

Human Pharmaceuticals in Wastewaters from Urbanized Areas of Argentina

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Abstract The study contributes with a first survey of pharmaceuticals in municipal wastewaters discharging into fresh and estuarine waters from areas with varying degrees of urbanization of Argentina. Analyses were done on the soluble fraction by HPLC–MS after SPE extraction. In all of the samples were detected caffeine and ibuprofen within the range of 0.9–44.2 and 0.4–13.0 µg/L, and lower levels of carbamazepine, atenolol and diclofenac between 0.2–2.3, 0.2–1.7 and <0.03–1.2 µg/L, respectively. Profiles of compounds were similar in all studied locations.

Keywords Carbamazepine · Caffeine · Ibuprofen · Emerging pollutants · Effluents

Emerging pollutants of concern, including pharmaceutical products, have recently gained the attention of the scientific community. The continuous introduction of pharmaceutical compounds to the environment is a potential threat to aquatic organisms, ecosystems and, ultimately, to human health. These compounds are biologically active and have been detected in large quantities and variety in the environment (Thomas and Hilton 2004; Gagné et al. 2006). After consumption, a fraction of the pharmaceuticals is excreted in the original form or as metabolites in urine or feces, reaching water systems, although in small quantities, continuously via wastewater effluents (Ankley et al. 2007). Concentrations of these compounds in effluents of the wastewater treatment

plants have been identified in receiving surface waters and they have even been also detected in potable water (Heberer 2002). In Argentina, there is no data on the presence of these contaminants in the environment. In recent years have been developed and optimized analytical sensitive methods for the determination of pharmaceutical compounds in environmental samples, allowing accurate quantification of concentrations (Kostopoulou and Nikolaou 2008; Baranowska and Kowalski 2012). Since environmental samples are complex matrices and the pharmaceuticals are found in low levels of concentrations, a pretreatment step is required for the extraction and concentration of the analytes for further analysis.

The aim of the present research was to study for the first time, the occurrence of five widely used pharmaceuticals in wastewater effluents from cities with varying degrees of urbanization of Argentina, establishing the prevalence, relative abundance and concentration range of the detected compounds.

Materials and Methods

Selection of the studied pharmaceuticals was done on the basis of a survey of medical prescriptions conducted in local pharmacies and hospitals. Caffeine was also included as a general tracer of sewage and also a stimulant compound (Table 1). The samples were collected at the outfall of six wastewater effluents. Sampling locations (Fig. 1) were: (1) Palo Blanco (PB) untreated effluent, (2) Chascomús (CH), (3) Guaminí (GUA), (4) Bell Ville (BV), (5) Monte Maíz (MM), (6) Río Tercero (RT), the latter five from primary treatment plants. Sampling sites correspond to sectors of different population levels, ranging 5,000 to half a million inhabitants. Samples were collected directly

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Table 1 Pharmaceuticals investigated

Pharmaceutical class	Pharmaceutical	CAS #	Empirical formula	Molar weight (Mw) g/mol
Analgesics and anti-inflammatories	Diclofenac	15307-86-5	C ₁₄ H ₁₁ NCL ₂ O 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 ₂ O ₃	266.3
Anticonvulsant	Carbamazepine	298-46-4	C ₁₅ H ₁₂ N ₂ O	236.3
Stimulants, sewer tracer	Caffeine	58-08-2	C ₈ H ₁₀ N ₄ O ₂	194.2

from the outfall and split in two glass bottles, one spiked with known standard concentrations of the target pharmaceuticals. A total of 60 mL of samples were filtered (47 mm cellulose membrane 0.45 μm pore size) immediately after arriving 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 5 mL of methanol (Pailler et al. 2009). Extracts were taken to dryness under gentle flow of nitrogen and resuspended in the mobile phase. Samples were analyzed using an 1100 Series LC-MSD VL G1956A (Agilent Technologies Inc., USA) equipped with an electrospray ionization (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® C18 (Phenomenex) 100 mm × 2.1 mm column, at 45°C. A gradient elution program was applied using a ternary mobile phase composed of solvent A: nanopure water, both using a mix of 10 mM ammonium acetate and 0.03 % formic acid as additive and solvent B: methanol/acetonitrile (1:1) in 5 mM ammonium acetate. The percentage of the organic phase varied along the 15 min gradient from 20 % to 80 % at a flow rate of 0.15 mL/min. Sample injection volume was 20 μL. 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 nebulization at a flow rate of 10 L/min, and 20 psi and 350°C for its drying. Nitrogen was also used as collision gas with capillary potential of 4,000 V in positive mode and 3,500 V in negative mode. Optimized collision induced dissociation (CID), for precursor and fragment ions are shown in Table 2. Programmed single ion monitoring mode (SIM) was employed for analyte quantification (Table 2). 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.

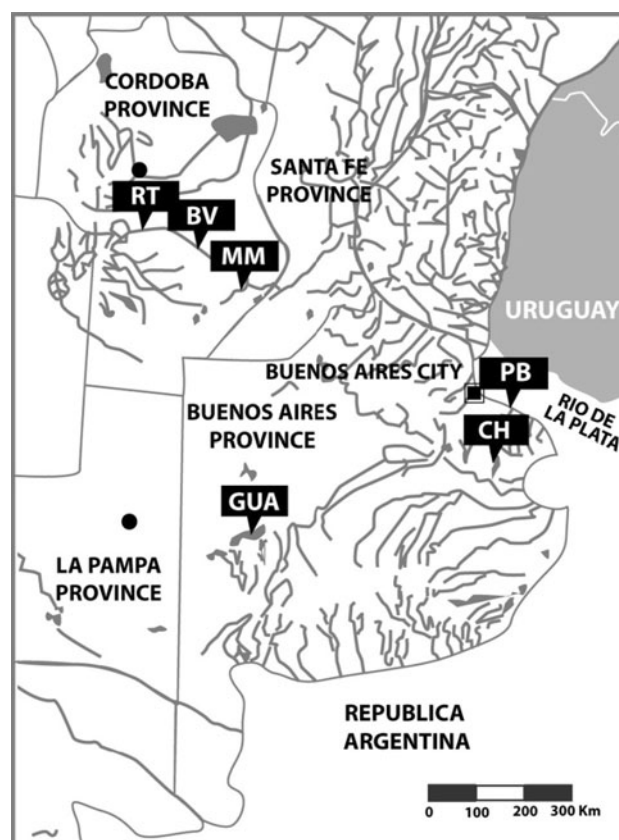


Fig. 1 Geographic location of sample collection places of wastewater effluents. BV Bell Ville, CH Chascomús, GUA Guaminí, MM Monte Maíz, PB Palo Blanco, RT Río Tercero

Results and Discussion

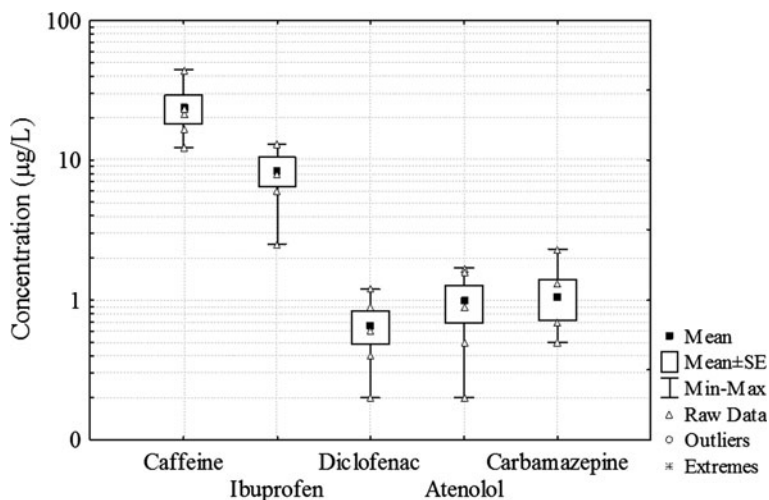
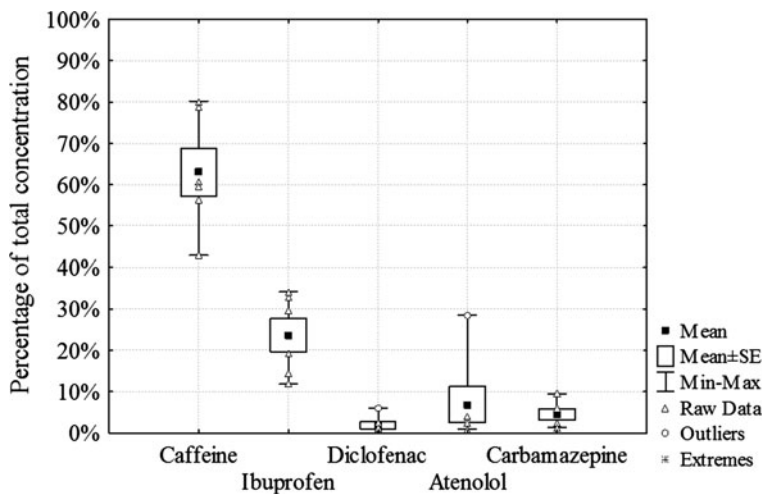
Recovery of tested compounds was over 90 % according results obtained during the implementation of the method (from sample extraction, including pretreatment steps, to final detection and quantification). The coefficient of variation (CV%) for $n = 5$, n being the number of replicate of the analytical process, was between 1 % and 10 %, including inter day tests. Detection limits, percentages of recovery and CV are detailed in Table 2. The used SPE method was versatile for the group of tested compounds.

The results of the analysis of the environmental samples are given in Figs. 2 and 3. All of the tested effluents showed

Table 2 ESI/MS optimized detector conditions for the analysis of pharmaceuticals by positive and negative ion modes, ionization mode (Ion mode)

	Ionization mode	Q(m/z), (CID,eV)	q(m/z), (CID, eV)	Rec %	RSD %	LOQ (ng/L)
Atenolol	ESI+	267 (130)	145 (180)	93.2	5.0	3.3
Caffeine	ESI+	195 (130)	138 (180)	142.4	4.2	9.4
Carbamazepine	ESI+	237 (120)	192 (210)	93.0	1.6	1.0
Ibuprofen	ESI-	205 (100)	159 (100)	97.4	3.8	15.0
Diclofenac	ESI-	294 (100)	250 (100)	91.1	10.5	5.0

Method validation: Recovery (Rec %) and relative standard deviation (RSD%) for five replicates. Limits of quantification (LOQ). Ion quantification, Q. Ion confirmation, q. Collision induced dissociation, CID (eV)

Fig. 2 Concentration ranges of the studied pharmaceuticals in the assessed wastewater effluents of Argentina**Fig. 3** Relative proportion of the studied pharmaceutical compound detected in wastewater effluents from urban areas of Argentina

the presence of pharmaceutical compounds. Caffeine and ibuprofen are the two compounds found in higher concentrations in all samples (Fig. 2). Atenolol, carbamazepine and diclofenac concentration profiles varied between discharges. Figure 3 shows the relative proportion of pharmaceutical compound detected in wastewater effluents from the studied urban areas. Over half of the total burden of the tested

pharmaceuticals corresponds to caffeine. Ibuprofen accounts approximately 25 %–30 %, and the rest of the tested compounds are below 10 %.

The presence in the environment of the detected pharmaceuticals has been also observed in other reports since over a decade (Ternes 1998). The results are also in agreement with a survey conducted by Santos et al. (2010)

based on 134 articles published between 1997 and 2009. As a consequence of differences between the study site population characteristics, it would have been expected differences in composition of the detected pharmaceuticals in the effluents, though the concentration of the majority of the studied compounds was very similar. This behavior has been already seen and analyzed in detail by Veach and Bernot (2011). The authors observed a similar composition in effluents from rural and urban areas.

Within the group of analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen and diclofenac were detected in all of the studied effluents. These two compounds are the most important found in wastewater effluents and surface waters in several reports (Ashton et al. 2004). Valcárcel et al. (2011) detected ibuprofen in rivers of Spain, being the most representative within the groups of NSAIDs. These authors also observed higher concentrations of atenolol than the rest of tested β -blockers. Detection of carbamazepine in the studied effluent samples was expected given its high persistence (Daneshvar et al. 2012). Heberer (2002) also observed relatively high concentrations in surface waters from Germany. Ternes et al. (2001) lists caffeine as a dominant compound in wastewater treatment plants and rivers of German treatment plants. Recent publications have shown that caffeine is detected in various environmental matrices and can be used as a marker for wastewater pollution (Daneshvar et al. 2012).

The study demonstrates for the first time in Argentina the presence of pharmaceutical compounds in sewers discharges from different urban areas. Caffeine, ibuprofen, carbamazepine, diclofenac and atenolol were detected in concentrations within the order of $\mu\text{g/L}$, indicating inputs of these compounds into surface waters of the region.

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