ANALYSIS OF PERFLUORINATED COMPOUNDS IN WHOLE BLOOD AND PLASMA USING ACQUITY UPLC™ AND WATERS QUATTRO PREMIER™ XE

Antonietta Gledhill[1], Anna Kärrman [2], Ingrid Ericson [2], Bert van Bavel [2], Gunilla Linström [2], Gordon Kearney [1]

[1]Waters Corporation, Atlas Park, Simonsway, Manchester, M22 5PP, United Kingdom, [2]Örebro Universitet, 701 82 Örebro, Sweden

Introduction

The worldwide ubiquitous occurrence of perfluorinated compounds (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].

Many papers describe the analysis of these compounds using HPLC and MS (MS and MS/MS). In this paper UPLC-MS/MS is employed: where the UPLC system runs 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.

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

This method has a solid-phase extraction (SPE) procedure followed by analysis on the ACQUITY Ultra Performance Liquid Chromatography (UPLC) and the Quattro Premier™ XE in negative ion electrospray mode (ES-MS/MS).

Results are comparable with other results that have been obtained in previous experiments using MS/MS with limits of detection ranging from 0.002-0.08 pg/uL in whole blood and plasma samples.

Materials and Methods

Compounds

PFBuS tetrabutylammonium-salt (≥ 98%), PFOS potassium-salt (≥ 98%), PFDA (>97%), PFHxA (≥ 97%) were purchased from Fluka (Steinheim, Germany). PFHpA (99%), PFNA (97%), PFOA (96%), PFUnDA (95%), were purchased from Aldrich (Steinheim, Germany and Milw. WI, USA). *7H*-PFHpA (98%) was purchased from ABCR (Karlsruhe, Germany). PFHxS (98%) was purchased from Interchim (Montlucon, France). $^{13}C_4$PFOS, $^{13}C_4$PFOA, $^{13}C_5$PFNA were from Wellington Laboratories (Ontario, Canada). HPLC grade solvents were used (Fisher Scientific UK)

Extraction Procedure[a]

0.5 mL plasma or whole blood was spiked with internal standards ($^{13}C_4$-PFOS and $^{13}C_4$-PFOA). After mixing, 2 mL 50 v/v% formic acid/water was added. The solution was sonicated for 15 minutes and centrifuged at 10,000 x g for 30 minutes. The supernatant was extracted using a Waters Oasis® WAX SPE column (200 mg / 2 mL) that was previously conditioned with 2 mL methanol and 2 mL water. The column was washed with 2 mL 40% methanol in water and dried with vacuum until visual dryness. Elution of the compounds was performed with 1 mL 2% ammonium hydroxide in methanol. The extract was evaporated under a gentle nitrogen stream to 0.5 mL and filtered using a 0.2 µm polypropylene filter into a vial. Recovery standards ($^{13}C_5$-PFNA and *7H*-PFHpA) were added.

[a] Based on Taniyasu et al.[10]

UPLC Method

ACQUITY UPLC™ System
Mobile phase A: 2mM Aq. ammonium acetate
Mobile phase B: Methanol + 2mM ammonium acetate
Mobile phase residue trap (MPRT)
Column: ACQUITY BEH C18 2.1 x 50mm, 1.7mm
Column temp: 50°C
Flow rate: 0.4mL/min
Injection volume: 10µL

UPLC Gradient

0.00 min:	70% A	30% B
0.50 min:	70% A	30% B
5.00 min:	10% A	90% B
5.10 min:	0% A	100% B
6.00 min:	0% A	100% B
7.00 min:	70% A	30% B
10.00 min:	70% A	30% B

MS Method

Waters Quattro Premier™ XE
Electrospray mode with negative polarity

Table 1: MRM conditions for each compound

RT	PFC	Parent	Daughter	Dwell	Cone Energy	Collision Energy
2.09	PFBuS	299.00	80.00	0.20	45.00	29.00
2.50	*7H*-PFHpA	345.00	281.00	0.20	16.00	10.00
2.81	PFHxA	313.00	269.00	0.20	15.00	10.00
3.45	PFHpA	363.00	319.00	0.05	16.00	10.00
3.51	PFHxS	399.00	80.00	0.05	45.00	35.00
3.90	PFOA	413.00	369.00	0.05	17.00	11.00
3.90	^{13}C-PFOA	417.00	372.00	0.05	17.00	11.00
4.26	PFNA	463.00	419.00	0.05	16.00	11.00
4.26	^{13}C-PFNA	468.00	423.00	0.05	16.00	11.00
4.28	PFOS	499.00	80.00	0.05	45.00	40.00
4.28	^{13}C-PFOS	503.00	80.00	0.05	45.00	40.00
4.56	PFDA	513.00	469.00	0.05	17.00	12.00
4.83	PFUnDA	563.00	519.00	0.05	18.00	12.00

Results and Discussion

Results and Discussion : I. Performance

Figure 1 illustrates the improved chromatography using UPLC compared to traditional HPLC. The run-time for these 13 compounds is 22 minutes in HPLC, but this is shortened to 5.0 minutes using UPLC.

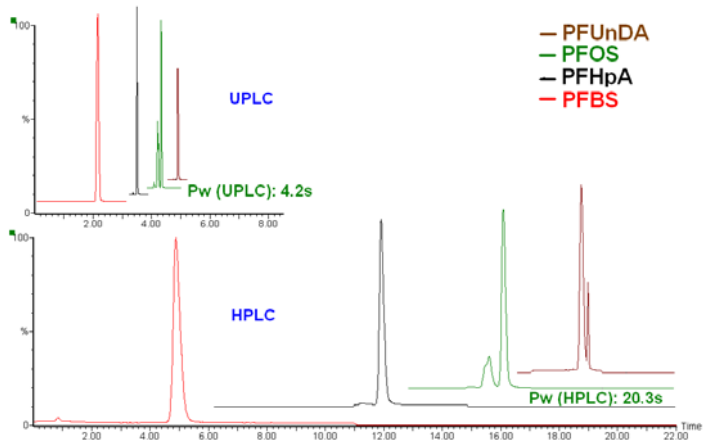

Figure 1: *Comparison of run-times & peak widths obtained using UPLC and HPLC for 13 perfluorinated compounds*

Results and Discussion: II. Recoveries
Table 2 shows the instrumental and the method detection limit. 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 blood was estimated from blood samples spiked at low concentrations and was defined as the concentration with a S/N ratio of 3:1 (see table 2).

Table 2: Detection limits for the experiment, in pg/µL

pg/ul	Detection limits		
	Instrument	Method - whole blood	Method - Plasma
PFBuS	0.0003	0.001-0.002	0.002
PFHxS	0.0006	0.002	0.003-0.005
PFOS	0.0035	0.035	0.018-0.025
PFHxA	0.0045	0.028-0.034	0.016-0.023
PFHpA	0.0016	0.008	0.009-0.011
PFOA	0.0031	0.038-0.044	0.017-0.023
PFNA	0.0021	0.013	0.018-0.035
PFDA	0.0110	0.026-0.032	0.067-0.083
PFUnDA	0.0022	0.018-0.024	0.032-0.042

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) (see figure 2) 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 could be used even if the bottles are sealed with PTFE lids. This reduces time for evaluation of solvents either between suppliers or from batch to batch.

It also allows the analyst ease of mind when running samples that contamination will not occur if the solvents are replenished during analysis.

Accumulation of these compounds can occur at the head of the column if the flow is stopped (where the source may be the methanol or components in the UPLC system). 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 run. A blank was always run before the start of next run.

Figure 2: Modification of UPLC system to reduce the interference of mobile phase PFC presence

Two procedure blanks that were prepared at different times were monitored. The procedure blanks showed more presence of the mobile phase PFC's than the solvent blanks, and in some cases the occurrence of the mobile phase PFC's observed may have come from the internal standards. Another explanation is that it may be due to the glassware / other sources that the solvents have been in contact with.

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Conclusion
A sensitive and rapid method using UPLC-MS/MS has been described. This method reduces the run-time of 22 minutes to under 5.0 minutes. The detection limits using 0.5 mL blood and plasma were between 0.002-0.04 pg/µL and 0.002-0.08 pg/µL respectively.

PFC's coming primarily from the mobile phase could be greatly reduced by inserting a mobile phase residue trap (MPRT) post-pump and pre-injector. This allows generic solvents to be used without compromising results of samples.

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