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**What is PFAS?**

PFAS is an abbreviation for per- and polyfluoroalkyl substances which are a large family of man-made chemicals that contain a carbon and fluorine atom backbone. How large is their family? We are talking about more than 5000 chemicals in this group. These chemicals have different functional groups, which can include other elements like oxygen, hydrogen, or sulfur.

**Why is PFAS so popular?**

PFAS are considered useful because they are resistant to heat, water, and oil. PFAS have been manufactured and used in a variety of industries around the globe, including in the United States since the 1940s[1]. PFAS can be found in:

Food packaged in PFAS-containing materials, processed with equipment that used PFAS, or grown in PFAS-contaminated soil or water.

Commercial household products, including stain- and water-repellent fabrics, nonstick products (e.g., Teflon), polishes, waxes, paints, cleaning products, and fire-fighting foams (a

major source of groundwater contamination at airports and military bases where firefighting training occurs).

Workplace, including production facilities or industries (e.g., chrome plating, electronics manufacturing or oil recovery) that use PFAS.

Drinking water, typically localized and associated with a specific facility (e.g., manufacturer, landfill, wastewater treatment plant, firefighter training facility).

Living organisms, including fish, animals and humans, where PFAS have the ability to build up and persist over time[1].

**Should we be concerned?**

Unfortunately, the answer is YES! It takes a very long time for these compounds to break down in the environment and they can accumulate over time. They also can accumulate in plants, animals, and in our bodies.

A recent biomonitoring study conducted by the National Health and Nutrition Examination Survey (NHANES) on a nationally representative sample of the U.S. population found that more than 98% of the people tested had multiple congeners of PFAS present in their

bodies[2].

**What can be done?**

Researchers are trying to understand the extent of PFAS contamination and develop new methods for PFAS detection and remediation. Biomonitoring of PFAS levels is very critical to evaluate their risk and further implement necessary regulations. Therefore, there is a raising need to develop tools that can accurately and precisely detect low levels of PFAS in biological fluids.

**Challenges**

PFAS chemicals can be found everywhere. They can travel through air or water. They also can accumulate and contaminate the LC systems used in analytical testing. It is very important to make sure the reported results are free of outside, ambient, and system-related PFAS contamination.

In addition, chromatographic separation of PFAS, including the compounds that contain both branched and linear isotopes is critical to ensure reliable and accurate quantification.

**Possible Solutions**

The following is a summary of some collaborative work between Phenomenex, Inc and Sciex Company.

In this method, a Phenomenex Luna C18(2) column (30 x 2 mm, 5 µm, 00A-4252-Y0) is installed between the autosampler and LC pumps to trap ambient and system-related PFAS compounds. This additional column serves as a delay column to isolate PFAS contamination leaching from the LC system components and minimize the risk of system-related PFAS interfering with real signals from the sample during the analytical run. High-density polyethylene or polypropylene consumables (Eppendorf tubes, pipette tips, HPLC vials, etc) were used to minimize PFAS contamination from external sources. In addition, the LC system was modified to reduce possible system contamination.

Phenomenex Gemini® C18 column (50 x 2 mm, 3 µm, 00B-4439-B0) at 25ºC on a SCIEX ExionLC™ AC System is utilized for separation, Figure 2 shows the chromatographic profile on an injection of the neat, 10 ng/mL standard solution containing the 22 PFAS. The choice of column, gradient, and optimized mobile phase composition resulted in the baseline separation that was needed to correctly distinguish all isomers. As seen in Figure 2, the delayed contamination peaks caused by the delayed column did not interfere with the PFAS sample peaks.

**Experimental Conditions and Results**

Figure 1 summarizes the chromatographic response following the addition of the delay column and shows the extracted ion chromatograms (XICs) for PFHpA (top row) and PFHxS (bottom row) before and after the hardware modifications were made on the LC system.

Figure 1A shows the background signal resulting from a blank sample injection before the system modifications were made. Sharp PFAS peaks resulting from the ambient LC system contamination were observed throughout the chromatograms, including at the retention times at which the sample peaks were expected.

Figure 1B shows the background signal of the same blank sample injection after the system modifications were made (including the addition of the **delay column**). This configuration eliminated the PFAS interference peaks at the expected analyte retention times and produced a much broader and delayed contaminant peak caused by the system-related PFAS that were held up by the delay column.

Figure 1C shows the XICs resulting from a 10 ng/mL injection of a sample containing all the PFAS in the panel with the system modification. The XICs show a sharp peak resulting from the PFAS in the samples followed by the same broad and delayed contamination peak shown in Figure 3B. The addition of the delay column and the modifications made to the LC system components together minimized the impact of system-related PFAS contamination and ensured the analytical integrity of this quantitative workflow.

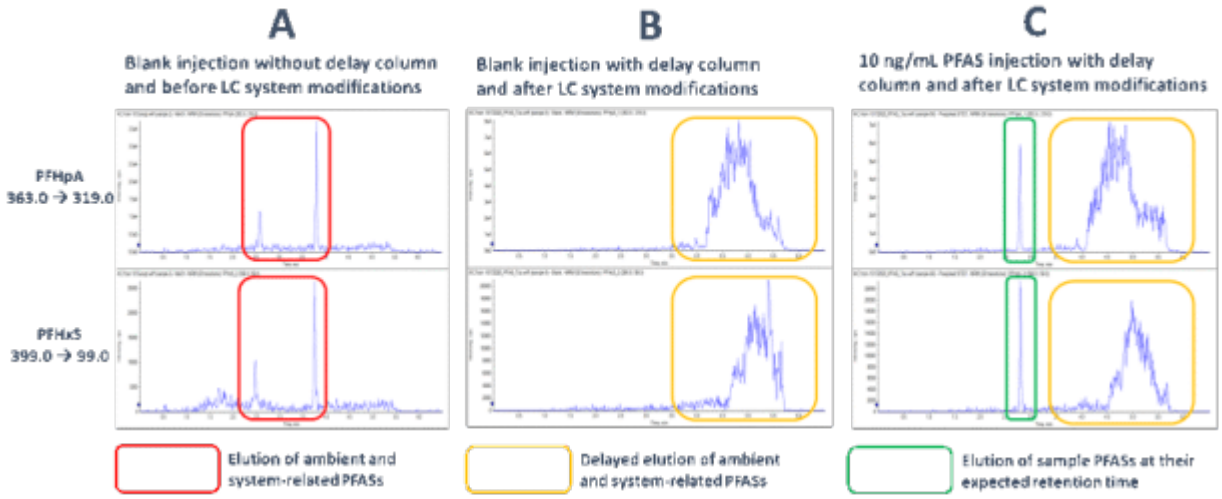


Figure 3. Benefits of using a delay column for PFAS analysis: XICs for PFHpA (top row) and PFHxS (bottom) showing (A) background and system-related contamination signals resulting from a blank sample injection before the system modifications were made showing, (B) broader and delayed contaminant peaks caused by the system-related PFAS that were held up following the addition of the delay column, and (C) sharp peaks resulting from the PFAS in the samples followed by the delayed and broad contamination peaks caused by the delayed column. The modifications made to the LC components significantly reduced the impact of system-related PFAS interferences and enabled accurate quantification of PFAS in serum samples.

Figure 2 shows the chromatographic profile on an injection of the neat, 10 ng/mL standard solution containing the 22 PFAS. The choice of column, gradient and optimized mobile phase composition resulted in the baseline separation that was needed to correctly distinguish all isomers including compounds with both branched and linear isotopes (PFOS and PFHxS). As seen in Figure 2, the delayed contamination peaks caused by the delayed

column did not interfere with the PFAS sample peaks.

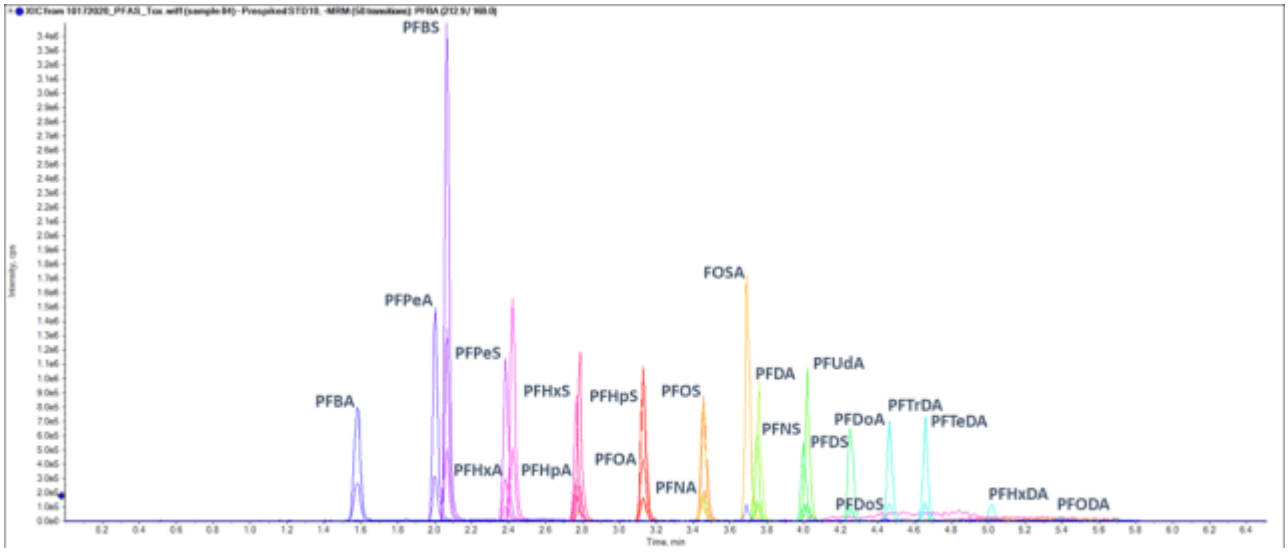


Figure 2. Chromatographic profile of the 22 PFAS monitored in this study:  Extracted ion chromatograms (XICs) resulting from the optimized data acquisition method using a 10 ng/mL neat standard mixture. The combination of the optimized mobile phase composition and column choice enabled baseline separation of the PFAS from the injected sample, including branched and linear isotopes such as PFOS and PFHXs.

**Commentary and Conclusion**

In the growing world of PFAS and their persistent presence in the environment and biological samples, it is very critical to develop methods to quantify these compounds accurately and precisely.  The choice of column and the delay column along with the

appropriate instrumentation has a significant role in accurate and precise analysis of PFAS compounds.

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***References***

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