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Faster Than the Speed of Life: A Rapid Screening Method for Select PFAS Compounds in Serum by RapidFire-MS

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Introduction

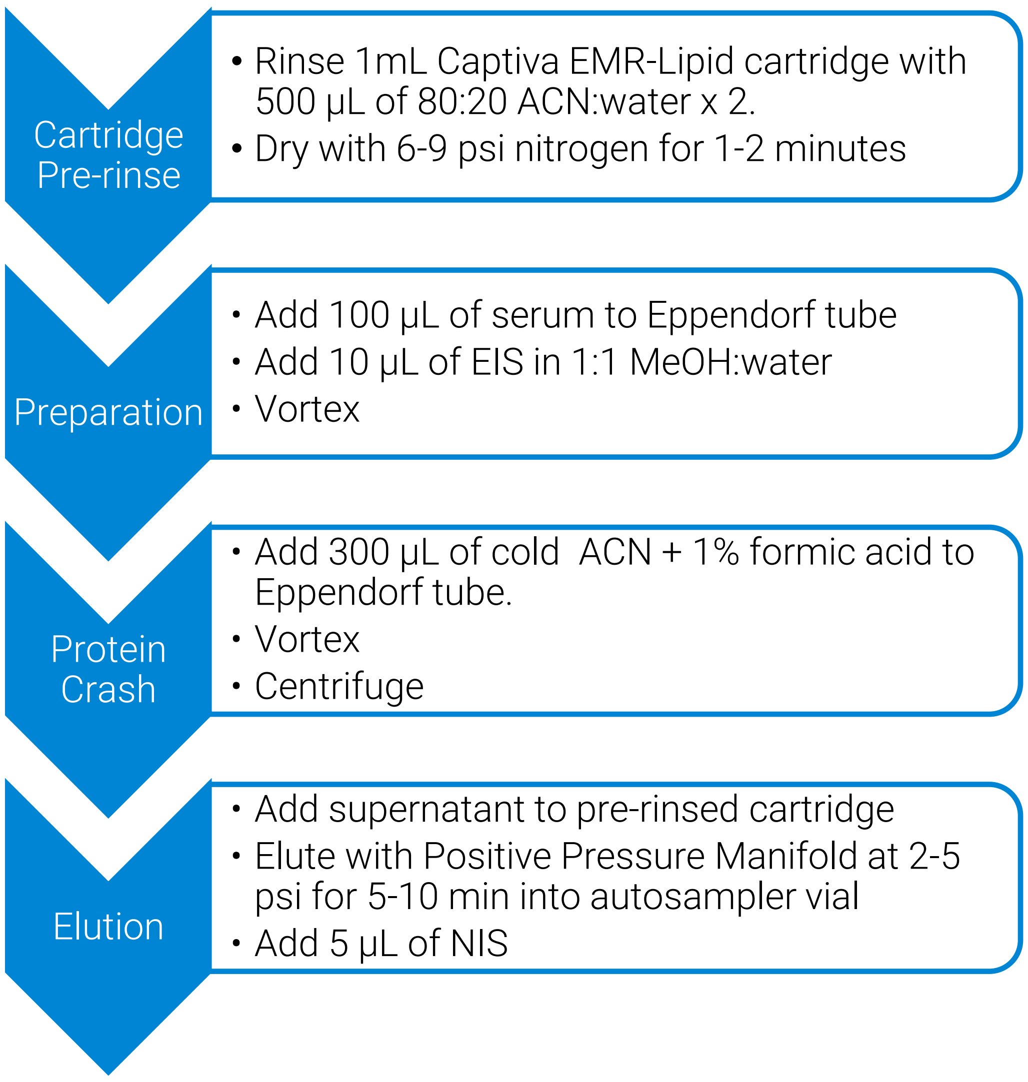
Per- and polyfluoroalkyl substances (PFAS) are a broad class of compounds with a high degree of fluorination used in a variety of industries and consumer products over the past seventy years. Because of their widespread use, as well as their persistence and poorly understood effects on the human body, laboratories have started to monitor their presence in biological fluids, including serum. Biological samples are commonly prepared with protein precipitation. Additional cleanup steps such as solid phase extraction (SPE) with either C18 or weak anion exchange (WAX) sorbent can be used for increased sensitivity and cleanup. Alternatively, a pass-through cleanup using Captiva EMR-Lipid removes proteins and phospholipids through filtration, which significantly reduces sample complexity.

Agilent RapidFire 400

Agilent 6495D

Experimental

Purchased serum was aliquoted into two sets of samples, one for protein precipitation (ppt) only and one for a protein precipitation plus Captiva EMR-Lipid based cleanup. For each preparation, 100 µL serum was aliquoted into a tube and spiked with 25 native and extracted isotopically labeled (EIS) compounds (EPA 533 list). The samples were vortexed for 1 minute and then crashed with 1% formic acid in cold ACN. They were vortexed for 3 minutes and then spun for 10 minutes to complete the crash. Samples undergoing the Captiva-EMR Lipid cleanup were then loaded onto a pre-rinsed cartridge and allowed to filter through (Figure 1). All samples were then spiked with the additional non-extracted isotopically labelled (NIS) compounds, vortexed, and transferred to a plate for analysis.



Cartridge Pre-rinse

- Rinse 1mL Captiva EMR-Lipid cartridge with 500 µL of 80:20 ACN:water x 2.
- Dry with 6-9 psi nitrogen for 1-2 minutes

Preparation

- Add 100 µL of serum to Eppendorf tube
- Add 10 µL of EIS in 1:1 MeOH:water
- Vortex

Protein Crash

- Add 300 µL of cold ACN + 1% formic acid to Eppendorf tube.
- Vortex
- Centrifuge

Elution

- Add supernatant to pre-rinsed cartridge
- Elute with Positive Pressure Manifold at 2-5 psi for 5-10 min into autosampler vial
- Add 5 µL of NIS

Figure 1. Extraction procedure for Captiva EMR-Lipid

The RF/TQ system consisted of a RapidFire 400 front end with a 6495D triple quadrupole mass spectrometer for detection. Samples were introduced into the mass spectrometer using a RapidFire cartridge for additional cleanup, and the total cycle time was about 12.5 seconds, with analytes detected in negative polarity. Specific method conditions are detailed in Tables 1 (RF) and 2 (MS).

Results and Discussion

Method Parameters

Cartridge Type	• C18, type C • C18 anion exchange, custom mixed mode	
Injection Volume	10 µL	
Buffer A	Water + 5 mM ammonium acetate	
Buffer B	MeOH	
Buffer C	MeOH + 0.1% sodium hydroxide	
Wash Solvents	**Aq**: Water **Org**: MeOH	
State Timings	State	Time (ms)
	1 (aspirate)	600
	2 (load/wash)	2000
	3 (extra wash)	0
	4 (elute)	6000
	5 (reequilibrate)	2500

Table 1. RF parameters.

Gas Temp	150 °C
Gas Flow	18 L/min
Nebulizer Pressure	20 psi
Sheath Gas Temp	390 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2500 V
Nozzle Voltage	0 V
Funnel setting	Standard/Fragile
Gain Factor	6
Dwell Time	1 ms

Table 2. Agilent JetStream ESI source parameters.

Early Development Work

Feasibility studies for detection of PFAS compounds in serum using the RapidFire started by looking for PFAS background in the instrument and in all steps of the workflow. Early testing demonstrated no detectable background from any stage of the process.

RapidFire method development tested a variety of cartridges for optimal binding of the PFAS compounds, as well as determining the best state times and solvent conditions. For the preliminary work, the C18 cartridge appeared to have the best results, while the optimized elution solvent was 5 mM ammonium acetate in methanol.

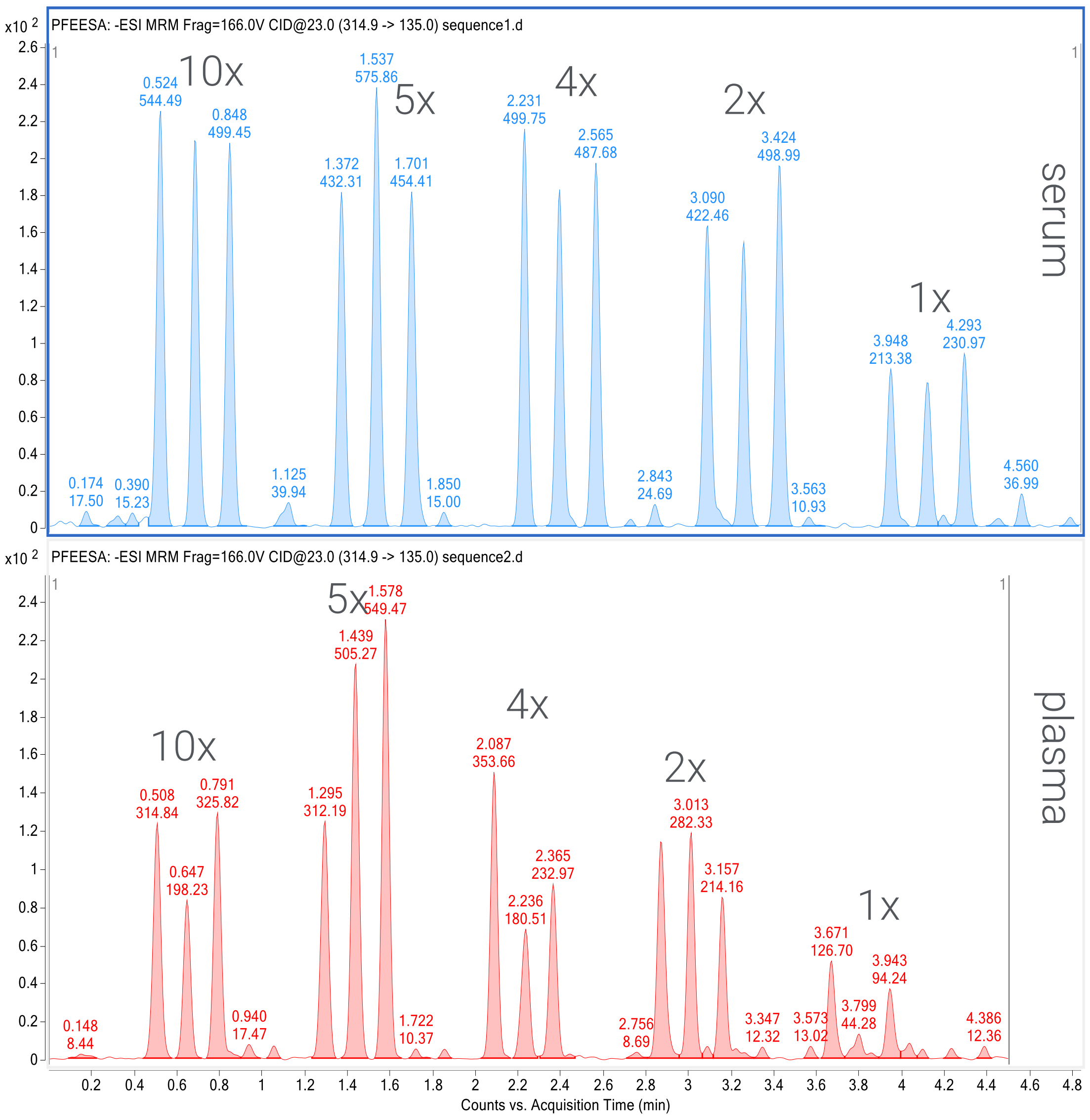


Figure 2. Effect of dilution on spiked samples prepared only with a protein precipitation. Dilution minimizes matrix suppression and allows for better analyte retention on the cartridge, improving sensitivity.

Early experiments were performed to understand the effects of dilution and sample prep on the overall sensitivity of the method. When only a protein precipitation preparation was employed, dilution had a significant positive effect on analyte sensitivity, as shown in Figure 2. However, when the sample prep routine utilized protein precipitation in combination with a Captiva EMR-Lipid cleanup, additional dilution was not necessary and in fact decreased overall sensitivity, as shown in Figure 3. This is attributed to the removal of extraneous matrix using the Captiva EMR-Lipid device, which has a unique selectivity for unbranched alkane chains, capturing phospholipids while allowing PFAS compounds to pass through the sorbent bed unretained.

Results and Discussion

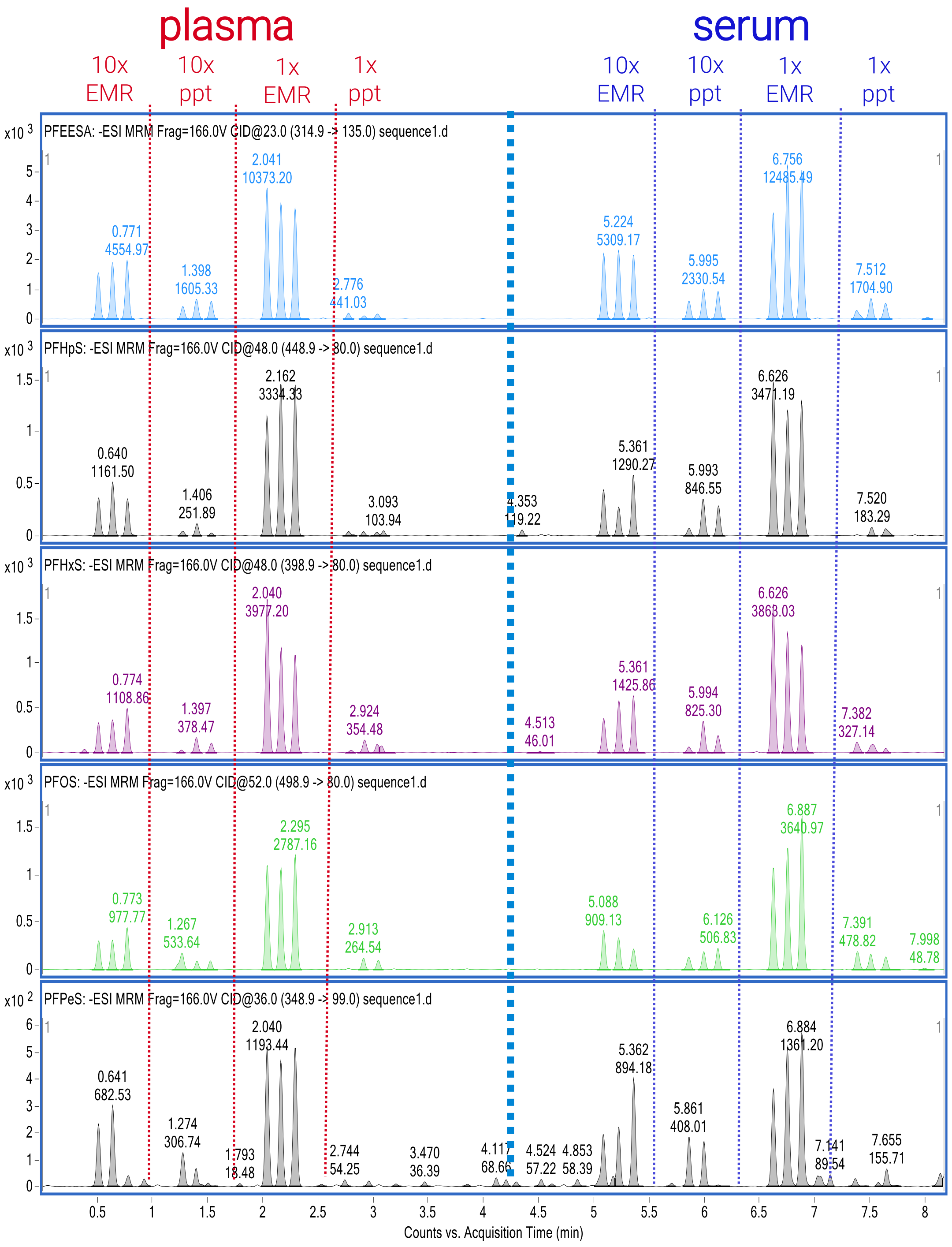


Figure 3. Effect of dilution on spiked matrix samples prepared using both the Captiva EMR-Lipid cleanup and ppt (EMR) compared to just ppt (ppt). Two dilutions were compared for each sample prep routine, and samples were injected in triplicate.

Custom Cartridge Work

This preliminary work was done using the C18 cartridge, but the cartridge had poor retention of the short-chain PFAS compounds (C5 and below). To address this issue, a cartridge was custom-packed with mixed mode material that included C18 and anion exchange resins. Further testing and method optimization for this specific packing material yielded better results across the board, but especially for the short-chain PFAS compounds. Sensitivity for most analytes was at or below 0.1 ppb, as summarized in Table 3, with a calibration range of 0.01-10 ppb tested for all analytes.

	LOQs (ppb)	
Compound	Mixed Mode	C18
11Cl-PF3OUdS	0.5	0.1
4-2 FTSA	0.05	0.1
6-2 FTSA	0.5	0.1
8-2 FTSA	0.01	0.05
9Cl-PF3ONS	0.1	0.5
DONA	0.5	0.5
HFPO-DA	0.05	0.05
NFDHA	0.5	0.5
PFBA	0.1	>10
PFBS	0.01	0.05
PFDA	1	0.5
PFDoDA	0.1	0.5
PFEESA	0.05	0.5
PFHpA	0.05	0.5
PFHpS	0.05	0.1
PFHxA	0.1	0.5
PFHxS	0.5	0.5
PFMBA	0.05	0.5
PFMPA	0.5	0.5
PFNA	0.05	0.5
PFOA	0.1	1
PFOS	0.05	0.05
PFPeA	1	10
PFPeS	0.01	0.01
PFUnDA	0.5	0.5

Table 3. LOQs for each analyte from the EPA 533 PFAS list as determined via two different RapidFire methods. The custom packed cartridge LOQs are summarized on the left and overall showed better results when compared to the traditional C18 cartridge (right), especially for short chain analytes (PFBA).

Conclusions

- LOQs for most PFAS analytes of interest in serum were 0.1 ppb or below
- Samples were injected every 12 seconds, allowing for high throughput analysis
- Custom mixed mode cartridge had best overall performance
- There was no background PFAS contribution from the RapidFire system

References

Protein Precipitation for Biological Fluid Samples Using Agilent Captiva EMR-Lipid 96-Well Plates; Zhao, L. and Juck, M.; Agilent Application Note 5991-9222EN, 2018.

https://www.agilent.com/en/promotions/asms

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