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Development of extraction method of pharmaceuticals and their occurrences found in Japanese wastewater treatment plants

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ABSTRACT

In this study, occurrence of 66 PPCPs (pharmaceuticals and personal care products) in liquid and solid phases of sewage sludge was elucidated. The extraction methods for the PPCPs from sludge were newly developed employing Pressurized Liquid Extraction (PLE) and Ultrasonic Solvent Extraction (USE). As an appropriate method, PLE using water (pH2), PLE using methanol (pH4), and USE using mixture of methanol and water (1/9,v/v, pH11) was found most effective because total recovery of most of the PPCPs indicated 40 to 130%. The developed extraction method with previously developed method for liquid phase analysis was applied to field survey at wastewater treatment plants (WWTPs) in Japan. 56 compounds were detected from the primary sludge and 61 compounds were detected from the excess sludge. The concentration was ranged between several ng/g and several μg/g. Solid-water distribution coefficient (Log K_d) ranged between 0.9 L/kg (Caffeine) and 3.7 L/kg (Levofloxacin) for primary sludge and between 1.4 L/kg (Sulpirid) and 4.3 L/kg (Mefenamic acid) for excess sludge.

1. Introduction

Pharmaceuticals have recently raised great public attention as emerging contaminants in the aquatic environment (Herberer, 2002; Kolpin et al., 2002; Harrison et al., 2006). Pharmaceuticals and personal care products (PPCPs) are used all over the world for human beings and veterinary. The users administrating pharmaceuticals excrete them and their metabolites and utilizing personal care products waste them after usage into wastewater. Many PPCPs are, therefore, discharged into the aquatic environment via wastewater treatment plants (WWTPs) if the WWTPs have less efficiency in their removal (Ternes, 1998). Since PPCPs are designed to have some biological effect even at low concentrations, they are concerned to cause adverse effect on the aquatic organisms and/or the occurrence of drug-resistant bacteria in the aquatic environment (Hernando et al., 2006). It is also a problem that regulation of PPCPs seems difficult since usage of PPCPs is quite beneficial for human health even if some toxicity to the aquatic ecosystem would be found. Therefore, PPCPs that are inevitably used and discharged from WWTPs to the aquatic environment should be further reduced from the view point of environmental protection in precautionary principle.

Currently, the research on the behavior and fate of PPCPs in the wastewater treatment process has been gradually increasing (Göbel et al., 2005a). However, there are still limited studies dealing their removal mechanics in wastewater treatment process. In general, two processes are responsible for PPCPs reduction in WWTPs; sorption and biodegradation. Without taking into any consideration of particulate phase sorbed onto sludge, their behavior in the WWTPs would be never understood. Furthermore, PPCPs included in sludge would cause concerns of their contamination of food, soil and groundwater in the environment if sludge utilization for fertilizers on agricultural land would be performed.

More than ten thousand of PPCPs are used in the all over the world, among which PPCPs should be concerned is still unknown. However, even the studies discussing the occurrence of PPCPs they dealt limited number of PPCPs except for several researchers (Kolpin et al., 2004; Gros et al., 2006; Westerhoff et al., 2005; Miao et al., 2004).

From these reasons, we set two objectives in this study; 1) develop simultaneous analytical method of various PPCPs in particulate content in sludge, 2) grasp the occurrence of the various PPCPs in water and solid phases in sewage sludge by applying the developed analytical method.

2. Methods

2.1. Target compounds

66 compounds were selected from the following view points: amount of usage in Japan, the frequency of their detection in the aquatic environment (Nakada et al., 2006; Sugishita et al., 2007; Sugishita et al., 2008) or WWTPs (Okuda et al., 2008); the toxicity to the algae or microorganism (Fukunaga et al., 2006; Fukunaga et al., 2007), analytical capability of the laboratory (Table 1). These compounds consist of 32

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Table 1
Target compounds and limit of quantification.

No.	Name	Abbr.	LOQ (μg/L)	Use/category	No.	Name	Abbr.	LOQ (μg/L)	Use/category
1	Azithromycin	AZM	0.19	Antibiotic	34	Fenoprofen	FNP	1.87	Analgesic
2	Clarithromycin	CAM	0.61	Antibiotic	35	Ibuprofen	IBP	–	Analgesic
3	Roxithromycin	RXM	0.24	Antibiotic	36	Indometacin	IDM	0.65	Analgesic
4	Tylosin	TYL	0.23	Antibiotic	37	Isopropylantipyrine	IPP	0.13	Analgesic
5	Ciprofloxacin	CPFX	1.02	Antibiotic	38	Ketoprofen	KTP	1.68	Analgesic
6	Enrofloxacin	ERFX	0.16	Antibiotic	39	Mefenamic acid	MFA	0.94	Analgesic
7	Levofloxacin	LVFX	1.31	Antibiotic	40	Naproxen	NPX	0.88	Analgesic
8	Norfloxacin	NRFX	0.51	Antibiotic	41	Crotamiton	CRT	0.24	Analgesic
9	Sulfadimethoxine	SDIME	0.21	Antibiotic	42	Diclofenac	DCF	2.19	Analgesic
10	Sulfadimidine	SDIMI	0.34	Antibiotic	43	Carbamazepine	CBM	0.16	Antiepilepsy
11	Sulfamerazine	SMERA	0.66	Antibiotic	44	Ifenprodil	IFP	0.23	Antiepilepsy
12	Sulfamonomethoxine	SMONO	1.64	Antibiotic	45	Phenobarbital	PBB	–	Antiepilepsy
13	Bezylpenicillin	BZPE	3.47	Antibiotic	46	Primidone	PRM	3.52	Antiepilepsy
14	Ceftiofur	CEF	15.41	Antibiotic	47	Atenolol	ATL	1.38	Antiarrhythmic
15	Chlortetracycline	CTC	9.54	Antibiotic	48	Disopyramide	DSP	0.19	Antiarrhythmic
16	Oxytetracycline	OTC	0.68	Antibiotic	49	Metoprolol	METOP	0.42	Antiarrhythmic
17	Tetracycline	TC	0.08	Antibiotic	50	Propranolol	PRP	0.19	Antiarrhythmic
18	Diclazuril	DCZ	1.38	Antibiotic	51	Diltiazem	DTZ	0.05	Blood-vessel dilator
19	Nicarbazin	NCB	0.69	Antibiotic	52	Dipyridamole	DPD	0.13	Blood-vessel dilator
20	Sulfamethoxazole	SMETH	0.55	Antibiotic	53	nalidixic acid	NLXA	0.30	Blood-vessel dilator
21	Trimethoprim	TRM	0.35	Antibiotic	54	Furosemide	FSM	0.64	Blood-vessel dilator
22	2-quinoxaline carboxylic acid	QCA	1.03	Antibiotic	55	Salbutamol	SBM	1.05	Bronchodilator
23	Chloramphenicol	CPH	1.17	Antibiotic	56	Theophylline	TEP	0.73	Bronchodilator
24	Thiamphenicol	TPH	–	Antibiotic	57	Clenbuterol	CLB	0.72	Bronchodilator
25	Griseofulvin	GRF	0.58	Antibiotic	58	Bezafibrate	BZF	1.16	Antilipidemic
26	Lincomycin	GRF	0.47	Antibiotic	59	Clofibric acid	CFB	0.42	Antilipidemic
27	Novobiocin	NVB	0.73	Antibiotic	60	Caffiene	CAF	0.48	Cardiac
28	Salinomycin	SAM	1.48	Antibiotic	61	Carbazochrome	CBZ	0.77	Hemostatic
29	Triclosan	TRC	–	Antibiotic	62	Cyclophosphamide	CYPP	0.66	Antitumor
30	Tiamulin	TIM	0.10	Antibiotic	63	N,N-diethyl-m-tolamide	DEET	0.11	Rejectant
31	Acetaminophen	ACEAM	0.84	Analgesic	64	p-Phenylphenol	PPP	–	Rejectant
32	Antipyrine	ATP	0.36	Analgesic	65	Pirenzepine	PZP	3.47	Peptic ulcer
33	Ethenzamide	ETZ	0.29	Analgesic	66	Sulpiride	SLP	0.05	Peptic ulcer

antibiotics such as Clarithromycin, 10 analgesic drugs such as Acetaminophen, 4 antiepilepsy drugs such as Carbamazepine, and the others such as Bezafibrate. All the compounds except Azithromycin and Levofloxacin were purchased from Wako Pure Chemical Company Ltd. to be prepared for standard solutions. Azithromycin and Levofloxacin were purchased from Fluka Chemicals. Clarithromycin, Sulfadimethoxine, and Sulfamonomethoxine were dissolved into acetone, Nicarbazin, Norfloxacin, Diclazuril were dissolved into N,N-Dimetyl Formamide, and all the other compounds were dissolved into methanol to prepare the stock solutions.

2.2. Analysis

All the samples were performed by using Solid-Phase Extraction (SPE) and the target compounds were analyzed with a Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) or an Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC/MS/MS). 20–100 mL of wastewater samples was filtered through a 1-μm glass fiber membrane filter (Whatman, GF/B) to separate dissolved and particulate phases. The SPE cartridge was conditioned using 3 mL of methanol and 5 mL of ultra-pure water. Samples were transferred to Oasis HLB cartridges (Waters, 200 mg bed, 6 cm^3 cartridge) at a flow rate of 10 mL/min. After drying the SPE cartridge using a vacuum pump, the elution was performed with 6 mL of methanol. The eluted solvent was evaporated to dryness by a gentle stream of nitrogen gas. The residue was dissolved in 1 mL of 0.1% formic acid–methanol mixture (85/15,v/v). The solution was analyzed by the LC/MS/MS or the UPLC/MS/MS.

In this study, two kinds of analytical devices were used; AQUITY UPLC (Waters) interfaced to Quattro micro API (waters) using Waters AQUITY UPLC BEH C_{18} (Waters, 2.1 mmφ × 100 mm, particle size:1.7 μm) as an analytical column, Agilent 1100 Series (Agilent) interfaced to API-4000 (Applied Biosystems) using Agilent Zorbax Eclipse XDB-C18 (Agilent, 2.1 mmφ × 150 mm, particle size:5 μm) was used as an analytical column. The limits of quantification for the target compounds by this analytical method were shown in Table 1.

2.3. Extraction

To decide the optimal extraction method, two extraction methods, ultrasonic solvent extraction (USE) and pressurized liquid extraction (PLE) methods, applying 16 extraction solvents were compared. According to the reports by Göbel et al. (2005a,b) and Hari et al. (2005), a ratio of methanol to water and pH of extraction solvents influence extraction efficiency of PPCPs from sludge. For this reason, 4 kinds of methanol concentration in the extraction solvent (water/methanol = 10/0, 9/1, 5/5, 0/10) and 4 kinds of pH (2, 4, 7, 11) were compared. In this study, pH was adjusted with hydrochloric acid and sodium hydroxide after mixing methanol and water. In most cases (Andersen et al., 2003; Gatidou et al., 2007), the compounds were extracted from the samples by USE. USE represents a simple and low-price approach. In a few cases (Hubert et al.,

Table 2
Overview of the WWTPs surveyed in this study.

WWTP	Process	Bloreactor	HRT (hr)	SRT (day)	Sampling season	Discharge amount (m^3/day)	Population
A	A-1	CAS with coagulation	5.6	18.4	Nov. 2007	57,000	99,000
	A-2	AO using carrier	2.8	14.2			
B	B-1	A2O	12.1	19	Nov. 2007	576,265	775,500
	B-2	AO	11.6	16			
	B-3	CAS	9.4	18			
C	C	AO with coagulation	10.9	17	Dec. 2007	50,000	236,000
D	D	AO with coagulation	14.1	13.1	Dec. 2007	9,500	33,900

CAS: conventional activated sludge process, AO: anaerobic–oxic process A_2O: anaerobic–anoxic–oxic process.

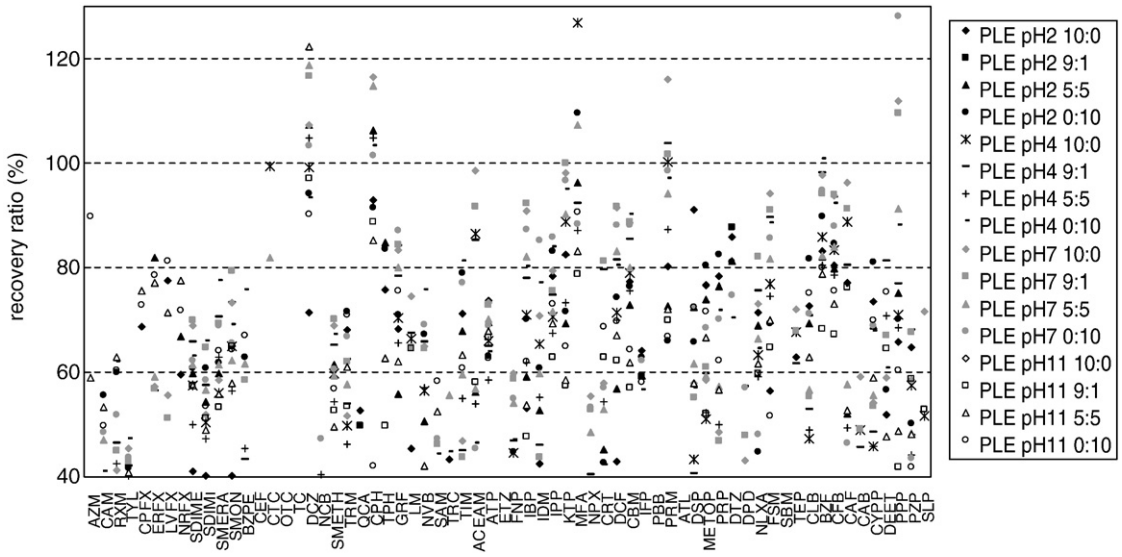

Fig. 1. Recovery ratio of the each compound for each extraction method (PLE). The ratio in the graph legends indicates the composition of the extraction solvent (water/methanol).

2000), PLE was also used. In using PLE, the samples are under high pressure and high temperature in small amount of solvent (Göbel et al., 2005a,b; Schlüsener et al., 2003; Golet et al., 2002; Smith, 2003). In this study, these two methods were compared.

The sludge sample was obtained by filtering 20 mL of primary sludge from a WWTP. We defined the optimal method to indicate the most proper recovery during extraction and pre-treatment process. The way to calculate the recovery is as follows: 6 same sludge samples for each extraction method were prepared. 3 samples were conducted without any operation before extraction (BL) and the other 3 samples were added with 50 μg of each compound before extraction (ADD). After extraction, cleanup was conducted with solid phase extraction (SPE) and measurement was conducted with LC/MS/MS or UPLC/MS/MS. Then, we compared the content of each PPCPs in the sludge between BL and ADD samples and calculated the recovery defined from the following Eq. (1):

$$\text{Recovery} = (\text{ADD} - \text{BL}) / \text{the amount of addition}. \quad (1)$$

For USE we used ultrasonic washing machine (SHARP UT-604F 600 W, 36–42 kHz). Each sample was extracted with 25 mL of an extraction solvent for 15 min at the first time, and for 10 min at the second and third times.

For PLE we used ASE-200 (Accelerated solvent extractor, Dionex). Each sample was transferred into 33 mL of an extraction cell and the airspace of the extraction cell was filled with glass beads. The extraction temperature of 100 °C and extraction pressure of 2000 psi were chosen for operating condition. Preheating period and static period were set 5 min and extracted 3 times. The total extraction solvent volume was 60% of the cell volume and was purged with nitrogen gas for 120 s to remove the solvent.

The extracted solvent was diluted with ultra-pure water so that the concentration of organic solvent was less than 5%. After addition of EDTA-2Na in 1 g/L, the diluted samples were performed with SPE followed by analysis by LC/MS/MS or UPLC/MS/MS.

2.4. Sampling at WWTP

Field surveys were conducted at 4 WWTPs in Japan to know the contents of the PPCPs sorbed to sewage sludge from November to December in 2007. All the WWTPs consist of primary settling tanks followed by the activated sludge process. Detailed information on

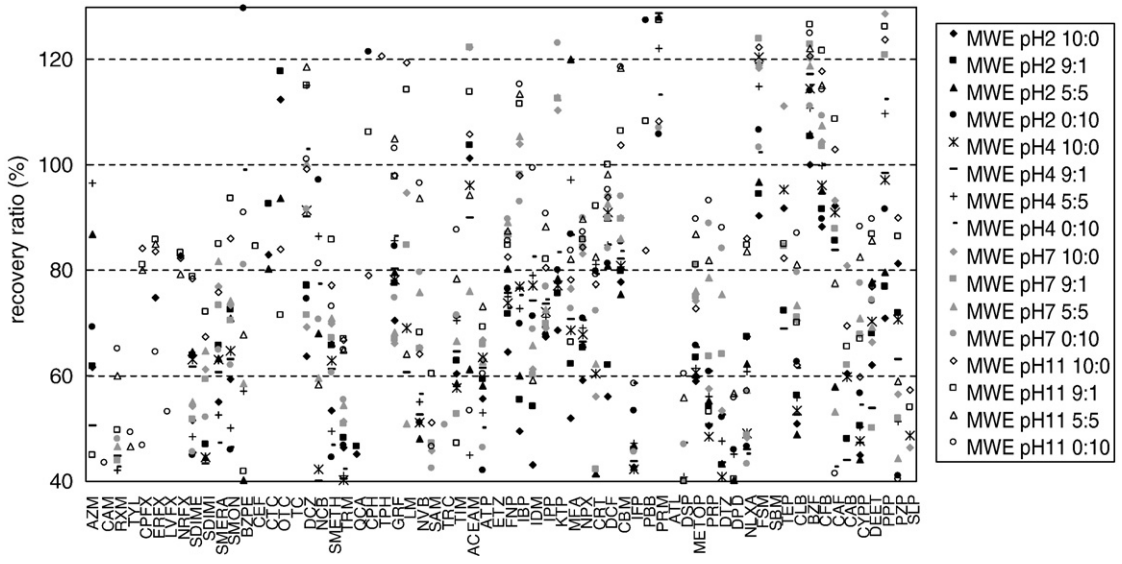

Fig. 2. Recovery ratio of the each compound for each extraction method (USE). The ratio in the graph legends indicates the composition of the extraction solvent (water/methanol).

Table 3
The number of the compounds whose averages of the recovery ratios were between 40% and 130% for each extraction and pretreatment method.

		PLE				USE			
		Water/menthol				Water/menthol			
		10/0	9/1	5/5	0/10	10/0	9/1	5/5	0/10
pH	2	39	–	38	41	41	40	40	40
	4	36	41	38	45	41	42	41	38
	7	45	45	40	44	39	39	38	39
	11	–	30	42	41	43	49	37	40

biological treatment processes such as HRT, SRT, sampling season, discharge amount, and population by each plant are listed in Table 2. We took 2 grab samples in each WWTP at noon; primary sludge and excess sludge. But at WWTP A, both samples were obtained by making 24-h composite samples. At WWTP B, the primary sludge sample couldn't be obtained.

3. Results and discussion

3.1. Extraction method

The optimal methods were selected by several groups depending on the compounds. First, the recovery ratio of each extraction and pretreatment method for each target compound was calculated from Eq. (1). Figs. 1 and 2 show the recovery ratios which were between 40% and 130% for each compound and each extraction method. In these figures, the value of recovery ratio extracted with USE was slightly higher than that with PLE for analgesic compounds, and antilipidemic compounds. Most of the recovery ratios of macrolide antibiotics (such as Azithromycin, Clarithromycin, Roxithromycin, and Tylosin), fluoroquinolone antibiotics (such as Ciprofloxacin, Enrofloxacin, Levofloxacin, and Norfloxacin) and sulfonamide antibiotics (such as Sulfadimethoxine, Sulfadimidine, Sulfamerazine, Sulfamonomethoxine ,and Sulfamethoxazole) were up to 90% in any extraction method. For analgesic compounds and sulfonamide antibiotics, the recovery ratio was relatively similar value for each method compared to other compounds. The number of the compounds whose recovery ratios were between 40% and 130% was shown in Table 3. The table indicates a matrix consisting of the two extraction methods of PLE and USE in 8 columns and the pH condition in 4 rows. The figures in the table indicates the number of the compounds which satisfied 40 to 130% of recovery ratios among the 66 PPCPs. From the results and each recovery ratio for each method, the optimal method was chosen. First, the method using USE with mixture of water and methanol (9/1,v/v) conditioned at pH11 was selected because the number of the compounds whose recovery ratios were between 40% and 130% was the largest. Then, for the compounds whose recovery ratio was low in the above method more appropriate methods and conditions were selected so as to satisfy the same recovery range. In the results, the method which uses the solvent with high content of methanol and the method which pH of the extraction solvent was low was selected.

The following optimal method consisting of 3 extraction and pretreatment methods was selected;

(i) the method using PLE with water conditioned at pH2
(ii) the method using PLE with methanol conditioned at pH4
(iii) the method using USE with mixture of water and methanol (9/1,v/v) conditioned at pH11.

The optimal extraction and pretreatment method was chosen for each compound from the above three methods and recovery ratio in the coincident method were shown in Fig. 3. In this figure, the ranges of the recovery ratios for the 32 extraction and pretreatment methods conducted in this study were also shown. The number of the combination was 32 because 2 extraction methods (PLE and USE), 4 solvent types and 4 pH conditions were tested as shown in Table 3. The circles plotted on the ranges are the recoveries of the selected methods. Based on the above three methods, all the compounds except Clarithromycin, Tetracycline, Atenolol, Ethenzamide, and Salbutamol, can satisfy 40 to 130% in the recovery. Therefore, we can conclude that the optimal extraction and pretreatment methods for the 66 kinds of the compounds were

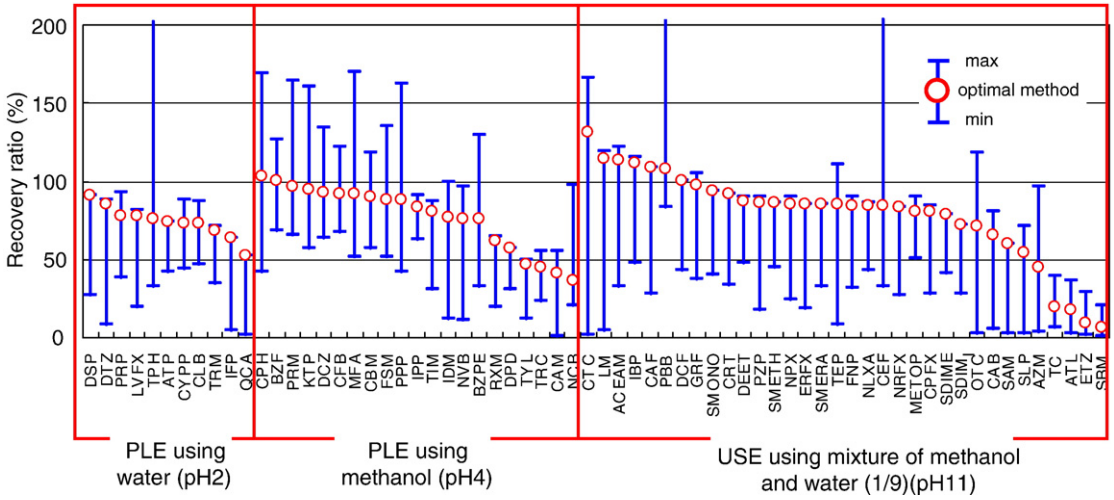

Fig. 3. The optimal extraction method for each compounds and each recovery ratio in that method and all the method.

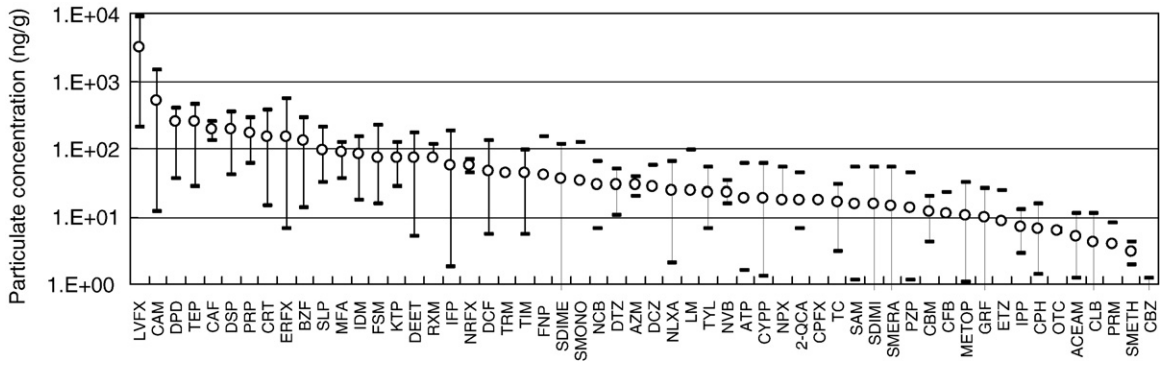

Fig. 4. Particulate concentrations of the PPCPs in the primary sludge.

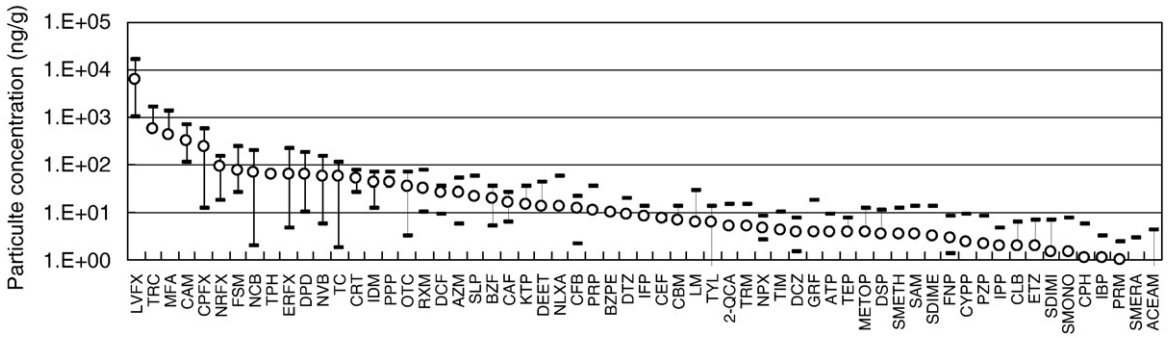

Fig. 5. Particulate concentrations of the PPCPs in the excess sludge.

developed. The recoveries of the 61 compounds extracted by the optimal method ranged between 40 and 130%.

The reason why the solvent with high content of water tended to be selected as appropriate extraction solvents for many compounds might be explained from the following three speculations. The first is that extracting with water can save the amount of solvent which pass through the cartridge during SPE because not so much amount of pure water was needed to dilute the extracted organic solvent. The second, since most PPCPs are relatively hydrophilic, more polar solvent is more suitable for extraction. The third, since water is one of the most polar solvent among the solvents, hydrophobic compounds in sludge tend to be less extracted, which results in decreasing ion suppression during measuring by LC/MS/MS.

In other reports, water was selected as one of the extraction solvent for the PPCPs. Kinney et al. (2006) have reported on the extraction method from sewage sludge for the 16 pharmaceuticals by using PLE with acetnitrile/water mixture (70/30, v/v). Göbel et al. (2005b) also reported that the optimal extracting solvent was mixture of methanol and water (1/1, v/v) for sulfonamide, macrolide, and Trimethoprim.

3.2. WWTP survey

3.2.1. PPCPs in the primary sludge

The dissolved and the particulate concentrations of the PPCPs in primary sludge at WWTP A, C, and D were measured. The particulate concentrations of the PPCPs in the primary sludge were shown in Fig. 4 which indicates the range and the mean of their concentrations among 3 WWTPs. As many as 56 out of 66 compounds were detected and the particulate concentration ranged from ng/g to μg/g where the content is express on unit dry weight. The compound indicating the highest concentration was Levofloxacin and that concentration was from 204 ng/g to 8680 ng/g (average 3110 ng/g). As for the particulate concentration, the compound indicating the second highest concentration was Clarithromycin (average 503 ng/g), which was followed by Dipyridamole (average 248 ng/g) and Theophylline (average 242 ng/g). The concentration of Norfloxacin (average54 ng/g) and Ciprofloxacin (average 17 ng/g) in the primary sludge samples in this study were much lower than those in other paper (Lindberg et al., 2006) which reported those concentration ranged from 1.7 to 4.2 mg/kg-dry and from 2.0 to 4.0 mg/kg-dry, respectively.

3.2.2. PPCPs in the excess sludge

The particulate concentration of the PPCPs in the excess sludge was shown in Fig. 5. As many as 61 out of 66 compounds were detected and the particulate concentration ranged from ng/g to dozens of μg/L. The compound indicating the highest concentration was also Levofloxacin (average 6310 ng/g), which was followed by Triclosan (average 573 ng/g), Mefenamic acid (average 433 ng/g), Clarithromycin (average 306 ng/g), and Ciprofloxacin (average 243 ng/g). There were many antibiotics such as Levofloxacin, Clarithromycin, Ciprofloxacin, and Norfloxacin (average 87 ng/g) found high concentrations in particle phase of the excess sludge. The concentration of Caffeine in the excess sludge (average 16 ng/g) was much lower than that in the primary sludge (average 193 ng/g). For this reason, Caffeine has the high biodegradation as Miao reported that Concentrations of caffeine were reduced by 99.9% (Miao et al., 2005).

Jacobs et al. (2005) reported that Triclosan may act as an endocrine disruptor and Orvos et al. (2002) also reported that Triclosan was shown to cause toxicity in aquatic organisms, especially algae. Therefore, the concentration of the Triclosan in the

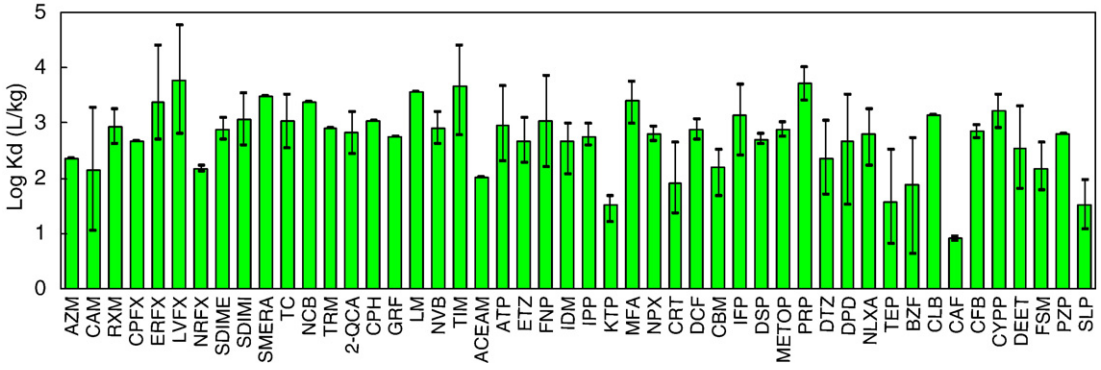

Fig. 6. Log K_d value of each compound in the primary sludge.

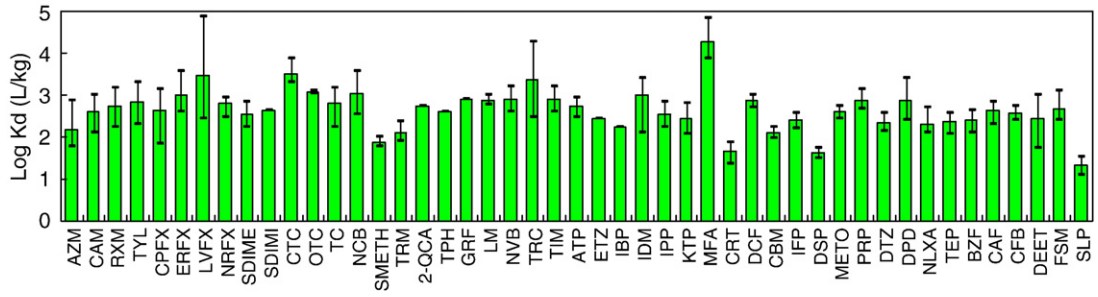

Fig. 7. Log K_d value of each compound in the excess sludge.

treatment process was needed to be monitored. The concentration of Norfloxacin and Ciprofloxacin in this study was more than ten times lower than those in other paper (Lindberg et al., 2006) which reported those concentration ranged from 1.4 to 1.7 mg/kg-dry from 2.2 to 2.7 mg/kg-dry respectively. The concentration of Carbamazepine which was not removed during the wastewater treatment (Clara et al., 2005) was 7 ng/g-dry in this study. The concentration of Tetracycline and Oxytetracycline in this study were 56 ng/g (average) and 34 ng/g (average), and these values were from 5 to 20, and from 2 to 50 times higher than those in the swine manure (Jacobsen and Sorensen, 2006) respectively. The concentrations of Caffeine, Clofibric acid (average 12 ng/g), Diclofenac (average 26 ng/g), Diltiazem (average 9 ng/g), Sulfamethoxazole (average 3 ng/g) were almost same value with the report by Spongberg and Witter (2008) which measured the concentration of the PPCPs in biosolids. But those of Ciprofloxacin, Clarithromycin in our study were around ten times higher than those in their study (Spongberg and Witter, 2008). On the other hand, the concentrations of Caffeine, Carbamazepine, Bezafibrate, Clofibric acid, and Diclofenac in our study were more than 5 times lower than those in the report which Nieto et al. (2007) measured sewage sludge. This trend for Caffeine, Clofibric acid, and Diclofenac was different with Spongberg and Witter (2008).

3.2.3. Solid-water distribution coefficient

Solid-water distribution coefficient (K_d) is defined a ratio of content in particle to that in water. K_d value of each PPCPs in primary sludge and excess sludge is shown in Figs. 6 and 7. The compound which had the highest K_d value was Levofloxacin (Log K_d = 3.8 L/kg) in the primary sludge and Mefenamic acid (Log K_d = 4.3 L/kg) in the excess sludge. On the other hand, the compound which had the lowest K_d value was Caffeine (Log K_d = 0.9 L/kg) in the primary sludge and Sulpirid (Log K_d = 1.4 L/kg) in the excess sludge. The K_d value of fluoroquinolone antibiotics was relatively higher than the other compounds.

There was no definite correlation between Log K_d and Log K_{ow} (octanol–water partition coefficient) (r^2 = 0.018). For this reason, many PPCPs exhibit ionic interactions with sludge which surface is negatively charged. For example, fluoroquinolone antibiotics have two pK_a value and can be positively charged, negatively charged, zwitterionic, or uncharged. Most of other compounds also have their own pK_a value and resulted in contributing the unexpectable content of PPCPs in the sludge. Kinney et al. (2006) also suggested that multiple mechanisms may be responsible for the incorporation of PPCPs into biosolids. The further study will be needed to determine the mechanism between PPCPs and sludge.

4. Conclusion

(1) Extraction method for the 66 PPCPs from primary sludge was newly developed. Combination of Pressurized Liquid Extraction using water (pH2), Pressurized Liquid Extraction using methanol (pH4) and Ultrasonic Solvent Extraction using mixture of methanol and water (1/9, v/v, pH11) was the most effective method satisfying recoveries of 40% to 130% for 61 PPCPs.
(2) The concentrations of as many as 56 compounds in the primary sludge and 61 compounds in the excess sludge were detected in Japan. The compound which had the highest concentration in primary sludge and excess sludge was Levofloxacin and the concentration was 3110 ng/g and 6310 ng/g respectively.
(3) The compound which had the highest K_d value was Levofloxacin in primary sludge and Mefenamic acid in excess sludge. On the other hand, the compound which had the lowest K_d was Caffeine in primary sludge and Sulpirid in excess sludge.

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