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Article

Development and Validation of Liquid Chromatography-Tandem Mass Spectrometry Methods for Determination of Beta-Lactams, Macrolides, Fluoroquinolones, Sulfonamides and Tetracyclines in Surface and Drinking Water from Rio de Janeiro, Brazil

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Two analytical methods for determination of five antibiotics classes in surface water and drinking water samples were developed and validated based on solid phase extraction followed by high-performance liquid chromatography coupled to tandem mass spectrometry. Two distinct chromatographic gradients were used according to the polarity of the different pharmaceuticals. The methods were applied for the quantification of 46 analytes belonging to beta-lactams, macrolides, fluoroquinolones, sulfonamides and tetracyclines classes. Validation results showed recoveries above 75% for the studied analytes in water samples. The method limits of detection calculated for the surface water and drinking water samples were, respectively, from 1 to 12 ng L^{-1} and from 0.15 to 20 ng L^{-1}. The method limit of quantification ranged from approximately 3 to 38 ng L^{-1} for surface water samples and from 0.5 to 64 ng L^{-1} for drinking water samples. The methods showed to be linear over the range of 25 to 1000 ng L^{-1} with coefficients of determination greater than 0.94. Amoxicillin, cephalexin and sulfamethoxazole as high as 105 ng L^{-1} were found in surface water and erythromycin, azithromycin and clarithromycin up to 35 ng L^{-1} could also be found in surface water. Clarithromycin, cefaclor, oxacillin, sulfamethoxazole and troleandomycin were detected in the lower range up to 10 ng L^{-1} in drinking water.

Keywords: antibiotics, surface water, drinking water, mass spectrometry, emergence pollutants

Introduction

Antibiotics represent one of the most used class of drugs worldwide[1] and correspond to the largest category of compounds used in human and veterinary medicine, as growth promoters or for therapeutic purposes.[2] As regards to antimicrobials used in human medicine, non-prescribed medicines are consumed at home, and prescribed ones are consumed in hospitals and clinics.[3] Individuals affected by infectious diseases use specific antibiotics, and after administration, the molecules are absorbed, distributed, metabolized partially, and finally excreted from the body. The metabolism eliminates substances in excess and other xenobiotics via a series of enzymatic biotransformations and converts them into more polar and hydrophilic compounds.[4] These substances were developed to be persistent, keeping its chemical properties, with a therapeutic purpose and after use, about 50 to 90% of a drug dose is excreted and persists in the environment.[5] The occurrence of antibiotics in the aquatic environment and drinking water (DW) has raised questions about impacts on the environment and public health. The adverse effects caused by pharmaceutical compounds include aquatic toxicity, development of resistance in pathogenic bacteria, genotoxicity and endocrine disorders.[6,7]

Several methods have been developed to extract antibiotics in water. Currently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the technique most widely used in analysis of drugs in complex environmental

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samples and have shown to be a sensitive analytical tool that leads to efficient results and lower detection (LOD) and quantification limits (LOQ) (in the range from μg L^{-1} to ng L^{-1}), and generate reliable data in the identification of several molecules.[8] LC-MS/MS comprises a separation and a detection technique that provides structural confirmation of the analyzed compounds.[8] A good chromatographic separation is advisable in order to reduce matrix effects, which usually results in suppression or, less frequently, signal enhancement.[8] To minimize matrix interference, water extracts are generally cleaned up and pre-concentrated by solid-phase extraction (SPE), mainly using HLB cartridges. The use of SPE cartridges may greatly influence the recoveries of target compounds.[8] Sample preparation is a crucial step in environmental analysis. It is highly influenced by the physical and chemical analytes properties and by matrices. The main objectives of sample preparation are to extract and concentrate the analytes of interest, removing sample matrix interferences for subsequent chromatographic analysis. The whole analytical procedure typically includes five steps: sampling, sample preparation, chromatographic separation, detection and data analysis. The most important part of the analytical process is sample preparation because it can take more than 80% of the total analysis time.[8]

Some information about contamination of Brazilian aquatic environment by antibiotics has been published in the form of dissertations and theses, but scientific papers are very scarce. The studies have been most accomplished in the southern and southeast of Brazil, and for this reason research in other geographical areas is necessary in order to obtain a complete panorama of the country.[9-14]

The watershed of Guandu River has a fundamental role for Rio de Janeiro metropolitan region where approximately 12.2 million inhabitants live. This watershed is very important because it is the only option for subsistence and development of the Metropolitan Region of the State of Rio de Janeiro. Its waters supply the second largest metropolitan region of the country, and for several productive sectors, such as the steel, petrochemical, clothing, food and beverage industries, among others, and also as a water body for the collection of domestic and industrial sewage.[15]

The aim of this study was to develop and validate a methodology to determine the antimicrobial residues of beta-lactams (BL), macrolides (MC), fluoroquinolones (FQ), sulfonamides (SF) and tetracyclines (TC) classes in river surface water (SW) and DW samples in the state of Rio de Janeiro (Brazil). These methods were developed based on US EPA method 1694[16] to determine pharmaceuticals in environmental samples by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The methods was applied for quantification of 46 analytes of BL, MC, FQ, SF and TC classes in nine SW and ten DW samples collected in the state of Rio de Janeiro (Figure 1).

Figure 1. Map of the state of Rio de Janeiro with the sampling locations.

Experimental

Chemicals and materials

Methanol (MeOH) HPLC grade was purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile (ACN) HPLC grade, hydrochloric acid (HCl) and formic acid (FOA) analytical grade were purchased from Merck (Darmstadt, Germany). Ascorbic acid (ASA), sodium hydroxide (NaOH) 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 (OTC), doxycycline hyclate (DC), hydrochloride salts of chlortetracycline (CTC), demeclocycline (DMC), dapsone (DAP), sulfacetamide (SCT), sulfadimethoxin (SDM), sulfamerazine (SFM), sulfamethazine (SMT), sulphaquinoxaline (SQN), sulfathiazole (STZ), tylosin tartarate (TYL), troleandomycin (TRO), erythromycin (ERY), cephapirin sodium salt (CPPN), ceftiofur (CFTF), cefoperazone (CFPZ), benzylpenicillin sodium salt (PENG), oxacillin sodium salt hydrate (OXA), moxifloxacin (MXF) and ofloxacin (OFX) were supplied from US Pharmacopeial Convention (Rockville, MD, USA). Amoxicillin tryhidrate (AMOX), ampicillin (AMPI), cefaclor (CFCL), cefadroxila (CFDX), cefalexin hydrate (CFLX), cefazolin (CFZL), clarithromycin (CLA), ciprofloxacin hydrochloride (CPF), norfloxacin (NOR), tetracycline hydrochloride (TC), sulfamethoxazole (SMZ) were chemical reference substances from the Brazilian Pharmacopeial Convention (Santa Maria, RS, Brazil). Methacycline (MTC), 4-epioxytetracycline (4-EOTC), 4-epitetracycline (4-ETC), 4-epichlortetracycline hydrochloride (4-ECTC), were acquired from Acros (Pittsburgh, PA, USA). Azithromycin dehydrate (AZI), roxithromycin (ROX), spiramycin (SPI), oleandomycin (OLE), tilmicosin (TILM), and cefquinome sulphate salt (CFQN) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Phenoxymethylpenicillin potassium salt (PENV), cloxacillin sodium salt hydrate (CLOX), dicloxacillin sodium salt hydrate (DCLOX) and nafcillin sodium salt (NAFC) were supplied from WHO Collaborating Centre for Chemical Reference Substances (Stockholm, Sweden). Desacetylcephapirin (DESAC) was supplied from Bristol-Myers Squibb (New York, USA). Ampicillin-d_5 (AMPID5) was purchased from Purity Grade Standards (San Francisco, CA, USA). Solid-phase extraction (SPE) was performed with 60 mg Oasis® HLB cartridges from Waters Corp. (Milford, MA, USA). Membrane filters of polyvinylidene fluoride (PVDF) with pore size 0.22 μm were purchased from Millipore (Billerica, MA, USA).

Standard solutions

Stock solutions of 1 mg L^{-1} were prepared in MeOH for MC, SF and TC, in ultrapure water for BL and in 0.03 mol L^{-1} NaOH for FQ. Stock solutions of DMC (1 mg L^{-1}) and AMPID5 (1 mg L^{-1}) were prepared using MeOH and ultrapure water, respectively. All stock solutions were stored at ≤ –70 °C.

DMC was used as internal standard/surrogate for SF and TC quantification. AMPID5 was used as internal standard for BL, MC and FQ.

Sampling and sample preparation

Water samples used for the development and validation

An aliquot of 250 mL of each water sample was collected in polypropylene bottles, identified and transported under refrigeration to the laboratory for analysis. DW samples were taken from the tap at the National Institute for Quality Control in Health/Oswaldo Cruz Foundation (Rio de Janeiro, RJ), from the residences in the city of Barra Mansa and from the city of São Gonçalo (Rio de Janeiro). SW samples were collected from some rivers that make up the Guandu system (Guandu and Queimados rivers), which is the main source of the DW supply for the greater metropolitan area of Rio de Janeiro and Parado River in the Lidice District of Rio Claro, in the state of Rio de Janeiro, Brazil. A total of six samples were collected for validation, according to Table 1. Water samples were first filtered using 8 μm paper filters from Whatman (England), followed by 0.22 μm PVDF membrane filters (Millipore, Billerica, MA, USA). Water samples were used for method development experiments. They proved to be blank samples in previous

Table 1. Sample collection for validation

Type	Surface water (SW)		Drinking water (DW)	
	Location	Sample	Location	Sample
1	Guandu River	SW1	National Institute for Quality Control in Health/Oswaldo Cruz Foundation	DW1
2	Queimados River	SW2	Barra Mansa	DW2
3	Parado River	SW3	São Gonçalo	DW3

analysis, for this reason they were used for all validation experiments.

Water samples collected for method application

The method was applied to analyze nine SW samples collected in June 2016 from Guandu River (Paraíba do Sul, Piraí, Macacos, Queimados, Guandu and Santana rivers, Guandu lagoon mouth, Guandu main dam and adductor to Ribeirão das Lajes River). Figure 1 shows sampling sites position. In addition, ten DW samples were collected in July 2016, from residences in Rio de Janeiro State (Barra Mansa, Belford Roxo, Resende, Rio de Janeiro and Volta Redonda cities).

The sample codes and GPS coordinates are listed in Table 2 referring to the SW samples. The objective of this investigation was to determine the selected antimicrobials residues in surface and drinking water in the state of Rio de Janeiro, Brazil.

SPE procedure

The following procedure was developed based on US EPA method 1694[16] and on a previously published method for TC and SF analysis in river SW.[12]

A 50 mL aliquot of each sample (SW and DW) was spiked at 100 ng L^{-1} (BL, MC, FQ, SF and TC) and spiked with 100 ng L^{-1} of internal standards/surrogate (DMC and AMPID5). Then, the samples were acidified to pH 2.5 with HCl, and 2 mL of 25 mg L^{-1} EDTA stock solution was added. For the sample DW, it was added 2 mL of 625 mg L^{-1} ASA to reduce any residual chlorine that had been added as a disinfectant. 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. The samples were percolated at a flow rate of approximately 3 mL min^{-1}. Cartridges were washed twice with 2 mL of ultrapure water and then dried under vacuum (–35 kPa) for 2 min. Antimicrobials were eluted with three portions of 2 mL methanol and one portion of 2 mL ACE, using gravity flow only. 4 mL aliquots of the eluate were transferred to two centrifuge tubes and evaporated to dryness under N_2 in a temperature up to 47 °C, using an evaporator with nitrogen flow (Pierce Reacti-Therm IIITM and Pierce Reacti VapTM III, Rockford, IL, USA). The dry residues were reconstituted with 1 mL of 0.1% FOA:MeOH (80:20, v/v) for TC and SF analysis (diluent 1) and 1 mL of MeOH:H_2O (65:35, v/v) for BL, MC and FQ analysis (diluent 2), vortexed for 30 s and filtered through a 0.22 µm polyvinylidene fluoride (PVDF) syringe filter into amber auto-sampler vials.

LC-MS/MS instrumentation

An LC-MS/MS system consisting in a Shimadzu Prominence HPLC instrument (Shimadzu, Kyoto, Japan) equipped with a solvent delivery pump (LC-20AD), a quaternary gradient kit, a membrane degasser (DGU-20A5), an auto-sampler (SIL-20AC), a column oven (CTO-20AC), 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 was used. Analyst® V1.4.2 LC/MS software was used for data acquisition. Positive electrospray ionization technique (ESI+) in multiple reaction monitoring (MRM) acquisition mode was used to monitor two ions for each substance. Nitrogen was employed as nebulizer gas (Gas 1, 40 psi), dryer gas (Gas 2, 40 psi), collision-activated dissociation (CAD) gas (6 a.u.) and Curtain™ gas (10 psi). Other parameters selected during automatic tuning were: ion spray potential = 5000 V; source temperature = 500 °C (SF and TC), 550 °C (BL, MC and FQ); entrance potential = 10 V. The column temperature

Table 2. Sampling site details and GPS coordinates (surface sample (SW) samples)

Sample	Location	Source	GPS coordinates
RPS-01	Barra do Piraí	Paraíba do Sul River	22°28'56.81"S/43°50'20.45"W
RPI-02	Piraí	Piraí River	22°37'41.90"S/43°53'49.22"W
RLL-03	Piraí	adductor to the Ribeirão das Lajes River	22°41'31.43"S/43°51'44.38"W
RMC-05	Paracambi	Macacos River	22°38'5.99"S/43°42'17.79"W
RSA-06	Japeri	Santana River	22°38'13.87"S/43°40'5.58"W
RGN-08	Queimados	Guandu River	22°43'40.35"S/43°38'26.18"W
RQM-10	Queimados	Queimados River	22°44'45.90"S/43°36'42.37"W
LGA-15	Nova Iguaçu	Guandu lagoon mouth	22°48'21.37"S/43°37'38.48"W
RGN-17	Nova Iguaçu	Guandu main dam	22°48'31.69"S/43°37'39.44"W

was set at 25 °C for TC and SF method and 35 °C for BL, MC and FQ method. The injection volume was 25 µL for both methods. The autosampler was set at 4 °C. An analytical column Pursuit™ RS C18 (100 × 2 mm id, 3 µm particle size, 200 Å) with a respective guard column (Agilent, Santa Clara, CA, USA) was used. Mobile phases A, B and C were prepared using H_2O, ACN and MeOH, respectively, all of them with 0.1% FOA. Injection volumes and the gradient elution programs were described in Table 3.[12,17]

Fragmentation studies with beta-lactams and fluoroquinolones 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 multiple reaction monitoring (MRM) acquisition mode was used to monitor two ions for each substance. MRM experiments for TC analysis in electrospray positive-ion mode (ESI+) were described by Spisso *et al.*;[17] for SF by Monteiro *et al.*;[12] for MC by Spisso *et al.*[18] and Costa *et al.*,[19] and the analytical conditions used were listed in Table 3.

Validation

The validation of optimized method was performed according to protocol for EPA[20] approval of new methods for organic and inorganic analytes in wastewater and DW. Validation method was further evaluated in terms of sensitivity, initial precision and recovery (IPR), intermediate precision and linearity.

Sensitivity (method limits of detection (LOD) and method limits of quantification (LOQ))

The method limits of detection (LOD) is calculated using seven replicates of river (SW) and drinking water (DW) samples spiked in concentration of 20 ng L^{-1}. The LODs were calculated by multiplying the standard deviation from the seven measurements by the Student's *t*-test value for six degree of freedom at 99% confidence level (3.143). The LOQ were calculated by multiplying 3.18 times the LOD.

Initial precision and recovery (IPR)

The IPR for each compound was determined spiking four replicates at 100 ng L^{-1} in three samples of water from different origins (DW1, DW2, DW3, SW1, SW2 and SW3). A total of twelve samples of each type (DW and SW) were analyzed. The spiked samples were proceeded by SPE and then analyzed by LC-MS/MS.

The overall recovery was obtained comparing the analyte response in the extract of water samples (SW and DW) post-extraction reconstituted with 1 mL of 100 ng L^{-1} solutions (BL, MC, FQ, SF, TC, DMC and AMPID5) prepared with respective dilution solvents, 1 mL of diluent 1 and 1 mL of diluent 2, and the theoretical concentration in the final extract assuming 100% SPE recovery. Precision was assessed with respect to repeatability (intraday precision) and intermediate precision.

Linearity

A six-point calibration set was freshly prepared by spiking varying levels of working standard solutions in ultrapure water. The analytical curves for all analytes in the concentration range from 25 to 1000 ng L^{-1} were constructed in order to quantify the analytes in the SW and DW samples.

Results and Discussion

Development of the LC-MS/MS method

MRM acquisition mode is the most suitable for quantification due to its sensitivity and specificity. Declustering potential (DP), collision energy (CE) and

Table 3. Gradient elution programs for sulfonamides (SF), tetracyclines (TC), beta-lactams (BL), macrolides (MC), fluoroquinolones (FQ)

TC and SF method					BL, MC and FQ method				
time / min	A[a] / %	B[a] / %	C[a] / %	Flow rate / (mL min^{-1})	time / min	A[a] / %	B[a] / %	C[a] / %	Flow rate / (mL min^{-1})
1	80	5	15	0.15	4.00	41	0	59	0.25
15	60	25	15	0.15	4.10	0	50	50	0.30
16	5	5	90	0.15	10.00	0	50	50	0.30
26	5	5	90	0.15	10.10	41	0	59	0.30
27	80	5	15	0.15	14.00	41	0	59	0.30
35	80	5	15	0.15	14.10	41	0	59	0.25
–	–	–	–	–	16.00	41	0	59	0.25

[a]A, B, C: mobile phases with 0.1% FOA prepared using H_2O, ACN and MeOH, respectively.

collision cell exit potential (CXP) values for MC, SF, TC, BL and FQ precursor/product ion pairs obtained in MRM mode are shown in Table 4. For BL and FQ, only protonated molecules $[M + H]^+$ were observed and selected as precursor ions, and no adducts were noted. The two most abundant fragment ions were monitored for each compound. For target analytes, the most abundant transition was used for quantification purposes, whereas the second was used to confirm the identity of the substances.

The transition ERY-H_2O could be monitored, because at pH below 7, ERY is immediately converted into its main degradation product ERY-H_2O.[21] All compounds showed a good chromatographic peak resolution.

Two chromatographic methods were developed to obtain an increase in substances sensitivity, because the physicochemical properties of the five antimicrobials classes analyzed were different. Both methods were used according to polarity and extraction of different pharmaceuticals.

SPE procedure

Sample preparation is a crucial step in environmental analysis. It is highly influenced by the physical and chemical properties of the studied analytes and the matrices. The main objectives are to concentrate the analytes in the sample, remove matrix interferences and prepare the analyte in the form suitable for subsequent chromatographic analysis. Usually, the sample preparation step includes adjusting the pH of the solution, plus the use of a chelator (EDTA) followed by an extraction procedure, extract treatment and final preparation for the following chromatographic analysis. In most of the methods presented in the literature, Oasis HLB® cartridge has been used. This cartridge usually works at a neutral pH. Because of their chemical composition (the combination of lipophilic divinylbenzene and hydrophilic *N*-vinylpyrrolidone polymers), they are capable of extracting acidic, neutral and basic compounds at a wide range of pH values including neutral pH.[8,21-26]

EDTA was used as a chelating agent, it is recommended in the analysis of antibiotic residues in environmental samples. A chelating agent was added to water samples, prior to extraction, in order to chelate metals that are found in water, making possible to achieve good extraction efficiencies.[21]

Ascorbic acid (ASA) was added to remove residual chlorine in DW, because it can react with some antibiotics, including CPF, CTC, DC, ERY, OTC, sulfamethoxazole and TC.[27,28] It is important to do the removal of free chlorine in water samples, because this fact leads to a more precise analysis and resulting in a reliable data, without affecting the stability of the antibiotics in water.

DMC and AMPID5 were used as surrogate standards, they were added to the samples before extraction and were also used for the quantification of the samples. Internal standard/surrogate was therefore added to the sample to compensate the losses originated from both the sample preparation procedure and from matrix effects.

Table 5 presents the comparison between the developed method and the 1694 US EPA method.[16] The lower amount of sample, consumables and time show that as a result, the developed method is faster and cheaper than the 1694 US EPA method.[16]

Validation

Drug residues are frequently detected and quantified in aquatic environments. Unreliable analytical data can lead to misinterpretation and wrong decision-making. Therefore, the validation of the analytical method is important to obtain a correct analysis of the possible effects of these compounds on human health, as well as on non-target organisms. Methods developed by laboratories, that is, non-standardized, should be validated. Therefore, the validation of the analytical method is an important step to assure the reliability of the results and hence to enable a correct analysis of the possible effects of these compounds on human health, as well as on non-target organisms.

Sensitivity (LOD and LOQ)

The LOD and LOQ were estimated from the injection of spiked real samples (SW and DW). Results for each matrix are presented in Table 6 (SW) and Table 7 (DW). LODs calculated for SW samples were from 1 to 12 ng L^{-1} and for DW samples were from 0.15 to 20 ng L^{-1}. LOQs ranged from approximately 3 to 38 ng L^{-1} for SW samples and from 0.5 to 64 ng L^{-1} for DW samples. It is worth mentioning that in the validated method, low LODs and LOQs were achieved for all antibiotics, even though low sample volumes were used for sample preconcentration. By reducing sample volume of complex samples such as river water samples, a decrease in matrix effects may be achieved.

IPR

The achieved recoveries for all target compounds ranged from 49 to 117% and from 50 to 110% for SW

Table 4. LC-MS/MS conditions for beta-lactams (BL), macrolides (MC), fluoroquinolones (FQ), tetracyclines (TC) and sulfonamides (SF)

Substance	Precursor ion (*m/z*)	Product ion (*m/z*)	DP[a] / V	CE[b] / V	CXP[c] / V
CTC	479.23	444.00	121	29	16
		462.01		23	16
DMC	465.21	448.10	106	25	16
		430.10		33	16
DC	445.31	428.10	96	27	16
		321.20		43	12
MTC	443.26	426.10	126	25	16
		201.10		49	16
OTC	461.20	426.20	52	29	34
		443.40		19	32
TC	445.27	410.10	126	27	16
		427.10		19	14
4-ECTC	479.22	462.00	91	25	16
		444.10		31	14
4-EOTC	461.19	426.20	77	29	16
		444.00		23	16
4-ETC	445.27	410.10	96	29	14
		427.10		19	16
DAP	249.30	156.00	156	21	20
		108.10		31	12
SCT	215.14	156.10	71	15	16
		108.00		29	14
SDM	311.16	156.30	141	29	10
		108.10		41	14
SFM	265.25	108.20	96	37	10
		156.20		25	10
SMT	279.21	124.10	111	37	16
		204.10		25	16
SMZ	254.18	156.10	116	23	16
		108.20		33	14
SQN	301.34	156.10	141	25	22
		108.10		39	12
STZ	256.30	156.10	91	21	16
		108.10		33	16
CFQN	529.09	134.10	101	21	14
		125.00		77	16
CFZL	455.09	323.10	71	15	24
		155.90		23	20
CFCL	368.06	106.10	86	33	14
		174.00		19	24
PENV	351.09	159.90	56	15	20
		113.90		45	20
AMPID5	355.11	111.00	111	27	18
		197.20		23	28
CPPN	424.06	152.00	106	33	24
		124.00		59	18
NAFC	415.16	199.10	96	21	16
		171.00		47	18
PENG	335.17	160.10	81	25	8
		176.00		29	22
CLOX	436.02	277.20	86	19	20
		160.20		21	10
CFLX	348.19	158.00	71	13	16
		106.20		41	14
OXA	402.09	159.90	101	19	16
		243.00		17	16
CFTF	524.00	241.00	121	23	16
		124.90		77	18
CFDX	364.09	114.10	71	27	14
		208.00		13	12
DCLOX	470.03	160.10	101	19	16
		311.10		21	22
AMPI	350.16	106.10	81	29	14
		114.00		41	16
CFPZ	646.09	143.00	111	45	20
		530.00		17	18
DESAC	382.096	111.10	106	63	14
		112.10		35	14
AMOX	366.10	114.00	91	31	14
		208.10		17	16
TYL	916.62	174.10	226	49	18
		772.40		39	24
SPI	422.37	174.00	126	29	30
		144.90		19	22
TRO	772.48	158.10	146	37	16
		586.20		25	20
OLE	688.39	158.20	136	35	20
		544.40		21	18
ROX	837.46	158.20	171	47	16
		679.50		29	22
TILM	435.34	174.00	106	33	22
		695.60		19	22
CLA	748.52	158.20	146	35	16
		590.20		25	20
ERY-H_2O	716.41	558.30	146	23	18
		158.10		41	14
AZI	749.56	158.10	80	35	12
		591.40		35	12
NOR	320.21	302.20	111	31	22
		231.10		53	14
CPF	332.21	231.10	106	49	18
		314.10		31	24
OFX	362.24	318.20	136	27	24
		261.20		39	20
MXF	402.21	384.10	126	31	28
		364.20		39	28

[a]Declustering potential; [b]collision energy; [c]collision cell exit potential. CTC: chlortetracycline; DMC: demeclocycline; DC: doxycycline hyclate; MTC: methacycline; OTC: oxytetracycline; TC: tetracycline hydrochloride; 4-ECTC: 4-epichlortetracycline hydrochloride; 4-EOTC: 4-epioxytetracycline; 4-ETC: 4-epitetracycline; DAP: dapsone; SCT: sulfacetamide; SDM: sulfadimethoxin; SFM: sulfamerazine; SMT: sulfamethazine; SMZ: sulfamethoxazole; SQN: sulfaquinoxaline; STZ: sulfathiazole; CFQN: cefquinome sulfate salt; CFZL: cefazolin; CFCL: cefaclor; PENV: phenoxymethylpenicillin potassium salt; AMPID5: ampicillin-d_5; CPPN: cephapirin sodium salt; NAFC: nafcillin sodium salt; PENG: benzylpenicillin sodium salt; CLOX: cloxacillin sodium salt hydrate; CFLX: cefalexin hydrate; OXA: oxacillin sodium salt hydrate; CFTF: ceftiofur; CFDX: cefadroxila; DCLOX: dicloxacillin sodium salt hydrate; AMPI: ampicillin; CFPZ: cefoperazone; DESAC: desacetylcephapirin; AMOX: amoxicillin tryhidrate; TYL: tylosin tartarate; SPI: spiramycin; TRO: troleandomycin; OLE: oleandomycin; ROX: roxithromycin; TILM: tilmicosin; CLA: clarithromycin; ERY: erythromycin; AZI: azithromycin dehydrate; NOR: norfloxacin; CPF: ciprofloxacin hydrochloride; OFX: ofloxacin; MXF: moxifloxacin.

Table 5. Comparisons between the developed method and the 1694 US EPA[16]

	Method developed	1694 US EPA method
Sample / mL	50	1000
EDTA	2 mL of 25 mg L^{-1}	500 mg
Cartridge	HLB 60 mg	HLB 1000 mg
Washing water / mL	4	10
Drying / min	2	5
Solvent MeOH / mL	6	18
Solvent ACE / mL	2	3
Chromatographic method	2	3

EDTA: ethylenediaminetetracetic acid.

and DW samples, respectively (Tables 6 and 7). Good performance with recoveries above 75% among 80% of the 46 analytes for the surface and drinking water sample was achieved. Only PENG samples showed recovery rates below of 40%. This fact can be explained by their instability in water, related to their chemical structure.[21] High recoveries obtained for fluoroquinolones can be explained by the retention of these antibiotics in acidic conditions.[21,24] Such recoveries are similar to those achieved by other studies depending on the analyte.[8,18-23]

In both matrices, the relative standard deviation (RSD) obtained are less than 58% for all analytes for repetitivity and repeatability, which is lower than values reported by

Table 6. Performance data for pharmaceuticals in surface water (SW)

Class	Compound	Recovery / %	Repetitivity (RSD / %; n = 6)	Repeatability (RSD / %; n = 12)	LOD / (ng L^{-1})	LOQ / (ng L^{-1})
Tetracycline (TC)	CTC	85	9	9	9	29
	DMC	88	12	12	7	23
	DC	82	9	7	10	32
	MTC	91	16	11	6	19
	OTC	92	12	12	5	16
	TC	91	10	17	10	32
	4-ECTC	89	14	15	2	6
	4-EOTC	78	8	13	9	27
	4-ETC	88	11	13	4	12
Sulfonamide (SF)	DAP	49	15	32	3	9
	SCT	89	17	47	3	9
	SDM	57	10	13	7	22
	SFM	80	10	31	5	16
	SMT	65	12	13	8	26
	SMZ	67	6	14	9	27
	SQN	62	14	17	6	20
	STZ	73	13	34	4	14
Fluoroquinolone (FQ)	CPF	97	19	58	12	38
	NOR	117	14	19	7	27
	OFX	109	22	31	6	18
	MXF	105	6	12	2	5
Beta-lactam (BL)	CFQN	104	32	18	3	8
	CFZL	104	12	6	4	13
	CFCL	93	23	19	1	3
	PENV	101	13	12	2	6
	CPPN	107	18	8	3	11
	NAFC	62	28	27	6	20
	PENG	33	22	37	8	24
	CLOX	91	18	10	10	33
	CFLX	84	21	11	4	13
	OXA	90	17	15	3	10
	CFTF	97	7	8	6	19
	CFDX	83	32	43	3	9
	DCLOX	88	16	11	10	33
	AMPI	100	26	19	10	33
	CFPZ	107	22	10	2	7
	AMOX	77	36	50	6	20
	DESAC	87	15	12	3	9
	AMPID5	97	14	8	2	7

Table 6. Performance data for pharmaceuticals in surface water (SW) (cont.)

Class	Compound	Recovery / %	Repetitivity (RSD / %; n = 6)	Repeatability (RSD / %; n = 12)	LOD / (ng L^{-1})	LOQ / (ng L^{-1})
Macrolide (MC)	TYL	73	26	17	8	25
	SPI	68	34	36	11	35
	TRO	84	28	17	4	14
	OLE	97	7	4	11	34
	ROX	86	18	28	5	16
	ERY	82	15	12	5	15
	TILM	71	49	41	5	14
	CLA	98	19	16	6	18
	AZI	92	15	12	4	11

LOD: limits of detection; LOQ: limits of quantification; CTC: chlortetracycline; DMC: demeclocycline; DC: doxycycline hyclate; MTC: methacycline; OTC: oxytetracycline; TC: tetracycline hydrochloride; 4-ECTC: 4-epichlortetracycline hydrochloride; 4-EOTC: 4-epioxytetracycline; 4-ETC: 4-epitetracycline; DAP: dapsone; SCT: sulfacetamide; SDM: sulfadimethoxin; SFM: sulfamerazine; SMT: sulfamethazine; SMZ: sulfamethoxazole; SQN: sulfaquinoxaline; STZ: sulfathiazole; CPF: ciprofloxacin hydrochloride; NOR: norfloxacin; OFX: ofloxacin; MXF: moxifloxacin; CFQN: cefquinome sulfate salt; CFZL: cefazolin; CFCL: cefaclor; PENV: phenoxymethylpenicillin potassium salt; CPPN: cephapirin sodium salt; NAFC: nafcillin sodium salt; PENG: benzylpenicillin sodium salt; CLOX: cloxacillin sodium salt hydrate; CFLX: cefalexin hydrate; OXA: oxacillin sodium salt hydrate; CFTF: ceftiofur; CFDX: cefadroxila; DCLOX: dicloxacillin sodium salt hydrate; AMPI: ampicillin; CFPZ: cefoperazone; AMOX: amoxicillin tryhidrate; DESAC: desacetylcephapirin; AMPID5: ampicillin-d_5; TYL: tylosin tartarate; SPI: spiramycin; TRO: troleandomycin; OLE: oleandomycin; ROX: roxithromycin; ERY: erythromycin; TILM: tilmicosin; CLA: clarithromycin; AZI: azithromycin dehydrate.

Table 7. Performance data for pharmaceuticals in drinking water (DW)

Class	Compound	Recovery / %	Repetitivity (RSD / %; n = 6)	Repeatability (RSD / %; n = 12)	LOD / (ng L^{-1})	LOQ / (ng L^{-1})
Tetracycline (TC)	CTC	85	18	23	11	34
	DMC	80	20	19	5	17
	DC	73	21	26	15	46
	MTC	86	11	26	12	39
	OTC	92	17	18	6	20
	TC	86	10	12	6	18
	4-ECTC	88	7	19	8	25
	4-EOTC	77	13	9	6	20
	4-ETC	88	11	12	6	19
Sulfonamide (SF)	DAP	50	30	30	16	52
	SCT	90	10	37	9	27
	SDM	59	17	18	9	29
	SFM	77	19	19	9	28
	SMT	66	13	13	8	26
	SMZ	66	11	12	11	34
	SQN	53	21	19	11	34
	STZ	80	13	30	7	23
Fluoroquinolone (FQ)	CPF	99	19	11	12	38
	NOR	99	14	10	3	11
	OFX	94	22	12	5	16
	MXF	101	6	7	2	6
Beta-lactam (BL)	CFQN	72	16	26	9	29
	CFZL	102	11	9	4	13
	CFCL	90	22	15	20	64
	PENV	105	10	5	8	24
	CPPN	97	7	12	3	11
	NAFC	66	19	19	7	23
	PENG	37	26	23	5	15
	CLOX	106	15	10	8	26
	CFLX	76	17	21	4	12
	OXA	97	10	8	7	22

Table 7. Performance data for pharmaceuticals in drinking water (DW) (cont.)

Class	Compound	Recovery / %	Repetitivity (RSD / %; n = 6)	Repeatability (RSD / %; n = 12)	LOD / (ng L^{-1})	LOQ / (ng L^{-1})
Beta-lactam (BL)	CFTF	94	10	15	9	29
	CFDX	65	27	29	4	11
	DCLOX	103	20	12	6	19
	AMPI	82	16	14	14	44
	CFPZ	102	8	9	6	20
	AMOX	84	25	33	5	16
	DESAC	99	8	9	3	10
	AMPID5	95	8	10	5	14
Macrolide (MC)	TYL	85	20	9	3	10
	SPI	62	35	30	4	12
	TRO	97	16	24	4	12
	OLE	110	12	4	5	17
	ROX	91	12	20	3	10
	ERY	85	23	21	0.15	0.5
	TILM	83	40	31	4	13
	CLA	101	9	10	4	11
	AZI	104	13	13	9	27

LOD: limits of detection; LOQ: limits of quantification; CTC: chlortetracycline; DMC: demeclocycline; DC: doxycycline hyclate; MTC: methacycline; OTC: oxytetracycline; TC: tetracycline hydrochloride; 4-ECTC: 4-epichlortetracycline hydrochloride; 4-EOTC: 4-epioxytetracycline; 4-ETC: 4-epitetracycline; DAP: dapsone; SCT: sulfacetamide; SDM: sulfadimethoxin; SFM: sulfamerazine; SMT: sulfamethazine; SMZ: sulfamethoxazole; SQN: sulfaquinoxaline; STZ: sulfathiazole; CPF: ciprofloxacin hydrochloride; NOR: norfloxacin; OFX: ofloxacin; MXF: moxifloxacin; CFQN: cefquinome sulfate salt; CFZL: cefazolin; CFCL: cefaclor; PENV: phenoxymethylpenicillin potassium salt; CPPN: cephapirin sodium salt; NAFC: nafcillin sodium salt; PENG: benzylpenicillin sodium salt; CLOX: cloxacillin sodium salt hydrate; CFLX: cefalexin hydrate; OXA: oxacillin sodium salt hydrate; CFTF: ceftiofur; CFDX: cefadroxila; DCLOX: dicloxacillin sodium salt hydrate; AMPI: ampicillin; CFPZ: cefoperazone; AMOX: amoxicillin tryhidrate; DESAC: desacetylcephapirin; AMPID5: ampicillin-d_5; TYL: tylosin tartarate; SPI: spiramycin; TRO: troleandomycin; OLE: oleandomycin; ROX: roxithromycin; ERY: erythromycin; TILM: tilmicosin; CLA: clarithromycin; AZI: azithromycin dehydrate.

1694 US EPA method[16] and high deviations may have occurred due to the validation of three different water source types. RSD values were acceptable, considering the specifications laid down by European Commission[29] and by Codex Alimentarius Commission.[30]

The internal standard/surrogate recoveries and standard deviations for antibiotics in both SW and DW water are presented in Tables 6 and 7, respectively.

Linearity

The linearity was evaluated with matrix-matched analytical curve at six concentration levels. The results showed good linearity over the range of 25 to 1000 ng L^{-1} with coefficient of determination (R^2) greater than 0.97 for SW and greater than 0.94 for DW.

Method application

The method was applied to the analysis of nine SW and ten DW samples. According to the results, showed in Table 8, compounds were found present in eight out of nine SW samples. Antibiotics were not detected only in the adductor to the Ribeirão das Lajes SW samples. The results showed levels of AMOX, CFLX and SMZ as higher as 105 ng L^{-1}. Also, concentrations of ERY, AZI, CLA up to 35 ng L^{-1} could be found in the river water.

CLA, CFCL, OXA, SMZ and TRO were detected in the lower range up to 10 ng L^{-1} in DW water (Table 9).

Figure 2 shows the MRM chromatograms of a contaminated SW samples with a maximum concentration of AMOX, CFLX, SMZ, ERY, AZI and CLA. The other antibiotics analyzed were below the method limits of detection (LOD).

Many compounds have been found worldwide in several different types of water. A recent review described that among 22 pharmaceuticals detected in SW around the world, about 13 are common in Brazil and other countries, being the most commonly detected antibiotics.[9] Studies conducted by Locatelli *et al.*[10] and Monteiro *et al.*[11] in rivers located in São Paulo, Brazil, showed that NOR, AMOX, CFLX, CPF, SMZ, TC, trimethoprim, OTC and florfenicol were determined with a concentration between 2.2 and 484 ng L^{-1} and in a river in Rio de Janeiro, Brazil, OTC and SMZ were detected in concentration between 44.1 and 467 ng L^{-1}, respectively.[12] In SW samples from Dilúvio

Table 8. River water results for surface water (SW) samples

Analyte	SW samples / (ng L^{-1})							
	Paraiba do Sul River	Macacos River	Queimados River	Guandu main dam	Piraí River	Guandu Lagoon	Santana River	Guandu River
AMOX	38.0	287.5	ND	ND	ND	ND	ND	ND
SMZ	DE	60.3	105.0	DE	DE	DE	DE	DE
CLA	DE	DE	39.2	DE	ND	ND	ND	ND
CFLX	ND	575.5	ND	ND	ND	ND	ND	ND
ERY-H_2O	ND	ND	DE	DE	DE	DE	ND	ND
AZI	ND	ND	35.9	ND	ND	ND	ND	ND

DE: detected (> limit of detection (LOD); < limit of quantification (LOQ)); ND: not detected (< LOD). AMOX: amoxicillin tryhidrate; SMZ: sulfamethoxazole; CLA: clarithromycin; CFLX: cefalexin hydrate; ERY: erythromycin; AZI: azithromycin dehydrate.

Table 9. River water results for drinking water (DW) samples

Analyte	DW samples / (ng L^{-1})					
	Rio de Janeiro	Belford Roxo	Barra Mansa	Volta Redonda	Resende	São Gonçalo
CFCL	DE	ND	ND	ND	DE	ND
SMZ	ND	DE	ND	ND	DE	DE
CLA	ND	DE	DE	ND	ND	ND
TRO	ND	ND	DE	ND	ND	ND
OXA	ND	ND	ND	DE	ND	ND

DE: detected; ND: not detected (below limit of detection). CFCL: cefaclor; SMZ: sulfamethoxazole; CLA: clarithromycin; TRO: troleandomycin; OXA: oxacillin sodium salt hydrate.

Creek in Porto Alegre, Brazil, SMZ, CPF, NOR and AZI were detected between 15.7 and 572 ng L^{-1}.[13]

The non-detection of TCs in water samples may be due to their strong adsorption on organic matter and, although TCs are very soluble in water and are weakly adsorbed by biomass, mechanisms like metal complexation likely played a significant role in the sorption of TCs into solids.[11,14] Similarly, FQs were not found in water, possibly because these molecules are strongly adsorbed by sediment, especially when the concentration of Ca^{2+} and Mg^{2+} is high.[14]

Conclusions

The analytical method developed and validated, based on SPE followed by LC-MS/MS analysis, for the simultaneous extraction of beta-lactams, macrolides, fluoroquinolones, sulfonamides and tetracyclines classes, showed good performance. The achieved recoveries for all target compounds ranged from 49 to 117% and from 50 to 110% for SW and DW samples, respectively. In both matrices, the obtained RSD are less than 58% for all analytes for repetitivity and repeatability, which is lower than values reported by 1694 US EPA method. As a result, a fast and cost-effective method was developed.

The developed and validated method in this study was applied to evaluate the occurrence of compounds in SW and DW from Rio de Janeiro. The results showed that several compounds are occasionally present at high levels, indicating that the evaluated rivers receive uncontrolled loads of wastewater of different sources and/or that these compounds are not efficiently removed in the wastewater treatment plant. The results highlight the worries related to the presence of these compounds in the environment, because of their possible ecotoxicological effects on non-target organisms and on human health arising from the food chain via the water cycle.

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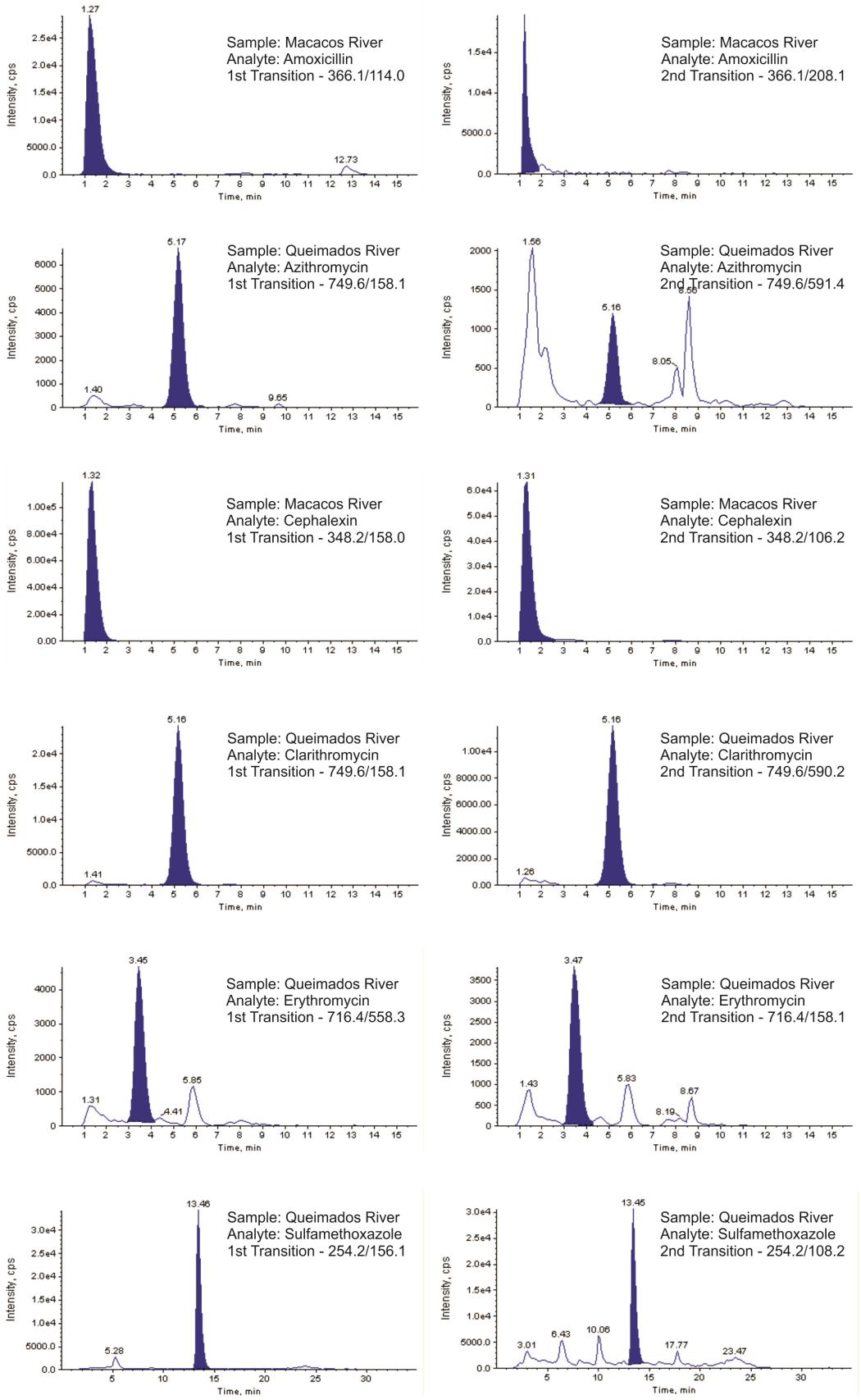

Figure 2. MRM chromatograms of the surface water samples with of amoxicillin, cefalexin, sulfamethoxazole, erythromycin, azithromycin and clarithromycin.

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