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Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquía River basin (Córdoba, Argentina)

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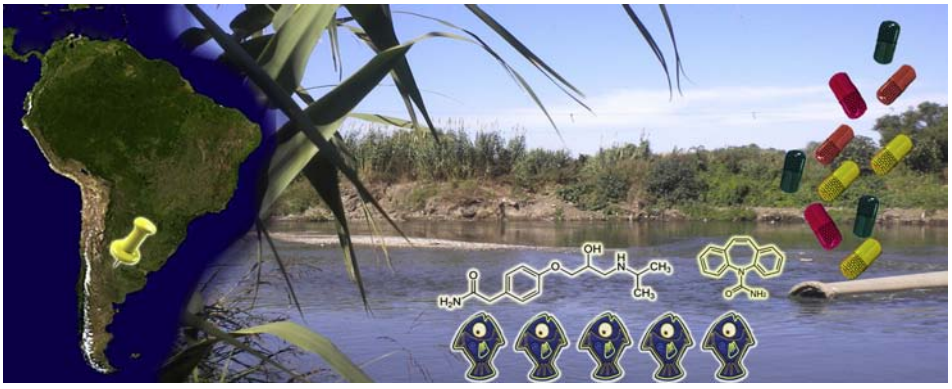
HIGHLIGHTS

- Pharmaceutical levels are reported for the first time in a river basin of Argentina.
- Atenolol, carbamazepine and diclofenac are the most frequently detected (sub μg L^{-1} levels).
- Laboratory atenolol and carbamazepine accumulation in *Gambusia affinis* is reported.
- Bioconcentration factors of atenolol and carbamazepine indicate low potential of bioaccumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

In South America, there is a lack of data concerning the occurrence and levels of pharmaceuticals in main rivers as well as their negative effects on the biota. Here we report the occurrence as well as the spatial and temporal variations of some common prescribed pharmaceuticals in the Suquía River basin (Córdoba, Argentina). We also report the bioconcentration of two of them in *Gambusia affinis*, a widely distributed fish species inhabiting the river basin. The influence of the wastewater treatment plant of Córdoba City was critical (up to 70 km downstream). Among 15 compounds analyzed, atenolol, carbamazepine and diclofenac were the most frequently detected (reaching sub μg L^{-1} levels), showing different distribution patterns. Bioconcentration of atenolol and carbamazepine was studied under laboratory controlled conditions. Estimated bioconcentration factors (BCFs) were: 0.13 and 0.08 L kg^{-1} upon exposure to 100 and 1000 μg L^{-1} atenolol in water, respectively; while BCFs were 0.7 and 0.9 L kg^{-1} when exposed to 10 and 100 μg L^{-1} carbamazepine, respectively. To the extent of our knowledge, this is the first report on pharmaceuticals in superficial waters of Argentina as well as the first report on the bioaccumulation of atenolol in whole body fish.

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1. Introduction

Pharmaceuticals are a class of emerging environmental contaminants that are being extensively and increasingly used in human and veterinary medicine. Tons of medicinal components (i.e. active principles and excipients) are produced annually worldwide (Christen et al., 2010; Santos et al., 2010; NISC, 2013) and, after consumption, excreted via urine or feces as either parent compounds or their metabolites. Wastewater treatment plants (WWTPs) are commonly not designed to eliminate micropollutants like pharmaceuticals. Depending on the WWTP nature and on the process design, the elimination rates range from <10% (e.g. atenolol and carbamazepine) to almost complete removal (e.g. propranolol) (Miège et al., 2009). Being continuously discharged into aquatic ecosystems, WWTP effluents have been recognized as the main source of human pharmaceuticals in the environment (Fent et al., 2006), reaching concentrations of ng L^{-1} to μg L^{-1} (Mompelat et al., 2009). Pharmaceuticals are of special concern in areas where treated effluent discharges contribute to a significant portion of the river flow, or to streams that are used for the production of drinking water (Garcia et al., 2012). Both are the case of the Suquía River basin, which is located in a semi-arid region (700–900 mm mean annual rainfall) of Argentina. The river drainage area covers approximately 7700 km^2, while almost 900 km^2 correspond to the drainage area of Córdoba City, a highly urbanized capital with 1.3 million inhabitants (Fig. 1). The ratio between the WWTP effluent flow (mean: 2.45 m^3 s^{-1} in the period 2011–2012) and the river flow (mean: 2.5 m^3 s^{-1} in the same period) is almost 1:1, which exceeds the purification capacity of the river (Mancini, 2012). Once in the aquatic environment, the exposure of biota to pharmaceuticals is of particular concern, since these compounds are manufactured with the intention of having a beneficial effect on human/animal health, which is not necessarily the same for aquatic organisms subjected to continual lifecycle exposure. Pharmaceuticals often have the same type of physico-chemical behavior, e.g. are lipophilic (to pass membranes) and persistant (to avoid the active principle from becoming inactive before having a curing effect) as other harmful xenobiotics; therefore, they have many of the necessary properties to bioaccumulate and provoke effects on aquatic or terrestrial ecosystems (Halling-Sorensen et al., 1998). Even though potential for accumulation of some pharmaceuticals has been addressed (Kuster et al., 2009), experimental bioconcentration test results are still scarce in the literature (e.g. atenolol). Given this background, the aim of the present study was: 1) to describe the presence and concentration of pharmaceuticals along the Suquía River basin, considering seasonal and spatial variations; and 2) to evaluate the bioconcentration of some already recognized ubiquitous pharmaceuticals found in this basin on the fish *Gambusia affinis*.

2. Material and methods

2.1. Chemicals and materials

Fifteen compounds were selected to be studied, considering different therapeutic classes of pharmaceuticals and steroid hormones, as follows: anti-inflammatory (diclofenac), β-blockers (atenolol, propranolol), antibiotics (ciprofloxacin, clarithromycin), diuretics–antihypertensive (enalapril, furosemide), antiepileptic (carbamazepine, oxcarbazepine), androgens (androstenedione, testosterone, dihydrotestosterone, methyltestosterone) and estrogens (17β-estradiol, estrone). They have different characteristics (consumption, physical and chemical properties, WWTP degradation, environmental behavior, etc.), covering a wide range of compounds that have been commonly reported by other studies (Khetan and Collins, 2007; Mompelat et al., 2009; Santos et al., 2010). Sodium diclofenac (DICL) was purchased from Parafarm (Buenos Aires, Argentina), atenolol (ATE), propranolol (PROP), ciprofloxacin (CIPR), clarithromycin (CLAR), enalapril (ENAL), furosemide (FUR), carbamazepine (CBZ), oxcarbazepine (OXCZ), androstenedione (AND), testosterone (T), dihydrotestosterone (DHT), methyltestosterone (MT), 17β-estradiol (E_2) and estrone (E_1) were purchased from Sigma-Aldrich (Buenos Aires, Argentina, purity ≥ 98%). Individual stock solutions at 1 mg/mL were prepared for most pharmaceuticals in methanol (HPLC grade). Calibration plots were obtained from fresh working solutions, prepared daily by proper dilution of solutions in the initial composition of HPLC mobile phase. All solutions were stored at −20 °C in the dark until use.

HPLC grade methanol was purchased from J.T. Baker (USA), Formic Acid 98–100% from Merck Química Argentina (Buenos Aires, Argentina) and ammonium acetate (puriss. p.a. for mass spectroscopy) from Fluka (Germany). Ultrapure water was obtained using a water purification system (Arium 611 UV system, Sartorius, Germany). Analytical grade hydrochloric acid and sodium hydroxide were from Anedra (Buenos Aires, Argentina). Cellulose filters (47 mm diameter, 0.45 μm pore size, Sartorius, Germany) and polyvinylidene fluoride membranes (PVDF 13 mm diameter, 0.22 μm pore size, Millipore, USA) were used

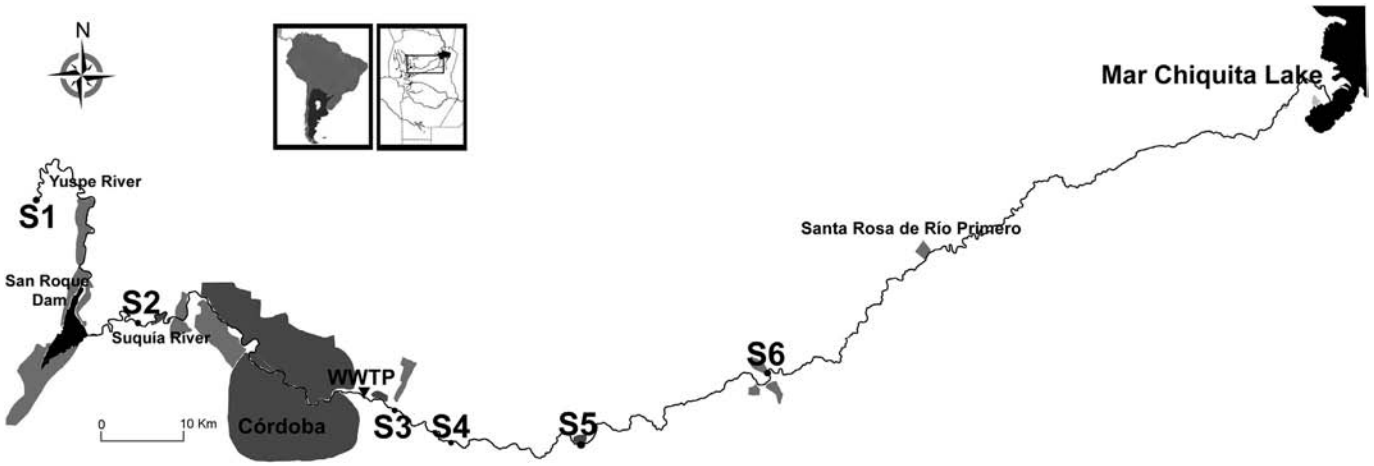

Fig. 1. Map of the Suquía River basin with sampling stations: S1: Río Yuspe; S2: La Calera; S3: Chacra de la Merced; S4: Villa Corazón de María; S5: Capilla de los Remedios; and S6: Río Primero. Freshwater superficial courses are represented in black. Areas of urban locations are depicted in the map under a gradient of gray colors according to density of population (from lighter to darker gray indicating increasing density of population).

for filtration. Polymeric reverse cartridges (Strata-X™, 500 mg/6 mL, Phemomenex, USA) were used for solid phase extraction (SPE).

2.2. Area of study and sampling procedure

The Suquía River basin is located in the province of Córdoba, Argentina (Fig. 1). The Suquía River begins at the San Roque Dam and, a few kilometers downstream, part of its water is diverted to the drinking water facilities of Córdoba City. Then the river flows for about 40 km across Córdoba City, receiving the WWTP discharge (from nearly 700,000 inhabitants). Thereinafter, the river crosses small towns, which add their sewage and run-off inputs and also use the river water for crop irrigation. The Suquía River discharges its water in the Mar Chiquita Lake, a Ramsar site (wetland of international concern included in the list of the Ramsar Convention) located 150 km downstream from Córdoba City (Fig. 1). The basin has a high flow period during the wet season (November to April), with a maximum flow of 17 $m^3\ s^{-1}$, whereas during the dry season (May to October) its flow can reach a minimum of 3 $m^3\ s^{-1}$. According to Mancini (2012) the flow of the river 0.7 km upstream the WWTP discharge oscillates between 1.35 and 4.92 $m^3\ s^{-1}$, while downstream the WWTP the river flow ranges from 3.6 to 7.7 $m^3\ s^{-1}$. Sampling sites were selected considering previous reports on the water quality of the basin (Wunderlin et al., 2001). The first sampling point (Site 1, 31°14′17.6″ South; 64°31′14.7″ West) is an already established reference site, Río Yuspe, located 30 km upstream from the San Roque Dam. Other 5 sampling locations were selected at different points of the basin considering the reported contamination gradient. Thus, next sampling area is located at the high basin, before the water supply pipeline of Córdoba City (La Calera; Site 2, 31°21′24.7″ South; 64°23′18.7″ West). Downstream Córdoba City, Chacra de la Merced (Site 3, 31°25′6.5″ South; 64°3′51.7″ West) is the first point downstream where the WWTP discharges into the river, followed by Villa Corazón de María (Site 4, 31°26′50.1″ South; 63°59′30.6″ West), Capilla de los Remedios (Site 5, 31°26′5.3″ South; 63°49′54.1″ West) and Río Primero (Site 6, 31°20′20.5″ South; 63°36′35.2″ West), nearly 70 km downstream the WWTP. Study sites were sampled twice during both dry and wet seasons (October 2011–July 2012 and March–April 2012, respectively). Two replicated water samples (without headspace) were taken in amber glass bottles (previously rinsed with ultrapure water) ca. 30 cm below the river surface, at the middle of the riverbed. Water temperature, pH and conductivity were measured in situ using WTW multiparametric equipment, previously calibrated at the laboratory (Multiline F/Set 3; American Public Health Association, [APHA] et al. (2005). Samples were ice-refrigerated and transported to the laboratory where they were filtered within 24 h using cellulose filters and stored at 4 °C until solid phase extraction step (within 72 h). Ammonia $[NH_4]^+$, nitrate $[NO_3]^-$ and nitrite $[NO_2]^-$ concentration as well as chemical oxygen demand (COD) were measured following APHA et al. (2005) methodologies.

2.3. Water sample analysis

2.3.1. Sample pre-treatment

The influence of pH (at values: 3, 6, 9) on the solid phase extraction of 15 studied pharmaceuticals was evaluated first. Higher recoveries were obtained at pH = 6 for most compounds. For that reason, NaOH 0.1 M or HCl 10% was added to filtered samples to adjust pH to this condition. Off-line solid phase extraction was carried out on a manifold, assisted by a vacuum pump. The methodology was optimized using river water samples spiked with studied pharmaceuticals. Strata-X® SPE cartridges (500 mg/6 mL) were conditioned with 10 mL methanol, followed by 10 mL ultrapure water at 1 mL min^{-1}. Then 400 mL of river water sample was loaded at 5 mL min^{-1}. Finally, cartridges were rinsed with 6 mL ultrapure water and air-dried for 20 min under vacuum. Analytes were eluted with 10 mL HPLC grade methanol. This procedure was carried out twice and both eluates from the same sample were combined. Combined eluates were completely dried under a gentle stream of nitrogen at 40 °C, considering previous reports using this temperature to get a fast evaporation (Ramirez et al., 2007; Zhang and Zhou, 2007). Finally, dried extracts were reconstituted in 400 μL methanol: ultrapure water, 15:85 (2000 × enrichment factor), vortexed, sonicated for 5 min and transferred to HPLC micro-insert vials after syringe filtration by 0.22 μm PVDF filters.

2.3.2. LC–MS

The analysis of pharmaceuticals in sample extracts was accomplished by high performance liquid chromatography coupled to mass spectrometry using a quadrupole time-of-flight analyzer, with an electrospray ionization source operated in either positive or negative modes (HPLC–ESI–qTOF, Agilent–Bruker Daltonics, USA). Detailed LC–MS analytical procedures can be found in the Supplementary data section. The precision of the method (in terms of peak areas) was expressed as the relative standard deviation (RSD) of replicate measurements. The RSD values obtained from run-to-run experiments ranged from 0.1 to 8% (3 successive injections of a standard solution) and for day-to-day from 2 to 21% (3 different days). The instrumental detection limits (IDLs) for most studied compounds ranged from 6 to 100 pg on-column, with the exception of 17β-estradiol and ciprofloxacin (with IDL of 120 and 500 pg on-column, respectively). The method detection limit (MDL) ranged from 0.1 to 16 ng L^{-1}. Quantification was done by external five-point calibration curves run in each sampling batch (5, 10, 100, 500, 1000 μg L^{-1} mixture of pharmaceuticals, dissolved in starting HPLC mobile phase, equivalent to 3.1 to 625 ng L^{-1} in river water sample), using linear regression analysis ($R^2 > 0.99$). The whole methodology recoveries for each compound, evaluated in river water, ranged from 50 to 83%. Ion suppression percentages ranged from 0 to 80%. Therefore, values reported correspond to the measured quantity (analyzed by HPLC–MS), corrected by both error sources (extraction procedure & cleanup + ion suppression). For that purpose, one sample from Río Primero (Site 6, 800 mL) was spiked with a standard mixture of the 15 studied pharmaceuticals at a final concentration of 100 ng L^{-1}, during each analytical batch. Concentrations here reported were corrected by the recovery factor obtained from this procedure. CIPR, OXCBZ, E_1 and DHT recoveries were lower than 70%, thus concentrations here reported for those compounds should be considered as semi-quantitative, following an approach similar to that used by Lindqvist et al. (2005). The optimized methodology was successfully applied to the quantification of pharmaceuticals in river water samples (Section 3.1.2) and fish tissue (Section 3.2).

2.4. Laboratory bioconcentration study

2.4.1. Fish

G. affinis (Poeciliidae, Cyprinodontiformes) is an interesting species to study due to its great invasive ability and the fact that it inhabits uncontaminated as well as polluted ecosystems (Hued and Bistoni, 2005; Grapputo et al., 2006). It has been used as an ecotoxicological model since it is easily maintained in laboratory conditions and it is widely distributed in different parts of the world (Orlando et al., 2005), including several locations along the Suquía River basin (Hued and Bistoni, 2005, 2007).

Adult *G. affinis* males (mean standard length: 23 ± 2 mm and mean body weight: 0.21 ± 0.07 g) were collected with a net from Site 2 (La Calera), where no pharmaceuticals were detected throughout this study, nor in preliminary works. Fish were transported to the laboratory into 20 L tanks and acclimatized to aquarium conditions for 1 month in 100 L aerated glass aquaria, fed twice a day ad libitum with commercial fish pellets (TetraMin, USA). One week before starting the bioassay, fish were acclimatized to aquarium water, 12:12 h light:dark photoperiod cycle and 21 ± 1 °C temperature. Twenty-four hours before the bioassay, fish were randomly separated in 5 L aquaria (1 fish per liter) and stopped feeding.

2.4.2. Exposure conditions

Five adult males were exposed during 96 h in aerated glass aquarium containing 5 L of aquarium water, supplied with 0.05% methanol (solvent control group), two groups were exposed to 10 or 100 μg L^{-1} carbamazepine (CBZ-group), while two independent groups were also exposed to 100 or 1000 μg L^{-1} atenolol (ATE-group). Each aquarium contained five individuals and two replicates were made for each of the five treatments. Higher concentrations of ATE were used since it has a lower K_{ow} compared to CBZ, therefore lower bioaccumulation would be expected. The stability of carbamazepine and atenolol in the aquarium water was previously assayed, reaching recoveries over 85% after 48 h. Therefore, during the experiments test solutions were half renewed every day, measuring the concentration of studied pharmaceuticals in the aquarium water before the renewal; thus, evaluating the actual concentration of the chemicals in the exposure medium. Dissolved oxygen (7.9 ± 0.3 mg L^{-1}), conductivity (720 ± 50 μS cm^{-1}), pH (8.3 ± 0.1) and temperature (19.8 ± 0.4 °C) were measured throughout the experiment. Fish were starved during the exposure in order to estimate the bioconcentration factors resulting exclusively from the uptake of dissolved pharmaceuticals. At the end of exposure, fish were ice-anesthetized, rinsed with ultrapure water, sacrificed by spinal cut and stored at −20 °C until analysis.

2.4.3. Tissue sample preparation

Approximately 1 g of whole fish (pool of 5 individuals), arising from each replicate, was placed into 50 mL polypropylene copolymer round-bottomed centrifuge tubes (Nalgene, USA) with 10 mL methanol for sample homogenization using a tissue homogenizer (ULTRATURRAX T18, IKA, Germany) set at 20,000 rpm. Then 10 mL HCl 0.05 M was added and samples were homogenized again. Homogenates were sonicated for 20 min at 30 °C (Chu and Metcalfe, 2007). Supernatants were separated by centrifugation for 15 min at 3000 *g*. The same procedure was repeated twice. Methanol was evaporated in a rotary evaporator at reduced pressure (Buchi, Switzerland), and pH was adjusted to 6 by adding NaOH 1 M. This allowed the precipitation of lipids, which were separated by centrifugation at 3000 *g* for 20 min. Supernatants were transferred to glass bottles and ultrapure water was added to reach a volume of 100 mL. These aqueous extracts were analyzed following the same procedure used for water samples (Section 2.3) with the exception of final reconstitution in 1000 μL of methanol:ultrapure water, 15:85. Recoveries from fish homogenates were previously evaluated for carbamazepine and atenolol, obtaining 40% recovery at 100 ng g^{-1} and 1000 ng g^{-1} wet weight, for both compounds and 80% recovery with respect to a standard prepared from a fish extract. As important ion suppression percentages were obtained using the described methodology (ca. 50% for both compounds), calibration curves were prepared from fish extracts (control group, free from pharmaceuticals), in the range of 5–1000 μg L^{-1} of CBZ and ATE, equivalent to 6.25–1250 ng g^{-1} in fish tissue; thus, allowing a more accurate quantification of both compounds.

2.4.4. Water analysis

Water samples from each treatment (including replicates) were analyzed by the same methodology used for river water determinations (Section 2.3).

2.4.5. Bioconcentration factor estimation

Bioconcentration factors (BCFs, in L kg^{-1} unit) at each exposure treatment were estimated as the ratio between the concentration of the corresponding pharmaceutical in whole fish (μg kg^{-1} wet weight) and the measured pharmaceutical concentration in water samples (μg L^{-1}).

2.5. Statistical analysis

Statistical analyses were performed using the Infostat Software Package (Di Rienzo et al., 2012). All values are expressed as mean ± standard deviation. Differences among sites and sampling periods were assessed by one way analysis of variance (ANOVA). Since parametric assumptions were not fulfilled, Kruskal–Wallis followed by multicomparison Dunn tests were used (P-value < 0.05 for significant differences). Values below the limit of detection were considered as 0 for ANOVA.

3. Results and discussion

3.1. Occurrence of pharmaceuticals in the Suquía River

3.1.1. Chemical characterization of water samples

Results of chemical analysis in water samples are presented in Table 1. In general, there are no significant differences between the values obtained during the dry and wet seasons, except for 6 parameters. Most of them correspond to S4, showing that this site presented temporal variability (seasonality). In both seasons, samples from Site 1 (Río Yuspe) and Site 2 (La Calera) presented better water quality conditions than sites located downstream from Córdoba City. This is in agreement with previous reports, since there are no evident anthropogenic sources of pollution in Site 1 (*quasi*-pristine area) and Site 2 is located upstream from the urban area. Conversely, the water quality of the Suquía River dramatically decreases downstream from Córdoba City (Sites 3 to 6). This behavior has been mentioned several times in previous reports (Pesce and Wunderlin, 2000; Monferrán et al., 2011; Merlo et al., 2011). During the dry season (Table 1), conductivity values show

Table 1
Chemical characterization of water samples in dry (1.a) and wet (1.b) season at sampling sites S1 to S6.

	Season	pH	C	$[NH_4]^+$	$[NO_2]^-$	$[NO_3]^-$	COD
S1	Dry	8.0 (0.7)	108^{a} (58)	0.6^{a} (0.7)	$<LOD^{a}$	2^{a} (2)	40 (44)
	Wet	8.4 (0.3)	88.5^{a} (5)	0.2^{a} (0.1)	$<LOD^{a}$	2^{a} (1)	123 (174)
S2	Dry	8.1 (0.6)	254^{ab} (69)	0.5^{a} (0.3)	0.04^{ab*}	4^{ab} (1)	66 (47)
	Wet	8.2 (0.4)	169^{ab} (11)	0.6^{ab}(0.1)	$<LOD^{a}$	3^{ab} (1)	213 (52)
S3	Dry	7.7	1544^{b}	34^{bc} (5)	5.1^{c*} (0.2)	14^{c*} (2)	837 (920)
	Wet	7.7	1237^{b}	11^{bc} (6)	0.08^{ab}	28^{c} (3)	520 (571)
S4	Dry	7.4 (0.3)	1523^{b*} (39)	42^{c*} (7)	1.0^{c} (0.5)	5.9^{bc*} (0.4)	603 (657)
	Wet	7.9 (0.3)	1202^{b} (87)	18^{c} (7)	0.5^{b} (0.3)	13^{bc} (2)	411 (409)
S5	Dry	7.8 (0.1)	1441^{b} (17)	28^{bc} (2)	0.5^{bc} (0.4)	6^{abc} (1)	223 (282)
	Wet	7.7(0.04)	1188^{b} (120)	21^{c} (10)	0.4^{b} (0.2)	12^{abc} (6)	883 (1342)
S6	Dry	7.7 (0.1)	1332^{b} (116)	8^{ab} (6)	0.6^{bc} (0.4)	22^{c} (7)	226 (310)
	Wet	7.9 (0.1)	1118^{b} (138)	4^{abc} (3)	0.4^{b} (0.1)	29^{c} (5)	316 (402)

$LOD_{[NO2-]}$: 0.04 mg L^{-1}.
Values are expressed as mean (standard deviation): pH in pH units, conductivity (C) in μS cm^{-1}; concentrations in mg L^{-1}. For each parameter, * indicates significant differences between seasons at each sampling site. Mean values with a common letter are not significantly different among sampling sites within the same season (P < 0.05).

this general pattern, increasing in Site 3 and maintaining this level up to Site 6. Pesce and Wunderlin (2000) identified ammonia and nitrite among the main pollutants associated with sewage pollution in this section of the river. In the present study, highest concentrations of ammonia were found in Sites 3, 4, and 5, decreasing towards Río Primero (Site 6). Ammonia concentration, measured downstream the city of Córdoba were above the Argentinean Environmental Water Quality Guideline for aquatic biota protection (0.06–0.60 mg L^{-1} NH_4^+) (AEWQG, 2003). A similar pattern was found for nitrites. Inversely, nitrate concentrations increased towards Rio Primero, probably as a consequence of the natural oxidation of both, ammonia and nitrites (Pesce and Wunderlin, 2000). Chemical oxygen demand (COD) results showed an increase after the WWTP discharge and attenuation downstream, even though differences among sites were not statistically significant. In the wet season (Table 1), even though the general tendency of all parameters remained the same, lower values were found, corresponding to the dilution effect caused by the high flow period.

3.1.2. Seasonal and spatial distribution of pharmaceuticals

Pharmaceutical concentrations are reported in Table 2. Values reported in rivers and streams receiving urban effluents from other countries are also shown in Table 2. Seven out of 15 measured compounds were below the detection limit throughout studied samples: propranolol, clarithromycin, furosemide, androstenedione, testosterone, methyltestosterone and 17β-estradiol. Conversely, eight out of 15 pharmaceuticals studied were found in river waters: ciprofloxacin, enalapril, estrone, dihydrotestosterone, oxcarbazepine, carbamazepine, atenolol and diclofenac. Moreover, concentrations of studied pharmaceuticals were always below the detection limit in samples from sites located upstream from Córdoba City (Sites 1 and 2). These results are relevant considering that S2 is near the intake of the drinking water facilities of Córdoba City. Thus, there is no evident risk for the city inhabitants of consuming pharmaceuticals through drinking water.

Conversely, water samples from all sites located downstream the city (Sites 3 to 6) had quantifiable amounts of pharmaceuticals, showing the negative impact of the WWTP discharge into the Suquía River. These results are in accordance with those obtained with chemical parameters (Section 3.1.1), showing the negative impact of WWTP on the water quality, raising concerns about its use for domestic or recreational activities. This finding reinforces the idea of wastewater urban effluents as important sources of pharmaceuticals in river waters (Silva et al., 2011; Ferrari et al., 2011). The percentage (frequency) of positive findings of pharmaceuticals in Suquía River samples (S2 to S6) is presented in Fig. 2. Among eight detected compounds, ciprofloxacin (CIPR) and enalapril (ENAL) were present during the dry season in two sites downstream the WWTP discharge (22% frequency in the Suquía River). Similar concentrations of ENAL and CIPR (Table 2) have been detected in the Ebro River and its tributaries (Spain) with 33–60% and 7–11% frequency, respectively (López-Serna et al., 2012).

Estrone (E_1) and dihydrotestosterone (DHT) were quantified during the wet season (Table 2). E_1 (33% frequency in the Suquía River) increased its concentration towards Site 6, while DHT was only present in the last monitoring station (Site 6, 22% frequency in the Suquía River). E_1 has been previously detected in superficial waters. Our current values are within the reported range (Table 2). Not only the conversion of 17β-estradiol (E_2) to E_1, in aerobic batch experiments with activated sludge, has been reported but also its transformation by microorganisms in water samples from United Kingdom rivers (Yin et al., 2002), explaining the difficulty to detect E_2. Moreover, log K_{oc} values of estrogens greater than 1 have been reported, indicating

Table 2
Concentration of studied pharmaceuticals (ng L^{-1}) reported as mean (standard deviation) and ranges (min–max) observed in sampling sites (S1–S6) during dry and wet seasons.

		S1	S2	S3		S4		S5		S6		Reported[a]
DICL	Dry	<LOD[b]	<LOD	124 (9)	117–130	100 (40)	62–136	91 (60)	34–145	43 (49)	<LOD-88	0.4–18740
	Wet	<LOD	<LOD	59[ab] (1)	58–59	68[b] (3)	65–73	71[b] (29)	44–102	7[a] (8)	<LOD-14	
ATE	Dry	<LOD	<LOD	338[ab] (22)	322–353	481[bd] (91)	372–581	389[bd] (70)	322–453	130[a] (139)	9–261	50–2225
	Wet	<LOD	<LOD	169[ab] (11)	161–177	279[b] (20)	255–302	312[b] (32)	289–359	31[a] (13)	19–43	
PROP	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
CIPR	Dry	<LOD	<LOD	<LOD		18 (20)	<LOD-35	18 (21)	<LOD-36	<LOD		23–1300
	Wet	<LOD	<LOD			<LOD		<LOD		<LOD		
CLAR	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
ENAL	Dry	<LOD	<LOD	<LOD		<LOQ		2 (2)	<LOD-4	<LOD		0.67–88
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
FUR	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
CBZ	Dry	<LOD	<LOD	27[ab] (5)	23–30	21[a] (2)	18–22	20[a] (4)	17–25	41[b] (6)	33–47	1.2–3090
	Wet	<LOD	<LOD	16[a] (1)	15–16	19[ab] (3)	16–21	65[bc] (34)	40–113	73[c] (39)	33–110	
OXCZ	Dry	<LOD	<LOD	<LOD		<LOD		19 (22)	<LOD-39	23 (27)	<LOD-51	51–255[c]
	Wet	<LOD	<LOD	<LOD		<LOD		18 (20)	<LOD-35	22 (25)	<LOD-44	
E_2	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
E_1	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOQ		4.29–113
	Wet	<LOD	<LOD	<LOD		<LOD		<LOQ[a]		6[b] (2)	5–8	
AND	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
T	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
MT	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		
DHT	Dry	<LOD	<LOD	<LOD		<LOD		<LOD		<LOD		55.3
	Wet	<LOD	<LOD	<LOD		<LOD		<LOD		7 (3)	4–10	

Mean values with a common letter are not significantly different among sampling sites within the same season (Kruskal Wallis test, $P \leq 0.05$).

[a] Revised literature: (Thomas and Hilton, 2004; Fent et al., 2006; Hernando et al., 2007; Vieno et al., 2007; Leclercq et al., 2009; Lei et al., 2009; Ribeiro et al., 2009; Pal et al., 2010; Gracia-Lor et al., 2011; Liu et al., 2011; Silva et al., 2011; Valcárcel et al., 2011; López-Serna et al., 2012; Osorio et al., 2012; Laane et al., 2013).

[b] <LOD: below limit of detection, for each compound being (ng L^{-1}) DICL: 0.5; ATE: 0.2; PROP: 0.5; CIPR: 16; CLAR: 0.3; ENAL: 0.1; FUR: 2; CBZ: 0.2; OXCZ: 3; E_2: 6; E_1: 2; AND: 0.8; T: 0.6; MT: 0.1; and DHT: 2.

[c] Concentrations reported in WWTP effluents (Leclercq et al., 2009).

[d] Indicates significant differences in seasons at each sampling site (Kruskal Wallis test, $P \leq 0.05$).

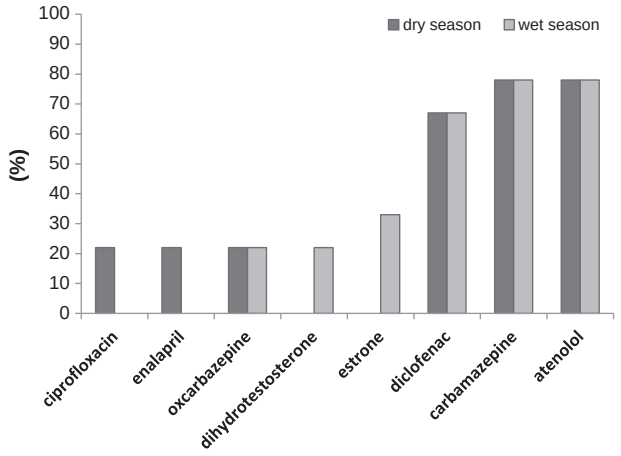

Fig. 2. Frequency of detected pharmaceuticals in Suquía River samples (calculated as percentage of positive findings in S2 to S6 samples within each season). [1] PROP, CLAR, FUR, E2, AND, T, MT: <LOD in all studied samples.

higher affinity of estrogens to sediments rather than the water phase (Lei et al., 2009). On the other hand, Liu et al. (2012) quantified DHT and E_1 in wastewater of dairy cattle farms, and E_1 in receiving stream waters up to 20.7 ng L^{-1}. In our case, the Site 6 is located at the beginning of the plain area of the eastern part of the Province of Córdoba, which has frequent presence of cattle farms. Therefore, these farms could be contributing to the steroid load into the Suquía River by runoff.

There are very few reports on the presence of androgens in surface waters. Liu et al. (2011) found DHT in Danshui River, China (impacted by WWTP effluent) in similar levels than those found in the present study. Even with low frequency, the detection of DHT during the present study contributes to the general background knowledge on the fate of androgens to the environment. The effects on the aquatic biota associated to the presence of DHT and E_1 should not be neglected, as Margiotta-Casaluci and Sumpter (2011) and González et al. (2012) reported endocrine disrupting effects on fish occurring at 20 ng L^{-1} and 1–10 ng L^{-1}, respectively.

From Site 3 (Chacra de la Merced) to Site 6 (Río Primero), the anti-inflammatory diclofenac (DICL), the antiepileptic carbamazepine (CBZ) and the β-blocker atenolol (ATE) were ubiquitous throughout the studied period. In agreement with their high frequencies of detection (67–78% in the Suquía River), these 3 compounds have been proposed in the literature as suitable indicators for tracking the presence of municipal sewage contaminations in surface waters (Heberer, 2002; Nakada et al., 2008). DICL reached up to 145 ng L^{-1}, while CBZ reached up to 113 ng L^{-1} at the Site 5. Both compounds have been reported in other surface waters of the world at similar levels (Table 2). Oxcarbazepine (OXCZ), another antiepileptic drug, was detected in both seasons at Sites 5 and 6. Although with low frequency (22%), this is, to our knowledge, the first report of OXCZ in river water samples. OXCZ has been previously reported in effluents (Leclercq et al., 2009) at the same concentration range found during this study in natural river waters.

The highest concentration of studied pharmaceuticals in the river corresponds to ATE, which reached 581 ng L^{-1} in the Site 4 (Villa Corazón de María).Osorio et al. (2012) reported similar concentrations in surface waters from Spanish river systems and Kasprzyk-Hordern et al. (2008) in rivers of the South Wales region (United Kingdom).

As regards seasonal comparison, higher concentrations of atenolol and diclofenac occurred during the dry season, although significant differences were only found for atenolol. Higher concentrations observed during the dry season could be explained by the lower river flows at this time of year. Concerning spatial distribution, significant differences were found among sampling sites for the 3 ubiquitous compounds (DICL, ATE and CBZ). The general trend for DICL and ATE was a decrease in the concentration of these pharmaceuticals as the distance from the WWTP increases (Table 2). ATE concentrations in both seasons followed the same pattern than ammonia (Table 1), reinforcing the idea of WWTP effluent as the main source for these pollutants. A different spatial distribution pattern was observed for carbamazepine, whose concentrations significantly increased in the Site 6 (Río Primero). As it was previously mentioned, the plain area of the basin begins towards Site 6, with lower river flow and more settling of suspended material. Lahti and Oikari (2011) measured pharmaceuticals in settleable particulate material of Finland Rivers receiving WWTP effluents, finding appreciable amounts of CBZ (3.2–19.1 ng g^{-1} dry weight). Vazquez-Roig et al. (2012) also mentioned the tendency of CBZ to be accumulated in sediments, since they found it with higher frequency in this compartment (100%) than in water (26%) or soil samples (39%) of the Pego–Oliva marsh (Spain). Given the capability of CBZ to sorb to settleable material, sediments from the Site 6 could possibly be acting as reservoirs of this compound, maintaining a constant source of CBZ to the water. These facts, along with its low removal efficiency by WWTPs (≤10%, Ternes, 1998), and its resistance to photodegradation in surface waters (Andreozzi et al., 2003; Yamamoto et al., 2009), could explain the increase in CBZ concentration in the Site 6. Moreover, OXCZ levels followed a similar distribution pattern than CBZ, probably given their structural similarities.

A NOEC of 0.5 μg L^{-1} for DICL was derived by Hoeger et al. (2005) based on histopathological and immunohistological effects, evaluated on various organs of brown trout at environmentally relevant concentrations. A similar situation is found for CBZ, since Ferrari et al. (2003) reported a PNEC of 0.42 μg L^{-1} for this compound, considering the risk quotient estimation on different taxonomic groups. Based on these results, a low risk scenario for fish in the Suquía River is expected, since the highest measured concentrations of DICL and CBZ found during this study are lower than the reported NOEC and PNEC, respectively. Nevertheless, it should be considered that these ecotoxicological values are derived from other species, which do not inhabit the Suquía River basin. So far, to our knowledge, the effects of pharmaceuticals on native species remain unknown.

Atenolol, on the other hand, has not yet been found to be toxic, even at μg L^{-1} levels (Cleuvers, 2005; Kim et al., 2009; Santos et al., 2010; Verlicchi et al., 2012). Nevertheless, Pomati et al. (2008) identified ATE among priority pharmaceuticals to be studied, considering its long-term effects on aquatic species, as it displayed statistically significant effects on prokaryotic and eukaryotic cells in environmentally relevant exposure levels (ng L^{-1}) when evaluated in a mixture of drugs.

3.2. Bioconcentration test

Among the pharmaceuticals most frequently detected in the Suquía River basin, as in most reported surface waters worldwide, atenolol (ATE) and carbamazepine (CBZ) were selected to study their bioconcentration in *G. affinis*. This decision was taken considering the lack of published data on the accumulation of ATE in fish, while the bioaccumulation behavior of CBZ has not yet been reported in *G. affinis*.

3.2.1. Measured test concentrations

The measured exposure concentrations in water samples for the 96 h bioconcentration test were: 12 ± 1 μg L^{-1}, 105 ± 4 μg L^{-1} for CBZ (nominal concentrations: 10 and 100 μg L^{-1}, respectively) and 94 ± 4 μg L^{-1}, 700 ± 200 μg L^{-1} for ATE (nominal concentrations: 100 and 1000 μg L^{-1}, respectively). Neither CBZ nor ATE was detected in control samples.

3.2.2. Bioconcentration factors (BCF)

Average CBZ and ATE concentrations (ng g^{-1} wet wt.) in *G. affinis* whole body tissue, during the 96 h bioconcentration test, are presented in Fig. 3. CBZ and ATE levels in fish from the control treatment were

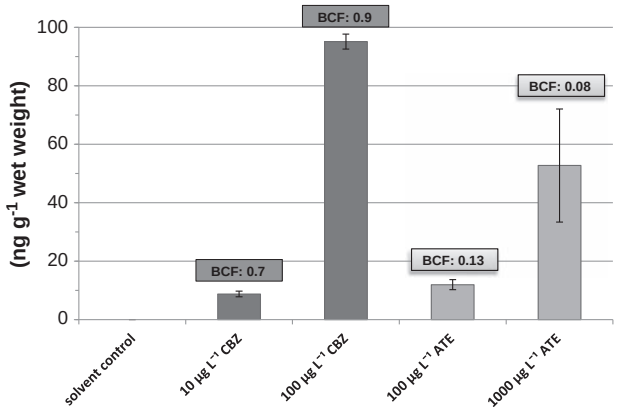

Fig. 3. Accumulation of carbamazepine (CBZ) and atenolol (ATE) in whole body fish (ng g^{-1} wet weight) under tested conditions. Bioconcentration factors (BCF) were calculated for each condition (numbers above bars, in L kg^{-1} unit).

below the limit of detection (2 and 4 ng g^{-1} wet wt., respectively). Both compounds were detected in whole body fish under exposure conditions. The average bioaccumulation of CBZ was 9 ± 1 and 95 ± 3 ng g^{-1} wet wt. (at 10 and 100 µg L^{-1} exposure levels), while the average bioaccumulation of ATE was 12 ± 2 and 53 ± 19 ng g^{-1} wet wt. (at 100 and 1000 µg L^{-1}, respectively). As Fig. 3 shows, fish exposed to CBZ and ATE showed a concentration-dependent bioaccumulation. In addition, at the same concentration of pure pharmaceuticals (100 µg L^{-1}), an almost eight times higher bioaccumulation of CBZ was observed in comparison with ATE (95 and 12 ng g^{-1} wet weight, respectively). Considering *n*-octanol/water partition coefficients (log K_{ow}), a higher bioaccumulation of CBZ is expected, since log $K_{ow,CBZ}$: 2.45 (Beausse, 2004) is 15 times higher than log $K_{ow,ATE}$: 0.16 (Hernando et al., 2007). Even though bioaccumulation of CBZ and ATE occurred in studied fish, estimated bioconcentration factors (BCFs) were ≤1 for both compounds, indicating low bioaccumulation potential. Moreover, BCF_{CBZ}: 0.7–0.9 L kg^{-1} was 8 times higher than BCF_{ATE}: 0.13–0.08 L kg^{-1}, as previously explained. To our knowledge, this is the first report on bioconcentration of ATE in fish tissue. Winter et al. (2008) reported a male fathead minnows (*Pimephales promelas*) plasma concentration of 0.0518 mg L^{-1} upon exposure to 3.2 mg L^{-1} ATE. Additionally, Cleuvers (2005) calculated BCFs based on log P (log K_{ow}) values for β-blockers; however, they could not calculate BCF for ATE because of its low K_{ow}. As Daughton and Brooks (2010) summarized, CBZ has a low propensity to bioconcentrate and most BCF derived in fish by different authors are <1. However, Garcia et al. (2012) reported laboratory tissue-specific BCF ranging 1.5–7.1 L kg^{-1}. The higher BCFs reported in that study could probably be derived from differences in evaluating BCF from specific organ tissues (potentially more concentrated in pharmaceutical residues) rather than whole body fish tissue.

Even though apparently no potential for bioconcentration was found for CBZ or ATE in *G. affinis*, both compounds were bioaccumulated by this species under the laboratory conditions used in our study after 96 h exposure. Given the background knowledge on toxicity, especially for CBZ, and the fact that fish are exposed to a pool of pharmaceuticals over a long period of life, more specific studies should be carried out to accurately assess the risk of these pharmaceuticals to non-target organisms.

4. Conclusion

Emerging organic contaminants are ubiquitous in the environment and South American rivers are not an exemption as it was demonstrated by the presence of many pharmaceuticals in the Suquía River basin.

WWTPs are not prepared to eliminate these compounds from domestic sewage as it was evidenced by the presence of 8 out of 15 compounds analyzed during this work, which were quantified up to 70 km downstream the WWTP discharge to the river.

Atenolol, carbamazepine and diclofenac were the most frequently detected compounds in both dry and wet seasons, with concentrations ≤ 1 µg L^{-1}. The possible effects of them on the native biota should be addressed in future research.

Although sporadically, the presence of E_1 and DHT in river waters evidence the need of more sensible methods to evaluate the possible presence of other estrogens and androgens, given the risks they pose to the aquatic biota.

The potential for bioaccumulation of ATE and CBZ in *G. affinis* was low at the concentrations tested during this work (10–1000 µg L^{-1}) in the laboratory. However, given the high frequency of detection of both compounds in the Suquía River, and in many other streams and rivers of the world, low level chronic exposure is a more realistic scenario, in addition to the presence of a mixture of pharmaceuticals. So far, field derived bioaccumulation factors should be estimated in future works to better understand the risk that these pharmaceuticals pose to the aquatic biota.

Conflict of interest

Dr. Daniel A. Wunderlin, BSc. María Eugenia Valdés, Dr. María Valeria Amé and Dr. María de los Ángeles Bistoni declare that we do not have any conflict of interest on submitting the MS *"OCCURRENCE AND BIOACCUMULATION OF PHARMACEUTICALS IN A FISH SPECIES INHABITING THE SUQUIA RIVER BASIN (CORDOBA, ARGENTINA)"*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.10.124.

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