Determination of isomer/enantiomers of perfluorooctanoic acid in river water sample by gas chromatography-mass spectrometry with chiral derivatization

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Introduction

Perfluorinated chemicals (PFCs) is an organofluorine compound containing only carbon-fluorine (C-F) bonds and carbon-carbon (C-C) bonds but also other functional group (carboxylate, sulfonate, or phosphonate). PFCs have been produced in large quantities since the late 1940s and were used ubiquitously in a wide range of consumer and industrial products[1]. Perfluorocarboxylic acids (PFCAs) are aliphatic carboxylic acids with a perfluoroalkyl moiety (C_nF_{2n+1}-) bonded to a carboxylic acid functional group (-COOH)[2]. Due to its hydrophobicity and stain-resistance, derivatives of PFCAs have been used as fluorosurfactants, treatment of fabrics, paper, lubricants, polishes, food packaging, shampoos and fire-fighting foams. These perfluorinated compounds have absolute advantages with chemical stabilities because of the high-energy bonds between carbon and fluorine[3]. However, these properties cause PFCAs to be stable for a long time; and PFCAs are leached from fluoropolymers through biodegradation, transformation reactions and thermolysis in the environment[4]. Therefore, PFCAs are regarded as environmentally persistent, bio-accumulative and potentially harmful to humans. Perfluorooctanoic acid (PFOA) is the most widely representative PFCAs and is routinely found in human samples[5]. In epidemiology studies, many reports suggest that there may be associations between PFOA exposure and human carcinogenicity, immunotoxicity, reproductive dysfunction and endocrine disruption[6-8].

PFCAs have been manufactured by four synthesis routes: electrochemical fluorination (ECF), fluorotelomer iodide oxidation, fluorotelomer olefin oxidation, and fluorotelomer iodide carboxylation. Historically, linear eight- or nine-carbon PFCAs are major component of commercial PFCAs products[9]. Since 1947, the ECF process manufactured majority of perfluorooctanoic acid and yields a composition of 78% linear and 22% branched isomers of PFOA[10]. Some of the branched isomers contain a chiral carbon center, which corresponds to two non-superimposable mirror image molecules, termed enantiomers. Most of manufactured or abiotically generated enantiomers are racemic mixtures having a (1:1) enantiomer ratio. Enantiomers are alike in their physical and chemical properties so that abiotic transformation such as leaching, volatilization and hydrolysis do not change the enantiomer ratio. In contrast, when they metabolized with enzymes and microorganism, they proceed enantioselectively, leading to a nonracemic mixture enantiomers and an alternation of the original enantiomer ratio[11]. As reported by Naile et al.[12], enantioselectivity usually results in differences in toxicity of the two enantiomers because enantiomers interact with other chiral molecules at different rates or bind with different strengths. Thus, enantiomers are useful as tracers of environmental sources and significant in assessment of human risks. To evaluate the situation of PFOA pollution in water samples, it is important to develop an accurate method for determination of isomers/enantiomers of PFOA.

In recent years, several literatures have reported that liquid chromatography tandem mass spectrometry (LC-MS/MS) is used for quantitative determination of PFCAs in environmental samples. However, LC-MS/MS could not separate them completely. Gas chromatography mass spectrometry (GC-MS), with its high chromatographic resolution and sensitivity, is another method for separation and quantitative. The PFOA is difficult to directly determine by GC-MS due to its strong polarity. Derivatization step is required to make them amenable to GC. Naile et al. derivatized PFOA to methyl esters and separate two enantiomers of PFOA (3m- and 4m-PFOA) using two chiral GC columns by GC-MS[12]. Various chiral columns for GC are available, but still not in routine use. Chiral separation with achiral columns can be alternative method for determination of PFOA enantiomers.

In this research, a rapid and simple chiral derivatization method has been developed for determination of isomers/enantiomers of perfluorooctanoic acid by gas chromatography-mass spectrometry with electron-capture negative ionization. The enantiomers of three of the chiral isomers (3m-PFOA, 4m-PFOA and 3,5 dm-PFOA), linear isomer and 6m-PFOA were completely separated by the achiral column instead of chiral column. This method was also applicable to analyze isomers/enantiomers of PFOA in water samples.

Materials and methods

Perfluorooctanoic acid (PFOA): Perfluorooctanoic acid (Technical Grade, T-PFOA), Linear, Perfluoro-3-methyl-PFOA, Perfluoro-4-methyl-PFOA , Perfluoro-5-methyl-PFOA , Perfluoro-6-methyl-PFOA, Perfluoro-4,5-dimethyl-PFOA , Perfluoro-3,5-dimethyl-PFOA were purchased from Wellington Laboratories Inc. (Guelph, Canada). , (S)-1-Phenylethanol, thionyl chloride and acetone (pesticide residue and PCB analysis grade) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Ultrapure water produced by Milli-Q purification system (Merck, Tokyo, Japan).

Water samples were obtained from a river in Chiba prefecture. River water samples were drawn from the surface using a tinplate bucket. Water samples were collected into polypropylene bottles and were delivered to the laboratory immediately after collection, then stored at 4 ℃ until analysis.

35 mL water sample was placed in a injection syringe. ^{13}C-labeled PFCs were added as a recovery surrogate (MPFAC-MXA; Wellington Laboratories Inc., Guelph, Canada). Water sample was concentrated using a solid phase extraction cartridge (Oasis WAX plus 225mg, Waters, Milford, MA, USA), the cartridge was conditioned with 1.5 mL 0.1M NH_4OH/methanol, after which 35 mL of water sample was passed through the cartridge. The cartridge was washed with 1.5 mL purified water and methanol, after which PFOA were eluted into glass tube with 2mL 0.1M NH_4OH/methanol. The eluate was dried under a N_2 stream at 60°C. The derivatization of PFOA was performed by reacting chiral chloroalkane with perfluorooctanoic acid to form carboxylate. Chiral derivatizing reagent, (S)-1-phenethyl chloride (PhEtCl)was generated by reacting (S)-1-phenylethanol with thionyl chloride, prior to use. The residue was dissolved in derivatizing solution, 5% PhEtCl and 100 μL acetone with 1 ng 11H-perfluoroundecanoic acid (11H-PFUnDA) as internal standard. Then the derivatized PFOA solution was injected to gas chromatography-electron capture negative ionization-mass spectrometry (GC-ECNI-MS) for analysis.

The analysis was performed on an Agilent 6890 gas chromatography, coupled with a 5937inert mass spectrometer (Agilent, USA). The injection volume was 1.0 μL in splitless mode and the injector

temperature was 300 °C. The chromatography separation was carried on an HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm thick film). The column temperature was programmed as follows: the initial oven temperature was set at 70 °C for 1min, then it was increased to 83 °C at 0.5 °C min^{-1}, and ramped to 120°C at 20 °C min^{-1}, then ramped to 300 °C at 30 °C min^{-1} and held for 1min.The total run time was 35.85 min. High purity helium was used as the carrier gas at a flow rate of 1mL min^{-1}. The GC-MS was run in selected-ion-monitoring mode (SIM), and Table 1 shows the retention time and mass spectrometric parameters.

Table 1. Retention times and mass spectrometric parameters of enantiomers and isomers of PFOA

	L-PFOA	3,5dm	3m	4m	5m	4,5dm	6m
Retention time (min)	21.5	19.7/19.9	19.6/19.9	21.2/21.4	21.6	22.9	22.4
Fragment ions (m/z)	413	350	350	350	413	350	413

Result and discussion

Optimization of the MS conditions was performed with individual isomer standard solutions. Selected ions were chosen based on the strongest peaks in the mass spectra of linear and branched isomer PFOA standards. Retention time and selected ion were shown in Table 1. For linear PFOA, 5m-PFOA and 6m-PFOA, the prominent ion were M-105, resulting from loss of -$HC(CH_3)C_6H_5$ from the parent ion. The other prominent ion of 3m-PFOA, 4m-PFOA, 4,5 and 3,5 dm-PFOA were M-168 resulting from loss of undefined fragment ion. The GC separation was performed on a HP-5MS column, and the temperature programming procedure shown in section of materials and method was carried out, which can reduce analysis time and overlap peaks. Especially, 3m-PFOA and 4m-PFOA have the same prominent ion, and a good chromatography resolution was achieved with different retention time. The chromatogram of linear and branched isomers of PFOA was shown in Fig.1.

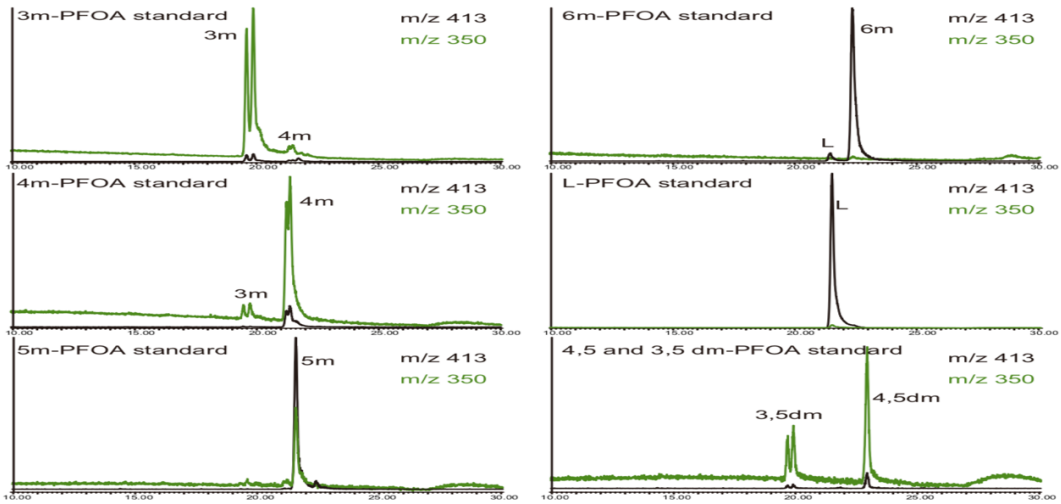

Fig.1 Chromatogram of PFOA standards: 3m-PFOA,4m-PFOA, 5m-PFOA,6m-PFOA,linear PFOA, and 4,5 and 3,5 dm-PFOA.

Because of the high boiling point and polarity of perfluorooctanoic acid, it is difficult to analyze PFOA using GC techniques directly. Thus, derivatization step of PFOA is required to make them amenable to

GC analysis. In this study, phenylethyl alcohol and thionyl chloride were used to generated chloroalkane. Then, PFOA are reacted with chloroalkane to form carboxylate. Compared to previously derivatization method which was performed by reacting the carboxylic acids with diazomethane to form methyl esters but 4m- and 5m-PFOA were overlapped[13], the developed derivatization method can separate 4m-PFOA and linear-PFOA effectively (shown in Fig.1) and the derivatives were sensitive in ECNI analysis. Extraordinary, under this derivatization method, enantiomers of 3m-PFOA ,4m-PFOAwere separated by the achiral column instead of chiral column. However, the two peaks of 5m-PFOA and linear were overlapped and the separation of the enantiomers of 5m-PFOA and 4,5dm -PFOA were not achieved as previously reported by Naile et al. This may indicate that branched CF_3 at apart from phenetyl group may have less conformational change and intramolecular interaction than 3m-PFOA diastereomers.

The developed method was applied to determine isomers/enantiomers in river water samples. Fig.2 depicts the chromatogram of well water sample. Isomers/enantiomers of PFOA, especially 3m-PFOA, 4m-PFOA, 6m-PFOA, as well as linear PFOA were determined in river water samples.

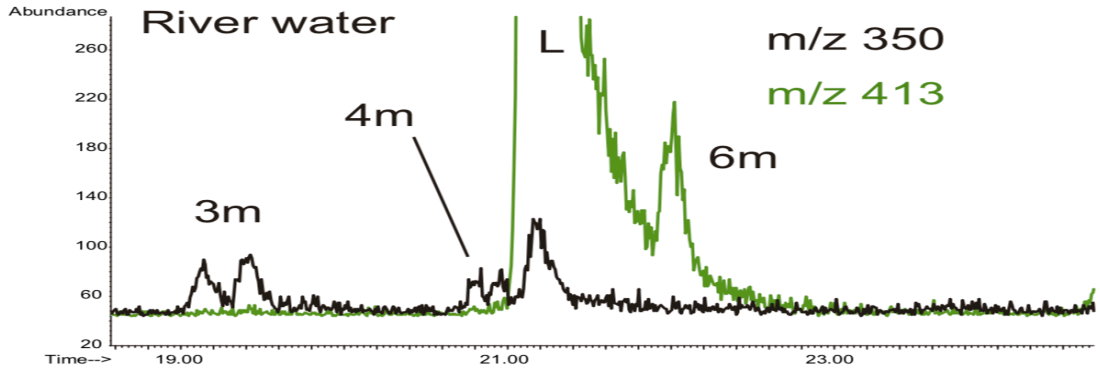

Fig.2 Chromatogram of the river water sample

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