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ANALYSIS OF PERFLUORINATED COMPOUNDS (PFCS) ON THE ACQUITY UPLC SYSTEM & THE QUATTRO PREMIER™ XE IN ES-MS/MS

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INTRODUCTION

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

Laboratory studies indicate that PFCs may disturb the fatty acid metabolism, affect the reproductive system and/or cause liver damage[10,11]. These effects together with their stability and bio-accumulative properties, suggest that PFCs are potentially harmful to humans and the environment. Therefore, accurate and reproducible determination of PFCs in environmental and human samples is a necessity but can pose many challenges[12]. Contamination from laboratory materials and instrumental parts are common problems.

The commercialization of high performance liquid chromatography-mass spectrometry (HPLC/MS) has facilitated the selective and sensitive analysis of PFC acids. This note describes a method using ultra performance liquid chromatography (UPLC®) and tandem mass spectrometry (MS/MS).

The Waters® ACQUITY UPLC®, launched in 2004, uses columns with 1.7 µm particle size that can operate at high pressure (maximum pressure of 15,000 psi). The combination of smaller particle size and higher pressure provide a fast, high resolution separation that increase sensitivity and minimize matrix interference.

In this study, 13 PFC's normally run using HPLC have been analyzed by UPLC. The HPLC method requires 22 minutes compared to a run-time of less than five minutes for UPLC. Faster run times not only increase the throughput of the instrument but also reduce method development time.

ACQUITY UPLC and Quattro Premier XE mass spectrometer.

This method includes an extraction procedure involving a solid-phase extraction (SPE) step using Waters Oasis® WAX columns followed by analysis on the ACQUITY UPLC and the Waters Quattro Premier™ XE in negative electro spray (UPLC/ES-MS/MS).

EXPERIMENTAL

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 Milwaukee, WI, USA). 7HPFHpA (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).

Extraction Procedure (based on Taniyasu et al.[13])

Waters Oasis WAX SPE Column

Conditioning parameters: 2 mL methanol, 2 mL 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 a Waters Oasis WAX SPE column (200 mg/2 mL).

Mobile Phase Residue Trap (MPRT)

Figure 1. Modification of UPLC system to reduce the interference of mobile phase PFC presence.

UPLC Method

Waters ACQUITY UPLC

Mobile phase A: 2 mM Aq. ammonium acetate

Mobile phase B: Methanol + 2 mM ammonium acetate Mobile phase residue trap (MPRT) (see Figure 1)

Column: ACQUITY BEH C_{18} 2.1 x 50 mm, 1.7 µm, (P/N 186002350)

Column temp.: 50 °C

Flow rate: 0.4 mL/min

Injection volume: 10 µL

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

The MRM transitions along with the cone voltages and collision energies are listed in Table 1.

Function 1 10.00-3.10						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
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
Function 2 3.10-3.85						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
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
Function 3 3.75-4.10						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
3.88	THPFOS	427.00	80.00	0.05	45.00	40.00
3.90	PFOA	413.00	369.00	0.05	17.00	11.00
3.90	13C-PFOA	417.00	372.00	0.05	17.00	11.00
Function 4 3.80-5.00						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
4.26	PFNA	463.00	419.00	0.05	16.00	11.00
4.26	13C-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	13C-PFOS	503.00	80.00	0.05	45.00	40.00
Function 5 4.40-5.20						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
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

Table 1. MRM transition parameters for PFCs in ES-.

RESULTS AND DISCUSSION

Figure 2 shows the full names and the abbreviations for the PFCs used in this report. Two internal standards were used in the quantification, ^{13}C-PFOA and ^{13}CPFOS Recovery standards were also included and these were 7HPFHpA and ^{13}CPFNA. Initial experiments used water and methanol without buffer, but it was found that although the compounds were retained on the column, in order to sustain good peak shape, the addition of 2 mM ammonium acetate was required in the mobile phases.

Perfluorinated compound	Abbreviation	Formula
perfluorobutanesulfonate	PFBuS	$C_4F_9SO_3$
perfluorohexanesulfonate	PFHxS	$C_6F_{13}SO_3$
perfluorooctanesulfonate	PFOS	$C_8F_{17}SO_3$
perfluorohexanoic acid	PFHxA	$C^5F_{11}CO_2H$
perfluoroheptanoic acid	PFHpA	$C_6F_{13}CO_2H$
perfluorooctanoic acid	PFOA	$C_7F_{15}CO_2H$
perfluorononanoic acid	PFNA	$C_8F_{17}CO_2H$
perfluorodecanoic acid	PFDA	$C_9F_{19}CO_2H$
perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}CO_2H$
Internal & Recovery Standards		
7H-perfluoroheptanoic acid	7H-PFHpA	$HC_6F_{12}CO_2H$
perfluorooctanesulfonate	13C-PFOS	$^{13}C_8F_{17}SO^3$
perfluorooctanoic acid	13C-PFOA	$^{13}C_7F_{15}CO_2H$

Figure 2. List of PFCs used in the experiment.

TargetLynx™, an application manager available with Waters MassLynx™ software, was used to process the data and quantify the amount of compounds present in the blank plasma and whole blood samples. Good linearity (r^2>0.99) is observed for all compounds. The PFOS calibration curve has been included in this report (Figure 3). A chromatogram from the PFC run using the ACQUITY UPLC/Quattro Premier XE is shown in Figure 4.

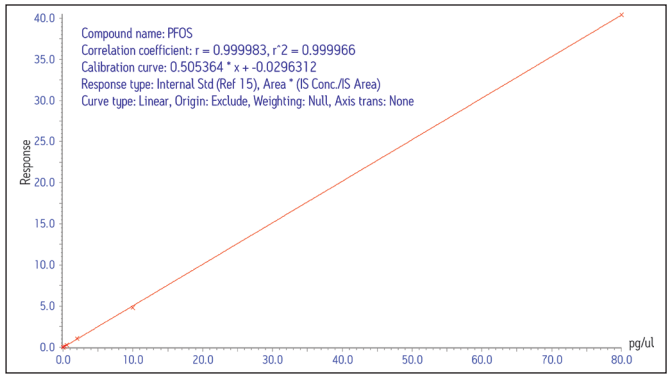

Figure 3. Calibration curve for PFOS covering the concentration range 0.01-80 ng/ml.

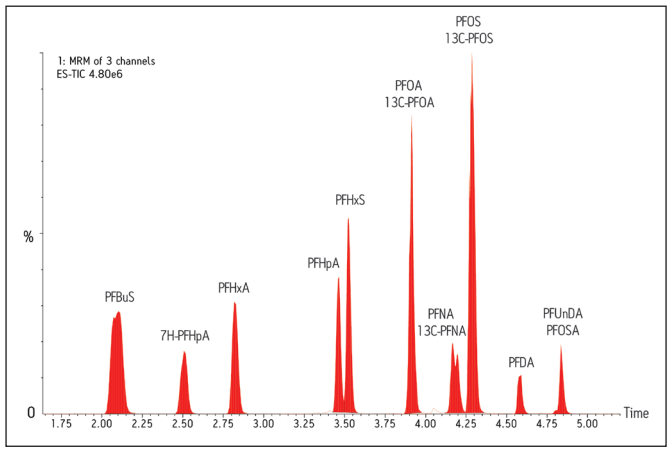

Figure 4. Chromatogram of a standard solution containing 13 PFCs using UPLC/MS/MS.

Performance

Figure 5 illustrates the improved chromatography with respect to run-time and peak width using UPLC compared to traditional HPLC (where the HPLC data was obtained using LC/MS and the UPLC data employs LC/MS/MS). The run time for these 13 compounds is 22 minutes with HPLC, but this is shortened to five minutes using UPLC.

A number of advantages result from the faster run times afforded by UPLC, including reduced method development times and smaller peak widths. In Figure 4, the peak width for PFOS has been reduced from 20.3 s to 4.2 s. This reduces the opportunity for co-elution, increases the chance that isomers will be separated and may improve sensitivity.

Recoveries

Table 2 shows the instrumental and the method detection limits. The instrumental detection limit was defined as the concentration required 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 an S/N ratio of 3.

Recovery and reproducibility of the extraction procedure were evaluated by adding known amounts of PFCs to whole blood and plasma samples in 5 replicates (Table 3: PART A). Recovery was calculated by comparing the obtained area in the sample extracts, corrected for volume, with the corresponding area, corrected for volume, in a standard solvent solution. Acceptable values are regarded as being between 70-130%. (For some of the longer chain PFCs, the values are slightly lower for the plasma samples. The standard deviation is also higher for these compounds, but they are generally more difficult to quantify than the shorter chain PFCs).

Possible suppression or enhancement of the signal in the electrospray ionization were evaluated (Table 3: PART B) by extracting unspiked whole blood and plasma according to the method and adding known amounts of PFCs to the extracts prior to injection (and post- SPE).

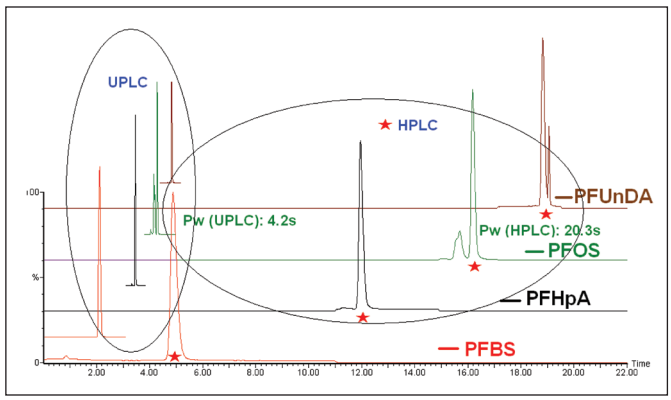

Figure 5. Comparison of run times and peak widths obtained using UPLC and traditional HPLC.

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

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

The obtained areas, corrected for volume, were then divided by corresponding areas in a standard solvent solution. A ratio >1 indicates that the sample matrix enhanced the signal and a ratio <1 indicates that the signal is suppressed by the sample matrix. These results are relatively close to 1 (ideal), except for PFUnDA in whole blood, which is high at 2.47.

PART A: Recovery and reproducibility							PART B: Matrix effects		
	Whole blood (n=5)			Plasma (n=5)				Whole blood (n=1)	Plasma (n=3)
	Average	Stddev	RSD%	Average	Stddev	RSD%		Average	Average
PFBuS	77%	0.045	6%	77%	0.031	4%	PFBuS	0.86	0.89
PFHxA	92%	0.035	4%	83%	0.027	3%	PFHxA	1.11	0.97
PFHpA	82%	0.039	5%	79%	0.011	1%	PFHpA	0.98	0.92
PFHxS	70%	0.045	6%	77%	0.037	5%	PFHxS	0.79	0.88
PFOA	82%	0.063	8%	94%	0.083	9%	PFOA	0.94	1.04
PFNA	86%	0.037	4%	99%	0.048	5%	PFNA	1.07	1.09
PFOS	78%	0.032	4%	65%	0.061	9%	PFOS	0.91	0.77
PFDA	92%	0.130	14%	70%	0.246	35%	PFDA	1.37	0.93
PFUnDA	124%	0.307	25%	64%	0.212	33%	PFUnDA	2.47	0.95

Table 3. Recovery and reproducibility of whole blood and plasma samples.

Contamination

Contamination is a known problem when analyzing for PFCs, and was prevalent for a number of compounds (Table 4). A set of experiments was designed to determine sources of contamination: 1) blank air injection + gradient run; 2) gradient run without injection; 3) methanol injection (solvent blank) and 4) procedure blank. Contamination for compounds PFOA and PFNA was found from the instrument pre-injector (either from the solvent used or instrument parts), and the injector; contamination eluted as a peak as the amount of methanol increased. To reduce contamination, a column was inserted post-pump and pre-injector, which allowed the contaminating peaks to be separated from the analytical peak.

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, solvent flow was left at 0.050 ml/min once the sequence had been run and until the next sequence was run.

Two procedure blanks prepared at different times were monitored. The procedure blanks showed more contamination than the solvent blanks, and in some cases the contamination observed may have come from the internal standards. A secondary explanation for incidents of slightly higher contamination may be glassware or other sources that had contact with the solvents.

Contamination from the extraction procedure was evaluated by including one MilliQ water sample (blank) for each set of 12 blood samples extracted.

Contamination from the instrument was evaluated by multiple methanol injections during the sample sequence.

CONCLUSION

A sensitive and rapid method for the analysis of PFCs using UPLC/MS/MS has been described. This method reduces the typical HPLC run-time of 22 minutes to less than five 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.

Further investigation is required to extend the complete method to include additional longer chain PFCs. The initial results for these compounds were not successful and have been omitted from this application note.

Contamination of blanks (pg/ul) – solvent and procedure				
	Solvent blank	Procedure blanks		
	Water: Methanol (65:35)	60103	20051027	Comments
PFBuS	✘	✔		
PFHxA	✘	~ 0.002	~ 0.002	
PFHpA	✘	~ 0.001	~ 0.001	
PFHxS	✘	➜	✘	
PFOA	~ 0.0065	~ 0.1	~ 0.4	
PFNA	~ 0.023	~ 0.05		About 50:50 from both the instrument: ISTD
PFOS	✘	✔		Could be contamination from the ISTD
PFDA	➜	✘	~ 0.05	
PFUnDA	✘	✘	✘	

✘ = not detected

✔ = found but not quantified

➜ = maybe traces

Table 4. Contamination of monitored PFCs 1) from the solvent/injector, and 2) from the procedure.

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