Method Optimization and Validation of PFAS in Human Serum Using Online SPE UHPLC-MS/MS

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INTRODUCTION

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are emerging environmental contaminants that have received much attention recently due to their persistence, abundance, and toxicity. Colloquially known as "forever chemicals," they have been extensively used in the production of hundreds of everyday products since their invention in the 1930s.[1] PFAS bioaccumulate in the human body. Although health effects have not been clearly defined, recent studies suggest a probable association between PFAS exposure and negative health outcomes.[2,3]

To date, human exposure studies have primarily focused on measuring a limited number of conventional PFAS in serum. However, growing research data indicates that PFOA and PFOS are present as branched and linear isomers whose accurate measurement is important to support health studies and source identification.[4] With the phasing out of conventional PFAS and the rise of newer replacements (such as GenX, ADONA, and 9Cl-PF3ONS), it is imperative to develop methods that accurately measure the emerging PFAS in the human body.

We recently optimized and validated what we believe is ***the first*** online SPE-UHPLC-MS/MS method for accurately identifying and quantifying 18 PFAS, including linear and branched PFOS and PFOA, GenX, ADONA, and 9Cl-PF3ONS in human serum. This new method uses a Chronos online SPE unit, coupled with a UHPLC (CHRONECT SPH1299) by Spark Holland and a Sciex 7500 mass spectrometer. The method is based on the CDC Method #6304.09; but has been significantly modified to operate under UHPLC conditions and greatly improved to give much lower LODs with recoveries >90%.

A high background encountered during the development was controlled by strategic selection of SPE cartridge, column, solvent adjustment, and cleaning protocol. The addition of a delay column helped to lower the background by separating contamination interference from the analytes.

OBJECTIVES

- Optimize an online SPE-UHPLC-MS/MS method to measure 18 PFAS in human serum based on CDC method 6304.09
- Determine the optimal transition, charge energy (CE), and collision energy (CXP) for each compound to improve sensitivity and selectivity using the Sciex 7500
- Optimize the column selection, mobile phases, gradient program, and flow rate to achieve the best separation with a UHPC system
- Determine the optimal injection volume to maximize sensitivity while decreasing amount of sample used
- Screen different solvents and SPE cartridges to decrease high background levels
- Validate the method for use in biomonitoring studies

REFERNCES

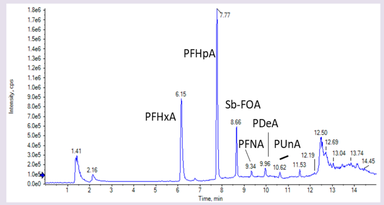
1. Plunkett, R.J. The History of Polytetrafluoroethylene: Discovery and Development. In High Performance Polymers: Their Origin and Development, In *Proceed. Symp.* Seymour, R.B., Kirshenbaum, G.S., Eds.; Elsevier: New York, NY, USA, 1987. High Performance Polymers at the ACS Meeting in New York, April 1986
2. *Agency for Toxic Substances and Disease Registry (ATSDR) website.* https://www.atsdr.cdc.gov/pfas/health-effects/index.html (accessed 2022-10-28)
3. Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. *J Expo Sci Environ Epidemiol.* **2019**, 29 (2), 131-147. doi: 10.1038/s41370-018-0094-1.
4. *Centers for Disease Control and Prevention (CDC) website.* https://www.cdc.gov/exposurereport/pfos_pfoa_calculation.html (accessed 2022-10-28)

METHOD COMPARISON

	CDC Method	Our Method
System	Symbiosis Online SPE with HPLC	Symbiosis Online SPE with UHPLC
Analysis Column	Merck KGaA Chromolith® High Resolution RP-18e HPLC column	Agilent Zorbax Rapid Resolution SB-C18 UHPLC column 150 mm and 1.8 micron
Mobile Phase A	20mM ammonium acetate buffer/acetonitrile (95:5), pH = 4	10mM ammonium acetate buffer/acetonitrile (95:5), pH = 4
Mobile Phase B	Acetonitrile	Acetonitrile
Flow Rate	1.0 to 1.5 mL/min	0.3 to 0.4 mL / min
Gradient	% B = 25% to 95% 0 to 11 min % B = 95% to 75% 11.01 - 13.01 min	% B = 28% to 95% 0 to 11 min % B = 95% to 28% 11.1 to 15.0 min
MS Instrument	Sciex 5500, 6500+, Qtrap 6500	Sciex Qtrap 7500
Ionization source	Negative Turbo Ion Spray (TIS)	Negative Turbo Ion Spray (TIS)
SPE Cartridge	HySphere C8-SE	CHROspe Polymer DVB
Injection Volume	550 µL	250 µL
Serum Volume	50 µL	25 µL

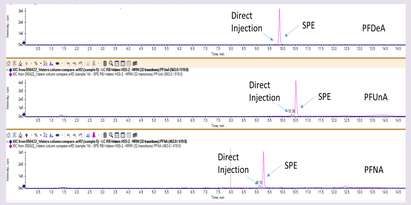
CHALLENGES

High Background



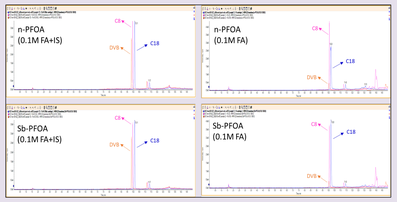
- Blank samples had analytes present
- ***6 analytes identified as major source of the high background***

Direct Injection and SPE



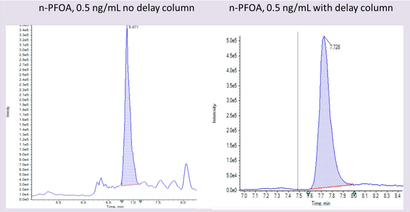
- Blank contamination higher when SPE used
- SPE program needs improvement
- ***Contamination is from the SPE cartridge***

SPE Cartridge Screening



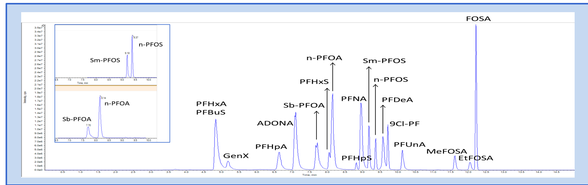
- IS remains constant regardless of cartridge
- Background analytes drop when DVB cartridge used
- ***DVB = better cartridge***

Delay Column



- No delay column = signal above background
- Delay column = background contamination removed
- ***Delay column improves sensitivity and selectivity***

UHPLC SEPARATION



- Most compounds are baseline separated
- Elution time < 13 min
- Linear and branched PFOS and PFOA are well separated

VALIDATION RESULTS

LOD, Range, Linearity

Analyte	LOD (ppb)	S/N (N=7)	Linear range (ppb)	Linearity (n=3)
9Cl-PF	0.01	605	0.05 - 20	0.998
ADONA	0.01	402	0.05 - 20	0.998
EtFOSAA	0.01	252	0.05 - 20	1.00
FOSA	0.01	1320	0.05 - 20	0.997
GenX	0.01	200.	0.05 - 20	0.997
MeFOSAA	0.01	124	0.05 - 20	0.999
n-PFOA	0.02	191	0.05 - 20	0.996
n-PFOS	0.05	254	0.05 - 20	0.999
PFBuS	0.01	248	0.05 - 20	0.999
PFDeA	0.03	266	0.1 - 20	0.995
PFHpA	0.03	214	0.1 - 20	0.992
PFHpS	0.01	338	0.05 - 20	0.998
PFHxA	0.02	98	0.05 - 20	0.999
PFHxS	0.01	134	0.05 - 20	0.999
PFNA	0.02	368	0.05 - 20	0.994
PFUnA	0.01	482	0.05 - 20	0.992
Sb-PFOA	0.03	691	0.1 - 40	0.996
Sm-PFOS	0.01	490.	0.05 - 20	0.999

Accuracy

	N = 6		
Analyte	0.25 ng/mL	2.5 ng/mL	10 ng/mL
9Cl-PF	105%	108%	105%
ADONA	102%	90.6%	99.7%
EtFOSA	102%	101%	107%
FOSA	105%	101%	103%
GenX	106%	95.5%	102%
MeFOSA	103%	99.0%	106%
n-PFOA	104%	93.3%	106%
n-PFOS	96.1%	96.6%	104%
PFBuS	103%	95.5%	105%
PFDeA	97.0%	96.2%	108%
PFHpA	112%	99.7%	107%
PFHpS	106%	107%	113%
PFHxA	110%	102%	108%
PFHxS	101%	98.2%	106%
PFNA	107%	91.6%	104%
PFUnA	98.5%	102%	111%
Sb-PFOA	106%	106%	114%
Sm-PFOS	98.6%	98.0%	103%

Precision

	3 days, N = 6		
	0.25 ng/mL	2.5 ng/mL	10 ng/mL
Analyte	%RSD	%RSD	%RSD
9Cl-PF	6.7	7.4	2.0
ADONA	4.8	4.6	12
EtFOSA	2.3	4.8	1.2
FOSA	4.0	7.4	5.8
GenX	9.6	6.0	3.0
MeFOSA	6.2	5.0	4.0
n-PFOA	5.0	7.0	13
n-PFOS	4.4	7.2	3.4
PFBuS	2.7	3.6	2.1
PFDeA	19	10.	9.5
PFHpA	7.1	17	3.7
PFHpS	6.8	5.5	7.0
PFHxA	7.3	3.9	5.2
PFHxS	5.0	3.7	3.4
PFNA	11	14	8.2
PFUnA	9.9	6.6	6.1
Sb-PFOA	14	15	14
Sm-PFOS	4.9	7.8	6.5

CONCLUSIONS

- A high background encountered during the development was controlled by selection of SPE cartridge, column and delay column, and solvent adjustment
- Improved LOD from 0.1 ng/mL to 0.05 ng/mL for most compounds (Sb-PFOA, PFDeA, and PFHpA = 0.1 ng/mL) compared to CDC 6304.09 with linearity R^2>0.99
- Decreased injection volume from 550 µL to 250 µL compared to CDC 6304.09 increasing sensitivity 4x.
- Excellent accuracy (91-114%) and precision (RSD=2-19%) were achieved
- This method has been used to support collaborative university research programs as well as the NJ Health and Nutrition Examination Survey (NJHANES)

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Disclaimer: Contents and conclusions presented here are solely the authors' and do not necessarily represent the views of the CDC