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Analysis of PFAS in Breast Milk: An Alternative Sample Prep

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are broadly grouped into several classes of over 9000 structurally different compounds used in a variety of industries and consumer products over the past eighty years. Because of their widespread use, as well as their persistence and poorly understood effects on the human body, laboratories have started to monitor their presence in several matrices, including breast milk, following guidelines for detection and sample prep set out by governmental agencies like the EPA. Solid phase extraction (SPE) using weak anion exchange (WAX) sorbent is typically employed in PFAS analysis, but an alternative sample preparation using simultaneous removal of proteins and phospholipids through filtration with Captiva EMR-Lipid significantly reduces complexity and cost while achieving similar results.

Agilent 6470 LC/TQ.

Experimental

Sample Prep

Breast milk was pooled and then split into two sets of aliquots, one for the traditional weak anion exchange (WAX) based sample preparation outlined in EPA 1633, and one for the Captiva EMR-Lipid based prep (Figure 1). For the set of samples designated for WAX, a protocol based off the draft method 1633 was followed. For the alternative preparation, two grams of milk was weighed out and 8 mL of acetonitrile was added prior to ultrasonication for 30 minutes. Samples were centrifuged for 5 minutes at 3000 rpm, and then 6 mL of supernatant was loaded onto the Captiva EMR-Lipid cartridge and allowed to filter through. This was followed by a 1.5 mL rinse of 80% acetonitrile. and then the eluate was dried down and reconstituted in 96% methanol.

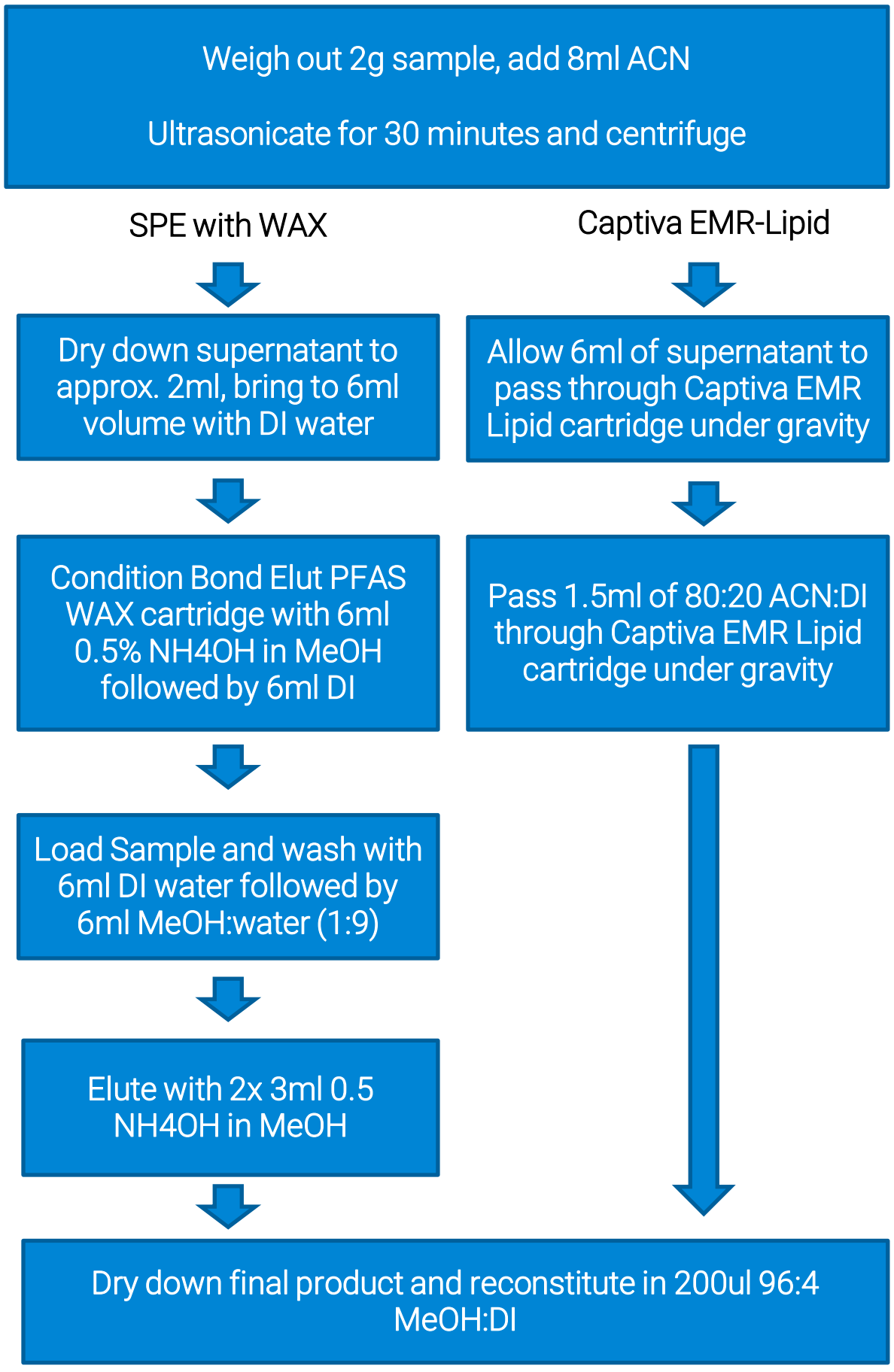

Figure 1. Process schematic comparing WAX protocol with Captiva EMR-Lipid protocol.

LC-MS/MS Analytical Method

The LC-MS/MS system consisted of a 1290 binary pump, a thermostatted multisampler, a temperature-controlled column compartment, and a 6470 triple quadrupole mass spectrometer. Separation conditions are given in Table 1. Modifications were made to the LC system to minimize or shift background PFAS from the system, including replacing tubing with PFC-free versions and installing a delay column in the pump.

Analytical Column	Agilent Zorbax EclipsePlus C18 RRHD, 2.1x100mm, 1.8 µm	
Injection Volume	5 µL	
Mobile Phase A	95% Water + 2 mM ammonium acetate + 5% acetonitrile	
Mobile Phase B	Acetonitrile	
Needle Wash	50:20:20:10 Isopropanol:Methanol:Acetonitrile :Water	
Autosampler Temp	4 °C	
Column Temp	40 °C	
Flow Rate	0.4 mL/min	
Gradient	Time 0.00 0.20 10.00	%B 2 2 95
Stop Time	12.20 min	
Post Time	2.00 min	

Table 1. LC parameters.

Gas Temp	230 °C
Gas Flow	8 L/min
Nebulizer Pressure	20 psi
Sheath Gas Temp	355 °C
Sheath Gas Flow	10 L/min
Capillary Voltage	2500 V
Nozzle Voltage	0 V

Table 2. Agilent JetStream ESI source parameters

Detection of all analytes was undertaken in multiple reaction monitoring (MRM) mode, and the phospholipid transitions were monitored for background levels. MS source conditions for the mass spectrometer are shown in Table 2. The total injection cycle time was approximately 15 minutes sample to sample. Data was acquired and analyzed using MassHunter software version 12.

Results and Discussion

Chromatography

Thirteen phospholipid transitions (Table 3) were monitored for background reduction when comparing the two sample prep methods. 40 PFAS compounds, along with 8 labeled internal standards and 35 surrogates were monitored as per the 1633 method.

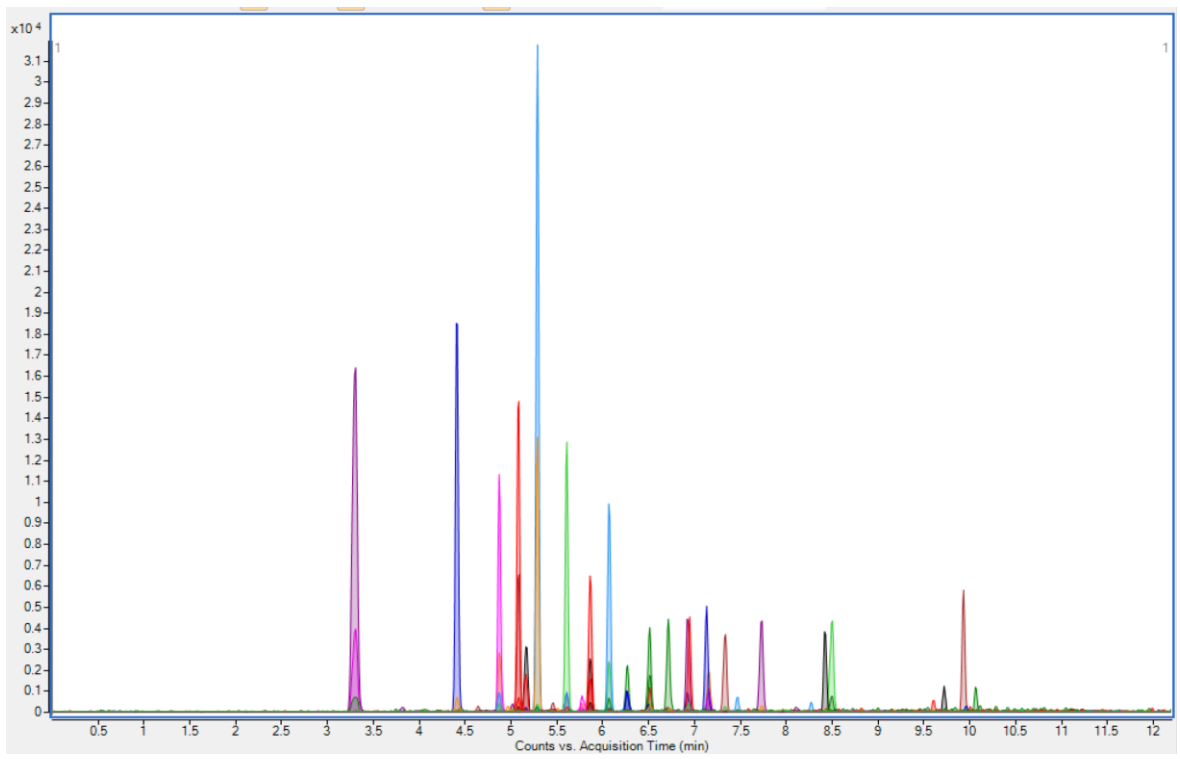

Figure 2. Representative chromatogram showing overlaid MRMs for each compound at 100 ppt.

Precursor Ion	Product Ion	Polarity
808.4	184.4	+
806.4	184.4	+
786.4	184.4	+
784.4	184.4	+
760.4	184.4	+
758.4	184.4	+
704.4	184.4	+
524.4	184.4	+
522.4	184.4	+
520.4	184.4	+
496.4	184.4	+
184.1	184.1	+
153.0	153.0	-

Table 3. Phospholipid transitions monitored to determine background reduction.

Results and Discussion

Sample prep development work demonstrated that the Captiva EMR-Lipid filtration significantly simplified the workflow while still allowing for excellent detection of the compounds of interest. This is due to the unique selectivity of the Captiva EMR-Lipid device for unbranched alkane chains, which differentiates between phospholipids and PFAS, capturing the former while allowing the latter to pass through the sorbent bed unretained. The phospholipid background was significantly reduced when implementing the EMR filtration prep compared to the WAX prep, as shown in Figure 3.

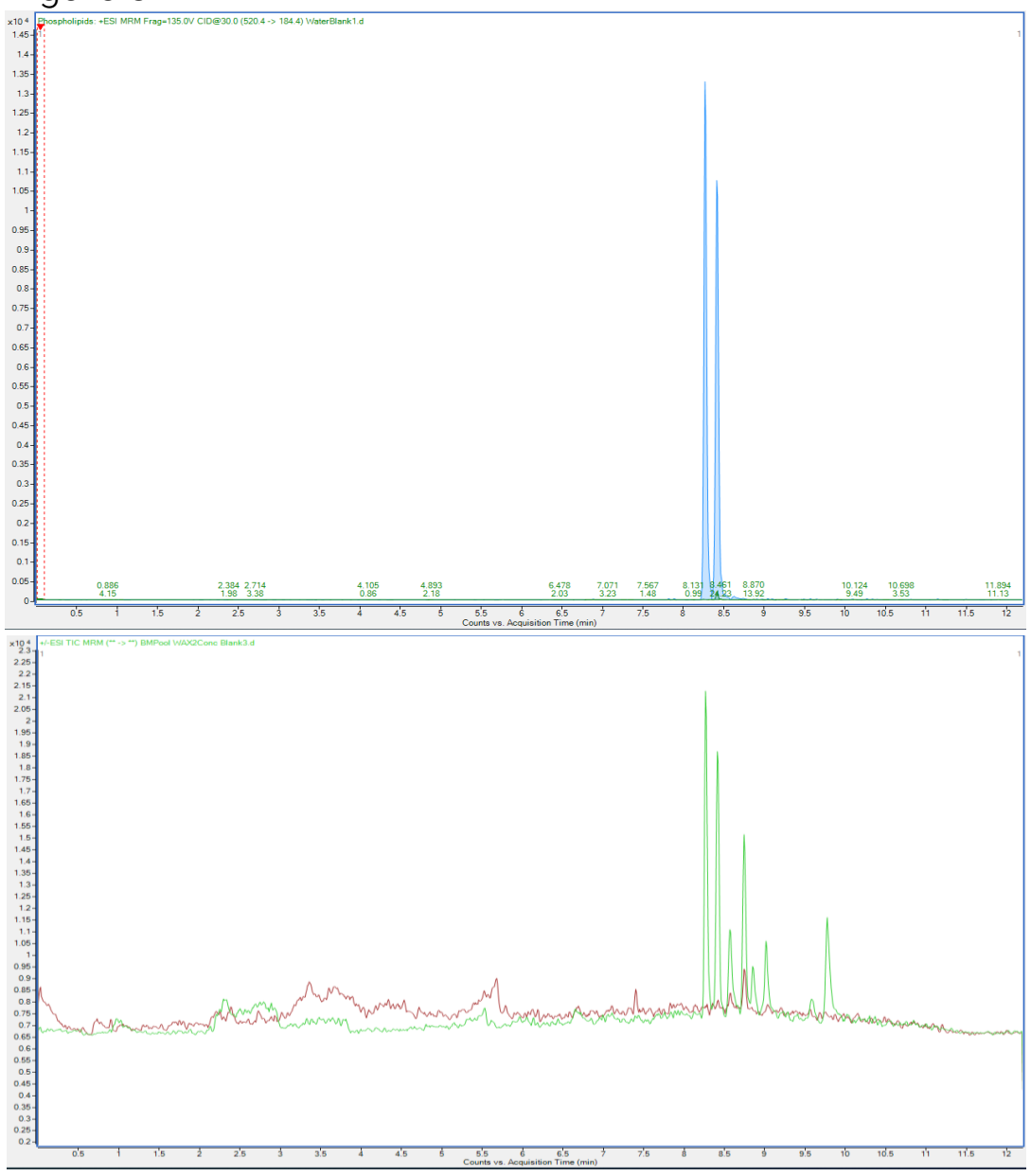

Figure 3. Overlaid chromatograms showing phospholipid background of the WAX prep (blue) compared to the Captiva EMR-Lipid prep (green). Bottom: Overlaid TICs of WAX (green) vs. EMR (brown).

One major advantage to the Captiva EMR-Lipid filtration prep is the time savings and fewer number of steps required, as shown in Figure 1. Preliminary experiments suggest this is a viable alternative workflow for PFAS analysis, as both protocols required a tenfold sample concentration, but the WAX process took significantly longer for no additional gain. Recoveries in early experiments were comparable for a few analytes or better by the Captiva EMR-Lipid process, as shown in Figure 4, and sensitivity was not an issue due to the concentration factor.

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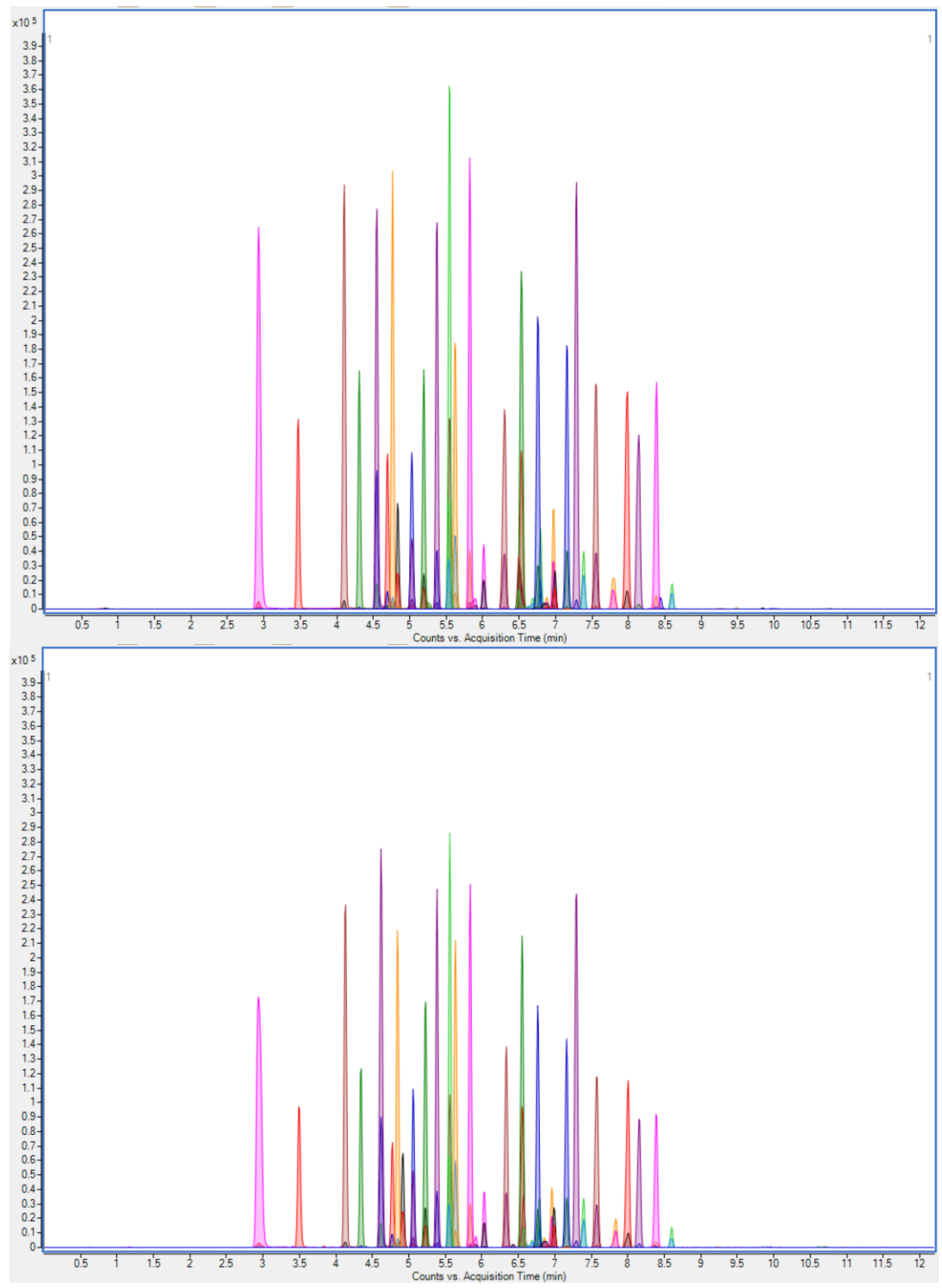

Figure 4. Comparison of responses from an EMR prep (top) and a WAX extraction (bottom). Scales are identical.

Conclusions

The simplified workflow utilizing the Captiva EMR-Lipid filtration cartridges showed significantly reduced background when compared to the traditional WAX SPE protocol outlined in the 1633 method. This workflow demonstrated better or comparable recoveries of the PFAS analytes of interest, and the workflow was more efficient, with fewer steps, leading to fewer chances of error and reducing the potential for contamination.

References

- Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, 3rd Draft Method 1633, US EPA, Dec. 2022.
- Per- and Polyfluoroalkyl Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS; Zheng, G. et al; *Environ. Sci. Technol.*. 2021, 55, 7510-7520.

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