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Risk evaluation of pharmaceutical residues in waste water from selected treatment plants in Gwangju, South Korea

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ABSTRACT

The occurrence and removal of three anti-inflammatory drugs (ibuprofen, diclofenac and ketoprofen), an anti-epileptic drug (carbamazepine), an antipyretic drug (acetaminophen), an antibiotic (sulfamethoxazole) and a lipid regulator (gemfibrozil) in influent and effluent samples from three wastewater treatment plants (WWTPs) excluding hospitals and industrialized locations in Gwangju were evaluated. Analytical determination was carried out by high performance liquid chromatography interfaced with a mass spectrometer (LC-MS). All pharmaceutically active compounds (PhACs), except diclofenac, were detected in wastewater influents and effluents. Diclofenac was detected in only one influent sample at a concentration of 7.8 ng/L. Other target compounds were detected at various ranges of concentrations*:* acetaminophen (7.4-12.9 ng/L), carbamazepine (0.4-35.0 ng/L), sulfamethoxazole (0.1-4.2 ng/L), ketoprofen (55.4-888.4 ng/L), gemfibrozil (16.16-17.1 ng/L), and ibuprofen (22.6-8330.9 ng/L). Removal rates of the pharmaceuticals ranged between 2.9 % to 100 %. Risk quotient, which was expressed as a ratio of the measured environmental concentration (MEC) and predicted no-effect concentrations (PNEC) were evaluated for green algae, fish and invertebrates. Majority of the pharmaceuticals, except acetaminophen, were found in effluent samples at sufficiently high concentrations that would pose adverse risk to aquatic organisms.

Keywords: emerging contaminants, pharmaceuticals, wastewater, risk assessment, WWTPs

INTRODUCTION

Reports on the detection of pharmaceutically active compounds (PhACs) in the environment have since spurred emerging concern [1-3]. These compounds are tagged "emerging" because they are not routinely monitored but have the potential of entering the environment to cause known or suspected adverse ecological and (or) human health effects. Of more concern is the fact that pharmaceuticals are developed to produce biological activity and therefore having them where they are not expected to be found (i.e. in untargeted media) simply makes them contaminants –hence potential pollutants. Also, they can be referred as "pseudo-persistent" contaminants for the reason that their transformation and/or removal rates are counter-balanced by continuous inputs into the environment [4].

The occurrence of pharmaceuticals in the environment is to a large extent dependent on the prevalent local diseases, treatment methods and market profiles which in turn lead to significant variations in pollution profiles between geographic locations/countries [5]. Even while several studies have been done on the potential environmental impacts of pharmaceuticals, it is also necessary to continually monitor their

concentrations in various environmental compartments especially where such data are scarce [6]. In doing so, the possibility of correlating potential impacts and the detectable concentrations in the environment will help in proper environmental risk assessment and management.

Several other studies on human pharmaceuticals with special focus on industrially impacted areas or on hospital wastewater treatment plants in the Korean environments have previously been reported [7,8]. However, studies on risk assessments of pharmaceuticals in wastewater samples from other sources (example, municipal effluents) within the Gwangju area of South Korea are limited.

The aim of the present study was to investigate the extent of PhACs residues in wastewater samples of municipal origins from three WWTPs in Gwangju, South Korea. The study further evaluates the the potential risks associated with the various concentrations of the pharmaceuticals with respect to environmental exposure.

MATERIALS AND METHODS

General Characteristics of WWTPs

In this study, the WWTPs were considered to be similar in characteristics as they were located within close distances from each other to suggest population of the same magnitude and similar treatment technologies (membrane bioreactor and membrane filtration) were employed. Furthermore, since the sampling campaign was done within a short period of time (within three weeks), wastewater samples collected from the three WWTPs were considered to be homogenous with negligible seasonal and temperature variations. This way, the samples collected were assumed to be replicate representation of WWTPs of the study area.

Sample Collection, Preservation and Preparation

Wastewater samples were collected between July and August 2013 from three municipal wastewater treatment plants denoted here as A, B and C. A total of twelve (12) grab samples were collected, two (2) from each influent and effluent points, into 1 litre amber bottles and stored at 4 ^{o}C until analysis. Upon reaching the laboratory, the two grab samples from each sampling points of the WWTPs were merged into composites. Field grab samples were preserved by adding 250 mg/L sodium thiosulphate ($Na_2SO_3S_2$). Sample filtration was done using Pall water filtration apparatus with 47 mm GF/F glass microfilter membrane into a clean amber bottle using a vacuum pump.

Extraction of Samples by SPE

Before the extraction, 2 g of EDTA and 10 ml of 0.25 M ammonium acetate solution were added to all filtered samples after which the pH value of each sample was adjusted to 6.95 ± 0.05 using 10% (w/v) NaOH and 10% (v/v) H_2SO_4. In addition to the wastewater samples, 400 ml deionized water each was used to prepare a blank and 2 spiked samples (300 µl of level 3 standard) according to method documented by [9, 10]. Target analytes were extracted using hydrophilic lipophilic balance (HLB) cartridges (Oasis HLB 6cc, 200mg) from Waters Corporation (Milford MA, USA). The HLB cartridges were preconditioned with 5mL of 10 % (v/v) methanol/water at 2 mL/min and then 400 mL of samples were loaded onto the SPE at 10 mL/min to extract target compounds, after which cartridges were rinsed with 10 mL water and 5mL 5% methanol at 2 mL/min. This was followed by cartridge drying by gentle nitrogen streaming for 30 min. The target compounds were then eluted from the SPE cartridge with 5 mL methanol at 1mL/min. Samples were concentrated to 1mL using TurboVap Concentration Evaporator Workstation (Life Sciences, USA) and transferred to vials before analysis.

LC-MS analysis

Analysis of pharmaceuticals was done using liquid chromatography-mass spectrometry (LC-MS). The LC component consisted of a high performance liquid chromatography (HPLC) – Waters Alliance 2695 while the MS system consisted of Micromass Quattromicro API triple stage quadrupole with multimode ionization mass spectrometer from Waters Alliance, Milford MA, USA. Chromatographic separation was carried out using a reversed phase analytical column SunFire^{TM} C_{18} –3.5µm (2.1×150mm)

from Waters, Milford, USA. The chromatographic conditions were as follows: column temperature of 40 ^{o}C, mobile phase solvents A (85% H_2O and 0.1% HCOOH) and B (15% CH_3CN and 0.1% HCOOH), flow rate 0.250 ml/min. Gradient programme: Isocratic 85% A for 1min, then to 15 % B for 3 min, increased linearly to 80 % B for 6 min and held for 3 min, then stepped to 100 % B for 8 min, and finally to 15 % B for 9 min.

The mass spectrometric detection was performed in both positive and negative modes of the electrospray ionization (ESI) interface and multiple reaction monitoring mode (MRM). The MS operating parameters were: capillary temperature 350 ^{o}C, source voltage 3.5 0kv, capillary voltage 29 V, drying gas flow rate 30l /min. The list of pharmaceuticals selected for analysis, their therapeutic class, structure and other properties is presented in Table 1. The target compounds were selected based on the fact that they are among the most commonly used pharmaceuticals in South Korea [8]. Percentage recoveries were determined using mixture of standards purchased from Smart Solutions (O2Si), USA. The lowest (32 %) recovery was recorded for acetaminophen while the highest was recorded for carbamazepine (126 %) (Table 2). The limit of detection (LOD) of each compound was defined as LOQ (limit of quantitation) multiplied by 3. The LOQ was calculated based on the standard deviation of the replicate measurements (STDev) and the slope of the calibration curve (SC) using the formula LOQ = 10*(STDev/SC). Concentrations below the LOD were assumed to be below detection limits (BDL).

Removal rates of PhACs

The average concentrations of pharmaceutical compounds found in effluents after treatment were compared with those obtained from for influents of the WWTPs. Removal rates of the monitored PhACs were calculated using the equation from [11], shown below:

$$(C_{inf} - C_{effl}) \times 100/C_{inf}$$

where C_{inf} is the averaged concentration of a pharmaceutical compound found in the influents of the three WWTPs and C_{effl} is the average concentration measured in effluents of the three WWTPs.

Risk Assessment of PhACs

The risk posed by certain contaminants in aquatic environment can be assessed through the calculation of risk quotients (RQ) as described elsewhere [12, 13]. An RQ value of a single contaminant for aquatic organisms was calculated from measured environmental concentrations (MEC) of the effluents and predicted no effect concentration (PNEC) using the equation shown below:

RQ = MEC/PNEC

PNEC values were calculated from lowest measured effective concentration (EC_{50}) for fish, green algae and invertebrate for each compound obtained from literature and the assessment factor of 1,000 following the equation below:

PNEC = EC_{50}/1000

An assessment factor of 1000 was introduced to account for extrapolation from intra- as well as inter-species/media variability in sensitivity [14]. Nevertheless, errors still exist while deriving the PNEC values since toxicity data are substantially influenced by several factors, including the lifecycle stage of the organism, properties of the surrounding environment, and the experimental conditions [15]. A commonly used risk ranking criteria was applied: RQ<0.1 means minimal risk, 0.1≤RQ<1 means medium risk, and RQ≥1 means high risk [14].

RESULTS AND DISCUSSION

Occurrence of PhACs in WWTPs

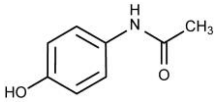
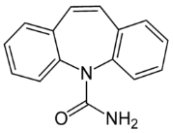
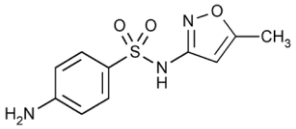
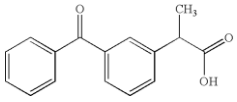
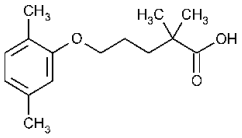
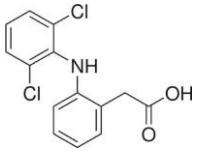
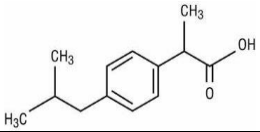
The chromatograms obtained from the LC-MS analysis of some samples are presented in in Fig. 1. The total concentrations of the pharmaceuticals found in the WWTPs are presented in Table 3. It can be seen in Table 3 and Figure 2 that ibuprofen tends to be more prevalent in the WWTPs followed by ketoprofen and acetaminophen.

Diclofenac was found only in the influent of WWTP-A at the concentration of 7.8 ng/L.

Diclofenac has been reported to be non-persistent in the aquatic environment, possessing a short half-life of <1 day and susceptible to photodegradation ($t_{1/2}$ = 4h) [20]. The available information on the environmental fate of diclofenac suggests that it is environmentally labile [11], explaining why it was found in only one out of all the samples analyzed. However, higher levels (3.54 ng/ml in influent and 2.56 ng/ml in effluent samples) were detected in some Polish wastewater treatment plants [21]. Other compounds targeted were detected at various ranges of concentrations *viz*: acetaminophen (7.4-12.9 ng/mL), carbamazepine (0.4-35.0 ng/mL), sulfamethoxazole (0.1-4.2 ng/mL), ketoprofen (55.4-888.4 ng/mL), gemfibrozil (16.16-17.1 ng/mL), and ibuprofen (22.6-8330.9 ng/mL).

In comparing the results with the survey carried out on the distribution of some of these pharmaceuticals in effluents of some Korean WWTPs by [8], it was observed that acetaminophen and gemfibrozil were detected within the ranges: 1.8-19 ng/L.

Table 1: Properties of pharmaceuticals targeted in this study

S/N	Pharmaceuticals	Abbreviation	Therapeutic group	Structure	pKa	logK_{ow}
1	Acetaminophen	ACT	Analgesic		9.4[a]	0.46[a]
2	Carbamazepine	CBM	Anti-epileptic		0.37[a]	2.45[a]
3	Sulphamethoxazole	SMX	Antibiotic		5.6[a]	0.89[a]
4	Ketoprofen	KEP	Analgesic/Anti-inflammatory		4.45[b]	3.12[b]
5	Gemfibrozil	GFC	Lipid regulator		4.45[c]	4.77[d]
6	Diclofenac	DCF	Analgesic		4.15[a]	0.70[a]
7	Ibuprofen	IBU	Analgesic		4.9[a]	3.97[a]

Sources: [a] [16], [b] [17], [c] [18], [d] [19].

Table 2: Limit of detection (LOD) and recoveries of target compounds

S/N	Compounds	LOD (ng/L)	% Recovery
1	Acetamidophen	0.5	32
2	Carbamazepine	0.004	126
3	Diclofenac	0.03	115
4	Gemfibrozil	0.06	110
5	Ibuprofen	0.4	74
6	Ketoprofen	0.06	124
7	Sulfamethoxazole	0.03	99

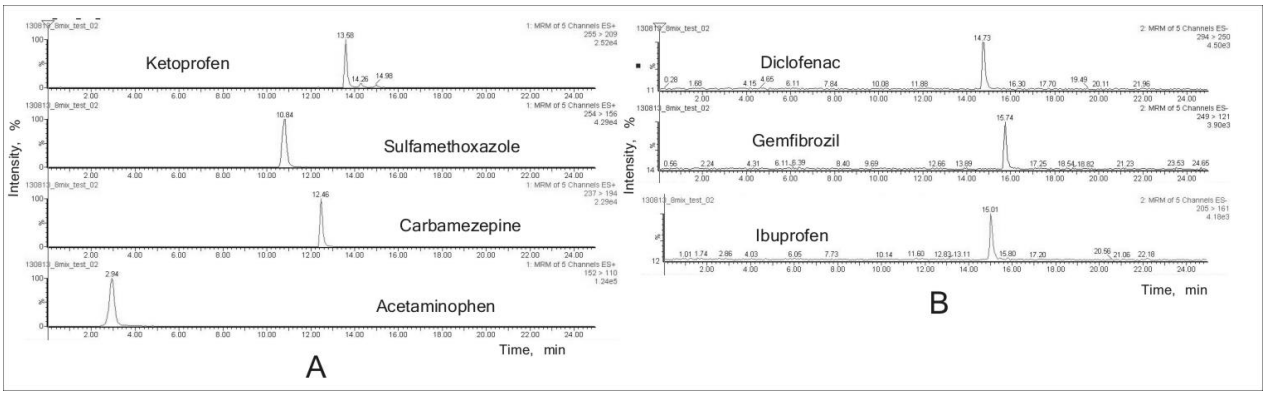


Fig. 1: Chromatograms of samples from WWTPs, showing compounds analyzed in positive (A) and negative (B) ESI modes with their retention times

Table 3: Total concentrations of detected compounds

Treatment plants	Measured concentrations of each compounds (ng/l)[a]						
	ACT	CBM	SMX	KEP	GEC	DCF	IBU
	Influent						
WWTP-A	BDL	0.4	BDL	BDL	17.1	7.8	84.9
WWTP-B	161.4	37.8	4.2	885.4	BDL	BDL	496
WWTP-C	38.5	BDL	BDL	BDL	BDL	BDL	8330.9
Total Influent	199.9	38.2	4.2	885.4	17.1	7.8	8911.8
	Effluent						
WWTP-A	BDL	BDL	BDL	BDL	16.6	BDL	22.6
WWTP-B	7.4	35	0.1	55.4	BDL	BDL	41.1
WWTP-C	12.9	BDL	BDL	BDL	BDL	BDL	0
Total effluent	20.3	35	0.1	55.4	16.6	BDL	63.7

[a]Samples were composite of two grab samples (n=2); BDL = below detection limit

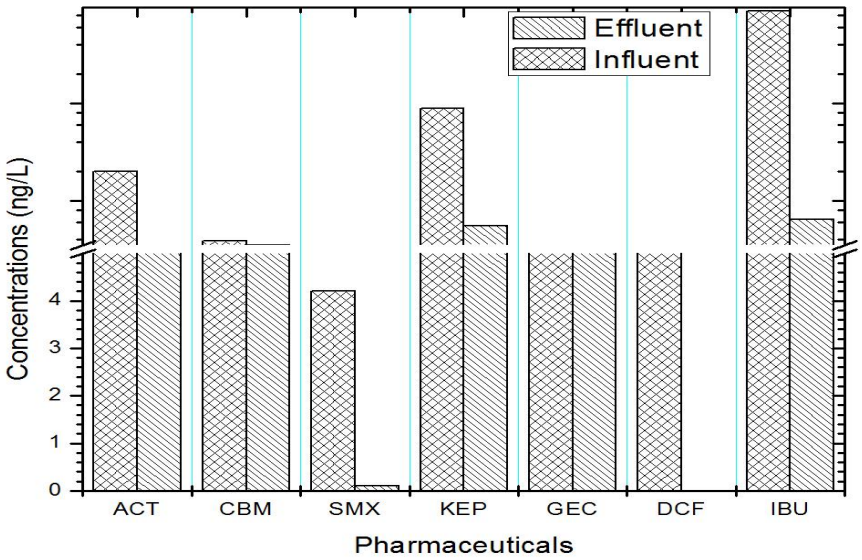


Fig. 2: Comparison of pharmaceuticals in influents and effluents of WWTPs of the three WWTPs

In terms of the frequency of occurrence of PhACs in the studied WWTPs, the prevalence of target compounds followed the order: WWTP-B>WWTP-A>WWTP-C in both influents and effluents (Table 3). Concentrations of pharmaceuticals reduced drastically in the effluents of the WWTPs.

Removal Rates of PhACs in the WWTPs

The removal rates of pharmaceutical compounds in each of the WWTPs were combined to obtain a representative their removal efficiencies per compound. The removal rates of compounds from WWTPs are shown in Figure 3. The results obtained for each target compound are comparable with data available in literature. The maximum removal rate for acetaminophen was 89.84 % comparable with 79 % reported by [7] while for others: carbamazepine (8.38 %) comparable with 8 % reported by [3], Ibuprofen (98.93 %) comparable with 98% reported by [22]; ketoprofen (93.74 %) comparable with 98 % reported by [23]. Gemfibrozil removal rate was very low (2.9 %), low removal rate (16-46 %) was also reported by [24]. Diclofenac removal rate was 100 % which is comparable to 98-100 % reported for conventional WWTPs by [25]. As previously said, diclofenac is environmentally labile. The maximum removal rate for sulfamethoxazole was 97.62%. In contrast, this value was far greater than that (55.6 %) reported by [26].

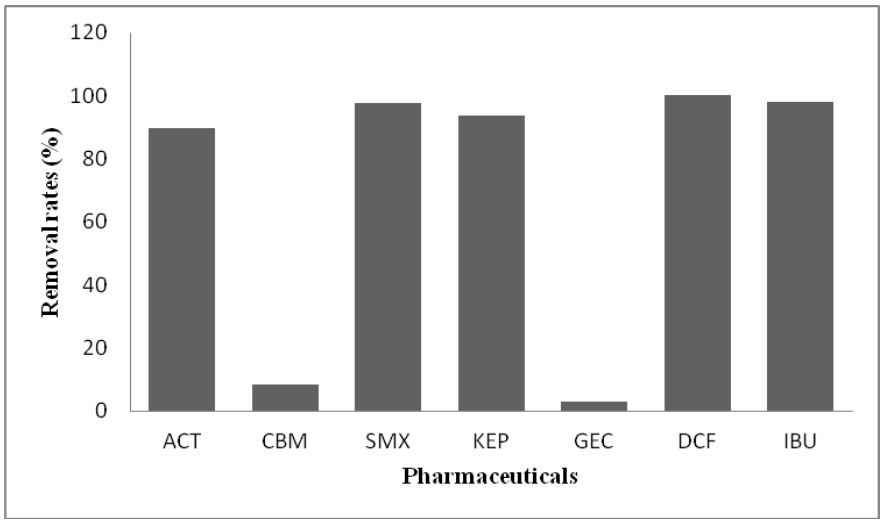


Fig. 3: Removal rates (%) of the targeted pharmaceuticals computed from an average data from the three WWTPs of the three WWTPs

Table 4: Calculation of risk quotients for green algae (a), fish (f), invertebrate (i) of pharmaceuticals in wastewater effluents from the three WWTPs

Pharmaceuticals	MEC (μg/L)	EC_{50} (μg/L)	PNEC (μg/L)	RQ (MEC/PNEC) (EC_{50}/1000)	Potential risk level
Acetaminophen	10.15	134000(a)[e]	134	0.08	Low
		378000(f)[e]	378	0.03	Low
Carbamazepine	17.50	33600(a)[g#]	33.6	0.52	Medium
		35400(f)[h]	35.4	0.49	Medium
		13800(i)[g]	13.8	1.27	High
Sulphamethoxazole	0.10	30(a)[j]	0.03	3.33	High
		562500(f)[h]	562.5	0.0002	Low
		15500(i)[j]	15.5	0.006	Low
Ketoprofen	55.40	32000(f)*	32	1.73	High
		164000(a)*	164	0.33	Low-Medium
Gemfibrozil	16.60	900(f)[k]	0.9	18.44	High
		4000(a)[k]	4	4.15	High
Diclofenac	BDL	-	-	-	-
Ibuprofen	31.85	7100(a)[m]	7.1	4.50	High
		173000(f)[n]	173	0.18	Low-Medium
		101200(i)[n]	101.2	0.31	Low-Medium

MEC: measured environmental concentration; BDL: Below detection limits; diclofenac was not detected in the effluents obtained from the three WWTPs. Values indicated with * mean that EC_{50} is estimated with ECOSAR. Data were taken from [27, 28].
[e] [29]; [g] [30]; [j] [31]; [h] [32]; [k] [14]; [m] [33]; [n] [1]; [#]blue-green algae.

Risk Assessment of PhACs

In this study, the environmental risk of pharmaceuticals in the wastewater effluents were evaluated since the effluents are most times disposed directly into nearby aquatic environments. Due to the different modes of action of pharmaceuticals, risk of individual target compounds were evaluated. The combined measured environmental concentration in effluents of WWTPs (MEC), predicted no effect concentrations (PNECs) and risk quotients (RQ=MEC/PNEC) to aquatic organisms are shown in Table 4. A commonly used risk ranking criteria was applied: RQ<0.1 means minimal risk, 0.1≤RQ<1 means medium risk, and RQ≥1 means high risk [14].

The potential risk level of acetaminophen was low to fish and green algae since RQ values were far less than unity. On the other hand, carbamazepine posed medium to high risk to the aquatic organisms. Sulfamethoxazole posed a high risk to green algae and medium risk to fish and invertebrates since RQ values were approaching unity. Ketoprofen posed significantly high risk to fish and low-medium risk to green algae. The potential risk level of gemfibrozil was high for both fish and algae. Ibuprofen posed a high risk to algae and low-medium risk to fish and invertebrates.

For the tested aquatic organisms for which risk quotients of target PhACs were lower that unity, the implication is that ecological risk expected would be minimal or nil. However, when the risk quotient is greater than one, it is considered that the aquatic organisms under study are exposed to some risks.

CONCLUSION

Samples from three wastewater treatment plants in the urban municipal areas of Gwangju in South Korea were analyzed by LC-MS after sample treatment by solid phase extraction (SPE). All pharmaceuticals, except diclofenac, were detected in wastewater influents and effluents. Ibuprofen was present in the highest concentration in both influent and effluent samples in spite of high removal rates achieved in all the WWTPs monitored. Removal efficiencies were in the range from 2.90 % (gemfibrozil) to 100 % (diclofenac). Targeted pharmaceutical compounds, except acetaminophen, were found in effluent samples at sufficiently high concentrations that would pose significant risk to aquatic organisms. This research work has revealed that apart from hospital effluents and environmental impacts due to industrial activities, significantly toxic concentration of pharmaceuticals may be released into the environment from municipal activities in a typical urban setting.

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