Application Note

Environmental

Large-Volume Dilute-and-Shoot Analysis of PFAS in Drinking Water Using the Altura PFAS Column

Authors

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Abstract

Per-and polyfluoroalkyl substances (PFAS) are a group of organic synthetic chemicals with widespread presence in the environment. Due to their persistence and potential health risks, PFAS have primarily become a major environmental concern, with stringent limits in drinking water. This creates demand for sensitive methods that have reduced sample preparation time while supporting high laboratory throughput. This application note presents a large-volume dilute-and-shoot liquid chromatography/mass spectrometry (LC/MS) method for selected legacy PFAS C4-C10 in drinking water using the Agilent 1290 Infinity II LC coupled to an Agilent 6495D triple quadrupole LC/MS and the Agilent Altura Poroshell 120 PFAS column (Altura PFAS column). The method injects 200 µL of water samples diluted in methanol. This reduces sample preparation time while maintaining good peak shape, linearity, and robustness. Method detection limits (MDLs) range from 0.37 to 1.15 ng/L with method accuracy and reproducibility at 75 to 99% and < 7% respectively, in matrix spiked samples. This workflow provides a practical, high-throughput option for routine drinking water laboratories focused on legacy PFAS analysis.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic fluorinated compounds valued for their oil- and water-repellent properties, but their environmental persistence and mobility have driven the development of increasingly stringent regulations for drinking water monitoring. Existing dilute-and-shoot methods, such as EPA 8327 and ASTM D7979, were originally designed for screening PFAS in nonpotable water. These methods typically rely on 10 to 30 µL injection volumes, setting practical quantitation limits appropriately 10 ng/L.[1,2]

We present a large-volume injection workflow that extends the capabilities of these methods toward trace PFAS quantitation in drinking water. Using the Agilent 6495D triple quadrupole LC/MS (LC/TQ) coupled with a 1290 Infinity II LC, we achieved injections of 200 µL of methanol-diluted water on the Altura PFAS column without requiring SPE concentration. The Altura PFAS column maintained peak symmetry and retention stability under these injection conditions. This configuration enabled a working calibration range of 0.25 to 25 ng/L, extending the low-end sensitivity substantially relative to published dilute-and shoot methods.[1,2]

This performance enhancement simplifies operations by removing labor-intensive extraction steps, while maintaining sensitivity required for regulatory guidelines thereby supporting high-throughput PFAS workflows suitable for routine drinking water analysis. The PFAS compounds analyzed were PFBS, HFPO-DA, PFHpA, PFHxA, PFHxS, PFOA, PFOS, PFNA, and PFDA. These compounds were selected due to their relevance to ongoing regulatory actions and proposed maximum contaminant level (MCL) considerations for drinking water.

Specific highlights of this experiment include:

- Minimized peak tailing and solvent effects with 200 µL injections water/methanol (1:1) injections.
- Increased throughput by elimination of solid phase extraction (SPE).
- Enhanced sensitivity of 6495D LC/TQ at low end (0.25 ng/L) for the dilute-and-shoot method in drinking water.

Experimental

Instrumentation

Agilent 1290 Infinity II LC coupled to Agilent 6495D LC/TQ system was utilized for the LC/MS/MS analysis. An Infinity II multisampler with an extended multidraw capillary tube (G7137-68307) was connected to the needle seat and injection valve with a union for large volume injection (200 µL). The data were acquired with MassHunter acquisition software version 12.2. All LC/MS parameters are shown in Table 1 and 2. PFAS multiple reaction monitoring (MRM) transition methods were imported from the PFAS MRM database (G1736AA) while labelled homologues were optimized to obtain correct MRM transitions (Table 3).

Table 1. LC conditions.

Parameter	Value
LC	Agilent 1290 Infinity II LC with other components: – Agilent 1290 Infinity II high-speed pump (G7120A) – Agilent 1290 Infinity II multisampler (G7167B) – Agilent 1290 multicolumn thermostat (G7116B)
Column	Agilent Altura Poroshell 120 PFAS, 2.1 × 50 mm, 2.7 µm (part number 227215-007)
Delay Column	Agilent Poroshell 120 PFAS delay column, 4.6 × 30 mm (part number 027403-007)
Column Temperature	50 °C
Mobile Phase	A) 2 mM ammonium acetate in H_2O B) 2 mM ammonium acetate in MeOH
Injection Volume	200 µL
Flow Rate	0.400 mL/min
LC Gradient	Time (min) %B 0.00 35 1.00 35 3.00 70 5.00 90 7.00 100 11.00 100 11.30 35 12.00 35
Stop Time	12.00 min
Post Time	2.00 min
Needle Wash Mode	Standard wash
Wash Solvent 1 (S1)	IPA:H_2O (90:10)

Table 2. MS method.

Parameter	Value
MS Source	Agilent Jet stream
Gas Temperature	200 °C
Gas Flow	11 L/min
Nebulizer	33 psi
Capillary Voltage	2,100 V
Sheath Gas Temperature	310 °C
Sheath Gas Flow	12 (L/min)
Nozzle Voltage	0 V

Table 3. MRM parameters.

Name	Full Name	CAS No.	Precursor Ion	Product Ion	RT (min)	Fragmentor (V)	Collision Energy (V)
$^{13}C_4$-PFBS	Perfluorobutanesulfonate ($^{13}C_4$)	—	302.9	80	6.08	166	44
$^{13}C_3$-HFPO-DA	Tetrafluoro-2-(heptafluoropropoxy) propanoic acid ($^{13}C_3$)	—	288	172	6.54	166	20
$^{13}C_3$-HFPO-DA			288	188	6.54	166	4
$^{13}C_6$-PFHxA	Perfluorohexanoic acid ($^{13}C_6$)	—	319	273.9	6.47	166	8
$^{13}C_6$-PFHxA			319	121	6.47	166	24
$^{13}C_6$-PFHxS	Perfluorohexanesulfonate ($^{13}C_6$)	2687960-06-9	405	99.3	6.77	166	50
$^{13}C_6$-PFHxS			405	80.4	6.77	166	50
$^{13}C_8$-PFOA	Perfluorooctanoic acid ($^{13}C_8$)	1350614-84-4	421	376	7.12	166	8
$^{13}C_8$-PFOA			421	172	7.12	166	20
$^{13}C_8$-PFOS	Potassium perfluoro-1-octanesulfonate ($^{13}C_8$)	—	507	99	7.36	166	52
$^{13}C_8$-PFOS			507	80	7.36	166	54
$^{13}C_9$-PFDA	Perfluorodecanoic acid ($^{13}C_9$)	—	522	476.6	7.69	166	20
$^{13}C_9$-PFDA			522	222.8	7.69	166	20
$^{13}C_9$-PFNA	Perfluorononanoic acid ($^{13}C_9$)	2283397-80-6	472	427	7.41	166	8
$^{13}C_9$-PFNA			472	223	7.41	166	16
PFBS	Perfluorobutanesulfonate	29420-49-3	298.9	99	6.08	166	34
PFBS			298.9	80	6.08	166	36
PFDA	Perfluorodecanoic acid	3830-45-3	513	469	7.69	166	16
PFDA			513	219	7.69	166	20
PFHpA	Perfluoroheptanoic acid	375-85-9	363	219	6.81	166	8
PFHpA			363	319	6.81	166	16
PFHxA	Perfluorohexanoic acid	2923-26-4	313	269	6.47	166	8
PFHxA			313	119	6.47	166	24
PFHxS	Perfluorohexanesulfonate, mixed isomers	3871-99-6	398.9	99	6.77	166	40
PFHxS			398.9	80	6.77	166	56
PFNA	Perfluorononanoic acid	375-95-1	463	419	7.41	166	8
PFNA			463	216	7.41	166	16
PFNA			463	169	7.41	166	20
PFDA	Perfluorodecanoic acid	3830-45-3	513	269	7.69	166	16
PFDA			513	219	7.69	166	20
PFOA	Perfluorooctanoic acid	335-67-1	413	369	7.12	166	8
PFOA			413	219	7.12	166	16
PFOA			413	169	7.12	166	16
PFOS	Perfluorooctanesulfonate, mixed isomers	1763-23-1	498.9	99	7.36	166	50
PFOS			498.9	80	7.36	166	54
HFPO-DA	Tetrafluoro-2-(heptafluoropropoxy) propanoic acid	13252-13-6	285	185	6.54	166	20
HFPO-DA			285	169	6.54	166	4
HFPO-DA			285	119	6.54	166	32

Sample preparation and calibration

Three different water types were tested: reagent water, Delaware tap water, and Pennsylvania treated water samples labeled prefilter, postfilter, and reverse osmosis (RO) were used for this analysis. An aliquot of 3 mL of each water sample was spiked at a concentration of 1 ng/L and 10 ng/L of native PFAS mix, respectively. The samples were amended with 10 ng/L of isotopically labeled PFAS mix and diluted with 3 mL of methanol. Ascorbic acid (10 µg/mL) was added to the mixture to dechlorinate the water samples. Next, 10 µL acetic acid was added to the total volume of diluted samples to improve peak shape.[3] The resulting mixture (6 mL) was vortexed for 2 minutes and passed through an RC filter (0.2 µm pore size, 25 mm). A calibration curve of targets was prepared at 0.25 to 25 ng/L, which is equivalent to 0.5 to 50 ng/L in water samples.

Results and discussion

Chromatograms

All nine PFAS compounds were separated using Agilent Altura Poroshell PFAS column. The chromatogram has gaussian peaks for all short and long chain C4–C10 perfluoroalkyl acids (PFAAs) at 200 µL injection as shown in Figure 1.

Linearity

A 1/x weighted linear regression curve setting was used for calibrating all the compounds. All analyzed PFAS shows $R^2 > 0.99$ suggesting the linear response of the method even at low sub-ppt levels with accuracy falling between 95 and 115% for all calibration levels. Calibration curve ranges from 0.25 to 25 ng/L in-vial corresponding to 0.5 to 50 ng/L in water samples. Calibration curve of all nine PFAS is shown in Figure 2.

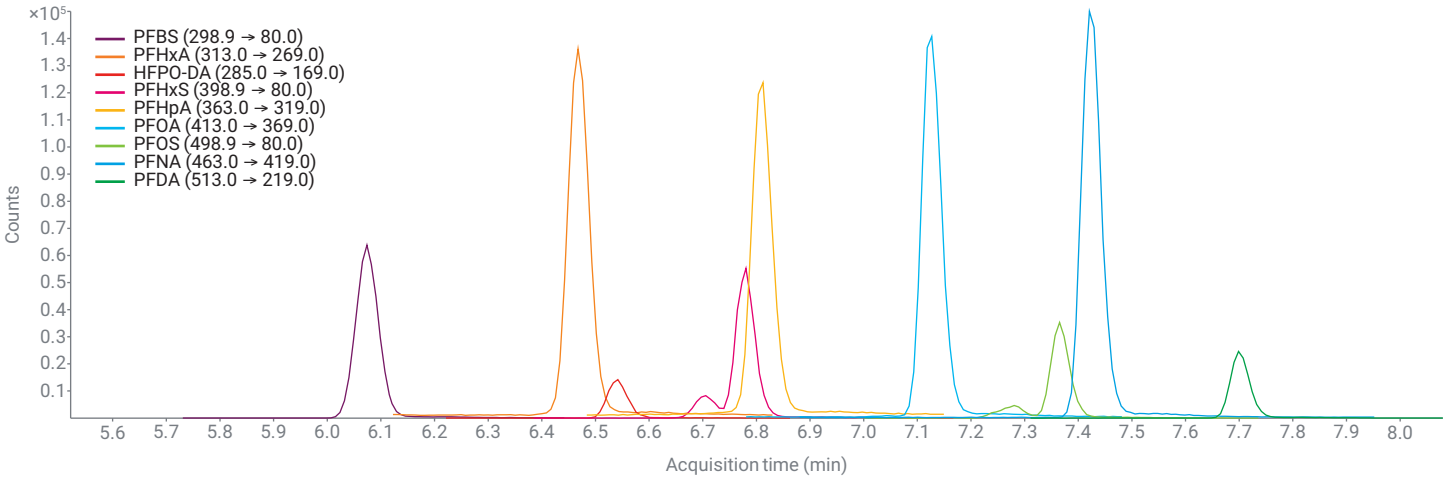

Figure 1. Chromatograms of all analyzed PFAAs at 25 ng/L for 200 µL injection on an Agilent Altura Poroshell PFAS column.

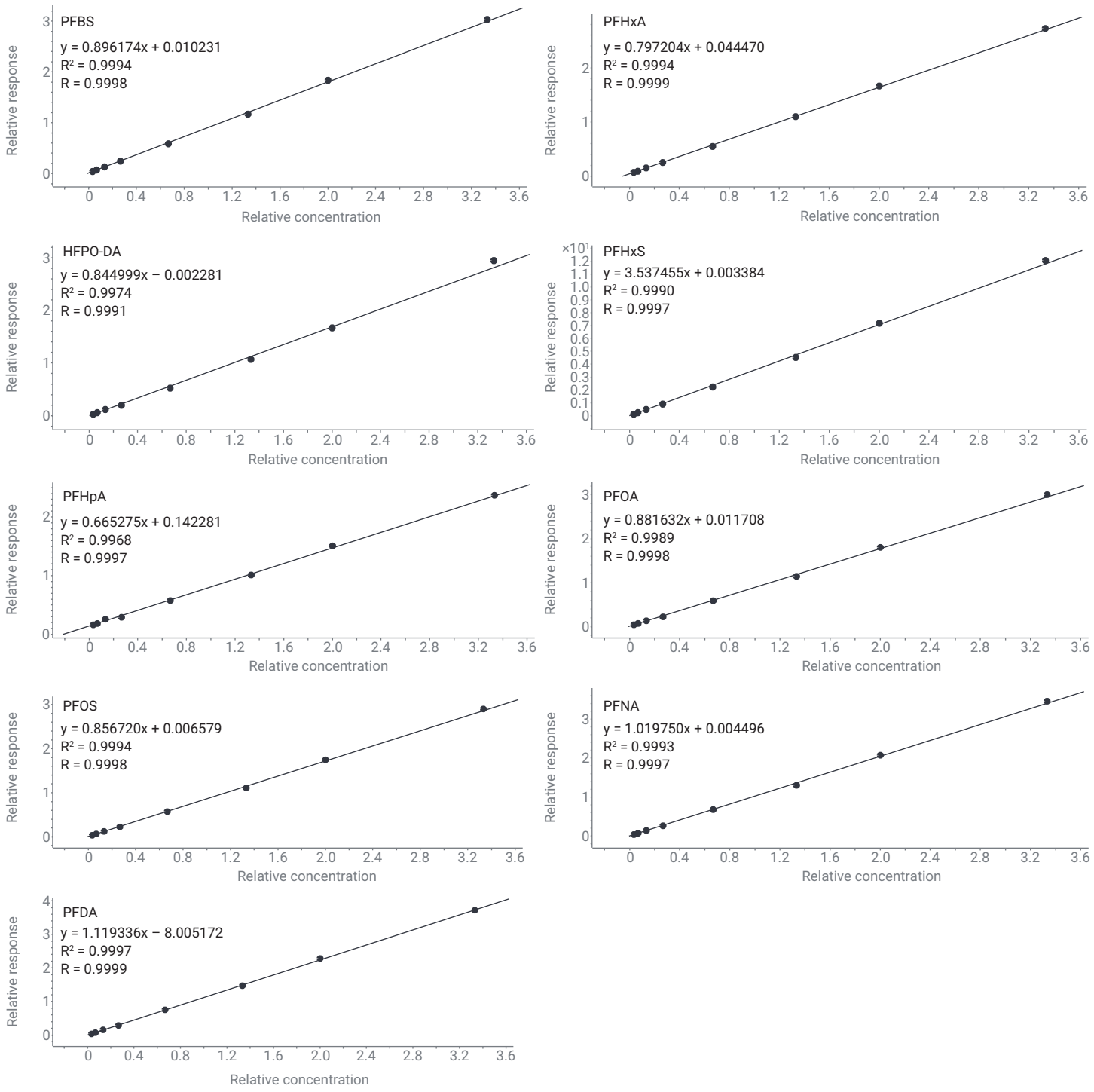

Figure 2. Calibration curve showing linearity of PFAS for all C4–C10 PFAAs and C6 PFECA (HFPO-DA) on an Agilent 6495D LC/TQ.

Column stability assessment

Column stability was assessed by analysing replicate spike samples of PFAS at sub-ppt levels (0.25 and 2 ng/L, respectively). Seven replicate injections of each concentration levels were run to monitor consistency in performance. All PFAS accuracy ranges from 82 to 108% while %RSD < 11% at 0.25 ng/L while at 2 ng/L, accuracy ranges from 87 to 106% and %RSD < 12%. Additionally, %RSD of RT is < 0.1 at both levels indicating excellent column stability.

Selected PFAS is shown in Figure 3. This confirms that the Altura Poroshell 120 PFAS column provides a reproducible and robust performance for trace-levels of PFAS under high volume injection conditions.

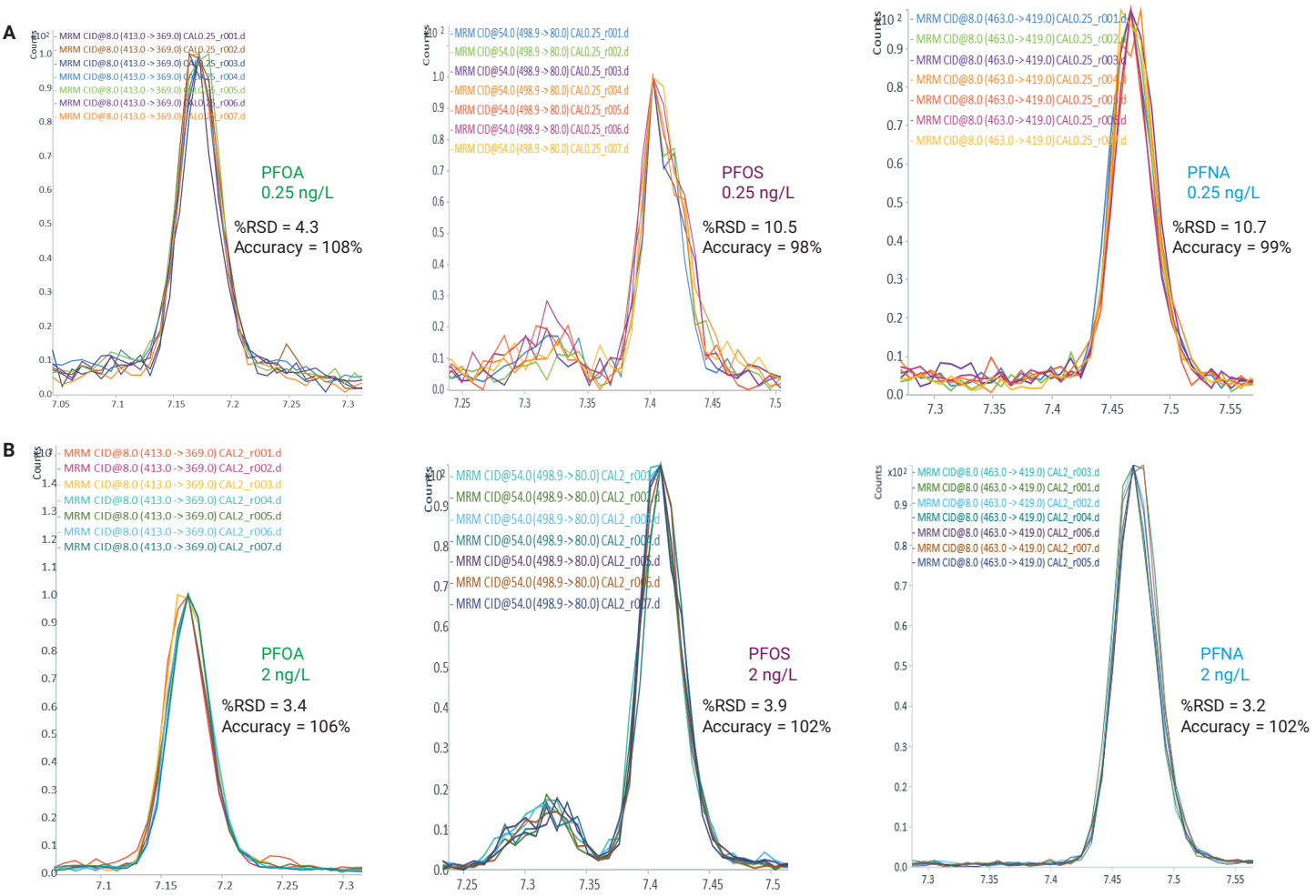

Figure 3. Stability of selected PFAAs at 0.25 ng/L (A) and 2 ng/L (B), respectively. **Note:** %RSD in the graph is based on abundance. The %RSD based on retention time is generally < 0.1 at both levels.

Method performance

At a low spiking level of 0.25 ng/L, seven replicates of method blanks were assessed for accuracy and precision. The method demonstrates excellent recovery (82 to 108%) and %RSD (< 11%) as shown in Figure 4A and 4B.

Overall, the results indicate that even at sub-ppt levels, the method provides reliable quantitation with consistent accuracy and precision across all native standards.

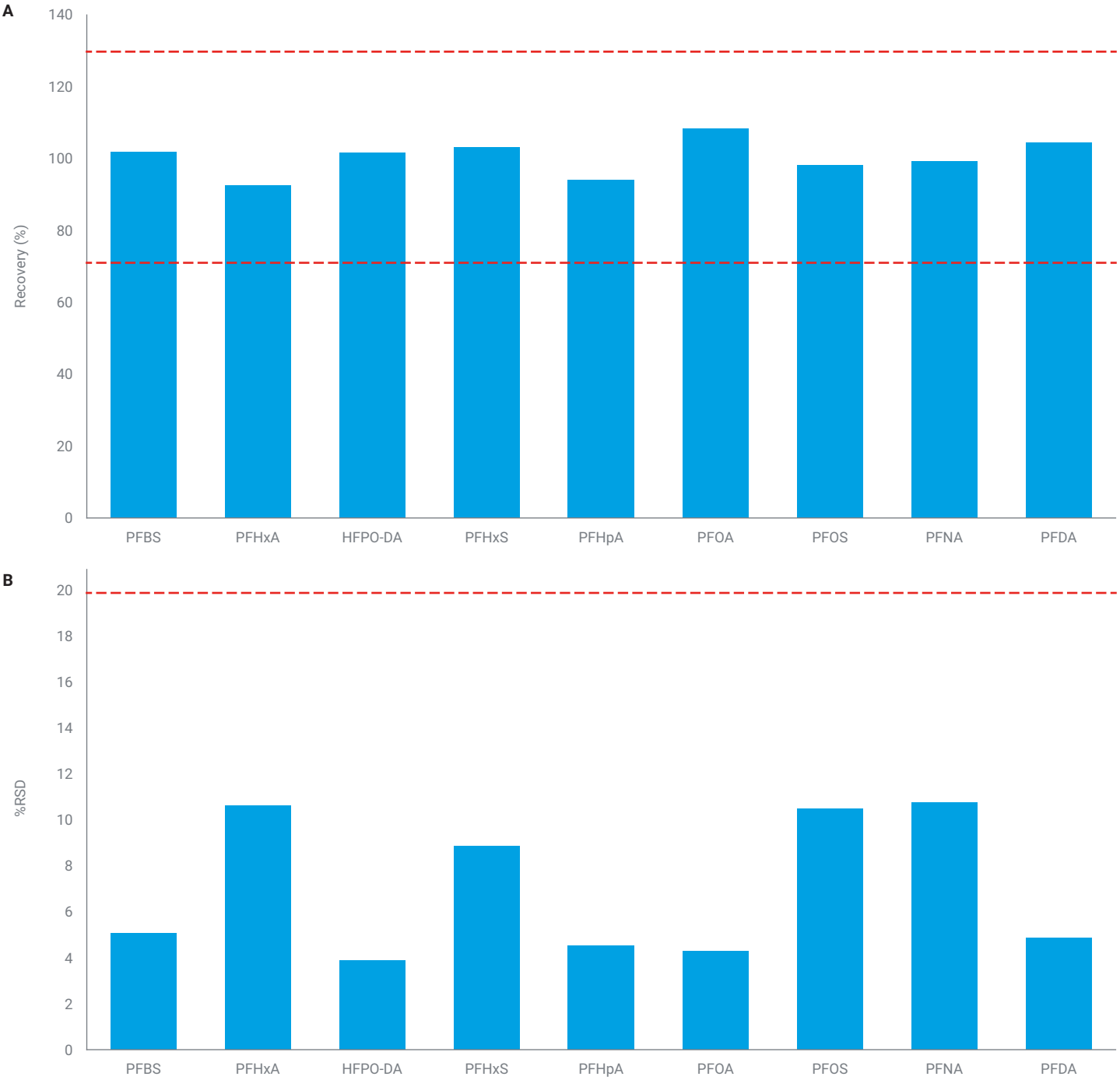

Figure 4. % Recovery (A) and %RSD (B) of native standards spiked at 0.25 ng/L in vials. Dotted lines indicate regulatory limits for recovery (70 to 130%) and ≤ 20% for %RSD.

Method detection limits (MDL)

MDL was performed to assess the sensitivity of the developed method. MDLs were calculated based on replicates of seven low-level spikes that are distinguishable from the method blanks.[4] The spiked samples (0.5 ng/L in MilliQ water) and method blanks were passed through the same sample treatment process. Where the MDL in blanks was higher than the lowest spiked, the blank MDL was used. MDL range from 0.37 to 1.15 ng/L, % recovery and %RSD are within the acceptable limits[1,2] as shown in Table 4 (70–130%). MDL calculation is based on the formula below:

$$MDL = SD \times t_{(n-1,\ 1-\alpha\ =\ 0.99)}$$

Where:

SD = the standard deviation of replicates samples

t = the t-values for a 99% confidence interval with n–1 degrees of freedom

Table 4. MDL of low- level spikes based on 200 µL injection. ***Note:** where the MDL in blanks were higher than the lowest spikes, the MDL in blank was used.

Compound	MDL (ng/L)	Calibration Range in ng/L (Samples)	% Recovery	%RSD
PFBS	0.50	0.5 – 50	102	5.1
PFHxA	1.01	0.5 – 50	93	10.6
HFPO-DA	0.37	0.5 – 50	102	3.9
PFHxS	0.86	0.5 – 50	103	8.8
PFHpA*	0.58	0.5 – 50	94	4.5
PFOA	0.44	0.5 – 50	108	4.3
PFOS	1.13	0.5 – 50	98	10.5
PFNA	1.01	0.5 – 50	100	10.7
PFDA	0.48	0.5 – 50	105	4.9

Quantitative performance of matrix spikes

Water samples from a Pennsylvania homeowner's in-house filtration system, namely prefilter, postfilter and reverse osmosis (RO) treated water, were used for the experiment. Tap water from Delaware was also used in addition to the listed water samples (Table 5). Each water sample were spiked at a low level (1 ng/L in water samples) and a mid level (10 ng/L in water samples) and passed subsequently through the sample treatment process. The matrix blanks only amended with isotopically labeled standard were run alongside and subtracted from the average of each matrix spikes (Table 5). All compounds analyzed with this method were within the expected range suggesting minimal matrix influence and excellent method performance in each of the water sample types. All the samples were processed in triplicates (n = 3) with %RSD < 7% indicating the reproducibility of the method in different drinking water types tested (Table 5).

Table 5. Method performance of 200 µL injection in different water samples. Low spikes were spiked at 0.5 ng/L equivalent to 1.0 ng/L in-samples while high-spikes were spiked at 5 ng/L equivalent to 10 ng/L in samples.

Sample	Spiking Level	Figures of Merit	PFBS	PFHxA	HFPO-DA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA
Tap Water	Tap blank	Conc (ng/L)	0.96	1.63	–	0.35	1.09	1.20	0.37	0.23	0.10
	Low spike	% Recovery	96	81	95	84	79	94	85	76	81
		%RSD	1.6	1.0	0.6	2.3	0.3	4.2	6.5	3.3	3.8
	High spike	% Recovery	88	86	92	91	91	80	93	90	86
		%RSD	0.2	3.6	4.8	1.4	0.2	1.5	1.3	0.2	0.2
Prefilter	Prefilter blank	Conc (ng/L)	1.70	1.90	–	0.50	1.02	2.31	1.36	0.36	0.18
	Low spike	% Recovery	93	77	84	95	93	76	97	78	82
		%RSD	0.6	0.4	1.5	0.6	0.6	1.4	2.2	3.8	1.7
	High spike	% Recovery	95	98	85	85	92	82	93	89	95
		%RSD	0.4	0.1	2.2	0.1	1.0	0.5	1.0	0.2	0.8
Postfilter	Postfilter blank	Conc (ng/L)	0.04	0.53	0.36	–	0.21	0.08	0.07	0.04	0.04
	Low spike	% Recovery	83	87	96	83	81	75	86	75	83
		%RSD	5.7	0.2	1.1	1.9	1.4	1.6	1.5	1.4	2.1
	High spike	% Recovery	93	90	94	94	81	84	95	90	96
		%RSD	1.6	0.5	1.5	1.2	0.6	0.2	0.5	0.9	0.4
RO	RO blank	Conc (ng/L)	0.06	0.11	–	–	0.70	0.07	–	0.05	0.08
	Low spike	% Recovery	88	87	91	97	93	76	99	88	75
		%RSD	0.3	2.5	2.4	3.4	4.4	1.1	0.7	1.1	0.2
	High spike	% Recovery	92	89	97	97	79	89	94	92	94
		%RSD	0.5	2.0	0.8	1.5	3.1	0.6	1.4	1.8	0.3

Conclusion

This study demonstrates a practical large-volume dilute-and-shoot workflow for the determination of PFAS in drinking water using the Agilent 1290 Infinity II LC coupled to the 6495D triple quadrupole LC/MS, and Agilent Altura Poroshell PFAS column. With 200 µL water samples diluted in methanol can be injected directly, eliminating the need for SPE concentration and significantly reducing sample preparation time. This method exhibits good sensitivity, accuracy, and reproducibility. The Altura PFAS column maintained consistent peak performance and retention stability even at low sub-ppt concentrations, supporting long-term reliability for trace-level PFAS monitoring. Overall, the findings highlight the method potential to support routine, high-sensitivity PFAS monitoring in drinking water laboratories.

Consumables

Consumables	Part Number
2 mL vials	5191-8121
Screw caps with septa	5191-8151
Extended seat capillary (500 µL)	G7137-68307
RC filters	5190-5110
InfinityLab PFAS analysis HPLC conversion kit	5004-0006
533 Native Analyte PDS	PFS-533-NAS
533 Isotope Dilution PDS	PFS-533-IDS

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