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Occurrence of Antimicrobials in River Water Samples from Rural Region of the State of Rio de Janeiro, Brazil

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Abstract

The occurrence of antimicrobials in the aquatic environment and drinking water has raised the question of their impact on the environment and public health. Animal production is one of the most expressive activities of Brazilian agribusiness. In order to ensure the productivity and competitiveness of the sector, the use of drugs for therapeutic and prophylactic purposes is a common practice. Due to the continuous release of antimicrobials into the environment, the aim of this study was to compare the frequency of detection of tetracyclines and sulfonamides in surface water collected from rural areas in Lidice District of Rio Claro, in the State of Rio de Janeiro, Brazil. An investigative study was conducted with 24 river water samples analyzed by high-performance liquid chromatography coupled to tandem mass spectrometry and the aim of this study was to determine residues of sulfonamides and tetracyclines based on the USEPA method 1694. The results indicated the presence of sulfamethoxazole and oxytetracycline concentrations at the ng·L^{-1} level. The applied method showed overall good performance with recoveries above 57%, method detection limits ≤ 7.17 ng·L^{-1}, method quantification limits ≤ 23.90 ng·L^{-1} and good linearity.

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1. Introduction

Animal production is one of the most expressive activities of Brazilian agribusiness. In order to ensure the productivity and competitiveness of the sector, the use of drugs for therapeutic and prophylactic purposes is a common practice. Concerning the drugs employed, the antimicrobial agents correspond to one of the most widely prescribed classes [1]-[7]. Antimicrobials are drugs used in human and veterinary medicine. They are applied as growth promoters and for therapeutic or prophylactic purposes [2]. Therefore, several classes, as aminoglycosides, β-lactams, fluoroquinolones, lincosamides, macrolides, tetracyclines and sulfonamides are widely used in veterinary medicine [8] [9].

There are several possible sources and routes for the occurrence of antimicrobials in the aquatic environment. As regards to human use antimicrobials, non-prescribed medicines are consumed at home, and other prescribed are consumed in hospitals and clinics. These drugs are metabolized and excreted partly in urine and faeces, then go to the sewage collection systems. Unused or surplus medicines, or out of date, can be discarded of in toilets, although this practice is not currently recommended. Hospital effluents can be treated separately or combined with municipal wastewater and then treated in wastewater treatment plants (WWTP). The consumption of veterinary antibiotics is held on farms, veterinary clinics and at home to treat pets [10]. There is a great difficulty in predicting the possible implications of the presence of antimicrobials in water and sewage, regarding to the environment and public health. Furthermore, some drugs such as antidepressants and antimicrobials may be prone to bioconcentration and bioaccumulation in aquatic organisms, particularly fish. Therefore, the presence of pharmaceuticals in environmental waters, especially in drinking water and pharmaceuticals industries effluents should be considered an important issue in terms of safety of human health [11].

Different adverse effects of pharmaceutical compounds have been described including aquatic toxicity, development of resistance in pathogenic bacteria, genotoxicity and endocrine disorders [12]-[14].

Several substances have attracted attention because of their impact on the environment, especially in aquatic one, due to their high stability and toxicity. Water treatment systems are not very effective to degrade this substance, which is an extremely important requirement for disposing such waste, preventing the risk of environment contamination [11] [15].

The integrity of human and environmental health is closely related to a good sanitation and health of the waters. According to the World Health Organization (WHO), about 85% of known diseases are waterborne, or are associated with water [16].

Pharmaceuticals compounds are present at low level ranging from ng$\cdot$L^{-1} to μg$\cdot$L^{-1}. A large number of analytical methodologies have been developed for the determination of antimicrobials residues in surface waters [17].

Sulfonamides (SF) are inexpensive antimicrobial used to treat bacterial infections with a broad spectrum of activity, employed in human and veterinary medicine [7]. Tetracyclines (TC) are widely used in veterinary medicine for both prophylactic and therapeutic purposes in food producing animals, besides in humans [2].

Several veterinary drug residue monitoring programs have been conducted worldwide, and sulfonamide and tetracycline residues have been detected in food of animal origin, proving the use of these drugs in veterinary medicine [18].

The aim of this study was to determine residues of sulfonamides and tetracyclines based on the United States Environmental Protection Agency—USEPA 1694 [19]. USEPA Method 1694 is intended to determine pharmaceuticals and personal care products (PPCPs) in environmental samples (aqueous, solid and biosolids matrices) by high performance liquid chromatography combined with tandem mass spectrometry (HPLC/MS/MS) using isotope dilution and internal standard quantitation techniques. 24 Surface water samples were collected from two rivers (Parado and Pedras) in the Lidice District of Rio Claro, in the state of Rio de Janeiro, Brazil, in August 2014 (**Figure 1**). The method was able to determine 14 analytes from tetracycline and sulfonamide classes: chlortetracycline, demeclocycline, doxycycline, metacycline, oxytetracycline, tetracycline, dapsone, sulfacetamide, sulfadimethoxin, sulfamerazine, sulfamethazine, sulfamethoxazole, sulphaquinoxaline and sulfathiazole.

Figure 1. Water map of Rio Claro city, in the State of Rio de Janeiro, highlighting Lidice District. Source: Rio Claro geography [20].

2. Material and Methods

2.1. Chemicals and Materials

Methanol (MeOH) for liquid chromatography was purchased from J.T. Baker (Phillipsburg, NJ, USA). LC-grade acetonitrile (ACN) and reagent grade hydrochloric acid, oxalic acid (OXA), formic acid (FOA) and acetone (ACE) were purchased from Merck (Darmstadt, Germany). Ethylenediaminetetracetic acid disodium dihydrate (EDTA) was acquired from Calbiochem (Gibbstown, NJ, USA). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Certified reference standards of oxytetracycline, doxycycline hyclate and hydrochloride salts of chlortetracycline, demeclocycline, dapsone, sulfacetamide, sulfadimethoxin, sulfamerazine, sulfamethazine, sulfamethoxazole, sulphaquinoxaline and sulfathiazole were available from US. Pharmacopeial Convention (Rockville, MD, USA). Tetracycline hydrochloride was a Chemical Reference Substance of the Brazilian Pharmacopeial Convention (Santa Maria, RS, Brazil). Methacycline was acquired from Acros (Pittsburgh, PA, USA).

Solid-phase extraction (SPE) was performed with 60 mg Oasis® HLB cartridges from Waters Corp. (Milford, MA, USA).

2.2. Sample Collection and Preparation

300 mL of each water samples were collected in polypropylene bottles during three consecutive Sundays (17, 24 and 31 August 2014), in the morning and in the afternoon, in the upper and lower sides of each river (two sampling points), a total of eight samples per day collection, according to the **Table 1**. Grab sampling procedures were applied in study. The lengths of the rivers Parado and Pedras are respectively 10 km and 13 km.

The 24 samples were sealed, identified and transported under refrigeration to the laboratory for performing analyses. An aliquot of 100 mL of the each sample was acidified to pH 2.5 with HCl, and then 100 mg of EDTA was added. 25 mL of this solution was applied to an Oasis HLB® cartridge previously conditioned with 3 mL of MeOH, 3 mL of ultrapure water and 3 mL of ultrapure water acidified to pH 2.5 with HCl. A manifold vacuum from Alltech (Deerfield, IL, USA) was used for SPE. Cartridges were washed twice with 2 mL of ultrapure water and then dried under vacuum (-35 kPa) for 2 min. Antimicrobials were eluted with two portions of 2 mL methanol and two portions of 2 mL ACE:MeOH (1:1, v/v), using gravity flow only. 1 mL aliquots of the eluate were transferred to two centrifuge tubes and evaporated to dryness under N_2 in a temperature up to 47.5°C, using an evaporator with nitrogen flow (Pierce Reacti-Therm III™ and Pierce Reacti Vap™ III, Rockford, IL, USA).

The dry residues were reconstituted with 1 mL of 0.01 mol·L^{-1} OXA:MeOH (80:20, v/v) for tetracycline analysis and 1 mL of 0.01 mol·L^{-1} FOA:MeOH (80:20, v/v) for sulfonamide analysis, vortexed for 30 s and

Table 1. Sample collection.

	Pedras River		Parado River	
	Upper Side	Lower Side	Upper Side	Lower Side
Morning	3 per day	3 per day	3 per day	3 per day
Afternoon	3 per day	3 per day	3 per day	3 per day
Total samples in 3 consecutive days	6	6	6	6

transferred to amber auto-sampler vials for the injection of 10 µL (TC) and 20 µL (SF) [3] [7].

In order to calculate method recoveries, 25 mL of a solution at 100 ng·L^{-1} (TC and SF) and 25 mL of ultra-pure water were used. Ultrapure water samples were spiked post-extraction and reconstituted with 1 mL of 100 ng·L^{-1} solutions (TC and SF) prepared in the respective dilution solvents, 0.01 mol·L^{-1} OXA:MeOH (80:20, v/v) for tetracycline analysis and 1 mL of 0.01 mol·L^{-1} FOA: MeOH (80:20, v/v) for sulfonamide analysis. Six point calibration curves in the range of 78 ng·L^{-1} to 780 ng·L^{-1} were constructed and fitted by weighted regression analysis using a factor of $1/x^2$ and were performed in order to quantify the analytes. Recovery corrections were applied in all samples for quantification.

2.3. LC/MS-MS Instrumentation

The LC-MS/MS system was a Shimadzu Prominence HPLC instrument (Kyoto, Japan) equipped with a quaternary pump (LC-20AD), a membrane degasser (DGU-20A5), an auto-sampler (SIL-20AC), a column oven (CTO-20AC) and a system controller (CBM-20A) interfaced to a triple quadrupole mass spectrometer (API5000, Applied Biosystems/MDS Sciex, Foster City, CA, USA) with a TurboIonSpray® ESI source. Analyst® V1.4.2 LC/MS control software was used. Maintained at 0.15 mL·min^{-1} at 25°C. The autosampler was set at 4°C. Positive electrospray ionization technique (ESI+) in Multiple Reaction Monitoring (MRM) acquisition mode was used to monitoring three ions for each substance. Nitrogen was employed as nebulizer and dryer gas (Gas 1 and Gas 2 = 40 psi), collision-activated dissociation (CAD) gas (6, arbitrary unit) and Curtain™ gas (10 psi). Other parameters selected during automatic tuning were: ionspray potential = 5000 V, source temperature = 500°C, entrance potential = 10 V, resolution Q1 and Q3 = unit, dwell time of 150 ms for each MRM transition. The analytical column was a Pursuit™ RS C18 (100 mm × 2 mm id, 3 µm particle size, 200 Å), with a respective guard column (Agilent, Santa Clara, CA, USA). Mobile phases A, B and C consisted of H_2O, ACN and MeOH, all of them with 0.1% FOA. Gradient elution programs were carried out for separating the antimicrobials, as described in **Table 2** [3] [7]. Column temperatures and injection volumes are also described in **Table 2**.

Multiple Reaction Monitoring (MRM) experiments for tetracycline analysis in electrospray positive-ion mode (ESI+) were described by Spisso *et al.* [3] and the employed conditions are listed in **Table 3**.

Fragmentation studies with sulfonamides for tuning the mass spectrometer were performed with mixed standard solutions at concentrations between 50 and 100 ng·mL^{-1} in MeOH: 1% FOA (50:50, v/v). ESI+ in MRM acquisition mode was used to monitor three ions for each substance.

3. Results and Discussion

3.1. Mass Spectrometry Conditions

Optimizing the mass spectrometer detection consists of adjusting several parameters relating to both ionization process in the ion source and ion transportation in the MS in order to maximize the response of the mass spectrometer for each analyte. MRM acquisition mode is the most suitable for quantification due to its sensitivity and specificity. Declustering Potential (DP), Collision Energy (CE) and Collision Cell Exit Potential (CXP) values for sulfonamide precursor/product ion pairs obtained in MRM mode are showed in **Table 3**. For sulfonamides, only protonated molecules $[M + H]^+$ were observed and selected as precursor ions, and no adducts were noted. The three most abundant fragment ions were monitored for each compound. For target analytes, the first transition was used for quantification purposes, whereas the second and the third ones to confirm the identity of the substances.

SFs showed a standard mass spectrum, due to the presence, in their structure, of a heterocycle attached to the

Table 2. Gradient elution programs for Sulfonamides (SF) and Tetracyclines (TC).

TC Method					SF Method				
Time (min)	% A	% B	% C	Flow rate (mL·min^{-1})	Time (min)	% A	% B	% C	Flow rate (mL·min^{-1})
1	80	5	15	0.15	1	80	5	15	015
15	60	25	15	0.15	15	60	25	15	0.15
16	5	5	90	0.15	16	5	5	90	0.25
26	5	5	90	0.15	20	5	5	90	0.25
27	80	5	15	0.15	26	5	5	90	0.15
35	80	5	15	0.15	27	15	5	15	0.15
-	-	-	-	-	35	15	5	15	0.15
Column temperature		15°C					25°C		
Injection Volume		10 μL					20 L		

sulfonamide moiety. The fragmentation scheme of protonated sulfonamides in positive-ion ESI has already been described in the literature and was characterized by m/z 92 [H_2NPhenyl]$^+$, m/z 108 [H_2NPhenylO]$^+$, m/z 156 [H_2NPhenylSO$_2$]$^+$, besides [M + H-93]$^+$ and [M + H-66]$^+$ [21].

All of the sulfonamides studied exhibited m/z 156 ion, except for sulfamethazine. The m/z 92 ion, although described in the literature, was not identified in the present study [21]-[25]. USEPA 1694 method did not present m/z 92 ion, but since only a single transition was used, it is not possible to conclude if it was not identified during the method development. Sulfamethazine spectrum showed m/z 204 ion which was also described by Verzegnassi *et al*. [21] for sulfadimidine corresponding to the fragment [M + H-93 + H_2O]$^+$. Since both substances have the same molecular weight, we concluded that the observed m/z 204 ion had the same identity.

3.2. LC Method Development

Two chromatographic gradients were employed, one for sulfonamides and another for tetracyclines. USEPA Method 1694 guidelines establishes that any chromatographic gradient may be applied for the separation of the pharmaceutical compounds as long as the last eluting peak has a retention time greater than that obtained in the standard USEPA method [19] [24]. Gradient elution for chromatographic separation of tetracyclines was the same described by Spisso *et al*. [3]. For sulfonamide separation, another gradient was developed based on that employed for tetracyclines, due to the physicochemical similarities. A reversed phase gradient using a C18 analytical column with 3 μm particles and mobile phases consisted of H_2O, ACN and MeOH, all with 0.1% FOA, was developed, as described in **Table 3**, and all of the sulfonamides showed a good chromatographic peak resolution and eluted in a total time of 16 min. In this method, tetracyclines elute up to 20.3 min and sulfonamides up to 15.2 min, whereas in the USEPA Method 1694 tetracyclines elute in a total time of 16.7 min and sulfonamides in 13.2 min.

3.3. River Water Analysis

Method recoveries were determined by analyzing samples of ultrapure water spiked in triplicate at 100 ng·L^{-1}. Recoveries were in the range of 57% - 92% and were similar to those described for USEPA 1694 method [19]. Recovery values with relative standard deviation (RSD) are reported in **Table 3** as well as method detection limits (MDL), method quantification limits (MQL) and regression coefficients (R^2) of calibration curves were constructed in the range of 78 ng·L^{-1} to 780 ng·L^{-1}. Comparing average concentrations obtained for each compound in the present study with average concentrations of USEPA 1694 method [19], all detected antimicrobials showed similar values, except for dapsone, sulfacetamide and sulphaquinoxaline that are not present in USEPA method. MDLs calculated for surface water were from 0.57 to 7.17 ng·L^{-1} and MQLs ranged from approximately 1.73 to 23.90 ng·L^{-1}.

Table 3. LC-MS/MS conditions for tetracyclines and sulfonamides.

Substance	Retention Time (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	DP (volts)	CE (volts)	CXP (volts)
			444.00		29	16
Chlortetracycline	17.65	479.23	462.01	121	23	16
			154.00		39	14
			448.10		25	16
Demeclocycline	14.75	465.21	430.10	106	33	16
			289.10		45	22
			428.10		27	16
Doxycycline	20.30	445.31	321.20	96	43	12
			154.20		41	12
			426.10		25	16
Metacycline	19.45	443.26	201.10	126	49	16
			145.20		75	16
			426.20		29	34
Oxytetracycline	10.92	461.20	443.40	52	19	32
			444.00		21	16
			410.10		27	16
Tetracycline	11.90	445.27	427.10	126	19	14
			154.20		37	12
			156.00		21	20
Dapsone	7.64	249.30	108.10	156	31	12
			110.20		37	14
			156.10		15	16
Sulfacetamide	3.40	215.14	108.00	71	29	14
			110.00		33	16
			156.30		29	10
Sulfadimethoxine	14.66	311.16	108.10	141	41	14
			245.20		27	16
			108.20		37	10
Sulfamerazine	4.85	265.25	156.20	96	25	10
			110.20		35	10
			124.10		37	16
Sulfamethazine	6.44	279.21	204.10	111	25	16
			108.20		41	12
			156.10		23	16
Sulfamethoxazole	10.50	254.18	108.20	116	33	14
			147.20		23	22
			156.10		25	22
Sulphaquinoxaline	15.23	301.34	108.10	141	39	12
			146.20		33	14
			156.10		21	16
Sulfathiazole	4.21	256.30	108.10	91	33	16
			101.10		33	18

It is worth mentioning that in this method, low MDLs and MQLs were achieved for antibiotics, even though low sample volumes were used for sample preconcentration. By reducing the sample volume of complex samples such as river water, matrix effects may be decreased. In fact, MDLs and MQLs obtained in this study were comparable to those obtained by USEPA 1694 method where a bigger volume had to be loaded in the SPE cartridge [19]. The proposed analytical method showed satisfactory performance characteristics in terms of linearity, repeatability, reproducibility, accuracy and sensitivity (**Table 4**).

According to the results shown in **Table 5**, only the Pedras River (lower side) showed the presence of antimicrobial substances, from both tetracycline class (oxytetracycline), and sulfonamide class (sulfamethoxazole). All other 12 studied compounds were not detected. Failure to identify the substances sought in the Parado River may be related to the shortage of location farms animal (cattle and poultry production) where the river flows. It was also observed that, only in the lower region of the Pedras River, substances were identified, probably due to be in the low region of reduced stream flow of the rivers, facilitating the accumulation of substances. Water samples were collected during three consecutive Sundays and variations in substance concentrations present in the samples collected in the morning and in the afternoon should be related both to management practice and weather conditions.

Sulfamethoxazole is a drug of sulfonamide class and is widely used in human and veterinary medicine. Sulfamethoxazole was detected in the Pedras River at concentrations up to 467.0 ng·L^{-1}. **Figure 2** shows MRM chromatograms of a contaminated water sample with a concentration of sulfamethoxazole estimated at 467.0 ng·L^{-1}, above the MQL of the proposed method. The presence of sulfamethoxazole was verified in four samples in the lower side of the Pedras River, in a total of six samples collected, having a frequency of 67% of contamination (**Table 5**).

Sulfamethoxazole is an indicative substance, expected to be present in groundwater which is influenced by wastewater, being relatively polar and persistent [26], according to one study about the environmental occurrence of pharmaceutical products in surface water and WWTP influents and effluents that has been performed in the frame of the project KNAPPE—Knowledge and Need Assessment on Pharmaceutical Products in Environmental Waters funded by the European Commission within the 6th Framework Programme [26] [27].

Table 4. Performance data for pharmaceuticals in surface waters.

Compound	% Recovery* EPA 1694 method (±RSD)	% Recovery** proposed Method (±RSD) (n = 3)	R^2 proposed method	MDL proposed method (ng·L^{-1})	MQL proposed method (ng·L^{-1})	Repeatability preliminary (±RSD) (n = 3)	Reproducibility preliminary (±RSD) (n = 6)
Chlortetracycline	95 (23)	57 (29)	0.9941	3.05	10.16	11	29
Demeclocycline	137 (2)	66 (5)	0.9973	1.22	4.08	4	5
Doxycycline	120 (1)	92 (8)	0.9889	1.99	6.62	11	11
Metacycline	-	84 (9)	0.9928	1.51	5.03	6	10
Oxytetracycline	149 (4)	87 (4)	0.9941	6.09	20.31	3	6
Tetracycline	125 (4)	78 (12)	0.9930	7.17	23.90	5	13
Dapsone	-	77 (12)	0.9958	0.67	2.24	3	14
Sulfacetamide	-	88 (5)	0.9933	1.63	5.42	6	6
Sulfadimethoxine	87 (3)	92 (3)	0.9944	0.57	1.90	4	5
Sulfamerazine	91 (1)	89 (8)	0.9965	0.52	1.73	6	9
Sulfamethazine	101 (6)	88 (5)	0.9948	0.71	2.36	2	6
Sulfamethoxazole	88 (4)	88 (8)	0.9974	2.62	8.74	8	9
Sulphaquinoxaline	-	90 (10)	0.9927	3.14	10.45	2	13
Sulfathiazole	77 (4)	80 (3)	0.9952	3.12	10.41	2	4

*Range = 15 to 75 ng·L^{-1}, depending on the analyte; **At 100 ng·L^{-1}.

Table 5. Concentration (ng·L^{-1}) of studied pharmaceutical drugs in river surface waters from Lidice.

Compound	Concentration (ng·L^{-1})				
	*Pedras River (lower side)	*Pedras River (upper side)	*Parado River (upper side)	*Parado River (lower side)	Contaminated samples (%)
Chlortetracycline	<MDL	<MDL	<MDL	<MDL	0
Demeclocycline	<MDL	<MDL	<MDL	<MDL	0
Doxycycline	<MDL	<MDL	<MDL	<MDL	0
Metacycline	<MDL	<MDL	<MDL	<MDL	0
Oxytetracycline	<MDL - 44.1	<MDL	<MDL	<MDL	67
Tetracycline	<MDL	<MDL	<MDL	<MDL	0
Dapsone	<MDL	<MDL	<MDL	<MDL	0
Sulfacetamide	<MDL	<MDL	<MDL	<MDL	0
Sulfadimethoxin	<MDL	<MDL	<MDL	<MDL	0
Sulfamerazine	<MDL	<MDL	<MDL	<MDL	0
Sulfamethazine	<MDL	<MDL	<MDL	<MDL	0
Sulfamethoxazole	> MDL - 467.0	<MDL	<MDL	<MDL	67
Sulphaquinoxaline	<MDL	<MDL	<MDL	<MDL	0
Sulfathiazole	<MDL	<MDL	<MDL	<MDL	0

This substance has been found worldwide in several different types of water. The highest antibiotic concentrations were detected at five points located along the Atibaia River watershed, in the State of São Paulo, Brazil. At these sampling point, sulfamethoxazole presented a maximum concentration of 109 ng·L^{-1} [25]. In Spain, sulfamethoxazole was detected at a maximum concentration of 119 ng·L^{-1}, in the Llobregat River and in the rivers in the area of Girona [15] [27] [28]. In Germany, sulfamethoxazole was determined with a concentration of 0.48 µg·L^{-1}, in the Lutter River, in Bielefeld [22]. Sulfamethoxazole has been frequently detected in other studies of pharmaceuticals, in surface waters from the rivers in USA at concentrations up to 150 ng·L^{-1} [29]-[33].

Studies conducted by Kasprzyk-Hordern *et al.* [34] in the Taff River, United Kingdom, and in the Warta River, Poland, showed that sulfamethoxazole was determined with a concentration between 26 and 60 ng·L^{-1}. The maximum value in surface waters for sulfamethoxazole in Portugal was 8.0 ng·L^{-1} in the Tejo and Zêzere rivers [14]. High concentrations of sulfamethoxazole were detected in the Nairobi River, Nigeria, and were related to the large amounts of consumption of this antibiotic in the country. The maximum concentration found in this river was 20 µg·L^{-1} [35].

Oxytetracycline is a broad spectrum antibiotic with a long history in veterinary medicine for the treatment and control of a wide variety of bacterial infections. A previous work accomplished in our laboratory had demonstrated that oxytetracycline was the most frequently detected substance from tetracycline group in milk samples in the metropolitan area of Rio de Janeiro [5]. The maximum concentration of oxytetracycline detected in the Pedras River was 44.1 ng·L^{-1}. **Figure 2** shows MRM chromatograms of a contaminated water sample with a concentration of oxytetracyline estimated at 44.1 ng·L^{-1}, above the MQL of the proposed method. Oxytetracycline was present in four samples in the lower side of the Pedras River, in a total of six samples collected, having a frequency of 67% of contamination (**Table 5**).

Tetracyclines can enter the aquatic environment via effluent discharge of sewage treatment plants (STPs), agricultural runoff, or disposal of unused drugs. In China, in surface water samples, oxytetracycline was detected at a level of 2.2 ng·L^{-1} [36].

Tetracyclines were detected in river waters around the world in a much lesser extent than sulfonamides.

4. Conclusions

The presence of pharmaceuticals in the environment raises many questions about the risk to the environment and
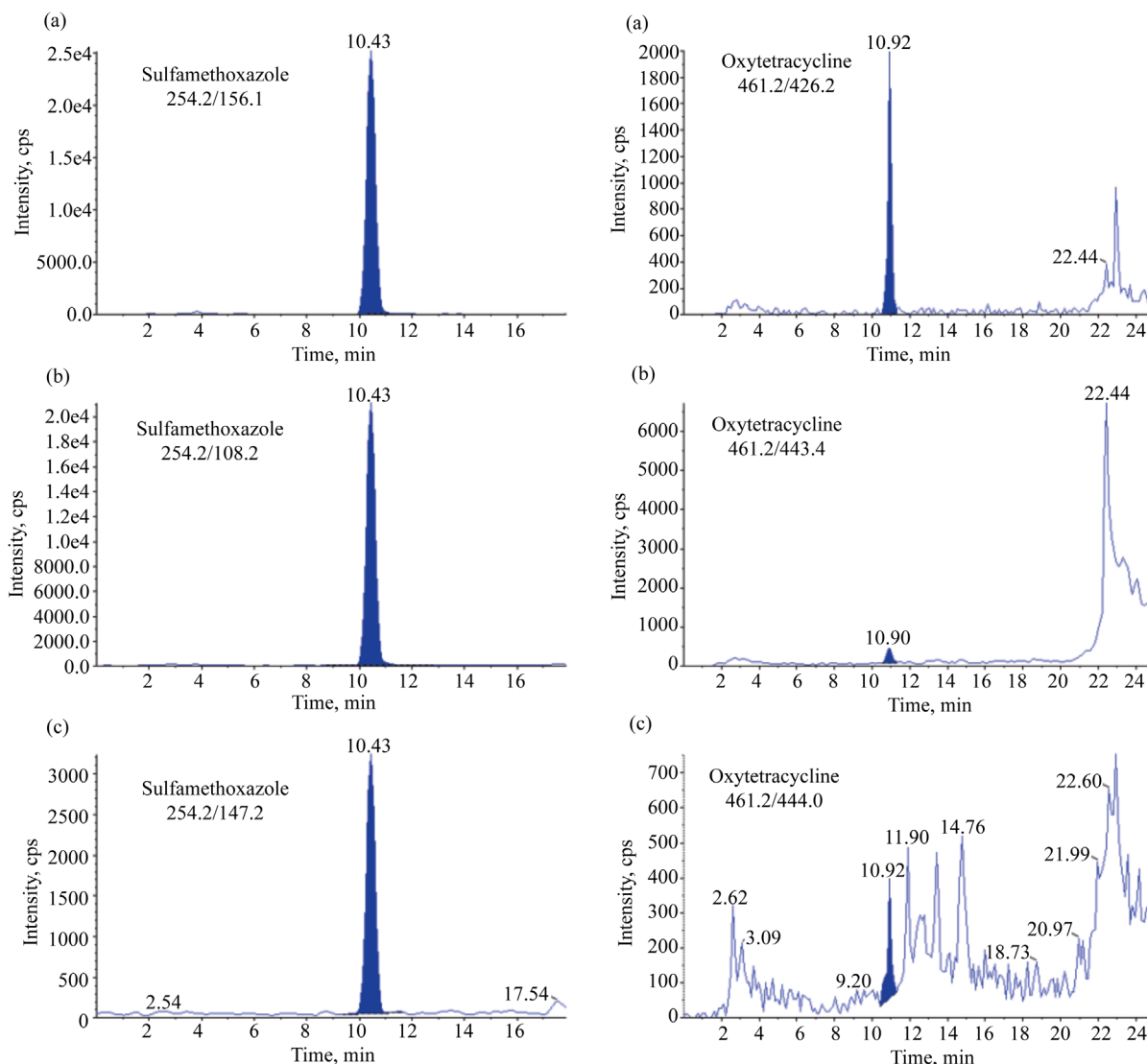
Figure 2. MRM chromatograms of a contaminated water sample with a concentration of sulfamethoxazole estimated at 467.0 ng·L^{-1} and of a contaminated water sample with a concentration of oxytetracycline estimated at 44.1 ng·L^{-1} (a) 1st transition; (b) 2nd transition; (c) 3rd transition.

human health. The occurrence of antimicrobials in the environment, especially in aquatic systems, has recently become a matter of concern. This study investigated the presence of sulfonamide and tetracycline residues in river water samples from Lidice District of Rio Claro, in the State of Rio de Janeiro, Brazil. The results confirmed the presence of sulfamethoxazole and oxytetracycline in one of the two rivers studied: the Pedras River. The compounds were present at the ng·L^{-1} level, with a maximum concentration of 467.0 ng·L^{-1} for sulfamethoxazole and 44.1 ng·L^{-1} for oxytetracycline.

The results presented of the proposed method for sulfonamides and tetracyclines were satisfactory and indicated that the approach was very promising for antibiotics determination in surface water. Therefore, the validation of methodology will be performed according to protocol for EPA approval of new methods.

The most important issues to worry about, related to the presence of these compounds in the environment, are the possibility that they may exert ecotoxicological effects on non-target organisms and that they may possibly enter into the human food supply via the water cycle.

Through this background, it is highly troubling that in Brazil there is not yet legislation considering drugs as pollutants, and the possible effects of their residues on health have not been evaluated by WHO so far.

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