

# PREVALENCE OF ISO-FORMS OF PERFLUORINATED CARBOXYLATES IN JAPANESE ENVIRONMENT

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## Introduction

Perfluorinated surfactants (termed “perfluorochemicals” or PFCs), such as PFOS (perfluorooctane sulfonate) or PFOA (perfluorooctanoate), are quite stable organic compounds with water and oil-repellant properties, and have been used extensively for variety of purposes, such as surface protection for textiles, carpets and packaging, surfactants for electroplating of metals and production of electronic devices and fluoropolymers, and active ingredients of fire-fighting foams. Due to the increase of toxicological information on these chemicals, however, their regulation started globally as well as in national levels; PFOS was listed in Annex B of the Stockholm Convention in 2009, and PFOA, its precursors and other perfluoroalkyl carboxylates with more than 8 carbons were the target of Stewardship Program (elimination until 2015) in US. Also their environmental monitoring has been conducted extensively in recent years.

During the analysis of PFCs in terrestrial environment of Japan by an LCMSMS technique, we frequently observed double peaks, i.e., appearance of a second peak which precedes the peak corresponding to the linear perfluoroalkyl carboxylates, such as PFOA, PFNA and PFDA<sup>1,2)</sup>. Both peaks appeared in the same parent / daughter ions combination not only by a triple-Q LC/MSMS but also by an LC/Q-high resolution TOF, with differences in the relative peak intensities among daughter ions. Here we report the identification of these preceding peaks by high resolution time-of-flight mass spectrometry combined with HPLC separation.

## Experimental<sup>2)</sup>

Mixed standards of PFCs (MPFCA-MXA and PFCA-MXB) and isomers of PFOS, PFOA (P1~P6MHpS) and PFNA (ipPFNA) were obtained from Wellington Lab. Inc. Acetonitrile (HPLC grade) was purchased from Kanto Chemicals Co. Other chemicals were selected among commercial reagents by checking the contamination levels of PFCs carefully. LCMSMS and LC-QTOF analysis were conducted with 1200 series LC (Agilent) combined with either 4000 QTRAP (Applied Biosystems) or 6530 (Agilent), respectively. Operation conditions were summarized in the table below.

### Analytical Conditions

#### [LC]

- \* Agilent 1200 series
- \* Column Zorbax XDBC18 (2.1x20mm+2.1x150mm, 3.5 $\mu$ m)
- \* Gradient A: 10mM CH<sub>3</sub>COONH<sub>4</sub> / B: CH<sub>3</sub>CN  
B (%) 40 (0 min) - 100 (15 min) - 100 (20 min) 0.2mL/min, 40 C
- \* Injection 10  $\mu$ L

#### [Q-TOF]

- \* Agilent 6530 (JS)
- \* Polarity Negative
- \* Ionization ES (Jet Stream)
- \* Drying gas N<sub>2</sub> (10L/min: 300 C)
- \* MSMS PFOA Precursor m/z=413, CE = 5 eV
- \* Mass Range 70 ~ 600 (m/z)
- \* Fragmentor 50 V
- \* Nebulizer N<sub>2</sub> (50 psi)
- \* Sheath gas N<sub>2</sub> (12L/min: 400 C)

#### [MSMS (Q-pole type)]

- \* 4000 QTRAP (Applied Biosystems)
- \* Polarity Negative
- \* Ionization ES
- \* Ion source voltage -4500 V
- \* Source Temp 400 C
- \* Nebulizer gas 40 psi
- \* Turbo gas 80 psi

## Results and Discussion

An example of the presence of second peak in each perfluoroalkyl carboxylate in the real environmental sample in Japan is shown in Figure 1. The upper figure shows the chromatogram of all the monitored ions, the second line shows a selected chromatogram of PFOA (blue) and PFNA (red) and the bottom line shows a chromatogram of the isotope labeled internal standard of PFOA and PFNA. As clearly shown in the middle chromatogram, both PFOA and PFNA show a second peak preceding the peak corresponding to the linear isomer standards.

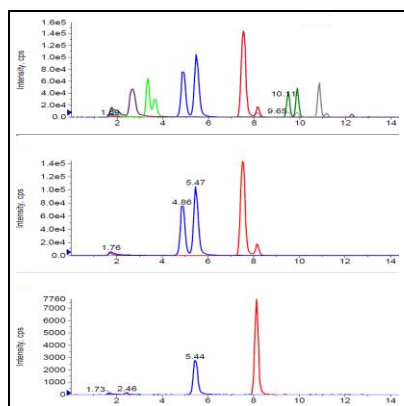


Figure 1 PFCs chromatogram of a river water

In Figure 2, the fragment pattern of the preceding peak of PFOA in the real water sample by LC-QTOF was compared with those of the commercial branched isomers. As shown in the figure, each of branched isomers of PFOA showed a characteristic fragment pattern based primarily on the structure. Typical dominant peaks include those corresponding to the [M-CO<sub>2</sub>H]<sup>-</sup> (m/z=368.98) the parent ion (412.97), C<sub>2</sub>F<sub>5</sub><sup>-</sup> fragment (118.99), C<sub>3</sub>F<sub>7</sub><sup>-</sup> fragment (168.99), and C<sub>4</sub>F<sub>9</sub><sup>-</sup> fragment (218.99). Apparently a molecule tends to brake in the middle of the linear structure (C<sub>3</sub>F<sub>7</sub> and C<sub>4</sub>F<sub>9</sub> for linear PFOA, and C<sub>2</sub>F<sub>5</sub> for 3-methyl-, 4-methyl-isomers) and also at the branched position (C<sub>3</sub>F<sub>7</sub> for 6-methyl-, and C<sub>4</sub>F<sub>9</sub> for 5-methyl-isomers) in the collision cell (Q2). The unknown peak in the lower second line shows the identical fragment pattern to the iso-form of PFOA (perfluoro-6-methylheptanoic acid) in the lowest line. This assignment was confirmed further by the LCMSMS analysis of a series of isomer standards and a sample.

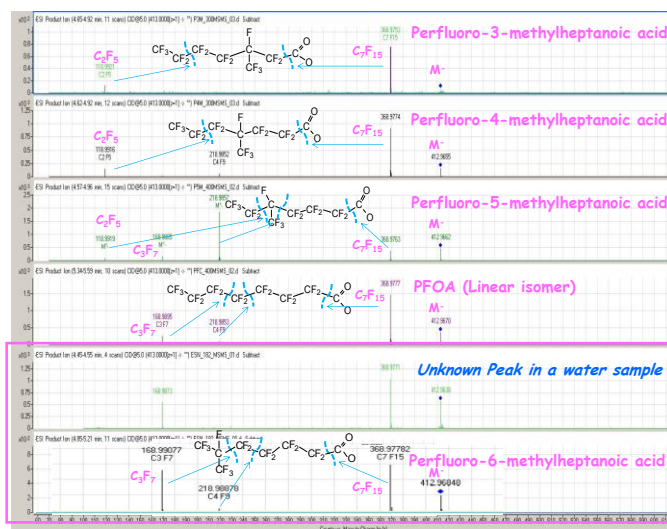


Figure 2 Fragment patterns of daughter ions of the observed unknown peak and various isomers of PFOA

Figure 3 shows the fragment pattern of both the preceding peak (upper) and the linear isomer (lower) of PFNA, PFDA and PFuDA, respectively in the real sample by LC-QTOF. It is clear from the figure that each of the preceding peak of three PFCs contains fairly strong fragment peak corresponding to  $C_3F_7^-$  structure commonly, while the other peaks corresponding to the linear isomers show only a trace peak in the position. This can be reasonably interpreted by the hypothesis that each of the preceding compounds contains perfluoro-isopropyl ( $(CF_3)_2-CF-$ ) structure at the terminal commonly (Figure 4).

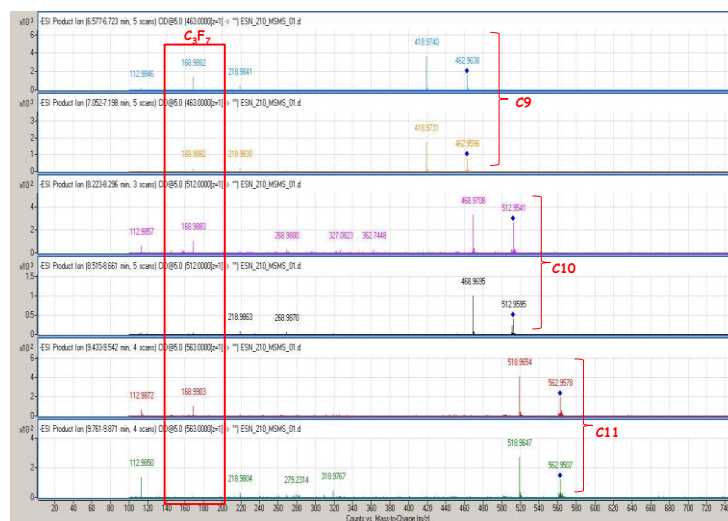


Figure 3 Fragment patterns of peaks corresponding to PFNA (C9) ~ PFuDA (C11) in a real water sample. In each group, lower: corresponding to linear isomer, upper: unknown preceding isomer (peak at 112.99 is a reference for accurate mass measurement)

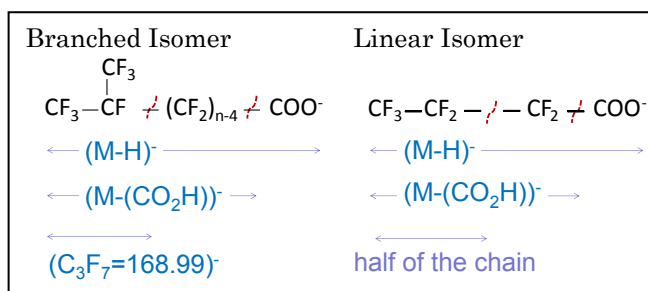


Figure 4 Estimated major fragmentation patterns of linear and branched isomers

In fact, LC-TOF (Agilent 1100+6240) analysis of linear and iso-form (perfluoro-7-methyloctanoic acid) of PFNA standards showed basically the same fragment patterns to the above result (Figure 5).

As shown in the present study, a branched isomer with perfluoro-isopropyl structure at the far end of the perfluoroalkyl chain from the carboxyl group (iso-form) were detected not only in PFOA but also in many other perfluoroalkyl carboxylates. Similar characteristic patterns, i.e., presence of an additional peak preceding the linear isomer position, were observed in many environmental samples, particularly in terrestrial environment, in Japan, indicating that iso-forms of perfluoroalkyl carboxylates are prevailing in Japanese environment. It should also be pointed out that iso-form / linear peak ratios tend to be higher in perfluoroalkyl carboxylates with odd-numbered carbon atoms than those with even-numbered ones, as typically shown in Figure 1.

There are reported two different synthetic route of PFOA, i.e., electrochemical fluorination and telomerization. The former process produces a mixture of branched isomers in addition to the linear isomer. On the other hand, the latter process is expected to produce linear isomer dominantly with trace impurities of different chain length (typically two carbon differences). This idea has been the basis of source identification / apportionment of PFOA in the environment. In Japanese environment, however, the presence of two isomer peaks in each compound has been reported occasionally.<sup>1, 2, 3)</sup> In fact, synthetic method of branched isomer containing perfluoro-isopropyl moiety was patented by a Japanese company<sup>5)</sup> and use of mixed perfluoroalkyl carboxylates containing both linear and iso-form was described in the literature.<sup>6)</sup> Based on the monitoring data, it seems that the iso-forms-containing commercial perfluoroalkyl carboxylates or their derivatives have been produced and used in considerable amounts in Japan. Further research will be needed to clarify the production amount and major usage of iso-forms or mixed PFCs, to reveal their levels in the environment not only in Japan but also in other countries, and to assess their potential risks to the wildlife as well as to human beings.

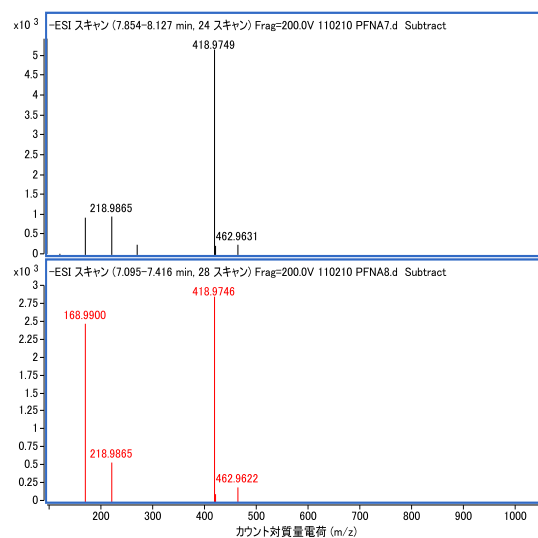


Figure 5 Fragment patterns of linear (upper) and iso-form (lower) of PFNA standards by LC-TOF

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