Eliminate the Impact of Instrument-Related PFAS Interferences by Using a Delay Column

- Traps and "delays" system-related PFAS, preventing interference.
- Allows accurate identification and quantitation of trace-level PFAS in samples.
- Highly retentive—no breakthrough even with extended equilibration times.

Poly- and perfluoroalkyl substances, or PFAS, are rapidly emerging as some of the most important environmental contaminants to monitor around the world. Their widespread use and environmental persistence make them truly a global issue. Concerns over possible health risks are driving environmental scientists to look for these compounds everywhere from penguins [1] in the south to polar bears [2] in the north, and many points in between, and they are discovering the extent of PFAS contamination is literally pole to pole [3,4]. However, one other concerning place where at least some of these compounds are present is inside the very instruments used for PFAS analysis of environmental samples. Background PFAS contamination can interfere with low-level quantification requirements, which are usually in the range of parts per trillion (ppt) or below. Instrument-related background contamination can stem from LC components that contain fluoropolymers (e.g., PTFE), which can leach into mobile phase solutions. Using tubing and containers without fluorochemicals and switching instrument components to PEEK or stainless steel mitigates some of the risk of background contamination, but it does not eliminate the impact of interferences on trace-level analysis, so another solution is required.

In a standard setup, instrument-related PFAS interferences move through the system with the mobile phase and collect at the head of the analytical column between sample injections. However, adding a second column, called a "delay column," into the mobile phase flow path immediately before the injector is an effective way catch the background PFAS before they reach the analytical column (Figure 1). In this scenario, when the sample is injected, only PFAS in the sample will focus at the head of the analytical column. Then, during the course of a gradient analysis, PFAS from the sample will begin to move through the analytical column like normal. The background PFAS trapped in the delay column during equilibration will be eluted and pass through the analytical column as well. However, the background PFAS will elute later than the sample PFAS because they entered the analytical column after the injected sample due to additional retention by the delay column prior to reaching the injector. In addition, the background PFAS never focus on the head of the analytical column because they are present in the flow path in continuum rather than as a discrete injection. The net effect is that by using a delay column you can differentiate instrument-related PFAS background contamination from target PFAS in a sample.

Figure 1: Installation Position of the PFAS Delay Column.

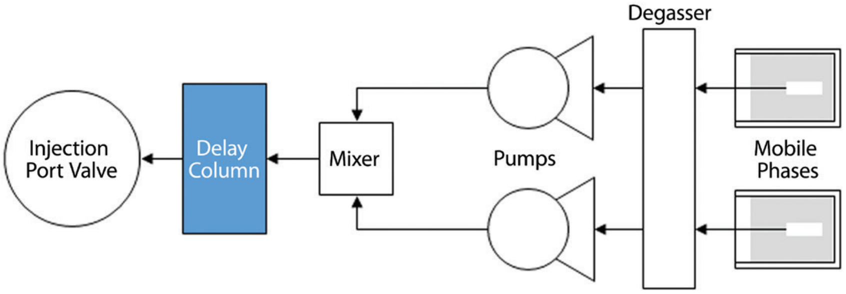
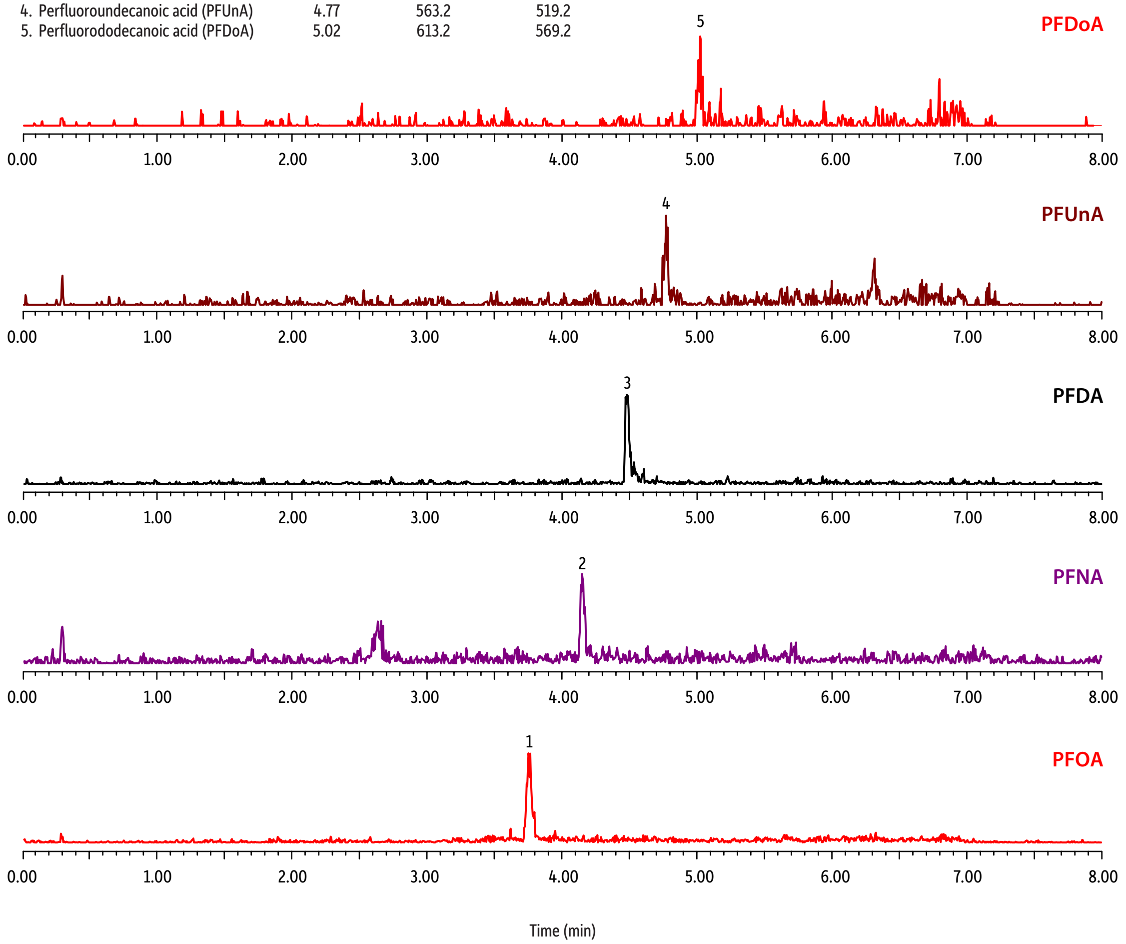


Figure 2 is an example of the kind of background contamination that interferes with accurate PFAS analysis. The system was equilibrated for 10 minutes without a delay column to allow instrument-related PFAS interferences to accumulate at the head of the analytical column before a blank injection. In this case, five potential target compounds were observed in the instrument blank. Which compounds are present and to what degree they are observed ultimately depends on the specific analytical setup. System-related PFAS contamination can be affected by factors such as instrument make and model, tubing and fitting materials, mobile phase solvent grade, and analytical method conditions.

Figure 2: System-related PFAS contamination is present in a blank sample analyzed without a delay column installed.

Peaks	t_R (min)	Precursor Ion	Product Ion
1. Perfluorooctanoic acid (PFOA)	3.75	413.2	369.1
2. Perfluorononanoic acid (PFNA)	4.15	463.2	419.2
3. Perfluorodecanoic acid (PFDA)	4.48	513.2	469.2
4. Perfluoroundecanoic acid (PFUnA)	4.77	563.2	519.2
5. Perfluorododecanoic acid (PFDoA)	5.02	613.2	569.2



LC_EV0549

Column	Raptor C18 (cat.# 9304252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	Blank injection without delay column after 10 minutes of equilibration.
Diluent:	80:20 Water:methanol
Inj. Vol.:	5 µL

Mobile Phase

A: Water, 5mM ammonium acetate

B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.50	0.4	5	95
6.51	0.4	80	20
8.00	0.4	80	20

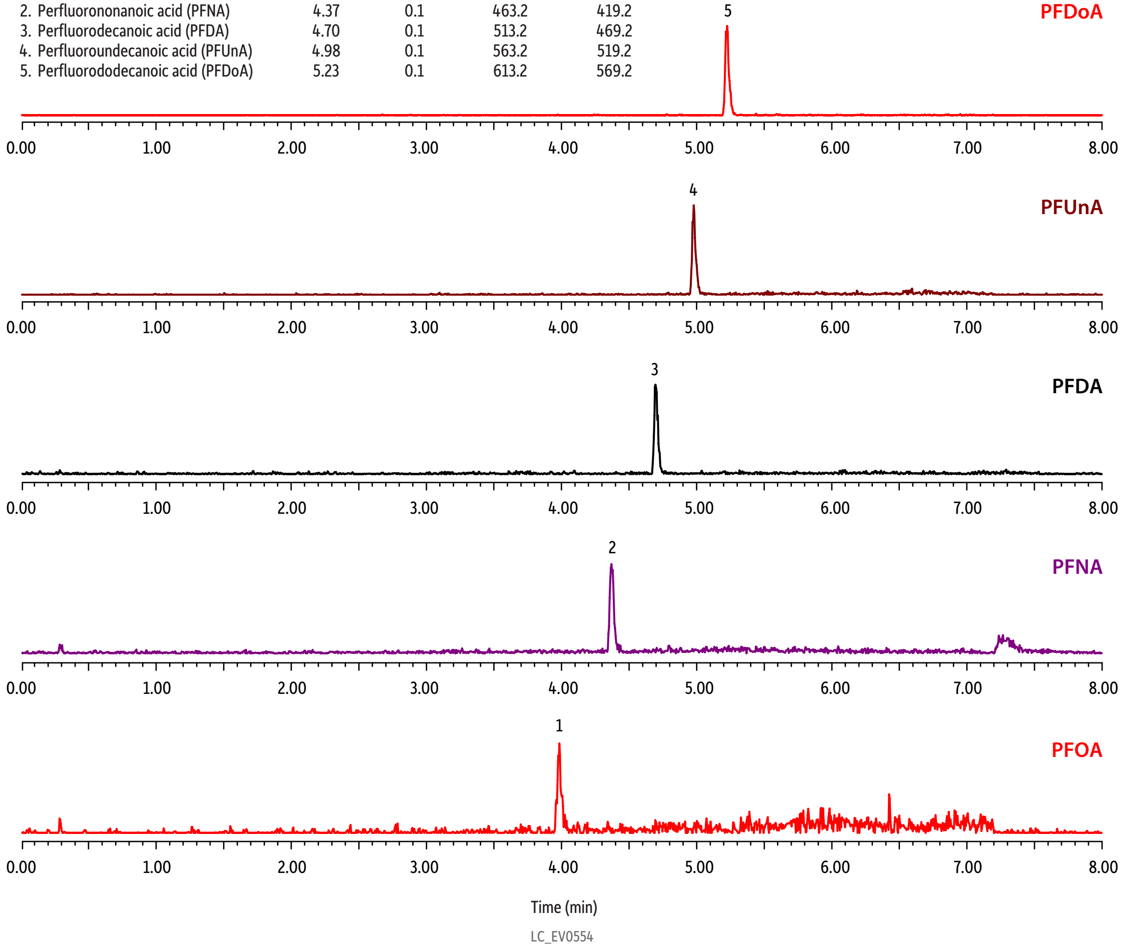
Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC

Adding a delay column to the flow path is an effective way to retain background-related PFAS interferences so they do not coelute with PFAS from the sample during analysis. When the experiment in Figure 2 was repeated with a delay column installed, there were no observable peaks in the blank injection at the retention time for each PFAS previously found, even after relatively long equilibration times. Instead, the background PFAS interferences from the instrument eluted in a wide band after the target retention time. Because band broadening occurs while the background PFAS compounds are being retained by the delay column during equilibration, the signal for background PFAS does not appear as a well-defined peak once the background PFAS elute. Instead, the background signal is observed as a region of slightly elevated baseline (Figure 4).

Since the delay column adds additional length to the flow path between the mixer and the injector, target analyte retention times also shift slightly because of the longer time it takes for mobile phase changes to reach the analytical column during a gradient analysis (Figure 3). The net effect of a PFAS delay column is demonstrated in Figure 4: target analyte retention times are distinct from the instrument-related PFAS elution region, so there is no interference from the instrument background during sample analysis. Note that the elution time regions for instrument-related PFAS shown in Figure 4 were determined by lengthening the equilibration times and observing the baseline deflection caused by a broad band of delayed instrument-related PFAS eluting from the analytical column.

Figure 3: The expected retention times for PFAS in samples with a delay column installed were established using a prepared standard.

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Perfluorooctanoic acid (PFOA)	3.99	0.1	413.2	369.1
2. Perfluorononanoic acid (PFNA)	4.37	0.1	463.2	419.2
3. Perfluorodecanoic acid (PFDA)	4.70	0.1	513.2	469.2
4. Perfluoroundecanoic acid (PFUnA)	4.98	0.1	563.2	519.2
5. Perfluorododecanoic acid (PFDoA)	5.23	0.1	613.2	569.2



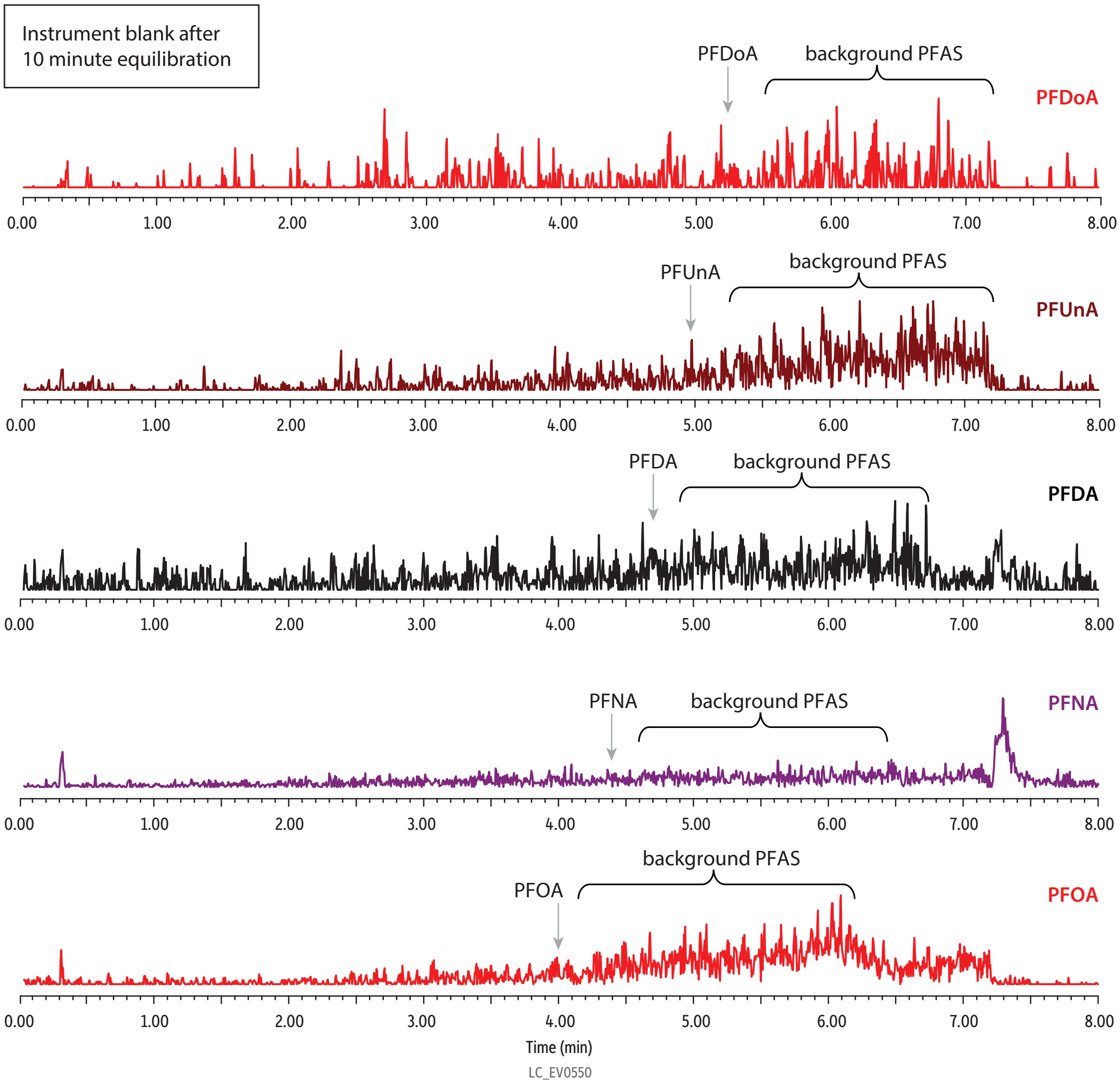
Column	Raptor C18 (cat.# 9304252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	
Diluent:	80:20 Water:methanol
Conc.:	0.1 ng/mL
Inj. Vol.:	5 µL

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

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.50	0.4	5	95
6.51	0.4	80	20
8.00	0.4	80	20

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

Figure 4: Using a delay column eliminates the impact of instrument-related PFAS interferences by delaying their elution until after PFAS from the sample have eluted. No sample PFAS were detected. Arrows indicate expected retention time.



	Peaks	Precursor Ion	Product Ion
1.	Perfluorooctanoic acid (PFOA)	413.2	369.1
2.	Perfluorononanoic acid (PFNA)	463.2	419.2
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4.	Perfluoroundecanoic acid (PFUnA)	563.2	519.2
5.	Perfluorododecanoic acid (PFDoA)	613.2	569.2

Column	Raptor C18 (cat.# 9304252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	Blank injection with delay column (cat.# 27854) after 10 minutes of equilibration.
Diluent:	80:20 Water:methanol
Inj. Vol.:	5 µL

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

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.50	0.4	5	95
6.51	0.4	80	20
8.00	0.4	80	20

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC

In summary, using a PFAS delay column can eliminate the detrimental impact of background PFAS interferences from instrument-related sources by retaining contaminants prior to the analytical column and eluting them only after the sample has been injected and the gradient elution has started. The elution delay between the target PFAS in the sample and the background PFAS from the LC system is enough to separate them sufficiently to allow for accurate quantification of the target compound in the sample.

References

[1] A. Schiavone, S. Corsolini, K. Kannan, L. Tao, W. Trivelpiece, et. al. Perfluorinated contaminants in fur seal pups and penguin eggs from South Shetland, Antarctica, Sci. Total., 407 (12) (2009) 3899-904. https://doi.org/10.1016/j.scitotenv.2008.12.058

[2] S. Tartu, S. Bourgeon, J. Aars, M. Andersen, et al. Diet and metabolic state are the main factors determining concentrations of perfluoroalkyl substances in female polar bears from Svalbard, Environ. Pollut., 229 (2017) 146-158. https://doi.org/10.1016/j.envpol.2017.04.100

[3] K. Rankin, S. Mabury, T, Jenkins, J. Washington, A North American and global survey of perfluoroalkyl substances in surface soils: Distribution patterns and mode of occurrence, Chemosphere, 161 (2016) 333-41. https://doi.org/10.1016/j.chemosphere.2016.06.109

[4] B. Gonzalez-Gaya, J. Dachs, J Roscales, G. Cabellero, B. Jiminez, Perfluoroalkylated Substances in the Global Tropical and Subtropical Surface Oceans, Environ. Sci. Technol., 48 (22) (2014) 13076-84. https://pubs.acs.org/doi/abs/10.1021/es503490z

Raptor C18 LC Columns (USP L1)

Description	cat.#
1.8 µm Columns	
50 mm, 2.1 mm ID	9304252

similar **phases**

Accucore C18, Accucore RP-MS, ACE UltraCore Super C18, Ascentis Express C18, Cortecs C18, Halo 2.7 C18, Kinetex C18, Nucleoshell RP 18, Poroshell EC-C18, Sunshell C18

PFAS Delay Column

Description	cat.#
5 µm Columns	
50 mm, 2.1 mm ID	27854

Column Characteristics:

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

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