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Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues

Rumi Tanoue [a], Kei Nomiyama [a,*], Haruna Nakamura [b], Terutake Hayashi [c], Joon-Woo Kim [a,d], Tomohiko Isobe [a], Ryota Shinohara [b], Shinsuke Tanabe [a]

[a] Center for Marine Environmental Studies (CMES), Ehime University, 2-5, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[b] Graduate School of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, 3-1-100, Tsukide, Kumamoto 862-8502, Japan
[c] Tochigi Prefectural Museum, 2-2 Mutsumi-cho, Utsunomiya, Tochigi 320-0865, Japan
[d] Monitoring and Analysis Division, Seamangeum Regional Environmental Office, 100 Seogok-ro, Wansan-gu, Jeonju-si, Jeollabuk-do 560-870, South Korea

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ABSTRACT

In the present study, a sensitive and accurate isotope dilution method was developed for the simultaneous determination of 17 polar pharmaceutical and personal care product (PPCP) residues ($\log K_{ow}$ = 1.40–5.74), including 14 pharmaceuticals and 3 personal care products, in biological organs and tissues. The proposed method involved enzymatic hydrolysis, followed by sequential clean-up using silica gel chromatography and gel permeation chromatography, and analysis via ultra-high-performance liquid chromatography with tandem mass spectrometry. This method yielded acceptable absolute recoveries (48–88%) and internal standard-corrected recoveries (90–130%) for 17 PPCPs. Method detection limits were between 0.0092 and 3.2 ng g^{-1} wet weight, and the limits of quantification were between 0.020 and 8.7 ng g^{-1} wet weight. The method can be used to readily detect the target compounds at trace levels while minimizing the required sample volume. The developed method was applied to the determination of 17 PPCPs in the liver and kidney of 17 birds collected from Japan and also in the plasma, liver, and brain of 7 cyprinoid fish from an effluent-dominated stream in Japan. Triclosan was detected in 5 of 11 fish-eating birds but not in non-fish-eating birds, suggesting the contamination of prey fish by the chemical. Non-steroidal anti-inflammatory drugs, antibacterial agents, and psychotropic agents were frequently detected in the fish tissues. In addition, 7 of the target compounds were found in fish brain. The median brain/plasma ratios of the psychotropic agents ranged from 1.6 (carbamazepine) to 12 (diphenhydramine), indicating high transportability to fish brain.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are constantly introduced into the environment through human activities and thus have been detected in various environmental media such as surface water and sediments. Because of their continuous loading to the aquatic environment through effluent discharges from wastewater treatment plants (WWTPs), PPCPs are considered as "pseudo-persistent" contaminants. In fact, various PPCPs have been detected in wild fish inhabiting wastewater discharge areas, reflecting their constant exposure [1–4]. Among PPCPs, personal care products (PCPs) with high lipophilicity, such as synthetic musks and organic UV filters, have been detected in not only fish but also marine mammals and waterfowls [5,6]. In contrast, the bioaccumulation potential of polar PPCPs including pharmaceutical chemicals has been considered insignificant. However, *in vitro* and *in vivo* studies [7–9] recently showed that certain pharmaceuticals, such as antidepressants and antihistamines, have greater bioaccumulation potentials in fish and shrimp than those in terrestrial mammalian species. These results suggest that bioaccumulation of pharmaceutical chemicals is not determined only by their chemical lipophilicity. Considering the fact, continuous exposure to PPCP residues and their adverse effects on fish and fish-eating birds (top predators of the freshwater food web) may become a great concern.

It has been reported that diclofenac (DF), a non-steroidal anti-inflammatory drug (NSAID), is the principal cause for the marked decrease in endemic vulture species in India, Pakistan, and Nepal [10]. Although DF was detected at relatively low concentrations (51–643 ng g^{-1}) in the kidney of wild vultures, these levels were

* Corresponding author. Tel.: +81 89 927 8196; fax: +81 89 927 8196.
E-mail addresses: keinomi@agr.ehime-u.ac.jp, keinomiyama@gmail.com (K. Nomiyama).

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Table 1
Physicochemical properties of selected PPCPs.

Compound	Abbreviation	Therapeutic class	CAS no.	Formula	MW	pK_a[a]	log K_{ow}[b]	log D_{ow} (pH 7.1)	log D_{lipw} (pH 7.1)	Water solubility (mg L^{-1})[c]
Pharmaceuticals										
Diclofenac	DF	NSAIDs	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	295.0	4.00	4.06	0.96	1.87	4.518
Ethenzamide	ETZ	NSAIDs	938-73-8	$C_9H_{11}NO_2$	165.1	8.79, 6.30	1.40	1.40	2.21	14,110
Ibuprofen	IBP	NSAIDs	15687-27-1	$C_{13}H_{18}O_2$	206.1	4.85	3.72	1.47	2.26	41.05
Indometacin	IND	NSAIDs	53-86-1	$C_{19}H_{16}ClNO_4$	357.1	3.80	3.11	−0.19	0.97	3.114
Mefenamic acid	MF	NSAIDs	61-68-7	$C_{15}H_{15}NO_2$	241.1	3.89	5.33	2.12	2.77	1.121
Bezafibrate	BZF	Antihyperlipidemic agent	41859-67-0	$C_{19}H_{20}ClNO_4$	361.1	3.83	3.46	0.19	1.27	1.224
Clofibric acid	CA	Clofibrate metabolite	882-09-7	$C_{10}H_{11}ClO_3$	214.0	3.37	2.72	−1.01	0.33	582.5
Fenofibric acid	FA	Fenofibrate metabolite	42017-89-0	$C_{17}H_{15}ClO_4$	318.1	3.10	3.99	−0.01	1.11	9.114
Sertraline	SER	Antidepressant agent	79617-96-2	$C_{17}H_{17}Cl_2N$	305.1	9.85	4.81	2.06	2.73	3.517
Norsertaline	NSER	Sertraline metabolite	91797-58-9	$C_{16}H_{15}Cl_2N$	291.1	9.73	4.89	2.26	2.88	10.61
Diphenhydramine	DPH	Antihistamine agent	58-73-1	$C_{17}H_{21}NO$	255.2	8.87	3.66	1.88	2.59	362.7
Carbamazepine	CBZ	Antiepileptic agent	298-46-4	$C_{15}H_{12}N_2O$	236.1		1.90	1.90	2.60	17.66
Crotamiton	CTM	Anti-itch agent	483-63-6	$C_{13}H_{17}NO$	203.1		3.10	3.10	3.54	195.3
Losartan	LS	Hypertensive agent	114798-26-4	$C_{22}H_{23}ClN_6O$	422.2	7.40, 4.12	3.57	3.39	3.77	0.8223
Personal care products										
Triclosan	TCS	Antibacterial agent	3380-34-5	$C_{12}H_7Cl_3O_2$	288.0	7.68	5.17	5.07	5.07	4.621
Triclocarban	TCC	Antibacterial agent	101-20-2	$C_{13}H_9Cl_3N_2O$	314.0	11.4	5.74	5.74	5.60	0.6479
N,N-diethyl-3-toluamide	DEET	Insect repellent	94271-03-1	$C_{12}H_{17}NO$	191.1		1.96	1.96	2.65	666

MW, monoisotopic mass; NSAIDs, non-steroidal snti-inflammatory drugs; log K_{ow}, logarithm of octanol–water partition coefficient; log D_{ow} (pH): logarithm of octanol–water partition coefficient at a given pH value; log D_{lipw} (pH): logarithm of liposome–water distribution coefficient at a given pH value.
[a] Estimated values, from database of Chemicalize. org: http://www.chemicalize.org
[b] Estimated values, from database of Chemspider (ACD/Labs' ACD/PhysChem Suite): http://www.chemspider.com.
[c] Estimated values, from database of Chemspider (EPISuite): http://www.chemspider.com.

sufficient to cause serious renal failure and mass mortality. An *in vivo* study observed that these residue levels were comparable to the LD_{50} of DF for the White-rumped Vulture (98–225 ng g^{-1} b.w.) [11]. On the other hand, the reported LD_{50} is 2–3 orders of magnitude lower than that for rats [12]. These results indicate that species of the class Aves can be considered as sensitive to NSAIDs. In addition, several studies have reported the contamination of vultures inhabiting the Indian subcontinent by NSAIDs [10,13]. However, no report is available on the comprehensive monitoring of polar PPCPs in birds.

Several analytical methods for the determination of multi-class pharmaceuticals in biological matrices have been developed in the last few years [14,15]. However, there is still room for improvement with respect to the sensitivity and accuracy of these analytical methods, particularly for samples from biological tissues (*e.g.*, liver) with rich undesirable matrices (*e.g.*, lipids and proteins) that can interfere with analysis. In fact, many studies have focused on the determination of pharmaceutical residues in whole fish or muscle tissues [16], although high residue levels of pharmaceutical chemicals in other tissues such as the liver and brain have been found in some studies [2,17], which may be a critical fact when considering their adverse effects. Silica gel and/or gel permeation chromatography (GPC) cleanup have been often required for the removal of background interference from samples with complex biological matrices [18]. However these approaches have rarely reported in the literature for relatively more polar compounds, such as pharmaceutical chemicals. Although Subedi et al. [19] previously tried to develop analytical methods based on pressurized-liquid extraction (PLE) combined with silica-gel cleanup, followed by GPC for 13 PCPs and 6 pharmaceutical chemicals, it resulted in the loss of 4 pharmaceutical chemicals due to their low recoveries. Huerta et al. [15] most recently previously reported an analytical method for the determination of 20 pharmaceutical chemicals in fish homogenates, liver, and muscle based on PLE and GPC. However, the accuracy and precision of the method varied for different fish matrices, suggesting a lack of versatility and robustness.

The aim of this study was to develop an accurate and sensitive analytical method for the simultaneous determination of 17 polar PPCPs, including 14 pharmaceuticals and 3 personal care products (log K_{ow} = 1.40–5.74, Table 1) in various biological tissues. Extraction after enzymatic hydrolysis and subsequent clean-up procedures [GPC, silica gel chromatography, and solid-phase extraction (SPE)] were optimized and validated. In addition, we provided reasonable rationale for selection of optimal alternative internal standards (ISs) and evaluated the efficacy of alternative ISs for correction of significant matrix effects when analogous ISs were not available for some PPCP. The proposed analytical method was subsequently applied to the determination of the target compounds in the liver and kidney of birds collected from Japan as well as in the plasma, liver, and brain of fish obtained from an effluent-dominated stream in Japan.

2. Materials and methods

2.1. Chemicals and materials

The analytical standards 2-ethoxybenzamide (ETZ), carbamazepine (CBZ), ibuprofen (IBP), triclocarban (TCC), and sertraline-d_3 hydrochloride (SER-d_3) were purchased from Sigma–Aldrich (St. Louis, MO, USA). DF, indomethacin (IND), mefenamic acid (MF), losartan potassium (LS), crotamiton (CTM), fenofibric acid (FA), (1R, 4S)-N-desmethyl sertraline hydrochloride (NSER), sertraline hydrochloride (SER), diphenhydramine hydrochloride (DPH), N,N-diethyl-3-toluamide (DEET), carbamazepine-d_{10} (CBZ-d_{10}), indomethacin-d_4 (IND-d_4), ibuprofen-$^{13}C_3$ (IBP-$^{13}C_3$), diclofenac-d_4 (DF-d_4), losartan-d_3 (LS-d_3), diphenhydramine-d_6 hydrochloride (DPH-d_6), N,N-diethyl-3-toluamide-d_6 (DEET-d_6), triclocarban-$^{13}C_6$ (TCC-$^{13}C_6$), and triclosan-$^{13}C_{12}$ (TCS-$^{13}C_{12}$) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Bezafibrate (BZF) and clofibric acid-d_4 (CA-d_4) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Triclosan (TCS) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and (1R,