# The Development of a High Throughput Solid Phase Extraction Method for PFAS in Human Serum Using an Automated Janus® Workstation

**Zhan Quan Li[1,2],** Linbin Zhong[2], Carrie Xu[2], Shawn O'Leary[2], Annie Lin[2], Collen Lestician[2], Diana Mathes[2] and Tina Fan[2]

1. APHL-CDC Public Health Laboratory Fellowship, 2. New Jersey Department of Health, Public Health and Environmental Laboratories, Environmental and Chemical Laboratory Services, Ewing, NJ 08628

## Introduction

Per- and Polyfluoroalkyl Substances (PFAS) are a large group of organic persistent pollutants that are of interest in human health studies. PFAS can be quantitated in human serum with ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Samples typically undergo solid phase extraction (SPE) before instrument analysis. The PFAS method used at the New Jersey Public Health Laboratories (NJPHL) employs an online automated sample micro-SPE prior to injection and chromatographic separation. Since these events occur sequentially, sample throughput is a limitation of this approach. We aim to develop and optimize this SPE process and automate the pre-sample preparation steps. This method will be validated for precision and accuracy. This new SPE method reduces the duration to prepare 48 samples from eight to two hours. Initial chromatographic peaks shapes were poor but was resolved by dissolving the sample in initial mobile phase conditions. Low analyte peak intensities were first observed because lower sample volumes were injected but increasing the injection volume from 40 µL to 100 µL fixed this problem. The new method produces acceptable calibration curves, the $R^2$ are between 0.991 to 0.998 for all analytes. The accuracy of matrix spiked samples is within ±30% of the nominal (accepted) value.

## Method

A SPE protocol was created using "WinPREP® for Janus®" software on a Janus® workstation. The protocol is built by combining pipetting orders on the list provided by the software and run on the Janus® application assistant program. The protocol combines 50 µL human serum sample, 50 µL internal standard and 200 µL acetonitrile. This mixture is centrifuged at 4,000 RPM for 20 minutes. 200 µL of the supernatant is loaded on the SPE plate (Oasis® WAX) and washed with 200 µL 1% formic acid and 1:3 acetonitrile: water solution. A conditioning step is then performed with 200 µL 2% ammonia in methanol, 200 µL methanol, and 200 µL 1% formic acid. The analytes are eluted with 50 µL methanol and 100 µL 2% ammonia in methanol. The samples are dried at 70 ℃ and reconstituted with 120 µL an initial mobile phase solution. The sample is injected into Sciex 7500 UHPLC-MS/MS instrument with UHPLC Guard 3PK column/InfinityLab ® PFC Delay column for LC-MS/MS analysis.

**Protocol Part 1: Sample Pre-preparation**

**1.** 50 µL Internal Standard
**2.** 50 µL Serum Sample
**3.** 200 µL Acetonitrile containing 1% Formic Acid




**Centrifuge : 4,500 rpm for 10 min**

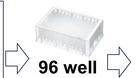
**Protocol Part 2: Condition**

**4.** 200 µL 2% (v/v) ammonia in methanol
**5.** 200 µL methanol
**6.** 200 µL Formic Acid

**SPE Plate** Activating vacuum manifold

**Protocol Part 3 Loading Sample**

**7.** 200 µL Supernatant from the part 1
**8.** 800 µL Formic Acid



**96 well Plate** Diluting Sample

**SPE Plate** Activating vacuum manifold

**Protocol Part 4: Wash Plate**

**9.** 200 µL 1% Formic Acid

**96 well Plate**

**SPE Plate** Activating vacuum manifold

**10.** 200 µL 1:3 acetonitrile : water solution

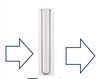
**SPE Plate** Activating vacuum manifold

**Protocol Part 5: Elution and Drying out**

**11.** 50 µL Methanol
**12.**100 µL 2%(v/v) ammonia/methanol

**SPE Plate** Activating vacuum manifold

**Collective Plate**



**Test Tube**

**Water Bath**

Drying out:
70°C
10 psi for 20 minutes

**Protocol Part 6: Reconstitution**

**13.**120 µL 72:28 Mobile Phase A: Mobile Phase B Solution

**Test Tube**

**vial**

**UHPLC-MS/MS system**

## Results

### Peak Shapes

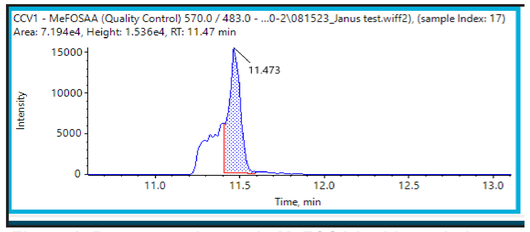



**Figure 1: Representative peak: MeFOSAA without drying down and reconstitution procedure**

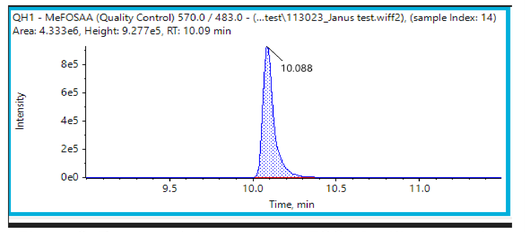



**Figure 2: Representative peak: MeFOSAA with drying down and reconstitution procedure**

### Calibration Curve and Correlation Coefficient

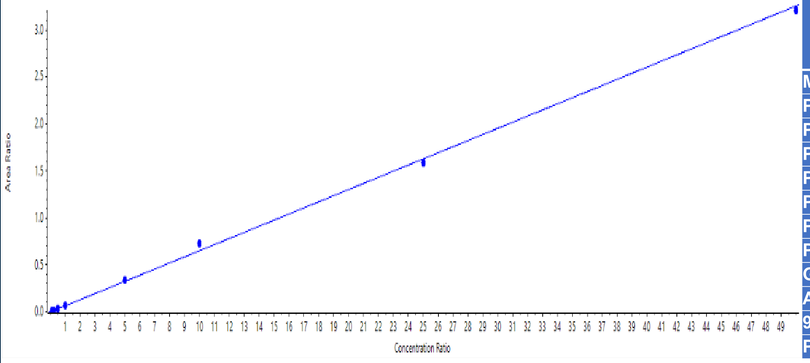



**Figure 3: MeFOSAA as a representative 10 points calibration curve (Automated off-line SPE method)**

| | R^2 volume of the new automated off-line SPE Method |
|---|---|
| MeFOSAA | 0.997 |
| PFHxS | 0.993 |
| PFHpS | 0.998 |
| PFOS | 0.993 |
| PFOA | 0.991 |
| PFNA | 0.992 |
| PFDeA | 0.992 |
| PFUnA | 0.996 |
| GenX | 0.997 |
| ADONA | 0.994 |
| 9Cl-PF | 0.996 |
| PFDoA | 0.995 |

**Table 1: R^2 Volume of the new automated off-line SPE Method**

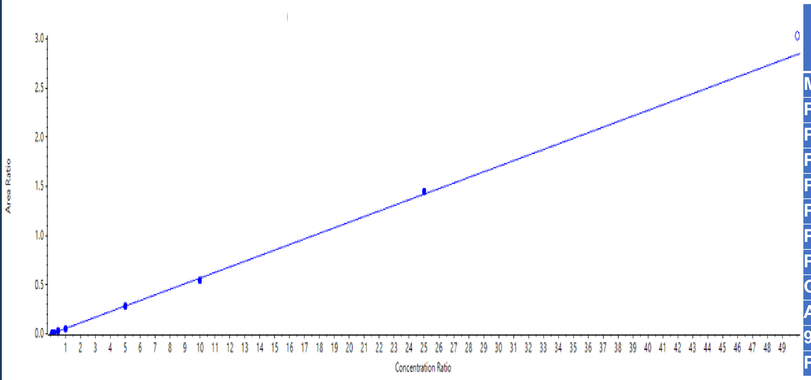



**Figure 4: MeFOSAA as a representative 10 points calibration curve (on-line SPE method)**

| | R^2 volume of the original on-line SPE Method |
|---|---|
| MeFOSAA | 0.999 |
| PFHxS | 0.999 |
| PFHpS | 0.997 |
| PFOS | 0.997 |
| PFOA | 0.998 |
| PFNA | 0.999 |
| PFDeA | 0.996 |
| PFUnA | 0.999 |
| GenX | 0.995 |
| ADONA | 0.992 |
| 9Cl-PF | 0.999 |
| PFDoA | 0.999 |

**Table 2: R^2 Volume of the original on-line SPE Method**

### Recovery Rate

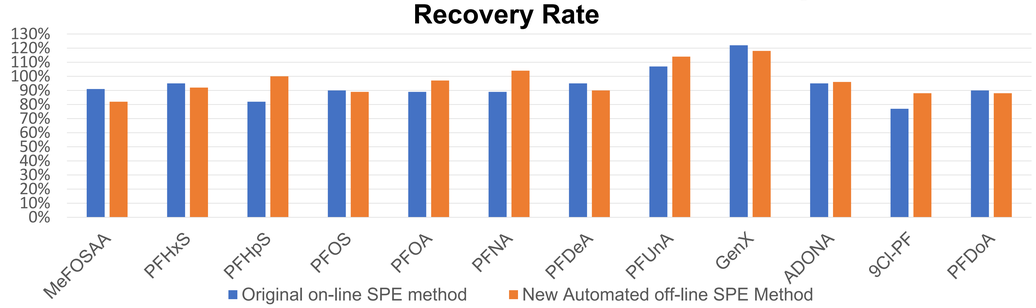



**Figure 5. Recovery Rate of 14 analytes with the original online SPE method and the new automated off-line SPE method**

## Conclusions

1. A new Janus protocol has been created for the new automated off-line SPE method.
2. The poor peak shape issue in the UHPLC-MS/MS analysis is a major challenge of the new method development. Drying out and reconstituting the analytes before the injection of the UHPLC-MS/MS system can improve the peak shape significantly (See Figure 1 and Figure 2.)
3. The new protocol has already been tested on the Janus. This new SPE method reduces the time it takes to prepare 48 samples from eight to two hours.
4. The test results (See Figure 3) showed that the new off-line SPE method produces acceptable calibration curves, the $R^2$ are between 0.991 to 0.998 for all analytes as the original on-line SPE method does (See Figure 4.)
5. According to the test result (See Figure 5), the new automated SPE method has the same recovery rate as the original on-line SPE method had. Like the original online SPE method, the accuracy of the matrix spiked samples is within ±30% of the nominal value. This means the usage of the automated off-line method will not decrease the accuracy and precision of the sample preparation.
6. We will continue to improve and test the protocol for the further method validation.
7. This method will be used in our biomonitoring projects for PFAS after method validation.

## References

1.Brandsch, Thomas. "Determination of PFAS in Water According to EU 2020/2184 and DIN 38407-42 Using Online SPE–LC–MS/MS." (2022).

2.Silva MJ, Samandar E, Preau JL Jr, Reidy JA, Needham LL, Calafat A. Automated solid-phase extraction and quantitative analysis of 14 phthalate metabolites in human serum using isotope dilution-high-performance liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 2005 Nov-Dec;29(8):819-24. doi: 10.1093/jat/29.8.819. PMID: 16374941.

3."Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS).*National Institute of Environmental Health Sciences,* ***https://www.niehs.nih.gov/health/topics/agents/pfc/index.cfm*** . Accessed 17 January 17, 2023.

4.Krista Y. Christensen, Michelle Raymond, Jon Meiman, Perfluoroalkyl substances and metabolic syndrome, International Journal of Hygiene and Environmental Health, Volume 222, Issue 1, 2019, Pages 147-153, ISSN 1438-4639,

https://doi.org/10.1016/j.ijheh.2018.08.014.

(https://www.sciencedirect.com/science/article/pii/S1438463918304024)

5.The Centers for Disease Control and Prevention. (2017-2018) Online Solid Phase Extraction-High Performance Liquid Chromatography-Turbo Ion Spray-Tandem Mass Spectrometry (online SPE-HPLC-TIS-MS/MS). Retrieved February 21, 2023, from Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) Laboratory Procedure Manual (cdc.gov)

6. Organtini KL, Rosnack KJ, Lame ME, Calton LJ. A Method for the Extraction and Analysis of PFAS from Human Serum Utilizing Weak Anion Exchange (WAX) Chemistry and Xevo TQ-S micro. Waters Corporation. 2021. A Method for the Extraction and Analysis of PFAS from Human Serum Utilizing Weak Anion Exchange (WAX) Chemistry and Xevo TQ-S micro (waters.com)

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