DEVELOPMENT OF PFCs HIGH SENSITIVITY ANALYSIS METHOD APPLIED RETENTION GAP TECHNIQUE WITH UPLC/MS/MS

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Abstract

Liquid chromatography/tandem MS/MS system is used to analyze Per fluorinated compounds (PFCs) without derivatization, quantifying trace levels of PFCs in samples unambiguously is challenging because of the widespread back ground PFCs contamination. Since PFCs are present in many conpornents of lab instruments, trace levels of PFCs can leach out. In addition, PFCs are also detected in common HPLC solvents and in lab water. Because background PFCs contamination is pervasive, quantifying trace levels of PFCs requires special care. With online removal of PFCs in mobile phase by solid phase extract ion (SPE) cartridge or technique applied retention gap, the contaminated PFCs can been reduced or shifted off.

Introduction

Per fluorinated compounds (PFCs) such as Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonate (PFOS) have been used for over 50 years in various applications that include surfactants, element in materials of chrome plating, fire fighting foam, photoresist on semiconductor manufacture and polymerization aid in synthesis polytetrafluoroethlene (PTFE), and fluorpolymers[1]. PFCs are extremely stable and not prone to environmental degradation. Long-chain PFCs such as PFOA and PFOS bioaccumulate in animals causing tumors and disturbing reproductive development[2]. Trace levels of PFCs have been measured in groundwater, wastewater treatment plants, lake water, the marine environment and even in the Arctic.

In recent reports studies, PFOA, PFOS and other PFCs have been detected at parts per billion levels in wildlife tissues and human serum.

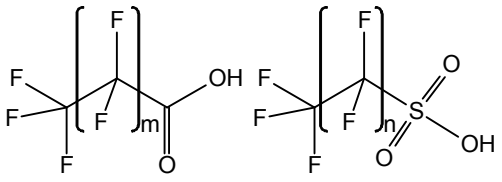

Figure 1. Chemical structures of PFCs : m=2, PFBA; m=3, PFPeA; m=4, PFHxA; m=5,PFHpA; m=6, PFOA; m=7, PFNA; m=8, PFDA; m=9, PFUdA; m=10, PFDoA; m=11, PFTrA; n=3, PFBS; n=5, PFHxS; n=7, PFOS; n=9, PFDS.

Literature reports on the potential impact of PFCs on human health and the environment indicate that this is a global concern. Consequently, there is an increased demand for rapid, sensitive and accurate analytical methods for the analysis of PFCs in environmental and biological matrics.

Materials and Methods

SPE:

OASIS HLB and SepPak AC2 used as SPE cartridges for on-line cleanup were obtained from Waters. The packing particle of OASIS HLB is reverse phase based hydrophilic-lipophilic balanced co-polymer structure and traps PFCs of long chain length. SepPak AC2 is packed the particle of activated carbon and irreversibly traps PFCs of short chain length.

PFCs Standard Solution:

"PFC-MXA" mixed Perfluorocarboxylic acid (PFCAs), "PFS-MXA" mixed Perfluoroalkyl sulfonate (PFASs) and "MPFAC-MXA" mixed surrogate (^{13}C labeled) of PFCAs were all obtained from Wellington Laboratory Inc and were used as standard solution. All standard solutions were diluted with Methanol that was HPLC grade (Kanto Chemical Co.,Inc.).. Ammonium acetate and Acetonitrile (HPLC grade) were purchased from Kanto Chemical Co.,Inc. and used as mobile phase.

LC–MS/MS analysis:

The LC apparatus was an Acquity Ultra Performance LC(Waters). All analytes were separated using a Waters Acquity UPLC BEH C18 column (50mm×2.1mm, 1.7um particle size) and Acquity UPLC BEH Shield RP18 column (100mm×2.1mm, 1.7um particle size). On the case of on-line cleanup of PFCs in mobile phase and method applied retention gap technique, analytical condition of UPLC/MS/MS is shown in Table 1.

Mass spectrometry was performed using a Waters TQ detector operated with an ESI interface in negative ionization mode. The capillary voltage was set at 0.5- 2.0 kV. The flow rates of desolvation gas and cone gas were set to 1000 and 20-50 L/h, respectively. The source temperature and desolvation gas temperature were held at 120 and 400 ◦C, respectively. Quantitative analysis was performed in multiselected reaction monitoring (MRM), and the one or two most abundant MRM transitions were listed in Table 2.

Results and Discussion

Method with on-line cleanup

Three SPE cartridges (reverse-phase, activated carbon and reverse-phase) were installed on the line of Mobile Phase A (Fig.2). PFBA, PFPeA and PFHxA in mobile phase A were irreversibly trapped on these cartridges and baseline can been shown low and stable noise level, additionally, the peaks of PFCs contaminated from LC system and mobile phase A can been disappeared (Fig 3). When SPE cartridge columns capacity were overloaded with contaminated PFCs, their peaks were appeared (Fig.4).

Table 1 Analytical conditions of UPLC/MS/MS

Table 1-1. UPLC/MS/MS method with on-line cleanup

UPLC Conditions

Column: UPLC BEH Shield RP18 2.1mmx100mm 1.7um

Mobile Phase A: water containing 2mM Ammonium Acetate

Mobile Phase B: Acetonitrile/ water in 2mM Ammonium Acetate (95/5)

Gradient table and Flow Rate

Flow rate (mL/min)	Time (min)	Conc. of A (%)	Conc. of B (%)	Gradient curve
0.4	Initail	60.0	40.0	6
0.4	4.0	5.0	95.0	6
0.4	5.0	60.0	40.0	11

Table 1-2. UPLC/MS/MS method with retention gap

UPLC Conditions

Column: ACQUITY UPLC BEH C18 2.1mmx50mm 1.7um

Retention gap: ACQUITY UPLC BEH C18 2.1mmx100mm 1.7um

Mobile Phase A: water containing 10mM Ammonium Acetate

Mobile Phase B: Acetonitrile

Gradient table and Flow Rate

Flow rate (mL/min)	Time (min)	Conc. of A (%)	Conc. of B (%)	Gradient curve
0.3	Initail	99.0	1.0	6
0.3	8.0	5.0	95.0	6
0.3	9.0	99.0	1.0	11

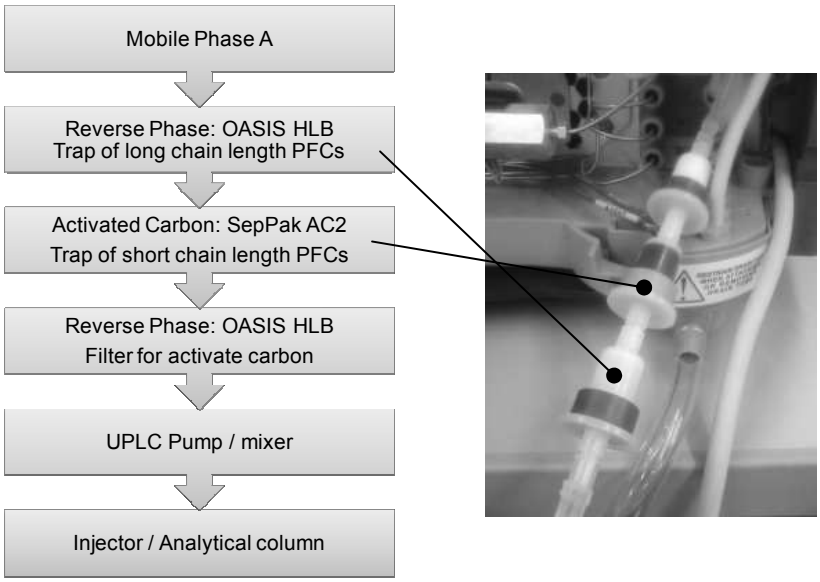

Fig.2 : Three SPE cartridges were installed on the line of Mobile Phase A

Table 2 Multi-selected reaction monitoring (MRM) transitions of PFCs

Table 2-1. MRM transitions of PFCAs

PFC	MRM	CV (V)	CE
PFBA	213.00 > 168.90	20	10
MPFBA	217.00 > 171.90	20	10
PFPeA	263.00 > 219.00	20	10
PFHxA	313.00 > 118.90	20	15
	313.00 > 268.70	20	10
MPFHxA	315.00 > 118.90	20	15
	315.00 > 269.70	20	10
PFHpA	363.00 > 168.70	20	20
	363.00 > 318.70	20	10
PFOA	413.00 > 168.70	20	20
	413.00 > 368.70	20	15
MPFOA	417.00 > 168.90	20	20
	417.00 > 371.70	20	15
PFNA	463.00 > 218.90	20	15
	463.00 > 418.70	20	10
MPFNA	468.00 > 218.90	20	15
	468.00 > 422.70	20	10
PFDA	513.00 > 218.90	20	20
	513.00 > 468.70	20	10
MPFDA	515.00 > 218.90	20	20
	515.00 > 469.70	20	10
PFUdA	563.00 > 268.90	25	20
	563.00 > 518.70	25	15
MPFUdA	565.00 > 268.90	25	20
	565.00 > 519.70	25	15
PFDoA	613.00 > 268.90	20	25
	613.00 > 568.90	20	10
MPFDoA	615.00 > 269.00	20	25
	615.00 > 569.90	20	10
PFTrDA	663.00 > 318.90	25	25
	663.00 > 619.10	25	10
PFTeDA	713.00 > 318.90	25	20
	713.00 > 669.10	25	10

Table 2-2. MRM transitions of PFASs

PFC	MRM	CV (V)	CE
PFBS	299.00 > 79.80	45	30
	299.00 > 98.80	45	35
PFHxS	399.00 > 79.80	50	40
	399.00 > 98.80	50	45
MPFHxS	403.00 > 83.80	50	40
PFOS	499.00 > 79.80	55	40
	499.00 > 98.80	55	45
MPFOS	503.00 > 79.80	55	40
	503.00 > 98.80	55	45
PFDS	599.00 > 79.80	65	55
	599.00 > 98.80	65	60

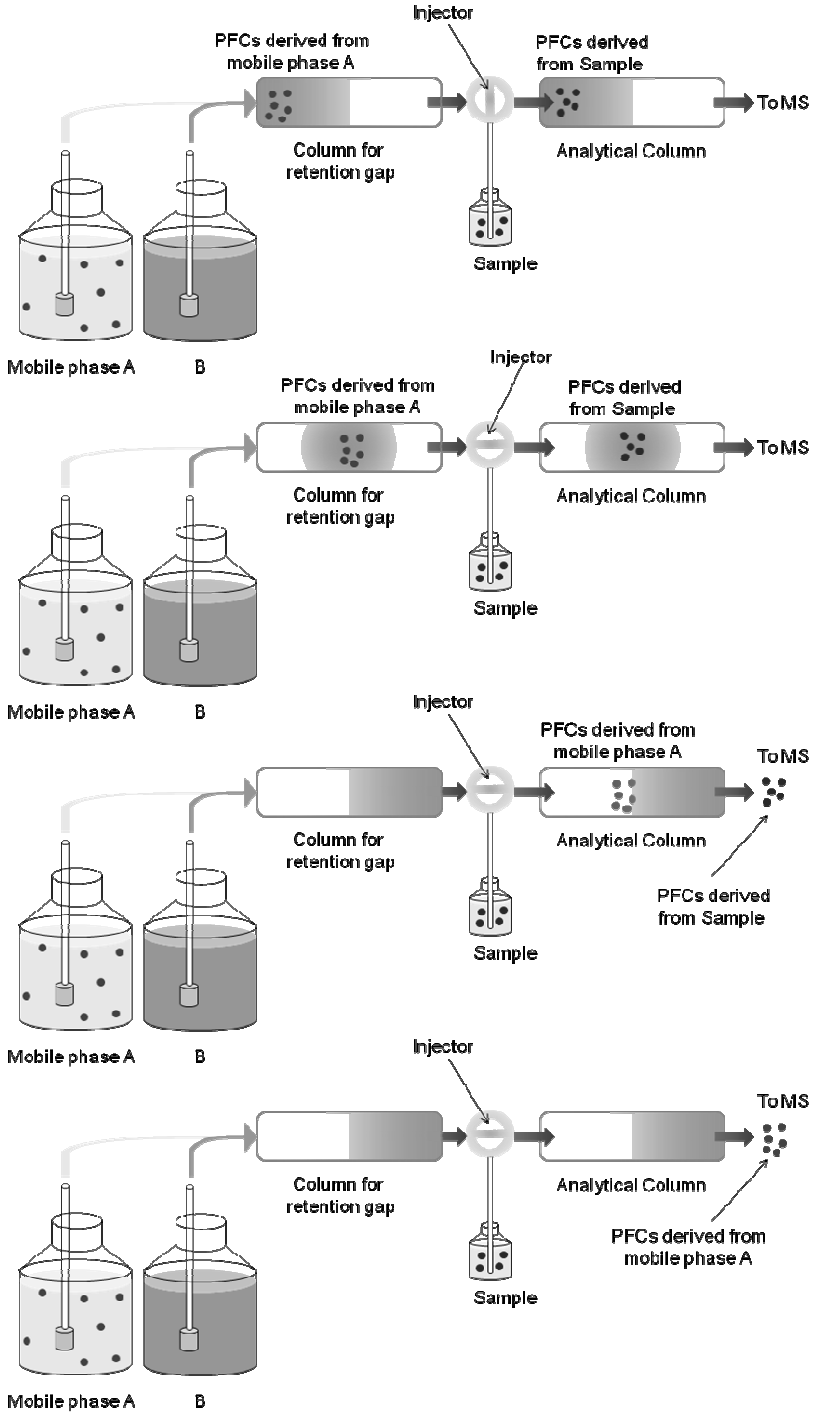

Fig.5 : Method applied retention gap technique

PFCs contaminated in mobile phase A are trapped with retention gap column installed before injector. Each contaminated PFCs elute on delay time for PFCs in sample

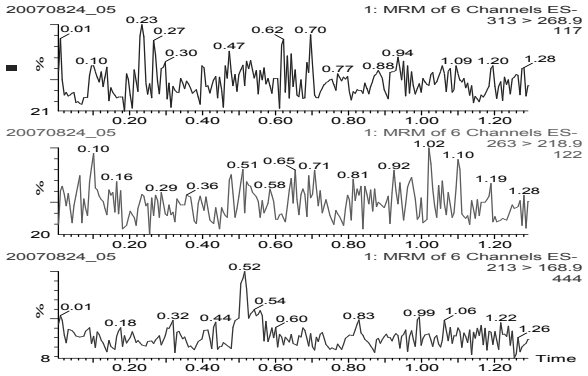

Fig.3: MRM chromatograms when Methanol were injected with SPE cartridges on the line of mobile phase A

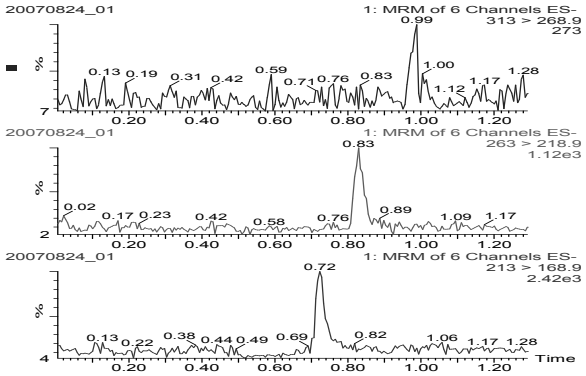

Fig.4: MRM chromatograms when SPE overloaded with contaminated PFCs

Method applied retention gap technique

PFCs contaminated in mobile phase A are trapped with retention gap column installed before injector. At the same time of sample injection, gradient program is started. Accuracy on quantitative analysis of PFCs can be improved with the time difference occurred between retention time of PFCs in sample and contaminated PFCs because contaminated PFCs are trapped on retention gap column (Fig.5). Fig. 6 shows MRM chromatograms of PFBA and PFBA in injected sample is detected as the peak that retention time is 2.81 minutes and the broad peak from 3.24 minutes is PFBA contaminated from mobile phase A .

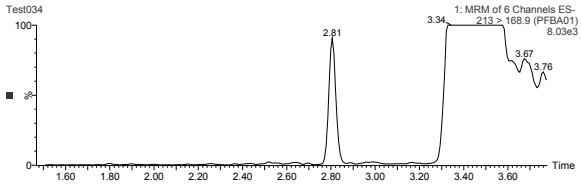

Fig. 6 MRM chromatograms of PFBA with method applied retention gap technique

Conclusion

UPLC/MS/MS method with higher sensitivity and separation efficiency was established for analyzing 11 PFCAs and 4 PFASs and was higher accuracy on quantitative analysis because PFCs in samples can be separated PFCs contaminated from mobile phase with retention gap column. The method applied retention gap technique has issue that the pressure is higher on LC pump because retention gap and analytical column are installed, but has the stability on continuous injection and higher reliability when compare with the method of on-line clean up mobile phase.

References

1. J W Washington, et al., J. Chromatogr. A, 1154, 111-120, 2007.
2. J M Flaherty, et al., J. Chromatogr. B, 819, 329-338, 2005.