Application Note

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Sensitive Analysis for Per- and Polyfluoroalkyl Substances (PFAS) in Whole Blood

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Abstract

This application note demonstrates a sensitive workflow for the extraction and analysis of 40 PFAS compounds in whole blood samples. Samples are extracted with Agilent Captiva EMR–Lipid 1 mL cartridges, then analyzed on an Agilent 1290 Infinity II LC coupled to an Agilent 6495 triple quadrupole LC/MS, demonstrating good reproducibility at low levels (0.03 to 0.67 ng/mL).

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a general class of anthropogenic compounds that contain a high degree of fluorination. The richness in carbon-fluorine bonds makes PFAS valuable in many industrial manufacturing processes, but it also enhances their resistance to degradation. These properties have led to their ubiquitous presence in the environment. Human exposure and PFAS body burden have been linked with many negative health effects[1], and research is ongoing.

Epidemiological studies rely on the quality of analytical data, which is complicated by the diversity of molecular structures in the PFAS class. Several methodologies for PFAS sample preparation in biological matrices have been reported, such as direct protein precipitation (PPT), weak anion exchange solid phase extraction (WAX SPE), and PPT followed by Captiva EMR–Lipid passthrough cleanup.[2]

This application note presents an optimized sample preparation with LC/MS detection that targets 40 PFAS compounds in whole blood. As whole blood samples contain high amounts of proteins, the sample volume and crashing solvent for protein precipitation (PPT) also required optimization. Sample preparation using PPT-only was compared with PPT followed by Agilent Captiva EMR–Lipid passthrough cleanup.

Experimental

Sample preparation for whole blood

Whole blood for method development was obtained from UTAK (44600-WB(F)). Native and isotopically labeled PFAS standards were purchased from Wellington Laboratories. All compounds listed in EPA 1633[3] were tested. The optimized sample preparation procedure for extracting PFAS from whole blood using an Agilent Captiva EMR–Lipid 1 mL cartridge (part number 5190-1002) is shown in Figure 1.

LC separation and MS parameters

An Agilent 1290 Infinity II binary pump LC coupled to an Agilent 6495C triple quadrupole LC/MS was used for the analysis of PFAS in whole blood. Background contamination was delayed with the Agilent InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100), as part of the full PFC-free HPLC conversion kit that was used. The background reduction details using this kit can be found in the Agilent technincal overview publication number 5994-2291EN. An injection program was employed to improve peak shape and sensitivity for early eluting compounds. Method details are shown in Tables 1 and 2.

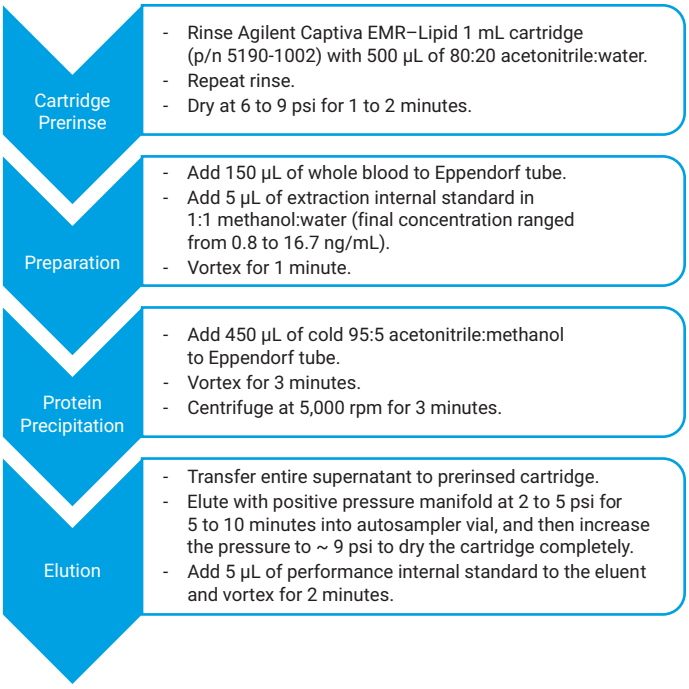

Figure 1. Sample preparation procedure for extracting PFAS from whole blood.

Table 1. Method parameters for the Agilent 1290 Infinity II LC.

Parameter	Setting
Needle Wash	1:1 Acetonitrile:isopropanol
Autosampler Temperature	10 °C
Injection Volume	5 µL of sample were injected, sandwiched between two 10 µL plugs of 0.1% acetic acid in water
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm column (p/n 695775-902)
Column Temperature	50 °C
Mobile Phase	A) 2 mM Ammonium acetate in water B) 95:5 Acetonitrile:water
Flow Rate	0.4 mL/min
Gradient	Time (min) %B 1.0 15 1.5 25 7.0 60 10.0 100 12.0 100 12.1 15
Stop Time	12.5 min
Post Time	3.0 min

Table 2. Method parameters for the Agilent 6495 triple quadrupole LC/MS.

Parameter	Setting
Ionization Mode	Negative
Drying Gas Temperature, Flow	150 °C, 18 L/min
Nebulizer Pressure	25 psi
Sheath Gas Temperature, Flow	390 °C, 11 L/min
Capillary Voltage	2,000 V
Nozzle Voltage	0 V
Delta EMV	200 V
HP/LP Funnel Voltages	90/80 V
MRM Transitions	Agilent PFAS MRM Database for triple quadrupole LC/MS (G1736AA)

Results and discussion

Sample preparation evaluation

PFAS compounds are common background contaminants. A preventive prerinsing of the cartridge twice with 80:20 acetonitrile:water provided consistent cleanliness through the sample preparation procedure, which is important for the targeted PFAS quantitation in whole blood.

Whole blood extraction volumes were evaluated between 100 and 300 µL, with crash solvent ratios three to five times the sample volume. An aliquot of 150 µL was selected to limit sample volume and still provide the required analytical sensitivity.

Previous work[4] has shown the optimal crashing solvent for whole blood protein precipitation to be 95:5 acetonitrile:methanol. For serum and plasma, the acidified crashing solvent, ACN with 1% formic acid, is typically used to assist the efficiency of protein precipitation. However, acidified solvent can extract more hemoglobin from whole blood, and thus is not recommended for whole blood protein precipitation. Instead, a precooled solvent of 95:5 ACN:MeOH was used to improve protein precipitation efficiency.

The method presented was performed with protein precipitation in an Eppendorf tube, centrifuged, and then passed through the Captiva EMR–Lipid cartridge into LC vials. Protein precipitation can be performed in the cartridge, reducing the required transfer steps.[4] However, in the cartridge format, sample mixing is difficult and can cause incomplete protein precipitation (especially for whole blood, which contains a higher abundance of proteins). Therefore, the offline PPT was used prior to Captiva EMR–Lipid passthrough cleanup. The in-well PPT on the Captiva EMR–Lipid 96-well plate is usually feasible, as samples can be mixed efficiently with the seal of a plate mat. However, for this study, after the preventive prerinsing step with 80:20 ACN:water, the wet sorbent did not hold the aqueous blood sample well until the addition of crashing solvent, resulting in the breakthrough of the whole blood sample. This caused the contamination of sample eluent. Therefore, offline PPT is still recommended even with the use of the Captiva EMR–Lipid plate.

Matrix removal using Captiva EMR–Lipid

Captiva EMR–Lipid sorbent removes lipids by size exclusion and hydrophobic interaction. Phospholipids compose a highly abundant lipid class that causes significant matrix effects in biological blood matrices such as plasma, serum, and whole blood.[4] Captiva EMR–Lipid passthrough cleanup has demonstrated that it can remove > 99% of phospholipids in biological fluid matrices.[5]

A full evaluation of matrix effects among solvent standards, protein precipitation (PPT) only, and PPT with Captiva EMR–Lipid cleanup was conducted. In the PPT-only treatment, matrix enhancement was observed for the late-eluting compounds, particularly MeFOSE, EtFOSE, PFTrDA, and PFTDA. MeFOSE evaluation is shown in Figure 2. For all compounds, solvent standards and the PPT with Captiva EMR–Lipid calibration curves were overlaid, demonstrating that the passthrough cleanup removed the matrix interfering components.

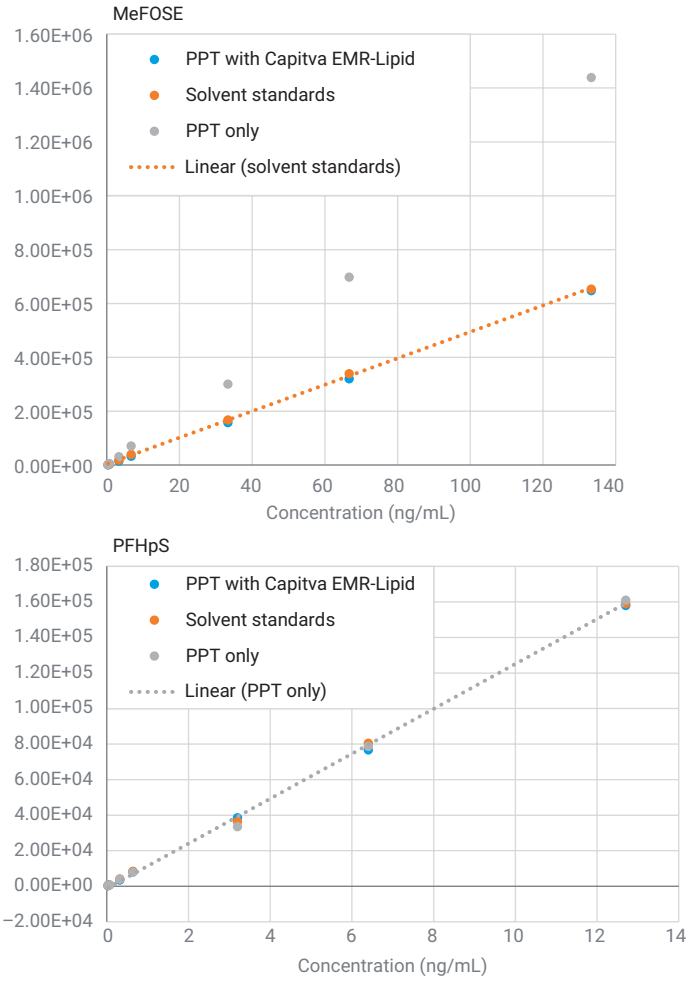

Figure 2. Calibration comparisons among three treatments. PFHpS shows no matrix enhancement, while MeFOSE shows matrix enhancement for PPT only.

LC injection program

Because the extracted sample was in a high percentage of acetonitrile, peak fronting for early eluting compounds was sometimes observed. Small injection volumes can address fronting but can also compromise method sensitivity. In Figure 3, the effect of creating an injection program to mix the extract with 20 µL of acidic water to improve the peak shape for better sensitivity and more reproducible quantitation is shown. Figure 4 shows the chromatogram of targets in a spiked sample using the optimized instrument method.

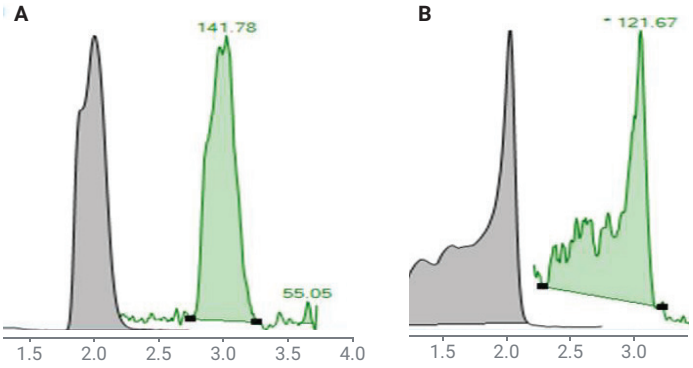

Figure 3. 13C4-PFBA (6.7 ng/mL) and PFMPA (0.07 ng/mL) chromatograms in the low level using the (A) injection program and (B) no injection program.

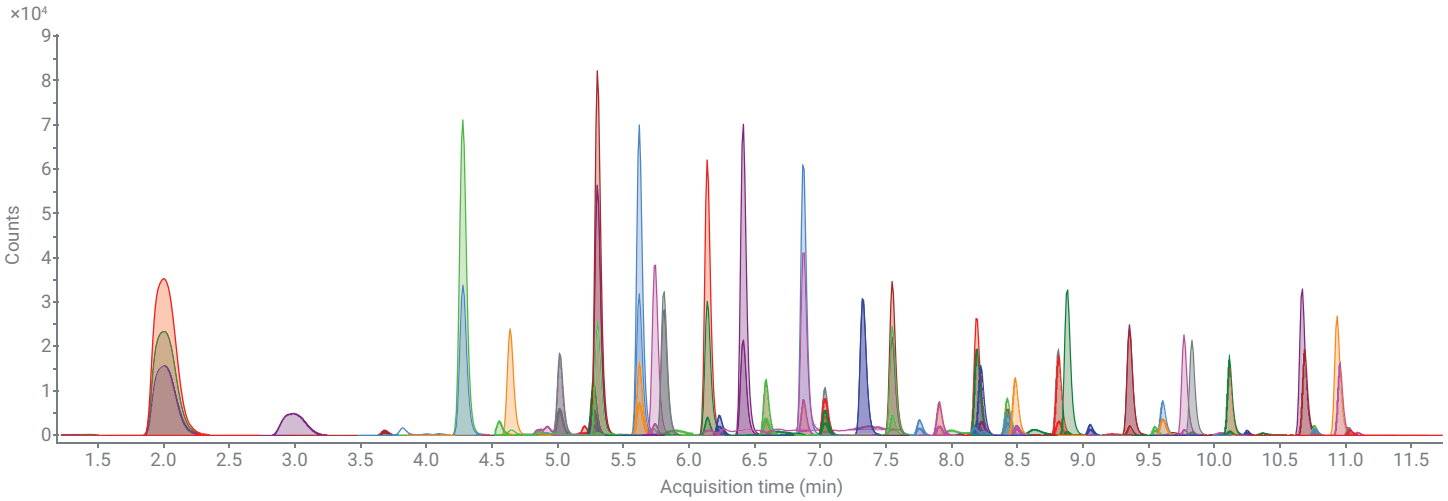

Figure 4. Full chromatogram of compounds.

Method performance

Table 3 shows the evaluated spiking range for all tested PFAS compounds. The blank matrix did have native concentrations of PFAS compounds present. Calibration curves generated from solvent standards and those from post-spiked extracted matrix standards were comparable. To accurately quantitate native concentrations, solvent standards were used. Extracted internal standard recovery using PPT followed by Captiva EMR–Lipid and PPT-only were compared. Error bars show standard deviation. Extraction standard recovery was determined by comparing performance in pre- and post-spike samples. Results are shown in Figure 5.

Recoveries were also compared to protein precipitation only. The recoveries between the two treatments were similar, indicating that for most compounds, losses took place during the crash and were not related to the Captiva EMR–Lipid passthrough cleanup.

Table 3. The tested range and reproducibility of evaluated compounds for PFAS in whole blood.

PFAS Analyte	Evaluated Range in Whole Blood (ng/mL)		RSD at Low Level in Whole Blood (n = 5)	Concentration in Blank Whole Blood (ng/mL) (RSD, n = 7)
PFMBA	0.07	26.7	4.7	
PFMPA	0.07	26.7	2.3	
NFDHA	0.07	26.7	14.9	
PFEESA	0.07	26.7	6.3	
N-MeFOSA	0.07	13.3	18.8	
N-EtFOSA	0.03	13.3	8.1	
MeFOSE	0.33	133.3	5.4	
EtFOSE	0.33	133.3	3.4	
HFPO-DA	0.07	26.7	14.3	
DONA	0.07	26.7	3	
9Cl-PF3ONS	0.07	26.7	7.6	
11Cl-PF3OUdS	0.07	26.7	7.1	
PFBA	0.13	53.3	6.5	
PFPeA	0.07	26.7	5.2	
PFHxA	0.03	13.3	12.8	
PFHpA	0.03	13.3	11.5	
PFOA	0.03	13.3	14.8	0.44, RSD = 6.1
PFNA	0.03	13.3	15	0.15, RSD = 10
PFDA	0.03	13.3	13.7	0.09, RSD = 11
PFUnDA	0.03	13.3	9.5	0.05, RSD = 7.5
PFDoDA	0.03	13.3	4.5	
PFTrDA	0.03	13.3	7.7	
PFTDA	0.03	13.3	11.3	
PFOSA	0.03	13.3	4.7	0.03, RSD = 12.5
N-MeFOSAA	0.07	13.3	15.7	
N-EtFOSAA	0.03	13.3	13.1	
PFBS	0.03	11.8	14.3	
PFPeS	0.03	12.5	18.6	
PFHxS	0.03	12.2	18.9	0.55, RSD = 7.7
PFHpS	0.06	12.7	12.8	
PFOS	0.03	12.4	10.5	1.38, RSD = 6.9
PFNS	0.06	12.8	20	
PFDS	0.03	12.9	6.8	
PFDoS	0.06	12.9	10.8	
4-2 FTSA	0.13	50.0	9.5	
6-2 FTSA	0.13	50.7	9.8	
8-2 FTSA	0.13	51.2	8.6	
3-3 FTCA	0.27	53.3	10.8	
5-3 FTCA	0.67	266.7	2.4	
7-3 FTCA	0.67	266.7	2.9	

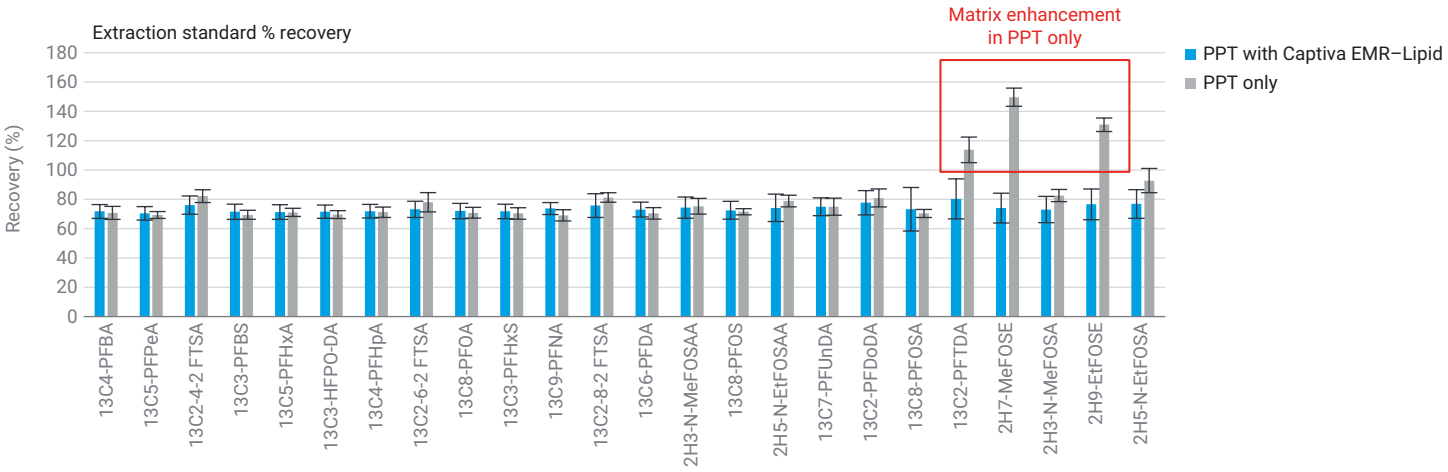

Figure 5. Extracted internal standard recovery via PPT followed by Agilent Captiva EMR–Lipid cleanup and PPT-only. Error bars show standard deviation.

Conclusion

This application note presents a workflow for the analysis of 40 PFAS compounds in whole blood. The analysis uses protein precipitation with Agilent Captiva EMR–Lipid cleanup and requires minimal sample volume. The sensitive analysis was performed with an Agilent 1290 Infinity II LC and an Agilent 6495 triple quadrupole LC/MS. Analytical method development was simplified, as all MRM transitions were provided by the Agilent PFAS MRM database for triple quadrupole LC/MS (G1736AA). The method showed good reproducibility at low PFAS levels (0.03 to 0.67 ng/mL) in whole blood samples.

References

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