People and PFAS: Quantitation in human serum and blood using volumetric absorptive microsampling (VAMS)

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This technical note describes the trace level analysis of per- and polyfluoroalkyl substances (PFAS) using only 60 µL of whole blood collected with volumetric absorptive microsampling (VAMS) devices (Figure 1). The sensitivity of the SCIEX 7500 system was used to achieve serum detection limits ranging from 0.1 to 1.0 ng/mL, which are sufficient for PFAS biomonitoring in the general population. National Institute of Standards and Technology (NIST) SRM 1957 samples that were collected using different sampling techniques were analyzed. These results demonstrated that samples collected with the VAMS method can be analyzed accurately and produce comparable results to samples collected by traditional PFAS serum sampling techniques. The use of VAMS for PFAS blood analysis overcomes many of the logistical obstacles common for traditional methods, such as the need for a trained phlebotomist for sample collection, sample storage and shipping costs.

Key features of the method for PFAS analysis of blood and serum samples

- Elevated sensitivity of the SCIEX 7500 system allowed for 100 pg/mL minimum detection levels, which are aligned with concentrations that are relevant for human health
- Excellent recovery for both serum and VAMS sample preparation approaches, with average recoveries of 88% and 99%, respectively
- The accuracy of the VAMS approach demonstrates an ability to support large-scale PFAS biomonitoring programs

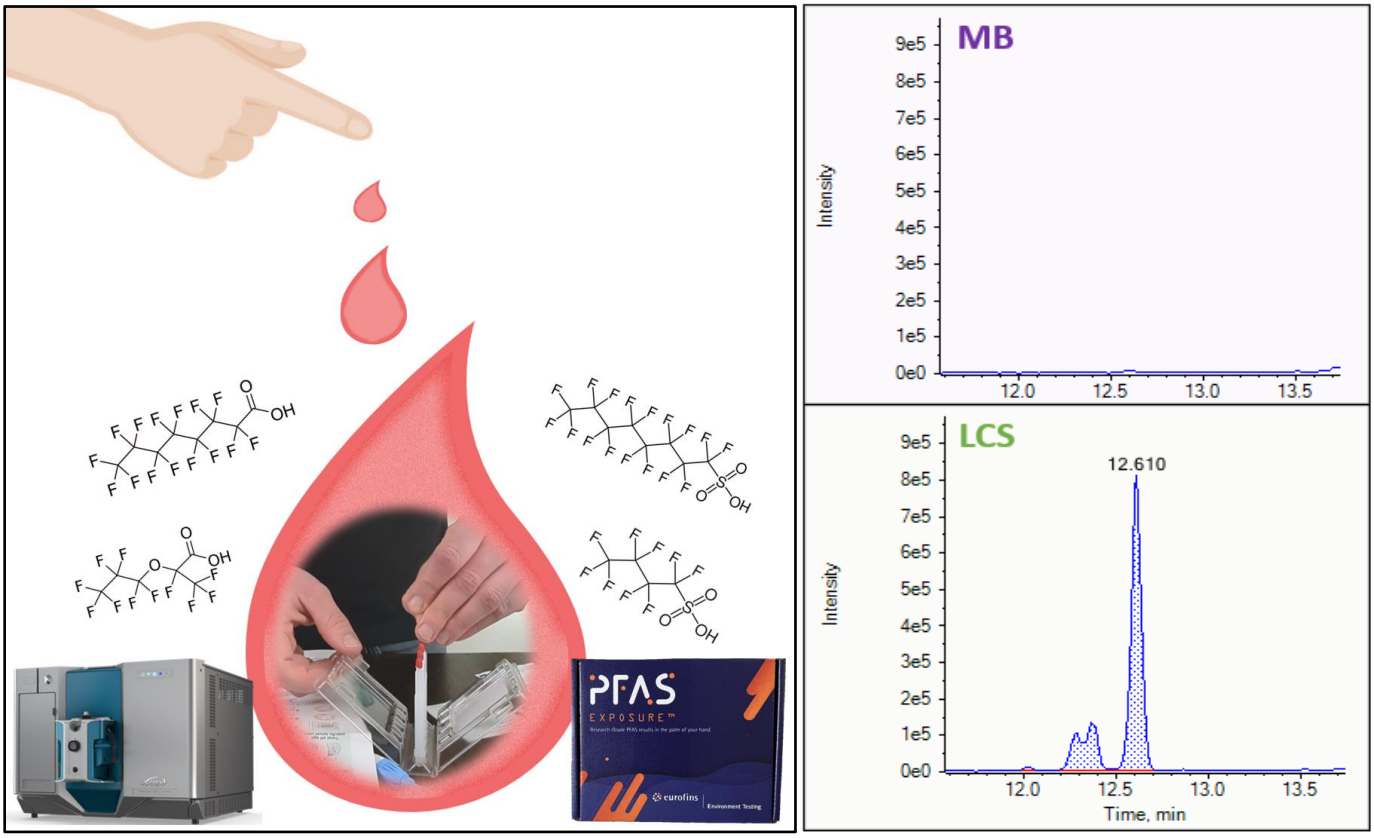

Figure 1. Graphical abstract (left) and comparison of PFOS content (right) in the method blank sample (MB, purple) and laboratory control sample (LCS, green).

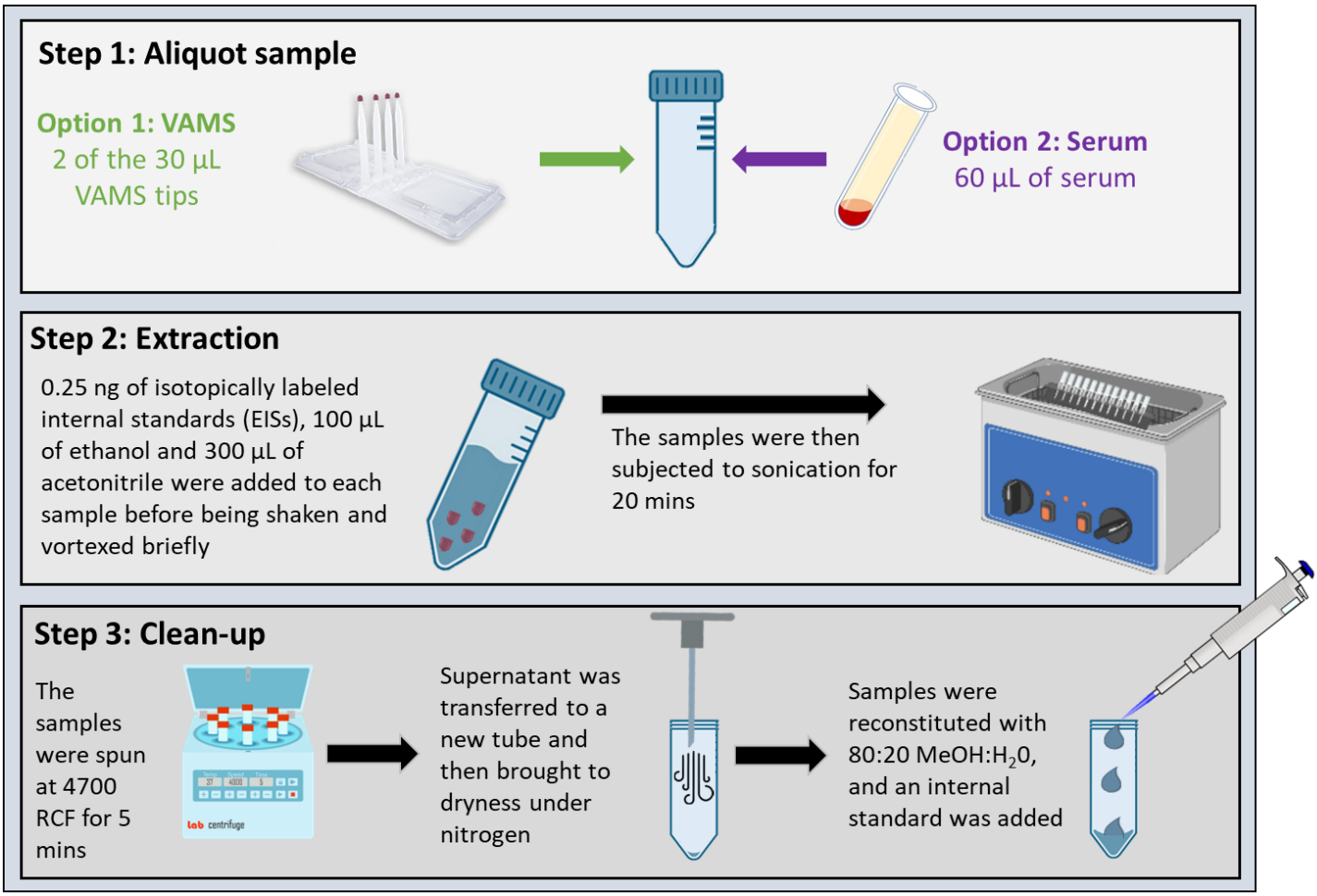

Figure 2. Simplified sample extraction procedure for both VAMS and serum samples.

Introduction

Bioaccumulation of PFAS in the human body due to environmental exposure is a growing public health concern.[1] PFAS are highly prevalent in the environment and in everyday consumer products, including our drinking water supply.[2,3] Therefore, quantitative tools capable of accurately and precisely detecting low levels of PFAS in biological fluids are needed to understand the impact of PFAS on the human body.

Traditionally, human serum or plasma has been used to measure human exposure to PFAS. However, the process of collecting and analyzing these samples requires a phlebotomist to draw blood, a dedicated laboratory to separate serum or plasma from whole blood and the transportation of samples on dry ice with proper biohazard protocols.[4–7] An alternative approach using dried blood spots (DBS) has been employed for several years to address these logistical challenges. However, there is a potential loss in accuracy associated with DBS due to the variability of blood volume on the filter paper and punch size.[4–7]

An alternative approach to traditional blood sampling is the use of VAMS.[8] These microsamples are collected with a hydrophilic polymer that provides a standardized 30 µL volume of whole blood for analysis, therefore improving accuracy and reliability. Due the small volume collected by VAMS, analytical sensitivity is extremely important to quantify PFAS at the relevant levels for human health. The objective of this study was to compare the VAMS-derived PFAS values to traditional serum measurements using the SCIEX 7500 system. The accuracy and robustness of each approach was assessed using both simulated and human-derived samples.

Methods

Sample preparation. The VAMS and serum samples were prepared for extraction separately, using a common method. The VAMS samples were measured by placing 2 of the VAMS tips containing 30 µL of sample in a polypropylene tube. In parallel, the serum samples were gently mixed and 60 µL was aliquoted into a separate polypropylene tube (Figure 2). From this point, the extractions followed identical procedures. Each sample was combined with 0.25 ng of isotopically labeled surrogates, 100 µL of ethanol and 300 µL of acetonitrile. The samples were then sonicated for 20 mins and centrifuged for 5 minutes. The supernatant of each sample was separated and transferred to new polypropylene tubes. The remaining precipitates were combined with 300 µL of methanol and shaken before undergoing centrifugation. The supernatants were separated and combined with the appropriate supernatant collected from the first extraction step. Finally, the samples were blown to dryness under nitrogen and reconstituted using 80:20 (v/v), methanol/water and an internal standard was added.

Liquid chromatography. Liquid chromatography was performed using a ExionLC system at a flowrate of 0.5 mL/min. The injected sample volume was 10 µL and was loaded onto a trap column (Luna NH_2, 3 µm, 50 × 2 mm, Phenomenex PN:00B-4377-B0). The trap column selectively retained the analytes of interest while directing aqueous and matrix interferences to waste. To facilitate the elution of the retained analytes, a valve switching mechanism occurred and pump C was engaged. Pump C back flushed/washed the analyte off the NH_2 column with 0.5% NH_4OH in 30:70, methanol/water at a flow rate of 0.5 mL/min. The sample was transferred to the analytical column, which was a Gemini C18 column (3 µm, 50 x 2 mm, Phenomenex PN:00B-4439-B0). Mobile phases A and B were water with 20mM NH_4OAc and methanol with 10mM NH_4OAc, respectively. Mobile phase B was held at 10% for 5 minutes then ramped to 45% by 5.1 minutes. Mobile phase B was ramped again to 80% over 6.9 minutes then finally brought to 99%, where it was held for 1.95 minutes.

Mass spectrometry. The samples were analyzed on a SCIEX 7500 system operated in negative ionization mode. The gas pressures used included CUR 40 psi, GS1 35 psi, GS 70 psi and CAD 10 psi. The source temperature was 325°C and the ion spray voltage was -1500 V. A total of 43 PFAS compounds were monitored using optimized MRM parameters and a minimum of 10 scans were required per peak (Figure 3).

Method detection limit study

The reporting limits and method detection limits (MDLs) were consistent for both serum and VAMS samples and were ≤0.5 ng/mL for each PFAS (Table 1). The MDLs were established following the guidelines set by the National Environmental Laboratory Accreditation Conference (NELAC) and 40 CFR Part 136.

The method described here involves processing a minimum of 7 spiked samples and 7 method blank samples. The samples designated for determining the MDL were prepared in 3 batches on separate calendar dates and were subsequently analyzed on 3 separate calendar dates. These samples were then analyzed alongside 7 laboratory blanks, all of which were analyzed on a minimum of 3 different days. The MDLs for both serum and VAMS samples ranged from 0.05 to 0.16 ng/mL. The PFOA, PFOS and PFHxS compounds had MDLs of 0.11 ng/mL, 0.081 ng/mL and 0.05 ng/mL respectively.

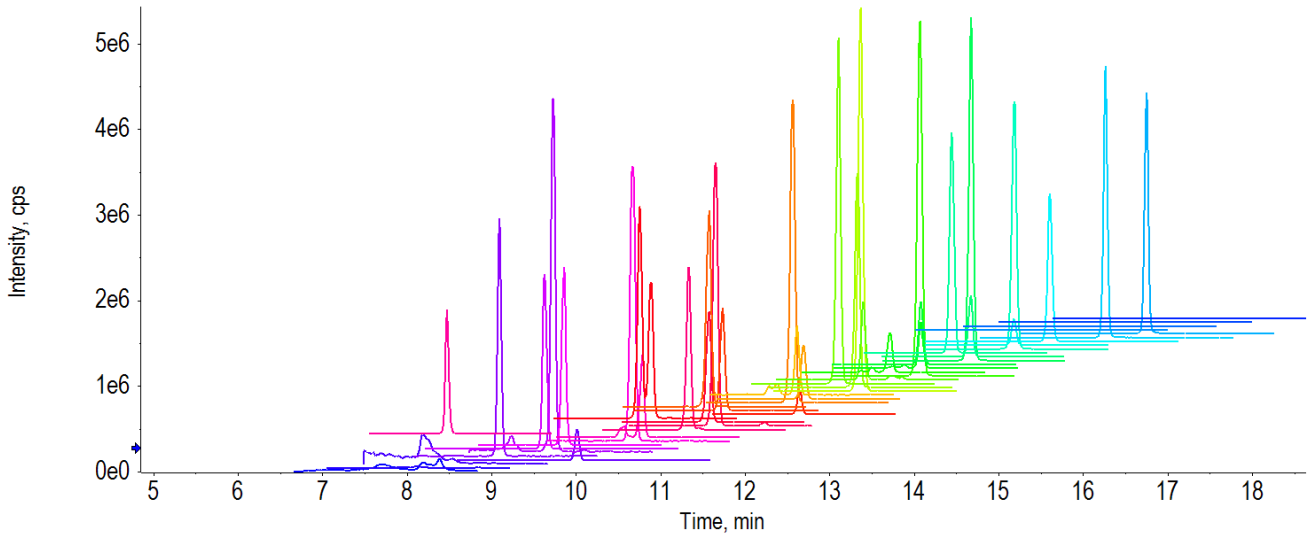

Figure 3. Example chromatogram showing the PFAS analyzed from a laboratory control sample (LCS).

Table 1. Method detection limits (MDL) for PFAS in serum and VAMS samples.

Compound Name	Acronym	MDL (ng/mL) Serum	MDL (ng/mL) VAMS
Perfluoroalkyl carboxylic acids (PFCAs)			
Linear perfluorooctanoic acid	Linear PFOA	0.11	0.11
Branched perfluorooctanoic acid	Branched PFOA	0.11	0.11
Perfluorohexanoic acid	PFHxA	0.27	0.27
Perfluoroheptanoic acid	PFHpA	0.05	0.05
Perfluorononanoic acid	PFNA	0.07	0.07
Perfluorodecanoic acid	PFDA	0.07	0.07
Perfluoroundecanoic acid	PFUnA	0.05	0.05
Perfluorododecanoic acid	PFDoA	0.05	0.05
Perfluorotridecanoic acid	PFTrDA	0.05	0.05
Perfluorotetradecanoic acid	PFTeA	0.05	0.05
Perfluorohexadecanoic acid	PFHxDA	0.05	0.05
Perfluorooctadecanoic acid	PFODA	0.061	0.061
Perfluoroalkyl sulfonic acids (PFSAs)			
Perfluorobutanesulfonic acid	PFBS	0.05	0.05
Perfluoropentanesulfonic acid	PFPeS	0.05	0.05
Perfluorohexanesulfonic acid	PFHxS	0.05	0.05
Perfluoroheptanesulfonic acid	PFHpS	0.05	0.05
Linear Perfluorooctanesulfonic acid	Linear PFOS	0.081	0.081
Branched Perfluorooctanesulfonic acid	Branched PFOS	0.081	0.081
Perfluorononanesulfonic acid	PFNS	0.08	0.08
Perfluorodecanesulfonic acid	PFDS	0.05	0.05
Perfluorododecanesulfonic acid	PFDoS	0.05	0.05
Perfluoroethylcyclohexane sulfonic acid	PFECHS	0.05	0.05
Fluorotelomer sulfonic acids			
4:2 Fluorotelomer sulfonic acid	4:2 FTS	0.05	0.05
6:2 Fluorotelomer sulfonic acid	6:2 FTS	0.17	0.17
8:2 Fluorotelomer sulfonic acid	8:2 FTS	0.05	0.05
10:2 Fluorotelomer sulfonic acid	10:2 FTS	0.05	0.05
Fluorotelomer phosphate diesters			
Bis(1H, 1H,2H,2H-perfluorooctyl) phosphate	6:2 diPAP	0.16	0.16
Bis(1H, 1H,2H,2H-perfluorodecyl) phosphate	8:2 diPAP	0.06	0.06
Bis(1H, 1H,2H,2H-perfluoroundecyl) phosphate	10:2 diPAP	0.05	0.05
(1H, 1H,2H,2H-perfluorooctyl-1H,1H,2H,2H-perfluorodecyl) phosphate	6:2/8:2 diPAP	0.07	0.07
Perfluoroalkyl sulfonamides			
Perfluorooctanesulfonamidoacetic acid	FOSA	0.05	0.05
N-methylperfluoro-1-octanesulfonamide	NMeFOSAA	0.05	0.05
N-Ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	0.06	0.06
Fluorotelomer carboxylic acid			
7:3 Fluorotelomer carboxylic acid	7:3 FTCA	0.06	0.06
Chlorinated perfluoroether sulfonic acid			
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl PF3OUdS	0.05	0.05
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl PF3ONS	0.05	0.05
Fluoroether Carboxylic and Sulfonic Acids			
4,8-dioxa-3H-perfluorononanoate	ADONA	0.05	0.05
Perfluoro-2-propoxypropanoic acid	GenX	0.05	0.05
2,2,3,3-tetrafluoro-3-((1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl)oxy)propanoic acid	Hydro EVE Acid	0.05	0.05
Perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy}ethanesulfonic acid	Hydro PS Acid	0.05	0.05
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	0.056	0.056
Perfluoro(2-ethoxyethane)sulphonic acid	PFEESA	0.05	0.05
Perfluoropolyether	PFPE 1	0.05	0.05
Perfluoro-4-methoxybutanic acid	PFMBA	0.05	0.05
1,1,2,2-tetrafluoro-2-[1,2,2,3,3-pentafluoro-1-(trifluoromethyl)propoxy] ethanesulfonic acid	R PSDCA	0.05	0.05

Method recovery study

Laboratory control samples (LCS) were aqueous solutions containing 4% bovine serum albumin that were spiked with isotopically labeled PFAS analogues and native PFAS. An LCS and a method blank (MB) sample were run every 20 samples. Recoveries ranged from 76% to 112% for the VAMS-collected samples, with an average recovery of 99% across all analytes (Figure 4). Recoveries for the serum samples, however, ranged from 69.7% to 148.3%, with an average recovery of 88.3%. These results suggest that a VAMS-based approach might improve the efficiency of the extraction of PFAS compared to the serum-based approach.

Analysis of NIST SRM 1957 Organic Contaminants in Non-Fortified Human Serum

An analysis of the NIST SRM 1957 Organic Contaminants in Non-Fortified Human Serum was performed to compare the accuracy and precision attainable using different sample collection techniques. Aliquots of NIST SRM 1957 were collected for analysis using either VAMS devices or calibrated pipettes to simulate earlier described VAMS and serum samples, respectively (Figure 5). Each measurement was performed in triplicate. The average recovery of all PFAS in the simulated VAMS sample was 100.4%, with a relative standard deviation (RSD) of 5.7%. When the serum sample was simulated, 87.3% of PFAS were recovered with an RSD of 2.6%. It is important to note that 13C labeled internal standards were added prior to the extraction process and used for quantitation. However, the serum from NIST SRM 1957 was not added to the vacutainers prior to analysis.

To further assess analytical reproducibility, we calculated the coefficients of variation (CVs) for duplicate samples and determined the average for each sample type (Figure 5). Simulated serum samples (n = 10 PFAS) exhibited average CVs >20% more frequently than simulated VAMS samples (n = 3 PFAS). Notably, there were no discernible patterns indicating higher CVs for PFAS with higher or lower detection frequencies.

Conclusions

The evaluation of the NIST SRM 1957 was conducted in triplicate using both the serum and VAMS collection methods. This experiment demonstrated consistent outcomes between the 2 approaches. The VAMS collection method exhibited greater accuracy, as evidenced by an average recovery of approximately 100% for the reported compounds. In contrast, the serum collection method displayed greater precision (RSD <3%) compared to the VAMS method (RSD <6%).

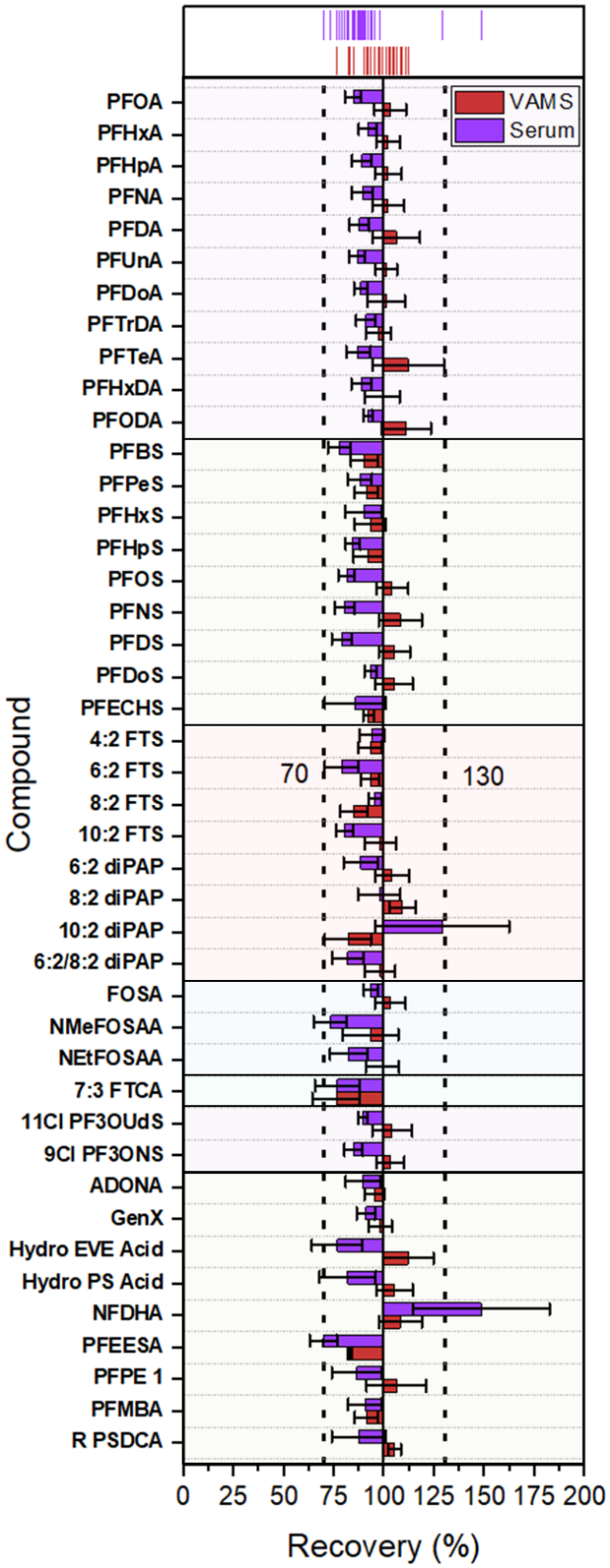

Figure 4. Compiled laboratory control sample (LCS) data for VAMS analytical batches.

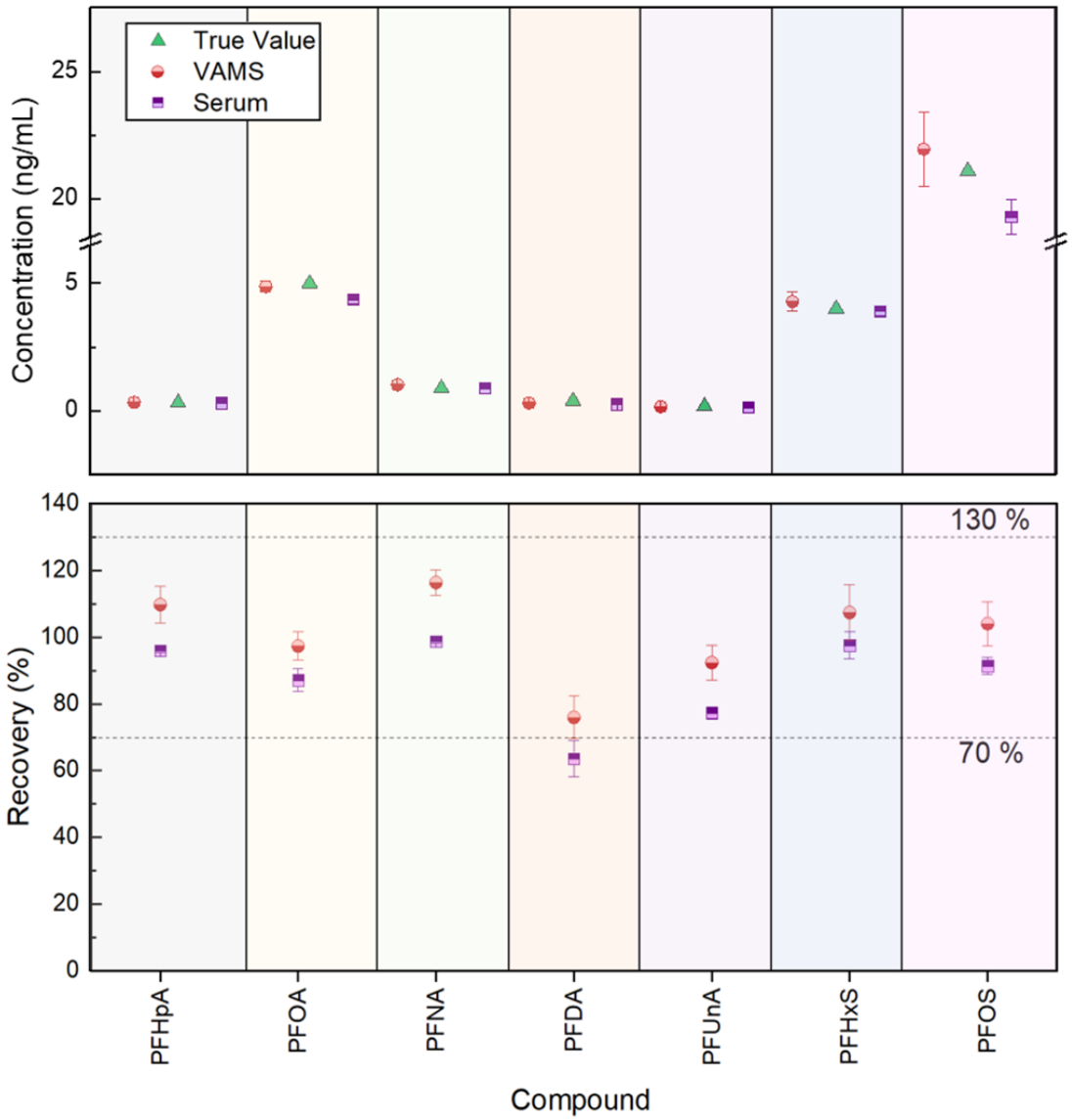

Figure 5. Evaluation of NIST SRM 1957. Three 60 µL aliquots of NIST SRM 1957 were extracted in triplicate.

The analytical method proved to be highly sensitive even with a small blood volume. The detection limits achieved through serum conversion, which ranged from 0.1 to 1.0 ng/mL, are adequately sensitive for biomonitoring purposes in the general population. This is particularly evident as median serum concentrations >1.0 ng/mL were observed for only 6 of the 15 investigated PFAS. Regardless, generating additional data using this method in conjunction with traditional serum measurements will help to understand the potential differential partitioning of emerging PFAS in serum, plasma and whole blood samples.

References

1. Hu, X. C.; Andrews, D. Q.; Lindstrom, A. B.; Bruton, T. A.; Schaider, L. A.; Grandjean, P.; Lohmann, R.; Carignan, C. C.; Blum, A.; Balan, S. A.; Higgins, C. P.; Sunderland, E. M. Detection of poly- and perfluoroalkyl substances (PFASs) in U.S. drinking water linked to industrial sites, military fire training areas, and wastewater treatment plants. Environ. Sci. Technol. Lett. 2016, 3, 344– 350.

2. Kurwadkar, S.; Dane, J.; Kanel, S. R.; Nadagouda, M. N.; Cawdrey, R. W.; Ambade, B.; Struckhoff, G. C.; Wilkin, R. Per-

and polyfluoroalkyl substances in water and wastewater: A critical review of their global occurrence and distribution. Sci. Total Environ. 2022, 809.

3. Andrews, D. Q.; Naidenko, O. V. Population-wide exposure to per- and polyfluoroalkyl substances from drinking water in the United States. Environ. Sci. Technol. Lett. 2020, 7, 931–936.

4. Poothong, S.; Papadopoulou, E.; Lundanes, E.; Padilla-Sánchez, J. A.; Thomsen, C.; Haug, L. S. Dried blood spots for reliable biomonitoring of poly- and perfluoroalkyl substances (PFASs). Sci. Total Environ. 2019, 655, 1420–1426.

5. Kato, K.; Wanigatunga, A. A.; Needham, L. L.; Calafat, A. M. Analysis of blood spots for polyfluoroalkyl chemicals. Anal. Chim. Acta 2009, 656, 51– 55.

6. Boyd Barr, D.; Kannan, K.; Cui, Y.; Merrill, L.; Petrick, L. M.; Meeker, J. D.; Fennell, T. R.; Faustman, E. M. The use of dried blood spots for characterizing children's exposure to organic environmental chemicals. Environ. Res. 2021, 195, 110796.

7. Ma, W.; Kannan, K.; Wu, Q.; Bell, E. M.; Druschel, C. M.; Caggana, M.; Aldous, K. M. Analysis of polyfluoroalkyl substances and bisphenol A in dried blood spots by liquid chromatography tandem mass spectrometry. Anal. Bioanal. Chem. 2013, 405, 4127– 4138.

8. Protti, M.; Mandrioli, R.; Mercolini, L. Tutorial: Volumetric absorptive microsampling (VAMS). Anal. Chim. Acta 2019, 1046, 32–47. DOI: 10.1016/j.aca.2018.09.004

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