[Note]

Development and Application of Simultaneous Analytical Method for Perfluorinated Compounds in Waste Samples by LC/MS/MS

Mihoko YOSHIDA[1], Shusuke TAKEMINE[2] and Chisato MATSUMURA[2]

[1] Water Environment Division, Hyogo Prefectural Institute of Environmental Sciences,
3-1-27, Yukihira-cho, Suma-ku, Kobe, Hyogo 654-0037, Japan, [2] Environmental Safety Division,

LC/MS/MS   (PFCs)

[1]   [2]   [2]

654-0037   3-1-27   [2]

The investigation was carried out to establish the simultaneous and high sensitive analysis for the perfluorinated compounds (PFCs) in waste sample.

In pretreatment of the waste, the extraction efficiency of three techniques; accelerated solvent extractor (ASE), soxhlet extraction and ultrasonic extraction were compared and examined. Operation blank test, recovery test spiked with mass labeled PFCs (MPFCs) and content examination in waste sample were experimented for 14 kinds PFCs.

As for the pretreatment method, the ultrasonic extraction method which had shown good results both in the operation blank and isotope spiked recovery tests were adopted. The PFCs in waste were extracted with methanol and separated as well as concentrated with nitrogen-gas and analyzed by LC/MS/MS determination. And the waste was used in solid form. PFCs were detected at retention time between 3.75~7.15 min by the technique which was established in this study. In addition, the chromatogram of waste sample shows that PFCs and isomer were detected at almost the same time without interference. Since the existence ratios of PFCs in various wastes are different, by establishing profile of each waste at different sources, this study will contribute to developing countermeasures that would prevent environmental contamination of PFCs at global level.

INTRODUCTION

The fluorochemical compounds such as perfluorooctanoate(PFOA) and perfluorooctane sulfonate(PFOS) are stain and water repellant. It is these superior properties that are widely applied to various fields in the modern society for pursuing comfortable and convenient life styles.

On the other hand, negative properties of PFCs such as hazardous, persistent and bioaccumulative properties have been concerned[1]. Thus, in May 9th, 2009, the 4th Conference of the Parties (COP4) for the Stockholm Convention on Persistent Organic Pollutants adopted perfluorooctane sulfonate and perfluorooctane sulfonyl fluoride (PFOS and PFOSF) in Annex B. In addition, these PFOS and PFOSF were designated as a class I specified chemical substances under the Law Concerning the Examination and

Regulation of Manufacture, etc., of Chemical Substances in Japan, and were subject to global regulation. Since the further production and use of these compounds will be strictly controlled, the widespread of the compounds into the environment would be reduced. However, PFCs products have still been widely stocked and used as well as disposed and discharged into our living environment. It is highly likely that the compounds will continue to present ubiquitously in whole society through the various environmental routes and media.

In recent years, many surveys and risk assessment have been carried out with regard to environmental contamination of PFCs in various media including atmosphere and water[2)-9)]. However, little information has been available with regard to the waste generated as a result of anthropogenic activities and transported to waste treatment facilities. PFCs are detected in high concentrations from leachate at some final disposal sites[10) 11)]. Thus, the investigation of PFCs in waste will be an important information source to develop countermeasures for PFCs pollution. In this study, sensitive method by using LC/MS/MS for the determination of PFCs in waste samples was developed and presented.

MATERIALS AND METHODS

1. Target PFCs, Reagents and Reagent preparation

The target substances of the development of our analytical method are 14 compounds. The target PFCs and octanol-water partition coefficient (log K_{ow}) are shown in Table 1. The target PFCs in this study were perfluorocarboxylic acids (PFCAs):10, perfluoro alkyl sulfonates (PFASs): 4 substances and mass-labeled PFCs (MPFCs) were mass-labeled PFCAs (MPFCAs):7, mass-labeled PFASs (MPFASs):2 for surrogate compounds. The former target PFCs used PFAC-MXB as standard substances, and the latter MPFCs used MPFAC-MXA as internal standard substances, these were all commercially available products. The both mixture solutions were manufactured by Wellington Laboratories Inc. The PFOA-$^{13}C_8$ used as internal standard was the product of Cambridge Isotope Laboratories. All compounds were linear chains. Methanol was originally for residual agrochemical analysis use obtained from Wako Junyaku Kogyo (Wako Pure Chemical Industries, Ltd.). No interference of the reagents with the measurement of substances was confirmed. The 100ug/L-standard solution for the calibration curve was prepared with 2000ug/L-PFAC-MXB in methanol.

Table 1　Target compounds (PFCs) and octanol-water partition coefficient (log K_{ow})

	compound	abbreviation	carbon number	structual formula	molecular weight	log K_{ow}
PFCAs	Perfluoropentanoic acid	PFPeA	5	CF3(CF2)3COOH	264.0	n.a.
	Perfluorohexanoic acid	PFHxA	6	CF3(CF2)4COOH	314.1	3.26
	Perfluoroheptanoic acid	PFHpA	7	CF3(CF2)5COOH	364.1	3.82
	Perfluorooctanoic acid	PFOA	8	CF3(CF2)6COOH	414.1	4.30
	Perfluorononanoic acid	PFNA	9	CF3(CF2)7COOH	464.1	4.84
	Perfluorodecanoic acid	PFDA	10	CF3(CF2)8COOH	514.1	5.30
	Perfluoroundecanoic acid	PFUnDA	11	CF3(CF2)9COOH	564.1	5.76
	Perfluorododecanoic acid	PFDoDA	12	CF3(CF2)10COOH	614.1	6.41
	Perfluorotridecanoic acid	PFTrDA	13	CF3(CF2)11COOH	664.1	n.a.
	Perfluorotetradecanoic acid	PFTeDA	14	CF3(CF2)12COOH	714.1	n.a.
PFASs	Perfluorobutane sulfonate	PFBS	4	CF3(CF2)3SO3H	300.1	n.a.
	Perfluorohexane sulfonate	PFHxS	6	CF3(CF2)5SO3H	400.1	n.a.
	Perfluorooctane sulfonate	PFOS	8	CF3(CF2)7SO3H	500.1	5.25
	Perfluorodecane sulfonate	PFDS	10	CF3(CF)9SO3H	600.1	n.a.

*n.a.: not available

Table 2   Characterization of waste samples

Sample No.	1	2	3	4	5	6
water content ratio (%)	37.0	11.1	34.4	0.9	52.3	57.7
pH	6.8	8.0	7.6	7.9	7.4	6.8
appearance	dewatered sludge	sludge	automobile shredder residue	shredded solid residue	sludge	sludge
color	black	white	black	gray	brown	white

2. Waste samples

The details of the characteristics of the waste samples such as water content, pH, and appearance are shown in Table2. The waste samples are dewatered sludge, automobile shredded residues (ASR), incombustible general solid wastes generated from effluent treatment facilities and incinerating process.

3. Equipment and instrumentation, LC/MS/MS analytical condition

The solid phase extract cartridge adopted Precep-C agri (short) (Wako Pure Chemical Industries, Ltd.). The target measurement substances were passed through the cartridge and adsorbed to the cartridge using a Sep-Pak Concentrator System Controller Plus (Waters) by 10 mL/min. The extraction solution was concentrated in TurboVap LV (Zymark) with nitrogen. Analytical conditions of LC/MS/MS are shown in Table 3. As for the ultrasonic generator, ULTRA SONIC AUTOMATIC WASHER (AS ONE Corporation) was used. A model 05PR-22 (Hitachi) was used as the centrifugal separator. DIONEX ASE-200 (DIONEX) was selected for the Accelerated Solvent Extractor (ASE). The pulverized wastes were prepared by vibrating sample mill (TI-100, HEIKO Co.), and sieved with 0.5mm-mesh after drying overnight at 50 degrees Celsius.

4. Development of PFCs analysis technique in waste and Examination of the preprocessing method

The following three extraction methods were compared and discussed for sample pretreatment: ASE, soxhlet extraction in Methanol for 12hours and ultrasonic extraction with Methanol. In each procedure, the operation blank test without interference from the waste and recovery test using real waste samples spiked with 1ng MPFCs were carried out at the same time. In preparing samples, 1ng of MPFCs was spiked to the waste sample as surrogate.

The sample solutions extracted by ASE or soxhlet extraction were concentrated to about 1ml with nitrogen injection. The PFCs adsorbed on the cartridge were then eluted with 2mL methanol. The eluent was concentrated to about 1mL using the TurboVap LV. The pretreated solution extracted by ultrasonic extraction was measured up to 10ml and TurboVap LV. The pretreated solution extracted by ultrasonic extraction was measured up to 10ml and 1mL aliquot of the 10ml extract was taken for determination. Then 1ng of PFOA-^{13}C$_8$ standard solution was spiked as internal standard to the obtained extract, as described above. And 5μL was injected into the LC/MS/MS for determination.

For the measurement of minimum detection limit (MDL), sample6 which contains little or no PFCs was taken. The waste was spiked with standard mixture methanol solution and adjusted to the final concentration of 2ng/g. The recovery test was repeated for 7 times and the results were used for calculating MDL by using the following equation.

$$MDL = 2 \times s \times t(n\text{-}1,0.05)$$

s : standard deviation

t(n-1,0.05) : t value of n-1 degree of freedom, level of significance 5%

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Table 3　　Analytical condition for PFCs with LC/MS/MS

LC condition

	Instrument	ACQUITY UPLC	(waters)
	Column	UPLC BEH C18	2.1×50mm
	Retention gap Column	UPLC BEH C18	2.1×100mm
	Mobile Phase	A : 10mM Ammonium Acetate aq　B : Acetonitrile	
	Gradient	0.0　　8.0 min	B : 1　　95%
		8.0　　8.1 min	B : 95　　1%
	Flow rate	0.3 mL / min	
	Column temp.	50	
	Injection volume	5μL	

MSMS condition

	Instrument	ACQUITY TQD	(waters)	
	Ionization Mode	ESI (-)	Source temp.	120
	Desolvation temp	300	Cappillary voltage	2kV
	Cone gas flow	20 L / hr	Desolvation gas flow	800 L / hr
	Collision gas flow	0.1 mL / min		

	Compound name		Precursor io	Product ion Qnt.	Idt. 1	Idt. 2	Cone Voltage	Collision Energy
PFCAs	PFPeA		263.00	219.00			18	9
	PFHxA		313.00	269.00	118.90		18	9
	PFHpA		363.00	318.90	169.00		18	11
	PFOA		413.00	368.90	169.00		18	11
	PFNA		463.00	418.90	169.00		18	13
	PFDA		513.00	468.90	219.00		21	13
	PFUdDA		563.00	518.80	269.00		18	15
	PFDoDA		613.00	568.90	168.90		24	15
	PFTrDA		663.00	618.80	169.00		24	17
	PFTeDA		713.00	668.70	169.00		24	15
MPFCAs	PFBA	$-{}^{13}C_4$	217.00	172.00			15	13
	PFHxA	$-{}^{13}C_2$	315.00	270.00			15	11
	PFOA	$-{}^{13}C_4$	417.00	371.90			27	11
	PFNA	$-{}^{13}C_5$	467.00	422.90			24	15
	PFDA	$-{}^{13}C_2$	515.00	469.80	219.00		21	14
	PFUdDA	$-{}^{13}C_2$	565.00	519.80	269.60		21	17
	PFDoDA	$-{}^{13}C_2$	615.00	569.90	269.50		24	14
PFASs	PFBS		299.00	79.90	98.90		51	37
	PFHxS		399.00	79.90	98.90	130.00	57	46
	PFOS		499.00	79.90	98.90	130.00	69	55
	PFDS		599.00	79.90	98.90	79.90	80	67
MPFASs	PFHxS	$-{}^{18}O_2$	403.00	83.90	103.00		55	47
	PFOS	$-{}^{13}C_4$	503.00	79.90	99.00		65	56

RESULTS AND DISCUSSION

1. Result of the pretreatment procedure

The results of operation blank by using the 3 methods are shown in Fig.1. High concentrations of PFHxA and PFHpA in PFCAs that exceeded 7ng/g-wet were detected by the ASE and soxhlet extraction. The result confirmed cross contamination in the operation process. The concentrations of the other PFCs and those of the PFCs extracted by ultrasonic extraction method were under 1ng/g-wet. The contamination level of PFCs was not as significant as to affect the analysis seriously. The result of the recovery test of the samples from 1 to 4 spiked with 1ng MPFCs is shown in Fig.2. The 3 color bar charts show the average recovery rate. The recovery rate of the

MPFHxA and MPFDoA by the ASE, soxhlet extraction, as well as MPFHxS by the ASE were under 60%, while the rest of the recovery rates show no significant differences among the extraction methods. Despite the use of wastes in various forms nd properties as target samples, the recovery rates of the samples from 1 to 4 were not significantly affected by the extraction methods used in the study. It is, therefore, concluded that extraction can be performed by either technique without a problem.
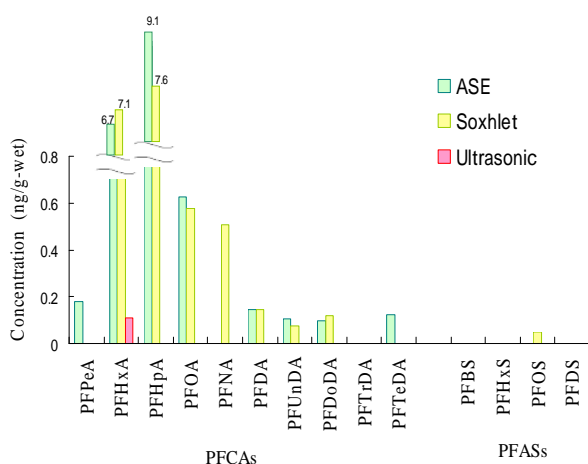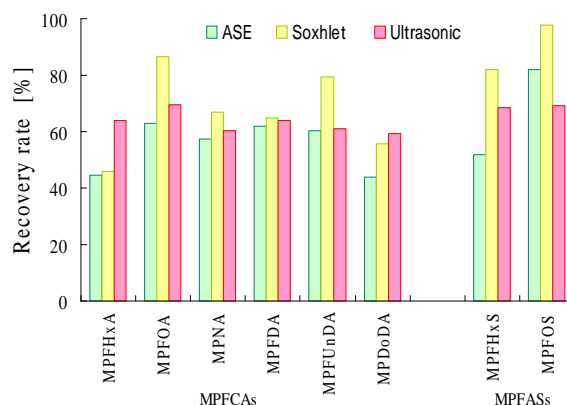
Fig.2 Recovery rate with MPFCs

2. Content examination in the waste sample

The results of content in waste sample1-4 with every method to compare extraction efficiency show in Fig.3.

The PFCs content were indicated to be deducted with the content obtained by the operation blank. In ultrasonic extraction method, each sample were analyzed repeatedly 3 times and showed the range of concentration (black bar line), the average (red bar charts). PFCs were detected from all waste samples.

As an example of PFCs analysis, the PFCAs chromatograms of the standard solution (20ng/L) and the sample1 are shown in Fig.4, and the PFASs chromatograms are shown in Fig.5, respectively. In the chromatogram of standard solution (left), PFCAs was detected between 3.75~7.15 min. The chromatograms of waste sample (right) show that PFCAs and isomer were detected almost at the same time without interferences. And the minimum detection limit values of PFCs obtained by ultrasonic extraction method were 1.4 - 3.9ng/g.

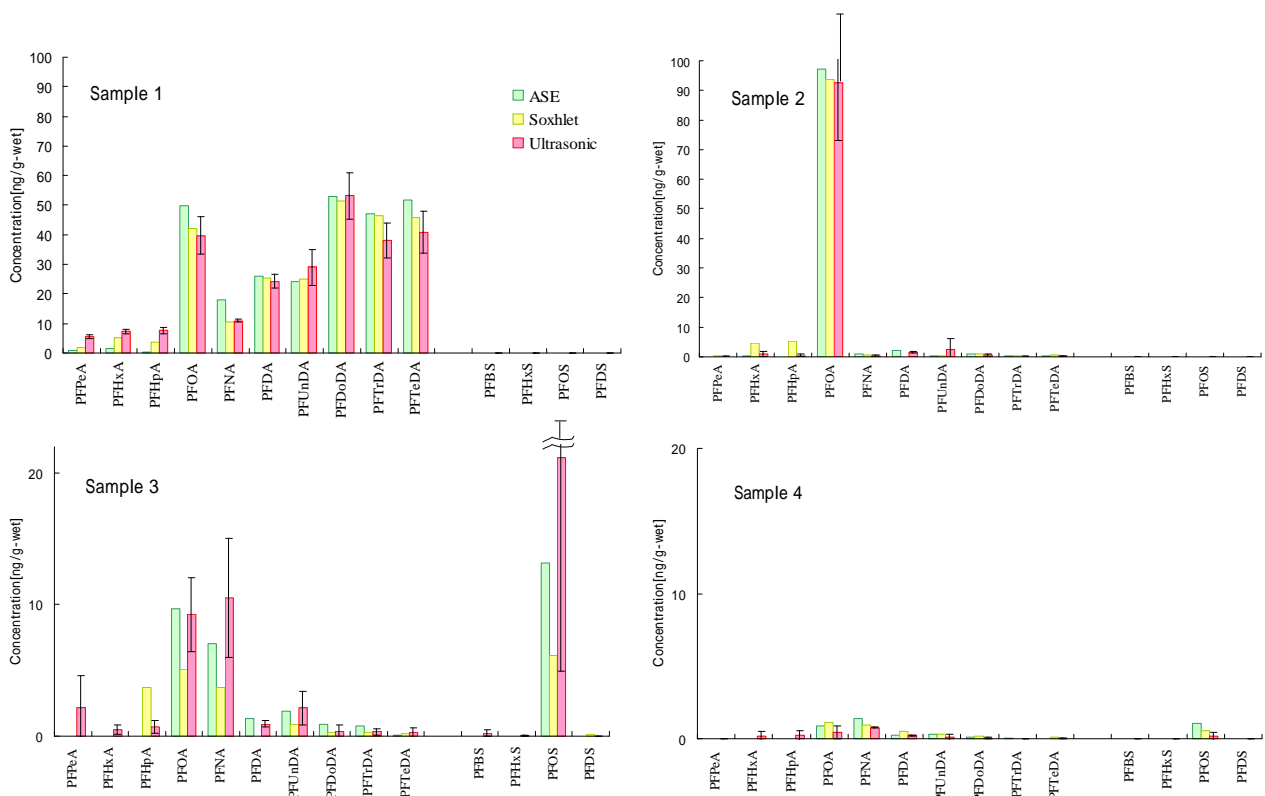Fig.1 PFCs concentration of operational blank

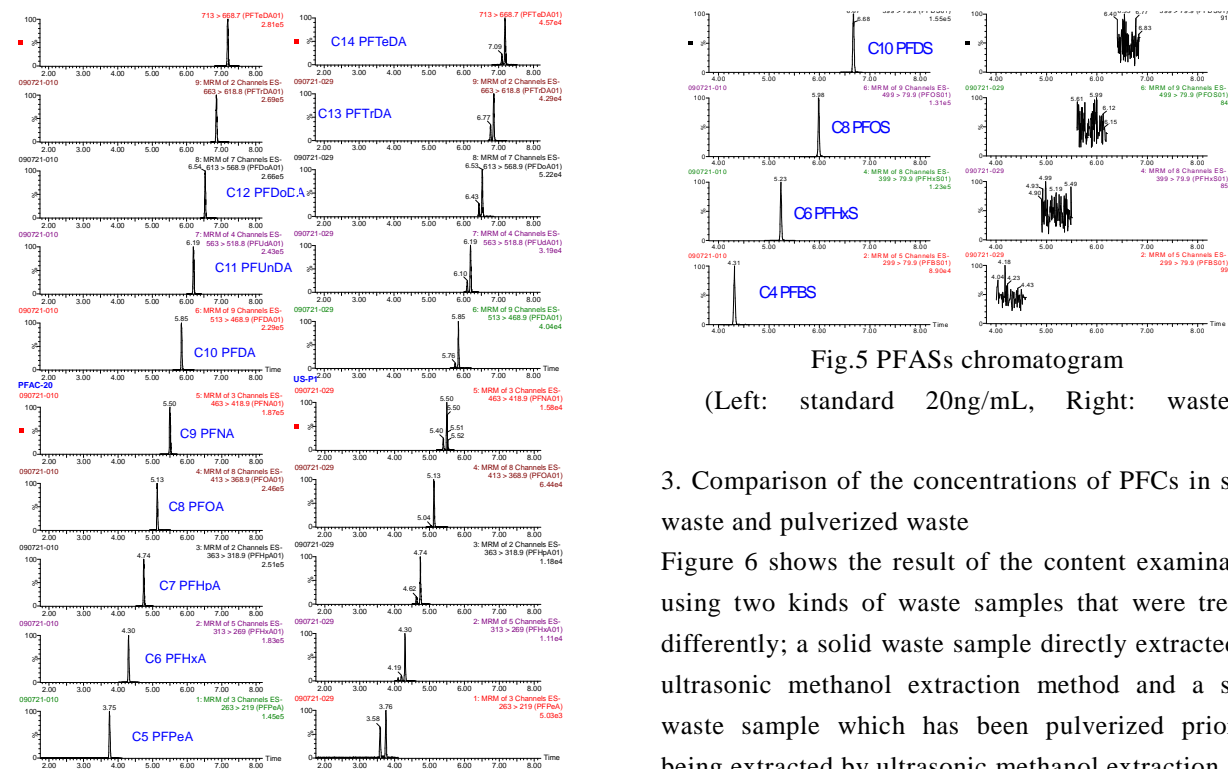Fig.3 PFCs concentration in waste sample comparing extraction between different 3 methods

Fig.4 PFCAs chromatogram
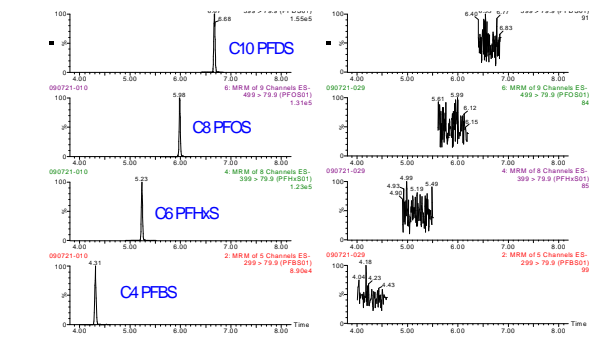(Left: standard 20ng/mL, Right: waste sample 1)

Fig.5 PFASs chromatogram
(Left: standard 20ng/mL, Right: waste

3. Comparison of the concentrations of PFCs in solid waste and pulverized waste

Figure 6 shows the result of the content examination using two kinds of waste samples that were treated differently; a solid waste sample directly extracted by ultrasonic methanol extraction method and a solid waste sample which has been pulverized prior to being extracted by ultrasonic methanol extraction method, as an example, sample1 and sample3. The concentrations of PFCs in the solid sample and the pulverized sample were nearly the same for waste sample1 (dewatered sludge). On the other hand, the waste sample3 (ASR) shows higher concentrations of PFCs in the solid state than the pulverized one.
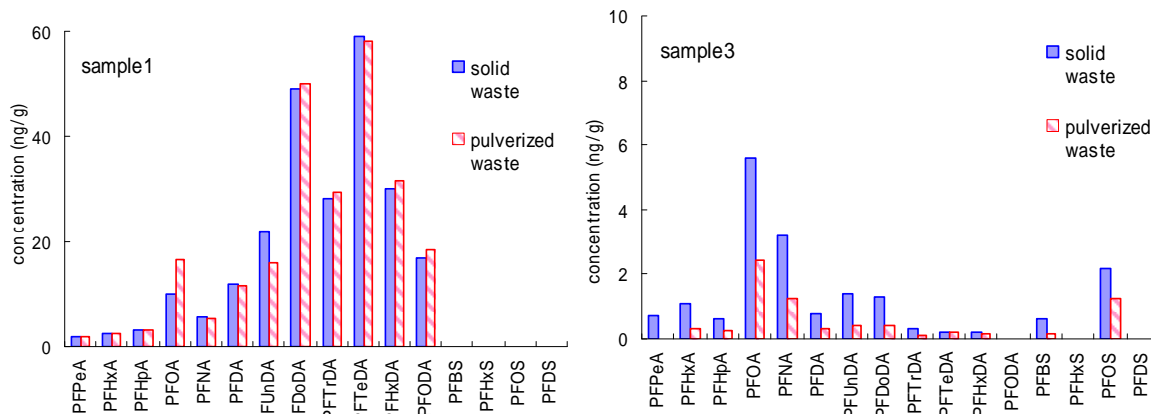
Fig.6 Comparison solid waste with pulverized waste

CONCLUSIONS

The analysis method for PFCs in waste samples was established.

Ultrasonic extraction method was employed, which gave good results as a pretreatment method, for both operation blank and isotope recovery tests. These analytical procedures are composed by methanol extraction, centrifugation, concentration with nitrogen-gas and determination by LC/MS/MS. And the waste was used in solid form.

PFCs were detected at retention time between 3.75~7.15 min without interferences.

MDL was 1.4 - 3.9ng/g. It was confirmed that this method was applicable for PFCs analysis in waste samples. As the result, the difference existence ratios of PFCs in various wastes was shown, establishing profile of each waste at different sources became possible.

The result indicates that the existence ratios of PFCs in various wastes are different, which leads to the possible establishment of a profile of each waste generated from different sources.

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PFC

14   PFCs

PFCs

LC/MS/MS

3.75   7.15

PFCs