ANALYSIS OF PERFLUOROALKYL SUBSTANCES (PFAS) AND METABOLITES IN BIOFLUIDS

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INTRODUCTION

Perfluoroalkyl substances (PFAS) are common, man-made, persistent environmental contaminants that are used in the production of many consumer and industrial products. While highly advantageous for their chemical properties, these analytes don't metabolize or degrade easily, leading to increased persistence in the environment. Exposure and bioaccumulation of some PFAS residues has been linked to harmful health effects including: cancer, obesity, immune and reproductive issues.

With their high environmental prevalence, known bioaccumulation, and toxicity, these PFAS compounds have gained increased attention as global contaminants. Thus, environmental monitoring and biomonitoring of PFAS has steadily increased. With its many benefits (e.g., multiplexing, selectivity, dynamic range and fast method development), liquid chromatography-mass spectrometry (LC-MS) is the most common and widely accepted technique for their determination and highly sensitive and selective quantification.

However, developing robust methodologies for PFAS analysis can be quite challenging. The broad family of PFAS compounds have diverse chemical properties. Some can experience poor solubility (particularly of the longer chain PFAS) while others are strong anionic species with the propensity to adhere to various surfaces that they may come in contact with (e.g., sample containers, pipette tips, extraction plates/cartridges, collection vials/plates, and LC-components). Confounding these challenges are the low expected concentration levels in both serum and environmental matrices. Finally, known contamination from LC instrument components, solvents and common laboratory consumables makes LC and sample preparation method development difficult.

This work described herein provides a single, simple method for the simultaneous quantification of multiple PFAS substances from serum. This method uses analytical scale sub-2µm LC and fast, selective sample prep in 96-well format to achieve an recovery and low matrix effects, while achieving LODs as low as 0.5-1.0 ng/mL.

METHODS

Sample Preparation

Concentrated PFAS standards (legacy and emerging) were prepared as a mix in methanol and then spiked into fetal bovine serum (100 µL). The pretreated sera samples were prepared for LC-MS/MS analysis using a Waters Oasis™ WAX µElution 96-well SPE extraction plate and the protocol described below.

Sample Extraction with Oasis WAX

Condition: 200 µL 5% NH_4OH in methanol followed by 200 µL methanol
Equilibrate: 200 µL water
Load sample: Entire diluted acid pretreated sample (~200 µL) was loaded onto the extraction plate
Wash 1: 200 µL acid in Water
Wash 2: 200 µL 0-10% acetonitrile or methanol in water
Elute: 2 X 25 µL 1-5% NH_4OH in 50-100% methanol or acetonitrile
Dilute: 50 µL 2 mM ammonium acetate in water
LC-MS analysis: 10 µL of prepared sample was injected for analysis.

LC-MS Conditions

LC-MS/MS quantification of PFAS analogues was performed using a Waters Xevo™ TQ-S triple quadrupole MS (ESI-). Chromatographic separation was achieved using an ACQUITY™ H-Class UPLC PLUS system fitted with the PFAS Analysis Kit using an ACQUITY UPLC™ BEH C_{18}, 2.1 x 100 mm, 1.7 µm column (35 °C) at flow rate of 0.3 mL/min. Mobile phases used for analysis consisted of Water: Methanol (95:5) containing 2 mM ammonium acetate (Mobile Phase A) and Methanol containing 2 mM ammonium acetate (Mobile Phase B). Total analysis time was 22 minutes.
A total of 37 PFAS analogues were monitored for quantification. MRM conditions for each PFAS compound was optimized using MassLynx™ Software and the QuanOptimize tool. Full details on the LC/MS method, can be found in Waters Application Note 720006471EN.

RESULTS

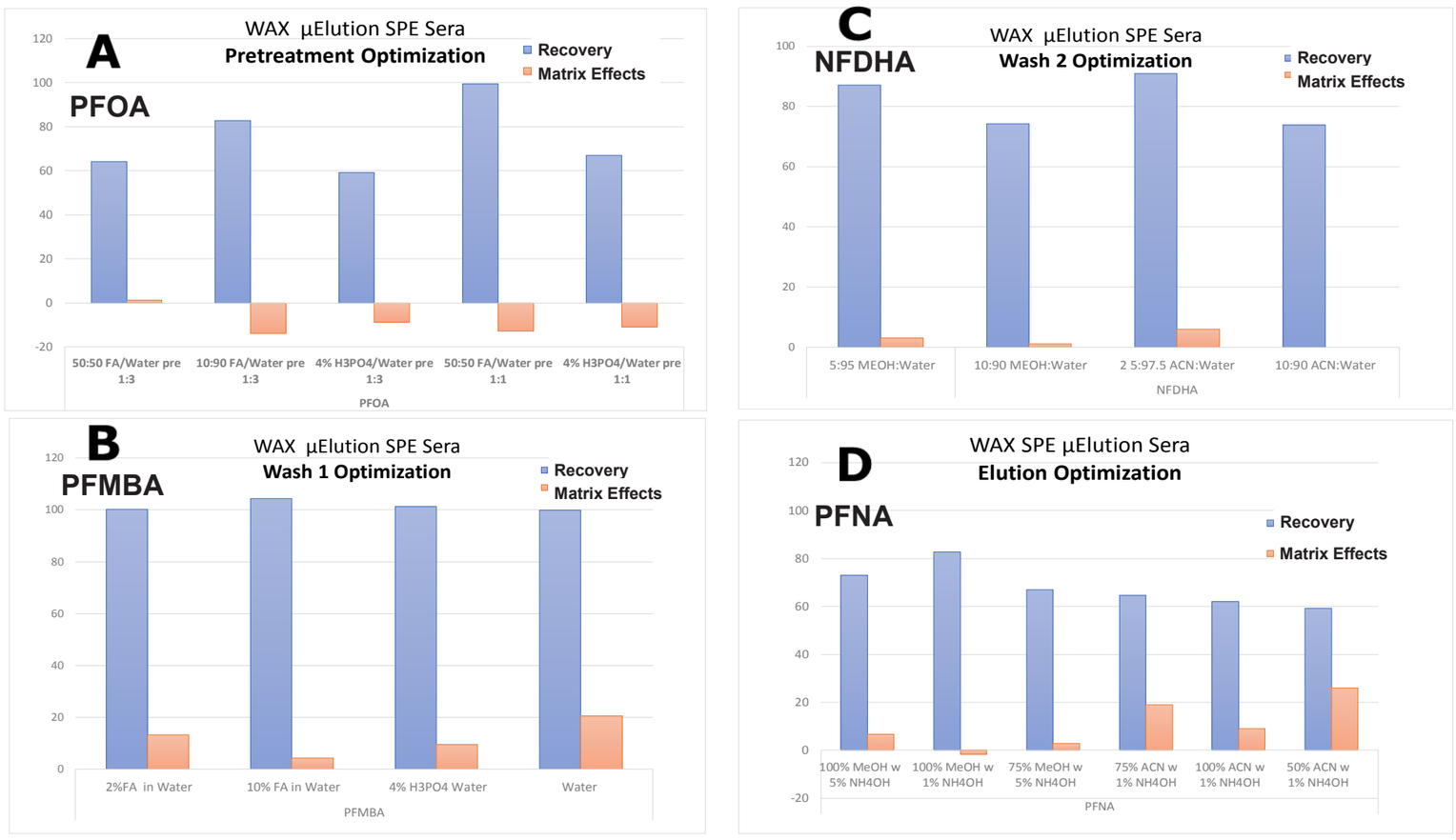

Figure 1. Representative PFAS sample preparation method development and optimization using mixed-mode WAX SPE in the 96-well format. *Best recovery with minimal matrix effects (ME) was seen when using 1:1 pretreatment with 50% formic acid (FA) or 4% H_3PO4 in water (**A**). Washing the sample with a strong acid (10 % FA or 4% H_3PO_4) maintained recovery and minimized MEs (**B**). MEs could be further reduced using a Wash 2 solution containing 2-10% organic without reducing recovery (**C**). Methanol containing 1-5% NH_4OH provided best overall recovery of the PFAS analytes, while providing high selectivity with MEs < 15% (**D**).*

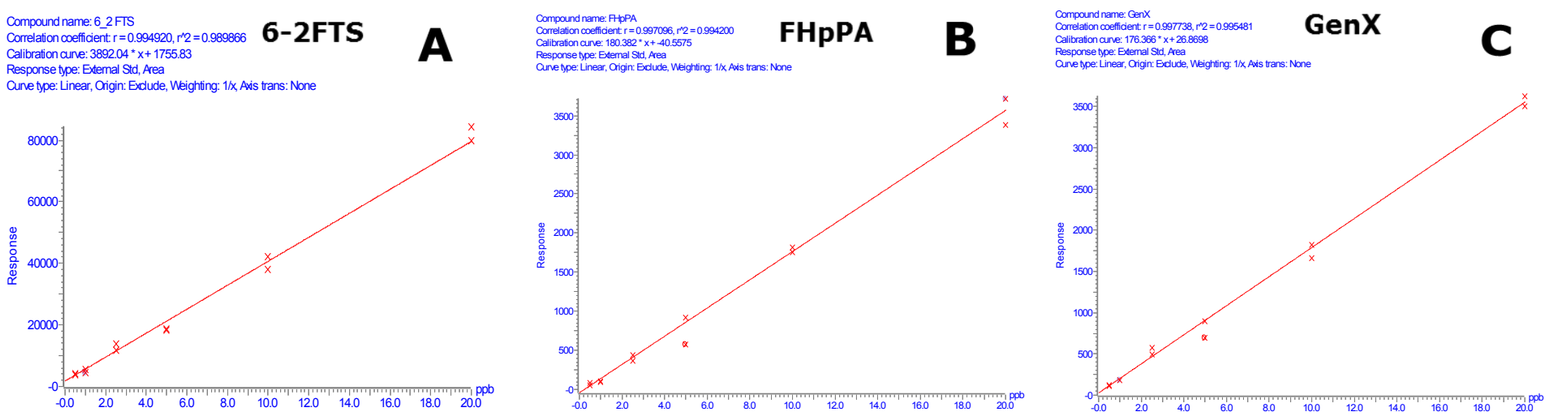

Figure 2. Representative matrix matched standard curves (0.5-20 ng/mL) for 6-2FTS (A), FHpPA (B), and Gen X (C) extracted from 100 µL bovine serum using mixed-mode WAX SPE in the 96-well format.

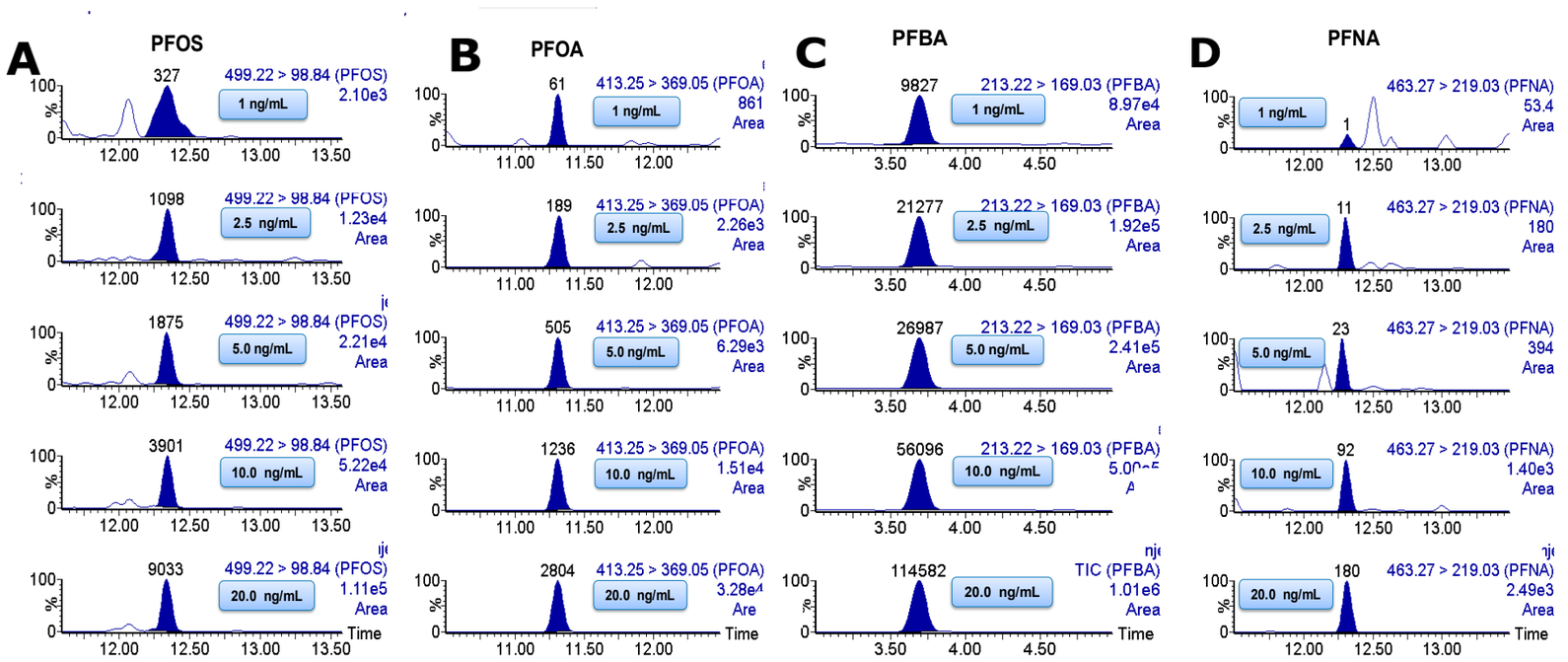

Figure 3. Representative LC-MS chromatograms, acquired using the ACQUITY H-Class UPLC and Xevo TQ-S Mass Spectrometer, for analysis of PFOS (A), PFOA (B), PFBA (C), and PFNA (D) extracted from 100 µL of spiked sera (1-20 ng/mL) using mixed-mode WAX SPE in the 96-well format.

CONCLUSIONS

- **An optimized SPE extraction method was developed for simultaneous extraction of PFAS analogs using a mixed-mode weak anion exchange polymeric sorbent, which afforded recovery and low matrix effects (Figure 1) ensuring sensitivity and selectivity of the assay for the diversity of polar and hydrophobic PFAS.**
- **Use of the µElution SPE plate format eliminated the need for evaporation, reducing losses due to volatility, adsorption and re-solubilization. Sample preparation with simple SPE was < 30 minutes.**
- **A selective UPLC method employing a sub–2µm polymeric reversed-phase column, provided resolution from closely related PFAS compounds and endogenous interferences, improving overall selectivity and sensitivity.**
- **Use of a PFAS LC analysis kit (e.g., PEEK mobile phase tubing and PFAS isolator column) successfully delayed any potential PFAS contribution from the LC system.**
- **Detection or quantification limits of 0.5-1.0 ng/mL were achieved for various PFAS compounds extracted from 100 µL of serum using a highly sensitive tandem quadrupole MS system.**
- **The method demonstrates it is fit-for-purpose for accurate measurement of PFAS compounds with high recovery and selectivity demonstrating its utility for monitoring exposure and potential health outcomes.**

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