

Analysis of PFASs in Sludge Fertilizers

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Key words PFASs, PFOS, PFOA, sludge, LC-MS/MS

We improved the PFOS and PFOA measurement methods to develop determination method of 16 PFASs in sludge fertilizers using liquid chromatograph/tandem mass spectrometer (LC-MS/MS). After the analytical portion was added the isotope performance extraction standard solution (20 ng/mL each of mass labeled PFOS and mass labeled PFOA), PFASs were extracted with methanol containing a small amount of formic acid. The extract was purified with weak anion exchange polymer cartridge column and graphite carbon cartridge column. PFASs were measured by LC-MS/MS. As a result of 3 replicate analysis in sludge fertilizer sample prepared to contain with PFASs at 1 µg/kg, 5 µg/kg and 50 µg/kg, the mean recoveries ranged from 62.4 % to 124.8 %. Their lower limit of quantification (*LOQ*) ranged from 0.5 µg/kg to 1 µg/kg (analytical sample). The following PFASs branched isomer peaks appeared on the chromatogram of the sample solution, which were PFHxS, PFOS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA and PFDoA. The proportions of the linear forms of PFOS and PFOA to the total amount including those of the branched form were the mean values of 83 % and 97 %, respectively.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a diverse group of human-made chemicals that include the perfluoroalkylsulfonic acids (PFSAs) such as the perfluorooctanesulfonic acid (PFOS) and the perfluoroalkylcarboxylic acids (PFCAs) such as the perfluorooctanoic acid (PFOA) (Table 1). PFASs bioaccumulate and are incredibly persistent in the environment due to the presence of the strong carbon-fluorine covalent bonds. Since PFOS and PFOA in the inflow water at the sewage treatment plant are transferred to suspensions and sludge in the process¹⁻³), we developed an analytical method for PFOS and PFOA and confirmed its validity⁴⁻⁶).

The toxicity profile of the Agency for Toxic Substances and Diseases (ATSDR)⁷) showed that PFOS, PFOA, PFHxS, PFDA and PFUdA are carcinogenic. In Europe, there is a trend to regulate PFHxS, PFOS, PFHxA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTTrDA, and PFTeDA⁸⁻¹⁰).

Therefore, we examined the measurable components of PFASs using the sample solution preparation procedure of the above methods⁴⁻⁶). And we analyzed PFASs in the sludge fertilizers, which were made by dehydrating or fermenting sludge generated in a sewage treatment plant, and in the dried microbes, which were produced as a by-product in food factories. Furthermore, we report the findings obtained on the analytical values of PFASs and their branched form isomers detected.

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Table 1 List of PFASs included in the resent study

Component	Abbreviation	Carbon ^{a)}	Chemical formula
Perfluoroalkylsulfonic acids	PFASs		
Perfluorobutanesulfonic acid	PFBS	C4	CF ₃ (CF ₂) ₃ SO ₃ H
Perfluoropentanesulfonic acid	PFPeS	C5	CF ₃ (CF ₂) ₄ SO ₃ H
Perfluorohexanesulfonic acid	PFHxS	C6	CF ₃ (CF ₂) ₅ SO ₃ H
Perfluoroheptanesulfonic acid	PFHpS	C7	CF ₃ (CF ₂) ₆ SO ₃ H
Perfluorooctanesulfonic acid	PFOS	C8	CF ₃ (CF ₂) ₇ SO ₃ H
Perfluorononanesulfonic acid	PFNS	C9	CF ₃ (CF ₂) ₈ SO ₃ H
Perfluorodenanesulfonic acid	PFDS	C10	CF ₃ (CF ₂) ₉ SO ₃ H
Perfluorododenanesulfonic acid	PFDoS	C12	CF ₃ (CF ₂) ₁₁ SO ₃ H
Perfluoroalkylcarboxylic acids	PFCAs		
Perfluorobutanoic acid	PFBA	C4	CF ₃ (CF ₂) ₂ COOH
Perfluoropentanoic acid	PFPeA	C5	CF ₃ (CF ₂) ₃ COOH
Perfluorohexanoic acid	PFHxA	C6	CF ₃ (CF ₂) ₄ COOH
Perfluoroheptanoic acid	PFHpA	C7	CF ₃ (CF ₂) ₅ COOH
Perfluorooctanoic acid	PFOA	C8	CF ₃ (CF ₂) ₆ COOH
Perfluorononanoic acid	PFNA	C9	CF ₃ (CF ₂) ₇ COOH
Perfluorodecanoic acid	PFDA	C10	CF ₃ (CF ₂) ₈ COOH
Perfluoroundecanoic acid	PFUdA	C11	CF ₃ (CF ₂) ₉ COOH
Perfluorododecanoic acid	PFDoA	C12	CF ₃ (CF ₂) ₁₀ COOH
Perfluorotridecanoic acid	PFTTrDA	C13	CF ₃ (CF ₂) ₁₁ COOH
Perfluorotetradecanoic acid	PFTeDA	C14	CF ₃ (CF ₂) ₁₂ COOH
Perfluorohexadecanoic acid	PFHxDA	C16	CF ₃ (CF ₂) ₁₄ COOH
Perfluorooctadecanoic acid	PFODA	C18	CF ₃ (CF ₂) ₁₆ COOH

a) Number of carbon

2. Materials and Methods

1) Samples

Eighty-six samples of sludge fertilizer and 10 samples of dried microbes were used as test products. The wet samples were pre-dried by the Testing Methods for Fertilizers. Each sample was ground to pass through a 0.5-mm mesh using a grinder (Retsch ZM 200) and thoroughly mixed. The ground samples were stored in closed in polyethylene bags at room temperature until analysis.

2) Apparatus

(1) High-Performance Liquid Chromatograph/Mass Spectrometer (LC-MS/MS): SHIMADZU LCMS-8045

Analytical Column: GL-Sciences InertSustain C18 (2.1-mm × 150-mm, 3-μm)

Guard column: GL-Sciences Inertsil ODS-SP HP (3.0-mm × 10-mm, 3-μm)

Delay column: GL-Sciences Delay Column for PFAS (3.0-mm × 10-mm)

(2) Ultrasonic generator: Yamato 8510 (Branson 8510 Ultrasonic Cleaner)

(3) Centrifugal separator: KUBOTA Table top centrifuge 4000

- (4) High speed centrifugal separator: AS ONE MCD-2000 (HSIANGTAI Microcentrifuge MCD-2000)
- (5) Solid Phase Extraction cartridges containing weak anion exchange (SPE-EX), mixed-mode polymeric sorbent: GL-Sciences InertSep MA-2 500 mg/6 mL, Waters Oasis WAX 6-cc (500 mg), Waters Oasis WAX for PFAS Analysis 6-cc (500 mg), phenomenex Strata-XL-AW 500 mg/6 mL
- (6) Solid Phase Extraction cartridges containing Graphite carbon (SPE-GC): GL-Sciences InertSep Slim GC 400 mg
- (7) Manifold: GL-Sciences, WATERS
- (8) Nitrogen concentrator: GL-Sciences Nitrogen spray branch unit
- (9) Vortex mixer: AS ONE Test tube mixer TRIO TM-2N
- (10) 7-mL Concentration Tube: GL-Sciences GL-SPE Concentration Tube (0.5-mL & 1.0-mL Graduated)
- (11) 15-mL centrifuge tube with a screw cap: Labcon Metal Free Centrifuge Tubes
- (12) 300- μ L vial for standard solution and sample solution: WATERS Polypropylene 12-mm \times 32-mm Screw Neck Vial, with Polyethylene Septumless Cap
- (13) 50-mL test tube with a screw cap: GL-Sciences DigiTUBEs
- (14) 1.5-mL ground-in stopper centrifuge tube: TreffLab Microtube Natural Click Cap
- (15) Pasteur pipette: CorningTM Borosilicate Glass Pasteur Pipets (146 mm), Corning Borosilicate Glass Pasteur Pipets (229 mm)

3) Reagent

- (1) Water: Ultrapure water purified using an ultrapure water production device (MILLIPORE Milli-Q Integral 5)
- (2) Methanol for extraction and Cleanup: Pesticide and residue and polychlorinated biphenyl analysis grade (FUJIFILM Wako Pure Chemical)
- (3) Methanol for cleaning test equipment: Analytical grade (FUJIFILM Wako Pure Chemical) (FUJIFILM Wako Pure Chemical)
- (4) Acetonitrile for LC-MS/MS: LC/MS grade (FUJIFILM Wako Pure Chemical)
- (5) Ammonia solution: Analytical grade (Approximately 28 % (w/w) in water as ammonia) (FUJIFILM Wako Pure Chemical)
- (6) Formic acid: Analytical grade (A compound purity 98 % (w/w) or greater) (KANTO CHEMICAL)
- (7) Ammonium acetate solution (1 mol/L): HPLC grade (FUJIFILM Wako Pure Chemical)
- (8) Ammonium acetate solution (10 mmol/L): The ammonium acetate solution (1 mol/L) was diluted 100 times with water.
- (9) Methanol-water (1+1): One volume of methanol was mixed with 1 volume of water.
- (10) Ammonia solution-methanol (1+100): One volume of Ammonia solution was mixed with 100 volumes of methanol.
- (11) PFASs standard solution: PFAC-MXC (Native standard solution containing PFASs shown in Table 2, 1.2 mL of methanol solution) (WELLINGTON). Then 1 mL of the PFAC-MXC was placed in a 10-mL volumetric flask, and methanol was added up to the marked line to prepare the PFASs standard

solution (200 ng/mL).

Table 2 Components of PFASs standard solution and their concentrations

Abbreviation	PFASs			PFCAs	
	Concentration (ng/mL)		coefficient (Acid/Salt)	Concentration (ng/mL)	
	as salt	as acid		Abbreviation	as acid
PFBS	2000	1770	0.885	PFBA	2000
PFPeS	2000	1880	0.940	PFPeA	2000
PFHxS	2000	1900	0.950	PFHxA	2000
PFHpS	2000	1910	0.955	PFHpA	2000
PFOS	2000	1920	0.960	PFOA	2000
PFNS	2000	1920	0.960	PFNA	2000
PFDS	2000	1930	0.965	PFDA	2000
—	—	—	—	PFUdA	2000
PFDoS	2000	1940	0.970	PFDoA	2000
—	—	—	—	PFTTrDA	2000
—	—	—	—	PFTeDA	2000
—	—	—	—	PFHxDA	2000
—	—	—	—	PFODA	2000

(12) $^{13}\text{C}_4$ -PFOS standard solution: MPFOS (Isotope performance standard solution containing mass labeled sodium perfluoro-1-[1,2,3,4- $^{13}\text{C}_4$]-octanesulfonate (50 $\mu\text{g/mL}$ as salt), 1.2 mL of methanol solution) (WELLINGTON). Then 1 mL of the MPFOS was placed in a 50-mL volumetric flask, and methanol was added up to the marked line to prepare the $^{13}\text{C}_4$ -PFOS standard solution (1 $\mu\text{g/mL}$).

(13) $^{13}\text{C}_8$ -PFOS standard solution: M8PFOS (Isotope performance standard solution containing mass labeled sodium perfluoro-1-[$^{13}\text{C}_8$]-octanesulfonate, 1.2 mL of methanol solution) (WELLINGTON). Then 1 mL of the M8PFOS was placed in a 50-mL volumetric flask, and methanol was added up to the marked line to prepare the $^{13}\text{C}_8$ -PFOS standard solution (1 $\mu\text{g/mL}$).

(14) $^{13}\text{C}_4$ -PFOA standard solution: MPFOA (Isotope performance standard solution containing mass labeled perfluoro-n-[1,2,3,4- $^{13}\text{C}_4$]-octanoic acid, 1.2 mL of methanol solution) (WELLINGTON). Then 1 mL of the MPFOA was placed in a 50-mL volumetric flask, and methanol was added up to the marked line to prepare the $^{13}\text{C}_4$ -PFOA standard solution (1 $\mu\text{g/mL}$).

(15) $^{13}\text{C}_8$ -PFOA standard solution: M8PFOA (Isotope performance standard solution containing mass labeled Perfluoro-n-[$^{13}\text{C}_8$]-octanoic acid (50 $\mu\text{g/mL}$), 1.2 mL of methanol solution) (WELLINGTON). Then 1 mL of the M8PFOA was placed in a 50-mL volumetric flask, and methanol was added up to the marked line to prepare the $^{13}\text{C}_8$ -PFOA standard solution (1 $\mu\text{g/mL}$).

(16) Isotope performance extraction standard solution: In a 50-mL volumetric flask were placed 10 mL each of $^{13}\text{C}_4$ -PFOS standard solution (1 $\mu\text{g/mL}$), $^{13}\text{C}_8$ -PFOS standard solution (1 $\mu\text{g/mL}$), $^{13}\text{C}_4$ -PFOA standard solution (1 $\mu\text{g/mL}$) and $^{13}\text{C}_8$ -PFOA standard solution (1 $\mu\text{g/mL}$), and methanol was added up to the marked line to prepare the mixed isotope performance standard solution (200 ng/mL). To prepare the isotope performance extraction standard solution (20 ng/mL), 10 mL of the mixed isotope performance standard solution (200 ng/mL) was placed in a 100-mL volumetric flask, and

methanol was added up to the marked line.

(17) Calibration standard solution (0.1 ng/mL-50 ng/mL): The mixed isotope performance standard solution (200 ng/mL) was diluted 10 times with methanol-water (1+1) to prepare the isotope performance standard solution (20 ng/mL). The PFASs standard solution (200 ng/mL) was diluted twice with water to prepare the PFASs standard solution (100 ng/mL). The PFASs standard solution (100 ng/mL) was diluted 10 times and 100 times with methanol-water (1+1) to prepare the PFASs standard solution (10 ng/mL) and PFASs standard solution (1 ng/mL), respectively.

To prepare calibration standard solution (10 ng/mL, 20 ng/mL and 50 ng/mL), each 1 mL, 2 mL and 5 mL of mixed standard solution (100 ng/mL) was put in 10-mL volumetric flasks, added 1 mL of isotope performance standard solution (20 ng/mL) to each of them, and add methanol-water (1+1) up to the marked line. Calibration standard solution (1 ng/mL, 2 ng/mL and 5 ng/mL) were prepared using the PFASs standard solution (10 ng/mL), and calibration standard solution (0.1 ng/mL, 0.2 ng/mL and 0.5 ng/mL) using the PFASs standard solution (1 ng/mL) in the same procedure.

(18) Isotope performance standard solution for study: The isotope performance extraction standard solution (20 ng/mL) was diluted 10 times with methanol to prepare the isotope performance standard solution (2 ng/mL) for study.

(19) PFASs standard solution for study: The PFASs standard solution (200 ng/mL) was diluted twice, 20 times, and 100 times with methanol to prepare the PFASs standard solution (100 ng/mL, 10 ng/mL and 2 ng/mL) for study, respectively. The PFASs standard solution (100 ng/mL) was diluted 100 times with methanol-water (1+1) to prepare the PFASs standard solution (1 ng/mL) for study.

The standard solutions, which were shown in paragraph number (11) to (19), transferred to a polypropylene tube with a screw cap and stored at less than 8 °C during the present study.

4) Preparation of sample solution

(1) Extraction

A 2.00 g of an analytical sample were placed in a 50-mL test tube (A) with a screw cap, and mixed 1 mL of isotope performance extraction standard solution. After the addition of 15 mL of methanol and 0.1 mL of formic acid as an extraction solvent, the solution was sonicated for 20 minutes. After the processed solution was centrifuged at 1700×g for 5 minutes, the supernatant was transferred into a 50-mL test tube (B) with a screw cap. Addition of an extraction solvent, sonicated and centrifugation were repeated 2 times, the supernatants were added up into the test tube (B), and methanol added to its 50-mL line to make an extract.

(2) Cleanup

A SPE-EX was previously washed sequentially with 5 mL of ammonia solution-methanol (1+100), 5 mL of methanol, then 5 mL of methanol-water (1+1). A SPE-GC was previously washed with 5 mL methanol.

In 15-mL centrifuge tube (C) was placed 5 mL of the extract and 5 mL of water, then the solution was mixed. In the cause of suspended solid or precipitate occurred, the mixture was centrifuged at 1700×g for 5 minutes. The mixture or the supernatant was put into the SPE-EX. Then the solution after washing the tube (C) with 5 mL methanol-water (1+1) was added to the SPE-EX. The SPE-EX was

washed with 5 mL of methanol twice and the PFE-GC was connected under the SPE-EX. The connected SPE was washed with 2 mL of ammonia solution-methanol (1+100). These effluents from the above operation were excluded. Then 4 mL of ammonia solution-methanol (1+100) was added to the connected SPE to elute PFASs, and the eluate was collected into a concentration tube (D). The eluate was evaporated to its 0.5-mL line at room temperature under gentle nitrogen gas flow. For the concentrate, 0.4 mL of water was added, mixed, and water was further added up to its 0.5-mL line. The solution was centrifuged at $10\ 000\times g$ for 5 minutes, the supernatant was used as a sample solution.

The flows of the procedure of the extraction and the cleanup are given in scheme 1-1 and scheme 1-2. A blank test solution was performed in the same procedure using another instrument.

5) Measurement

The standard solution for each calibration curve and sample solution were chromatographed by HPLC as shown in Table 3-1. Mass spectra were taken on an MS/MS system equipped with an orthogonal spray interface as shown in Table 3-1, and the fragment ions for PFASs were quantitatively analyzed using the triple-quadrupole analyzer operated in the multiple-reaction-monitoring (MRM) mode as shown in Table 3-2. The peak area ratios were calculated by dividing the peak areas of PFASs and PFCAs peak by the peak areas of PFOS and PFOA of isotope performance, respectively. The total amounts of PFOS and PFOA, including the isomers of those branched forms, were calculated using the combined area of their linear and branched form peaks. The peaks area ratios of sample solution were compared with them of calibration standard solution, to thereby determine the content of PFASs in the analytical sample.

Table 3-1 Operating conditions of LC-MS/MS

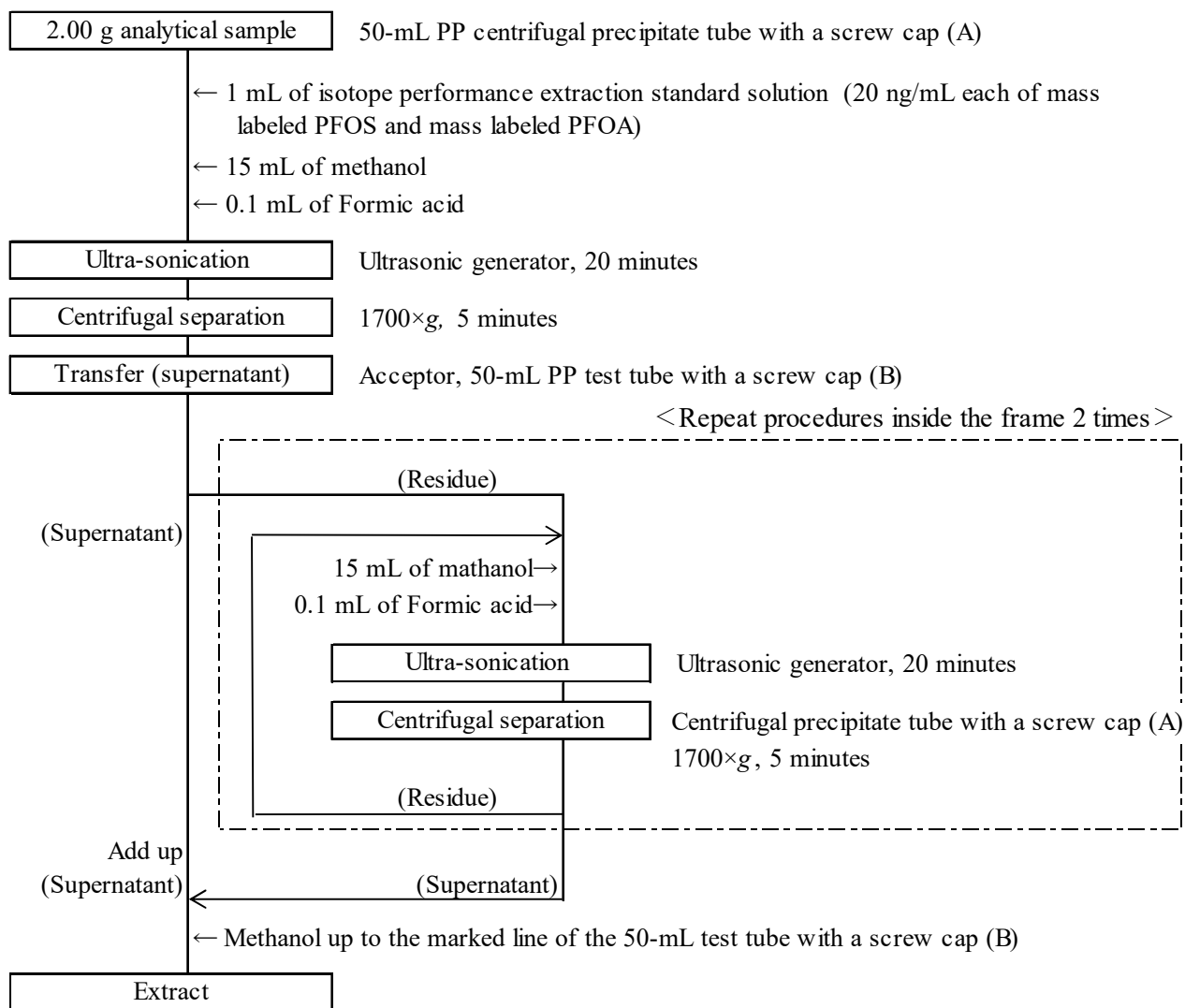
[HPLC conditions] SHIMADZU Nexera Series	
Delay column:	Delay Column for PFAS (3.0 mm I.D., 10 mm L)
Guard column:	Cartridge Guard Column E Inertsil (3.0 mm I.D., 10 mm L, 3 μm)
Analytical column:	InertSustain C18 (2.1 mm I.D., 150 mm L, 3 μm)
Mobile phase (A):	A: 10 mmol/L ammonium acetate solution B: Acetonitrile
Gradient program:	0 min (20 %B)→2.0 min (20 %B)→15 min (100 %B)→ 16 min (100 %B)→16.1 min (20 %B)→21 min (20 %B)
Flow rate:	0.3 mL/min
Column temperature:	40 °C
Injection volume:	5 μL
[MS conditions] SHIMADZU LCMS-8045	
Ionization:	Electrospray ionization (ESI)
Mode:	Positive
Probe voltage:	-1 kV
DL temperature:	200 °C
Heat block temperature:	300 °C
Interface temperature:	300 °C
Nebulizing gas flow:	3 L/min
Drying gas flow:	5 L/min
Heating gas flow:	15 L/min

Table 3-2 MRM parameters for detection of PFASs

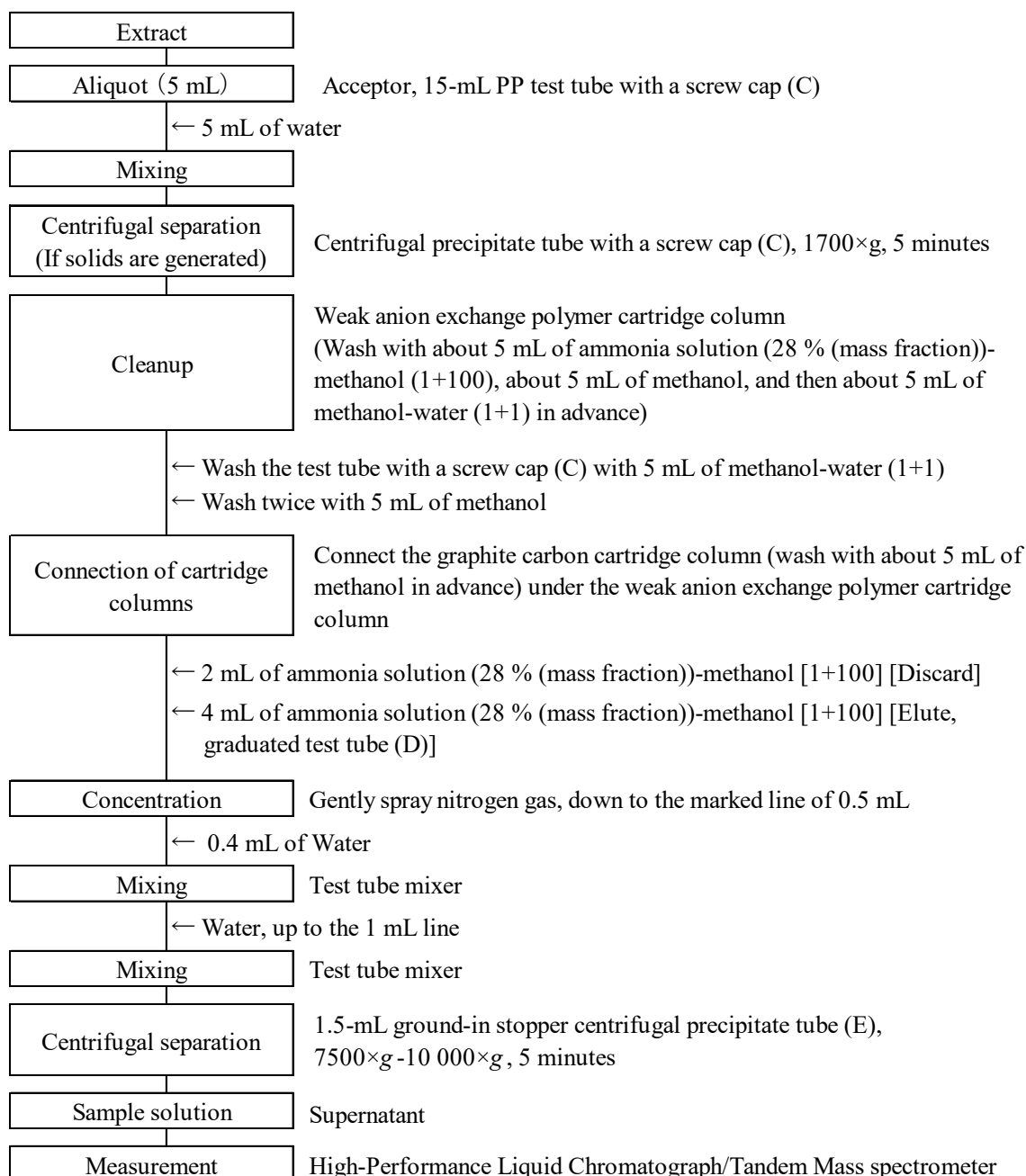
Abbreviation	For determination			For validation		
	Precursor	Product	Collision	Precursor	Product	Collision
	ion <i>m/z</i>	ion <i>m/z</i>	energy (eV)	ion <i>m/z</i>	ion <i>m/z</i>	energy (eV)
PFBS	298.9	80	33	298.9	99.0	28
PFPeS	348.9	80	44	348.9	98.9	33
PFHxS	399	80	44	399.0	99.0	35
PFHpS	448.9	80	51	448.9	98.9	38
PFOS	498.8	80	54	498.8	98.9	44
¹³ C ₄ -PFOS	502.8	80	52	502.8	98.9	45
¹³ C ₈ -PFOS	506.8	80	54	506.8	99.0	46
PFNS	548.9	80	55	548.9	99.0	46
PFDS	598.9	80	55	598.9	99.0	51
PFDoS	698.9	80	55	698.9	98.9	55
PFBA	212.9	169	10	—	—	—
PFPeA	262.9	219	8	—	—	—
PFHxA	312.9	269	8	312.9	119.0	19
PFHpA	362.9	319	9	363.0	169.0	16
PFOA	413	169	18	412.8	369.0	10
¹³ C ₄ -PFOA	416.9	169	18	416.8	372.0	10
¹³ C ₈ -PFOA	420.9	172	19	421.1	376.0	9

Table 3-2 Continued

Abbreviation	For determination			For validation		
	Precursor ion <i>m/z</i>	Product ion <i>m/z</i>	Collision energy (eV)	Precursor ion <i>m/z</i>	Product ion <i>m/z</i>	Collision energy (eV)
PFNA	463	419	10	463.0	219.0	17
PFDA	512.9	468.9	11	512.9	269.0	18
PFUdA	562.9	518.9	12	562.9	269.0	18
PFDoA	612.9	569.1	12	612.9	169.1	26
PFT _r DA	662.9	618.9	13	662.9	169.0	28
PFT _e DA	712.9	668.9	14	712.9	169.1	30
PFH _x DA	812.8	768.9	15	812.8	219.0	27
PFODA	912.8	868.8	16	912.8	219.0	32



Scheme 1-1 Flow sheet for PFASs in sludge fertilizers (Extraction procedure)



Scheme 1-2 Flow sheet for PFASs in sludge fertilizers (Cleanup and measurement procedures)

3. Results and discussion

1) Chromatographic identification of PFASs

For the separation of PFASs, HPLC was fitted with the analytical columns tested in the previous report⁴⁻⁶⁾ and operated according to the HPLC conditions (Table 3-1) indicated for the application¹¹⁾ of that column. The monitor ions and collision energies (Table 3-1 and Table 3-2) of all PFASs were tentatively set with reference to the LC-MS/MS (SHIMADZU LCMS-8050) application¹²⁾, and further optimized using the PFASs standard solution (200 ng/mL).

Fig. 1-1 and Fig. 1-2 show MRM chromatogram of a calibration standard solution at 50 ng/mL for all native target compounds included in PFAC-MXC (Table 2) and a sample solution, respectively. In

the former chromatogram, it was confirmed that the peak of PFASs at a high concentration was sharp. In the latter chromatograms, it was confirmed that the peaks of the branched isomer form and linear form of PFOS and PFOA are selectivity that can be quantified respectively.

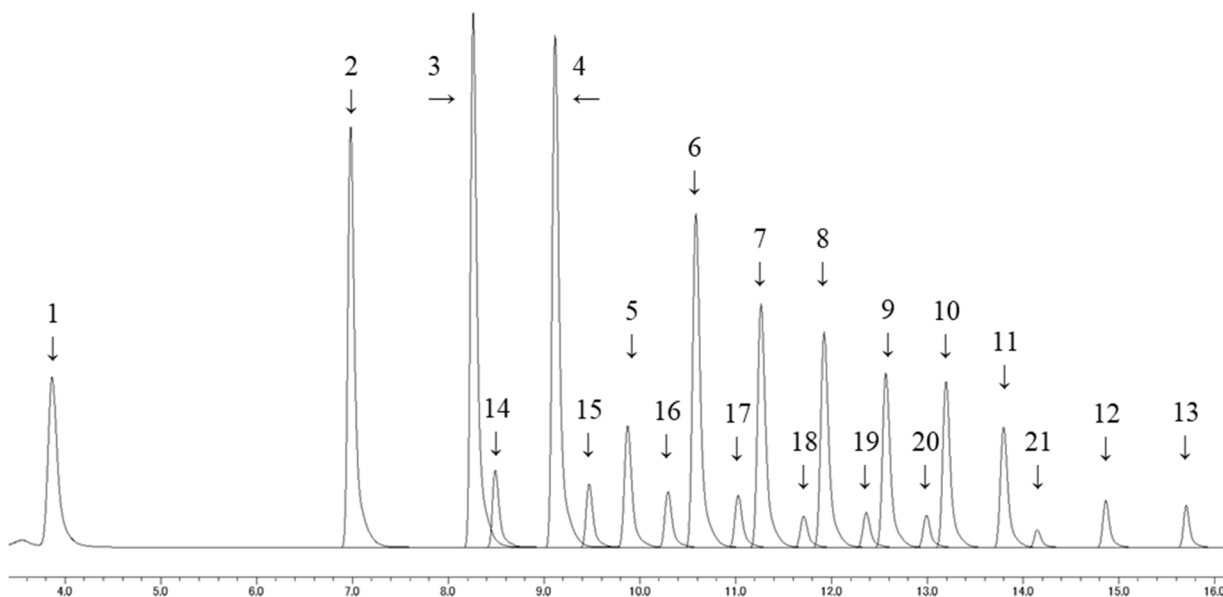


Fig. 1-1 MRM chromatogram of PFASs in a mixed standard solution, with each PFAS at 50 ng/mL

Peak position (↓ → ←)

1. PFBA 2. PFPeA 3. PFHxA 4. PFHpA 5. PFOA 6. PFNA 7. PFDA 8. PFUdA
9. PFDoA 10. PFTrDA 11. PFTeDA 12. PFHxDA 13. PFODA 14. PFBS 15. PFPeS
16. PFHxS 17. PFHpS 18. PFOS 19. PFNS 20. PFDS 21. PFDoS

Condition of LC-MS/MS As shown in Table 3-1 and Table 3-2.

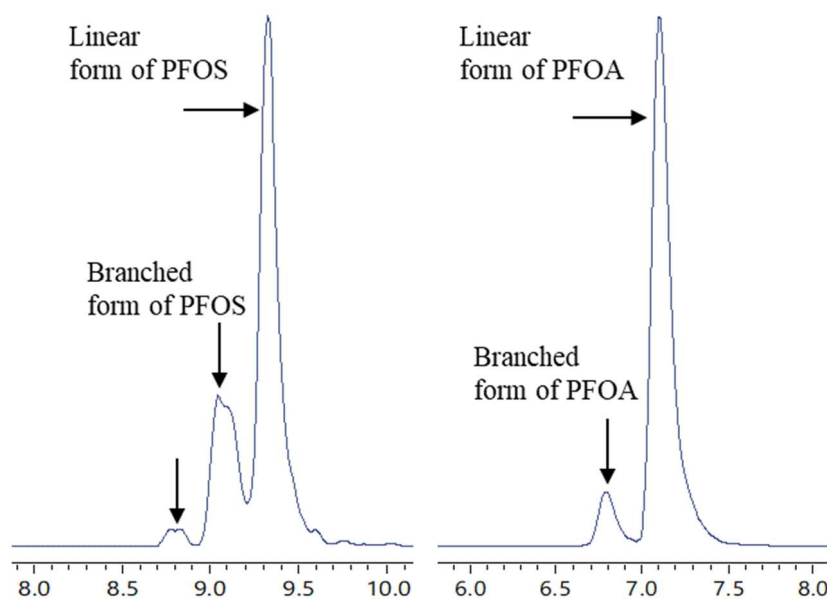


Fig. 1-2 MRM chromatograms of PFOS and PFOA in a sample solution extracted Sludge fertilizer

Condition of LC-MS/MS As shown in Table 3-1 and Table 3-2.

Calibration was performed for all PFASs targets using an eighteen-point calibration curve of the peak area ratios, which measured twice ranging from 0.1 ng/mL - 50 ng/mL. Then the following peak area ratios of PFASs were calculated¹³⁾: each PFASs / ¹³C₄-PFOS; each PFASs / ¹³C₈-PFOS; each PFCAs / ¹³C₄-PFOA; each PFCAs / ¹³C₈-PFOA. The linearity of the curve was determined using a 1/x² weighting factor and not forcing through zero. The regression coefficients and coefficients of determination obtained by these procedures were shown in Table 4. As an example, the calibration curves for the isotope performance standard peak area ratios of PFOS and PFOA were drawn in Fig.2-1 and Fig.2-2, respectively. In addition, their standard residuals were depicted in Fig.3-1 and Fig.3-2, respectively.

Excellent linearity was obtained with coefficients of determination (r^2) greater than 0.99 for 18 PFASs excluding PFDoS, PFHxDA, and PFODA. The origin was in the confidence interval (95%) of the intercept of those calibration curves. These results indicated that the calibration curves of those PFASs conformed to the standards of Testing Methods for Fertilizers (2021)⁶⁾. However, peaks of PFDoS, PFHxDA, and PFODA weren't detected in the low-level calibration standard solution.

Table 4 Calibration curve

Analytes		Isotope abbreviation ^{a)}	Linear equation ^{b)}		Confidence interval of a ^{c,d)}		Coefficient of determination ^{e)} r^2
Abbreviation	Range (ng/mL)		Inclination b	Intercept a	Lower limit	Upper limit	
PFBS	0.1 - 50	¹³ C ₄ -PFOS	0.852	0.008	-0.018	0.033	0.997
		¹³ C ₈ -PFOS	0.949	0.001	-0.028	0.030	0.997
PFPeS	0.1 - 50	¹³ C ₄ -PFOS	0.655	-0.005	-0.030	0.020	0.993
		¹³ C ₈ -PFOS	0.727	-0.008	-0.036	0.020	0.993
PFHxS	0.1 - 50	¹³ C ₄ -PFOS	0.583	0.001	-0.023	0.026	0.991
		¹³ C ₈ -PFOS	0.647	0.000	-0.028	0.027	0.991
PFHpS	0.1 - 50	¹³ C ₄ -PFOS	0.593	0.015	-0.009	0.039	0.994
		¹³ C ₈ -PFOS	0.659	0.014	-0.013	0.040	0.994
PFOS	0.1 - 50	¹³ C ₄ -PFOS	0.425	-0.012	-0.018	0.006	0.998
		¹³ C ₈ -PFOS	0.454	-0.003	-0.019	0.013	0.994
PFNS	0.1 - 50	¹³ C ₄ -PFOS	0.477	-0.008	-0.021	0.005	0.996
		¹³ C ₈ -PFOS	0.529	-0.010	-0.026	0.065	0.999
PFDS	0.1 - 50	¹³ C ₄ -PFOS	0.422	0.009	-0.004	0.022	0.995
		¹³ C ₈ -PFOS	0.467	0.009	-0.002	0.015	0.999
PFDoS	0.1 - 50	¹³ C ₄ -PFOS	0.229	-0.002	-0.021	0.016	0.969
		¹³ C ₈ -PFOS	0.254	-0.003	-0.023	0.017	0.970

a) Calibration standard solution containing 2 ng as each isotope performance PFOS and PFOA

b) Linear equation $y=bx+a$

c) Confidence interval 95%

d) Recommended criteria shown in Testing Methods for Fertilizers (2021) (lower limit $\leq 0 \leq$ upper limit)

e) Coefficient of determination usable as calibration curve shown in Testing Methods for Fertilizers (2021) ($r^2 \geq 0.99$)

f) Unable to create calibration curve

Table 4 Continued

Analytes		Isotope abbreviation ^{a)}	Linear equation ^{b)}		Confidence interval of a ^{c,d)}		Coefficient of determination ^{e)} r^2
Abbreviation	Range (ng/mL)		Inclination b	Intercept a	Lower limit	Upper limit	
PFBA	0.1 - 50	¹³ C ₄ -PFOA	0.337	-0.003	-0.013	0.007	0.995
		¹³ C ₈ -PFOA	0.293	-0.004	-0.015	0.007	0.993
PFPeA	0.1 - 50	¹³ C ₄ -PFOA	0.695	0.002	-0.015	0.019	0.997
		¹³ C ₈ -PFOA	0.603	-0.001	-0.017	0.016	0.996
PFHxA	0.1 - 50	¹³ C ₄ -PFOA	0.844	0.007	-0.015	0.029	0.997
		¹³ C ₈ -PFOA	0.732	0.003	-0.022	0.028	0.994
PFHpA	0.1 - 50	¹³ C ₄ -PFOA	0.860	0.013	-0.007	0.033	0.997
		¹³ C ₈ -PFOA	0.745	0.008	-0.003	0.019	0.999
PFOA	0.1 - 50	¹³ C ₄ -PFOA	0.518	0.002	-0.009	0.013	0.998
		¹³ C ₈ -PFOA	0.450	0.000	-0.012	0.011	0.997
PFNA	0.1 - 50	¹³ C ₄ -PFOA	0.606	0.014	-0.005	0.033	0.995
		¹³ C ₈ -PFOA	0.450	0.000	-0.012	0.011	0.997
PFDA	0.1 - 50	¹³ C ₄ -PFOA	0.495	0.013	-0.004	0.031	0.994
		¹³ C ₈ -PFOA	0.524	0.009	0.000	0.017	0.999
PFUdA	0.1 - 50	¹³ C ₄ -PFOA	0.446	0.016	-0.007	0.038	0.990
		¹³ C ₈ -PFOA	0.390	0.011	-0.003	0.026	0.994
PFDoA	0.1 - 50	¹³ C ₄ -PFOA	0.406	0.014	-0.001	0.029	0.993
		¹³ C ₈ -PFOA	0.351	0.010	-0.002	0.018	0.997
PFTrDA	0.1 - 50	¹³ C ₄ -PFOA	0.300	0.007	-0.005	0.018	0.993
		¹³ C ₈ -PFOA	0.258	0.005	-0.002	0.011	0.997
PFTeDA	0.1 - 50	¹³ C ₄ -PFOA	0.228	-0.004	-0.014	0.007	0.990
		¹³ C ₈ -PFOA	0.197	-0.004	-0.011	0.003	0.994
PFHxDA	0.1 - 50	¹³ C ₄ -PFOA	— ^{g)}	—	—	—	—
		¹³ C ₈ -PFOA	—	—	—	—	—
PFODA	0.1 - 50	¹³ C ₄ -PFOA	—	—	—	—	—
		¹³ C ₈ -PFOA	—	—	—	—	—

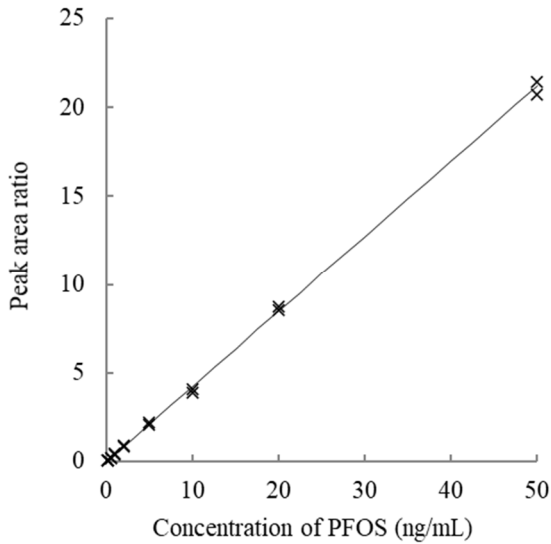


Fig. 2-1 PFOS calibration curve

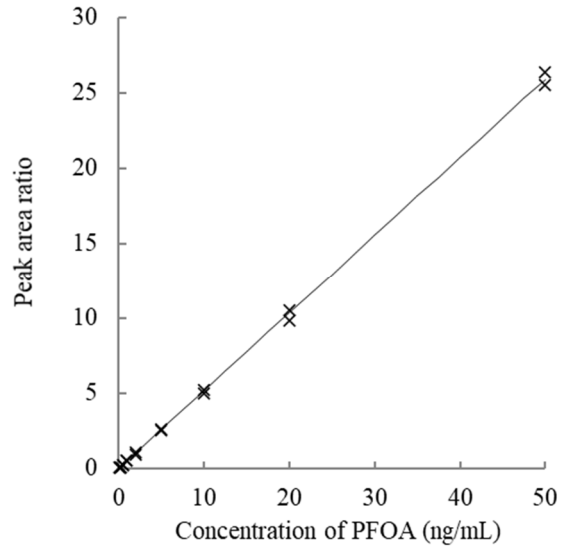


Fig. 2-2 PFOA calibration curve

×: Plot of peak area ratio of native to isotope performance analyte at each native analyte concentration (Concentration of isomer performance analytes: Each 2 ng/mL)
 Solid line: Regression line

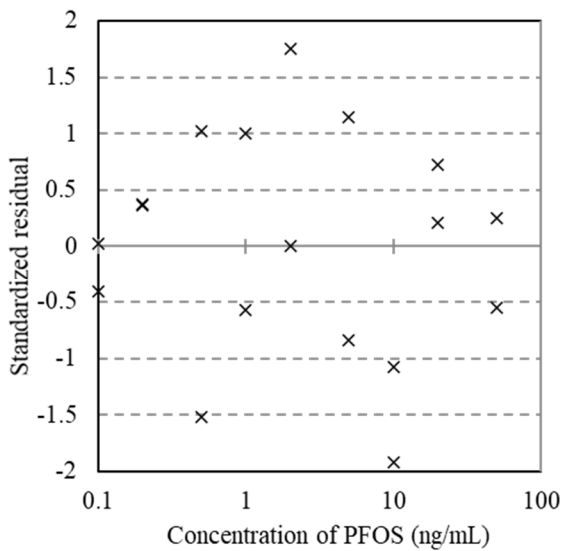


Fig.3-1 Evaluation of PFOS calibration curve

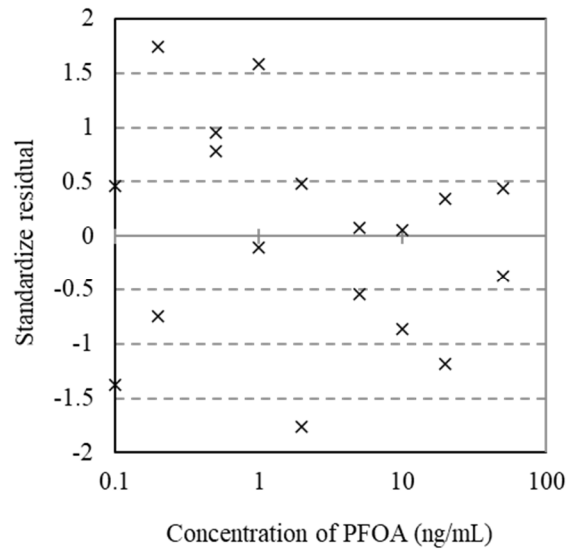


Fig.3-2 Evaluation of PFOA calibration curve

×: Plot of the standardized residual of peak area ratio at each native analyte concentration

2) Confirmation of cleanup operation

The following tests were conducted to identify PFASs to which the PFOS and PFOA procedure⁶⁾ for purifying extracts using weak anion exchange polymer cartridge columns and graphite carbon cartridge columns were applicable. In a pre-prepared weak anion exchange polymer cartridge was sequentially placed 10 mL of PFASs standard solution at each 1 ng/mL prepared with methanol-water (1+1), 5 mL of methanol-water (1+1) and 10 mL of methanol. After connecting the graphite carbon cartridge columns, the operation of adding 1 mL of elution solvent to the column was repeated 8 times, and the effluent was collected in each concentration tube. To each effluent was added 1 mL of the isotope

performance standard solution (2 ng/mL), and the mixture was concentrated and diluted with water to prepare a sample solution.

Table 5 shows the content of PFASs in each effluent fraction according to the above procedure using four types of weak anion exchange polymer cartridge columns, which are InertSep MA-2, Oasis WAX, Oasis WAX for PFAS Analysis and Strata-XL-AW. Each PFAS was eluted with the effluent fraction of 2 mL to 6 mL, and the recoveries of 16 PFASs excluding PFTrDA and PFTeDA was 72.3 % to 119.7 %. The recoveries of PFTrDA and PFTeDA were below 70 %, so these compounds were excluded from future studies.

Table 5 Elution pattern of PFASs^{a)} from the cleanup column^{b)}

Abbreviation	The name of the cleanup column at the top ^{c)}	Elution amount of PFASs (ng)								Recovery (%)	
		Fraction volume (mL)									
		0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	Total ^{d)}	
PFBS	InertSep MA-2	– ^{e)}	–	–	7.4	1.4	–	–	–	8.8	88.0
	Oasis WAX	–	–	–	0.3	8.5	0.5	–	–	9.3	92.8
	Oasis WAX(P) ^{f)}	–	–	–	0.7	7.1	2.5	–	–	10.3	103.4
	Strata-XL-AW	–	–	1.8	7.0	0.2	–	–	–	9.0	89.9
PFPeS	InertSep MA-2	–	–	0.2	8.1	1.3	0.1	–	–	9.7	97.1
	Oasis WAX	–	–	0.5	8.9	0.5	–	–	–	10.0	100.0
	Oasis WAX(P)	–	–	0.9	8.4	1.9	0.1	–	–	11.4	114.2
	Strata-XL-AW	–	–	2.5	7.3	0.2	–	–	–	9.9	99.0
PFHxS	InertSep MA-2	–	–	0.3	8.5	1.1	–	–	–	9.9	99.0
	Oasis WAX	–	–	1.0	8.5	0.5	–	–	–	10.1	100.6
	Oasis WAX(P)	–	–	0.7	8.9	1.1	–	–	–	10.7	106.9
	Strata-XL-AW	–	–	2.7	6.7	0.1	–	–	–	9.6	95.8
PFHpS	InertSep MA-2	–	–	0.4	8.4	0.8	–	–	–	9.7	97.0
	Oasis WAX	–	–	1.7	7.9	–	–	–	–	9.6	96.0
	Oasis WAX(P)	–	–	0.5	8.3	0.8	–	–	–	9.6	96.3
	Strata-XL-AW	–	–	2.9	6.4	–	–	–	–	9.3	93.2
PFOS	InertSep MA-2	–	–	0.7	7.9	0.8	–	–	–	9.4	93.8
	Oasis WAX	–	–	2.4	7.0	0.1	–	–	–	9.6	95.6
	Oasis WAX(P)	–	–	0.7	8.3	0.4	–	–	–	9.3	93.3
	Strata-XL-AW	–	–	2.8	6.1	0.1	–	–	–	9.1	91.0
PFNS	InertSep MA-2	–	–	0.8	8.4	0.6	–	–	–	9.8	98.1
	Oasis WAX	–	–	3.0	6.0	0.1	–	–	–	9.1	91.0
	Oasis WAX(P)	–	–	1.0	7.7	0.5	–	–	–	9.2	91.8
	Strata-XL-AW	–	–	3.1	5.8	0.2	–	–	–	9.0	90.3
PFDS	InertSep MA-2	–	–	1.0	8.1	0.5	–	–	–	9.5	95.0
	Oasis WAX	–	–	3.1	5.8	–	–	–	–	8.9	88.8
	Oasis WAX(P)	–	–	1.2	7.2	0.4	–	–	–	8.8	88.0
	Strata-XL-AW	–	–	2.7	5.7	0.1	–	–	–	8.4	84.5

a) Each PFASs was loaded with 20 ng.

b) Cleanup column with the graphite carbon cartridge column (InertSep Slim GC) connected under the anion exchange polymer cartridge column

c) The anion exchange polymer cartridge column

d) Total amount of PFASs eluted from 2 mL to 6 mL

e) Less than the lower limit (0.1 ng / mL) of calibration curve

f) Oasis WAX for PFASs Analysis

Table 5 Continued

Abbreviation	The name of the cleanup column at the top ^{c)}	Elution amount of PFASs (ng)								Recovery (%)	
		Fraction volume (mL)									
		0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8		Total ^{d)}
PFBA	InertSep MA-2	—	—	1.6	8.3	—	—	—	—	9.9	98.6
	Oasis WAX	—	—	3.0	7.9	—	—	—	—	10.9	109.1
	Oasis WAX(P)	—	—	1.4	8.9	1.6	—	—	—	12.0	119.7
	Strata-XL-AW	—	—	3.1	6.5	—	—	—	—	9.7	96.5
PFPeA	InertSep MA-2	—	—	2.3	6.7	—	—	—	—	9.0	89.9
	Oasis WAX	—	—	4.0	5.8	—	—	—	—	9.9	98.6
	Oasis WAX(P)	—	—	2.1	7.8	1.3	—	—	—	11.2	112.2
	Strata-XL-AW	—	—	3.5	5.5	—	—	—	—	8.9	89.4
PFHxA	InertSep MA-2	—	—	3.3	5.8	—	—	—	—	9.1	91.1
	Oasis WAX	—	—	5.0	4.8	—	—	—	—	9.8	98.2
	Oasis WAX(P)	—	—	3.1	7.2	1.1	—	—	—	11.3	113.3
	Strata-XL-AW	—	—	3.9	4.9	—	—	—	—	8.8	88.2
PFHpA	InertSep MA-2	—	—	4.1	5.0	—	—	—	—	9.1	91.3
	Oasis WAX	—	—	6.0	4.0	—	—	—	—	10.0	99.7
	Oasis WAX(P)	—	—	3.7	6.5	0.5	—	—	—	10.7	107.1
	Strata-XL-AW	—	—	4.2	4.7	—	—	—	—	8.9	89.4
PFOA	InertSep MA-2	—	—	4.8	4.4	—	—	—	—	9.2	91.8
	Oasis WAX	—	—	6.4	3.5	—	—	—	—	9.9	99.1
	Oasis WAX(P)	—	—	4.2	5.3	—	—	—	—	9.5	94.9
	Strata-XL-AW	—	—	4.2	4.3	—	—	—	—	8.5	84.8
PFNA	InertSep MA-2	—	—	5.1	3.7	—	—	—	—	8.8	87.8
	Oasis WAX	—	—	6.6	2.8	—	—	—	—	9.4	94.5
	Oasis WAX(P)	—	—	4.2	4.5	—	—	—	—	8.7	87.0
	Strata-XL-AW	—	—	4.4	4.3	—	—	—	—	8.7	87.2
PFDA	InertSep MA-2	—	—	5.5	3.6	—	—	—	—	9.1	91.3
	Oasis WAX	—	—	6.9	2.6	—	—	—	—	9.6	95.5
	Oasis WAX(P)	—	—	4.6	4.2	—	—	—	—	8.9	88.5
	Strata-XL-AW	—	—	4.4	4.0	—	—	—	—	8.4	83.9
PFUdA	InertSep MA-2	—	—	5.4	3.3	—	—	—	—	8.8	87.6
	Oasis WAX	—	—	7.0	2.3	—	—	—	—	9.3	92.7
	Oasis WAX(P)	—	—	4.5	3.9	—	—	—	—	8.4	83.9
	Strata-XL-AW	—	—	4.3	3.8	—	—	—	—	8.1	81.0
PFDoA	InertSep MA-2	—	—	4.3	3.0	—	—	—	—	7.2	72.3
	Oasis WAX	—	—	5.7	1.9	—	—	—	—	7.6	75.9
	Oasis WAX(P)	—	—	4.0	3.3	—	—	—	—	7.4	73.7
	Strata-XL-AW	—	—	3.7	3.7	—	—	—	—	7.4	74.0
PFTrDA	InertSep MA-2	—	—	2.5	3.0	—	—	—	—	5.5	54.7
	Oasis WAX	—	—	3.7	1.8	—	—	—	—	5.5	54.8
	Oasis WAX(P)	—	—	3.2	3.1	—	—	—	—	6.3	63.4
	Strata-XL-AW	—	—	3.0	3.8	—	—	—	—	6.8	67.7
PFTeDA	InertSep MA-2	—	—	—	2.9	—	—	—	—	2.9	29.0
	Oasis WAX	—	—	0.7	2.1	—	—	—	—	2.8	28.1
	Oasis WAX(P)	—	—	0.9	2.8	—	—	—	—	3.7	37.3
	Strata-XL-AW	—	—	0.7	3.5	—	—	—	—	4.2	41.9

3) Performance characteristics

Accuracy was evaluated by determining PFASs in three samples of sludge fertilizer with equivalent amounts of 1 µg/kg, 5 µg/kg and 50 µg/kg, respectively. Table 6 shows the results of each analysis using four different types of weak anion exchange polymer cartridge columns. The average recoveries of PFASs of 16 components was 62.4 % to 124.8 %, and all of them conformed to the criteria of accuracy shown in Testing Methods for fertilizers (2021)⁶.

Table 6 Result of recovery test

Analytes		InertSep MA-2		Oasis WAX		Oasis WAX (P) ^{a)}		Strata-XL-AW	
Abbreviation	Concentration ^{b)} (µg/kg)	Mean ^{c)} (µg/kg)	Recovery ^{d)} (%)	Mean (µg/kg)	Recovery (%)	Mean (µg/kg)	Recovery (%)	Mean (µg/kg)	Recovery (%)
PFBS	50	47.5	95.1	50.6	101.1	49.2	98.5	48.3	96.6
	5	4.87	97.3	4.68	93.6	4.79	95.8	5.10	102.1
	1	0.74	73.6	0.84	84.0	0.91	91.0	0.86	86.1
PFPeS	50	47.3	94.5	54.6	109.2	53.3	106.6	51.5	103.0
	5	4.95	99.0	5.12	102.4	5.19	103.8	5.44	108.9
	1	0.73	72.7	0.66	66.3	0.89	89.3	0.86	86.2
PFHxS	50	49.6	99.1	53.6	107.1	52.5	105.0	49.9	99.9
	5	5.00	100.0	5.00	99.9	5.13	102.7	5.60	112.0
	1	1.00	99.9	0.77	77.0	0.88	88.0	0.66	65.9
PFHpS	50	51.4	102.9	53.4	106.7	52.9	105.9	51.5	103.0
	5	5.32	106.4	5.04	100.9	5.06	101.2	5.41	108.1
	1	0.87	86.6	0.73	72.5	0.63	63.3	0.76	76.5
PFOS	50	52.3	104.6	49.4	98.7	50.6	101.1	49.7	99.5
	5	5.43	108.6	4.91	98.3	4.85	97.0	4.93	98.6
	1	0.99	98.8	0.91	91.3	0.88	88.2	0.94	94.4
PFNS	50	48.4	96.8	35.1	70.2	38.0	76.0	39.7	79.5
	5	5.21	104.3	3.87	77.5	4.01	80.3	3.89	77.7
	1	0.89	88.9	0.90	90.5	0.92	91.6	0.91	91.2
PFDS	50	45.2	90.4	35.5	71.0	36.8	73.6	36.7	73.4
	5	4.90	98.0	3.36	67.2	4.05	80.9	3.24	64.7
	1	0.85	85.0	0.79	79.2	0.82	82.1	0.74	74.5
PFBA	50	53.5	107.0	50.5	101.0	50.9	101.8	50.9	101.7
	5	5.65	113.1	5.50	110.0	5.80	116.0	5.92	118.5
	1	1.04	103.5	1.16	115.5	1.01	100.5	0.89	89.1
PFPeA	50	50.7	101.4	48.0	96.0	48.4	96.7	47.9	95.8
	5	5.33	106.6	4.95	99.0	5.13	102.6	5.19	103.8
	1	1.16	116.0	1.18	118.2	1.24	123.9	1.25	124.8
PFHxA	50	49.4	98.8	48.5	97.0	49.0	97.9	48.6	97.1
	5	5.06	101.1	5.18	103.7	5.43	108.7	5.45	109.0
	1	1.04	103.6	0.94	93.5	0.97	97.4	0.97	97.4

a) Oasis WAX for PFASs Analysis

b) The concentration of PFASs spiked to the sample

c) Mean value ($n=3$)

d) Tolerance range of recovery rate shown in Testing Methods for Fertilizers (2021)

(70 %-120 % for 50 ng/mL of the analyte, 60 %-125 % for 5 ng/mL and 1 ng/mL of the analyte)

Table 6 Continued

Analytes		InertSep MA-2		Oasis WAX		Oasis WAX (P) ^{a)}		Strata-XL-AW	
Abbreviation	Concentration ^{b)} (µg/kg)	Mean ^{c)} (µg/kg)	Recovery ^{d)} (%)	Mean (µg/kg)	Recovery (%)	Mean (µg/kg)	Recovery (%)	Mean (µg/kg)	Recovery (%)
PFHpA	50	48.8	97.5	48.6	97.1	48.6	97.3	48.6	97.3
	5	4.99	99.7	5.02	100.4	5.15	103.0	5.18	103.5
	1	1.06	106.4	1.01	101.5	1.05	104.9	0.98	98.2
PFOA	50	46.4	92.9	49.1	98.2	49.7	99.4	49.8	99.6
	5	5.30	106.0	5.31	106.3	5.22	104.5	5.18	103.7
	1	0.99	99.2	1.06	105.8	1.07	106.7	1.01	101.3
PFNA	50	49.2	98.3	44.1	88.1	45.6	91.2	46.4	92.8
	5	5.02	100.4	4.65	92.9	4.67	93.4	4.64	92.9
	1	1.09	109.1	1.10	109.6	1.13	112.9	0.98	97.9
PFDA	50	48.7	97.4	36.8	73.6	39.1	78.1	42.2	84.3
	5	4.98	99.6	3.74	74.7	3.97	79.3	3.71	74.2
	1	0.85	85.0	0.88	88.0	0.92	91.5	0.95	95.5
PFUdA	50	45.6	91.1	37.3	74.5	42.9	85.9	46.8	93.7
	5	4.88	97.5	3.50	70.1	3.98	79.7	3.64	72.9
	1	0.74	74.0	0.65	65.2	0.69	68.8	0.69	69.5
PFDoA	50	38.5	76.9	35.7	71.5	46.2	92.3	46.1	92.1
	5	4.45	89.0	3.12	62.4	3.46	69.1	3.19	63.8
	1	0.70	70.2	0.74	73.8	0.77	77.5	0.73	73.2

The lower limit of quantification was estimated from the following parameters described in Annex A of Testing Methods for Fertilizer (2021)⁶⁾: i) Standard deviation of analytical values of seven samples to which 1 µg/kg of each PFAS was added to sludge fertilizer. ii) Residual of calibration curve (0.1 ng/mL-2 ng/mL). iii) The signal-to-noise (SN) ratio of the peaks of each PFAS in the chromatogram of the sample solution.

Table 7 shows the estimated lower limit of quantification (*LOQ*) of PFASs of 16 components calculated by each procedure. The largest values of the *LOQ* calculated by each procedure were in the range of 0.5 µg/kg to 1 µg/kg, which were used as the *LOQ* for monitoring PFASs. Then the average recoveries of PFASs of 16 components were 72.6 % to 114.8%, and all of them conformed to the criteria of accuracy shown in Testing Methods for Fertilizers (2021)⁶⁾.

Table 7 Result of estimation of the lower limit of quantification

Abbreviation	Replicate test ^{a)}						Calibration curve ^{a)}		SN ratio ^{a)}		Estimation of LOQ
	Concentration ^{b)} (µg/kg)	Mean ^{c)} (µg/kg)	s_r ^{d)} (µg/kg)	Recovery (%)	LOQ ^{e)} (µg/kg)	LOD ^{f)} (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	
PFBS	1	0.81	0.10	81.4	1	0.4	0.3	0.1	0.4	0.1	1
PFPeS	1	0.94	0.14	93.8	1	0.5	0.7	0.3	0.6	0.2	1
PFHxS	1	1.04	0.08	104.5	0.8	0.3	0.7	0.3	0.4	0.1	0.8
PFHpS	1	0.86	0.04	85.7	0.4	0.2	0.3	0.1	0.5	0.1	0.5
PFOS	1	0.98	0.05	98.4	0.5	0.2	0.5	0.2	0.3	0.1	0.5
PFNS	1	0.90	0.14	89.9	1	0.6	0.5	0.2	0.4	0.1	1
PFDS	1	0.85	0.14	85.2	1	0.5	0.9	0.4	0.4	0.1	1
PFBA	1	1.03	0.11	103.0	1	0.4	0.3	0.1	1	0.3	1
PFPeA	1	1.15	0.05	114.8	0.5	0.2	0.4	0.2	0.7	0.2	0.7
PFHxA	1	1.03	0.07	102.6	0.7	0.3	0.7	0.3	0.5	0.2	0.7
PFHpA	1	1.04	0.04	104.1	0.4	0.2	0.6	0.3	0.3	0.1	0.6
PFOA	1	0.98	0.04	98.0	0.4	0.1	0.5	0.2	0.1	0.04	0.5
PFNA	1	1.09	0.04	109.2	0.4	0.2	0.7	0.3	0.4	0.1	0.7
PFDA	1	0.97	0.10	97.4	1	0.4	1	0.5	0.2	0.06	1
PFUdA	1	0.95	0.11	94.9	1	0.4	1	0.5	0.2	0.05	1
PFDoA	1	0.73	0.08	72.6	0.8	0.3	1	0.6	0.2	0.05	1

a) The Procedure shown in Testing Methods for Fertilizers (2021)

b) The concentration of PFAS spiked the sample

c) Mean value ($n=7$)

d) Repeatability standard deviation

e) Lower limit of quantitation

f) Lower limit of detection

4) Branched chain isomers analysis

In the quantification of PFASs, it is required to evaluate the combined amount of linear and branched chain isomers²⁾, so each combination amount of these about PFOS and PFOA was calculated.

Fig.4 shows a chromatograms of sample solutions with peaks of branched form isomer of PFASs, which are PFHxS, PFOS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA and PFDoA. The retention time of the peaks of these isomers was shorter than that of their linear peaks, as reported by Benskin et al¹⁴⁾ and Jin et al¹⁵⁾. For PFOS and PFOA showing peaks of branched form in all samples, the relationships between the analytical value of the linear form and the total amount including that of the branched form, respectively. Their relationships were found to have a strong correlation since the correlation coefficient of these regression formulas was 0.999. In addition, these plots were distributed around the regression lines with the inclinations of 0.797 for PFOS and 0.942 for PFOA in both the low and high concentration ranges. The proportions of the linear forms of PFOS and PFOA were the mean values of 83 % and 97 %, respectively, and were comparable to those in the reports by NAKAYAMA et al¹⁶⁾ and Fang et al¹⁷⁾.

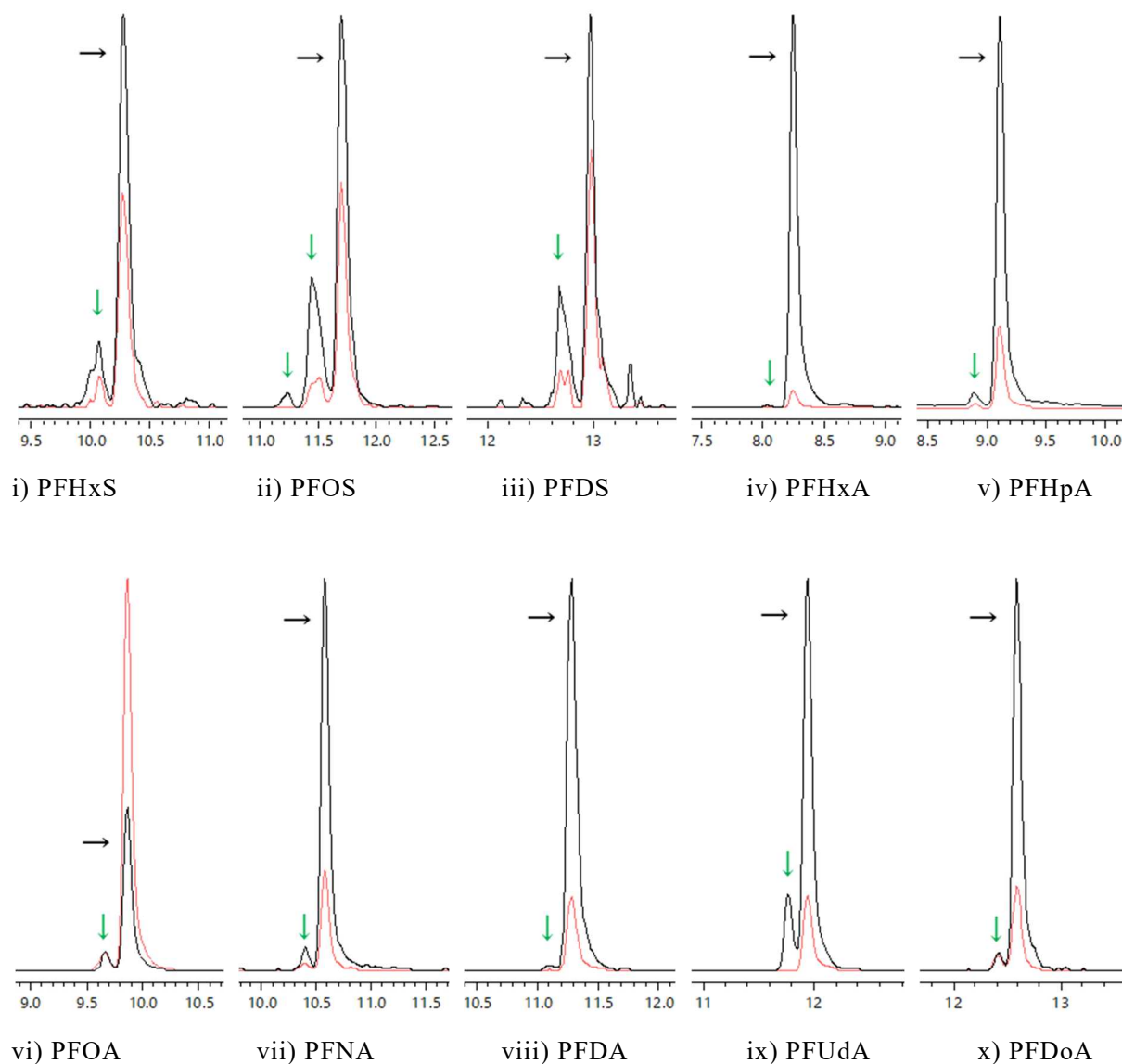


Fig.4 Example of MRM chromatogram of sample solution in which branched isomers are detected

Black line drawing: MRM chromatogram with product ion for determination

Red line drawing: MRM chromatogram with product ion for validation

→: Linear form of PFAS

↓: Branched form of PFAS

4. Conclusion

A LC-MS/MS method with a purification procedure using dual SPEs was developed for the determination of 16 PFASs in sludge fertilizers and dried microbes. The accuracy and sensitivity of the method were sufficient for application to the present study.

As a result of analyzing 86 samples of sludge fertilizers and 10 samples of dried microbes, PFASs in the analytical sample were detected. These PFASs were also detected in studies of their behavior in

sewage treatment plants and in their investigation reports in rivers and lakes.

The following PFASs branched isomer peaks appeared on the chromatogram of the sample solution, which were PFHxS, PFOS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA and PFDoA. The proportions of the linear forms of PFOS and PFOA to the total amount including those of the branched form were comparable to those in the reports by NAKAYAMA et al and Shuhong et al.

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