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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.