Desalination and Water Treatment
www.deswater.com
32 (2011) 49–56
August
1944-3994 / 1944-3986 © 2011 Desalination Publications. All rights reserved.
doi: 10.5004/dwt.2011.2677

Occurrence of β-blockers and β-agonists in hospital effluents and their receiving rivers in southern Taiwan

Tsung-Hsien Yu, Angela Yu-Chen Lin*, Xiao-Huan Wang, Cheng-Fang Lin

Graduate Institute of Environmental Engineering, National Taiwan University, 71, Chou-Shan Rd., Taipei 106, Taiwan, R.O.C. Tel. +886 (2) 3366-4386; Fax +886 (2) 2392-9828; email: yuchenlin@ntu.edu.tw

Received 13 July 2010; Accepted in revised form 1 January 2011

ABSTRACT

The occurrence of pharmaceuticals in aquatic environments has been of increasing concern in developed and developing countries. The major sources of pharmaceuticals in aqueous systems are waste streams from hospital effluents and excretion (in both metabolized and un-metabolized forms) from humans and animals. This study investigated the occurrence and distribution of four β-blockers (propranolol, atenolol, metoprolol and acebutolol) and four β-agonists (tulobuterol, salbutamol, clenbuterol and ractopamine) from three hospital effluents and four rivers in southern Taiwan. Analysis was performed via solid-phase extraction and liquid chromatography/tandem mass spectrometry. All targets were detected at least once in all collected samples. The most frequently detected compounds were propranolol, atenolol, acebutolol, and ractopamine, which were found in >70% of the collected samples. 83% of water samples contained three or more target compounds. Metoprolol was found in relatively higher concentrations (up to 592 ng/L) in one hospital effluent, although most target compounds were detected in ng/L-range. The concentrations of β-blockers and β-agonists observed here were comparable to those reported in previous studies around the world.

Keywords: Pharmaceuticals; β-blockers; β-agonists; Solid-phase extraction; Liquid chromatography/tandem mass spectroscopy

1. Introduction

Over the past decade, the ubiquitous occurrence of pharmaceuticals and personal care products (PPCPs) in the aquatic environment has raised significant concerns around the world [1–3]. β-blockers and β-agonists both are substantial medicinal classes. β-blockers are used to treat cardiac arrhythmias, hypertension, and angina and provide cardio protection after myocardial infarction; β-agonists are used to treat cardiogenic shock, acute heart failure, bradyarrhythmias, asthma and chronic obstructive pulmonary disease. In 2005, more than 2.7×10^8 doses of β-blockers and β-agonists were recorded by Taiwan's National Health Research Institutes (NHRI).

β-blockers and β-agonists have been reported to cause adverse effects on aquatic organisms. Previous studies have detected β-blockers at their half-maximum effective concentration (EC_{50}) in *Daphnia magna* (μg/L range) [4,5]. Marvin and Voulvoulis [6] observed mortality for *Guinea-pig trachea* after exposure to clenbuterol at a concentration of 12 μg/L.

Previous studies detected β-blockers and β-agonists in waste streams from wastewater treatment plants (WWTPs), sewage treatment plants (STP), hospitals and

* Corresponding author.

Presented at the Third International Conference on Challenges in Environmental Science & Engineering, CESE-2010 26 September – 1 October 2010, The Sebel, Cairns, Queensland, Australia

drug production facilities [3,7–9]. For instance, atenolol has been detected in hospital effluents at levels up to 2260 ng/L [3]. The presence of β-blockers and β-agonists in STP effluents has also been confirmed. Removal by conventional biological treatments seems to be inefficient, since these species are found in significant amounts in STP effluents and surface waters [9–11]. Vieno et al. [9] reported β-blockers in STP effluents at levels up to 1600 ng/L and in river water at 3–107 ng/L.

Nevertheless, little information exists on the occurrence of β-blockers and β-agonists in the aquatic environment in Taiwan. In this study, the main objectives were to: 1) establish an analytical method for four β-blockers (propranolol, atenolol, metoprolol and acebutolol) and four β-agonists (tulobuterol, salbutamol, clenbuterol and ractopamine) using solid-phase extraction followed by liquid chromatography tandem mass spectroscopy (LC-MS/MS), and 2) investigate the occurrence and distribution of target pharmaceuticals in three hospital effluents and four rivers in southern Taiwan.

2. Materials and methods

2.1. Standards and reagents

All target pharmaceutical standards were of high purity grade (>96%). Atenolol (100%), metoprolol tartrate (100%), acebutolol hydrochloride (100%), salbutamol (99%), clenbuterol hydrochloride (99%), and sulfuric acid (96.3%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tulobuterol hydrochloride (98.6%), atenolol-d_7 (98.1%), and clenbuterol-d_9 (99.1%) were obtained from Fluka (Buchs, Switzerland). Propranolol hydrochloride (99%) was obtained from US Pharmacopeia (Rockville, MD, USA). LC-grade methanol and disodium ethylenediaminetetraacetate (EDTA-2Na) were purchased from Mallinckrodt Baker (Phillipsburg, PA, USA). ACS-grade formic acid was obtained from Riedel-de Haën (Seelze, Germany).

Individual stock standard solutions were prepared in methanol and stored in amber glass bottles at –20°C. Standard mixtures were prepared by appropriate dilution from the stock solutions before each analytical run.

2.2. Site descriptions

The rivers and hospital effluents sampling sites are depicted in Fig. 1, which shows the four major rivers in southern Taiwan (the Jishuei, Yanshuei, Gaoping, and Donggang Rivers). Water samples were collected along the four rivers and from nearby hospitals in southern Taiwan, with sites denoted as follows: from the Jishuei River (J-1, J-2, J-3 and J-4), Yanshuei River (Y-1, Y-2, Y-3 and Y-4), Gaoping River (G-1, G-2, G-3, G-4, G-5 and G-6), Donggang River (D-1, D-2, D-3, D-4, D-5, D-6 and D-7), and three hospitals (H-1, H-2 and H-3).

The length of the Jishuei River, Yanshuei River, Gaoping River and Donggang River is 65, 41, 171 and 44 km. The drainage area of each is 379, 340, 3257 and 472 km^2, respectively. Their average annual runoff is 0.53, 0.30, 8.45 and 1.12 km^3, respectively [12]. These figures indicate that the Gaoping River is the biggest in southern Taiwan. Wastewater effluents from three hospitals were also sampled. Each hospital (H-1, H-2 and H-3) contains 946, 107 and 259 beds, respectively. The number of inpatients in each hospital was 17954, 435 and 1105, while the number of outpatients (including emergency cases) was 299734, 27610 and 50946 [13].

2.3. Sample collection and preparation

Triplicate grab samples were collected from each site in one-liter amber glass bottles and stored in ice-packed coolers during transport. Eight milliliters of 0.125 M EDTA-2Na were added before sample collection. A total of 21 surface water samples and 3 hospital effluents were collected in July 2009.

All water samples were vacuum-filtered through 0.22-μm cellulose acetate membrane filters (Advantec, Toyo Roshi Kaisha. Ltd., Japan), acidified to pH 4.0 using 1 M sulfuric acid, and stored at 4°C until analysis. For solid phase extraction (SPE), Oasis MCX cartridges with 150 mg of sorbent and 6 mL capacity (Waters, Milford, MA, USA) were preconditioned with 6 mL of methanol and 6 mL of deionized (DI) water. Aliquots of 200 mL water samples were spiked with 40 μL of 1 mg/L atenolol-d_7 and clenbuterol-d_9 as internal standards for β-blockers and β-agonists and loaded to the cartridges with a flow rate of 3–6 mL/min. After sample passage, cartridges were rinsed with 6 mL of DI water and dried with an air stream for 5 min. After drying, analytes were eluted with 6 mL of an ammonium hydroxide-methanol solution (5:95, v/v). The eluates were collected and evaporated to dryness with a nitrogen stream. 0.4 mL of 25% aqueous methanol was added and filtered through a 0.45-μm PVDF membrane filter before LC-MS/MS analysis.

2.4. LC-MS/MS analysis

The concentration of the analytes was analyzed using an Agilent 1200 module (Agilent, Palo Alto, CA, USA) equipped with a ZORBAX Eclipse XDB-C18 column (Agilent, Palo Alto, CA, USA, 150×4.6 mm, 5 μm). 0.1% formic acid (v/v) in DI water as mobile phase A and 0.1% formic acid (v/v) in methanol as mobile phase B were used as the binary gradient with a flow rate of 1.0 mL/min. Twenty microliters of sample were injected and eluted out of the column within 8 min. For measurement, a gradient elution program started with 0% of mobile phase B for 0.5 min, increased to 40% from 0.5 to 2.0 min, to 60% from 2.0 from 4.5 min, to 95% from 4.5 to 5.0 min, remained at 95% until 6.0 min, decreased to 0% from 6.0 to 7.0 min and reached completion at 0%. All target compounds were

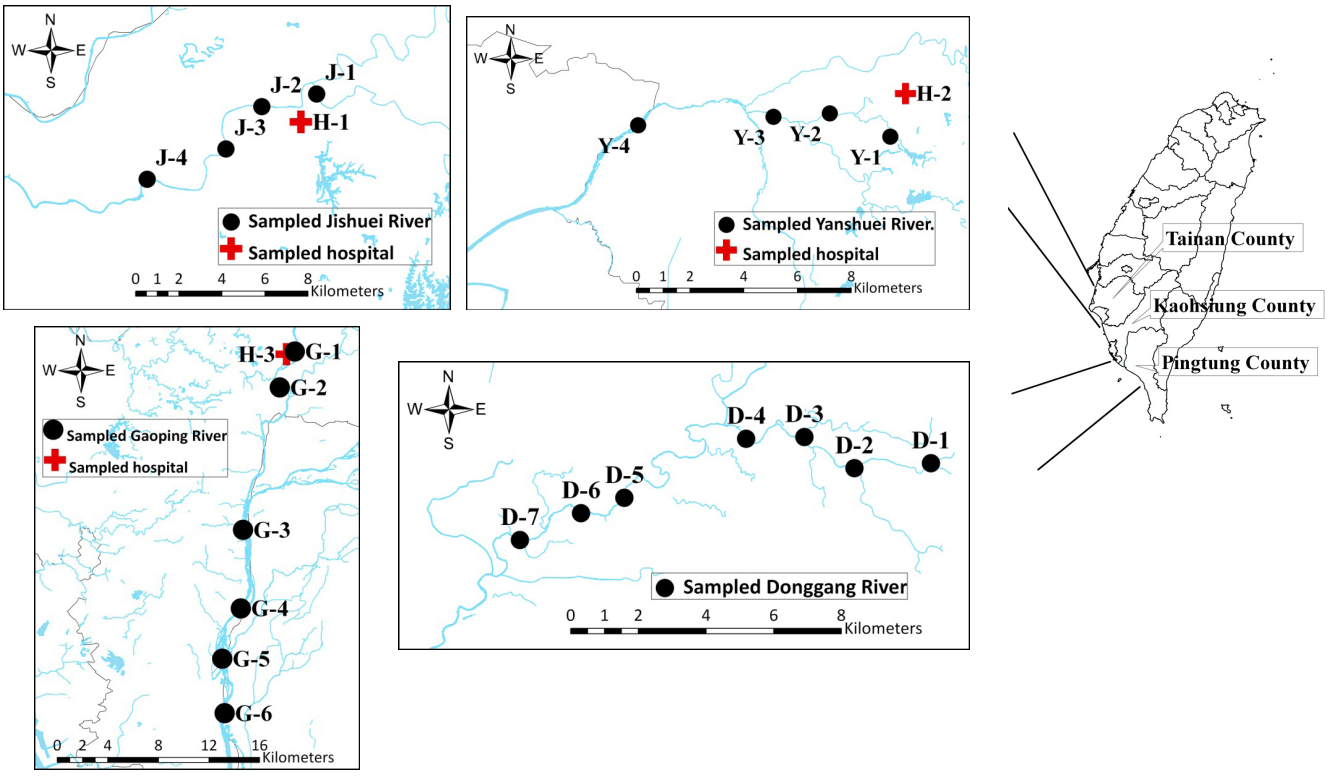

Fig. 1. Sampling sites in southern Taiwan: Jishuei River (J-1, J-2, J-3 and J-4), Yanshuei River (Y-1, Y-2,Y-3 and Y-4), Gaoping River (G-1, G-2, G-3, G-4, G-5 and G-6), Donggang River (D-1, D-2, D-3, D-4, D-5, D-6 and D-7) and three hospitals (H-1, H-2 and H-3).

eluted out of the column within 8 min. The autosampler was operated at room temperature.

For mass spectrometric measurements, the analytes were measured by a Sciex API 4000 mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface. All analyses were performed in positive mode. Multiple reaction monitoring (MRM) mode was used to acquire the ions with a dwell time of 50 ms. The mass spectrometer conditions were as follows: ion spray voltage: 5.5 kV, curtain gas: 10 L/h, nebulizer gas: 50 L/h, turbo gas: 50 L/h, heated capillary temperature: 500°C, and collisionally activated dissociation: 5.

After selecting the precursor and product ions by MS/MS, they were optimized with four key parameters: declustering potential, entrance potential, collision energy, and collision cell exit potential by direct injection of the pure standards to the MS/MS compartment. Detailed MS/MS parameters for target compounds are listed in Table 1.

2.5. Validation of the analytical procedure

Identification of β-blockers and β-agonists was performed with HPLC–MS/MS in MRM mode, using the two highest characteristic precursor ion/product ion transition pairs. Compounds were identified using the LC retention time ±30% of the retention time of a standard.

Recovery experiments were performed on DI and river water spiked with 50 ng/L standard mixtures to estimate this method's recovery, which was determined by comparing the concentrations of the spiked DI and river water before and after SPE extraction. The results are shown in Table 2. Atenolol-d_7 and clenbuterol-d_9 were used as the internal standards for quantification.

Two standard calibration curves showing that the 0.1–100 μg/L and 100–1000 μg/L ranges were constructed by spiking target pharmaceuticals into 25% aqueous methanol. The linearity of the calibration curves was ≥0.997, which was estimated by fitting a linear mode, least-squares regression analysis ($y = a + bx$). The method detection limits (MDLs) were determined with the minimum concentration of analytes in the linear range of calibration curves and a signal-to-noise ratio of at least 10:1 in DI water.

3. Results and discussion

3.1. Analytical method validation

All eight target compounds were detected by HPLC-MS/MS. The best separation was obtained using 0.1% formic acid (v/v) in DI water and 0.1% formic acid (v/v) in methanol as mobile phase. After selecting the precursor ions of target compounds, product ions were selected

Table 1
MS/MS parameters for target compounds in multiple reaction monitoring mode with positive ionization

Compounds	Retention time	Precursor ion	Product ion	MS/MS parameters			
	(min)	(m/z)	(m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
β-blockers							
Propranolol	6.91	260	116	45	10	25.5	8.8
			183			24.3	12.1
Atenolol	4.23	267	145	45	10	36.0	10.9
			190			26.0	10.3
Metoprolol	5.61	268	116	40	10	25.5	8.9
			191			24.3	14.3
Acebutolol	5.52	337	116	45	10	29.5	9.7
			319			24.0	8.9
Atenolol-d7	4.22	274	123	25	10	29.0	9.0
			145			36.0	10.0
β-agonists							
Tulobuterol	5.87	228	154	31	10	23.6	12.6
		230	156			22.7	12.2
Salbutamol	4.23	240	148	40	10	25.8	9.2
			222			15.2	13.5
Clenbuterol	5.44	277	132	32	10	37.1	8.3
			168			40.7	8.4
Ractopamine	5.10	302	164	31	10	23.2	10.5
			284			17.0	9.5
Clenbuterol-d9	5.41	286	204	25	10	23.0	45.0
			268			16.0	20.0

DP: declustering potential; EP: entrance potential; CE: collision energy potential; CXP: collision cell exit potential.

Table 2
Relative and absolute recoveries in DI water and river water, method detection limits (MDLs), and linearity (correlation coefficient) for target compounds

Compounds	MDLs (ng/L)	Relative recovery ± SD (%)		Absolute recovery ± SD (%)		Linearity *r*
		DI water (*n* = 6)	River water (*n* = 3)	DI water (*n* = 6)	River water (*n* = 3)	
Propranolol	0.6	83.2 ± 6.0	84.0 ± 2.5	76.0 ± 4.4	72.9 ± 4.1	0.9994[a] 0.9995[b]
Atenolol	1.2	103.2 ± 4.6	105.7 ± 1.5	93.2 ± 3.2	89.9 ± 4.2	0.9997 0.9995
Metoprolol	0.6	89.6 ± 4.7	95.3 ± 0.5	81.1 ± 4.2	86.9 ± 4.4	0.9993 0.9997
Acebutolol	0.6	93.3 ± 7.9	107.3 ± 3.1	82.8 ± 3.8	90.9 ± 2.0	0.9998 0.9994
Tulobuterol	1.0	33.8 ± 9.2	63.3 ± 5.1	31.2 ± 6.3	61.8 ± 4.0	0.9979 0.9981
Salbutamol	0.6	107.2 ± 2.6	100.4 ± 3.7	100.6 ± 4.1	96.9 ± 3.5	0.9999 0.9970
Clenbuterol	0.4	89.8 ± 7.0	86.9 ± 1.2	85.0 ± 4.5	85.2 ± 3.9	0.9995 0.9996
Ractopamine	0.6	99.2 ± 6.6	90.8 ± 1.0	95.3 ± 3.2	87.1 ± 1.7	0.9998 0.9992

[a]linear range 0.1–100 ng/L; [b]linear range 100–1000 ng/L.

by their higher signal. Precursor ions and product ions of target compounds are indicated in Table 1 and were selected by similar criteria in the literature [14–17].

For SPE pretreatment, atenolol-d_7 and clenbuterol-d_9 were used as the internal standards for quantification of β-blockers and β-agonists. Recoveries in DI water and river water samples are presented in Table 2. Recoveries achieved for all target compounds ranged from 83.2% to 107.2% (DI water) and from 84.0% to 107.3% (river water), with the exception of tulobuterol, which demonstrated lower recoveries (33.8 and 63.3% for DI and river water, respectively). It is possible that the physicochemical properties of tulobuterol differ from those of the other target β-agonists and that the internal standard clenbuterol-d_9 was unable to be calibrated to make good recovery possible. The MDLs for spiked DI water were in the range of 0.4–1.2 ng/L (as shown in Table 2). The linear range and the precision and accuracy results are presented in Tables S1 and S2 (Supplementary materials section).

3.2. Occurrence of target pharmaceuticals

The concentrations of pharmaceuticals in river water samples were plotted on a logarithmic scale (Fig. 2) and summarized for hospital effluents (Table 3).

For the four selected β-blockers, the detection frequencies were 81% for propranolol and 71% for atenolol, metoprolol and acebutolol in 21 river water samples, while concentrations ranged from 0.6 to 3.0, from 2.1 to 61.9, from 0.6 to 10.6, and from 0.6 to 6.4 ng/L, respectively. In river samples, atenolol was detected at the highest concentration (61.9 ng/L) in the Donggang River. The other results indicated that most β-blockers were found at lower concentration (< 10 ng/L) in southern Taiwan's rivers at levels comparable to those reported from different countries [15,17,18]. Gros et al. [17] also reported that these four β-blockers were measured at ng/L level in surface water and that atenolol was present at relatively high concentrations (up to 250 ng/L).

Table 3
Occurrence of target compounds in waste streams from three hospitals

Compounds	H-1	H-2	H-3
	ng/L ± SD		
Propranolol	366.0 ± 11.1	ND	ND
Atenolol	94.1 ± 3.9	ND	5.8 ± 0.3
Metoprolol	581.3 ± 16.8	ND	0.8 ± 0.2
Acebutolol	97.5 ± 6.1	0.6 ± 0.1	0.7 ± 0.1
Tulobuterol	ND	ND	ND
Salbutamol	1.8 ± 0.1	ND	ND
Clenbuterol	ND	ND	ND
Ractopamine	0.9 ± 0.0	0.7 ± 0.2	ND

ND: not detected

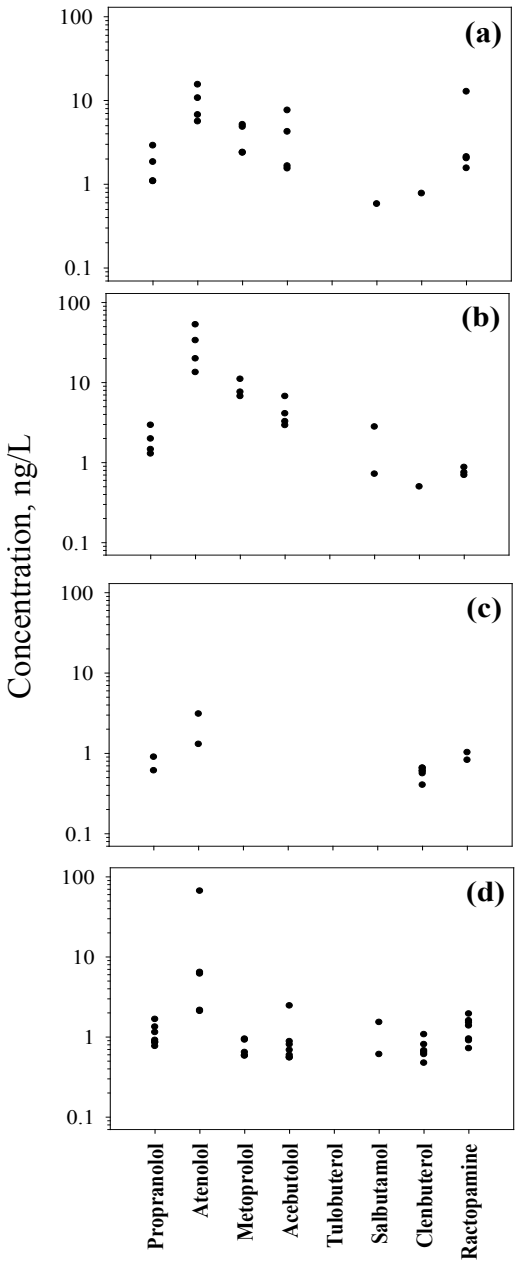

Fig. 2. Occurrence of target compounds from (a) Jishuei River (b) Yanshuei River (c) Gaoping River and (d) Donggang River.

The concentrations of β-blockers in effluents from hospitals upstream of the Jishuei, Yanshuei, and Gaoping Rivers were investigated. The results are given in Table 3. The concentrations of the four β-blockers in hospital effluents ranged from 94.1 to 581.3 ng/L in H-1, 0.6 ng/L in H-2 and 0.7–5.8 ng/L in H-3. Metoprolol was measured at the highest concentration (up to 581.3 ng/L). Concentrations of these four β-blockers in H-1 were relatively

high and corresponded to the greater number of hospital beds, inpatients, and outpatients (946, 17954 and 299734, respectively) served by H-1 compared to the two other hospitals.

For the four β-agonists, the detection frequencies in rivers were 9% for tulobuterol, 29% for salbutamol, 71% for clenbuterol, and 81% for ractopamine in the 21 river samples collected. Each was found at only trace concentrations in the four rivers, ranging from 1.4 to 1.6, from 0.6 to 2.5, from 0.4 to 1.5 and from 0.7 to 12.0 ng/L, respectively. Previous studies have also reported β-agonists in surface water in ng/L levels [19,20]. Calamari et al. [19] reported that salbutamol in two rivers was surveyed at ranges from 1.14 to 2.48 ng/L. Kasprzyk-Hordern et al. [10] found that salbutamol occurred in surface water at low concentration (< 3 ng/L). Detectable concentrations of these four β-agonists were found in hospital effluents from H-1 and H-2. Results for both the river samples and hospital effluents were near or below MDLs. Lin et al. [3] also found that the occurrence of 5 β-agonists in hospital effluents was at low concentration (up to 38 ng/L).

Previous studies reported that pharmaceuticals were detected in hospital effluents at relatively high concentrations (up to μg/L level) [2,3,8], and were then discharged to downstream rivers. Concentrations of these pharmaceuticals in effluents from hospitals and drug production facilities are not yet subject to regulation.

Currently, there are limited studies reporting adverse effects of these pharmaceuticals on aquatic organisms. The half-maximum effective concentration (EC_{50}) of β-blockers was observed in *Daphnia magna* to be in the μg/L range [4,5]. Marvin and Voulvoulis [6] observed increased mortality in *Guinea-pig trachea* after exposure to clenbuterol at 12 μg/L levels. The predicted non-effect concentrations (PNECs) of propranolol (730 ng/L), atenolol (77,700 ng/L), metoprolol (7,900 ng/L) and salbutamol (240,000 ng/L) have been estimated by previous studies [21–23]. Although selected pharmaceuticals have been detected at low concentrations in the effluents of hospitals and river waters, further experiments should be conducted to identify the potential risk they pose to the health of humans and wildlife.

4. Conclusion

This is the first study to document the composition and distribution of β-blockers and β-agonists in southern Taiwan's aqueous environments. All targets were detected at least once in all collected samples. The most frequently detected compounds were propranolol, atenolol, acebutolol, and ractopamine, which were found in > 70% of the collected samples. Eighty-three percent of water samples contained three or more target compounds. Metoprolol was found in relatively higher concentrations (up to 592 ng/L) in one hospital effluent, while most of the target compounds were detected in the ng/L range. Overall, waste streams from hospitals represent an important source of pharmaceuticals to the receiving water bodies.

Acknowledgments

Financial support was generously provided by the Environmental Protection Administration, Executive Yuan, Taiwan, ROC, under Contract No. EPA-98-E3S4-02-02.

References

[1] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton, Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: A national reconnaissance, Environ. Sci. Technol., 36 (2002) 1202–1211.

[2] A.Y.C. Lin and Y.T. Tsai, Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities, Sci. Total Environ., 407 (2009) 3793–3802.

[3] A.Y.-C. Lin, T.-H. Yu and C.-F. Lin, Pharmaceutical contamination in residential, industrial, and agricultural waste streams: Risk to aqueous environments in Taiwan, Chemosphere, 74 (2008) 131–141.

[4] B. Ferrari, R. Mons, B. Vollat, B. Fraysse, N. Paxeus, R. Lo Giudice, A. Pollio and J. Garric, Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment?, Environ. Toxicol. Chem., 23 (2004) 1344–1354.

[5] D.B. Huggett, B.W. Brooks, B. Peterson, C.M. Foran and D. Schlenk, Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (b-blockers) on aquatic organisms, Arch. Environ. Con. Tox., 43 (2002) 229–235.

[6] O.V. Martin and N. Voulvoulis, Sustainable risk management of emerging contaminants in municipal wastewaters, Philos. T. R. Soc. A., 367 (2009) 3895–3922.

[7] A.Y.-C. Lin, T.-H. Yu and S.K. Lateef, Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan, J. Hazard. Mater., 167 (2009) 1163–1169.

[8] M.J. Gómez, M. Petrovic, A.R. Fernandez-Alba and D. Barcelo, Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters, J. Chromatogr. A, 1114 (2006) 224–233.

[9] N. Vieno, T. Tuhkanen and L. Kronberg, Elimination of pharmaceuticals in sewage treatment plants in Finland, Water Res., 41 (2007) 1001–1012.

[10] B. Kasprzyk-Hordern, R.M. Dinsdale and A.J. Guwy, The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters, Water Res., 43 (2009) 363–380.

[11] M. Gros, M. Petrovic and D. Barcelo, Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the ebro river basin (northeast spain), Environ. Toxicol. Chem., 26 (2007) 1553–1562.

[12] Water Resources Agency, in, Ministry of Economic Affairs, Taiwan.

[13] Bureau of National Health Insurance, Taiwan, 2008.

[14] J. Blanca, P. Munoz, M. Morgado, N. Mendez, A. Aranda, T. Reuvers and H. Hooghuis, Determination of clenbuterol, ractopamine and zilpaterol in liver and urine by liquid chromatography tandem mass spectrometry, Anal. Chim. Acta., 529 (2005) 199–205.

[15] N.M. Vieno, T. Tuhkanen and L. Kronberg, Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in

recipient rivers using solid phase extraction and liquid chromatography-tandem mass spectrometry detection, J. Chromatogr. A, 1134 (2006) 101–111.
[16] L.D. Williams, M.I. Churchwell and D.R. Doerge, Multiresidue confirmation of [beta]-agonists in bovine retina and liver using LC-ES/MS/MS, J. Chromatogr. B, 813 (2004) 35–45.
[17] M. Gros, M. Petrovic and D. Barcel, Development of a multiresidue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters, Talanta, 70 (2006) 678–690.
[18] D. Bendz, N.A. Paxeus, T.R. Ginn and F.J. Loge, Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje River in Sweden, J. Hazard. Mater., 122 (2005) 195–204.
[19] D. Calamari, E. Zuccato, S. Castiglioni, R. Bagnati and R. Fanelli, Strategic survey of therapeutic drugs in the Rivers Po and Lambro in northern Italy, Environ. Sci. Technol., 37 (2003) 1241–1248.
[20] B. Kasprzyk-Hordern, R.M. Dinsdale and A.J. Guwy, Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography-positive electrospray ionisation tandem mass spectrometry, J. Chromatogr. A, 1161 (2007) 132–145.
[21] C. Carlsson, A.K. Johansson, G. Alvan, K. Bergman and T. Kuhler, Are pharmaceuticals potent environmental pollutants? Part I: Environmental risk assessments of selected active pharmaceutical ingredients, Sci. Total Environ., 364 (2006) 67–87.
[22] M. Cleuvers, Initial risk assessment for three beta-blockers found in the aquatic environment, Chemosphere, 59 (2005) 199–205.
[23] O.A.H. Jones, N. Voulvoulis and J.N. Lester, Aquatic environmental assessment of the top 25 English prescription pharmaceuticals, Water Res., 36 (2002) 5013–5022.

Supplementary materials

Table S1
The linear ranges, correlation coefficient, regression equation, response factor (RF) and relative standard deviation (RSD) of the calibration curves

Compound	Linear range (μg/L)	Correlation coefficient (r)	Regression equation	RF	RSD (%)
Propranolol	0.1~100	0.9994	$y = 0.0299x + 0.00151$	3.3	16.6
	100~1000	0.9995	$y = 0.0265x + 0.351$	2.8	4.6
Atenolol	0.1~100	0.9997	$y = 0.0186x + 0.00121$	1.6	9.3
	100~1000	0.9995	$y = 0.0134x + 0.303$	6.2	7.1
Metoprolol	0.1~100	0.9993	$y = 0.0273x + 0.00122$	2.6	5.3
	100~1000	0.9997	$y = 0.0228x + 0.236$	2.4	3.3
Acebutolol	0.1~100	0.9998	$y = 0.0605x + 0.00611$	5.7	9.1
	100~1000	0.9994	$y = 0.0432x + 1.11$	4.7	7.4
Tulobuterol	0.1~100	0.9979	$y = 0.0214x + 0.00104$	1.7	4.6
	100~1000	0.9981	$y = 0.012x + 0.556$	1.4	12.4
Salbutamol	0.1~10	0.9999	$y = 0.0283x + 0.00123$	3.2	17.7
	5~250	0.9970	$y = 0.0225x + 0.0516$	2.6	11.6
Clenbuterol	0.1~100	0.9995	$y = 0.00776x + 0.000288$	0.4	3.5
	100~1000	0.9996	$y = 0.00392x + 0.0528$	0.4	4.5
Ractopamine	0.1~100	0.9998	$y = 0.0107x + 0.000264$	1.1	10.4
	100~1000	0.9992	$y = 0.0078x + 0.317$	0.9	12.0

Table S2
Results of the accuracy test on different days

		Day 1			Day 2			Day 3			
	Spike conc. (ng/L)	Mean conc. (ng/L)	Accuracy (%)	CV (%)	Mean conc. (ng/L)	Accuracy (%)	CV (%)	Mean conc. (ng/L)	Accuracy (%)	CV (%)	MDL (ng/L)
Propranolol	50	34.6	–30.9	3.0	29.8	–40.4	2.9	25.7	–48.6	3.0	0.3
Atenolol	50	50.7	1.3	1.5	52.7	5.3	6.1	51.4	2.7	4.0	0.9
Metoprolol	50	45.3	–9.3	3.2	48.9	–2.1	2.1	44.5	–11.0	1.0	1.0
Acebutolol	50	52.8	5.7	6.4	52.3	4.7	2.3	49.2	–1.6	1.9	1.0
Tulobuterol	50	38.6	–22.8	0.8	32.9	–34.2	4.3	33.6	–32.8	7.5	1.5
Salbutamol	50	54.8	9.7	0.6	51.5	3.0	2.8	54.0	8.0	1.0	0.2
Clenbuterol	50	49.0	–2.0	1.3	41.6	–16.8	2.5	41.8	–16.5	2.1	1.2
Ractopamine	50	47.3	–5.4	5.7	47.7	–4.5	9.1	49.2	–1.7	3.5	0.2

CV: coefficient of variance