ANALYSIS OF PERFLUORINATED COMPOUNDS USING UPLC & MS/MS DETECTION

Waters

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OVERVIEW

A rapid and sensitive method for the analysis of 12 perfluorinated compounds (PFCs) using UPLC/MS/MS is described. This method reduces a typical HPLC run-time of 22 minutes to under 5 minutes.

Solid-phase extraction (SPE) is used to extract the PFCs from blood and plasma samples which are analysed using ACQUITY Ultra Performance LC (UPLC) and a Quattro Premier™ XE in negative ion electrospray mode (ES-MS/MS).

The results are comparable to other published data using MS/MS; with experimental limits of detection ranging from 0.001-0.17 pg/µL in whole blood and plasma samples.

The presence of mobile phase residue PFCs is discussed and the use of a mobile phase residue trap (MPRT) is suggested.

INTRODUCTION

The worldwide ubiquitous occurrence of PFCs in the environment and in human blood has in recent years raised researchers and authorities attention[1-7]. These compounds are both hydrophobic and hydrophilic, which are properties that make them frequently used for treatment of carpets, fabric, leather, protection of paper and food packaging and also as performance chemicals in plastic production, fire-fighting foam, polish, cleaners and insecticides[8,9].

This poster shows results using UPLC-MS/MS: UPLC systems run at much higher pressures than traditional HPLC (maximum operating pressure is 15,000 psi / ~1000 Bar) providing the advantage of much faster run-times without compromising the selectivity.

For this experiment a new method for the UPLC was set up and the amount of PFC's in whole blood and plasma samples were calculated.

METHOD

SPE Method - based on Taniyasu et al [10]

SPE Cartridge: *Waters Oasis WAX SPE Column*

Conditioning parameters:2mL methanol, 2mL water

Wash: 2 mL 40% methanol in water (vacuum) until dry

Elute: 1 mL 2% ammonium hydroxide in methanol.

Evaporate extract under a gentle nitrogen stream to 0.5 mL

Filter using a 0.2 µm polypropylene filter into a vial

Add recovery standards ($^{13}C_5$-PFNA and 7H-PFHpA).

0.5 mL plasma or whole blood

Internal standards ($^{13}C_4$-PFOS and $^{13}C_4$-PFOA).

Mix well and add 2 mL 50 v/v% formic acid/water.

Sonicate for 15 minutes Centrifuge at 10,000 x g for 30 minutes.

Take the supernatant and extract using Waters Oasis WAX SPE column (200 mg / 2 mL)

Figure 1. Modification of UPLC system to reduce the interference from mobile phase PFCs.

UPLC Conditions

LC System: Waters Acquity Ultra Performance LC

LC Column: Waters Acquity UPLC BEH RPC18 2.1 x 50 mm, 1.7 µm.

Column temp: 50°C

Mobile phase: A: 2mM aqueous ammonium acetate
B: 2mM ammonium acetate in methanol

Mobile phase residue trap (MPRT) (see Figure 1)

Time (min)	Flow Rate (µL /min)	% A	% B	Curve
Initial	400	70	30	Initial
0.50	400	70	30	6
5.00	400	10	90	6
5.10	400	0	100	6
5.50	400	0	100	6

MS/MS CONDITIONS

MS System: Quattro Premier XE

Ion Mode: Negative ion ESI

Cone Voltage: 15-45kV (Compound dependent)

Collision Energy: 10-40eV (Compound dependent)

Source Temp: 120ºC

Desolvation Temp: 400ºC

MRM Data: See table below

Ret. Time (min)	PFC	Precursor Ion	Product Ion	Dwell Time (s)	Cone Voltage (V)	Collision Energy (eV)
2.09	PFBuS	299	80	0.20	45	29
2.50	7H-PFHpA	345	281	0.20	16	10
2.81	PFHxA	313	269	0.20	15	10
3.45	PFHpA	363	319	0.05	16	10
3.51	PFHxS	399	80	0.05	45	35
3.88	THPFOS	427	80	0.05	45	40
3.90	PFOA	413	369	0.05	17	11
3.90	^{13}C-PFOA	417	372	0.05	17	11
4.26	PFNA	463	419	0.05	16	11
4.26	^{13}C-PFNA	468	423	0.05	16	11
4.28	PFOS	499	80	0.05	45	40
4.28	^{13}C-PFOS	503	80	0.05	45	40
4.56	PFDA	513	469	0.05	17	12
4.83	PFUnDA	563	519	0.05	18	12
4.72	PFDS	599	80	0.05	45	45
5.06	PFDoDA	613	569	0.05	18	13
5.42	PFTDA	713	669	0.2	19	14

RESULTS AND DISCUSSION I. CHROMATOGRAPHY

Figure 2 shows the retention times for the PFCs analysed. Base-line separation of the compounds was achieved using this UPLC method

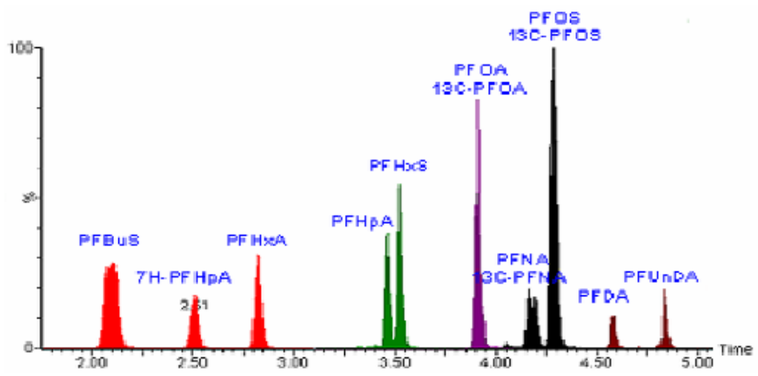

Figure 2. UPLC Chromatogram of PFCs in negative ESI MRM mode

Figure 3 illustrates the improved chromatography using UPLC compared to traditional HPLC. UPLC had a significant effect on the peak width: 4.2 s compared to 20.3 s using HPLC for PFOS. It also reduced the analysis time from 22 mins to 5.5 mins.

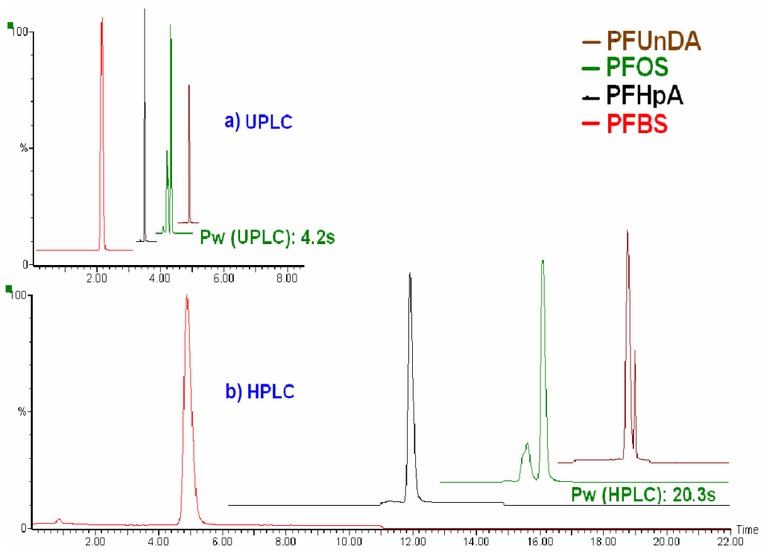

Figure 3. A comparison of two chromatograms a) UPLC & b) HPLC for four of the compounds (same time scale)

The calibration curve obtained for PFOS from 0.01-80.0 pg/µL is shown in Figure 4. The other compounds showed similar calibration curves.

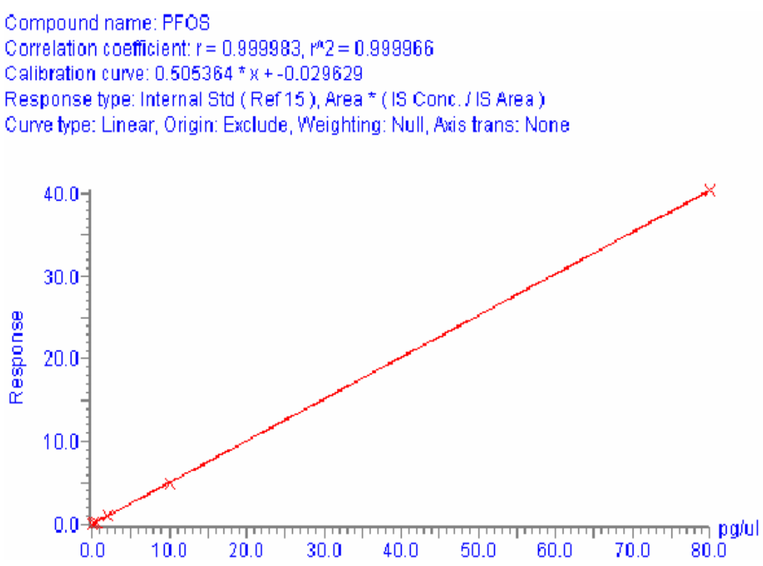

Figure 4. Calibration curve for PFOS from 0.01-80.0 pg/µL

RESULTS AND DISCUSSION II. DETECTION LIMITS

The table below shows the instrumental and method detection limits.

The instrumental detection limit was defined as the concentration needed to produce a signal to noise ratio of 3:1.

The method detection limit for 0.5 ml plasma / blood was also estimated from plasma / blood samples spiked at low concentrations and was defined as the concentration with a S/N ratio of 3:1.

PFC	Detection limits (pg/µL) Instrument	Method — Plasma	Method — Whole Blood
PFBuS	0.0003	0.002	0.001
PFHxA	0.0045	0.016	0.028
PFHpA	0.0016	0.009	0.008
PFHxS	0.0006	0.003	0.002
PFOA	0.0031	0.017	0.038
PFNA	0.0021	0.018	0.013
PFOS	0.0035	0.018	0.035
PFDA	0.0110	0.067	0.026
PFUnDA	0.0022	0.032	0.018
PFDS	0.0060	0.174	0.086
PFDoDA	0.0086	0.024	0.033
PFTDA	0.0100	0.024	0.042

RESULTS AND DISCUSSION III. MOBILE PHASE PFC'S

Introduction of perfluorinated compounds into the system not arising from the sample is a known problem when analyzing for PFCs, and was prevalent for a few of the compounds. A source of the compounds PFOA and PFNA was found to be from the mobile phase pre- injector; the additional presence of the PFC's eluted as a peak as the amount of methanol increased.

To reduce this effect, a mobile phase reside trap (MPRT) was inserted post-pump and pre-injector, and this allowed the non-analytical peak to be separated from the analytical peak.

By utilising the MPRT, standard HPLC grade solvents were used, with no additional contamination to the sample peak.

Accumulation of these compounds occurred at the head of the column when flow was stopped. To prevent this from occurring solvent flow was left at 0.050 mL/min once the sequence had been run and until the next sequence was started. A blank was always run before the start of the next run.

CONCLUSIONS

12 PFCs were monitored using UPLC and MS/MS. Analysis time for these compounds has been successfully reduced from 22 minutes to 5 minutes without compromising the chromatography or the limits of detection.

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