Chromatography Solutions

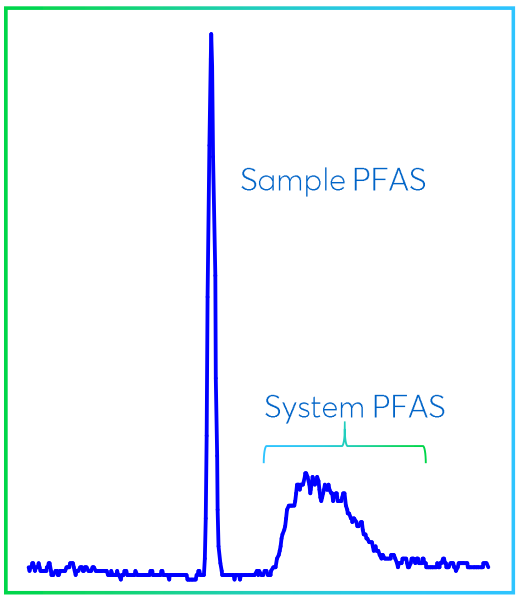
Technical note #035

Use of the Avantor® ACE® PFAS Delay Column to resolve background system interference in the LC-MS/MS determination of PFAS

INTRODUCTION

The ubiquitous use of poly and perfluoroalkyl substances (PFAS) in a wide range of industrial, commercial and consumer products, combined with their chemical inertness and longevity, means that widespread environmental PFAS contamination now exists on a global scale. Concerns over their impact to human health and the environment means that monitoring PFAS levels within natural and human environments is increasingly important.

One of the most powerful analytical techniques used for PFAS determination is LC-MS/MS. However, due to the presence of background PFAS within the laboratory environment, strict laboratory practices must be implemented to reduce the potential for inadvertent sample contamination or contamination of the chromatographic system which leads to inaccurate quantification. This technical note outlines one of the mandatory precautions that must be taken when using LC-MS/MS; the use of a PFAS Delay column to chromatographically separate native sample PFAS from



Avantor® ACE®

interfering background LC system PFAS peaks.

WHAT ARE PFAS?

Poly and perfluoroalkyl substances are rapidly emerging as one of the most important environmental contaminants for monitoring globally. PFAS comprise a large and complex group of synthetic substances, containing a fluorinated carbon backbone, which were first developed in the 1930's. Example PFAS chemical structures are shown in Figure 1. PFAS have been extensively manufactured and utilised since the 1940's, due to their useful physical properties, which include; oil, water and stain repellency, friction reduction and high thermal and chemical stability.[1] These advantageous properties have resulted in their widespread use in many industry sectors, firefighting applications and a wide range of consumer goods (e.g. stain repellents for clothing, carpets and upholstery, outdoor clothing and equipment, food packaging, etc.), amongst others. Over the past 80 years, the release of PFAS components into the natural environment, combined with their inherent chemical stability (which makes them highly persistent and has led to PFAS commonly being referred to as "forever chemicals"), has resulted in widespread documented environmental contamination in water, air, fish and soil.[1,2]

Their widespread use means that a variety of routes for human exposure to PFAS exist, including contaminated water sources, certain industrial processes, fire extinguishing foams, food, food packaging and household and personal care products. Additionally, many PFAS have the potential to bioaccumulate and biomagnify up food chains, potentially leading to high concentrations within the body over time.[3]

In recent decades, studies have linked exposure to certain levels of some PFAS to a variety of adverse health effects in humans.[4,5,6] Over the last 20 years, reduction in the manufacture and use of certain PFAS, identification of alternative substances and increasingly stringent regulatory restrictions of some PFAS have been implemented to begin to tackle the problem. Restrictions imposed by regulatory authorities are likely to increase substantially in the future. For example, PFOA and PFHxS are included Annex A of the Stockholm Convention to eliminate their use, whilst PFOS is listed in Annex B (restriction),[7] perfluorinated carboxylic acids (C9-C14) were restricted in the EU/EEA from February 2023,[8] and the European Chemicals Agency recently published a proposal to restrict over 10,000 PFAS substances under REACH regulations.[9] The need to monitor PFAS levels in the environment is therefore become becoming increasingly important.[4]

Increasing regulatory control over allowable PFAS concentrations in matrices such as drinking water means that highly sensitive analytical techniques that can achieve detection limits in the sub-parts per trillion level are typically required.[10] Liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) is widely used for the determination of PFAS in a variety of matrices due to the high level of sensitivity and specificity that can be achieved. The use of appropriate sample preparation techniques allows sample pre-concentration to achieve the low detection levels required.

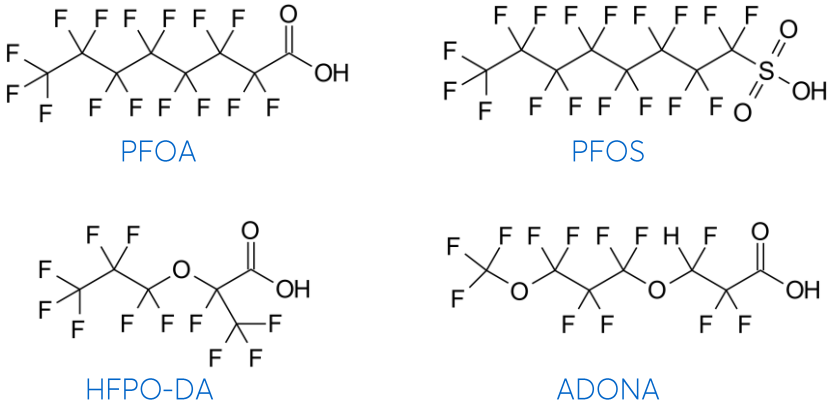


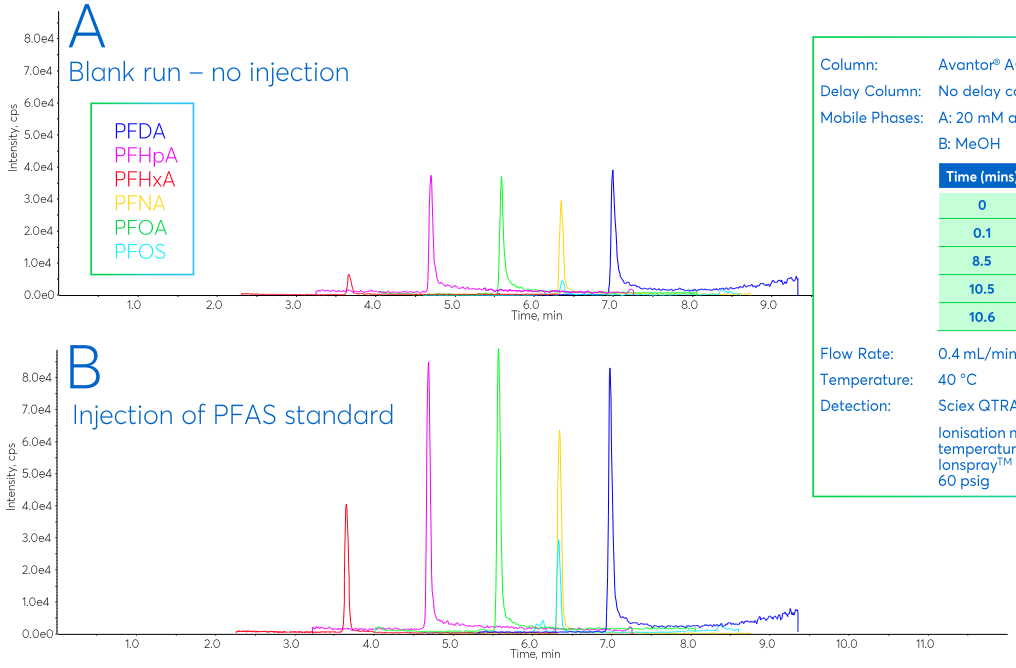
Figure 1: Example structures for some PFAS compounds.

SPECIAL CONSIDERATIONS FOR THE ANALYSIS OF PFAS

When testing for PFAS, special consideration must be given to the potential for background interference that may occur from PFAS derived from the laboratory environment, equipment and consumables used during sample collection, processing and subsequent analysis. The selection of consumables manufactured from appropriate materials, elimination of any PTFE containing materials from equipment, selection of appropriate laboratory solvents and reagents and methodical testing of individual workflow stages, together with implementation of rigorous laboratory practices aimed at reducing the potential for background contamination, are all vitally important.

The LC-MS system itself must also be considered as a source of PFAS contamination. LC-MS/MS analysis of PFAS typically employs a reversed-phase analytical column, operated under gradient conditions. Components within the flow path, such as solvent line tubing, degasser and pump components, along with the mobile phase, are all potential sources of background PFAS that can accumulate on the analytical column during mobile phase pre-gradient equilibration. During gradient analysis, these background components will be eluted from the column and will co-elute with sample analytes, thereby causing inaccurate quantification.

Figure 2a shows MRM chromatograms for PFAS components that were detected during a blank gradient run performed on a contaminated LC-MS system. These background PFAS were found to primarily originate from the solvent line tubing (which is often manufactured from PTFE or FEP), but may also originate from the degasser and pump modules, or the mobile phase. During a sample run (Figure 2b), the background system PFAS components were eluted with identical retention times to corresponding sample peaks and are therefore indistinguishable. Exchanging any PTFE or FEP tubing in the flow path for PEEK is highly recommended. Additionally, the use of a PFAS delay or trap column is a highly effective approach for eliminating interference from background PFAS and is typically regarded as mandatory.



Column:	Avantor® ACE® UltraCore 2.5 SuperC18, 100 x 2.1 mm
Delay Column:	No delay column used
Mobile Phases:	A: 20 mM ammonium acetate (aq) B: MeOH

Time (mins)	% B
0	5
0.1	40
8.5	90
10.5	90
10.6	5

Flow Rate:	0.4 mL/min
Temperature:	40 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS system. Ionisation mode: ESI, negative mode; Source temperature: 450 °C; Curtain gas: 30 psig; Ionspray™ source voltage: -4500 V; Ion source gas: 60 psig

Figure 2: A: LC-MS/MS MRM chromatograms for a blank gradient run on a column allowed to equilibrate under the initial gradient conditions for 3 minutes. The MRM transitions for PFAS components observed are displayed. B: The same MRM transitions for the injection of a standard mix, demonstrating the same retention time of sample and system PFAS components. Co-elution of system background PFAS with native sample PFAS will clearly result in inaccurate quantification.

USE OF THE AVANTOR® ACE® PFAS DELAY COLUMN

The Avantor® ACE® PFAS Delay Column is packed with a highly retentive reversed-phase material, designed to provide maximum retention of background system PFAS. The column should be plumbed into the LC system immediately before the sample injector and analytical column, as shown in Figure 3. When situated in this position, any background PFAS contamination within the mobile phase, solvent lines, degasser and pump components will be effectively retained on the PFAS Delay Column prior to sample analysis. Once the sample analysis begins, background PFAS that have accumulated on the delay column will be eluted from PFAS Delay Column under gradient conditions, but will elute later than the corresponding sample PFAS.

Figure 4 shows the result of a blank run on the same system as shown in Figure 2A, but with an Avantor® ACE® PFAS Delay Column installed. The background PFAS have been retained on the delay column and now elute significantly later than in Figure 2A, as a broad peak. For reference, the elution times for sample PFAS are denoted by arrows in Figure 4. The Avantor® ACE® PFAS Delay Column clearly retains background system PFAS beyond the expected retention time of native sample PFAS, eliminating the potential for interference.

The importance of using the PFAS Delay Column is best illustrated by examining the data obtained for one PFAS component, in this case PFOA. Figure 5A shows a blank run (no injection), performed on a contaminated LC-MS/MS system. Background PFOA has accumulated on the analytical column and is eluted during the gradient

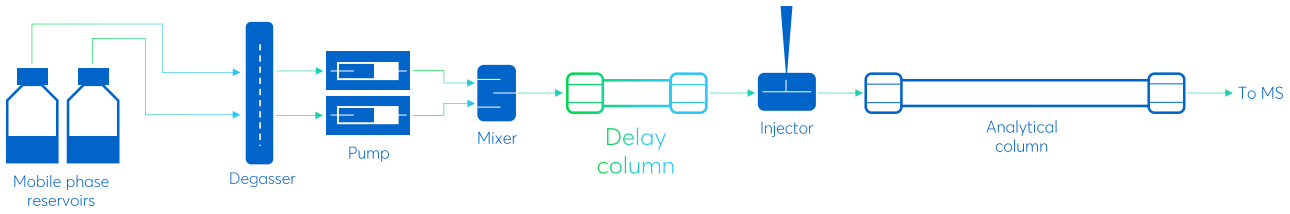
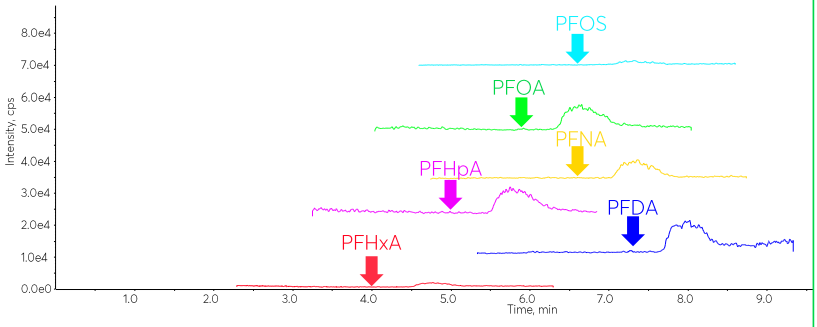


Figure 3: Schematic of a typical LC-MS/MS system. The Avantor® ACE® PFAS Delay Column should be plumbed into the flow path after the degasser, pump and mixer and immediately before the autosampler/injection port of the LC system.



Column:	Avantor® ACE® UltraCore 2.5 SuperC18, 100 x 2.1 mm
Delay Column:	Avantor® ACE® PFAS Delay Column, 50 x 2.1 mm
Mobile Phases:	A: 20 mM ammonium acetate (aq) B: MeOH

Time (mins)	% B
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Flow Rate:	0.4 mL/min
Temperature:	40 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS system. Ionisation mode: ESI, negative mode; Source temperature: 450 °C; Curtain gas: 30 psig; Ionspray™ source voltage: -4500 V; Ion source gas: 60 psig

Figure 4: LC-MS/MS MRM chromatograms (displayed offset on y-axis) for a blank gradient with an Avantor® ACE® PFAS Delay Column installed. The LC-MS system and analytical conditions were the same as those used in Figure 2. The system PFAS are held up by the delay column and elute as a broad peak and are retained longer than the sample peaks (expected elution times of sample peaks are denoted with arrows).

at 5.5 minutes. When a sample containing PFOA is injected (Figure 5B), the background PFOA co-elutes and cannot be distinguished from the sample PFOA, leading to inaccurate quantification and broader asymmetric (tailed) shaped peaks.

Figure 5C shows a blank gradient run on the same system with an Avantor® ACE® PFAS Delay Column installed. The background system PFOA now elutes much later as a broad peak. Injection of a PFAS standard with the PFAS Delay Column installed (Figure 5D) reveals that the sample PFOA elutes earlier than the background system PFAS, in a clean region of the baseline and is readily distinguished, therefore ensuring the integrity of analytical data obtained. Additionally, improved peak shape is also apparent. It is also worth noting that sample PFAS are retained marginally longer in Figure 5D than Figures 5A and 5B, which results from a ~100 µL increase in LC dwell volume with the delay column installed.

The Avantor® ACE® PFAS Delay Column clearly provides a simple solution to safeguard against background system PFAS that could interfere with analyte quantification. It is highly recommended that the PFAS Delay Column is installed on every LC-MS system used for the determination of PFAS, even systems that have been nominally shown to be PFAS free. This is because it is possible for background PFAS levels to vary over time due to factors such as mobile phase batch and source, cleaning or exchange of solvent bottles and variation in the general PFAS background of the laboratory.

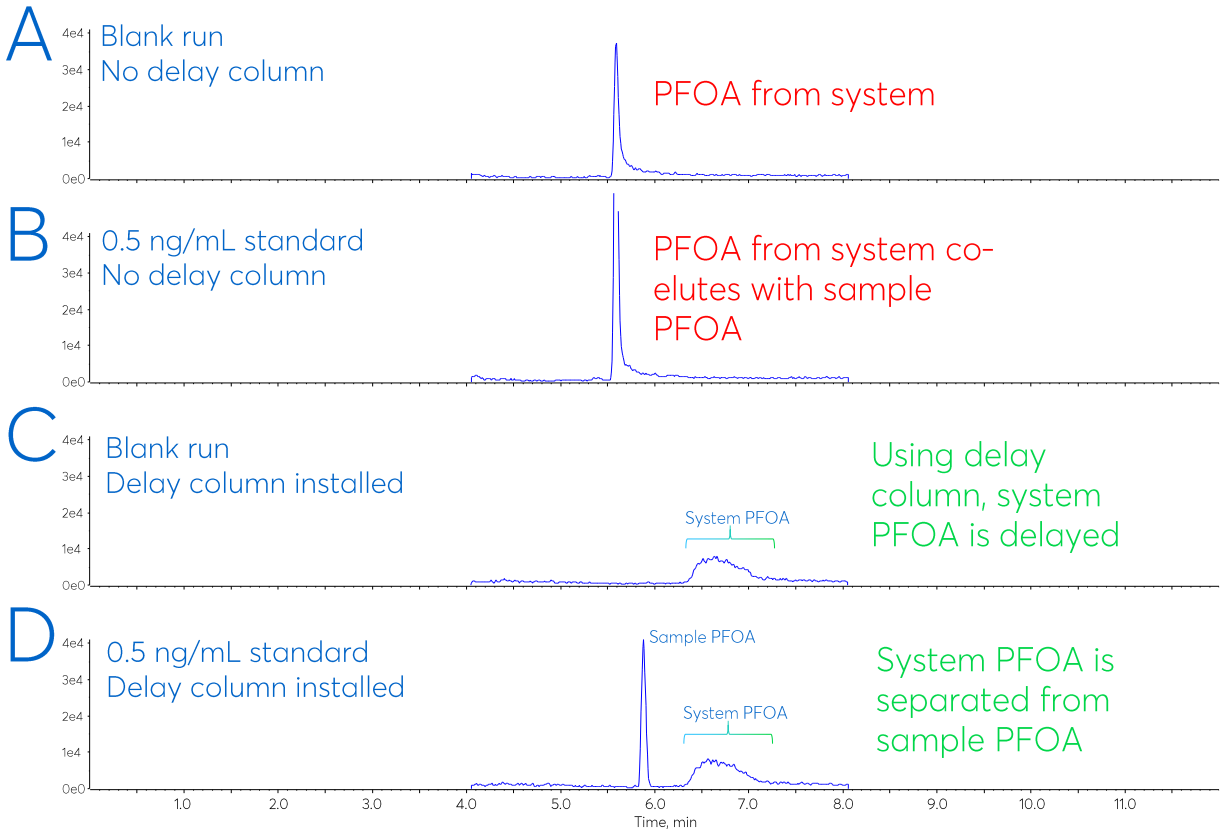

Figure 5: Demonstration of the effectiveness of the Avantor® ACE® PFAS Delay Column for the determination of PFOA. Method conditions: as per Figure 4.

CONCLUSIONS

Increasing concerns around the detrimental impact of PFAS on the environment and human health are resulting in increasingly lower detection limits being required for the analysis of PFAS by LC-MS/MS. This means that it is vital to eliminate any impact from interfering background PFAS components to ensure valid analytical data is obtained. The widespread use of PFAS unfortunately means that the LC-MS/MS system itself can be a significant source of background PFAS contamination during analysis. The data presented in this technical note demonstrates that the Avantor® ACE® PFAS Delay Column provides a simple solution for eliminating such interferences. Background PFAS contamination from the LC-MS system is effectively separated from native sample PFAS, thereby ensuring the integrity of the analytical data.

REFERENCES

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ORDERING TABLE

Product	Details	Size	Part Number
Avantor® ACE® PFAS Delay Column	PFAS Delay Column	50 x 2.1 mm	ACE-PFASD-0502