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Analytical method development for widely targeted perfluoroalkyl acids (PFAAs) and their precursors in plasma using multi-gradient eluent system by LC-MS/MS

ASMS 2017 WP-131

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Introduction

As perfluoroalkyl acids (PFAAs) had been widely used because of their excellent surfactant. While the product and usage of perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOS-F) were restricted by the Stockholm Convention in 2009, many kinds of precursors which cause PFAAs by decomposition keep being used because they are not restricted.
LC-MS/MS analysis methods for PFAAs have been developed, where a high selectivity analysis is available, but it was very difficult to implement the simultaneous analysis of PFAAs and their precursors since the wideness of their chemical property in terms of hydrophilic / hydrophobic. This time, we developed the analytical method for widely targeted PFAAs and their precursors in plasma using multi-gradient eluent system by LC-MS/MS.

Materials and Methods

A standard mixture of PFCAs (C4-18), PFASs (C4-10), FOSAs, FTSs and PAPs, total 28 compounds, were diluted to working concentrations in methanol. Plasma sample was pretreated by WAX cartridge (final solvent: 0.05 % NH3, 0.15 % HCOOH, 80 % MeOH). Separation was achieved using Oasis WAX column (2 mmID. x 20 mmL) and Triart C18 column (2 mmID. x 100 mmL) maintained at 40 °C on a UHPLC system (Shimadzu corporation, Kyoto, Japan), configured by four pumps and two 6-port valves. 500 uL of sample was inserted 250 uL water and injected. Data acquisition was performed on triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Corporation, Kyoto, Japan). All samples were analyzed by multiple reaction monitoring (MRM).

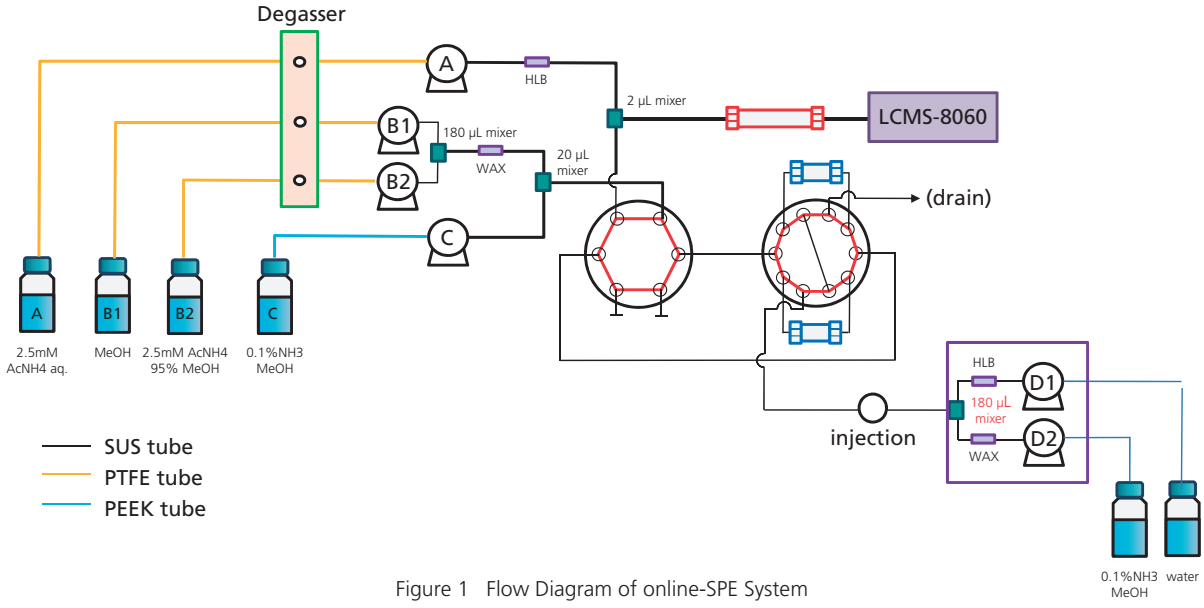

Figure 1 Flow Diagram of online-SPE System

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HPLC conditions	
Analytical Column	: YMC Triart C18 (100 mm L x 2.1 mm I.D., 3 µm)
Trap Column	: Oasis WAX (20 mm L x 2 mm I.D.)
Mobile Phase	: A: 2.5mM Ammonium Acetate in Water (Scrubber Column: OASIS HLB, 20 mm L x 2.1 mm I.D) : B: 2.5mM Ammonium Acetate in Methanol (Scrubber Column: OASIS WAX, 20 mm L x 2.1 mm I.D) : C: 0.1%NH_4OH in MeOH
Column temperature	: 40 °C
Injection vol.	: 500 µL

LCMS conditions	
Ionization	: heated ESI negative
Nebulizing Gas Flow	: 3 L / min
Drying Gas Pressure	: 10 L / min
Heating gas flow	: 10 L / min
DL Temperature	: 150 °C
BH Temperature	: 400 °C
Interface Temperature	: 250 °C

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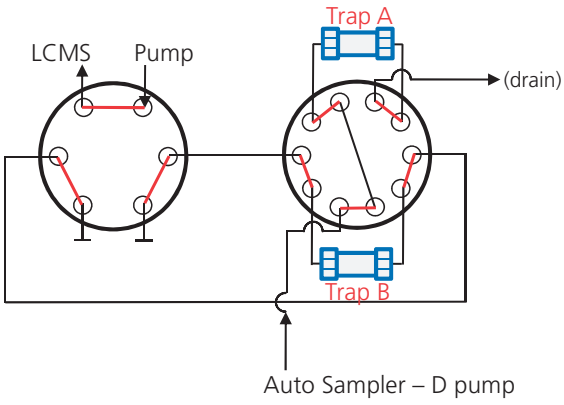
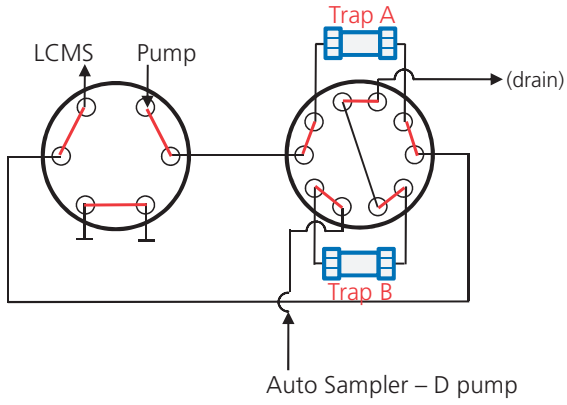
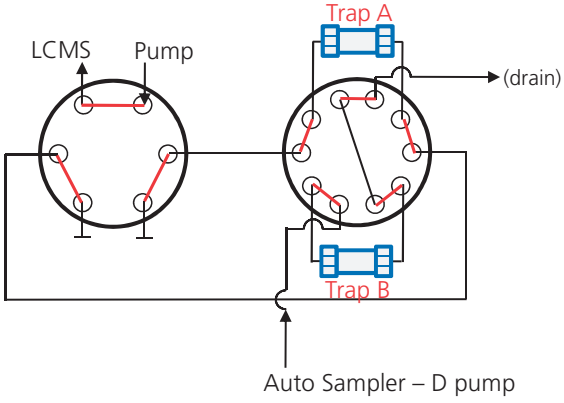
1) sample loading to Trap A



2) analysis using Trap A (washing Trap B)



1) sample loading to Trap B



2) analysis using Trap B (washing Trap A)

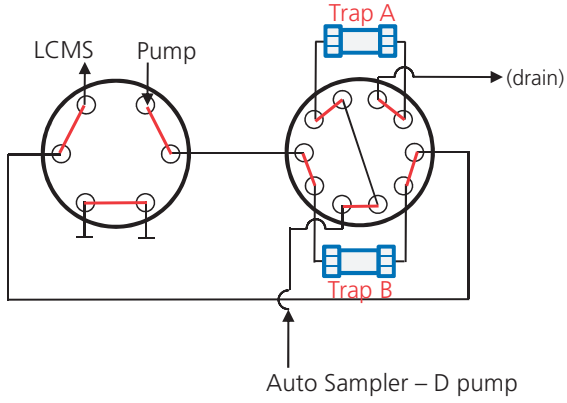


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Table 1 Calibration Range and LOD of 28 PFAAs

Compounds Name	Abbrev.	Range (pg) On column	System Blank CAL0 (pg)	LOD (ng/mL)
Perfluorobutanoic acid	PFBA	1.5-600	1	0.12
Perfluoropentanoic acid	PFPA	1.5-600	0.75	0.09
Perfluorohexanoic acid	PFHxA	1.5-600	0.75	0.09
Perfluoroheptanoic acid	PFHpA	1.5-600	0.6	0.072
Perfluorooctanoic acid	PFOA	1.5-600	0.6	0.072
Perfluorononanoic acid	PFNA	1.5-600	0.38	0.045
Perfluorodecanoic acid	PFDA	1.5-600	0.38	0.045
Perfluoroundecanoic acid	PFUnA	1.5-600	0.5	0.06
Perfluorododecanoic acid	PFDoA	1.5-600	0.38	0.045
Perfluorotridecanoic acid	PFTrDA	1.5-600	0.38	0.045
Perfluorotetradecanoic acid	PFTeDA	1.5-600	0.5	0.06
Perfluorohexadecanoic acid	PFHxDA	1.5-600	0.75	0.09
Perfluorooctadecanoic acid	PFODA	1.5-600	0.6	0.072
Perfluorobutane sulfonate	PFBS	1.5-600	None	<0.04
Perfluorohexane sulfonate	PFHxS	1.5-600	0.3	0.036
Perfluoroheptane sulfonate	PFHpS	1.5-600	None	<0.04
Perfluorooctane sulfonate	PFOS	1.5-600	None	<0.04
Perfluorodecane sulfonate	PFDS	1.5-600	None	<0.04
N-Methyl perfluorooctane sulfonamidoacetic acid	MeFOSAA	1.5-600	None	<0.04
N-Ethyl perfluorooctane sulfonamidoacetic acid	EtFOSAA	1.5-600	None	<0.04
N-Methyl perfluorooctane sulfonamide	MeFOSA	1.5-600	None	<0.04
N-Ethyl perfluorooctane sulfonamide	EtFOSA	1.5-600	None	<0.04
4:2 Fluorotelomer sulfonic acid	4:2FTS	1.5-150	None	<0.04
6:2 Fluorotelomer sulfonic acid	6:2FTS	1.5-150	None	<0.04
8:2 Fluorotelomer sulfonic acid	8:2FTS	1.5-150	None	<0.04
6:2 Polyfluoroalkyl phosphoric acid diester	6:2diPAP	1.5-600	None	<0.04
8:2 Polyfluoroalkyl phosphoric acid diester	8:2diPAP	1.5-600	None	<0.04
Sodium bis-[2-(N-ethylperfluorooctane-1-sulfonamide)ethyl] phosphate	diSAmPAP	1.5-600	None	<0.04

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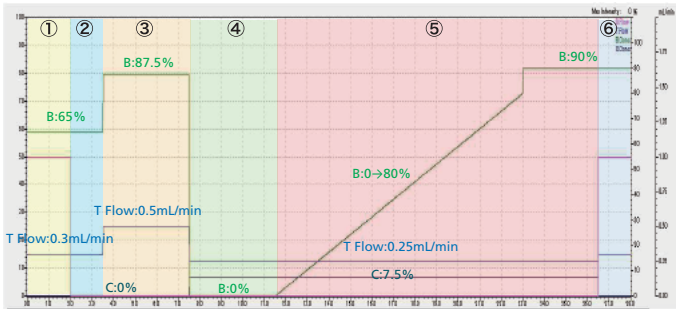
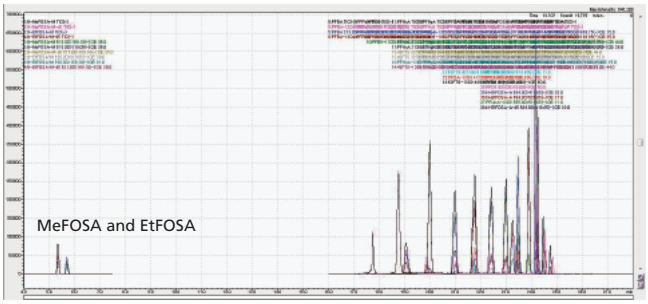
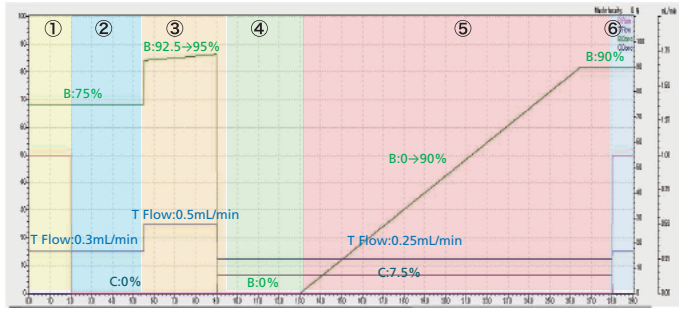
[method A]





[method B]



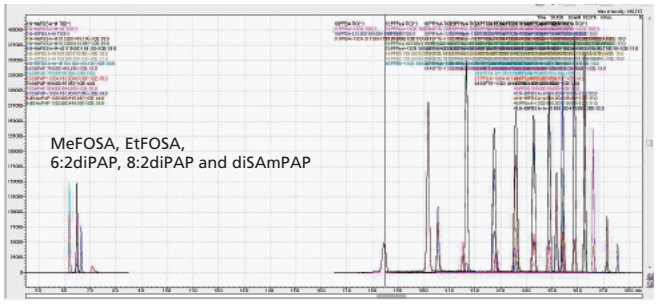


Figure 2　Gradient Program of “method A” and “method B”, and MRM Chromatograms of 28 PFAAs

Results

All of compounds were successfully ionized by negative electrospray ionization (ESI) mode.
Samples were injected and loaded on trap column (WAX) with 65 % 2.5 mM NH4Ac 95 % MeOH. MeFOSA and EtFOSA were hardly retained by this condition, so these two compounds were eluted through trap column and analysis column (ODS) to mass spectrometer. After these two compounds were detected, other 26 compounds were eluted by tertiary guradient; A: 2.5 mM NH4Ac aq., B: 2.5 mM NH4Ac MeOH, C: 0.1 % NH3 MeOH, B 0 % C 7.5 % to B 80 % C 7.5%. Analysis time was set less than 30 minutes and all compounds were eluted with excellent separation. Plasma matrix samples were tested and some compounds detected with ion suppression or ion enhancement, but this method resulted good repeatability.

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Table 2 IS Recovery of 28 PFAAs comparing "method A" and "method B"

Compounds	IS recovery (%)	
	Method A	Method B
PFBA	58.2	35.6
PFPA	83.1	77.1
PFHxA	85.2	79.6
PFHpA	84.5	76.6
PFOA	96.8	88.6
PFNA	86.4	78.6
PFDA	94.1	77.7
PFUnA	89.5	78.0
PFDoA	82.3	71.4
PFTrDA	44.6	66.5
PFTeDA	44.6	66.5
PFHxDA	46.8	50.0
PFODA	46.8	50.0

Compounds	IS recovery (%)	
	Method A	Method B
PFBS	96.0	93.9
PFHxS	73.7	62.0
PFHpS	92.3	86.7
PFOS	92.3	86.7
PFDS	92.3	86.7
MeFOSAA	71.6	57.2
EtFOSAA	81.1	65.3
MeFOSA	82.7	76.3
EtFOSA	70.9	69.4
4:2FTS	44.7	23.9
6:2FTS	173.6	132.4
8:2FTS	142.6	80.3
6:2diPAP	78.7	72.0
8:2diPAP	71.8	159.9
diSAmPAP	71.8	159.9

Conclusions

We developed the analytical method for widely targeted PFAAs and their precursors in plasma using multi-gradient eluent system by LC-MS/MS.

Acknowledgement

We thank Dr. Shoji F. Nakayama and Mitsuha Yoshikane (National Institute for Environmental Studies, Japan) for his help and valuable suggestions.

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First Edition: June, 2017

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