Integrating the Analysis of Ultrashort-Chain PFAS

Method Development for Simultaneous Analysis of Ultrashort-Chain, Alternative, and Legacy PFAS

By Shun-Hsin Liang and Mike Chang

Abstract

A simple direct injection LC-MS/MS method was developed and evaluated for the simultaneous analysis of ultrashort-chain, alternative, and legacy per- and polyfluoroalkyl substances (PFAS) in various water samples. This method is recommended for labs wanting to use a single procedure to analyze compounds from all three PFAS categories in potable and non-potable waters.

Introduction

LC-MS/MS methods for the analysis of legacy short-chain (C4, C5) and long-chain (>C5) per- and polyfluoroalkyl substances (PFAS) based on reversed-phase (RP) chromatography are well established. With proper modification, these methods often can also be used for LC-MS/MS analysis of alternative PFAS, such as HFPO-DA (GenX) and ADONA, which are perfluoroalkyl ether carboxylic acids used as PFOA substitutes. Similarly, F-53B is a PFOS alternative produced in China that contains two polyfluoroalkyl ether sulfonate components, 9Cl-PF3ONS and 11Cl-PF3OUdS, which are included as analytes in the updated EPA 537.1 method. However, current LC methods may not be suitable for the analysis of newly trending ultrashort-chain (C2, C3) PFAS, mainly due to their insufficient retention on typical RP columns.

While the use of short-chain PFAS (PFBA and PFBS) is intentional, numerous studies have shown the ubiquitous occurrence of C2 and C3 PFAS in aqueous environmental samples [1,2]. These include trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), perfluoroethane sulfonate (PFEtS), and perfluoropropane sulfonate (PFPrS). It was shown that PFPrA is the predominant PFAS (up to 45% of total detectable PFAS) in rain and snow samples collected from the U.S., France, and Japan [3]. To date, there is no definitive identification of contamination sources and levels for these ultrashort-chain PFAS, but a recent study detected PFEtS and PFPrS in aqueous film-forming foams (AFFFs) and groundwaters from 11 military bases in the U.S. (often used for fire department training exercises) [4], indicating AFFF firefighting foam may be a source of ultrashort-chain PFAS.

Currently, methods that allow the desired combined analysis of ultrashort-chain PFAS with alternative and legacy PFAS are very rare. To fill this gap, we developed a procedure for the simultaneous quantification of a wide range of chain lengths and structures including C3, C4, C8, and alternative PFAS in a variety of water samples.

Experimental

Sample Preparation

In a polypropylene vial, 250 µL water sample aliquots were mixed with 250 µL of 40:60 reagent water:methanol and 5 µL of internal standard solution (5 ng/mL of $^{13}C_2$-PFHxA, $^{13}C_2$-PFOA, $^{13}C_4$-PFOS in methanol). The vial was capped with a polyethylene cap and injected for analysis.

Calibration Standards and Quality Control Samples

Calibration standards were prepared by fortifying reagent water (Optima LC-MS water) with ten PFAS analytes across a range of 5–400 ng/L. The calibration standard solutions were then processed as described in the sample preparation procedure.

Fortified Water Samples

Samples of tap water collected from the Restek facility and three diverse water types (Chicago river water, groundwater, and publicly owned treatment works [POTW] effluent water) supplied by the U.S. EPA were included in this study. Each water type was fortified at 10 (20 ppt for PFPrA) and 80 ppt in duplicate per batch with a total of three batches being prepared and analyzed on different days. Unfortified and fortified water samples were processed through the sample preparation procedure for chromatographic analysis and quantification.

Instrument Conditions

LC-MS/MS analysis of ultrashort-chain PFAS concurrently with alternative and legacy PFAS was performed using a Raptor C18 analytical column and a Shimadzu Nexera X2 HPLC coupled to a SCIEX 4500 MS/MS. A PFAS delay column (cat.# 27854) was installed between the pump mixer and the injector in order to prevent coelution of any instrument-related PFAS with target analytes in the sample. Instrument conditions were as follows and analyte transitions are provided in Table I.

Analytical column:	Raptor C18 2.7 µm, 100 mm x 3.0 mm (cat.# 9304A1E)	
Delay column:	PFAS delay column (cat.# 27854)	
Mobile phase A:	5 mM ammonium acetate in water	
Mobile phase B:	Methanol	
Gradient	Time (min)	%B
	0.00	20
	7.00	95
	9.00	95
	9.01	20
	11.0	20
Flow rate:	0.25 mL/min	
Run time:	11 min	
Injection volume:	10 µL	
Column temp.:	40 °C	
Ion mode:	Negative ESI	
Ion spray voltage:	-2000	
Source temp.:	450 °C	

Table I: Ion Transitions for LC-MS/MS Analysis of Ultrashort-Chain PFAS Concurrently with Alternative and Legacy PFAS.

Peak ID	Compound	Precursor Ion	Product Ion	Internal Standard
1	PFPrA	162.9	119.0	$^{13}C_2$-PFHxA
2	PFBA	212.8	169.0	$^{13}C_2$-PFOA
3	PFPrS	248.8	79.6	$^{13}C_2$-PFHxA
4	PFBS	298.8	79.9	$^{13}C_2$-PFHxA
5	$^{13}C_2$-PFHxA	314.9	270.0	—
6	HFPO-DA	285.0	168.9	$^{13}C_2$-PFOA
7	ADONA	376.9	250.7	$^{13}C_2$-PFOA
8	PFOA	413.1	368.9	$^{13}C_2$-PFOA
9	$^{13}C_2$-PFOA	415.0	370.0	—
10	PFOS	498.8	80.0	$^{13}C_4$-PFOS
11	$^{13}C_4$-PFOS	503.0	80.0	—
12	9Cl-PF3ONS	530.8	350.7	$^{13}C_4$-PFOS
13	11Cl-PF3OUdS	630.7	451.0	$^{13}C_4$-PFOS

Results and Discussion

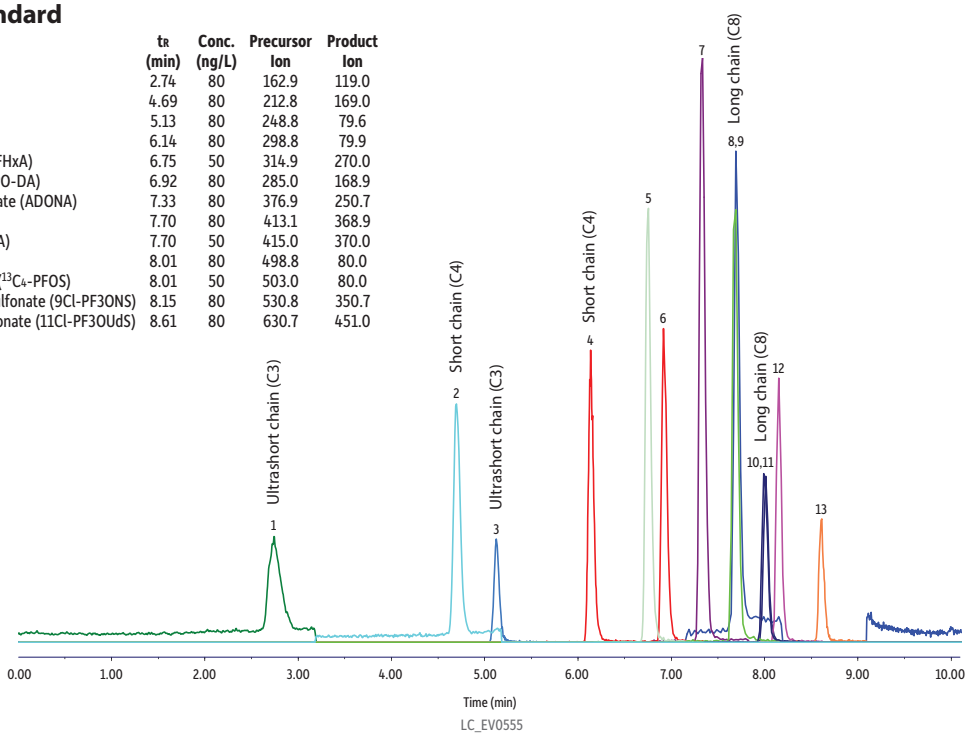
Chromatographic Performance

Analyte peak shapes, retention, and intensity were similar across the reagent water and field water samples. There was a higher baseline for the PFPrA signal in all field water samples (Figure 1), but this did not have a negative impact on peak integration and quantification of PFPrA. No matrix interference was observed for all water samples prepared by two-fold dilution.

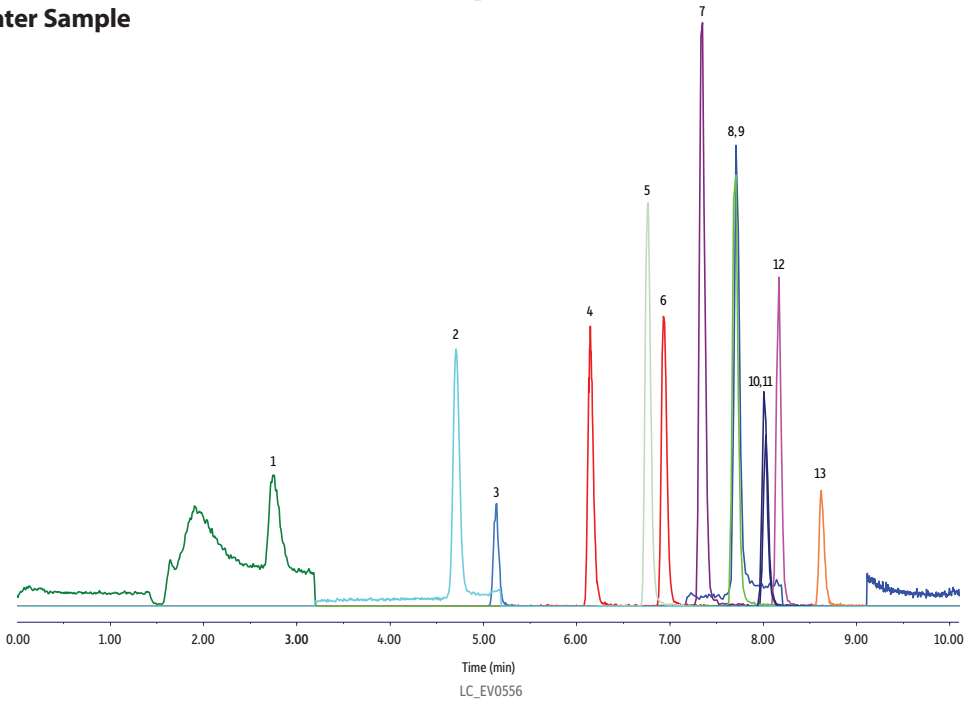
Figure 1: Good chromatographic peak shapes and adequate retention were obtained for the simultaneous LC-MS/MS analysis of ultrashort-chain PFAS along with alternative and legacy PFAS in various reagent and field water samples.

80 ppt Reagent Water Standard

Peaks	t_R (min)	Conc. (ng/L)	Precursor Ion	Product Ion
1. Perfluoropropanoic acid (PFPrA)	2.74	80	162.9	119.0
2. Perfluorobutanoic acid (PFBA)	4.69	80	212.8	169.0
3. Perfluoropropanesulfonic acid (PFPrS)	5.13	80	248.8	79.6
4. Perfluorobutanesulfonic acid (PFBS)	6.14	80	298.8	79.9
5. Perfluoro-*n*-[1,2-$^{13}C_2$]hexanoic acid ($^{13}C_2$-PFHxA)	6.75	50	314.9	270.0
6. Hexafluoropropylene oxide-dimer acid (HFPO-DA)	6.92	80	285.0	168.9
7. Ammonium 4,8-dioxa-3H-perfluorononanoate (ADONA)	7.33	80	376.9	250.7
8. Perfluorooctanoic acid (PFOA)	7.70	80	413.1	368.9
9. Perfluoro-[1,2-$^{13}C_2$]octanoic acid ($^{13}C_2$-PFOA)	7.70	50	415.0	370.0
10. Perfluorooctanesulfonic acid (PFOS)	8.01	80	498.8	80.0
11. Perfluoro-[1,2,3,4-$^{13}C_4$]octanesulfonic acid ($^{13}C_4$-PFOS)	8.01	50	503.0	80.0
12. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)	8.15	80	530.8	350.7
13. 11-Chloroeicosafluoro-3-oxanonane-1-sulfonate (11Cl-PF3OUdS)	8.61	80	630.7	451.0



80 ppt Fortified Groundwater Sample



Column
Dimensions: 100 mm x 3 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Temp.: 40 °C

Sample
Conc.: 80 ppt
Inj. Vol.: 10 µL

Mobile Phase
A: Water, 5 mM ammonium acetate
B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.25	80	20
7.00	0.25	5	95
9.00	0.25	5	95
9.01	0.25	80	20
11.0	0.25	80	20

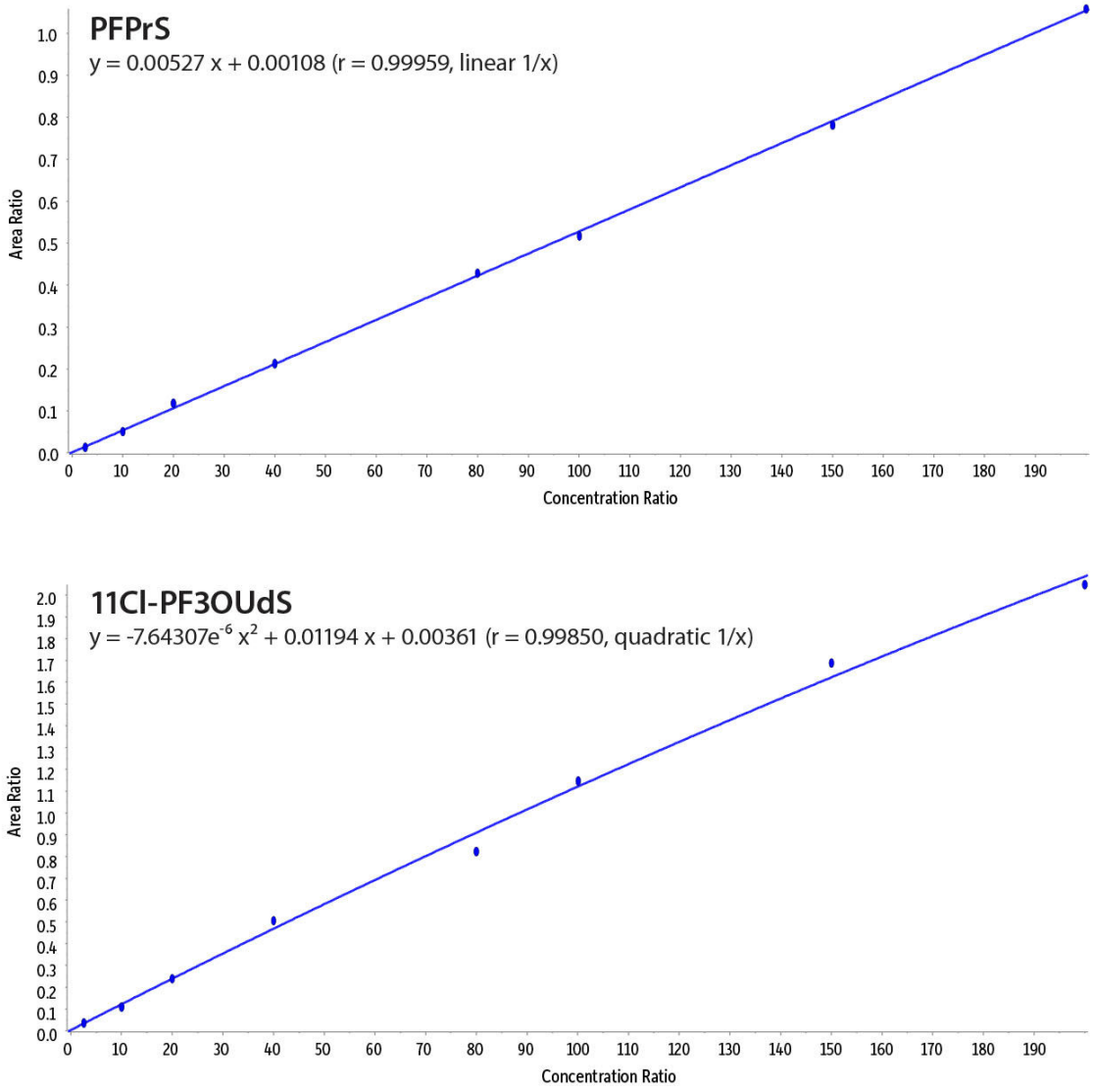
Detector MS/MS
Ion Mode: ESI-
Mode: MRM
Instrument UHPLC
Notes A PFAS delay column (cat.# 27854) was installed between the pump mixer and the injector.

In a polypropylene vial, 250 µL of ground water sample (fortified at 80 ppt) was mixed with 250 µL of 40:60 reagent water:methanol and 5 µL of internal standard solution (5 ng/mL of $^{13}C_2$-PFHxA, $^{13}C_2$-PFOA, $^{13}C_4$-PFOS in methanol). The vial was capped with a polyethylene cap prior to analysis.

Linearity

In this LC-MS/MS method for the simultaneous analysis of ultrashort-chain PFAS along with alternative and legacy PFAS, the calibration range was 10-400 ppt for PFPrA and 5-400 ppt for all other analytes. All compounds showed acceptable linearity with r values ≥ 0.999 and deviations of <20%. 11Cl-PF3OUdS is the only analyte that was quantified using a quadratic regression (1/x weighted) standard curve. All other analytes were quantified using a 1/x weighted linear regression (Figure 2).

Figure 2: Standard Curves



Accuracy and Precision

The unfortified water samples showed various levels of C3, C4, and C8 PFAS with no detectable PFPrS, ADONA, HFPO-DA, 9Cl-PF3ONS, and 11Cl-PF3OUdS (Table II). For accuracy (%recovery) calculation, the measured amount of analyte in the fortified sample was adjusted based on the unfortified concentration. Table III shows the accuracy and precision results calculated across all three batches of data. Method accuracy was demonstrated by recovery values that were within 20% of the nominal concentration for both fortified sample levels, as well as for the LLOQ concentration standard prepared in reagent water. The %RSD was <15%, indicating acceptable method precision.

Table II: PFAS Concentrations in Unfortified Water Samples.

	Detected Concentration (ng/L)									
	PFPrA	PFBA	PFPrS	PFBS	HFPO-DA	ADONA	PFOA	PFOS	9Cl-PF3ONS	11Cl-PF3OUdS
Tap Water	ND	1.1	ND	ND	ND	ND	ND	ND	ND	ND
River Water	ND	1.6	ND	ND	ND	ND	ND	ND	ND	ND
Groundwater	9.0	3.4	ND	2.6	ND	ND	ND	ND	ND	ND
POTW Water	11.7	10.6	ND	3.0	ND	ND	15.0	6.0	ND	ND

Table III: Accuracy and Precision.

	Average %Recovery (%RSD)								
	Tap Water		River Water		Groundwater		POTW Water		Reagent Water
Conc. (ng/L)	10*	80	10*	80	10*	80	10*	80	5** (LLOQ)
PFPrA	96.9 (11.0)	105 (3.91)	105 (6.57)	95.4 (6.84)	92.0 (9.54)	99.4 (7.40)	94.2 (5.29)	87.2 (8.18)	103 (10.9)
PFBA	99.3 (9.19)	108 (1.81)	108 (5.20)	110 (1.70)	104 (8.21)	108 (6.68)	108 (8.12)	97.1 (8.17)	97.9 (12.0)
PFPrS	100 (4.24)	107 (3.14)	103 (6.71)	105 (2.64)	105 (8.48)	109 (6.68)	109 (5.65)	103 (9.28)	99.1 (8.59)
PFBS	101 (5.20)	106 (1.84)	99.7 (7.54)	105 (2.10)	100 (6.57)	106 (2.82)	103 (1.93)	97.8 (5.85)	96.0 (8.75)
HFPO-DA	96.2 (7.86)	102 (4.64)	96.2 (4.99)	105 (3.94)	95.0 (3.59)	101 (8.92)	92.9 (4.87)	90.3 (7.77)	99.3 (8.54)
ADONA	101 (6.23)	106 (3.82)	97.6 (6.36)	106 (2.32)	98.4 (2.68)	105 (4.08)	98.2 (7.09)	98.2 (7.09)	102 (10.3)
PFOA	105 (8.65)	105 (3.70)	108 (12.1)	107 (3.63)	108 (9.66)	105 (5.26)	99.9 (10.5)	94.5 (7.24)	100 (9.05)
PFOS	99.3 (2.10)	108 (4.24)	112 (1.87)	107 (4.93)	101 (2.96)	102 (2.31)	104 (4.46)	98.3 (5.82)	94.3 (8.85)
9Cl-PF3ONS	95.6 (4.60)	106 (5.93)	105 (5.37)	110 (8.20)	97.2 (4.52)	107 (7.41)	101 (6.52)	99.8 (4.89)	98.8 (5.47)
11Cl-PF3OUdS	114 (8.78)	112 (8.91)	102 (15.0)	91.5 (2.34)	96.7 (5.99)	105 (15.2)	115 (2.67)	103 (8.45)	105 (8.04)

*20 ng/L for PFPrA
**10 ng/L for PFPrA

Conclusion

A robust LC-MS/MS method for quantitating a diverse range of PFAS of various chain lengths and structures utilizing a simplified direct injection approach was successfully evaluated in different water sample matrices. Using a Raptor C18 (2.7 µm) 100 x 3.0 mm column with a PFAS delay column, the analytical method was demonstrated to be fast, rugged, and sensitive with acceptable accuracy and precision. This method is suitable for labs needing to report an extended list of PFAS compounds, including the analysis of ultrashort-chain PFAS, for potable and non-potable water testing.

References

[1] S. Taniyasu, K. Kannan, L.W.Y. Yeung, K.Y. Kwok, P.K.S Lam, N. Yamashita, Analysis of trifluoroacetic acid and other short-chain perfluorinated acids (C2-C4) in precipitation by liquid chromatography-tandem mass spectrometry: comparison to patterns of long-chain perfluorinated acids (C5-C18), Anal. Chim. Acta. 619 (2008) 221-230.

[2] J. Janda, K. Nodler, H-J. Brauch, C. Zwiener, F.T. Lange, Robust trace analysis of polar (C2-C8) perfluorinated carboxylic acids by liquid chromatography-tandem mass spectrometry: method development and application to surface water, groundwater, and drinking water, Environ. Sci. Pollut.R. 26 (2018) 7326-7336.

[3] K.Y. Kwok, S. Taniyasu, L.W.Y. Yeung, M.B. Murphy, P.K.S. Lam, Y. Horii, K. Kannan, G. Petrick, R.K. Sinha, N. Yamashita, Flux of perfluorinated chemicals through wet deposition in Japan, the United States, and other countries, Environ. Sci. Technol. 44 (2010) 7043-7049.

[4] K.A. Barzen-Hanson, J.A. Field, Discovery and implications of C2 and C3 perfluoroalkyl sulfonates in aqueous film-forming foams and groundwater, Environ. Sci. Technol. Lett. 5 (2015) 95-99.

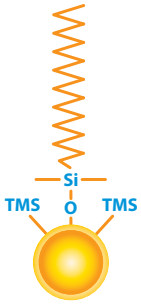
Raptor C18 LC Columns (USP L1)

Chromatographic Properties

When you need a general-purpose LC column, don't just grab any C18. Choose the speed, efficiency, and long-lasting ruggedness of the Raptor C18. This traditional end-capped C18 offers the highest hydrophobic retention of any Raptor phase, and it is compatible with a wide range of mobile phases from moderately acidic to neutral (pH 2–8). Whether for food safety or environmental or bioanalytical analyses, this phase offers consistently excellent data quality in less time across myriad reversed-phase applications, matrices, and compound classes. To lower costs and improve profitability, you need columns to last longer, data to be reproducible, and existing HPLC and UHPLC instrumentation to run faster. Get there with the only general-purpose C18 that gives you *Selectivity Accelerated*.

Column Characteristics:

- Stationary Phase Category: C18, octadecylsilane (L1)
- Ligand Type: End-capped C18
- Particle: 1.8 µm, 2.7 µm, or 5 µm superficially porous silica (SPP or "core-shell")
- Pore Size: 90 Å
- Carbon Load: 9% (1.8 µm), 7% (2.7 µm), 5% (5 µm)
- End-Cap: yes
- Surface Area: 125 m^2/g (1.8 µm), 130 m^2/g (2.7 µm), or 100 m^2/g (5 µm)
- Recommended Usage:
  - pH Range: 2.0–8.0
  - Maximum Temperature: 80 °C
  - Maximum Pressure: 1,034 bar/15,000 psi* (1.8 µm), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)



* For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.

Properties:

- Compatible with moderately acidic to neutral mobile phases (pH 2–8).
- Excellent data quality in food, environmental, bioanalytical, and other applications.

Switch to a C18 when:

- You need a general-purpose column for reversed-phase chromatography.
- You need to increase retention of hydrophobic compounds.

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9304232	—	—
50 mm	9304252	930425E	—
100 mm	9304212	930421E	—
150 mm	9304262	—	—
2.7 µm Columns			
30 mm	9304A32	9304A3E	9304A35
50 mm	9304A52	9304A5E	9304A55
100 mm	9304A12	9304A1E	9304A15
150 mm	9304A62	9304A6E	9304A65
5 µm Columns			
30 mm	—	930453E	—
50 mm	9304552	930455E	9304555
100 mm	9304512	930451E	9304515
150 mm	9304562	930456E	9304565
250 mm	—	—	9304575

Column Characteristics:

- Particle: 5 µm, spherical, fully porous
- pH Range: 2.5 to 8
- Maximum Temperature: 80 °C
- Maximum Pressure: 1,034 bar/15,000 psi

PFAS Delay Column

Chromatographic Properties

Per-and polyfluorinated alkyl substances (PFAS) are a group of widely used industrial compounds that resist degradation and have become ubiquitous in the environment and in human samples from around the world. Because of their inertness, PFAS are used in tubing and other liquid-contacting parts of an HPLC system where they can leach into the mobile phase and interfere with sample analysis. This contamination is particularly problematic when analyzing at trace levels, such as at the ppt health advisory levels for drinking water. With many labs analyzing at single-digit ppt levels and striving for sub-ppt level detection, PFAS contamination from HPLC components can prevent accurate identification and quantitation of PFAS in samples. To eliminate this issue, Restek's new PFAS delay column traps and "delays" system-related PFAS, preventing them from interfering with sample analysis. This delay column is a universal solution that can be used with any type of HPLC or UHPLC system up to 15,000 psi (1034 bar) and with any analytical column (fully porous or superficially porous).

Length	2.1 mm ID cat.#
5 µm Columns	
50 mm	27854

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