

Analysis of per/polyfluoroalkyl substances (PFASs) in drinking water using the Agilent Ultivo triple quadrupole LC/MS

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Abstract

Per/Polyfluoroalkyl substances (PFASs) are a class of compounds of growing environmental concern. The United States Environmental Protection Agency (US EPA) has issued drinking water health advisories for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) at 70 ng/L combined. Several states have issued lower public health guideline values for certain PFASs. This Application Note describes the analysis of 17 PFASs, including the 14 PFASs listed in US EPA Method 537, using a method developed for the Agilent Ultivo triple quadrupole LC/MS. The method, including extraction, was performed in accordance with US EPA Method 537. All analytes were detected at levels lower than specified by the US EPA Method, in a shorter analytical run time, and with half the injection volume.

Introduction

Per- and polyfluoroalkyl substances (PFASs) are man-made compounds widely used as surfactants, fire retardants, waterproofing, and nonstick and nonstain agents. Their unique properties make them persistent and ubiquitous in the environment and in animals. Research suggests that PFASs can cause reproductive and developmental problems, adverse effects on the liver, kidney, the immune system, and changes in cholesterol. Studies have shown that PFASs with carbon chains longer than seven carry the most risk for bioaccumulation¹.

Consequently, the United States Environmental Protection Agency (US EPA) has issued drinking water health advisories for two PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), at 70 ng/L combined. Several states, including New Jersey and Minnesota, have lower public health guideline values, varying from 14–35 ng/L for certain PFASs including PFOA and PFOS in water. US EPA Method 537 describes a method for the analysis of 14 PFASs in drinking water using solid phase extraction (SPE) and LC/MS/MS². However, several other PFASs are currently in use, and warrant monitoring.

This Application Note describes the analysis of 17 PFASs, including the 14 PFASs listed in US EPA Method 537 (Table 1), using a method developed on the Agilent Ultivo triple quadrupole LC/MS. All analytes were detected at levels substantially lower than those specified in the US EPA Method, in less than 22 minutes.

The Ultivo is unbelievably powerful and remarkably small. Its Triad Technologies (T³): Vortex Collision Cell, Cyclone Ion Guide, and VacShield innovations provide the sensitivity, robustness, reliability, and performance required for the challenges of high-throughput sample analysis. The Ultivo is also the first stackable Agilent triple quadrupole LC/MS, incorporating the mass spectrometer into the liquid chromatograph stack, reducing its footprint.

Experimental

Solutions and standards preparation

All PFAS standards were procured from Wellington Laboratories (Ontario, Canada) as solutions ranging 2–50 mg/L in acetonitrile or methanol. The standards were diluted in 96:4 (v/v) methanol:water to obtain the concentrations required for the calibration standards. LC/MS grade methanol and acetonitrile were purchased from Honeywell (Muskegon, MI), while ammonium acetate was obtained from Sigma-Aldrich (St. Louis, MO). Ultrapure water was sourced from a Millipore system with 18.2 MΩ purity.

Sample preparation

In accordance with US EPA Method 537, the spiked water samples and real drinking water samples (all 250 mL) were extracted using Agilent SampliQ Weak Anion Exchange (WAX) cartridges (p/n 5982-3667). Extraction conditions were similar to those described in US EPA Method 537. The extract was concentrated to dryness with nitrogen in a heated water bath, then adjusted to a 1 mL volume with 96:4 (v/v) methanol:water. Internal standards (IS) were then added.

LC/MS/MS analysis

An Agilent 1290 Infinity II high speed pump coupled to an Agilent Ultivo triple quadrupole LC/MS was used for LC/MS/MS analysis.

Because PFASs are routinely used in manufacturing processes, it is possible to have LC/MS/MS system contamination caused by solvents, tubing, fittings, filters, and other parts used in the routine operation of a liquid chromatograph. Fluoropolymers are used in all major (U)HPLC systems, and can be a potential source of PFASs contamination during LC/MS/MS analysis. To trap PFASs in the pump system, 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 avoid possible problems with PFASs adherence to glass vials, 1.0 mL crimp-top polypropylene vials (p/n 5182-0567, 100/pk) and snap-caps made with polypropylene (p/n 5182-0542, 100/pk) were used.

For more information on potential sources of PFASs contamination in LC systems and solutions to significantly reduce PFASs background interferences from Agilent 1260 Infinity II or Agilent 1290 Infinity II systems, see Agilent publication 5991-7863EN³.

Table 1. PFASs and internal standards (IS) analyzed, with corresponding triple quadrupole dMRM acquisition parameters. Compounds not in the scope of the US EPA Method 537 are highlighted in blue.

Compound	Acronym	Precursor ion (m/z)	Product ion (m/z)	RT (min)	Fragmentor (V)	Collision energy
Perfluorotetradecanoate	PFTeDA	712.9	669	14.8	100	7
Perfluorotetradecanoate	PFTeDA	712.9	169	14.8	100	23
Perfluorotridecanoate	PFTrDA	663	619	14.3	91	7
Perfluorotridecanoate	PFTrDA	663	169	14.3	100	23
Perfluorododecanoate	PFD _o A- ¹³ C ₂	614.9	570	13.9	79	4
Perfluorododecanoate	PFD _o A	613	569	13.9	79	4
Perfluorododecanoate	PFD _o A	613	268.7	13.9	100	15
Perfluorodecylsulfonate	PFDS	598.9	99	12.2	100	45
Perfluorodecylsulfonate	PFDS	598.9	80	12.2	100	60
N-Ethyl-N-((heptadecafluorooctyl)sulfonyl)glycine	N-EtFOSAA	584	525.9	13.3	115	15
N-Ethyl-N-((heptadecafluorooctyl)sulfonyl)glycine	N-EtFOSAA	584	418.9	13.3	115	15
N-((Heptadecafluorooctyl)sulfonyl)-N-methylglycine	N-MeFOSAA	570	482.9	13.0	115	12
N-((Heptadecafluorooctyl)sulfonyl)-N-methylglycine	N-MeFOSAA	570	418.9	13.0	115	15
Perfluoroundecanoate	PFUdA- ¹³ C ₂	565	520	13.3	73	4
Perfluoroundecanoate	PFUdA	563	519	13.3	73	4
Perfluoroundecanoate	PFUdA	563	218.7	13.3	100	15
Perfluorooctanoate	PFDA- ¹³ C ₂	514.9	469.9	12.7	81	3
Perfluorooctanoate	PFDA	513	469	12.7	81	3
Perfluorooctanoate	PFDA	513	218.7	12.7	100	12
Perfluorooctylsulfonate	PFOS- ¹³ C ₄	502.9	99	11.9	100	38
Perfluorooctylsulfonate	PFOS- ¹³ C ₄	502.9	80	11.9	100	38
Perfluorooctylsulfonate	PFOS	498.9	99	11.9	100	38
Perfluorooctylsulfonate	PFOS	498.9	80	11.9	100	38
Perfluorononanoate	PFNA- ¹³ C ₅	468	423	12.75	66	3
Perfluorononanoate	PFNA	463	419	11.9	66	3
Perfluorononanoate	PFNA	463	169	11.9	66	13
Perfluorooctanoate	PFOA- ¹³ C ₄	416.9	371.9	11.0	69	3
Perfluorooctanoate	PFOA	413	369	11.0	69	3
Perfluorooctanoate	PFOA	413	169	11.0	69	9
Perfluorohexylsulfonate	PFHxS- ¹⁸ O ₂	403	103	10.0	100	34
Perfluorohexylsulfonate	PFHxS	398.9	99	10.0	100	34
Perfluorohexylsulfonate	PFHxS	398.9	80	10.0	100	37
Perfluoroheptanoate	PFHpA	362.9	319	9.9	72	0
Perfluoroheptanoate	PFHpA	362.9	169	9.9	72	9
Perfluorohexanoate	PFHxA- ¹³ C ₂	315	270	8.5	70	6
Perfluorohexanoate	PFHxA	313	268.9	8.5	70	6
Perfluorohexanoate	PFHxA	313	119	8.5	70	14
Perfluorobutylsulfonate	PFBS	298.9	98.9	7.0	100	22
Perfluorobutylsulfonate	PFBS	298.9	80	7.0	100	34
Perfluoropentanoate	PFPeA	263	218.9	6.5	60	6
Perfluorobutanoate	PFBA- ¹³ C ₄	217	172	3.9	60	6
Perfluorobutanoate	PFBA	213	168.9	3.9	60	6

Table 2 provides the LC conditions. Due to the enhanced sensitivity of the Ultivo, 5 μ L, as opposed to 10 μ L, sample extracts, as described in US EPA Method 537, were directly injected into the LC system.

Table 3 provides the Agilent Jet Stream (AJS) ionization source parameters and MS conditions. The compounds were analyzed using dynamic multiple reaction monitoring (dMRM). Table 1 provides the triple quadrupole dMRM acquisition parameters for each PFAS compound monitored. All parameters, including precursor ion, product ion, fragmentor voltages, and collision energies were optimized for each analyte. The retention time window monitored was 2 minutes, and the Agilent Ultivo triple quadrupole LC/MS cell accelerator voltage (CAV) was set to 9 V for all analytes.

Agilent MassHunter Acquisition Software C.01 was used for instrument control and data acquisition. Agilent MassHunter Quantitative Analysis Software (B.09) with Quant-My-Way capability was used for data processing and analysis.

Calibration curve linearity was evaluated over the course of 1 month. Relative standard deviation (%RSD) of responses for 11 continuous calibration verification (CCV) samples at the 1 ppb level was calculated for a batch analyzed over 26 hours.

Table 2. LC conditions.

Parameter	Value												
Liquid chromatograph system	Agilent 1290 Infinity II high speed pump (G7120A) Agilent 1290 Infinity II multisampler with cooler (G7167B) Agilent 1290 Infinity II multicolumn thermostat (G7116B)												
Delay column	Agilent Eclipse Plus C18, 4.6 \times 50 mm, 3.5 μ m (p/n 959943-902)												
Analytical column	Agilent ZORBAX Eclipse Plus C18, 3.0 \times 50 mm, 1.8 μ m (p/n 959757-302)												
Injection volume	5 μ L												
Column temperature	50 $^{\circ}$ C												
Mobile phase	A) 5 mM ammonium acetate in water B) 5 mM ammonium acetate in 95 % MeOH												
Flow rate	0.4 mL/min												
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>10</td> </tr> <tr> <td>0.5</td> <td>10</td> </tr> <tr> <td>2.0</td> <td>30</td> </tr> <tr> <td>14.0</td> <td>95</td> </tr> <tr> <td>14.5</td> <td>100</td> </tr> </tbody> </table>	Time (min)	%B	0.0	10	0.5	10	2.0	30	14.0	95	14.5	100
Time (min)	%B												
0.0	10												
0.5	10												
2.0	30												
14.0	95												
14.5	100												
Run time	16.5 minutes												
Post time	5.5 minutes												

Table 3. AJS ESI source parameters and MS conditions.

Parameter	Value
Mass spectrometer	Agilent Ultivo triple quadrupole LC/MS with Agilent Jet Stream ESI source
Ionization mode	Negative
Gas temperature	230 $^{\circ}$ C
Gas flow	5 L/min
Nebulizer	15 psi
Capillary	2,500 V
Sheath gas temperature	350 $^{\circ}$ C
Sheath gas flow	12 L/min
Nozzle voltage	0 V

Results and discussion

Chromatographic performance

US EPA Method 537 describes a 35 to 37-minute analytical run time. The MRM chromatogram of the 1 ppb standard solution (Figure 1) shows that good chromatographic peak shapes and signal-to-noise ratio (S/N) can be obtained in 16.5 minutes. This time is less than half the time described in the US EPA method.

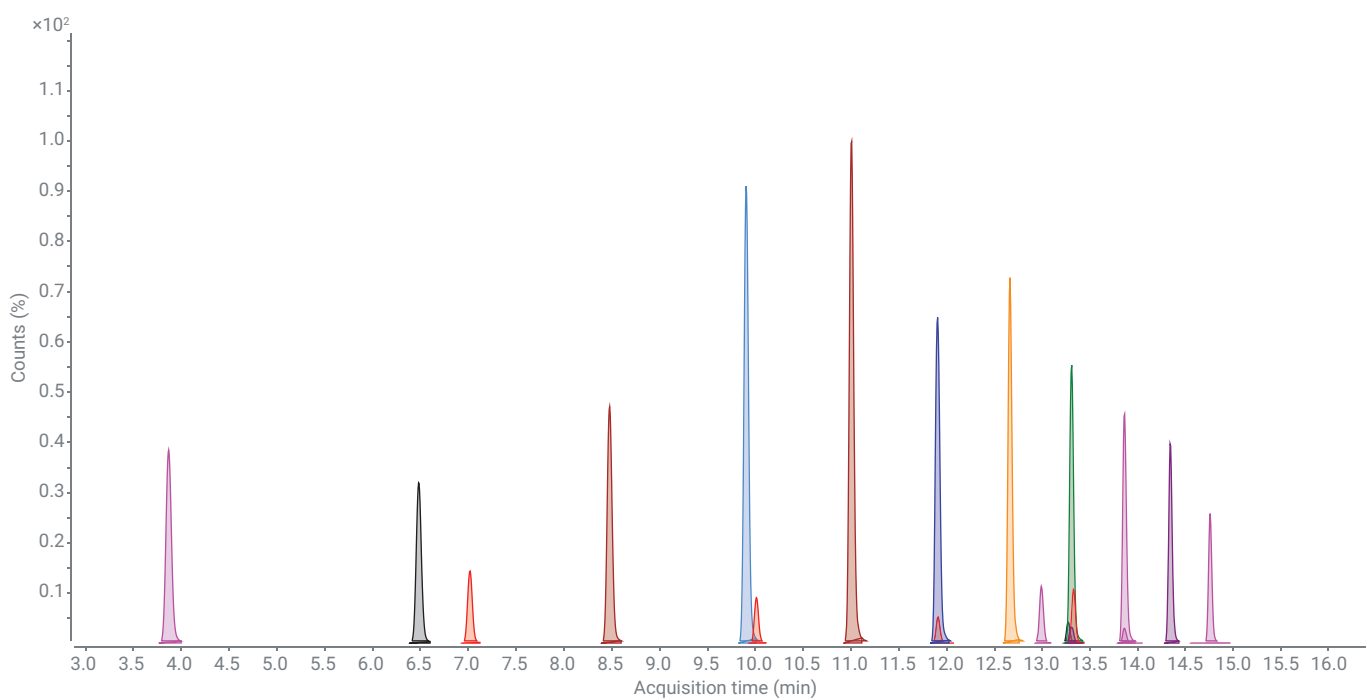


Figure 1. MRM Chromatogram of the 1 ppb standard solution for the 17 PFASs analyzed.

Linearity and reproducibility of response (%RSD)

Linear calibration curves with $R^2 > 0.99$ were obtained for all 17 PFASs analyzed (Table 4). The R^2 calculation was based on the average of three, eight-point calibration curves from 0.2 to 100 ppb (0.2, 0.5, 1, 5, 10, 25, 50, and 100 ppb) run over the course of 1 month. Linearity over the month tested was highly stable.

US EPA Method 537 requires injection and analysis of the calibration sample after every 10 samples. The %RSD response for the 11 CCV samples at the 1 ppb level were calculated for a batch spanning 26 hours. All analytes except one (N-EtFOSAA) were below 5 %RSD (Table 4). Figure 2 shows that the relative response, uncorrected by ISs, was stable across the 11 CCV samples analyzed over 26 hours.

Table 4. Linearity (R^2) and %RSD for continuous calibration verification (CCV) samples at 1 ppb over 26 hours.

Compound	R^2	CCV RSD (%)
PFBA	0.9965	2.1
PFPeA	0.9996	2.7
PFBS	0.9964	3.1
PFHxA	0.9988	3.0
PFHpA	0.9985	3.4
PFHxS	0.9995	1.7
PFOA	0.9971	1.2
PFOS	0.9991	1.9
PFNA	0.9811	3.7
PFDA	0.9941	3.6
N-MeFOSAA	0.9999	3.6
PFDS	0.9999	2.3
PFUdA	0.9940	2.3
N-EtFOSAA	0.9996	5.2
PFDoA	0.9974	3.6
PFTTrDA	0.9984	3.5
PFTeDA	0.9986	3.2

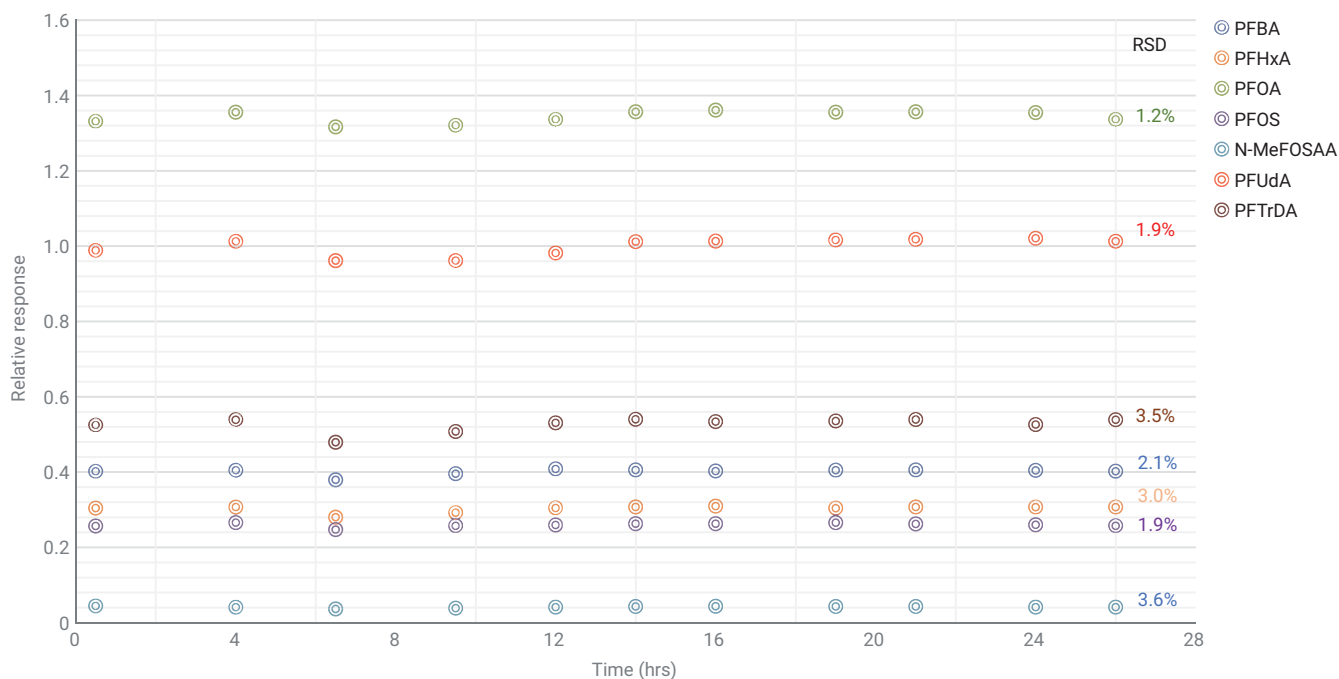


Figure 2. Relative response (uncorrected by ISs) for seven of the PFASs in the 11 CCV samples over 26 hours.

Analysis of real water samples

Drinking water samples from California, USA were analyzed for the 17 PFASs using this method. Figure 3 shows that PFBS, PFOS, PFOA, and PFNA were detected at 10, 4, 0.6, and 8, ng/L, respectively, in one of these samples. The Ultivo triple quadrupole LC/MS was

able to detect the presence of PFASs at low ng/L levels after US EPA Method 537 sample extraction and below the most stringent US state requirements. This demonstrates that the method provides good sensitivity and robustness for the analysis of real water samples.

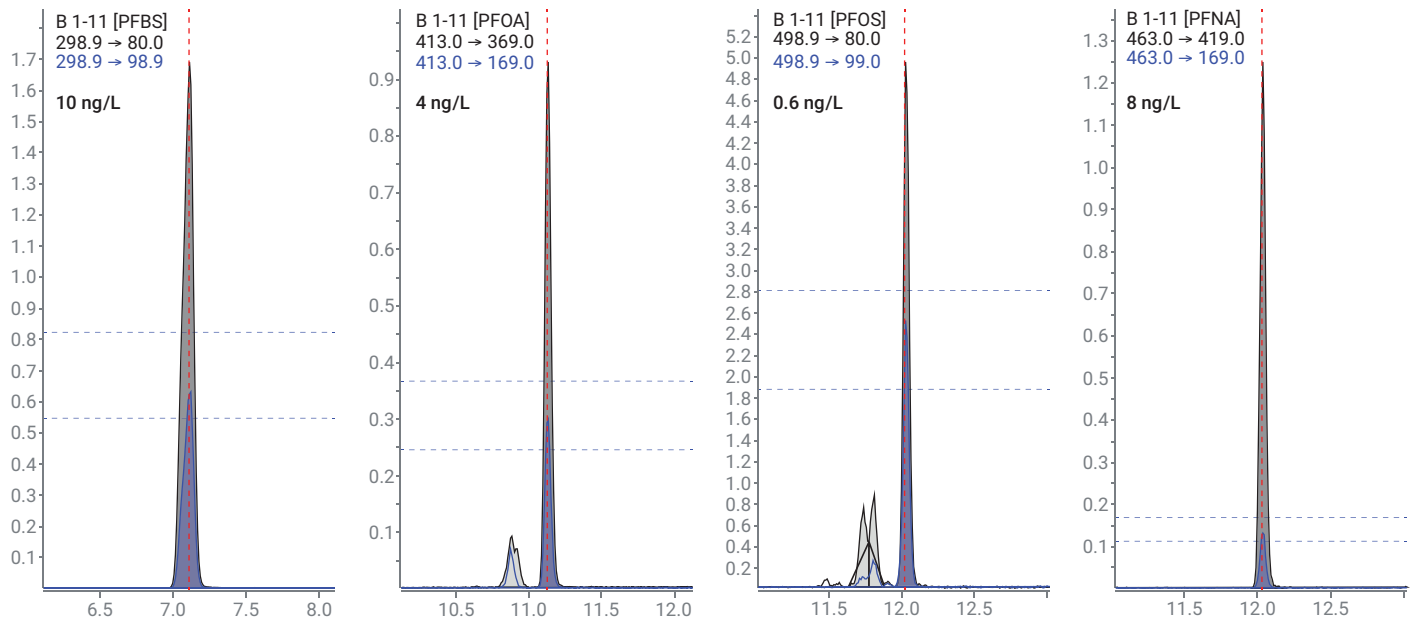


Figure 3. Four PFASs detected at low ng/L levels in a real water sample.

Conclusion

This Application Note presents the analysis of 17 PFASs, including the 14 PFASs listed in US EPA Method 537, using an Agilent Ultivo triple quadrupole LC/MS. All PFASs were detected at levels substantially lower, and with greater speed and lower injection volumes, than specified in the US EPA Method.

The Ultivo triple quadrupole LC/MS provided sensitive, reliable, and robust quantification of PFASs in water. Analysis of the PFASs at 1 ppb yielded ideal chromatographic peak shapes and good S/N. Response for the PFASs at eight concentration levels (0.2 to 100 ppb) proved stable over the course of 1 month with an R^2 greater than 0.99. Analysis of CCV samples per EPA Method 537 yielded %RSDs of response below 5 % for all but one analyte across a batch spanning 26 hours.

References

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