Application Note

Environmental

Reliable Ultra Short Chain PFAS Analysis in Water and Landfill Groundwater

Using the Agilent Altura Poroshell PFAS column and LC/MS/MS

Authors

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Abstract

The Agilent Altura Poroshell PFAS column, used in combination with the Agilent InfinityLab Poroshell 120 PFAS delay column and the Agilent 6495D triple quadrupole LC/MS, delivers a powerful solution for analyzing ultrashort-chain PFAS in complex environmental matrices. The column provides stable retention times, strong run-to-run reproducibility, and dependable performance across both high-salt synthetic water and real-world groundwater samples. With effective interference management and robust detection of the most challenging early-eluting PFAS, this workflow offers environmental laboratories a reliable, streamlined approach for ultrashort-chain per- and polyfluoroalkyl substances (PFAS) analysis.

Introduction

The increasing detection of ultrashort-chain PFAS in environmental waters[1] has created a pressing need for highly sensitive, reliable analytical methods capable of separating and quantifying these challenging compounds. Because ultrashort PFAS, such as TFA and DFA, show little retention on typical C18 columns, their analysis requires a specialized analytical and delay column, optimized mobile phases, and rigorous contamination control.

This application note evaluates the performance of the Altura Poroshell PFAS column (Altura PFAS column) paired with an Agilent 1290 Infinity III LC system and 6495D LC/TQ for the analysis of ultrashort-chain PFAS across laboratory-prepared matrices and real-world groundwater samples. The study presents detection limits, retention-time stability under ionic-strength conditions, matrix-spike recovery and precision in synthetic water, and field-sample results from landfill groundwater. Groundwater samples were also analyzed by an external collaborator following EPA 1633A[2], and the PFBA results were compared. Synthetic-water experiments were designed to specifically challenge the method under high-salt conditions, while groundwater samples from seven landfills provided insight into real-world interferences and matrix behavior.

Experimental

Solutions and standards

Table 1 summarizes the compounds included in the study along with their respective suppliers. Acetic acid and ammonium acetate were obtained from Sigma-Aldrich, while LC/MS-grade acetonitrile, methanol, and all other chemicals were sourced from VWR. Milli-Q water was used for mobile phase preparation, and reverse osmosis (RO) water was used to prepare calibration standards, spikes, and blanks. Laboratory synthetic (LS) water was based on a formulation suggested in EPA 557[3] to verify performance in samples with high anion concentration. LS was prepared with sodium salts to achieve the following concentrations: 20 mg/L nitrate, 150 mg/L bicarbonate, 250 mg/L chloride, and 250 mg/L sulfate.

Samples

Samples consisted of groundwater collected as part of routine landfill monitoring activities (Figure 1). Extraction results following EPA 1633A[2] of the same samples were provided by an external collaborator.

Table 1. Compounds used in the present study.

Compound Name	Abbreviation	Compound Formula	CAS Number	Supplier
$^{13}C_2$-TFA	13C2-TFA	$[^{13}C]_2HF_3O_2$	–	Cambridge Isotopes
$^{13}C_3$-PFPrA	13C3-PFPrA	$[^{13}C]_3HF_5O_2$	–	Wellington Labs
Pentafluoroethanesulfonic acid	PFEtS	$C_2F_5SO_3H$	354-88-1	Wellington Labs
Trifluoromethanesulfonic acid	PFMeS	CF_3SO_3H	1493-13-6	Wellington Labs
Perfluoro-2-methoxyacetic acid	PFOMAA	$C_3F_5O_3H$	674-13-5	Cambridge Isotopes
Pentafluoropropionic acid	PFPrA	$C_3F_5O_2H$	422-64-0	Cambridge Isotopes
Perfluoropropanesulfonic acid	PFPrS	$C_3F_7SO_3H$	423-41-6	Wellington Labs
Trifluoroacetic acid	TFA	$C_2F_3O_2H$	76-05-1	Cambridge Isotopes
Difluoroacetic acid	DFA	$C_2H_2F_2O_2$	381-73-7	Sigma Aldrich
Heptafluorobutyric acid	PFBA	$C_4HF_7O_2$	375-22-4	Wellington Labs
Bis(trifluoromethane)sulfonimide lithium salt/trifluoromethanesulfonyl (TFSI)	HQ115/TFSI	$C_2F_6LiNO_4S_2$	90076-65-6	Accustandard

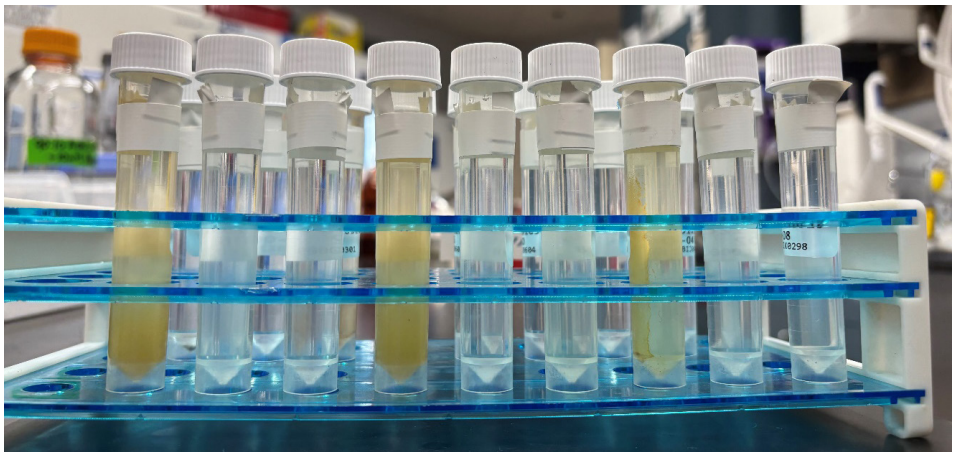

Figure 1. Groundwater samples from landfill monitoring wells.

Sample preparation

Samples were vortexed, and 1.2 mL was aliquoted into Eppendorf tubes and centrifuged for 10 minutes at 135,000 rpm. Supernatant (1 mL) was transferred into LC vials, and a mix of two labeled analogs was added at a concentration of 0.1 ng/mL.

LC/TQ conditions

Analysis was performed using a 1290 Infinity III LC system consisting of an Agilent 1290 Infinity III high-speed pump (G7120A), an Agilent 1290 Infinity III Multisampler (G7167B), and an Agilent 1290 Infinity III Multicolumn Thermostat (G7116A). The LC system was modified using an Agilent InfinityLab PFC-free HPLC conversion kit (part number 50040006) with the Agilent InfinityLab PFC delay column (p/n 5062-8100) replaced with the Agilent InfinityLab Poroshell 120 PFAS delay column (p/n 027403-007). Chromatographic separation was performed with an Altura PFAS column. Full separation details are provided in Table 2.

MS/MS acquisition and transitions

The LC system was coupled to a 6495D LC/TQ equipped with an Agilent Jet Stream source. The source conditions for the 6495D are shown in Table 3 and were optimized to minimize the TFA baseline. Compound transitions are provided in Table 4 and were optimized for 6495D using MassHunter Optimizer. Data acquisition and analysis were carried out with Agilent MassHunter Workstation software.

Table 2. Chromatographic conditions.

LC Conditions	
Column	Agilent Altura Poroshell PFAS 2.1 × 100 mm, 2.7 µm (p/n 227210-007)
Delay Column	Agilent InfinityLab Poroshell 120 PFAS delay column , 4.6 × 30 mm (p/n 027403-007)
Column Temperature	40 °C
Injection Volume	25 µL
Autosampler Temp	20 °C
Standard Wash	1:1 methanol:water, 5 s flush
Mobile Phase	A) 0.1% acetic acid in water B) 90:10 acetonitrile:water + 10 mM ammonium acetate
Flow Rate	0.5 mL/min
Gradient Program	Time (min) B (%) 0 10 0.5 10 2 40 6 40 10 98 12 98 12.1 10
Stop Time	12.50 min
Post Time	4.00 min

Table 3. Source conditions for Agilent 6495D triple quadrupole LC/MS.

Parameter	Setting
Gas Temperature	240 °C
Drying Gas Flow	18 L/min
Sheath Gas Temperature	350 °C
Sheath Gas Flow	11 L/min
Nebulizer	20 psig
Vcap	2,000 V
Nozzle	0 V

Table 4. Compound transitions.

Compound	RT	Precursor *m/z*	Product *m/z*	Fragmentor (V)	CAV (V)	CE (V)	iFunnel Mode	Ionization
13C2-TFA	5.25	115	69.9	166	5	14	Fragile	Negative
13C3-PFPrA	7.514	166	121	166	5	10	Fragile	Negative
PFEtS	8.602	199	98.9	166	5	30	Standard	Negative
PFEtS	–	199	79.9	166	5	34	Standard	Negative
PFMeS	6.729	149	98.9	166	5	30	Standard	Negative
PFMeS	–	149	79.9	166	5	30	Standard	Negative
PFOMAA	8.097	179	85	166	5	14	Fragile	Negative
PFPrA	7.515	163	119	166	5	10	Fragile	Negative
PFPrS	9.236	249	98.9	166	5	30	Standard	Negative
PFPrS	9.236	249	79.9	166	5	36	Standard	Negative
TFA	5.25	113	68.9	166	5	14	Fragile	Negative
HQ-115/TFSI	9.937	279.9	210.9	166	5	20	Fragile	Negative
HQ-115/TFSI	–	279.9	146.9	166	5	30	Fragile	Negative
HQ-115/TFSI	–	279.9	77.9	166	5	40	Fragile	Negative
DFA	4.185	95	51	166	5	18	Fragile	Negative

Results and discussion

Contamination control and detection limit

Initial tests indicated the presence of background TFA in all samples. Although instrument blanks were clean, TFA was consistently detected in method blanks. Because the laboratory is a mixed-use space, complete elimination of ambient TFA was not feasible. Accordingly, the method detection limit (MDL) evaluation was adjusted by elevating the TFA spike concentration to clearly distinguish it from persistent background levels. MDLs were determined in accordance with EPA procedures.[4] All compounds were spiked at 0.01 ng/mL, apart from TFA, which was spiked at 0.1 ng/mL.

Figure 2 presents the calculated detection limits ranging from 0.004 to 0.054 ng/mL for TFA. For both TFA and PFMeS, the final detection limits were determined based on their concentrations in the method blanks, as these background levels exceeded the values calculated from the spiking experiments.

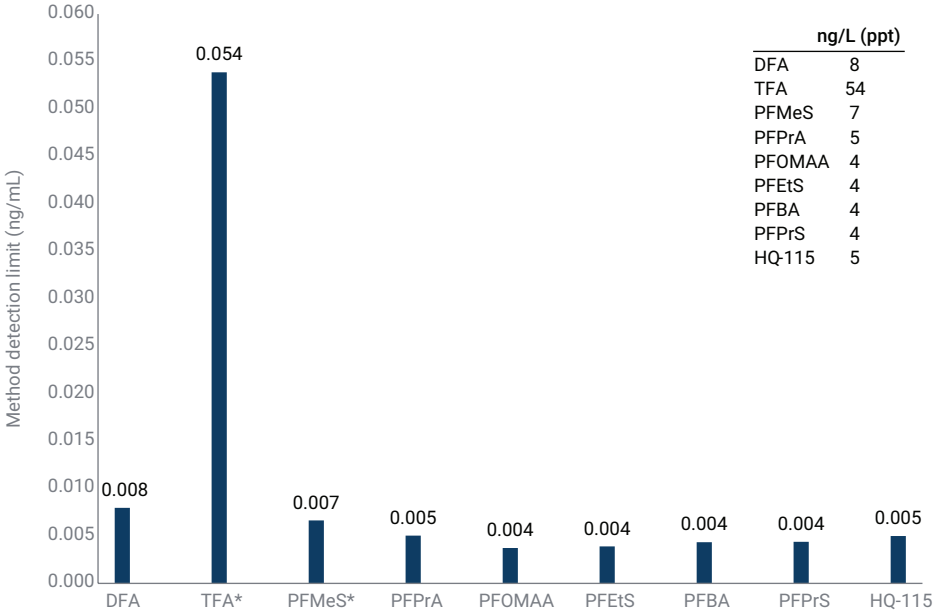

Figure 2. Method detection limit in ng/mL. * Indicates that the MDL was determined by blank levels (1 ng/mL = 1,000 ng/L).

Retention time stability under ionic-strength conditions

Synthetic water was evaluated to confirm retention time (RT) stability and peak shape under increased ionic strength conditions. Figure 3 compares chromatograms generated in standard reverse-osmosis (RO) water with those obtained in the synthetic water. Peak shapes remained Gaussian, and the largest retention time shift observed was a 0.1-minute forward shift for PFPrA in the synthetic water, indicating consistent performance in an ionic matrix.

Spike recovery and precision

Synthetic water was spiked at three levels to evaluate method reproducibility and recovery. The lowest spiking level was 0.01 ng/mL (0.1 ng/mL for TFA), followed by 1.5× and 2× the lowest level. With the exception of DFA and TFA, relative standard deviations were excellent (< 15%), and recoveries fell within the 70% to 130% range. Results are shown in Figure 4 for all compounds with the exception of DFA.

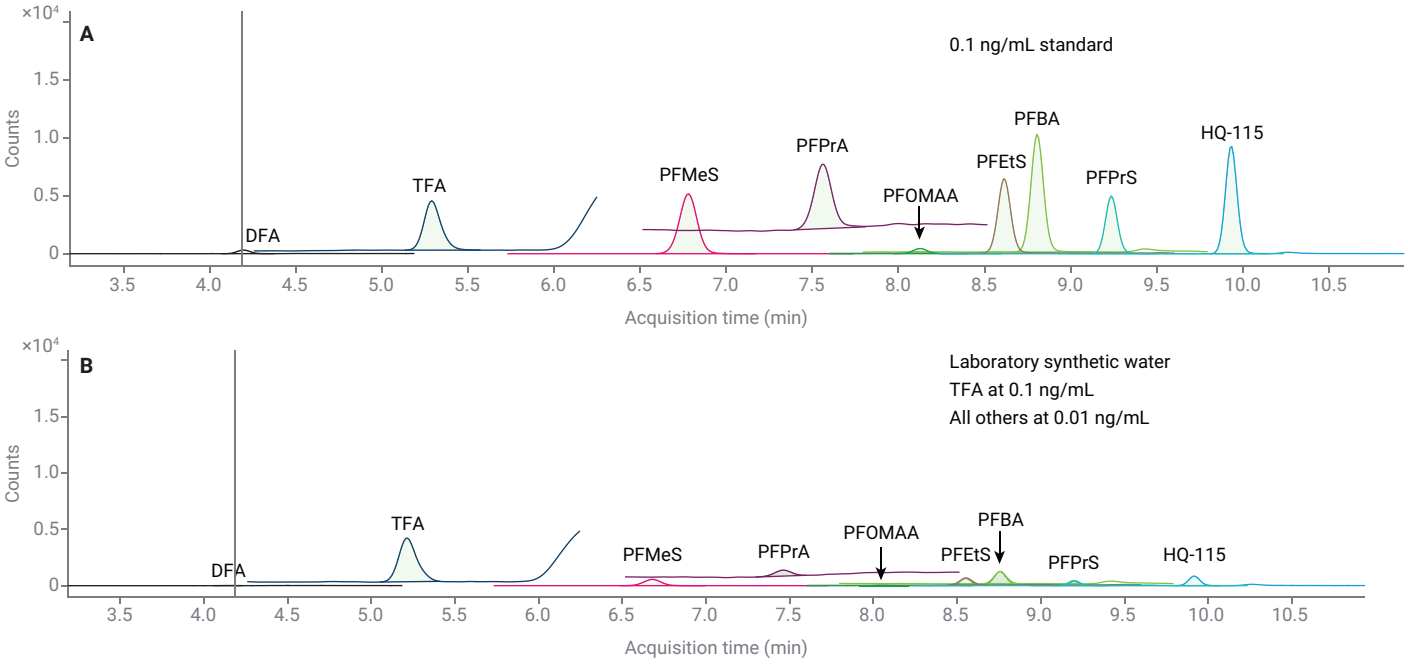

Figure 3. (A) Chromatogram of ultrashort PFAS in a 0.1 ng/mL calibration standard. (B) Chromatogram in laboratory synthetic water at 0.01 ng/mL for all compounds except TFA (0.1 ng/mL).

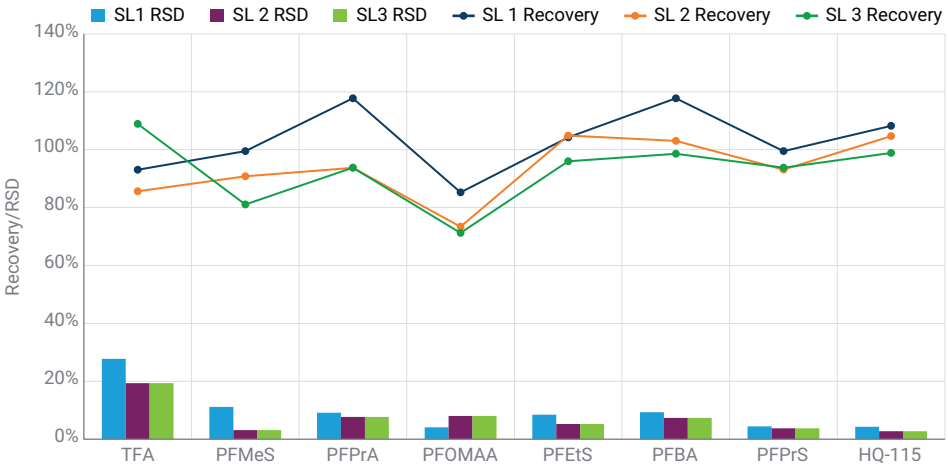

Figure 4. Recovery and RSD of replicates (n = 4) in laboratory synthetic water.

DFA exhibited poor recovery, likely due to matrix suppression caused by the high salt content of the synthetic water. Because no isotopically labeled analogue for DFA was available, quantitation was corrected using $^{13}C_2$-TFA, which negatively affected both accuracy and reproducibility.

The isotopically labeled TFA analogue showed good analytical performance for TFA; however, episodic background contamination persisted even after the spiking level was increased. In the lowest spiking set, one replicate displayed elevated background level, increasing the replicate RSD. To mitigate this issue, we recommend preparing samples in a laboratory space where TFA has never been used during any laboratory processes, such as using it as a mobile-phase additive.

Field sample analysis: landfill groundwater

A range of groundwater samples collected for landfill monitoring were analyzed from multiple sites across seven different landfills, and the results are presented in Figure 5. TFA was detected in every sample followed in frequency by PFPrA, PFMeS, DFA, and PFBA. Compared with the laboratory synthetic water, these groundwater samples exhibited less variation in retention time, with RTs matching the calibration standards exactly. Internal standard performance was also consistent and reliable recoveries between 66% and 99%.

PFBA analysis overlapped between EPA 1633A and the direct-injection method presented here. Because EPA 1633A includes SPE extraction and concentration, lower detection levels were achieved compared with the direct-injection workflow. To enable a consistent comparison, concentrations slightly below the MDL for the direct-injection method were reported for PFBA down to 3 ng/L. Concentrations for the same set of samples were compared, as shown in Figure 6, and good comparability between the methods across the concentration range evaluated. In addition, PFBA results generated using the Altura and Poroshell PFAS columns showed a strong linear correlation ($r = 0.84$, $p < 0.001$), indicating that the two columns (one from an external collaborator) track PFBA levels consistently in the tested matrices. As expected, these correlations demonstrate agreement in overall trends.

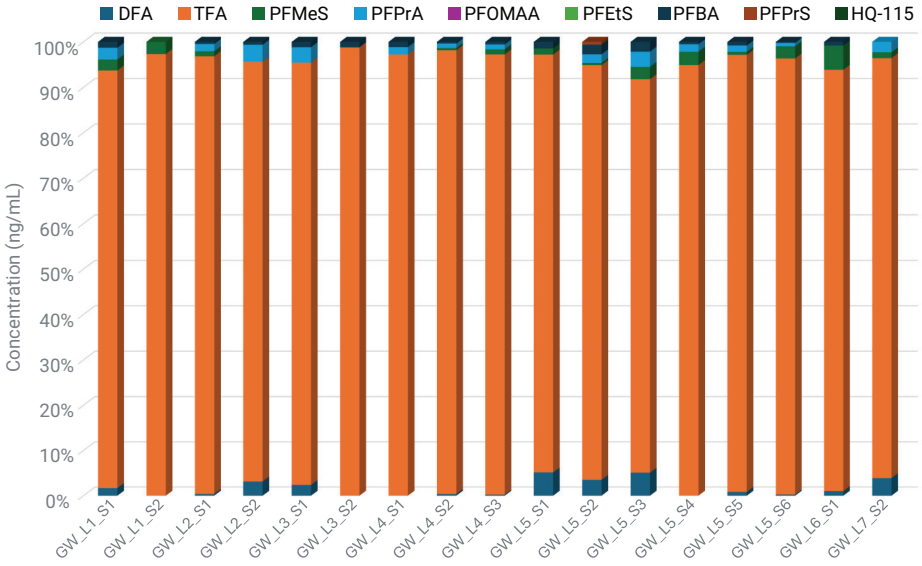

Figure 5. Sample results from landfill groundwater monitoring wells. Landfills are indicated by LX and then site number.

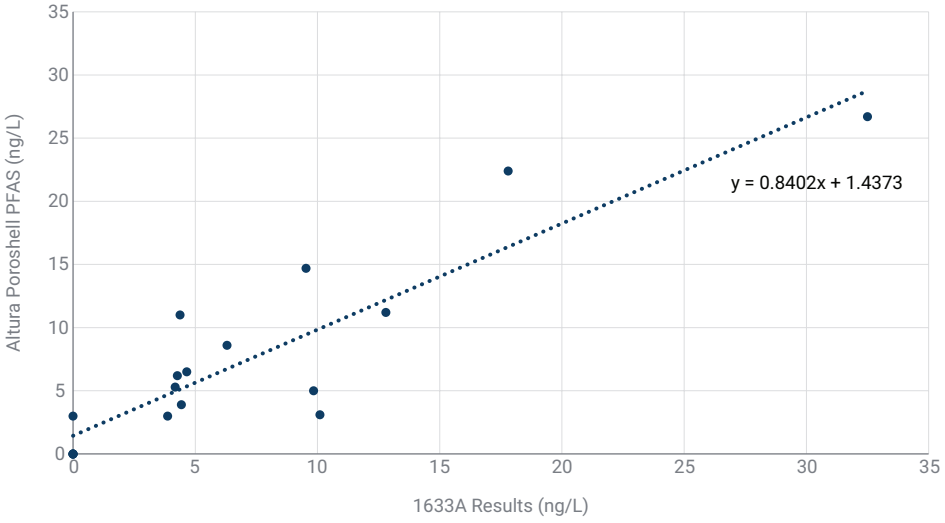

Figure 6. Graph comparing PFBA results from 1633A analysis and method presented using the Agilent Poroshell PFAS column.

Conclusion

The Agilent Altura Poroshell PFAS column paired with Agilent LC/TQ technology delivers stable chromatography for ultrashort-chain PFAS in both RO water and a high-ionic-strength synthetic water. Calculated detection limits in this study were in the low-ng/L range, and TFA and PFMeS were blank-limited under the conditions evaluated because method-blank levels exceeded spike-based estimates. Retention-time stability was maintained across ionic strength, with a maximum observed shift of 0.1 min (PFPrA). In synthetic water matrix spikes, most analytes met 70% to 130% recovery with < 15% RSD, except for DFA and TFA; DFA performance was impacted by high-salt matrix effects and the lack of a DFA isotopically labeled analog (surrogate correction used $^{13}C_2$-TFA). Field testing of groundwater from seven landfills showed retention times matching calibration standards and consistent internal-standard behavior.

References

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