An Automated Sample Preparation Approach for the Determination of Per and Polyfluoroalkyl Substances (PFAS) from Human Biological Fluids using UHPLC-MS/MS

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Introduction

Exposure to per and polyfluoroalkyl substances (PFAS) has been linked with changes in metabolism, increased cholesterol, and high incidence of some forms of cancer. PFAS pose particular challenges in the analytical laboratory due to their ubiquitous nature. Environmentally, PFAS are of concern because of their high persistence (forever chemicals), bioaccumulation and slow elimination, with potential impacts on human and environmental health. This poster presents an automated and robust, high-sensitivity method for the clean-up of 31 PFAS compounds from various biological fluids.

Experimental

31 PFAS standards were purchased from Wellington Laboratories (Guelph, Canada). The suite contains 10 classes of PFAS, varying by functionality, including: carboxylic acids, sulfonic acids and telomers, sulfonamides, and ethoxy compounds.

Reagents

Reagents were purchased from Merck Life Science (Gillingham, UK). LC/MS grade solvents were from Rathburn Chemicals Ltd. (Walkerburn, UK). Water (18.2 MΩ.cm) was drawn fresh daily from a Direct-Q 5 water purifier (Merck Life Science UK, Watford, UK). Blood, plasma, and serum were purchased from Welsh Blood Service (Pontyclun, UK). Urine samples were donated by healthy anonymized human volunteers.

Sample Preparation

Polymeric SPE:
EVOLUTE® Express ABN 96-well plate, 30 mg (600-0030-PX01) or EVOLUTE® Express WAX 96-well plate, 30 mg (604-0030-PX01). Serum was spiked with standards and pre-treated 1:3 v/v, 2% formic acid.

Multifunctional sorbent clean-up:
ISOLUTE® PLD+ for PFAS 96-well plate, 50 mg (919-0050-P01). Serum or whole blood was spiked with standards and used without pre-treatment.

Processing conditions:
96-well plates were processed using a Biotage® VacMaster™-96 or a Biotage® Extrahera™ Classic (Figure 1).

Figure 1. *Biotage® Extrahera™ Classic Automated Sample Preparation Workstation.*

Table 1. Optimized Extraction Protocols.

Processing	ABN (30 mg, 1 mL steps)	WAX (30 mg, 1 mL steps)	PLD+ for PFAS (50 mg)
Condition	2% NH_4OH/MeOH & MeOH		-
Equilibrate / Crash	H_2O		700 µL ACN
Load	400 µL pre-treated serum or urine		100 µL matrix
Wash 1	0.1% HCOOH	50 mM NH_4AC pH 5	-
Wash 2	10% MeOH (aq)	MeOH	-
Elute	500 µL MeOH	500 µL 2% NH_4OH /MeOH	-
Post-elute	Evaporate 40 min, 25 L min^{-1}, 40° C		-
Reconstitution	200 µL 50% MeOH/H_2O		dilute 1:1 v/v 20 mM NH_4AC

LC/MS Conditions

Instrument: Shimadzu Nexera UHPLC using fluoropolymer-free tubing and a pre-injector trap column coupled to an AB Sciex 5500 triple quadrupole MS system.

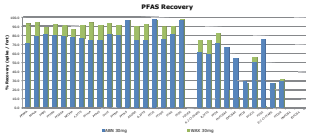
Column: Avantor ACE Ultracore SuperC18, 2.5 µm (50 x 2.1 mm)
Mobile Phase: A: 5 mM ammonium acetate (aq)
Mobile Phase B: methanol (MeOH)
Injection volume: 5 µL
Flow rate: 0.4 mL min^{-1}
Gradient: 30% B to 95% B (0.1 min to 6.5 min)
Source parameters (TurboIonspray):
IS: -3500 V **TEM:** 500 °C
CUR: 40 psi **GS1:** 40 psi **GS2:** 60 psi
MRM transitions: Optimized MRM transitions were selected using the most intense precursor ions in negative ionization mode, available on request.

Results

SPE

PFAS were extracted from serum using polymer-based reversed phase and corresponding mixed-mode weak anion exchange (WAX) chemistries in 30 mg 96-well plate formats. Reverse-phase chemistry recovery was below 50% for some long-chain PFAS with high RSD. Modification of load, wash, and elution steps did not demonstrate improvements in method performance (Figure 2).

Figure 2. *PFAS recovery profiles from serum using ABN 30 mg and WAX 30mg 96-well plates, spike 20 pg.*

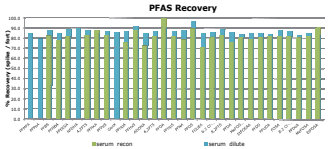


WAX SPE demonstrated comparable method performance to reverse-phase SPE. Short-chain PFAS recoveries were typically above 95%, with low RSD and matrix factors closer to 1. No improvement to WAX recovery was demonstrated from method modifications.

Multifunctional Sorbent Extraction

The suite was extracted using a multifunctional clean-up sorbent (ISOLUTE® PLD+ for PFAS). Evaporation/reconstitution of extracts demonstrated no recovery of some short-chain PFAS (Figure 3).

Figure 3. *PFAS recovery comparing evaporation to a dilute and inject workflow using 50 mg FWP, spike 160 pg.*



RSD were over 10% for over half the extracted suite. Optimum extraction was obtained using a solvent first protocol in a 1:7 ratio with unmodified acetonitrile. A dilute/inject strategy with dilute ammonium acetate demonstrated consistent extraction recoveries above 80% across our target suite from serum and whole blood matrices (Figure 4).

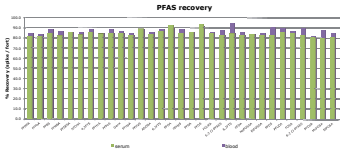


Figure 4. *PFAS recovery profiles from serum and whole blood using PLD+ for PFAS 96-well plate, spike 160 pg.*

The majority of RSD were below 5%. Matrix factors between 0.8 and 1.3 were demonstrated for most analytes (data not shown).

Matrix Interferences

The multifunctional sorbent demonstrates excellent matrix reduction, removing >99.9 % phospholipids (Figure 5).

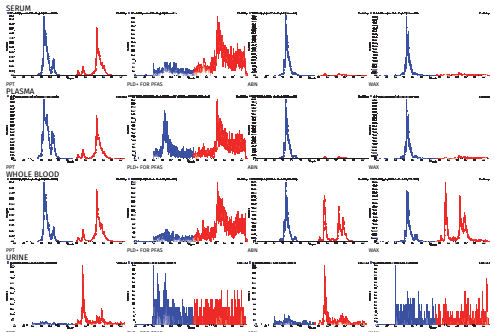
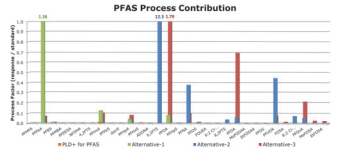


Figure 5. *Comparative phospholipid profiles for PFAS extraction*

Low PFAS residues are vital to ensure results are reproducible and free from product-derived interference. ISOLUTE® PLD+ for PFAS demonstrates background well below reportable limits (Figure 6).

Figure 6. *PFAS residues resulting from processing, response relative to 1.6 ng mL^{-1}.*



Background levels of PFAS in pooled and individual donor matrices tested were in the order of 1 ng mL^{-1}.

Automation

The optimized extraction method demonstrated comparable method performance when transferred to the Extrahera™ automated sample preparation platform (methods on request).

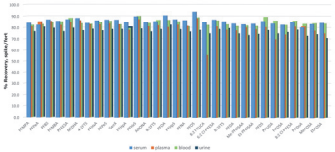
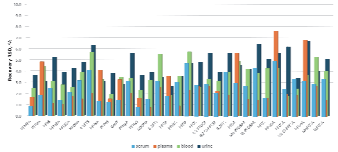


Figure 7. *Extrahera™ automated extraction recovery from multiple matrices, PLD+ for PFAS, spike 160 pg.*

Typical automated extraction recoveries were 75 to 95% for all matrix types (Figure 7). Recovery of 8:2-FTUCA from plasma was 56%, attributed to matrix effects. The majority of automated RSD were < 5% (Figure 8), slightly higher from whole blood and urine matrices.

Figure 8. *Extrahera™ automated extraction RSD from multiple matrices, PLD+ for PFAS spike 160 pg.*



The optimized ISOLUTE® PLD+ for PFAS extraction protocol demonstrates low PFAS background using Extrahera™ automation (Figure 9). Care must be taken to minimize lab cross-contamination.

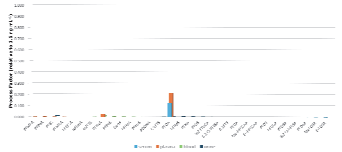


Figure 9. *Extrahera™ automated extraction, PFAS residue contribution, PLD+ for PFAS spike 160 pg.*

Calibration and LOQ

The Extrahera™ automated sample preparation platform demonstrated sub ng mL^{-1} LOQs for all analytes across our target suite when extracted from multiple matrices (Table 2) at clinical levels similar to those demonstrated from NIST SRM 1957. Calibration curves were linear over the extracted range (0.1 to 100 ng mL^{-1}) demonstrating coefficients of determination (r^2) above 0.995 for all targets.

Table 2. Method performance PLD+ for PFAS, extracted LOQ and linearity, 100 µL matrix, Extrahera™ Classic, others available on request.

PFAS Class	Serum LOQ, ng mL^{-1}, r^2	Plasma LOQ, ng mL^{-1}, r^2	Blood LOQ, ng mL^{-1}, r^2	Urine LOQ, ng mL^{-1}, r^2
Carboxylic acids (alkane/alkene)	0.1-0.4 0.9980-0.9996	0.1-0.4 0.9974-0.9984	0.1-0.4 0.9986-0.9994	0.1-0.4 0.9978-0.9986
Ethoxy**carboxylic acids**	0.1 0.9980-0.9996	0.1 0.9978-0.9984	0.1 0.9988-0.9994	0.1 0.9980-0.9984
Sulfonic acids	0.1-0.4 0.9990-0.9992	0.1-0.4 0.9960-0.9982	0.1 0.9986-0.9994	0.1 0.9976-0.9984
Telomeric/**ethoxy** sulfonic acids	0.1-0.4 0.9976-0.9994	0.1-0.4 0.9968-0.9978	0.1-0.4 0.9982-0.9992	0.1-0.4 0.9976-0.9986
Sulfonamides (and substituted)	0.1 0.9980-0.9998	0.1 0.9974-0.9990	0.1 0.9980-0.9994	0.1 0.9976-0.9990
Cl-substituted ethoxy sulfonates	0.1 0.9978-0.9988	0.1 0.9976-0.9986	0.1 0.9980-0.9988	0.1 0.9976-0.9978

Conclusion

- ISOLUTE® PLD+ for PFAS demonstrates high PFAS recovery and sensitivity with low matrix factors and good repeatability.
- ISOLUTE® PLD+ for PFAS demonstrates enhanced cleanliness compared to dilute-and-shoot methodology leading to more robust methods. Our approach demonstrates more consistent PFAS recovery compared to other sample preparation techniques.
- Use of the Biotage® Extrahera™ automated sample preparation platform demonstrates comparable data to a manual vacuum-based method, helping to improve sample throughput.

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