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Application Note

A Semi-Automated Extraction of PFAS from Human Serum and Plasma Using Otto SPEcialist

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Human biomonitoring projects have identified per- and polyfluorinated alkyl substances (PFAS) as a group of persistent environmental compounds commonly found in humans. Sample preparation for these compounds requires solid phase extraction (SPE) and can be performed in a 96 well plate. A previous method optimized the extraction and SPE clean up protocol for human serum using a negative pressure SPE manifold. With the introduction of the Otto™ SPEcialist positive pressure manifold, the PFAS method was transferred to this device and evaluated for accuracy and repeatability in both human serum and plasma. The Otto SPEcialist performed similar to, and in some cases better than, the negative pressure manifold in terms of accuracy. Otto SPEcialist also was shown to have high precision with low %RSD values for replicate extractions of both human serum and plasma.

Benefits

- Otto SPEcialist provides a semi-automated positive pressure extraction method increasing efficiency,

reproducibility, and accuracy of results

- Addition of the Otto SPEcialist, enhances and improves the total PFAS analysis workflow for human biomonitoring and exposure studies

Introduction

Large scale biomonitoring studies, such as the CDC's National Health and Nutrition Examination Survey (NHANES)[1] and European Human Biomonitoring Initiative (HBM4EU)[2] have identified numerous PFAS compounds in human biofluids. The best representative matrices of human exposure to PFAS have been either serum or plasma since blood derived matrices contain the highest concentrations of PFAS, thus requiring only a small sample size for sensitive analysis.[3]

Previously, a 96 well plate-based extraction method using weak anion exchange (WAX) was optimized for extraction of PFAS from human serum.[4] The method was optimized and run using a negative pressure SPE manifold. As an alternative to using a negative pressure manifold, positive pressure SPE provides a more efficient and reliable solution.

The Otto SPEcialist is a semi-automated software driven positive pressure sample preparation device. Positive pressure SPE is advantageous over a negative pressure approach due to the equal application of pressure across all wells/cartridges making sample loading and elution more uniform. Additionally, positive pressure does not suffer from the maximum pressure limits of negative pressure utilizing a vacuum that can severely affect flow rates when cartridges become clogged. Finally, the Otto SPEcialist is programmable to automatically adjust pressure settings during the extraction process, which improves not only efficiency, but reproducibility as well. The goal of the following work was to transfer the previous extraction method onto the Otto SPEcialist, as well as to evaluate the method for human plasma.

Results and Discussion

The same SPE protocol and instrument methods described in Waters Application Note 720007114 were used for

the evaluation of the Otto SPEcialist.[4] In addition to human serum, the Otto SPEcialist was also used to extract human plasma to determine if the serum method was applicable to plasma as well.

NIST standard reference materials (SRMs) for human serum (NIST 1957) and human plasma (NIST 1950) were used to initially measure the method performance. NIST 1957 contains certified levels of seven non-fortified PFAS compounds ranging from 0.172–21.1 µg/kg, whereas NIST 1950 contains certified levels of six non-fortified PFAS compounds ranging from 0.182–10.43 µg/kg. Calculated concentrations and the % bias compared to the NIST certified value can be seen in Figure 1. The experimental values in both SRMs using the Otto extraction provided accurate calculated concentrations within 12% in serum and within 10% in plasma, with the exception of PFDA which had a 30% bias. The high bias in serum for PFDA could be due to the low tolerance on uncertainty for this certified value. The serum SRM was also compared to previous results using a manual negative pressure manifold with the results being very comparable or even slightly lower in bias for the Otto SPEcialist.

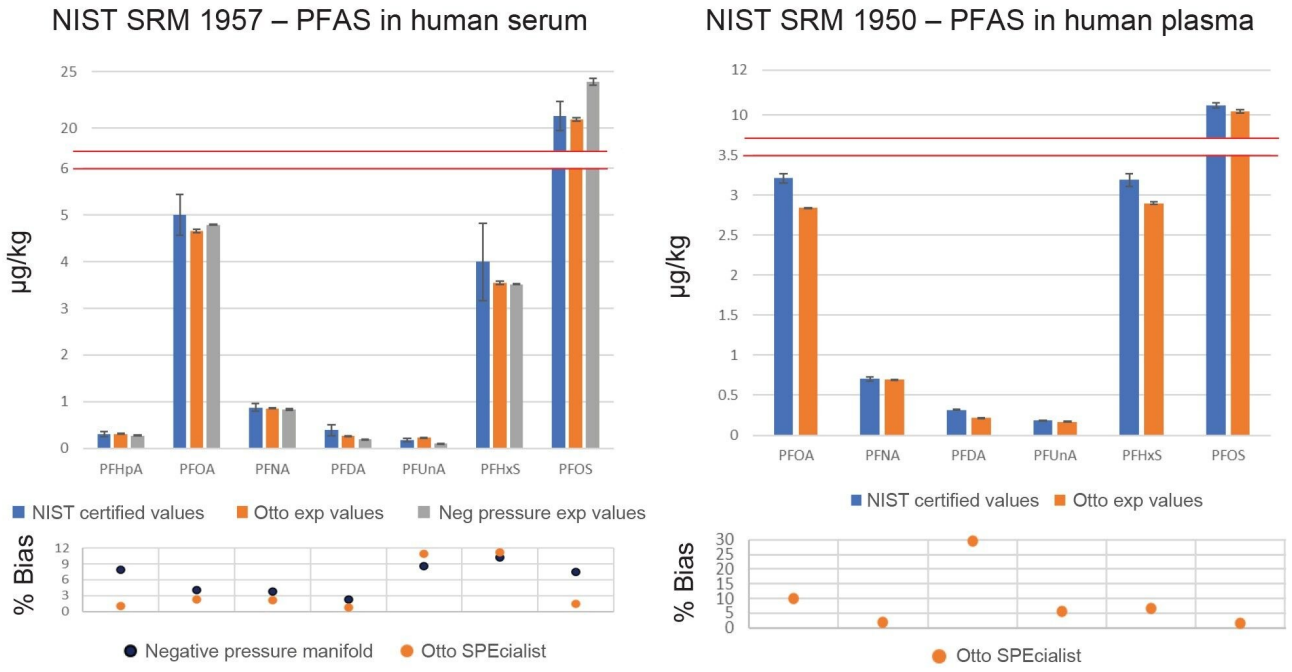

Figure 1. Analysis results for four replicates of the NIST 1957 (left) and NIST SRM 1950 (right) standard reference materials. (Top) Concentration values determined in the SRM sample. (Bottom) Calculated percent bias of the observed values from the NIST certified values.

Additional experiments were run to evaluate a broader range of PFAS by spiking pooled lots of human serum

and plasma with 27 PFAS. Accuracy and precision are detailed in Table 1 for three replicates of each matrix spiked at a concentration of 4 µg/kg (equivalent to 1 ng/mL injected concentration after SPE). The spiking experiment in serum was extracted using both the manual negative pressure manifold and OttoSPEcialist. Overall, the precision was better using the Otto SPEcialist, demonstrated by %RSD values below 10%, except for a few outlying compounds that were below 16% RSD. Accuracy of calculated concentration was also high for the Otto SPEcialist with all but two compounds having an accuracy greater than 80% in both matrices.

	Plasma		Serum			
	Otto SPEcialist		Otto SPEcialist		Negative pressure manifold	
Compound	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
PFBA	N/A	N/A	94	12	N/A	N/A
PFPeA	84	1.1	94	1.1	96	4.3
PFHxA	93	1.1	94	1.9	86	3.2
PFHpA	86	2.3	100	0.8	79	12
PFOA	96	1.8	91	0.4	80	8.1
PFNA	96	1.7	97	1.6	95	10
PFDA	97	4.5	100	4.0	88	4.6
PFUnDA	100	3.4	98	5.7	90	7.4
PFDoDA	97	6.6	98	0.8	87	3.2
PFTriDA	68	7.0	72	3.9	71	13
PFTreDA	100	3.8	100	5.5	78	2.2
PFBS	98	2.1	99	2.8	86	4.3
PFPeS	95	2.2	100	2.4	88	3.5
PFHxS	95	5.4	84	1.7	98	18
PFHpS	84	5.0	87	3.0	80	4.7
PFOS	95	6.9	81	0.7	90	21
PFNS	96	3.8	91	8.1	95	5.5
PFDS	90	3.2	90	2.0	98	14
GenX	98	1.2	100	6.5	87	4.8
ADONA	85	2.6	83	2.3	98	3.1
9ClPF3ONS	99	7.9	97	3.2	91	1.6
11ClPF3OUdS	92	5.3	80	11	93	6.1
4_2 FTS	92	13	98	4.5	96	19
8_2 FTS	78	9.6	84	16	72	19
FOSA	96	3.9	97	4.1	87	2.8
NMeFOSAA	87	9.3	90	9.1	98	23
NEtFOSAA	91	5.4	95	9.0	93	9.4

Table 1. Accuracy and %RSD measurements of calculated concentration for three replicates of human serum and plasma spiked with 4 µg/kg (1 ng/mL in well injected concentration) PFAS extracted using the Otto SPEcialist and negative pressure manifold. N/A denotes the measurement is not available due to high background interference.

Conclusion

A previously established method for PFAS extraction from human serum evaluated using a negative pressure SPE manifold was successfully transferred onto the semi-automated Otto SPEcialist positive pressure extraction system. In addition, the method was shown to be suitable for the extraction of PFAS from human plasma as well. The system was evaluated using two NIST SRMs, which produced accurate results that agreed closely with the NIST certified values. An extended suite of 27 PFAS were also spiked into human serum and plasma samples and extracted using the Otto SPEcialist which produced accurate and precise results. The use of the Otto SPEcialist for the analysis of human biofluids for PFAS can provide laboratory efficiency while maintaining confidence in high quality results.

References

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3. Vorkamp K, ***et al***. Biomarkers, Matrices and Analytical Methods Targeting Human Exposure to Chemicals Selected for a European Human Biomonitoring Initiative. ***Environment International***. 146 (2021).
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