Screening of pharmaceuticals in surface water bodies of the Pampas region of Argentina

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Abstract: Pharmaceuticals have been included among emerging pollutants due to their continuous input in aquatic environments by wastewater discharges. The aim of the present study was detecting and analysing concentration levels of five commonly used pharmaceuticals in surface water bodies of the Pampas Region of Argentina. Analyses were done on the soluble fraction by Liquid Chromatography with Mass Spectrometry (HPLC-MS) after Solid-Phase Extraction (SPE) extraction. Results show the presence of pharmaceuticals in 10 out of 11 sampling sites, detecting higher concentrations in sites closer to wastewater discharges. Compounds more commonly found and at higher concentrations were caffeine and ibuprofen, with maximum detected levels of 13.32 and 9.66 µg/L, respectively. Highest detected concentrations of carbamazepine and atenolol were 0.63 and 0.55 µg/L, respectively. The compound less frequently detected and at lower levels of concentration was diclofenac.

Keywords: pharmaceuticals; atenolol; carbamazepine; ibuprofen; caffeine; diclofenac; emerging pollutants; surface water; environmental health.

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1 Introduction

Pharmaceutical compounds in the environment became a pollution concern in the last decade. In Argentina, although pollution of surface waters by metals, hydrocarbons, agrochemicals and other major contaminants has been more extensively investigated, information on pharmaceuticals in the environment is very scarce (Elorriaga et al., 2012, 2013).

Large variety of pharmaceuticals are being used and after consumption are not completely metabolised by the organisms, being partly excreted in the parental form or as metabolites in urine and faeces, usually reaching surface water bodies as continuous inputs from wastewater discharges (Ankley et al., 2007). Treatment technologies do not completely eliminate pharmaceuticals, varying the rate of elimination depending on the type of compound and treatment plants (Tixier et al., 2003; Carballa et al., 2004; Ashton et al., 2004). Concentrations in aquatic environments were detected in the range of nanogram/litre to microgram/litre (Andreozzi et al., 2003; Yoon et al., 2010, Crouse et al., 2012). It has been demonstrated that wastewater discharges are the main sources of aquatic pollution by pharmaceuticals (Heberer, 2002). Analysis of wastewater discharges from several sites of Argentina has shown concentrations of pharmaceuticals in the order of the microgram/litre. Caffeine and ibuprofen in highest concentrations (up to 44.2 and 13.0 μg/L, respectively) and lower levels of carbamazepine, atenolol and diclofenac (up to 2.3, 1.7 and 1.2 μg/L, respectively; Elorriaga et al., 2013).

A diversity of chemical compounds of different physicochemical properties and persistence belong to the group of pharmaceuticals (Yamamoto et al., 2009). The complex environmental matrixes and low concentration levels do not allow direct detection, being usually necessary sample pre-treatments to purify and concentrate the analytes (Fatta et al., 2007; Kostopoulou and Nikolaou, 2008). The analytical methods for detection and quantification varied in the literature, including Gas Chromatography with Mass Spectrometry (GC-MS), Liquid Chromatography with Mass Spectrometry (HPLC-MS) and Liquid Chromatography with Mass in Tandem (LC-MS/MS; Ternes

et al., 2001; Hao et al., 2007; Zhang and Zhou, 2007; Petrovic et al., 2010). Also Liquid Chromatography with Diode Array Detection (LC-DAD) has been used (Gil García et al., 2009).

The objective of the present research is reporting results of the screening of pharmaceutical compounds in surface water bodies of the Pampas Region of Argentina in areas influenced by wastewater inputs.

2 Materials and methods

Selection of the five studied pharmaceuticals (Table 1) was done on the basis of a survey of medical prescriptions, use and expenditures conducted in 2010 by the Argentinean National Health Service on 3300 individuals (unpublished). Caffeine, a compound present in both pharmaceuticals and drinks, was also included.

Eleven surface water samples from streams and rivers, ponds or lakes from the provinces of Buenos Aires and Córdoba in the Pampas Region were studied (Table 2 and Figure 1). Sampling sites correspond to sectors of different population levels, ranging 5000 to half a million inhabitants. The influence of the effluent discharges on the water bodies varied from each site. The sampling sites were classified according to the distance to discharge in two groups taking a limit of 1 km to sort them. Samples were collected and split in two glass bottles, one spiked with known standard concentrations of the target pharmaceuticals.

Water samples were filtered (47 mm cellulose membrane 0.45 μm pore size) immediately after in the laboratory and kept at 4°C. Solid-Phase Extraction (SPE) was conducted using Oasis HLB® cartridges (60 mg/3 ml from Waters Corp.) preconditioned with methanol and nanopure water and eluted with methanol (Pailler et al., 2009). Extracts were taken to dryness under nitrogen flow and resuspended in the methanol/nanopure water (1 : 1). Samples were analysed using an 1100 Series LC-MSD VL G1956A (Agilent Technologies Inc., USA) equipped with an Electrospray Ionisation (ESI) interface. Chromatographic separation of caffeine, atenolol and carbamazepine was achieved following EPA method 1694 (USEPA, 2007), modified by using a Kinetex® PFP (Phenomenex) 100 mm × 2.1 mm column, at 25°C. Diclofenac and ibuprofen were chromatographically resolved using a Kinetex® C_{18} (Phenomenex) 100 mm × 2.1 mm column, at 45°C. A gradient elution programme was done with nanopure water with ammonium acetate and formic acid, and methanol/acetonitrile. The MS analysis was performed using an ESI interface operated in positive mode for caffeine, atenolol and carbamazepine, and negative mode for diclofenac and ibuprofen. Nitrogen was used for sample nebulisation and also as collision gas. Optimised collision induced dissociation for precursor and fragment ions were done according Elorriaga et al. (2013). Programmed single ion monitoring mode was employed for analyte quantification. Retention time and fragment/precursor ratio was used for identification and the external standard method for the quantification of each pharmaceutical. Data acquisition and analysis were performed using LC/MSD Agilent ChemStation. Solvents used were HPLC grade. Standards of pharmaceuticals were over 98% purity.

Table 1 Pharmaceuticals investigated

Pharmaceutical class	*Pharmaceutical*	*CAS No.*	*Empirical formula*	*Molar weight* (*Mw*) (g/mol)
Analgesics and anti-inflammatory	Diclofenac	15307-86-5	$C_{14}H_{11}NCL_2O_2$	295.0
	Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.3
β-blockers	Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.3
Anticonvulsant	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.3
Stimulants, sewer tracer	Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2

Table 2 Sample site localisations and codes of the sampling points of the monitored surface waters of Argentina

Sample code	*Name of site*	*Sampling site*	*Town*	*Province*	*Wastewater discharge less than 1 km away*
S1	Guaminí	Stream	Guaminí	Buenos Aires	Yes
S2	El Gato	Stream	La Plata	Buenos Aires	No
S3	El Gato	Stream	La Plata	Buenos Aires	Yes
S4	El Gato	Stream	La Plata	Buenos Aires	Yes
L1	Chascomús	Lake	Chascomús	Buenos Aires	Yes
L2	Monte Grande	Lake	Monte Grande	Buenos Aires	No
L3	Jume	Lake	Marco Juárez	Córdoba	No
R1	Río de la Plata	Estuary	Berisso	Buenos Aires	Yes
R2	Río de la Plata	Estuary	Berisso	Buenos Aires	No
R3	Río Tercero	River	Río Tercero	Córdoba	No
R4	Río Luján	River	Luján	Buenos Aires	No

3 Results

Recovery of tested compounds was over 90% according to results obtained during the implementation of the method. The coefficient of variation for $n = 5$ (n being the number of replications for the whole analytical procedure), was between 1% and 10%, including inter day tests. Details of obtained quantification limits (QL) (µg/L) were, atenolol: 0.003; caffeine: 0.009; carbamazepine: 0.001; ibuprofen: 0.015; diclofenac: 0.005.

The results of the analysis on the environmental samples are given in Figure 2(a) and (b). Ten out of the 11 studied sites show the presence of at least one of the tested pharmaceuticals. Figure 2(a) shows the detected compounds from sampling sites <1 km away of the wastewater discharge and Figure 2(b) those located further than 1 km. Caffeine and ibuprofen are the two compounds found at higher concentrations in the samples. In samples closer to wastewater discharges caffeine appears more frequently and at higher concentrations, though in samples from further than 1 km the tendency of both compounds reverts, being ibuprofen most prevalent. Diclofenac was the compound detected at lower concentration levels. Table 3 shows that surface water samples closer to wastewater discharges exhibit presence of all tested compounds, with the exception of

diclofenac not detected in one of the sites. In samples from locations farther from discharges the frequency of appearance is variable, atenolol the least frequent, and caffeine and ibuprofen the most frequent.

Figure 1 Geographic location of studied surface water bodies in the Pampas Region of Argentina

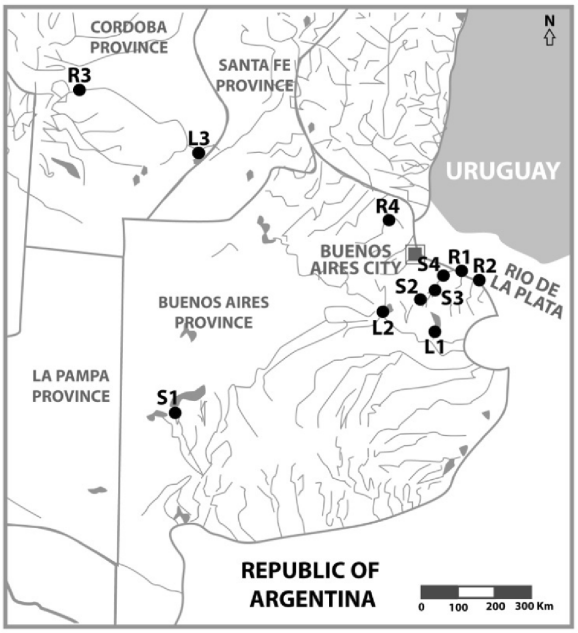
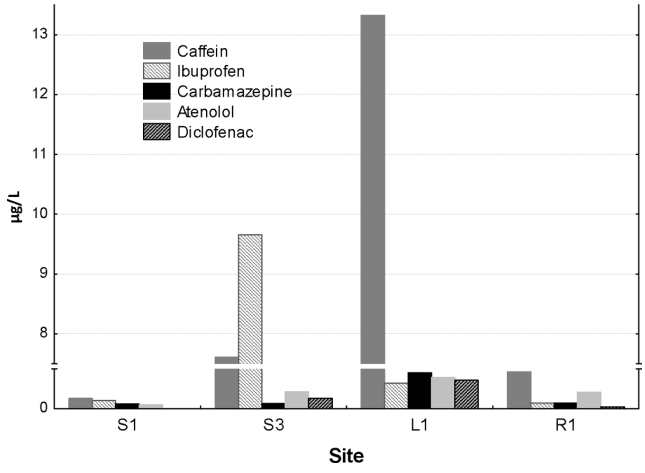
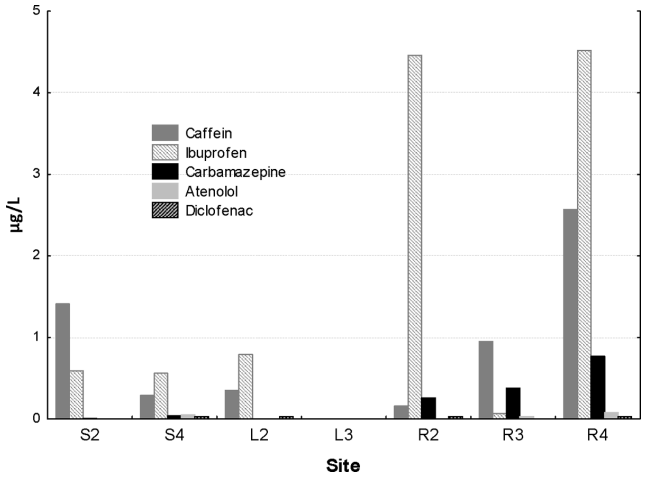


Figure 2 Concentrations of the studied pharmaceuticals in surface waters. (a) Presence of wastewater discharges less than 1 km away and (b) wastewater discharges further than 1 km away. Data is given in µg/L



(a)

Figure 2 Concentrations of the studied pharmaceuticals in surface waters. (a) Presence of wastewater discharges less than 1 km away and (b) wastewater discharges further than 1 km away. Data is given in µg/L (continued)



(b)

Table 3 Frequency, mean levels and maximum values found in surface waters of Argentina

	Wastewater discharges further than 1 km			*Wastewater discharge less than 1 km*		
Compound	*Frequency*	*Mean* (µg/L)	*Maximum* (µg/L)	*Frequency*	*Mean* (µg/L)	*Maximum* (µg/L)
Atenolol	3/7	0.05	0.08	4/4	0.30	0.55
Caffeine	6/7	0.86	2.57	4/4	5.44	13.32
Carbamazepine	5/7	0.36	0.38	4/4	0.22	0.63
Ibuprofen	6/7	2.58	4.52	4/4	2.59	9.66
Diclofenac	4/7	–	QL	3/4	0.34	0.50

QL: Quantification Limit.

4 Discussion

The presence in the environment of the tested pharmaceuticals has been documented in the literature for over a decade (Ternes, 1998). Our results are comparable with studies carried in aquatic environments from different parts of the world (Santos et al., 2010). The pharmaceuticals detected at highest concentrations in the studied surface waters correspond to those most consumed in Argentina according the mentioned survey of the Health Ministry. The compounds detected in the studied surface waters of the Region had been also observed in wastewater discharges of the studied sampling areas (Elorriaga et al., 2013).

The analysis detected, among the group of analgesics and non-steroid anti-inflammatory, ibuprofen as one of the compounds most frequently found and at highest concentrations. This compound had been detected in wastewaters and surface waters according to several reports (Thomas and Hilton, 2004; Valcárcel et al., 2011). Additionally, in Argentina ibuprofen is one of the most consumed pharmaceuticals. According the Ministry of Health survey over 40% of the people consume analgesics, anti inflammatory and/or antipyretic, ibuprofen being the most frequently used (~38% of total pharmaceuticals from this group).

Detection of carbamazepine in the tested samples is not unusual, considering that is a compound frequently found at high environmental concentrations according to Heberer (2002). Additionally, it is known that carbamazepine is persistent in the aquatic environment (Andreozzi et al., 2002). Though the half life in the environment of carbamazepine is higher than atenolol (Yamamoto et al., 2009), both compounds are usually found at similar levels in the environment since atenolol is more consumed than carbamazepine (according the previously mentioned survey, atenolol is the second most consumed in Argentina, within the group for heart therapy and arterial pressure). Additionally, ibuprofen half life is lower than atenolol and carbamazepine, indicating according our results that high consume of ibuprofen determines its environmental concentrations rather than its stability.

The appearance of the tested compounds nearer to wastewater discharges is in agreement with reports by Ankley et al. (2007). Usually, caffeine and ibuprofen are the two most abundant compounds, caffeine being more prevalent. Consequently, several reports have proposed that caffeine would be a good tracer for wastewater pollution (Daneshvar et al., 2012). Within the studied sites (Figure 2(a)), it is important to note that S1 and R1 exhibit low concentrations of the tested pharmaceuticals. S1 corresponds to a discharge into a small stream from an urban area below 3000 inhabitants. Contrarily, R1 corresponds to a wastewater discharge from a city of at least 100-fold inhabitants higher, though flowing into the Rio de la Plata estuary, with a very high dilution rate. The high concentration levels of pharmaceuticals detected in the Chascomús Lake (L1) and El Gato stream (S3) are associated to much lower dilution capacities of both surface water bodies.

Caffeine and ibuprofen are the two compounds detected at higher concentrations in sampling sites from further than 1 km away of the discharge (Figure 2(b)), though ibuprofen is prevalent over caffeine. At further distances, both compounds are still being detected. Jume lake (L3), within the same group of samples, is surrounded by agricultural areas, and was not expected to be under the influence of wastewater discharges. Also, from this group, the water sample with highest concentrations corresponds to R4 of Río Luján. This basin has been previously studied due to high pollution rate along the water course (Peluso et al., 2013), with evidences of wastewater inputs.

Along the El Gato stream there are several storm water, wastewater and industrial discharges (Ronco et al., 2007), in agreement with the high levels of pharmaceuticals detected along the basin (S2, S3 and S4). The S3 sampling point is located in its middle course, where higher urban density and productive activity are settled, exhibiting the highest levels of pharmaceuticals.

The observed concentrations of pharmaceuticals in different types of surface water environments were a result not only of the population served by sewers associated discharges, but also the dilution capacity of each water body. The European Medicines Agency requests before introducing pharmaceuticals in the market, an Environmental

Risk Assessment process that involves finding Predicted Environmental Concentrations (PEC). If the $PEC_{SURFACEWATER}$ value is equal to or ~0.01 μg/L, then further evaluation is requested. In our study concentration levels in surface waters of the pharmaceuticals are above this reference value, pointing towards the need for further assessments on the fate and effects of pharmaceuticals in aquatic environments of the region. The study demonstrates for the first time in Argentina the presence of pharmaceutical compounds in surface waters of the Pampas Region of Argentina. Caffeine, ibuprofen, atenolol, carbamazepine and diclofenac were detected in concentrations within the order of μg/l.

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