Application Note

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Analysis of Per/Polyfluoroalkyl Substances (PFAS) in Drinking Water by EPA 537.1 and EPA 533 Using the Agilent Ultivo Triple Quadrupole LC/MS

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Abstract

This application note highlights the validation of EPA methods 537.1 and 533 for per/polyfluoroalkyl substances (PFAS) in drinking water in a commercial lab setting. The analytes were all separated using Agilent Poroshell EC-120 columns in both methods. Peak asymmetry factors were within EPA guidelines while achieving a run time of less than 10 minutes, allowing significant throughput gains over the EPA method run times. Recovery of all analytes following the solid-phase extraction of 250 mL samples was between 70 to 130% following protocols outlined in EPA methods 537.1 and 533. The method reporting levels were calculated according to EPA guidelines. Levels were lower than 2 ng/L and below those listed in the EPA method single lab validation despite using lower injection volumes than stated.

Introduction

PFASs have unique chemical properties. The use of these chemicals in products in daily life has become convenient and essential. PFASs have been used in commercial, industrial, and personal-care products since the 1940s, and their presence in the environment is ubiquitous.[1] They are persistent, bioaccumulative, and toxic.[2] Hence, regulatory and government agencies such as the US Environmental Protection Agency (EPA), European Chemical Agency (ECHA), and others are looking to limit their presence in the environment.

In the US, currently there are two standard methods used to monitor and quantify PFASs in drinking water. EPA 537.1 analyzes 18 PFAS compounds using solid phase extraction (SPE) followed by liquid chromatography (LC) coupled to tandem mass spectrometry (LC/MS/MS) for low ng/L detection in drinking water. In 2019, the US EPA released a new method, EPA 533, to analyze 25 PFAS compounds. The method included analysis of shorter chain (C-chain <6) and some newer or emerging PFASs in drinking water, using SPE and LC/MS/MS. This application note provides second-lab validation data for both these methods using SPE and an Agilent 1290 Infinity II LC coupled to an Agilent Ultivo tandem quadrupole MS system to match and exceed EPA method requirements.

Experimental

Sample preparation

A set of 250 mL samples of drinking water were treated with antimicrobial and chlorine quenching agents as described in EPA methods 537.1 and EPA 533. They were spiked with isotopically labeled surrogate standards. The samples were then extracted using SPE following the protocol described in the respective EPA methods. The extracts were evaporated to a final volume of 1 mL, spiked with internal standards, and stored for LC/MS analysis. For EPA method 537.1, final 1 mL extracts were in 96/4 methanol/water, while EPA 533 extracts were in 80/20 methanol/water for better recoveries of the shorter chain PFASs. Details of the sample preparation protocol along with optimized recoveries and expected reproducibility and accuracy can be found in other Agilent application notes.[3,4]

Standard preparation

The standard stock was diluted appropriately to obtain a calibration solution of the following concentrations: 30, 20, 10, 5, 1, and 0.5 ng/mL. Each was prepared in a methanol/water mix consistent with EPA methods 537.1 or EPA 533.

Instrumentation

Analysis was performed using a 1290 Infinity II LC equipped with a high speed pump coupled to an Agilent Ultivo triple quadrupole LC/MS. The LC was configured with a 20 µL injection loop and a multisampler. To avoid PFAS contamination and background from solvents and the LC system, a delay column was used, listed in Table 1, and details of the setup have been described in another Agilent application note.[5]

Tables 1 and 2 display the LC and MS parameters.

Table 1. LC parameters.

Parameter	EPA 537.1	EPA 533
Liquid Chromatograph	Agilent 1290 Infinity II	Agilent 1290 Infinity II
Delay Column	Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 4 µm (p/n 699770-902T)	Agilent Poroshell 120 EB-C18, 2.1 × 50 mm, 4 µm (p/n 699770-902T)
Analytical Column	Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (p/n 699675-902)	Agilent Poroshell 120 SB-C18, 2.1 × 50 mm, 1.9 µm (p/n 699675-902)
Column Temperature	55 °C	55 °C
Mobile Phase	A) 0.1% Acetic acid in H_2O B) Methanol	A) 20 mM Ammonium acetate in H_2O B) Methanol
Total Run Time	9 min	9 min
Post Time	2.5 min	2.5 min
Injection Volume	4 µL	7 µL
Flow Rate	0.7 mL/min	0.7 mL/min

Table 2. MS parameters.

Parameter	EPA 537.1	EPA 533
Mass Spectrometer	Agilent Ultivo triple quadrupole LC/MS	Agilent Ultivo triple quadrupole LC/MS
Ionization Mode	Negative ESI	Negative ESI
Capillary Voltage	3500 V	3500 V
Nozzle Voltage	500 V	500 V
Nebulizer Pressure	45 psi	45 psi
Drying Gas Temperature	300 °C	300 °C
Drying Gas Flow Rate	9.0 L/min	9.0 L/min
Sheath Gas Temperature	260 °C	260 °C
Sheath Gas Flow Rate	11.0 L/min	11.0 L/min

MS acquisition method

Dynamic MRM (dMRM) acquisition using Agilent MassHunter (version 1.1) was performed. All data processing was done using MassHunter quantitative software with Quant-My-Way templates. Table 3 denotes the optimized transitions and compound parameters for the PFASs run in EPA 537.1 and 533 on the Ultivo triple quadrupole LC/MS.

Table 3. Compound-specific parameters.

Method	Compound Name	ISTD	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	Collision Energy (V)
537.1	PFTeDA	No	712.9	668.5	100	12
537.1	PFTeDA	No	712.9	169	60	0
537.1	PFTrDA	No	663	618.7	101	8
537.1	PFTrDA	No	663	169	101	15
Both	11Cl-PF3OUdS	No	631	451	70	38
Both	11Cl-PF3OUdS	No	631	85	70	30
533	PFDoA-13C2	Yes	614.9	570	104	5
Both	PFDoA	No	613	268.7	100	7
Both	PFDoA	No	613	568.9	79	12
537.1	Et-FOSAA-D5	No	589	419	115	15
537.1	Et-FOSAA	No	584	525.9	115	15
537.1	Et-FOSAA	No	584	419	115	15
537.1	Me-FOSAA-D3	Yes	573	418.9	115	19
537.1	Me-FOSAA	No	570	482.9	115	15
537.1	Me-FOSAA	No	570	418.9	115	12
533	PFUnA-13C7	No	570	525	98	8
Both	PFUnA	No	563	519	83	8
Both	PFUnA	No	563	218.7	100	15
Both	9Cl-PF3ONS	No	531	351	100	28
Both	9Cl-PF3ONS	No	531	351	98	20
Both	9Cl-PF3ONS	No	531	83	98	30
533	8-2 FTS-13C2	Yes	529	80.9	173	56
533	8-2 FTS	No	527	506.8	173	28
533	8-2 FTS	No	527	81	173	56
533	PFDA-13C6	Yes	519	474	102	5
537.1	PFDA-13C2	No	514.9	469.9	91	8
Both	PFDA	No	513	468.6	91	8
Both	PFDA	No	513	218.7	100	8
533	PFOS-13C8	Yes	507	80	210	50
Both	PFOS-13C4	Yes	502.9	80	110	46
Both	PFOS	No	498.9	99	100	46
Both	PFOS	No	498.9	80	100	46
533	PFNA-13C9	Yes	472	427	66	5
Both	PFNA	No	462.9	418.9	76	8
Both	PFNA	No	462.9	169	76	17
533	PFHpS	No	448.9	98.7	170	44
533	PFHpS	No	448.9	79.7	170	52
533	6-2 FTS-13C2	Yes	429	81	161	48
533	6-2 FTS	No	427	407	116	24
533	6-2 FTS	No	427	81	116	48
533	PFOA-13C8	Yes	421	376	79	8
Both	PFOA-13C2	Yes	415	370	79	8
Both	PFOA	No	412.9	368.9	79	8
Both	PFOA	No	412.9	169	79	17
533	PFHxS-13C3	Yes	401.9	99	164	45
Both	PFHxS	No	398.9	99	80	42
Both	PFHxS	No	398.9	80	110	45

Results and discussion

The compounds were separated sufficiently chromatographically, and the target peaks were well resolved with peak asymmetry factors meeting and exceeding EPA method criteria. The separation was done with the Agilent Poroshell EC-120 columns for both EPA methods. The columns are superficially porous and sub-2 µm in diameter, allowing sharp peaks at high flow rates without much backpressure. As a result, the chromatography met EPA requirements while the analytical method was shortened to less than 10 minutes run-time with a 2.5-minute post-time for both EPA 533 and 537.1. The use of high flow rates allowed significantly increased throughput compared to the EPA methods, which have run times of more than 20 minutes. Representative chromatograms of the PFASs in EPA 537.1 and EPA 533 analyzed during the lab validation are shown in Figures 1 and 2.

Calibration curves were generated using a linear fit with 1/x weighting, and the curve was forced through zero as required by both the EPA methods. Excellent linearities, with R^2 >0.99, were obtained in this study for all analytes in both EPA 533 and 537.1 with a minimum of five calibration standard points used for each compound.

Method	Compound Name	ISTD	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	Collision Energy (V)
Both	ADONA	No	377	251	95	1
Both	ADONA	No	377	84.9	95	38
533	PFHpA-13C4	Yes	367	322	66	5
Both	PFHpA	No	362.9	319	82	1
Both	PFHpA	No	362.9	169	82	17
533	PFPeS	No	349	99	110	45
533	PFPeS	No	349	80	110	45
533	4-2FTS-13C2	No	329	309	113	20
533	4-2FTS	No	327	307	113	20
533	4-2FTS	No	327	80.9	113	28
533	PFHxA 13C5	Yes	318	272.9	66	5
537.1	PFHxA 13C2	No	314.9	269.9	70	4
533	PFEESA	No	315	135	103	20
533	PFEESA	No	315	69.1	103	56
Both	PFHxA	No	313	268.6	70	4
Both	PFHxA	No	313	119	70	22
533	PFBS-13C3	Yes	302	98.9	133	29
Both	PFBS	No	298.9	98.9	110	30
Both	PFBS	No	298.9	80	110	42
533	NFDHA	No	295	201	83	0
533	NFDHA	No	295	85	83	24
Both	HFPO-DA-13C3	Yes	287	169	100	1
Both	HFPO-DA	No	285	185	100	12
Both	HFPO-DA	No	285	169	100	1
533	PFMBA	No	279	235	68	0
533	PFMBA	No	279	85	68	4
533	PFPeA-13C5	Yes	268	223	59	4
533	PFPeA	No	263	219	59	0
533	PFMPA	No	229	185	89	8
533	PFMPA	No	229	85	89	8
533	PFBA-13C4	Yes	217	171.7	62	4
533	PFBA-13C3	Yes	216	171.7	62	4
533	PFBA	No	213	168.7	62	4

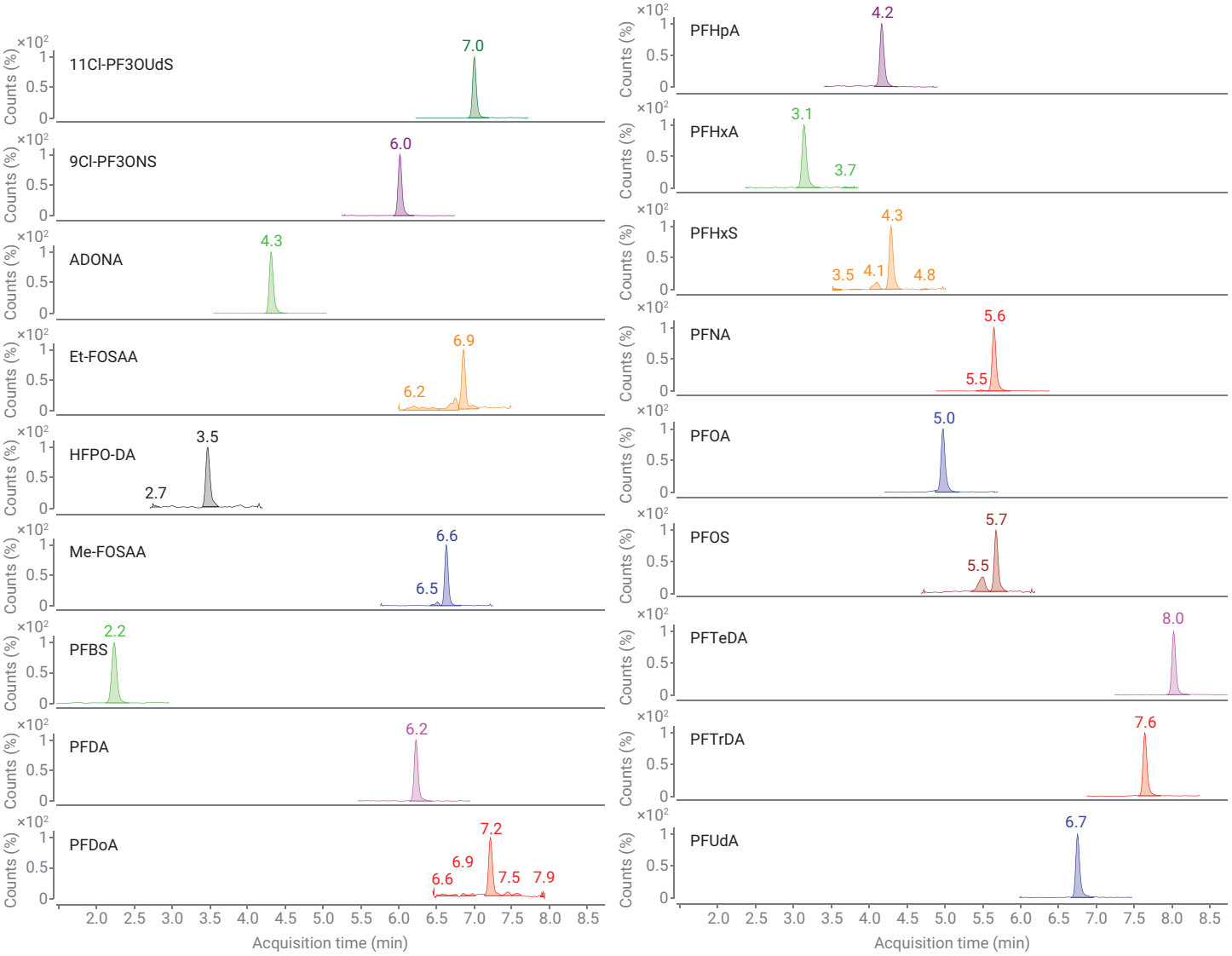

Figure 1. MRM chromatogram of 18 PFAS analyzed in EPA 537.1 at 2 ng/L spiked in drinking water

Blank contamination

In this study, the use of a delay column was found to sufficiently reduce background PFAS contamination below method reporting levels for both EPA methods. In addition, a carryover study was performed to determine if any PFAS contamination was seen in a blank standard, after the highest concentration calibration standard was run. In most cases, no carryover was observed, while a few compounds had minor carryover. The minor carryover was at least five times lower than the lowest calibration standard. The LC/MS/MS sensitivity at these low levels (5x less than 2 ng/L in sample) attests to the high sensitivity of the triple quadrupole LC/MS and are typically not required for labs running EPA 537.1 and EPA 533.

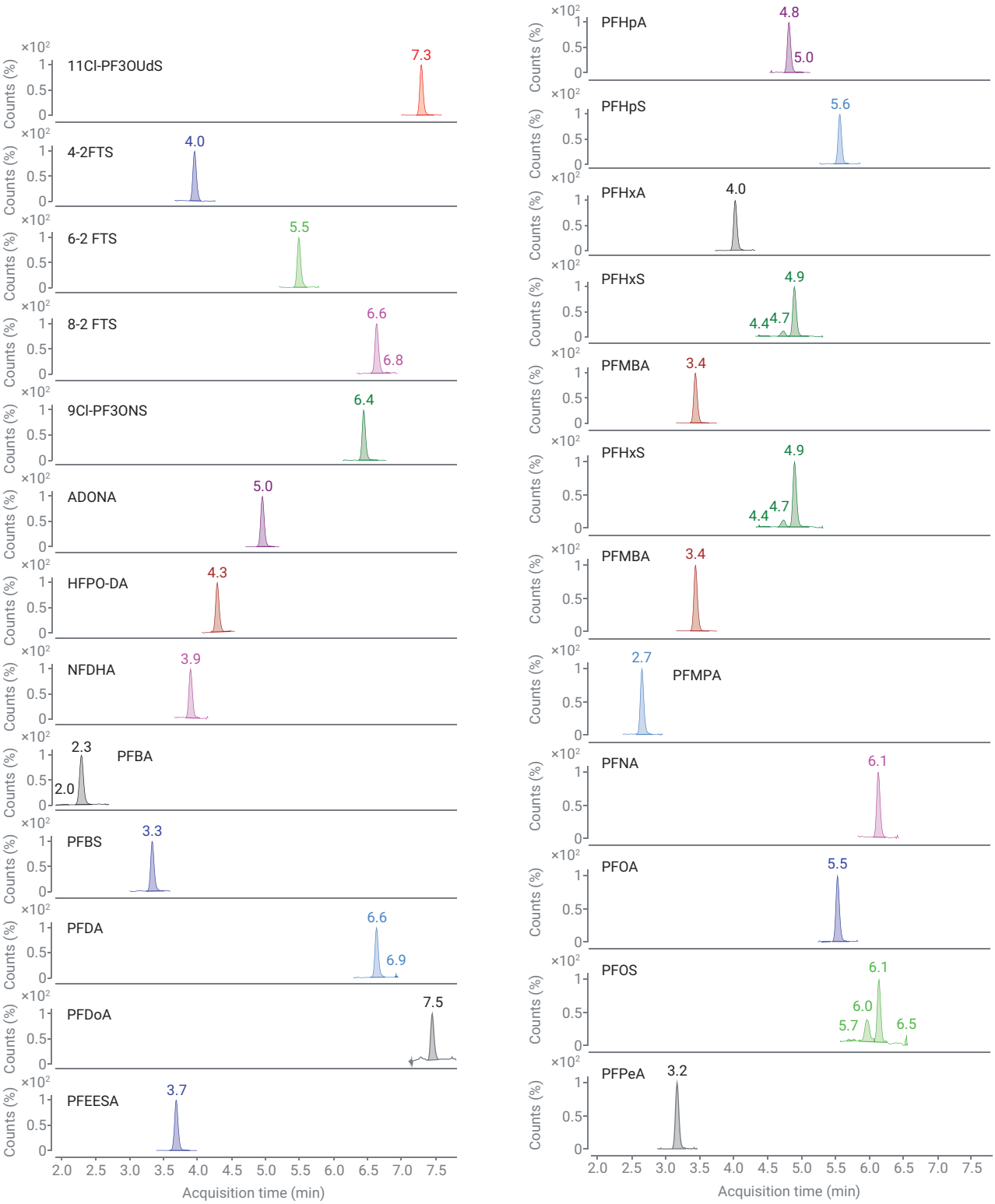

Figure 2. Extracted MRM chromatogram (quant transition) of 25 PFAS in lab fortified blank for EPA 533 at 1 ng/L

Recovery and reproducibility

The recovery and reproducibility of the entire method were assessed by spiking a known concentration of all analytes into a 250 mL sample of drinking water. The sample was appropriately preserved and fortified with internal standards corresponding to EPA 537.1 or 533. Five reagent and drinking water samples were spiked with 4 ng/L each of all analytes listed in EPA 537.1 and 533. These samples were taken through SPE and analyzed on the Ultivo triple quadrupole LC/MS. The mean recoveries and relative standard deviations (RSDs) are listed in Table 4. Recoveries were well within the 70 to 130% range required by the EPA methods. The RSDs were <20% in all cases for reagent and finished drinking water for all analytes tested in EPA 533 and 537.1

Method reporting levels

The method detection levels (MDLs) were calculated using eight replicates, spiked at progressively lower concentrations until recovery was between 50 to 150% as determined in the EPA methods for all analytes in both EPA 533 and 537.1. The spiked samples were taken through SPE extraction, evaporation, and analysis on the triple quadrupole LC/MS. MDLs for

Table 4. Recovery and RSD (%) of PFAS tested by EPA 533 and 537.1 in reagent water and finished drinking water.

	Reagent Water				Finished Drinking Water			
	EPA 533		EPA 537.1		EPA 533		EPA 537.1	
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBA	97%	2%	–	–	92%	3%	–	–
PFMPA	79%	5%	–	–	77%	3%	–	–
PFPeA	91%	3%	–	–	92%	5%	–	–
PFBS	96%	4%	109%	5%	95%	5%	106%	8%
PFMBA	94%	3%	–	–	92%	6%	–	–
PFEESA	93%	6%	–	–	91%	5%	–	–
NFDHA	93%	8%	–	–	98%	7%	–	–
4:2 FTS	96%	6%	–	–	91%	10%	–	–
PFHxA	93%	5%	119%	9%	90%	3%	116%	8%
PFPeS	95%	5%	–	–	96%	7%	–	–
HFPO-DA	92%	12%	116%	11%	93%	6%	111%	10%
PFHpA	81%	9%	–	–	85%	4%	–	–
PFHxS	92%	8%	102%	7%	100%	8%	114%	5%
ADONA	90%	2%	112%	8%	87%	1%	109%	8%
6:2 FTS	101%	15%	–	–	104%	3%	–	–
PFOA	89%	7%	101%	5%	89%	5%	117%	7%
PFHpS	92%	5%	–	–	85%	10%	–	–
PFNA	95%	6%	115%	6%	95%	6%	113%	8%
PFOS	94%	9%	98%	6%	100%	6%	103%	7%
8:2 FTS	109%	3%	–	–	97%	8%	–	–
9Cl-PF3ONS	110%	17%	110%	6%	122%	10%	109%	9%
PFDA	93%	6%	112%	6%	93%	6%	110%	8%
PFUnA	94%	4%	–	–	89%	3%	–	–
11Cl-PF3OUdS	81%	7%	106%	7%	77%	8%	109%	10%
PFDoA	96%	12%	101%	14%	100%	14%	96%	17%
Me-FOSAA	–	–	126%	8%	–	–	122%	9%
Et-FOSAA	–	–	120%	6%	–	–	118%	8%
PFHpA	–	–	122%	6%	–	–	126%	7%
PFTeDA	–	–	97%	9%	–	–	102%	9%
PFTrDA	–	–	103%	5%	–	–	110%	9%
PFUnA	–	–	98%	4%	–	–	104%	9%

all analytes were lower than 1 ng/L in both EPA 533 and 537.1, pointing to the excellent sensitivity of the Ultivo triple quadrupole LC/MS. These detection levels were much lower than those reported in the EPA methods. This sensitivity was achieved by injecting lower amounts of extract than specified in both EPA methods. Smaller injections enable sharper peak shapes and lower amounts of matrix compounds loaded onto the analytical LC column and MS/MS, allowing less contamination and potentially longer intervals before MS source cleaning.

Conclusion

The Agilent Ultivo triple quadrupole LC/MS system demonstrated superior sensitivity to quantify PFASs at levels needed for EPA methods 533 and 537.1. The performance in a commercial lab setting indicated that the method had lower reporting levels than required in the EPA method. The use of Agilent Poroshell EC-120 columns with a delay column gave good separation. The method delivered better than required peak asymmetry factors to resolve and quantify all PFASs without background levels to hamper analysis. The separation had much faster run times than shown in the EPA methods. When 250 mL samples were extracted with SPE, the recoveries in reagent water and finished drinking water for both EPA methods exceeded EPA method guidelines. The 1290 Infinity II LC coupled to the Ultivo triple quadrupole LC/MS is ideally suited for the analysis and quantification of PFASs in drinking water.

Table 5. Method detection limits for all PFAS analyzed in EPA 533 and EPA 537.1.

	EPA 533	EPA 537.1
Compound	MDL (ng/L)	MDL (ng/L)
PFBA	0.44	–
PFMPA	0.28	–
PFPeA	0.24	–
PFBS	0.26	0.67
PFMBA	0.33	–
PFEESA	0.13	–
NFDHA	0.42	–
4:2FTS	0.36	–
PFHxA	0.39	0.67
PFPeS	0.41	–
HFPO-DA	0.44	0.81
PFHpA	0.27	
PFHxS	0.4	0.79
ADONA	0.27	–
6:2FTS	0.67	–
PFOA	0.29	0.39
PFHpS	0.44	
PFNA	0.41	0.62
PFOS	0.44	0.55
9Cl-PF3ONS	0.48	0.82
8:2FTS	0.49	–
PFDA	0.27	0.68
PFUnA	0.41	–
11Cl-PF3OUdS	0.16	0.63
PFDoA	0.59	0.45
Me-FOSAA	–	0.82
Et-FOSAA	–	0.91
PFHpA	–	0.54
PFTeDA	–	0.62
PFTrDA	–	0.41
PFUnA	–	0.56
ADONA	–	0.46

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