Method validation of per- and polyfluoro alkyl substances (PFAS) in human plasma using high pressure liquid chromatography mass spectrometry (LC-MS/MS)

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Introduction

Per- and polyfluoroalkyl substances (PFAS) have been manufactured for 60 years, and their properties, utility, environmental impact, human exposure, and potential adverse health outcomes are reported.[1] The quantitation of PFAS in human serum liquid chromatography tandem mass spectrometry (LC-MS/MS) method are developed.[2]

This work aims to evaluate modifications to include human plasma in an already validated bioanalytical method for testing 12 PFAS in human serum. Human serum and plasma are similar, and latter differ only that contains fibrinogen and EDTA from the collection tube. Human serum and plasma have been tested for PFAS using the same method.[3] The FDA provided relevant guidance for this validation, which states: "changes in matrix within species (e.g., switching from human plasma to human blood or changes to the species within the matrix (e.g., switching from rat plasma to mouse plasma)".[2] Under these parameters, a partial validation is satisfactory and will include inter-assay accuracy and precision.

Method

Fifty µl plasma, 25 µl internal standards and 525 µl 0.10 M formic acid were combined, mixed and purified using C_8 cartridges (2 x 10 mm x 3.5 µm) by online solid phase extraction. The analytes were separated by liquid chromatography using an Agilent XDB-C_8 column (3 x 100 mm x 3.5 µm) and detected using a Sciex 6500 TQ Qtrap under schedules multiple reaction monitoring mode.

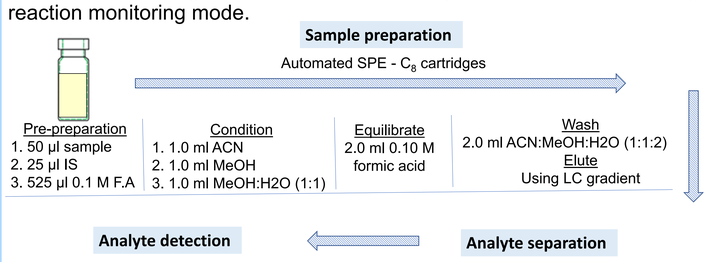


Fig 1. Analyte detection by LC-MS/MS Instrument Sciex Qtrap 6500 and spark Holland SPE/HPLC system.

	Time (min)	Flow ul/min	0.10M NH_4OAc (%)	ACN & MeOH (1:1) (%)
1	00:01	0.80	50	50
2	01:00	0.80	50	50
3	01:30	0.80	35	65
4	10:00	0.80	20	80
5	10:30	0.80	5	95
6	11:30	0.80	5	95
7	11:31	0.80	50	50
8	15:00	0.80	50	50

Table 1. Analyte separation using HPLC gradient program on a Agilent XDB-C_8 column (3 x 100 mm x 3.5 µm).

The accuracy and precision were be determined by repeated measurements (n=8) at 2.0 ng/ml and 10 ng/ml target concentrations in human plasma. One batch was acquired per day and consisted of two set of samples. The accuracy and inter-day precision are presented in **table 2**.

Results

Separation and calibration

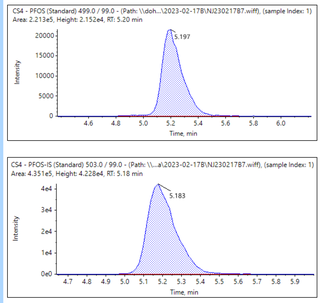
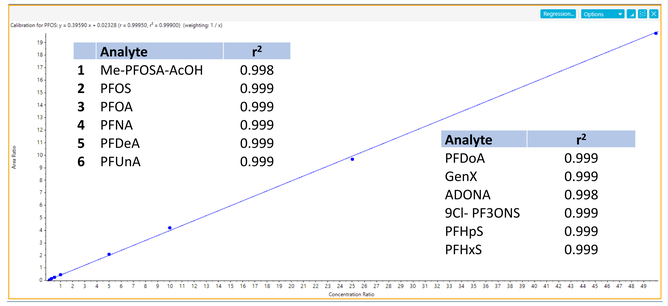


Fig 2. Representative, separated PFOS and IS peaks.



	Analyte	r^2
1	Me-PFOSA-AcOH	0.998
2	PFOS	0.999
3	PFOA	0.999
4	PFNA	0.999
5	PFDeA	0.999
6	PFUnA	0.999

Analyte	r^2
PFDoA	0.999
GenX	0.999
ADONA	0.998
9Cl- PF3ONS	0.999
PFHpS	0.999
PFHxS	0.999

Fig 2. PFOS as a representative 8 point calibration curve (0.05 – 100 ng/ml) and analyte r^2 values.

Accuracy

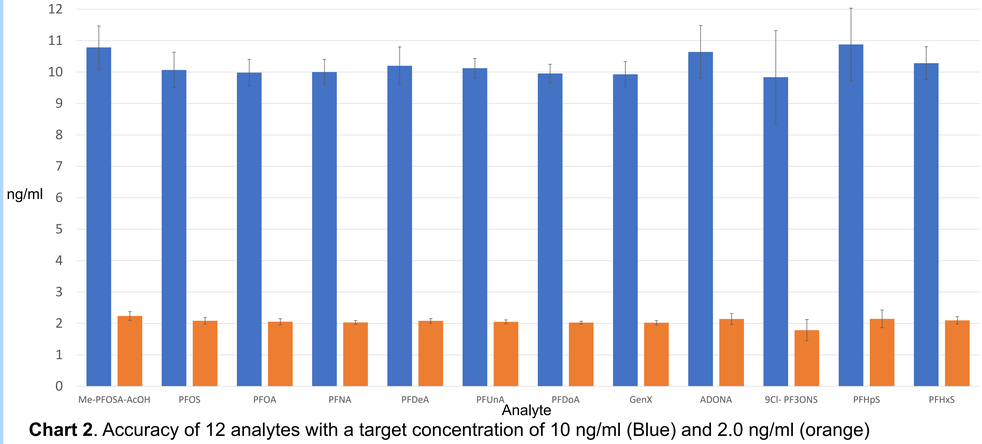


Chart 2. Accuracy of 12 analytes with a target concentration of 10 ng/ml (Blue) and 2.0 ng/ml (orange)

Precision

Analyte	RSD (n=8, 2.0 ng/ml)	RSD (n=8, 10 ng/ml)
Me-PFOSA-AcOH	6%	6%
PFOS	5%	6%
PFOA	5%	4%
PFNA	3%	4%
PFDeA	4%	6%
PFUnA	3%	3%
PFDoA	2%	3%
GenX	3%	4%
ADONA	8%	8%
9Cl- PF3ONS	19%	15%
PFHpS	13%	11%
PFHxS	6%	5%

Table 2. Precision of analytes spiked at 2 ng/ml and 10 ng/ml (n=8) analyzed over 4 days.

Conclusions

The accuracy and precision were determined for 12 PFAS in human plasma using an already validated bioanalytical method for human serum. All 12 analytes were baseline separated and the calibration curve for most analytes have r^2 values of 0.999 or except for Me-PFOSA-AcOH, which have r^2 values of 0.998. The accuracy for 2.0 ng/ml an 10 ng/ml spiking levels are presented in **chart 2**. The recovery for each analyte for both levels fell within ± 30% of the spiked concentrations and most analytes were within 10 %. The mean spike recovery is between 100 % - 109 %, and 98 % - 107 % for 2.0 ng/ml and 10 ng/ml respectively. The standard deviation is excellent for both concentrations.

The inter-day precision was determined at 2.0 ng/ml and 10 ng/ml target concentration levels. The RSD for samples spiked at 2.0 ng/ml and 10 ng/ml are 6% or less for 9 out of 12 analytes. The RSD for ADONA, 9Cl-PF3ONS and PFHpS are worse, and are between 8 – 19 %, this is because these analytes do not have matching internal standards (see **table 2**). This study confirms the results of previously published LC-MS/MS methods that permits the analyzed serum and plasma for PFAS using the same method. Published methods differ from this method, in the sample preparation, where protein crash was preformed followed by SPE using weak anionic exchange cartridge. This method uses C_8 SPE cartridges to accomplish sample purifications after sample dilution in 0.1 M formic acid.

Further improvement if the method will be focused on eliminating resource intense SPE procedures. Others have published method on the quantitation of PFAS in human serum by direct injection after protein crash.

References

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2. Ye, X.; Prikel, L.J.; Center for disease control and prevention. Perfluoroalkyl and Polyfluoroalkyl Substances (cdc.gov) (accessed 02/21/23).
3. Fregerio, G.; Cafagna.; Polledri, E.; Mecdante,R.; Fustinoni, S.; Development and validation of and LC-MS/MS method for the quantitation of 30 legacy and emerging per-and polyfluoroalkyl substances (PFASs) in human plasma, including HFPO-DA, DONA and cC604. Anal Bioanal Chem. **2022**. 414, 1259 -1278

Acknowledgements

This study was supported by the New Jersey State Government. Thank each colleague in the Chemical Terrorism Lab and other NJ State Biomonitoring team members for their endless support.

This poster is also partially funded by the CDC's State Biomonitoring Cooperative Grant (Grant #: NU88EH001326).