Wellington), the 12 mixes (prepared above), and 900 µL of ACN.
- High Calibrator solution: 10 ng/mL (27 compounds) + 200 ng/mL (FTA-e's) was prepared by mixing:
    - 100 µL of 100 ng/mL 27-Mix
    - 100 µL of 2 µg/mL FTA-MXA
    - 800 µL of 96 % methanol
- 1:2 Serial dilutions of high calibrator in 96 % MeOH

Table 1. d-MRM Transitions for all Compounds Studied

Compound	Precursor ion	Product ion	RT (min)	Fragmentor	Collision energy
4-2 FTS	327.0	80.9	9.17	125	36
4-2 FTS	327.0	306.9	9.17	125	20
6-2 FTS	427.0	406.8	11.80	125	24
6-2 FTS	427.0	79.9	11.80	125	40
6-2 FTUA	357.0	292.9	11.02	70	12
6-2 FTUA	357.0	242.9	11.02	70	40
8-2 FTS	527.0	506.8	13.51	170	28
8-2 FTS	527.0	80.9	13.51	170	40
8-2 FTUA	457.0	392.9	13.00	85	12
8-2 FTUA	457.0	342.9	13.00	85	40
FDEA	577.0	493.0	14.44	140	10
FDEA	493.0	493.0	14.44	73	21
FHEA	377.0	313.0	11.06	140	6
FHEA	377.0	293.0	11.06	140	15
FOEA	477.0	413.0	13.03	140	10
FOEA	477.0	393.0	13.03	140	10
FOSA	497.9	77.9	14.07	125	36
FOSA	497.9	47.9	14.07	100	100
N-EtFOSAA	584.0	525.9	14.19	115	20
N-EtFOSAA	584.0	418.9	14.19	115	20
N-MeFOSAA	570.0	482.9	13.84	115	16
N-MeFOSAA	570.0	418.9	13.84	115	20
PFBA	213.0	168.9	3.50	60	8
PFBS	298.9	98.9	7.82	100	29
PFBS	298.9	80.0	7.82	100	45
PFDA	513.0	469.0	13.52	81	4
PFDA	513.0	218.7	13.52	100	16
PFDoA	613.0	569.0	14.73	79	5
PFDoA	613.0	268.7	14.73	100	20

Compound	Precursor ion	Product ion	Ret time (min)	Fragmentor	Collision energy
PFDS	598.9	99.0	14.14	100	60
PFDS	598.9	80.0	14.14	100	80
PFHpA	362.9	319.0	10.76	72	0
PFHpA	362.9	169.0	10.76	72	12
PFHpPA	441.0	336.9	12.99	90	8
PFHpPA	441.0	316.9	12.99	90	24
PFHpS	448.9	98.7	11.90	100	44
PFHpS	448.9	79.7	11.90	100	52
PFHxA	313.0	268.9	9.32	70	8
PFHxA	313.0	119.0	9.32	70	18
PFHxS	398.9	99.0	10.87	100	45
PFHxS	398.9	80.0	10.87	100	49
PFNA	463.0	419.0	12.75	66	4
PFNA	463.0	169.0	12.75	66	17
PFNS	548.9	98.9	13.50	165	40
PFNS	548.9	79.9	13.50	165	40
PFOA	413.0	369.0	11.85	69	4
PFOA	413.0	169.0	11.85	69	12
PFOS	498.9	99.0	12.76	100	50
PFOS	498.9	80.0	12.76	100	50
PFPeA	263.0	218.9	7.21	60	8
PFPeS	348.9	98.9	9.58	135	40
PFPeS	348.9	79.9	9.58	135	40
PFTeDA	713.0	669.0	15.64	100	9
PFTeDA	712.9	169.0	15.64	100	30
PFTrDA	663.0	619.0	15.21	91	9
PFTrDA	663.0	169.0	15.21	100	30
PFUdA	563.0	519.0	14.17	73	5
PFUdA	563.0	218.7	14.17	100	20

Instrument performance

The instrument performance was determined by running experiments to determine linearity, accuracy, precision, and instrument detection limits, presented in Table 2. The linearity of a calibration curve was evaluated using six points, evaluating for fit, ignoring the origin, and using 1/x weighting. The R^2 values ranged from 0.992 to 1.000, which satisfied EPA requirements (ASTM D7979-15) of R^2 >0.98 for all compounds (see Table 2).

- Linear range = 0.08 ng/L equivalent (0.1 pg on-column) to 2.5 ng/L equivalent (3.13 pg on-column)
- FTA-e's linear range = 12.5 ng/L equivalent (15.6 pg on-column) to 400 ng/L equivalent (500 pg on-column)
- **Accuracy:** The accuracy of each point included in the calibration curve ranged from 76 % to 120 %, meeting the ASTM D7979-15 requirement of 70–130 %.
- **IDL:** Instrument detection limit (IDL) was based on continued 2x dilutions of the linearity series to a low of 0.02 ng/L equivalent (0.025 pg on-column). The FTA-e low was 0.39 ng/L equivalent (0.49 pg on-column) with an S/N > 3.
- **EPA LOD:** Limit of detection (LOD) was the calculated pg on-column based on the EPA 537 injection volume of 10 µL and reported LODs.
- **Precision:** Repeat injections of 1.25 ng/L equivalent (0.313 ng/mL in vial – 1.6 pg on column). FTA-e at 25 ng/L equivalent (6.25 ng/mL in vial – 31 pg on-column). Precision is expressed as %RSD of accuracy.

Table 2. Instrument Performance

Name	Compound group	RT	R^2	6470 IDL (pg)	EPA LOD (pg on-column)	Precision at 1.6 pg on-column, FTA-e at 31 pg on-column (%)
PFBA	Acid	4.11	1.000	0.025		4
PFPeA	Acid	7.17	1.000	0.025		5
PFHxA	Acid	9.26	0.998	0.025	4.00	5
PFHpA	Acid	10.72	0.999	0.025	1.25	7
PFOA	Acid	11.83	0.997	0.200	4.25	3
PFNA	Acid	12.74	1.000	0.100	1.75	8
PFDA	Acid	13.51	0.999	0.100	1.75	6
PFUdA	Acid	14.16	0.997	0.200	7.00	6
PFDoA	Acid	14.73	0.996	0.200	2.75	10
PFTrDA	Acid	15.22	0.999	0.025	5.50	8
PFTeDA	Acid	15.65	0.999	0.050	4.25	6
FOSA	FOSA	14.08	1.000	0.025		8
N-MeFOSAA	FOSAA	13.85	0.992	0.100	16.25	4
N-EtFOSAA	FOSAA	14.18	0.999	0.050	10.50	7
FHEA	FTA-e	11.06	1.000	16.000		7
FOEA	FTA-e	13.04	0.999	8.000		9
FDEA	FTA-e	14.43	0.996	16.000		15
PFHpPA	FTA-p	12.98	1.000	0.200		4
4-2 FTS	FTS	9.12	0.998	0.200		7
6-2 FTS	FTS	11.78	0.996	0.200		9
8-2 FTS	FTS	13.50	0.994	0.400		14
6-2 FTUA	FTUA	10.99	0.999	0.025		5
8-2 FTUA	FTUA	12.99	0.999	0.025		8
PFBS	Sulfonate	7.77	1.000	0.025	7.75	4
PFPeS	Sulfonate	9.53	0.998	0.025		6
PFHxS	Sulfonate	10.83	0.999	0.025	5.00	4
PFHpS	Sulfonate	11.88	0.999	0.025		7
PFOS	Sulfonate	12.75	0.999	0.025	3.50	8
PFNS	Sulfonate	13.49	0.993	0.200		11
PFDS	Sulfonate	14.13	0.994	0.100		4

Results and Discussion

Chromatography

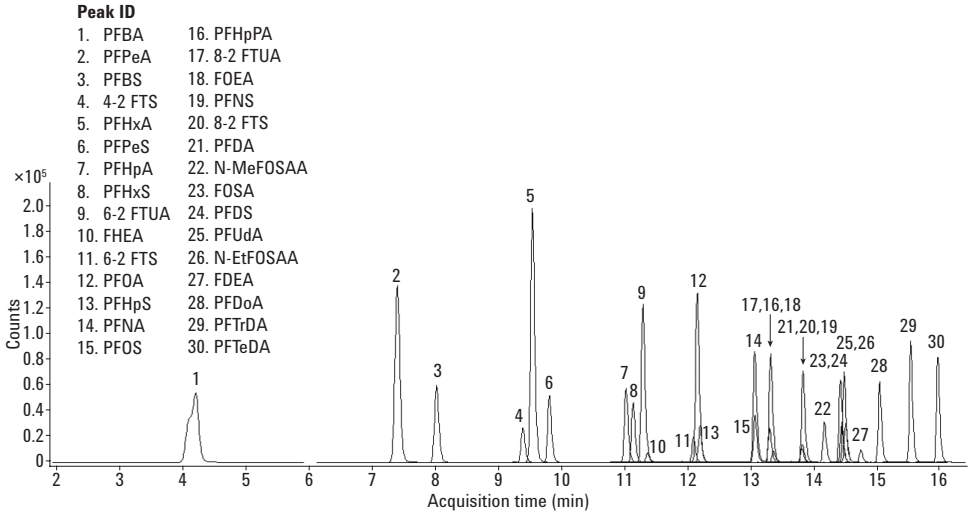


Figure 1. All compounds 20 ng/L equivalent (25 pg on-column), except FTA-e, at 400 ng/L equivalent (500 pg on-column).

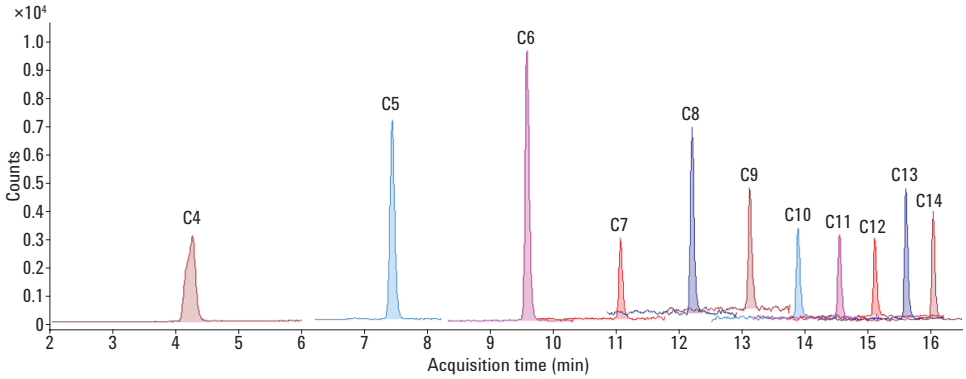


Figure 2. Acids at 1.25 ng/L equivalent (1.6 pg on-column).

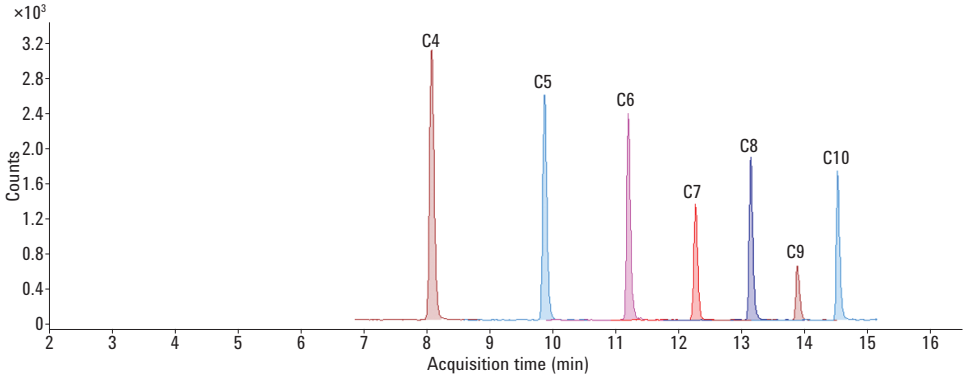


Figure 3. Sulfonates at 1.25 ng/L equiv (1.6 pg on-column).

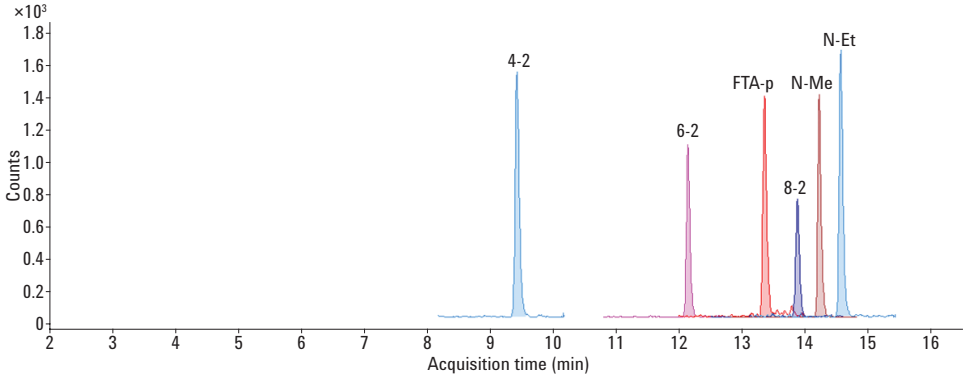


Figure 4 FTS's, FTA-p, and FOSAAs at 1.25 ng/L equivalent (1.6 pg on-column).

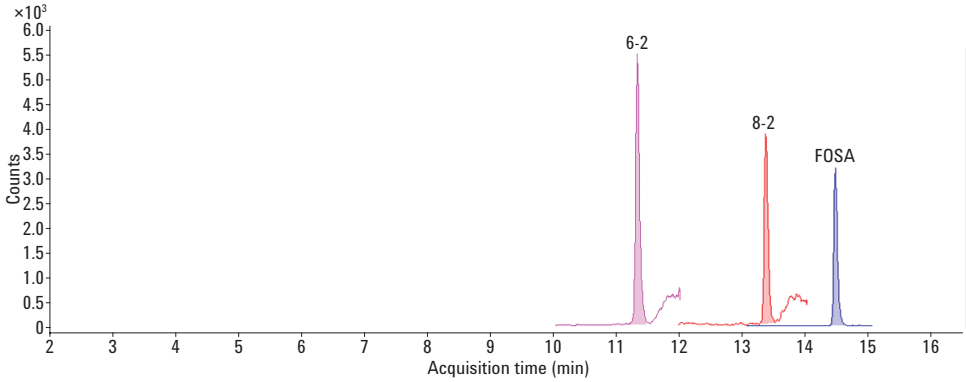


Figure 5. FTUAs and FOSA at 1.25 ng/L equivalent (1.6 pg on-column). The high baseline following peak elution is from delayed retention of system background.

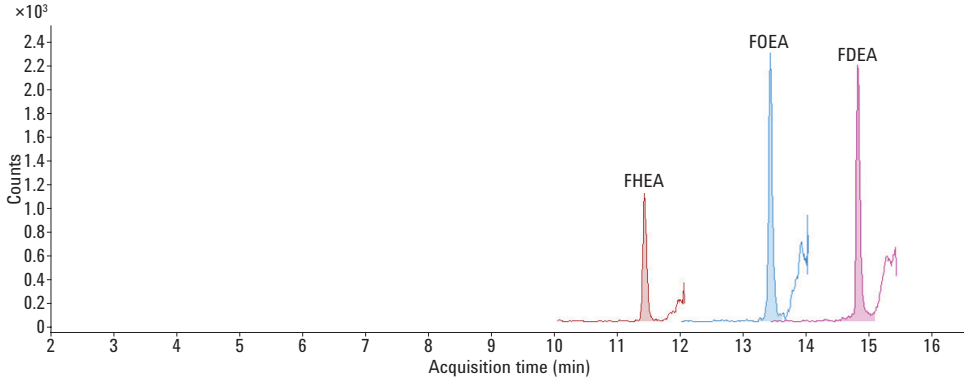
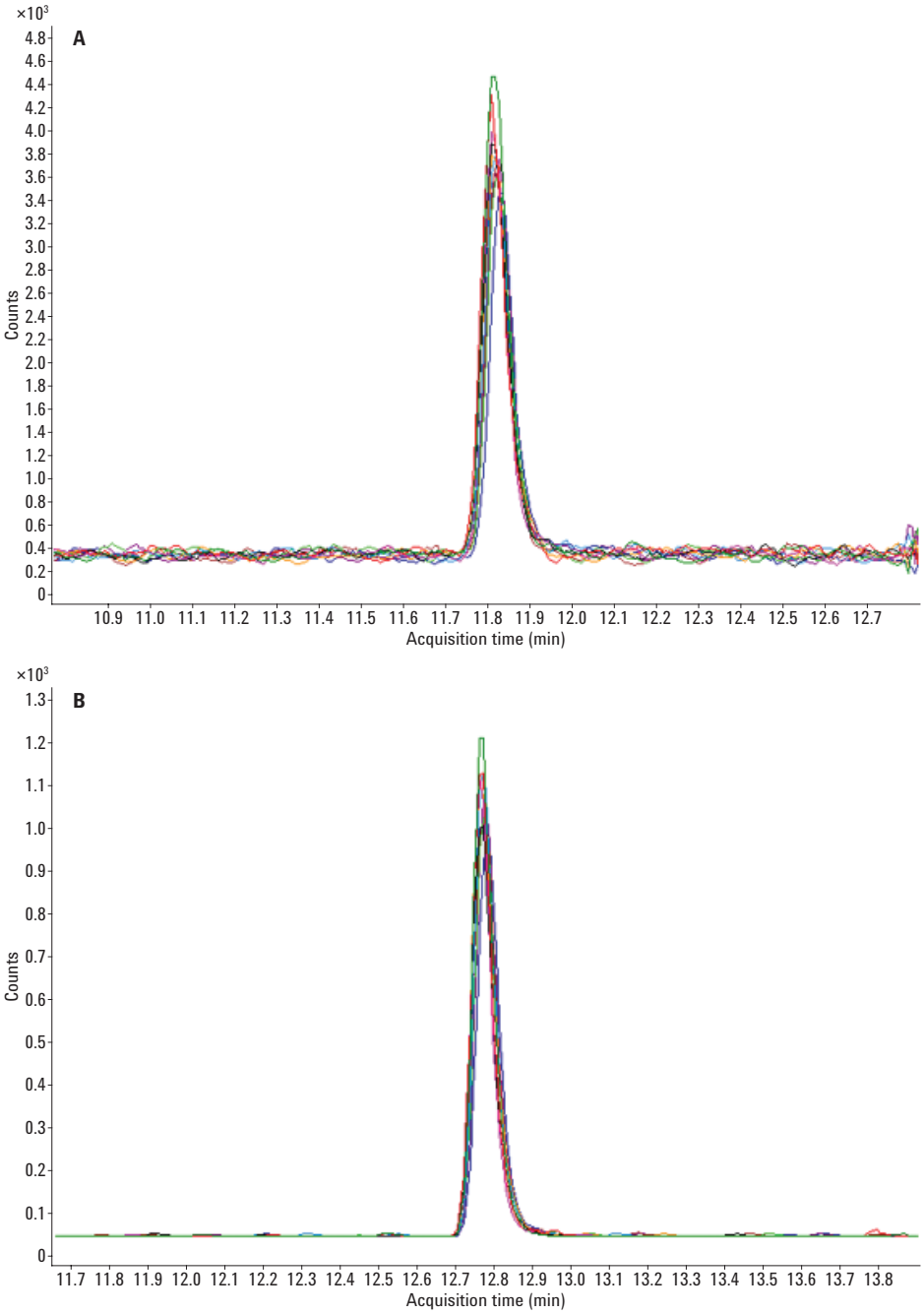


Figure 6. FTAs at 25 ng/L equivalent (31.3 pg on-column).

Instrument precision



0.78 pg on-column		
Injection	**PFOA**	**PFOS**
1	13,190	3,927
2	15,325	4,120
3	13,210	4,257
4	13,902	3,973
5	14,605	4,283
6	15,679	4,550
7	14,908	4,017
8	15,065	3,939
9	16,150	4,296
10	17,517	4,515
Average	**14,955**	**4,188**
RSD	**8 %**	**5 %**

Figure 7. Instrument precision at 0.63 ng/L equivalent (0.78 pg on-column).

Sensitivity

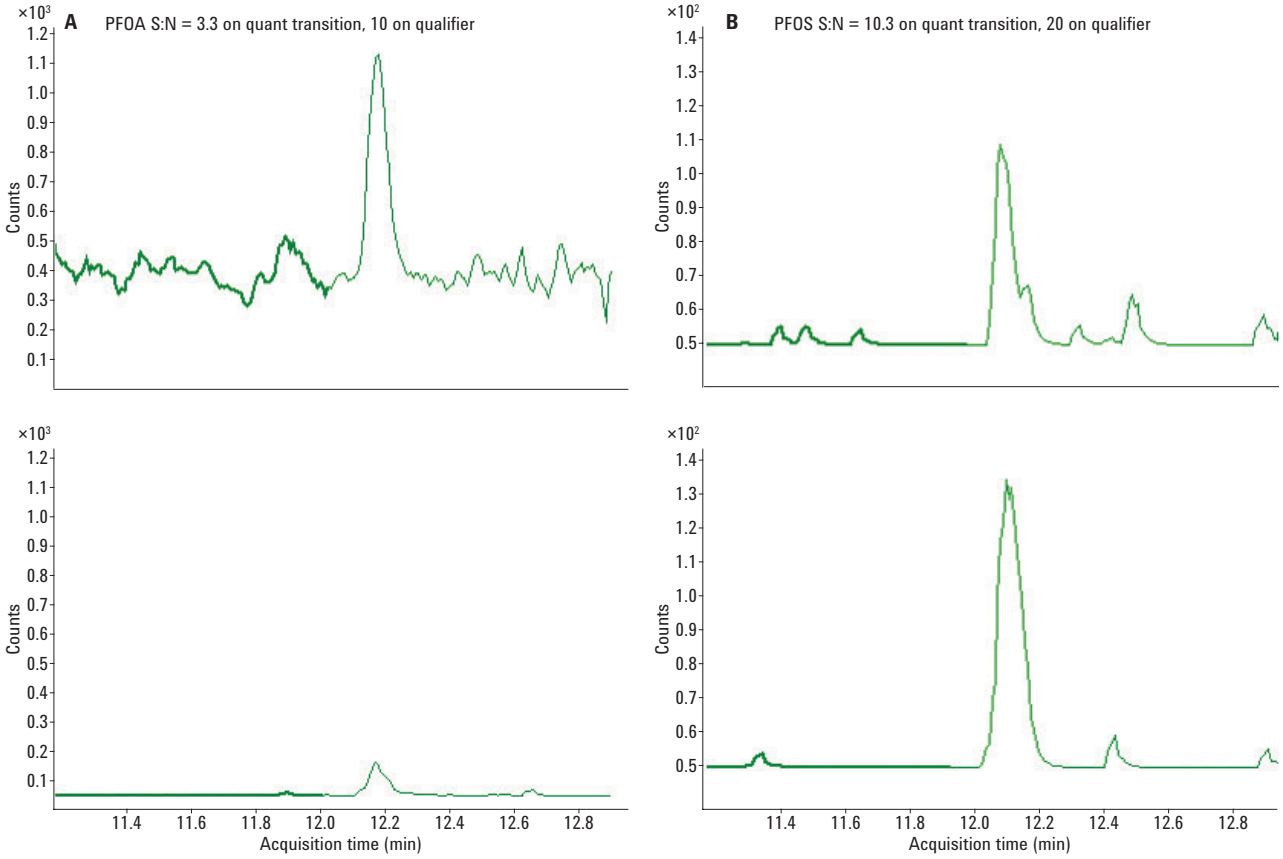


Figure 8. PFOA (A) and PFOS (B), 5-µL injection of 0.08 ng/L equivalent (0.02 ng/mL) = 100 fg on-column.

Background contamination

System contamination can be a major hurdle in PFAS analysis. For this work, a delay column (Agilent Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm) was installed after the mixing valve, and before the autosampler to trap PFASs in the pump system.

Another major source of contamination came from the PTFE septa, more specifically, pierced septa. To resolve this issue, and avoid possible problems with PFAS adherence to glass vials, polyethylene vials and caps were used.

For details on reduction in PFAS contamination caused by the instrument and guidance on eliminating potential sources of contamination, refer to Agilent publication 5991-7863EN [1].

Figure 9 shows the system contamination separated by the delay column for a selection of example compounds. Table 3 lists the compounds affected by background contamination.

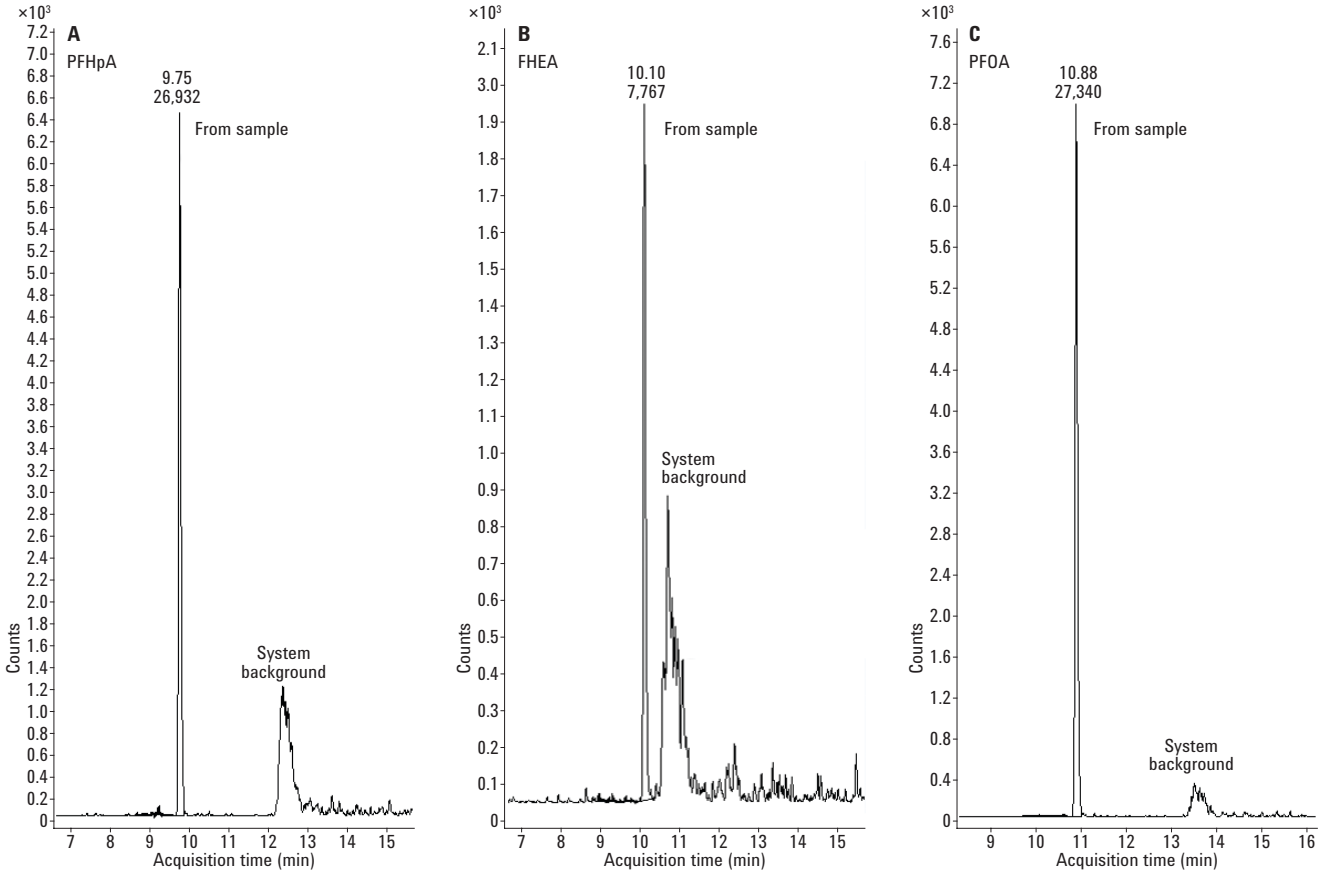


Figure 9. Background separated with delay column.

Table 3. Compounds Affected by Background Contamination

Compound	Acronym	Class	Fluorinated C chain	EPA 537 or extended	Background Contamination?	Source	Action	Separated or removed?
Perfluorobutanoate	PFBA	Acid	C4	Extended	Yes	Viariable contamination	Poly vials, caps, delay column	<Cal 1
Perfluoropentanoate	PFPeA	Acid	C5	Extended	Yes	PTFE Septa in caps and binary pump	Poly vials, caps, delay column	Yes
Perfluorohexanoate	PFHxA	Acid	C6	537	Yes	Binary pump + more	Delay column	Yes and <Cal 1
Perfluoroheptanoate	PFHpA	Acid	C7	537	Yes	Binary pump	Delay column	Yes
Perfluorooctanoate	PFOA	Acid	C8	537	Yes	Binary pump	Delay column	Yes
Perfluorononanoate	PFNA	Acid	C9	537	Yes	Binary pump	Delay column	Yes
Perfluorodecanoate	PFDA	Acid	C10	537	Yes	Binary pump	Delay column	Yes
Perfluoroundecanoate	PFUdA	Acid	C11	537	Yes	Binary pump	Delay column	Yes
Perfluorododecanoate	PFDoA	Acid	C12	537	Yes	Binary pump	Delay column	Yes
Perfluorotridecanoate	PFTrDA	Acid	C13	537	Yes	Binary pump	Delay column	Yes
Perfluorotetradecanoate	PFTeDA	Acid	C14	537	No			
Perfluorooctanesulfonamide	FOSA	FOSA	C8	Extended	No			
N-Ethyl-N-((heptadecafluorooctyl) sulfonyl)glycine	N-EtFOSAA	FOSAA	C8	537	No			
N-((Heptadecafluorooctyl) sulfonyl)-N-methylglycine	N-MeFOSAA	FOSAA	C8	537	No			
2-Perfluorodecyl ethanoic acid	FDEA	FTA-e	C10	Extended	Yes	Binary pump	Delay column	Yes
2-Perfluorohexyl ethanoic acid	FHEA	FTA-e	C7	Extended	Yes	Binary pump	Delay column	Yes
2-Perfluorooctyl ethanoic acid	FOEA	FTA-e	C8	Extended	Yes	Binary pump	Delay column	Yes
3-Perfluoroheptyl propanoic acid (FHpPA)	PFHpPA	FTA-p	C7	Extended	No			
4:2 Fluorotelomer sulfonate	4-2 FTS	FTS	C4	Extended	No			
6:2 Fluorotelomer sulfonate	6-2 FTS	FTS	C6	Extended	Yes	PTFE Septa in caps	Polyethylene vials and caps	Yes
8:2 Fluorotelomer sulfonate	8-2 FTS	FTS	C8	Extended	No			
2H-Perfluoro-2-octanoic acid (FHUEA)	6-2 FTUA	FTUA	C6	Extended	Yes	PTFE Septa in caps and binary pump	Poly vials, caps, delay column	Yes
2H-Perfluoro-2-decanoic acid (FOUEA)	8-2 FTUA	FTUA	C8	Extended	Yes	Binary pump	Delay column	Yes
Perfluorobutylsulfonate	PFBS	Sulfonate	C4	537	No			
Perfluoropentylsulfonate	PFPeS	Sulfonate	C5	Extended	No			
Perfluorohexylsulfonate	PFHxS	Sulfonate	C6	537	No			
Perfluoroheptylsulfonate	PFHpS	Sulfonate	C7	Extended	No			
Perfluorooctylsulfonate	PFOS	Sulfonate	C8	537	Yes	Solvents or carryover	Delay column	Yes
Perfluorononylsulfonate	PFNS	Sulfonate	C9	Extended	No			
Perfluorodecylsulfonate	PFDS	Sulfonate	C10	Extended	No			

Reference

1. T. Anumol, *et al.* Recommended Plumbing Configurations for Reduction in Per/Polyfluoroalkyl Substance Background with Agilent 1260/1290 Infinity (II) LC Systems, *Agilent Technologies Application Note*, publication number 5991-7863EN.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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