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Mass-balance-model-based evaluation of sewage treatment plant contribution to residual pharmaceuticals in environmental waters

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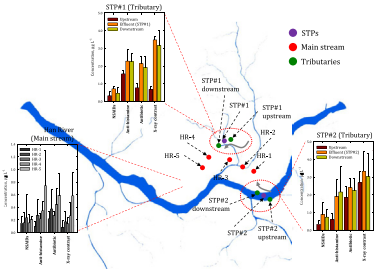
HIGHLIGHTS

- Mass balance model evaluating STPs contribution to water pollution by PPCPs.
- On-line SPE-LC-MS/MS based analytical method for quantifying PPCPs.
- PPCPs discharged by STPs flowing into main river.
- Minimization of PPCPs discharge from STPs required to preserve clean natural water.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a total of 52 pharmaceuticals in the influent and effluent of two sewage treatment plants (STPs) and in the receiving waters were quantified with an analytical method using on-line solid phase extraction coupled to liquid chromatograph-tandem mass spectrometry. 36 out of the 52 pharmaceuticals were detected in the influent and effluent of the STPs at quantifiable levels; influent and effluent concentrations ranged 1 ng L^{-1} to 30 μg L^{-1} and 3 ng L^{-1} to 3 μg L^{-1}, respectively. They were also detected from the receiving waters (both tributaries and main river); their concentrations ranged from 1 to 310 ng L^{-1}. A simple mass balance model was applied for the pharmaceutical data measured for the STPs, tributaries, and the main river to demonstrate the contribution of the STPs to the pollution of the streams and the main river. The average ratio of the model estimations and the measured concentrations was calculated 97% for the downstream of the STPs, while that for the main river was 89%. This modeling result clearly demonstrates that many pharmaceuticals flowing into an STP are not degraded and discharged to a nearby river, affecting the whole water body, and that the STP is the only source of the pollutants. While their discharge into STPs should be avoided. In addition, development of new technologies capable of completely degrading them is desirable.

1. Introduction

The presence of residual pharmaceuticals in stream, groundwater, lake, and even drinking water has been reported worldwide (Furlong et al., 2017; Kay et al., 2017; Yao et al., 2018). Many kinds of

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pharmaceuticals are being produced and consumed at a large amount, so their leak into water environment has drawn attention from the public. Therefore, good pharmaceutical production and disposal practices from the sustainability point of view are being sought for in the developed countries. In addition, aggressive sustainable conservation plans are being implemented in the countries to minimize the introduction of pharmaceuticals into the environment; therefore, further strengthen regulations and guidance on pharmaceuticals in natural waters are expected in near future (Benson et al., 2017; Küster and Adler, 2014; Paranychianakis et al., 2015).

At present, sewage is the most important among various sources of pharmaceuticals detected in the water environment (Luo et al., 2014). Pharmaceuticals in sewage are only partially decomposed in a sewage treatment plant (STP) before they are discharged into the environment. Therefore, a great deal of attention has been paid to how many pharmaceuticals would be in wastewater and how much of them could be removed within an STP for the past two decades. How the chemicals remaining in the treated wastewater would affect the receiving water body also has been of public concern.

A number of papers have reported a variety of pharmaceuticals in wastewater and in the environment. Most of them simply monitored dozens or hundreds of pharmaceuticals in the influent and effluent of STPs (Baker and Kasprzyk-Hordern, 2013; Sonya et al., 2015). Some of them assessed the contribution of different dischargers to the pharmaceutical concentrations of the influent to their STPs, while others compared the effluent of STPs and receiving water bodies. In general, the contribution of an STP to the concentrations of trace organic compounds like residual pharmaceuticals in a receiving river is evaluated by comparing their discharged amounts (Archer et al., 2017; Azuma et al., 2016) and their concentrations (Kay et al., 2017; Patrolecco et al., 2015) at a sampling location of the river. In recent studies, mass-balance approaches have been applied to estimate the contribution of residual pharmaceuticals in the effluent of an STP to the receiving water body (Fairbairn et al., 2016), or in-stream attenuations of pharmaceuticals along a river (Hanamoto et al., 2018). These studies provided useful information about the relation between an STP and its receiving water body. However, they analyzed the compounds in samples collected from only a few locations, so the impacts of mass loading from the STP on the receiving water were not clearly illustrated. A more in-depth and systematic study is required in which concentrations of pharmaceuticals at the point where STP effluent is discharged and its upstream and downstream locations are quantified to show the dynamics of the compounds along the stream. In order to track these emerging contaminants along a stream and design a control or management practice for them, an easy analytical method should be available that can be used for accurately assessing their extent of decomposition in an STP or dynamics in receiving water (Ratola et al., 2012).

Gas chromatograph/mass spectrometry (GC/MS) along with liquid-liquid extraction has been utilized to quantify residual pharmaceuticals in the environment since the early 1990s (Pietrogrande and Basaglia, 2007). Using these methods, however, quantification of trace pharmaceuticals (under ppb level) is not possible, mainly due to their high detection range and complicated sample pretreatment steps (especially for wastewater or sludge samples). Since solid-phase-extraction (SPE) followed by liquid chromatograph-tandem mass spectrometry (LC-MS/MS) was introduced, trace pharmaceuticals at sub-ppb levels in various water matrixes have been quantified (Cahill et al., 2004; US EPA, 2007; Vanderford et al., 2003). The SPE technique facilitates effective removal of interferences from such sample matrices as wastewater and superior extraction and concentration of target compounds. However, clean-up of an SPE column and sample extraction through the column are manually carried out, so routine monitoring of target pharmaceuticals in the environment cannot be easily done, especially when the physico-chemical characteristics of target analytes are diverse: e.g., acid-base dissociation, polarity, and hydrophobicity (Pavlović et al., 2007). The manual sample-pretreatment is not only labor-intensive but also often reduces the precision of the analysis.

Recently, on-line SPE methods have been introduced as a way to analyze multi-residues, in which sample pretreatment for residual organic compounds (e.g., pharmaceuticals, pesticides, phenols, phthalates, and perfluorinated chemicals) in water is simplified and automated (Casado et al., 2018; Kim et al., 2018; Idder et al., 2013). The US Geological Survey (USGS) successfully applied an on-line SPE method followed by LC-MS/MS for quantifying 35 pharmaceuticals in ground water (Meyer et al., 2007); samples were loaded on the pretreatment unit only after a simple filtration. A similar method was successfully applied for the quantification of steroid hormone compounds in the effluent of a STP (Guedes-Alonso et al., 2015).

In this study, an on-line SPE column with a switching valve system, which is automated for higher analytical accuracy, coupled to LC-MS/MS was applied for analyzing 52 pharmaceutical compounds in river waters and in the influent and effluent of two STPs (i.e., STP#1 and STP#2) and the contribution of the STPs to the water pollution of the receiving water bodies by the compounds was evaluated. In order to evaluate the contribution of STPs to the concentration of each target compound in river waters, a mass balance model considering only hydraulic dilution was applied for the distribution of pharmaceutical in the downstream of the effluent-discharging point of each STP.

2. Experimental

2.1. Chemicals and reagents

Fifty-two standards used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), Fluka (St. Louis, MO, USA), USP (Rockville, MD, USA), EDQM (Strasbourg, France), and MCE (Monmouth Junction, NJ, USA). Acetaminophen-D_4, ciprofloxacin-D_8, and sulfadimethoxine-$^{13}C_6$ were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as surrogate standards. Acetonitrile, methanol, and water of MS grade were purchased from Honeywell (Muskegon, MI, USA). Formic acid and of MS grade, and disodium ethylene diamine tetra acetic acid (Na_2EDTA) of ACS reagent grade were purchased from Sigma-Aldrich. All the stock solutions of target compounds were prepared at a concentration of 100 mg L^{-1} in water or methanol. Structures and physico-chemical properties of 52 target pharmaceuticals studied in this paper are provided in Table S1 (see Supplementary material).

2.2. Sample collection and preparation

In this study, sample collection points were carefully determined to effectively evaluate the contribution of STPs to pharmaceutical pollution of the water environment. Water samples were collected seven times from April 10th to April 28th, 2017 in the spring seasons along Han River and its two tributaries in Seoul, Korea (Fig. 1). There was no precipitation during sampling events; 5 sites along Han River (HR-1~5), 4 sites along the two tributaries (J-up, J-down, T-up, and T-down). Additionally, influent and effluent samples of two STPs; one is located by Tributary J (denoted as STP#1) and the other is Tributary T (denoted as STP#2).

STP#1 has a treatment capacity of 945,000 m^3 d^{-1} and treats mainly sewage discharged from residential areas. It has two main

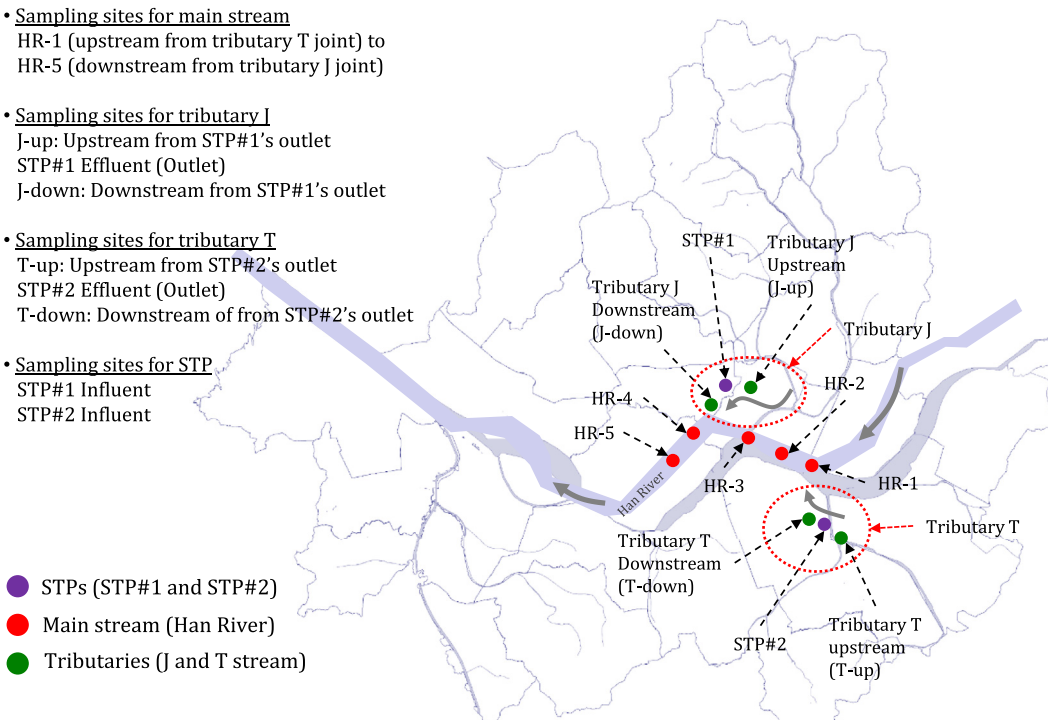

Fig. 1. Sampling sites around STP#1 and STP#2, including those on the main stream of Han River (five red circles) in Seoul, Korea. STP#1 located tributary J and STP#2 located tributary T stream (two violet circles) were taken samples from influent and effluent. Tributary samples indicate by the for green circles which are up- and downstream of a 500 m distance from STP's outlet connected J and T stream. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

processes; one is the Modified Ludzack Ettinger (MLE; Anoxic/Oxic) treating 670,000 $m^3\ d^{-1}$ and the other is the Anaerobic/Anoxic/Oxic (A^2O) process treating 275,000 $m^3\ d^{-1}$. In the case of STP#2, it treats wastewater discharged from both residential and commercial areas. Its main process is the MLE process and has a treatment capacity of 565,000 $m^3\ d^{-1}$.

At each site, a water sample of 2 L was manually collected into a dark amber glass bottle. Once water samples were collected, they were immediately transported in coolers to the university laboratory where total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), ammonium, phosphate, nitrate, and nitrite content were immediately analyzed following Standard methods (Table S2; APHA, 2012). The rest of the samples were sealed and stored at 4 °C in a dark fridge.

Before SPE-LC-MS/MS analysis was performed, 10 mL of each water sample was filtered with a 0.2 μm polyvinylidene fluoride syringe filter (Advantec, Tokyo, Japan). Then, methanol (10%, v/v) and formic acid (0.1%, v/v) were added to the filtrate along with 4 mg Na_2EDTA. The standard solutions for the development of a calibration curve were prepared in the same manner.

2.3. On-line SPE-LC-MS/MS analysis

Quantitative analysis of target compounds in water samples was performed using an SPE-LC-MS/MS system consisting of LC-MS/MS (LCMS-8050™, Shimadzu, Kyoto, Japan) and an on-line SPE column with a switching device. A schematic diagram for the analytical system is presented in Fig. 2. In this study, a methylcellulose-immobilized column was adopted to effectively remove the matrix interferences. Prepared water samples of 300 μL each were injected into an SPE column (Shim-pack MAYI-ODS; 10 mm × 2.0 mm in diameter, 50 μm particles; Shimadzu, Kyoto, Japan) and eluted at a flow rate of 0.4 mL min^{-1} using 0.1% formic acid in water. 0.1% formic acid (in water) was selected as the extraction solvent, considering the dissociation of acidic compounds among the target analytes. The optimal extraction flow rate and time to trap the target compounds were determined to be 0.4 mL min^{-1} and 2.5 min, respectively (Fig. S1). Once the target compounds were trapped, the pretreatment column was back-flushed with a solution of 0.1% formic acid in water/acetonitrile/methanol/isopropanol (v/v/v/v = 1/1/1/1) by switching the flow-selection valve on or off. Then, the column was stabilized and gotten ready for the analysis of the next sample.

The fifty-two pharmaceuticals in water samples were separated using an ACE5 C18-PFP column (150 mm × 2.1 mm in diameter, 5.0 μm particles; Advanced Chromatography Technologies, Scotland, UK) installed in the LC. The samples were eluted through the column at a flow rate of 0.2 mL min^{-1} with the column-oven temperature maintained at 40 °C. The gradient program of the mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B) was as follows. In the beginning, the mobile phase was kept at 10% B for 2.5 min, followed by a linear gradient to 100% B over 10.5 min, and kept for an additional 4 min. Finally, the mobile phase was returned to 10% B over 3 min to make the column ready for the next injection. All target analytes were quantified in a multiple-reaction monitoring (MRM) mode using electrospray ionization. The flow rates of the nebulizing gas (N_2), drying gas (N_2), and capillary heating gas (dry air) were 3, 10, and 10 L min^{-1}, respectively. The capillary, desolvation, and heating block temperatures were maintained at 300, 250, and 400 °C, respectively. The MRM parameters were optimized for collision energies (see Table S3 for more information). All the samples were analyzed three times.

Since the entire extract is injected into the analytical column, the sensitivity can be increased to the target concentration with <1 mL of sample (Andrade-Eiroa et al., 2016). However, at the same

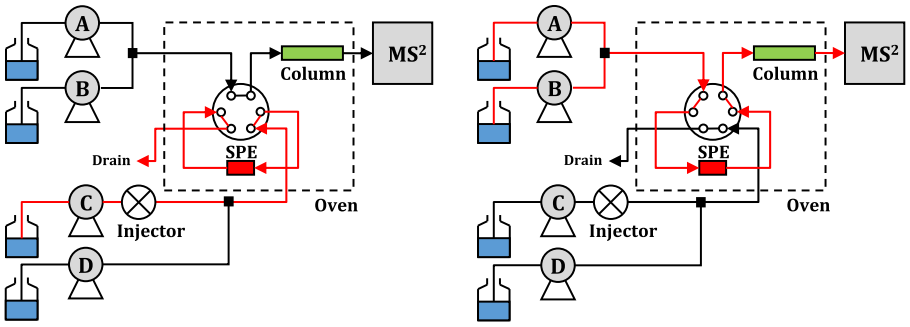

Fig. 2. Configuration of the on-line SPE-LC-MS/MS system. I. separation column equilibration (pump A and B); II. sample loading and trapping (pump C); III. SPE desorption (pump A and B) and cleaning (pump D).

time, direct injection of the sample can lead to contamination or clogging of the SPE column. Thus, it is very important to select an SPE column that can effectively remove the matrix interferences, while accurately trapping the target compounds. The bonded phases of octadecylsilane (ODS or C_{18}), hydrophilic-lipophilic-balanced (HLB) polymer and alkyl bonded silica have been used for manufacturing an SPE column; the separation phase has particles with the size of 2–25 μm with pores of 10–13 nm. In order to determine multi-class pharmaceuticals with different chemical properties, optimized operation conditions for the on-line SPE method should be considered.

2.4. Method validation

The linearity of calibration curve, recovery efficiency, repeatability, and method detection limits (MDLs) for each target compound were evaluated to validate the suitability of the final optimized method based on the on-line SPE-LC-MS/MS. The MDL for a target compound was determined by multiplying the Student *t*-value at the 99% confidence level by the standard deviation of seven replicates of a standard solution (0.005 or 0.05 μg mL^{-1}). Both recovery and repeatability were calculated based on the analytical results for samples spiked with the standards at three levels. The calibration range for each target analyte was determined based on the sensitivity of the analytical instrument for the chemical. For the recovery tests, samples at three levels (0.05, 0.4 and 0.8 μg L^{-1}, or 10 times higher level) were prepared by spiking each of 52 pharmaceuticals into the ultrapure water directly; seven replicates were made for each level. Similarly, samples for repeatability test of the developed method was prepared by spiking standards into water, and then were analyzed for calculating % relative standard deviation (%RSD; n = 7) for each analyte.

2.5. Setting up mass balance model

A simple dilution or mass balance model (eq. (1)) was set up using the MS Excel 2016 (Microsoft Inc., Redmond, WA, USA) for the target compounds in the upstream of a tributary and in the effluent discharged from an STP to estimate their concentration in the downstream. The model was applied only for the 36 pharmaceuticals detected in the current study. The calculated concentrations of the target compounds in the downstream of the two tributaries (i.e., J-down and T-down) were, then, compared with the measured values, which are presented as % ratio (eq. (2)).

$$C_{i,\,c,T-down} = \frac{C_{i,T-up} \times Q_T + C_{i,eff} \times Q_{STP}}{Q_T + Q_{STP}} \quad (1)$$

$$Mass\ balnce\ (\%) = \frac{C_{\,i,m,\,T-down}}{C_{i,\,c,T-down}} \times 100 \quad (2)$$

where $C_{i,T\text{-}up}$ and $C_{i,m,T\text{-}down}$ are the concentration of a pharmaceutical in the upstream and downstream of a tributary, respectively. $C_{i,eff}$ is the concentration of a pharmaceutical in the STP effluent, and Q_T and Q_{STP} are the flow rate of a tributary and of the effluent from an STP, respectively. Lastly, $C_{i,c,T\text{-}down}$ is the calculated concentration of a pharmaceutical in the downstream of a tributary.

2.6. Data analysis and calculation

Statistical analysis and graphical presentation of the results obtained in this study were performed using the MS Excel 2016 and the SigmaPlot 13 (Systat Software Inc., Chicago, IL, USA), respectively. The calculation of the mass balance for predicting the distribution of pharmaceuticals in the downstream of the STPs was carried out using the MS Excel.

3. Results and discussion

3.1. Optimization of on-line SPE-LC-MS/MS

In order to simultaneously analyze 52 pharmaceutical residues in the environmental water and wastewater, the on-line SPE-LC-MS/MS system should be optimized. First, the linearity and signal stability of the method were evaluated using standard solutions of target pharmaceuticals. In general, a good linearity and sensitivity could be developed for all the compounds except quinolones and tetracyclines. In the case of quinolones and tetracyclines, signal stability tended to deteriorate during a batch sequence in which a standard sample was diluted with water and injected (data not shown). The phenomenon was attributed to the chemical property of the target compounds; because of the zwitterionic characters of quinolones (Golet et al., 2002) ($pK_{a,COOH} = 6.0–6.2$, $pK_{a,NH2} = 7.6–8.7$ for ciprofloxacin, enrofloxacin, ofloxacin, and pefloxacin) and tetracyclines (Huq et al., 2006) ($pK_{a,zwitterion} = 6.5–6.6$ for chlortetracycline, oxytetracycline, and tetracycline), these compounds tend to dissociate at a circum-neutral pH and be adsorbed to metal-based surface or particles. Thus, formic acid was added to prevent dissociation of tetracycline and quinolone and their binding to divalent metallic cations such as Fe^{2+}, Ca^{2+}, and Mg^{2+} in the samples. Then, Na_2EDTA was added as a

chelator to facilitate enhanced trapping of the compounds by the SPE. However, when the methanol content of a sample increased over 10%, the trapping efficiencies of the SPE column for some compounds such as acetaminophen, and iopromide deteriorated.

The mobile phase of the column for separating the 52 pharmaceuticals was selected for better peak shapes and sensitivity following the method presented in our previous study with off-line SPE (Hong et al., 2015); in short, 0.1% formic acid in water and acetonitrile were used as the mobile phase. Using the mobile phase, target compounds could be better retained on the SPE column. In order to reduce matrix interference and improve the trapping efficiency of the SPE column, the sample loading rate was varied within the range of 0.4–2 mL min^{-1} (Fig. S1). As a result, the best efficiency was obtained when the SPE column was loaded at 0.4 mL min^{-1} for 2.5 min. Especially, hydrophilic compounds (e.g., amoxicillin, acetaminophen, cimetidine, and iopromide) (Table S1), which have a low log *P* (approximately < 1) and a relatively high water-solubility, tend to be less retained on a reversed-phase SPE column. In general, an on-line SPE method is loaded at a relatively high speed for the purpose of sufficient removal of interfering substances. If the sample loading rate is slow, macro-molecules (e.g., humic substances, biological fluids, surfactants, and polymers, etc.) present in the water or wastewater might clog the pore of the SPE column and the LC separation column. Due to these limitations, an on-line SPE method is rarely applied for wastewater or turbid stream water samples.

In this study, to overcome the limitations mentioned above, we used an SPE column the surface of which was modified with hydrophilic polymers to have holes which prevent clogging of the column by macro–size hydrophilic non–target molecules (Fig. S2). In short, when a sample is being loaded, large molecules are not held by the stationary phase of the SPE column. However, smaller molecules were easily trapped by the stationary phase. Moreover, the optimized trapping time was sufficient to remove matrix interferences from the SPE column (Fig. S1). After the trapping and extraction, the SPE was washed using an SPE washing solvent made of 0.1% formic acid in water/acetonitrile/methanol/isopropanol (v/v/v/v = 1/1/1/1) as a cleaning step.

Influent samples of an STP#1 were loaded on the optimized SPE column at the flow rate of 0.4 mL min^{-1}; the loading volume was 300 μL (Fig. S1). Based on the UV absorption data, sample matrix-interferences were not retained in the SPE column and almost completely released within 2.5 min (approximately 99%) (injection volume of <300 μL). On the other hand, when the flow rate was increased over 0.4 mL min^{-1}, the recovery of several compounds (e.g., acetaminophen, amoxicillin, atenolol, cimetidine, iopromide, and vancomycin, etc.) with a relatively short retention time was rapidly reduced about <50%.

Sample eluent and its volume, trapping and extraction conditions, and valve switching time were optimized. Under the optimal condition, the linearity (R^2) and sensitivity of the proposed method were all > 0.99 and in the level of a few ng L^{-1} or even less, respectively. Therefore, the optimized on-line SPE method could be utilized in the routine analysis of pharmaceuticals in wastewater and river waters in this study.

3.2. Method validation

To examine the validity of the proposed method, the linearity of calibration curves, recovery (%), MDL, and repeatability (% RSD, $n = 7$) were evaluated (Table S4). Re-analysis of the whole batch was carried out when the accuracy measured for the sample which was prepared for each batch of 20 samples and spiked with a surrogate was less than 75%.

The MDLs of the proposed method ranged from 1 to 80 ng L^{-1} for 32 compounds, while those for the rest (21 compounds) were below 1 ng L^{-1}. These MDLs were comparable to or better than those obtained using off-line-SPE-based analytical methods (Hong et al., 2015). These MDLs were also comparable with those reported in the literature about the analytical methods based on-line SPE-LC-MS/MS. Idder et al. (2013) applied an on-line SPE–LC–MS/MS method for analyzing 40 pharmaceuticals in drinking and surface water samples and reported limits of detection (LODs) of 0.7–15 ng L^{-1} with a sample injection of 1 mL. Similar or somewhat better LODs (0.1–1.4 ng L^{-1}) were obtained in studies where 24 priority pesticides, hormones, or pharmaceuticals included in European Union (EU) Directive 2013/39/EU (European Commission, 2013) and EU Decision 2015/495 (European Commission, 2015) were analyzed with on-line SPE–LC–MS/MS; in the study, the volume of sample injection was 10 mL.

The calibration curves of all the target pharmaceuticals reveal that a goodness of fit (R^2) > 0.99 was significant at a confidence level of 95%. The recoveries of all the target pharmaceuticals ranged from 81 to 113% except for enrofloxacin; that of which ranged from 65 to 92%. The repeatability of the analytical method was also excellent; it was calculated to be below 19% for the low level and 10% for the higher levels. Results obtained from the samples spiked with surrogate standards showed reproducibility of <20%: acetaminophen-D_4, 87.6 ± 13.2%; ciprofloxacin-D_8, 86.4 ± 17.1%; and sulfadimethoxine-$^{13}C_6$, 96.4 ± 10.8%.

In summary, the test result for the method validation demonstrated that the proposed analytical method based on on-line SPE-LC-MS/MS can easily and accurately evaluate residual pharmaceuticals in various waster matrixes including sewage.

3.3. Occurrences of selected pharmaceuticals in wastewater and their receiving water

Water samples were collected at five locations along Han River (HR-1 to HR-5), two along a tributary flowing from the northern side of the river (J-up and J-down), and two along a tributary from the southern side of it (T-up and T-down). In addition, samples were collected from the inlets and outlets of two STPs (Fig. 1). The collected samples were analyzed for the 52 target pharmaceutical compounds and the result is summarized in Table S5. Thirty-six out of the 52 target chemicals were detected at quantifiable levels both in the influent and effluent of the two STPs. The detected pharmaceuticals were grouped and summed-up according to their purpose of use and are presented in Fig. 3. In general, since the influents of both STP#1 and STP#2 are domestic wastewater, the levels of the pharmaceutical compounds in the influents were similar.

Acetaminophen, caffeine, cimetidine, and iopromide (an X-ray contrast agent) were all found at > 1 μg L^{-1} in the influent. Noticeably, the concentrations of iopromide in the influent and effluent of the two STPs were as high as 748–2462 ng L^{-1} and 1121–2809 ng L^{-1}, respectively; since there are several major hospitals in the drain areas of the STPs, such a high concentration of iopromide could be expected. Although non-steroidal anti-inflammatory drugs (NSAIDs; a group of drugs that reduce pain, fever, and inflammation) were also found at high concentrations in the influent sewage (31–34,979 ng L^{-1}), most of them were removed in the STPs. However, as these NSAIDs, which are sold without a prescription for domestic use in Korea, continuously flow into STPs via municipal wastewater, the impact of these pharmaceuticals on the microbial activity in the STPs should be further investigated. On the other hand, antihistamine, β-blocker, antibiotics, x-ray contrast medium, anti-neuropathic, lipid regulator, and antiviral drugs also were partially removed in the STPs; a significant amount of the compounds remained in the effluent. Atenolol, propranolol (β-

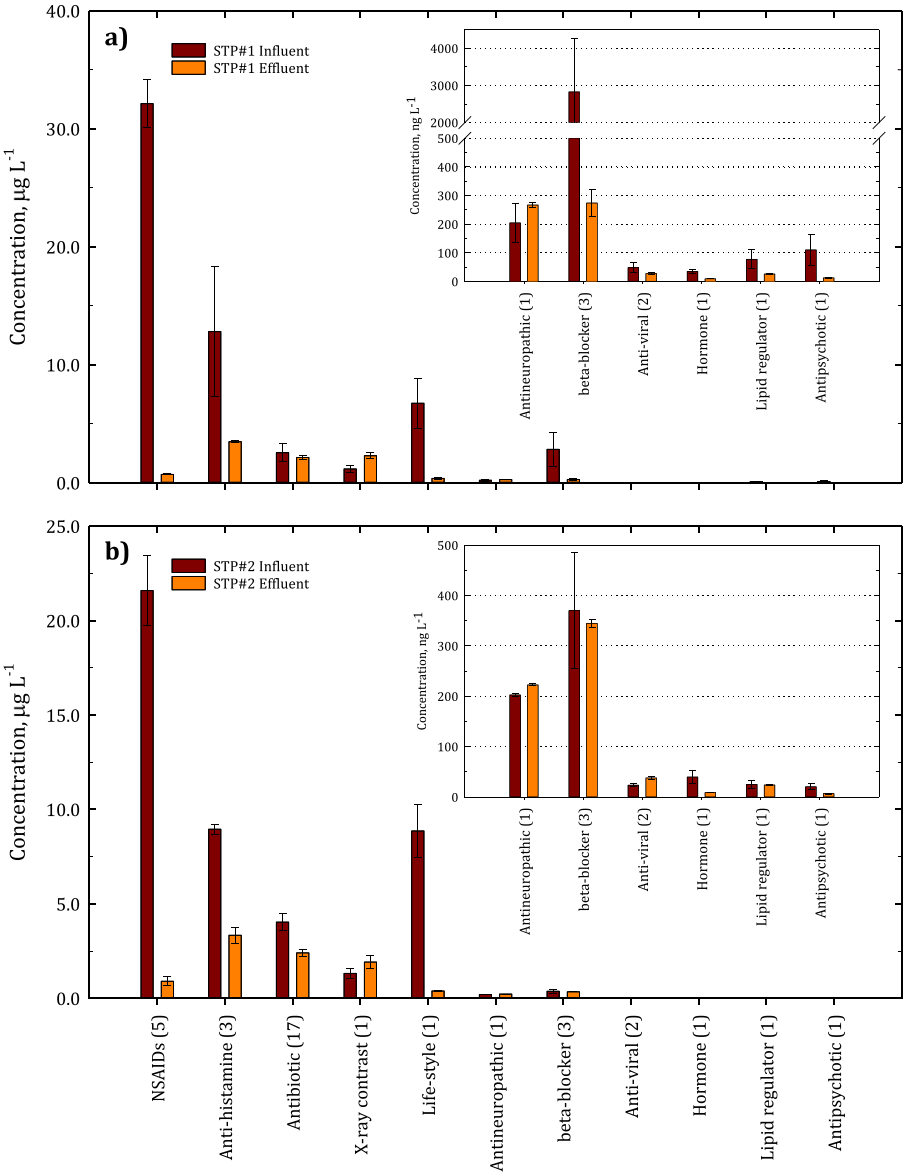

Fig. 3. Influent and effluent concentration by pharmaceutical classification in (a) STP#1 and (b) STP#2 located in Fig. 1. Bars and error bars indicate seven samples means and standard errors of classifications of target pharmaceuticals ($n = 7$). The classifications of target pharmaceuticals in this study were as follows: i) NSAIDs (5); acetaminophen, diclofenac, ibuprofen, ketoprofen, and naproxen, ii) Anti-histamine (3); chlorpheniramine, cimetidine, and diphenhydramine, iii) Antibiotic (17); cefradine, cephalexin, ciprofloxacin, clarithromycin, enrofloxacin, erythromycin, lincomycin, ofloxacin, oxolinic acid, roxithromycin, sulfachlorpyridazine, sulfadimethoxine, sulfamethoxazole, sulfathiazole, tetracycline, trimethoprim, and vancomycin, iv) X-ray contrast (1); iopromide, v) Life-style (1); caffeine, vi) Antineuropathic (1); carbamazepine, vii) beta-blocker (3); atenolol, metoprolol, and propranolol, viii) Anti-viral (2); oseltamivir and its metabolite oseltamivir acid, ix) Hormone (1); testosterone, x) lipid regulator (1); gemfibrozil, and xi) Antipsychotic (1); quetiapine.

blocker), and gemfibrozil (lipid regulator), which are used to treat high blood pressure and hyperlipidemia, were detected in both influent and effluent samples; their concentrations in influent and effluent in total were 25–559 ng L^{-1} and 18–327 ng L^{-1}, respectively. The mean concentrations of carbamazepine (an antineuropathic or antiepileptic agent) ranged from 203 to 295 ng L^{-1} and 236–309 ng L^{-1} in the influent and effluent samples, respectively. Of the pharmaceuticals in the antibiotics family, only quinolones (ciprofloxacin and ofloxacin), macrolides (clarithromycin, lincomycin, and roxithromycin), sulfamethoxazole, tetracycline, and vancomycin were detected at sub-μg L^{-1} levels. This result is consistent with our previous observations (Hong et al., 2015).

Although the sampling was performed in warm spring (April), oseltamivir and its metabolome, i.e., oseltamivir acid, which are used as an antiviral agent for influenza, were detected from both influent and effluent of the two STPs; the concentrations of oseltamivir in the influent and effluent were 3–25 and 5–10 ng L^{-1}, respectively. In general, it could be easily conjectured that their levels in the winter would be much higher because antiviral drugs are usually prescribed in the winter. Therefore, their concentrations in the influent and effluent of STP#1 were measured in the following winter. As expected, their levels were much higher; the average concentrations of oseltamivir and oseltamivir acid were 270 and 1127 ng L^{-1} in the influent and were 322 and 1228 ng L^{-1} in the effluent, respectively.

Comparing to those of the influent to the STPs, the pharmaceutical concentrations of Han River and its two tributaries were one or two magnitudes lower mainly due to dilution in the river

water (Table S5). For better illustration, the levels of target pharmaceuticals in the upstream and downstream of the two tributaries where the STPs are located are compared in Fig. 4. For both tributaries (i.e., Tributary J and T), water samples collected from the downstream of each STP showed similar or higher pharmaceutical levels than the respective upstream, indicating the adverse impact of the STPs.

For clearly demonstrating the adverse contribution of the STPs to the water pollution by the pharmaceuticals, a simple dilution or mass balance model (eq. (1)) was applied for the 36 pharmaceuticals detected in the upstream of a tributary and in the effluent discharged from an STP to estimate their concentration in the downstream. The estimated concentrations of the pharmaceuticals in the downstream of the two tributaries (i.e., J-down and T-down) were, then, compared with the measured values, which are presented as % ratio. As a result, the average ratio of the predicted and measured values of pharmaceutical levels in the downstream was 97% (Fig. 5). This result strongly indicates that treated wastewater discharged from the STPs contributes to the pharmaceutical levels of the downstream of the tributaries (i.e., J-down and T-down).

We also estimated the concentrations of pharmaceuticals in the samples collected from HR-5 by making a mass balance with the concentration data for the pharmaceuticals detected at HR-1 and the tributaries of Han River (T-down and J-down) and the result was depicted in Fig. 6. As shown in the figure, most of the pharmaceutical groups in total increased from the upstream to the downstream of Han River (HR-1 to −5). Since the upstream from HR-1 is protected as a water source conservation area, the inflow of pollutants near the site is relatively limited.

Interestingly, the predicted concentrations of the pharmaceuticals at the HR-5 were reasonably close to the measured ones; the average ratio of the predicted and measured concentrations was calculated 89%. Therefore, it was concluded that the tributaries fed with pharmaceutical compounds by the STPs are negatively affecting the whole Han River. In short, this result clearly demonstrates that residual drugs are continuously released from STPs and

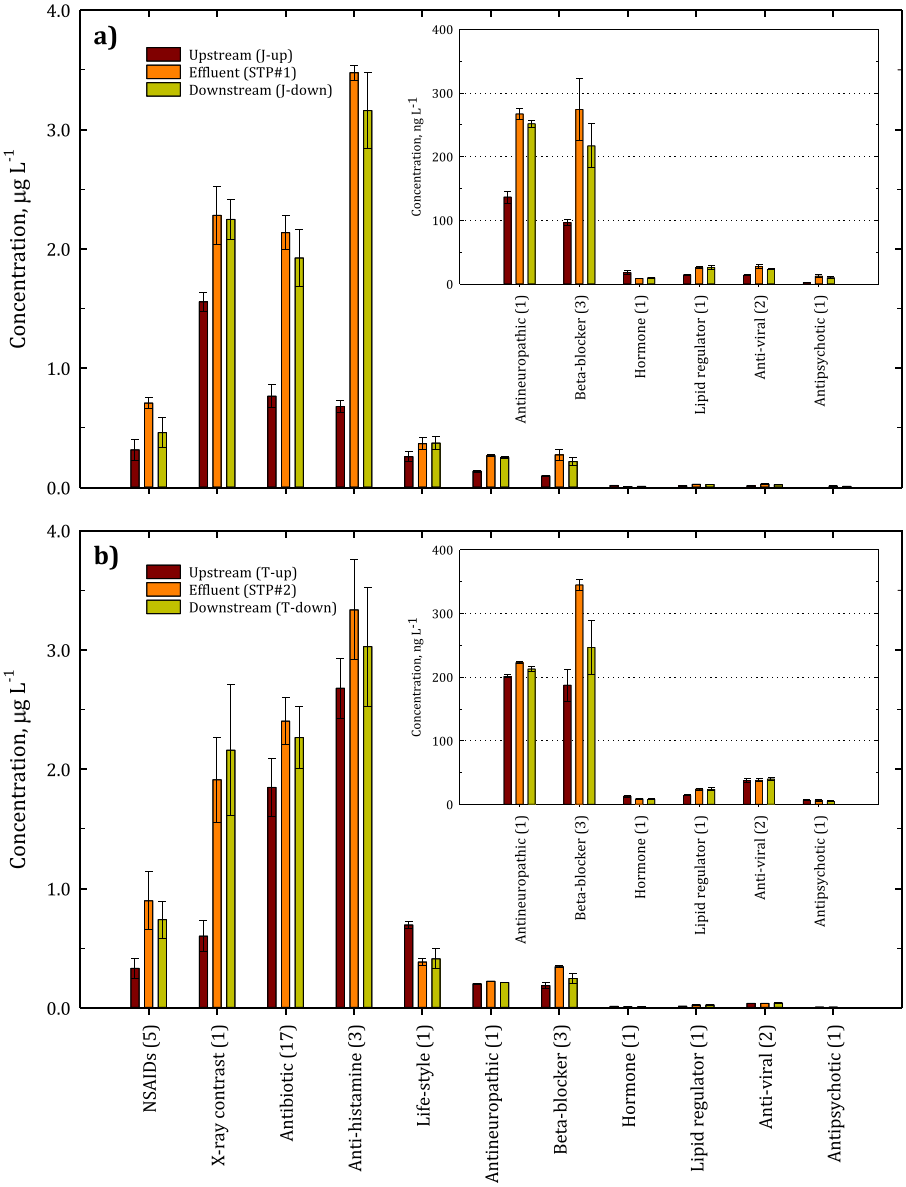

Fig. 4. The concentration profiles by pharmaceutical classes from upstream to downstream including effluent from treated sewer of STP#1 and STP#2 located in (a) tributary J and (b) T (see Fig. 1). Bars denote means and error bars denote standards error ($n = 7$).

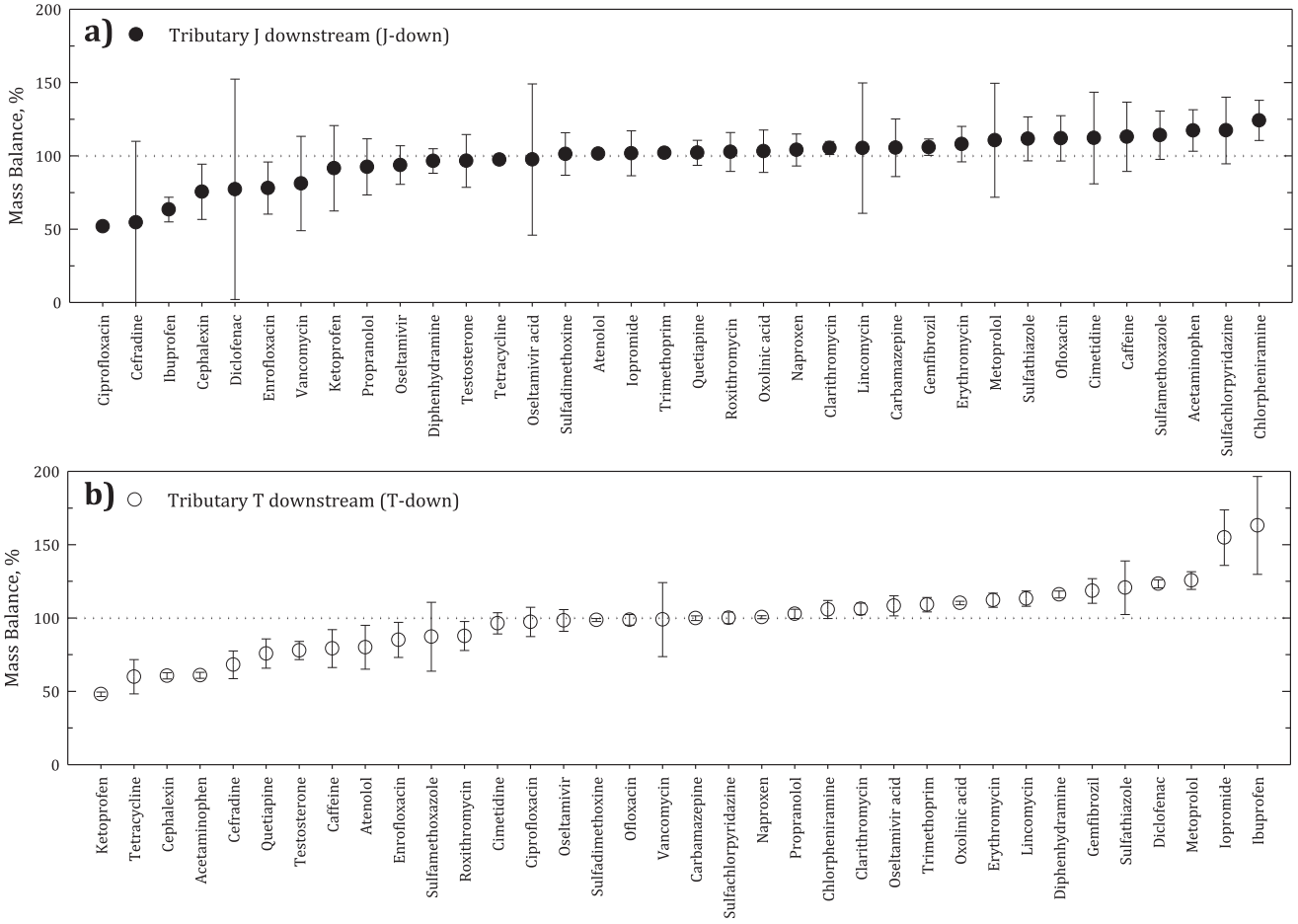

Fig. 5. Mass balances of each target pharmaceuticals in the downstream of tributary J and T. Mass balance means a ratio of measured and calculated concentrations by eq. (2) at (a) a downstream of STP#1 (J-down) and (b) STP#2 (T-down). Circles indicate a means of mass balance and error bars indicate standard errors ($n = 7$).

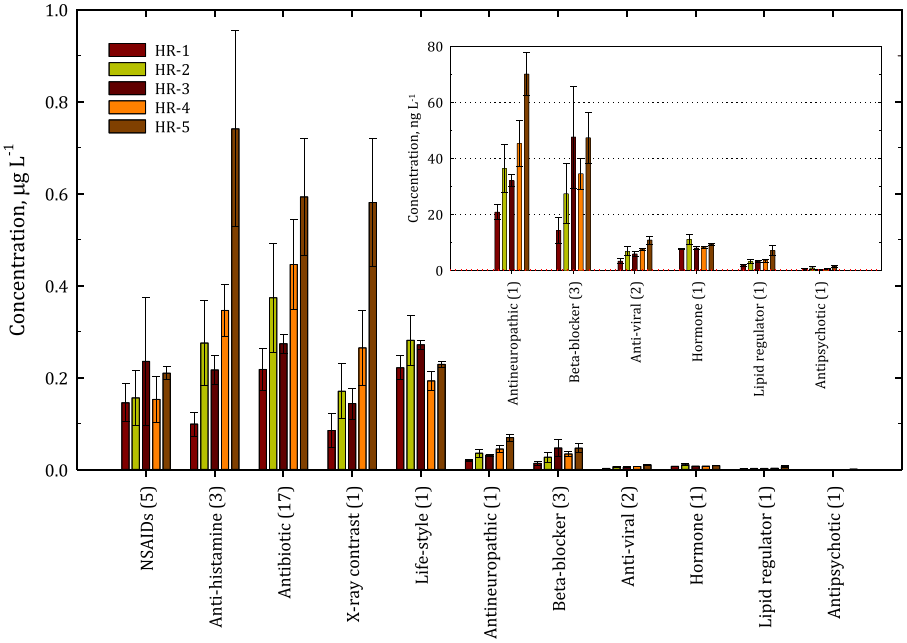

Fig. 6. Comparison of concentration profiles by pharmaceutical classes detected along Han River (HR-1 to HR-5). Bars denote means and error bars denote standards error ($n = 7$).

are the main cause of pharmaceutical pollution in a main stream. In addition, it also shows the pharmaceutical discharge by the STPs should be well controlled to protect the whole river from the impact by the compounds.

Roberts et al. (2016) reported a strong linear correlation between traditional chloride ion and pharmaceuticals in the receiving water of the STP and reported that the concentration of pharmaceuticals in the stream is likely to depend only on upstream conservative mixing. Similarly, Fairbairn et al. (2016) reported the amount of 16 pharmaceuticals and pesticides flowing into the mixed watershed through STPs, and their measured and predicted values showed good agreement with $R^2 = 0.881$ ($p < 0.001$). Hanamoto et al. (2018) reported on the in-stream attenuation of pharmaceuticals in river based on the mass balance approach and estimated drug contamination sources according to catchment populations. The systematic approach based on the mass balance model should predict the temporal and spatial behavior of pharmaceuticals in the aquatic environment and would be a powerful tool for estimating the sources of residual pharmaceuticals flowing into the river ecosystem.

4. Conclusion

In the present study, an easy analytical method based on an on-line SPE-LC-MS/MS system was applied to quantify various classes of pharmaceuticals in influent and effluent wastewater of two large STPs and in the natural water (i.e., Han River and its two tributaries) in Seoul, Korea. More importantly, the contribution of the wastewater discharged from the STPs to the pharmaceutical pollution of the natural water was demonstrated using a simple mass balance model. Relatively high levels of NSAIDs, antihistamines, and antilipidemic agents (at concentrations above the sub-μg L^{-1}) were found in the influent of the two STPs. Oseltamivir, antiviral agents, and various antibiotics that were detected in the STPs' influent at tens to hundreds ng L^{-1} were also continuously detected in the effluent. The pharmaceutical compounds under the study were also detected in the tributary streams of Han River at approximately one or more magnitude lower levels than those in STPs, mainly due to hydraulic dilution, which was verified by the mass balance model. The mass balance model also clearly demonstrated that most of the pharmaceuticals found in the Han River were originated from the STPs upstream of each tributary and STPs are not able to effectively treat them.

To prevent pollution of the water environment by pharmaceuticals, therefore, new technologies able to completely degrade the compounds should be developed urgently. In addition, a good management practice for pharmaceuticals should be applied to minimize their flowing into a sewer.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.03.046.

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