ANALYSIS OF PERFLUOROOCTANE SULFONATE ISOMERS IN SERUM BY IN-PORT ARYLATION-GAS CHROMATOGRAPHY-NEGATIVE CHEMICAL IONIZATION-MASS SPECTROMETRY

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Introduction

Perfluorooctane sulfonate (PFOS) and related chemicals (perfluorochemicals, PFCs) have been used in various applications.[1] PFOS was designated as persistent organic pollutants under Stockholm convention and poses public health concern because of their persistence and bioaccumulation in the environment and organisms.

PFOS manufactured by electrochemical fluorination consists of linear and branched perfluoroalkyl chains. Isomer profiles of PFOS in samples are thought to reflect those in contamination sources, which is valuable information for source tracking. In addition, PFOS isomers have different biological half-lives and toxicities in mammals. Hence, analysis of isomers is required for accurate exposure and risk assessment.

PFOS has been analyzed mainly by liquid-chromatography and tandem mass-spectrometers (LC-MS/MS). In the analysis, isomers are separated on LC and their specific transition in collision induced dissociation.[2,3] Several branched isomers are still co-eluted in LC such as 6-methyl and 2-methyl isomers. Isomer specific CID can distinguish co-eluted isomers, but the product ions are not sensitive compared with m/z 80.

On the other hand, several reports showed a gas-chromatographic separation of PFOS isomers.[4,5] In GC analysis, derivatization of PFOS is required, and laborsome. In-port derivatization using tetrabutyl ammonium salt successfully analyzed 11 PFOS isomers in technical mixture and environmental samples, but the sensitivity was not so high as in LC-MS/MS method.[5]

Aryl sulfonate has a stability to nucleophiles and is generally synthesized from sulfonyl chloride and aryl alcohol, and however, it requires time-consuming process to prepare PFOS chloride. In-port derivatization of sulfonate has been conducted for PFOS, alkane sulfonate and alkyl benzene sulfonate using quaternary ammonium salts[5,6] while quaternary aryl ammonium compounds are rarely available from commercial sources. Diaryliodonium salts has been reported to arylate sulfonic acid in mild and metal-free conditions,[7] which can be applied in in-port derivatization of PFOS.

In the present study, PFOS isomers and related chemicals were analyzed by in-port arylation-gas chromatography-mass spectrometry with negative chemical ionization. PFCs in serum samples were also quantified by this method.

Materials and methods

Technical mixture of PFOS, 11 individual PFOS isomers, and native and mass-labelled PFCs mixtures (PFAC-24PAR and MPFAC-MXA) were purchased from Wellington Laboratories (Guelph, ON, Canada). Bis(4-tert-butylphenyl)iodonium hexafluorophosphate (BtBPI) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Serum samples were from NIST standard reference material (SRM) 1957 and the archived sample from Japanese females in Kyoto Human Specimen Bank (Koizumi et al., 2009) with approval in Kyoto University Ethics Committee (R1478).

Standard solutions were prepared and diluted in methanol. BtBPI powder was washed by hexane and prepared at 1%(w/v) in acetone.

GC-MS conditions were optimized by analyzing standard solutions. The solution was divided and dried under nitrogen stream. Then, 50 µL BtBPI solution was added and injected to GC (Agilent 6890GC). Capillary column DB-5MS (30m length, 0.25 mm i.d., 0.25 µm film thickness) was used. Injection volume was 1 µL. Inlet temperature was maintained at 300 ℃. Pulsed splitless injection (30 psi for 1 min) was employed and, vent line was opened at 1 min. Helium gas was used as a carrier gas at flow rate of 1 mL/min. Oven temperature was maintained at 70 ℃ for 1 min and ramped at 5 ℃/min to 130 ℃, 2℃/min to 150℃, and 30℃/min to 240℃. Transfer line temperature was 280 ℃.

For serum analyses, 50 µL of samples were divided to 1.5 mL polypropylene tubes and mixed with 10 µL of surrogate compounds (50 pg/µL in methanol), followed by 200 µL acetonitrile. Solutions were vortexed for 30 s and centrifuged at 12000 rpm for 1 min. Supernatant was transferred to 5 mL glass tube and mixed with 1 ng of 11H-perfluoroundecanoic acid to measure recoveries of mass-labeled compounds and derivatization efficiency. Sample extracts were then dried under nitrogen stream and reconstituted in 50 µL 1% BtBPI solution to GC vial. In sample analyses, 1 µL BtBPI solution was injected in split mode (split ratio: 10) after sample run to reduce possible carry-over between samples.

Results and discussion:

Standard solution of technical mixture of PFOS were analyzed in full-scan mode with EI and NCI. In EI analysis by LR-MS, the molecular ion [M]+ of linear PFOS tert-Bu-phenyl ester was observed as m/z 632 (Fig. 1). Major fragments ions were m/z 617 $[M–CH_3]^+$ (base peak), 525 $[M–CH_3–H_2SO_3]^{+\cdot}$, 169, 149, 131, 119 and etc. HR-MS analysis also predicted corresponding elemental composition, $C_{17}H_{10}F_{17}O_3S$ at m/z 617.0014. Elution order of isomers was confirmed by individual isomer standards. The signal amplitudes of isomers were similar to composition of them determined by 19F-NMR. For other PFCs, similar signals attributable to tert-Bu-phenyl esters were found.

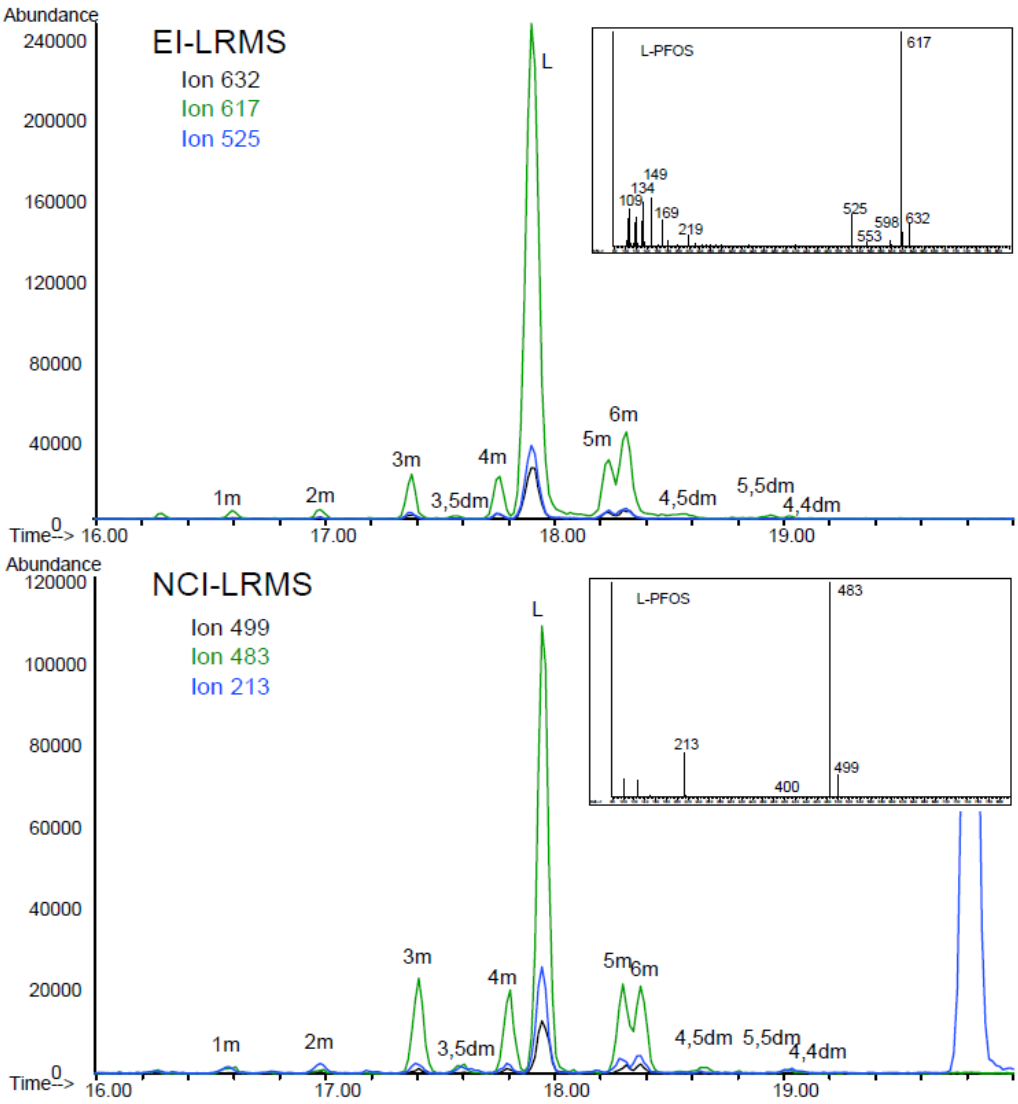

Figure 1. Chromatograms of T-PFOS derivatives in EI and NCI modes. Major fragment ions were plotted. Mass spectra of L-PFOS of them were depicted on the upper right corner.

In NCI analysis, a base fragment ion of m/z 483 was detected at the same retention time of linear PFOS (Fig. 1), which suggested a cleavage of the ester to sulfinate anion ($C_8F_{17}SO_2^-$) and tert-Bu-phenoxy radical (tert-BuC6H4O•). Other fragments of m/z 499 and 213 were assigned to sulfonate anion ($C_8F_{17}SO_2O–$) and [tert-$BuC_6H_4SO_3]^-$, respectively. Analysis by HR-MS showed possible elemental composition, $C_8F_{17}O_2S$ at m/z 482.9476. Among PFOS isomers examined, m/z 483 was base peak except for 2m-PFOS and 5,5dm-PFOS. Isomers of PFHxS were examined in PFAC-24PAR standard solution containing linear, 1m, 2m, 3m and 4m-PFHxS and 5 peaks were detected at m/z 383 and 213 (Fig. 2). While individual standard solutions of branched PFHxS were not available, based on the relative amplitude of signals and elution order of them, 1m, 2m, 3m and 4m-PFHxS were putatively assigned. Technical mixture of PFOA was also analyzed with individual isomer standards (Fig. 2). L-PFOA and 4m-PFOA were co-eluted in this GC condition. PFOA esters predominantly gave m/z 397, suggesting a cleavage of the ester to perfluorooctanoyl anion ($C_7F_{15}CO^-$) and tert-Bu-phenoxy

radical (tert-$BuC_6H_4O^{\bullet}$). Other fragments, m/z 378 and 350 were observed, but the intensity was much smaller than m/z 397. For other perfluorinated sulfonic acids (PFSAs), $[M–BuC_6H_4O]^-$ and $[tert\text{-}BuC_6H_4SO_3]^-$ were observed as same as in PFOS. For PFCAs, $[M–BuC_6H_4O]^-$ was a predominant ion.

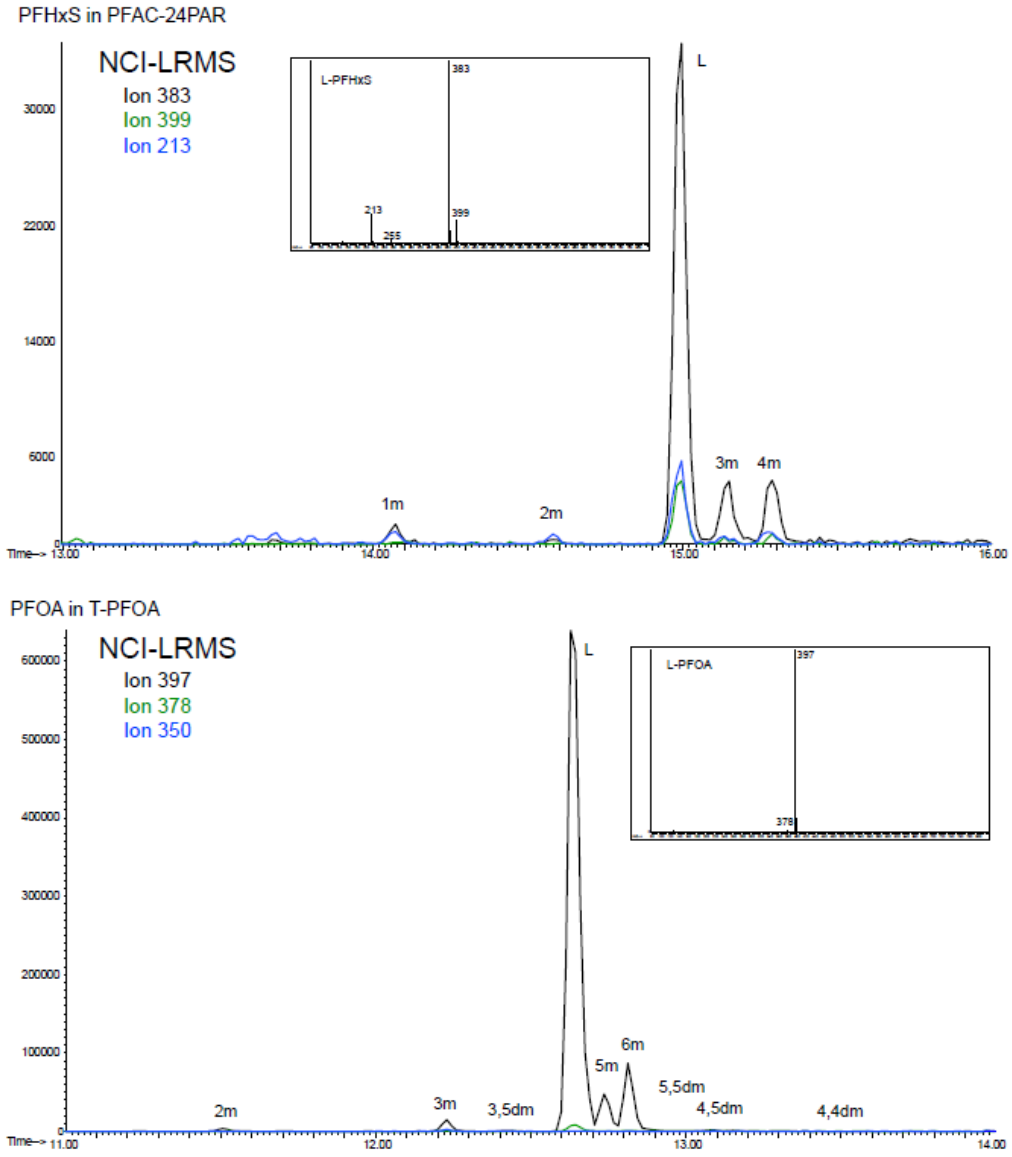

Figure 2. Chromatograms of PFHxS in 24PAR and T-PFOA derivatives in NCI mode. Major fragment ions were plotted. Mass spectra of L-PFOS of them were depicted in the windows.

Using the method above, we analyzed serum samples. The MDLs for serum samples ranged from 0.01–1 ng mL^{-1}. The mean recoveries (%, n=7) were from 80% to 100%. Typical chromatograms in analysis of serum sample from Japanese female in early 2002 is shown in Fig. 3. 50 µL of serum samples was enough to determine PFOS isomers in current method. Analysis of NIST SRM 1957 showed comparable results for PFOS isomers reported by Riddel et al. and Salihovic et al.[2,3] For other PFCs, similar values were obtained as in LC method and benzyl ester derivatization GC method.[8,9] Compared with T-PFOS, SRM 1957 had higher proportion of 6m, 5m, and 4m-PFOS (Fig. 3). Karrman et al. also reported higher composition branched isomers in serum samples from Sweden, Australia, and UK.[10] On the other hand, a serum sample from Japanese female showed smaller ratio of 6m and 3m-PFOS to L-PFOS than T-PFOS.

In conclusion, the method in this report can provide isomer composition in biological samples with sufficient sensitivity in small volume of samples.

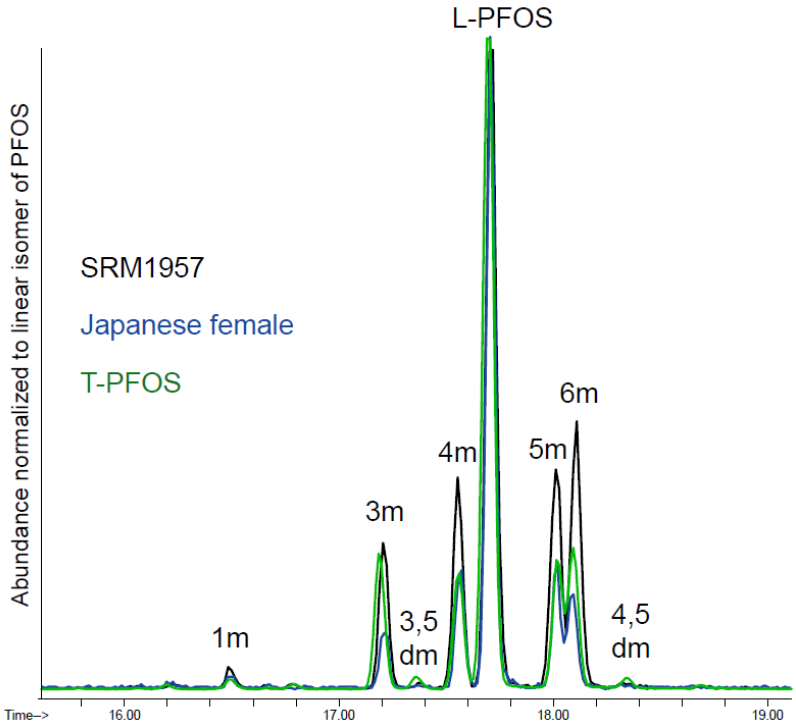

Figure 3. Chromatograms of serum samples and T-PFOS derivatives in NCI mode. m/z 483 was selected to plot chromatograms. Abundances were normalized to linear isomer of PFOS.

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References:

1. Kissa, E. (2001) Fluorinated Surfactants and Repellents, 2nd Ed. Surfactant science series. Marcel Dekker: New York.
2. Salihovic S, Kärrman A, Lind L, Lind PM, Lindström G, van Bavel B. (2015) Perfluoroalkyl Substances (PFAS) Including Structural PFOS Isomers in Plasma from Elderly Men and Women from Sweden: Results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS). *Environ. Int.* 82: 21–27.
3. Riddell N, Arsenault G, Benskin JP, Chittim B, Martin J W, McAlees, A, McCrindle, R. Branched. (2009) Perfluorooctane Sulfonate Isomer Quantification and Characterization in Blood Serum Samples by HPLC/ESI-MS(/MS). *Environ. Sci. Technol.* 43 (20), 7902–7908. https://doi.org/10.1021/es901261v.
4. Langlois, I, Berger, U, Zencak, Z, Oehme, M. (2007) Mass Spectral Studies of Perfluorooctane Sulfonate Derivatives Separated by High-Resolution Gas Chromatography. *Rapid Commun. Mass Spectrom.* 21 (22), 3547–3553.
5. Chu, S, Letcher, R. J. (2009) Linear and Branched Perfluorooctane Sulfonate Isomers in Technical Product and Environmental Samples by In-Port Derivatization-Gas Chromatography-Mass Spectrometry. *Anal. Chem.* 81 (11), 4256–4262. https://doi.org/10.1021/ac8027273.
6. Field, J. A, Miller, D. J, Field, T. M, Hawthorne, S. B, Giger, W. (1992) Quantitative Determination of Sulfonated Aliphatic and Aromatic Surfactants in Sewage Sludge by Ion-Pair/Supercritical Fluid Extraction and Derivatization Gas Chromatography/Mass Spectrometry. *Anal. Chem.* 64 (24), 3161–3167.
7. Jalalian, N, Petersen, T. B, Olofsson, B. (2012) Metal-Free Arylation of Oxygen Nucleophiles with Diaryliodonium Salts. *Chem. - A Eur. J.* 18 (44), 14140–14149. https://doi.org/10.1002/chem.201201645.
8. Keller, J. M, Calafat, A. M, Kato, K, Ellefson, M. E, Reagen, W. K, Strynar, M, O'Connell, S, Butt, C. M, Mabury, S. A, Small, J, et al. (2010) Determination of Perfluorinated Alkyl Acid Concentrations in Human Serum and Milk Standard Reference Materials. *Anal Bioanal Chem.* 397: 439–451.
9. Harada, K. H, Hitomi, T, Niisoe, T, Takenaka, K, Kamiyama, S, Watanabe, T, Moon, C. S, Yang, H. R, Hung, N. N, Koizumi, A. (2011) Odd-Numbered Perfluorocarboxylates Predominate over Perfluorooctanoic Acid in Serum Samples from Japan, Korea and Vietnam. *Environ Int.* 79: 314-9.
10. Karrman, A, Langlois, I, van Bavel, B, Lindstrom, G, Oehme, M. (2007) Identification and Pattern of Perfluorooctane Sulfonate (PFOS) Isomers in Human Serum and Plasma. *Environ Int.* 33: 782–788.