Suggestion on a Procedure to Identify Non-targeted Per – and Polyfluoroalkyl Substances (PFASs) Based on Fragmentation Flagging by Liquid Chromatography-Ion Mobility-Quadrupole-Time of Flight Mass Spectrometry (LC/IM-QTOF)

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Introduction

Per- and polyfluoroalkyl substances (PFASs) are a group of anthropogenic organic fluorinated compounds. Recently, PFASs have been a growing concern about adverse effects on water environment and human health. However, it is difficult to understand the occurrence of unknown PFASs because a lot of various type of PFASs have been used and discharged in the environment. It has been needed to develop a new quick and simple method to understand how many unknown PFASs exist in environmental samples. In previous study, non-target analyses of PFASs based on insource fragmentation flagging in which fluoroalkyl group was recognized as common fragment ions was suggested to utilize for estimation of chemical formula[1,2]. Next, it is considered to be important to develop a procedure for non-target analyses by fragmentation flagging. To identify a specific precursor ion, an additional selection technique is necessary, as fragmentation flagging cannot refine the candidate ions enough. Main objective of this study is to suggest a procedure for identification of non-targeted PFASs based on fragmentation flagging by Liquid Chromatography-Ion Mobility-Quadrupole-Time of Flight Mass Spectrometry (LC/IM-QTOF).

Materials and methods

Research overview was shown in **Fig.1**. In this study, two approaches were conducted to identify suspected PFASs peaks by fragmentation flagging. First of all, Targeted MSMS analyses were conducted for standard chemicals of 44 PFASs by using LC/IM-QTOF (Agilent) in order to create a database on fragment ions of PFASs. 12 PFCAs, 3 perfluoroalkyl sulfonic acids (PFSAs), 5 polyfluoroalkyl phosphate esters (PAPs), 6 fluorotelomer alcohols (FTOHs), 3 fluorotelomer sulfonic acids (FTSs), 5 fluorotelomer carboxylic acids (FTCAs), 3 fluorotelomer unsaturated carboxylic acids (FTUCAs), 3 perfluorooctanesulfonamide (FOSAs), 2 Perfluorooctanesulfonamidoacetic acids (FOSAAs), 2 perfluorooctanesulfonamide ethanol (FOSEs) were targeted in this study. Target analyses were conducted with 4 different collision energies (CE), namely, 0 V, 10 V, 20 V and 40 V by jet-stream ionization negative mode. In addition, previous literatures[3-8] were utilized to add more PFASs fragment ions to the database. Fragmentation flags were selected from fragment ions in the

Fig.1 Research overview
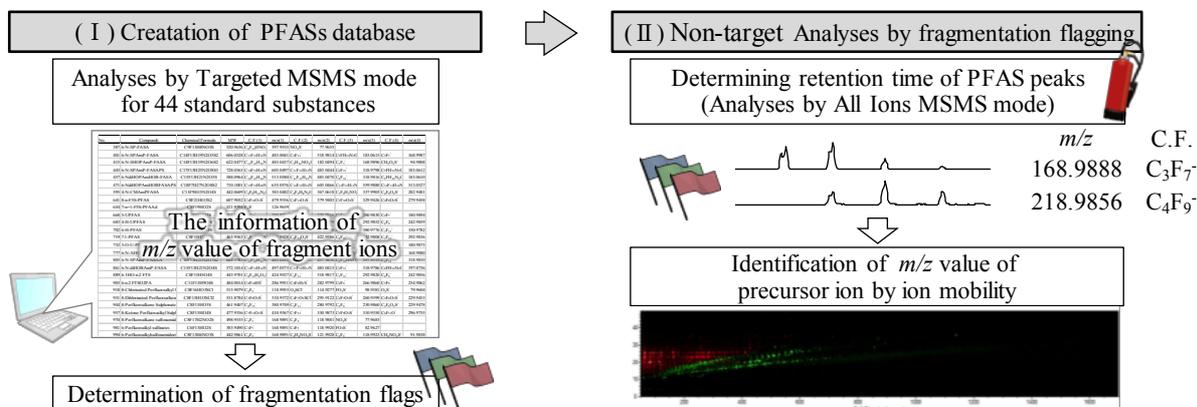
database. Secondly, non-target analyses were conducted by applying fragmentation flagging approach to an acquired total ion chromatogram of Aqueous Film-Forming Foam (AFFF) sample. The TIC was acquired in the range between 50-1700 m/z by All Ions MSMS analyses by using LC/IM-QTOF. Extracted ion chromatograms (EIC) were acquired at m/z value of fragmentation flags in the range of $\pm$ 0.01 Da. The EIC data was used to determine RT of the peaks which were suspected as PFASs. Detailed procedure is discussed in results and discussion (Ⅱ). Finally, fragmentation flags found in the RT range of $\pm$ 0.01 minutes were grouped as a flag set. Derived m/z value of precursor ions by determining each flag set was identified utilizing separation with drift time by ion mobility.

Results and discussion:

(Ⅰ) *Development of PFASs database*

PFASs database and determination of fragmentation flags were shown in **Fig.2**. The numbers of total PFASs in database were 1,301 basically C4-16 polyfluoroalkyl substances, and they were classified 82 groups. In this study, 31 kinds of the peaks of PFASs (12 PFCAs, 3 PFSAs,

Fig.2 PFASs databse and Determination of fragmentation flags

3 diPAPs, 3 FTSs, 2 FTCAs, 3 FTUCAs, 3 FOSAs, 2 FOSAAs) were observed by Targeted MSMS analyses and added to the database. The other information of m/z value of fragment ions for 42 PFASs were updated to the database according to previous literatures[3-8]. As a result, 73 PFASs which have m/z value of fragment ions were archived. Fragmentation flags were determined such as $C_2F_5^-$, $C_3F_5^-$, $C_6F_9^-$, $C_2F_5O^-$, $CF_2O_3S^-$, O_3S^-.

(Ⅱ) *Non-target analyses by fragmentation flagging*

Procedure of non-target PFASs analyses by fragmentation flagging for an AFFF sample was shown in **Fig.3**. In this study, fragmentation flags were categorized into four types, (*1*) 120 types of $C_nF_m^-$, (*2*) 121 types of $C_nHF_m^-$, (*3*) 123 types of $C_nF_mO^-$ and (*4*) 130 types of $C_nF_mO_3S^-$. It was possible to add a new category (*N*), if there is needed to add more fragmentation flags having C and F. In addition, others (not including C nor F, for example, O_2S^-) were categorized to (*X*). It was used to estimate chemical formula. In (*1*) RTs of the peaks which were detected at more than an arbitrary value of abundance (in this case 1×10^4) were extracted. They were named as t_1, t_2, t_3, t_4, t_5, t_6, t_7, t_8, t_9. When more than two fragmentation flags were found at a specific RT, they might be fragment ions from the same precursor ion. Similarly, t_{10}, t_{11}, t_{12}, t_{13}, t_{14}, t_{15} were extracted in (*2*), (*3*), (*4*) and t_{16}, t_{17}, t_{18}, t_{19} were extracted in (*X*). In the next steps, t_x with same RT was regrouped to T_y such as $T_1=t_1$, $T_2=t_2=t_{10}=t_{16}$, $T_3=t_3=t_{11}=t_{17}$, $T_4=t_4=t_{12}=t_{18}$, $T_5=t_5=t_{19}$, $T_6=t_6=t_{13}$, $T_7=t_7=t_{14}$, $T_8=t_8$ and $T_9=t_9=t_{15}$. At this point, T_y derived from only category (*X*) was ignored in further steps as they do not pose a possibility of fluoroalkyl group. 9 Flag sets were determined at 9 RTs, namely, T_1=3.881 min. (Flag set$_1$), T_2=4.448 min. (Flag set$_2$), T_3=4.454 min. (Flag set$_3$), T_4=5.017 min. (Flag set$_4$), T_5=5.367 min. (Flag set$_5$), T_6=6.629 min. (Flag set$_6$), T_7=7.186 min. (Flag set$_7$), T_8=8.922 min. (Flag set$_8$) and T_9=9.090 min. (Flag set$_9$). As an example, Flag set$_3$ was discussed as follows. There were 5 fragmentation flags in Flag set$_3$ in (*1*) : m/z =254.9856 ($C_7F_9^-$), m/z =266.9869 ($C_8F_9^-$), m/z =280.9824 ($C_6F_{11}^-$), m/z =304.9824 ($C_8F_{11}^-$), m/z =411.97844 ($C_9F_{16}^-$). They were suspected as fragment ions of PFASs. Next, RTs indicated by fragmentation flag sets were approached to find a precursor ion of a PFAS.

m/z value of precursor ions needed to be acquired from low fragmentation at CE 0 V. However, m/z value of precursor ions is difficult to identify from the mass spectrum acquired from low fragmentation at CE 0 V because candidate ions remains a lot. In this study, further selection was performed by drift time of ion mobility. Identification of m/z value of precursor ion by ion mobility was shown in **Fig.4**. The signals of low fragmentation (CE 0 V) were in green. The signals of high fragmentation (CE 40 V) were in red. The signals of substances were separated by drift time based on their collision cross section.
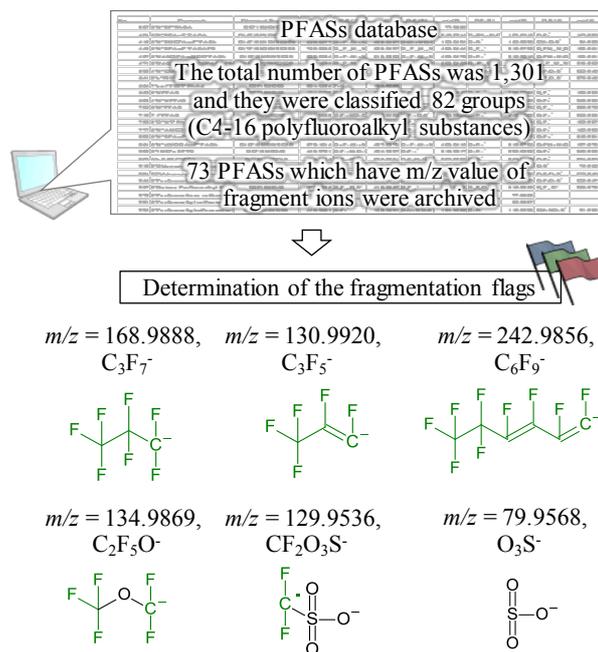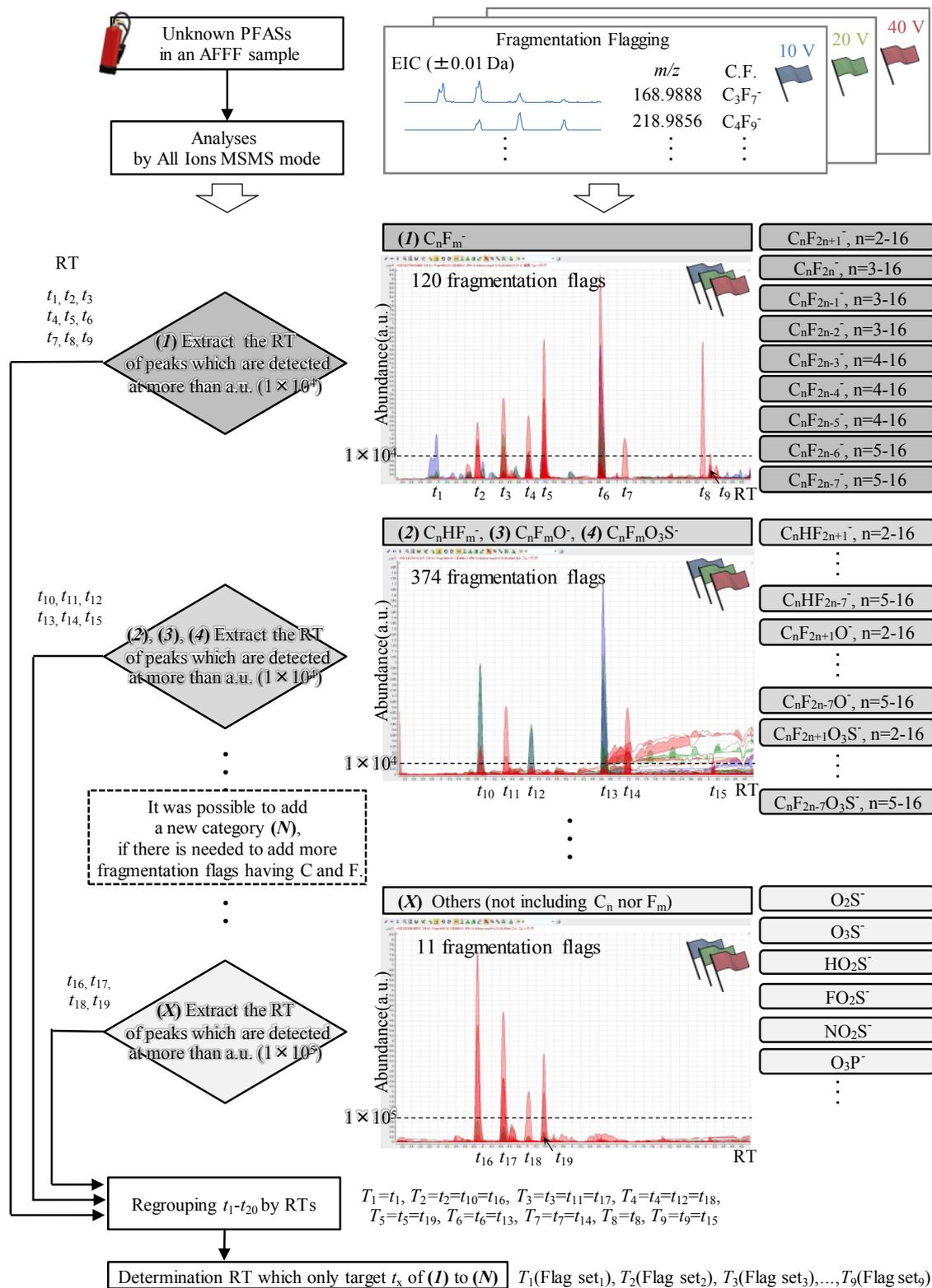
Fig.3 Procedure of non-target PFASs analyses by fragmentation flagging for an AFFF sample
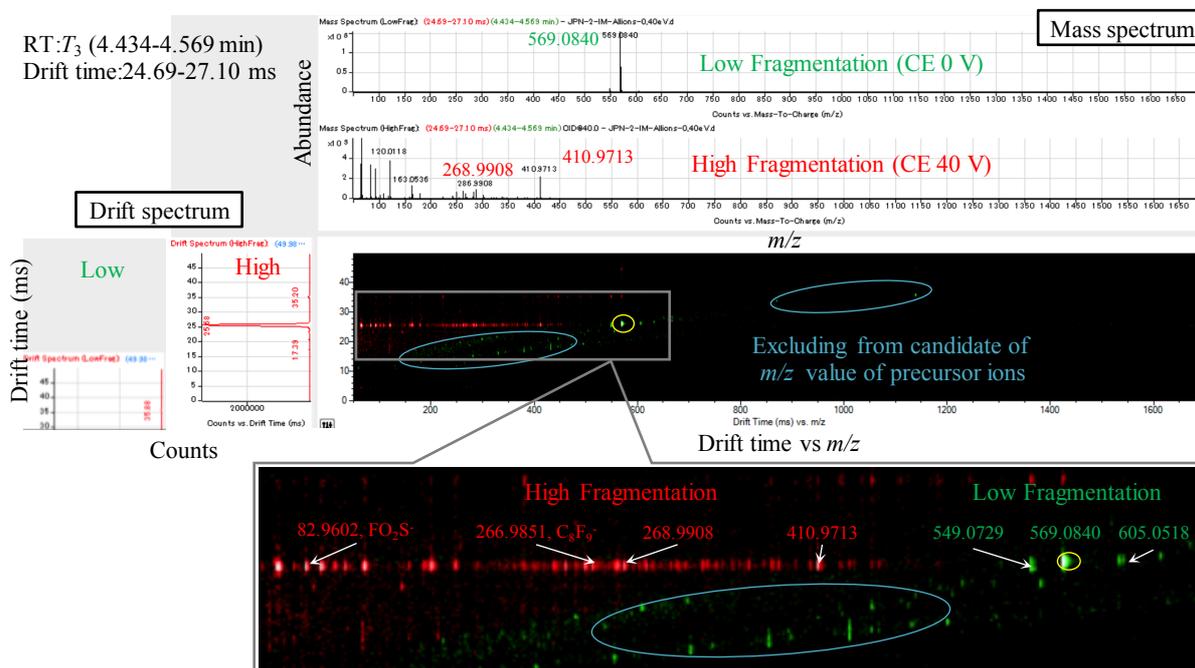
Fig.4 Identification of m/z value of precursor ion by Ion mobility (Flag set$_3$)

As an example, a precursor ion identification was demonstrated by using Flag set$_3$ as follows. As a result of high fragmentation, there were many obvious signals indicated at drift time 24.69-27.10 ms. Among them, signals at m/z =82.9602 (FO$_2$S$^-$), 266.9851(C$_8$F$_9^-$) matched with corresponding fragmentation flags in the database. Thus, this drift time range was applied to find signals from the result of low fragmentation. Consequently, the obvious signals were found at m/z =549.0729, 569.0840, 605.0518. In addition, high aboundance of mass spectrum was observed at m/z =569.0840, it was identified as precursor ion of Flag set$_3$. The same procedure were applied to other Flag sets, namely, m/z =312.9700 (Flag set$_1$), m/z =426.9753 (Flag set$_2$), m/z =458.9535 (Flag set$_4$), m/z =511.0732 (Flag set$_1$), m/z =425.9811 (Flag set$_6$), m/z =835.9561 (Flag set$_7$), m/z = 907.0222 (Flag set$_8$), m/z = 771.9876 (Flag set$_9$). It is needed to estimate the chemical formula and structure for next steps.

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