Determination of Antibiotics in Water Samples from Anil River, in Northeast of Brazil

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Abstract: There is a growing concern in monitoring the occurrence of antibiotics in the environment because even in low concentrations they may further increase resistance of pathogenic bacteria. Because the low concentrations in water samples (ng.L^{-1} or lower), it is necessary to incorporate a concentration procedure prior to analysis by a chromatographic technique. This study aims to determine the presence of fluoroquinolones drugs such as levofloxacin (LEV), ciprofloxacin (CIP), enrofloxacin (ENR), sarafloxacin (SAR) and norfloxacin (NOR) in water samples from Anil River, located in Sao Luis, Maranhao, northeast of Brazil, using solid phase extraction as preconcentration procedure and analyses by liquid chromatography. LEV, NOR and CIP were found in concentrations of ng.L^{-1}. ENR and SAR were not detected. These results confirm the contamination by domestic wastes and demonstrate the importance of effective actions against contamination in environment by sewage, since they could have compounds such as fluoroquinolones that may cause a strong negative impact to aquatic organisms.

Key words: fluoroquinolones drugs, Anil River, solid phase extraction, liquid chromatography

1. Introduction

The presence of pharmaceuticals in the aquatic system is not a new issue. They can enter in the environment through human and industrial processes wastes [1, 2]. Antibiotics are an important group of these compounds, which have been found especially in water samples [3]. They have been extensively used in human and veterinary applications to treat bacterial infections and/or as growth promoters in animal agriculture and aquaculture industry [4]. There is a growing concern in monitoring the occurrence of antibiotics in the environment because even in low concentrations they may further increase resistance of pathogenic bacteria. In this study, it will be considered a special group of antibiotics: the fluoroquinolones

(FQ's). They are commonly used because of your widespread spectrum activity against a number of both Gram-positive and Gram-negative bacteria [3].

Because the low concentrations of pharmaceuticals in water samples (ng.L^{-1} or lower), it is necessary to incorporate a concentration procedure prior to analysis by a chromatographic technique. An extraction method commonly used is the solid phase extraction (SPE) in which the target analytes from aqueous matrix are extracted after passing through a cartridge containing solvent. In order to remove the undesirable compounds extracted with analytes, an organic solvent is used to wash the cartridge. Afterward, a selective solvent is used to extract the compounds that will be used in the chromatographic analysis [5, 6]. The SPE is a practical method, allows automation, has high sensitivity and does not have problem with emulsion formation [7].

This study aims to determine the presence of FQ's drugs such as levofloxacin (LEV), ciprofloxacin (CIP),

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enrofloxacin (ENR), sarafloxacin (SAR) and norfloxacin (NOR) in water samples from Anil River, located in Sao Luis, Maranhao, northeast of Brazil, using SPE as preconcentration procedure and analyses by liquid chromatography (LC).

2. Material and Methods

2.1 Chemicals and Reagents

Antibiotic standards of levofloxacin, ciprofloxacin, enrofloxacin, sarafloxacin and norfloxacin in purity of > 98% were purchased from Sigma Aldrich (USA). The chemical structures of these drugs are shown in Fig. 1. Chromatographic grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck (Germany). Phosphoric acid, sodium phosphate monobasic (NaH_2PO_4) and formic acid were purchased from Isofar (Brasil) and dichloromethane (DCM) was obtained from Proquimios (Brasil). Ultrapure water was obtained using a Milli-Q Direct 8 purification system from Millipore (USA).

Stock solutions of CIP, ENR, SAR and LEV were prepared in methanol and NOR was in acetonitrile. The solutions were prepared in concentrations of 100 mg.L^{-1} and stocked in dark at -20°C. In order to construct the analytical curve, the solutions of standards were mixed in methanol in a concentration range of 10-300 µg.L^{-1}.
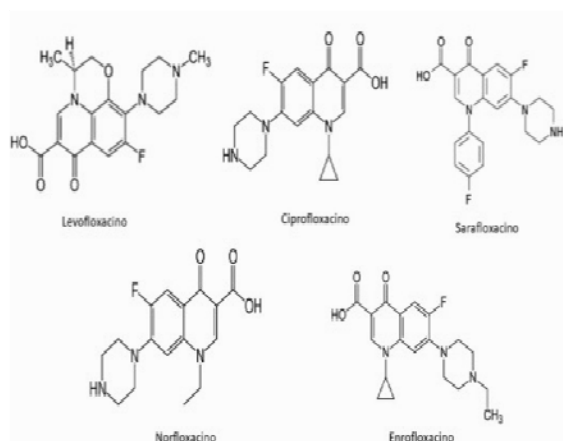

Fig. 1   Chemical structures of LEV, CIP, SAR, NOR and ENR.

2.2 Sample Collection

Water samples from Anil river were collected in a place with coordinates (-2.549270, -44.232179) as shown in Fig. 2. The samples for determination of antibiotics were placed in amber glass bottles of 1L, packed in a box thermal insulation and transported to the laboratory at Federal Institute of Maranhao in order to be stored at 2°C. Additional physicochemical parameters such as water temperature, pH, conductivity, dissolved oxygen, total dissolved solids and total suspended solids were measured in situ at the sampling sites during 36 hours with a break of 1 hour between each measurement.

2.3 Sample Extraction

Water samples were adjusted to pH 3 with phosphoric acid and filtered with a nylon filter (size 0.45µm) prior to extraction procedure. Extraction was performed as follows: HLB Oasis cartridges were conditioned with 2×2.5 mL of DCM + 2× 2.5 mL of MeOH + 3×3 mL of a phosphoric acid solution (pH 3). 200 mL of sample were gently loaded into the cartridges. Afterward, a solution of MeOH 5% adjusted to pH 2.5 with phosphoric acid was used to wash the cartridges. The elution of target analytes was performed with 3×2 mL of formic acid 2% in MeOH.
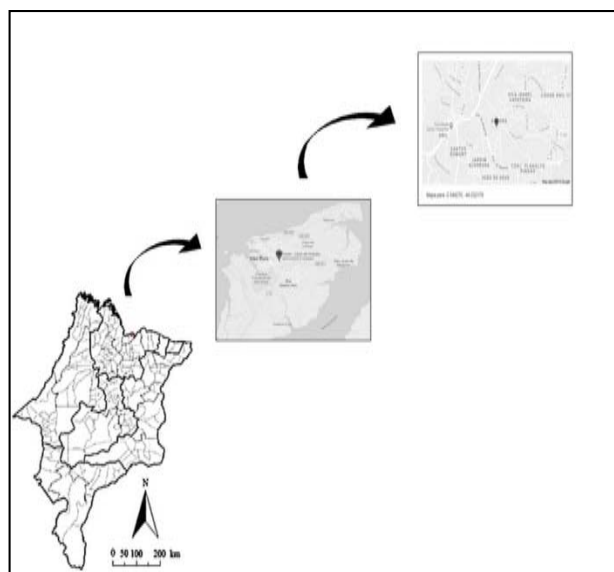

Fig. 2   Samples collection point.

The sample extract was dried with nitrogen and then reconstituted with 1mL of 30% MeOH/70% buffer (0.04 M NaH_2PO_4, pH3).

2.4 LC Analysis

The antibiotics analyses were conducted in gradient mode. Mobile phases A and B are methanol and buffer (0.04 M NaH_2PO_4, pH3), respectively. The gradient program used is as follows: 0-7.5 min, 20% A; 7.5-9 min, 25% A; 9-25 min, 35% A; 25-28 min, 20% A. The flow rate of mobile phase was kept at 1.2 mL.min^{-1} and temperature column was at 35°C. Injection volume of analytical solution was 10 μL. The monitoring wavelength was 280nm (excitation) and 450nm (emission) for all the target compounds.

2.5 Instruments

The analyses were performed using a LC 20-AT liquid chromatograph system (Shimadzu, Japan), a column oven (CTO-10AS) and a fluorescence detector (RF-20A). Chromatographic separation was carried out using a Luna C18 column (250 mm × 4.6 mm; 5 μm). Relevant data acquisition and processing were accomplished with Shimadzu *LC solution* software. The measurement of pH and temperature on Anil River was performed using the Fisher Scientific Accculmet AP72 multiparameter and for conductivity and total solids the Fisher Scientific Accculmet AP75 multiparameter was used. The analysis of dissolved oxygen was carried out with the Hanna HI 9146 Oximeter.

3. Results and Discussion

3.1 Analytical Curve

Table 1 shows the equations and linear correlation coefficients based on analytical curves. The method used in this study exhibit a good linearity for all compounds in a concentration range of 10-300 μg.L^{-1} because the linear correlation coefficients have values higher than 0.99 [12].

Table 1   Equations and linear correlation coefficients based on analytical curves of target analytes.

Fluoroquinolone	Analytical curve equation	R^2
LEV	Y = 1725,6 X-4391.5	0.9981
NOR	Y = 13560 X-105432	0.9975
CIP	Y = 6830 X-48370	0.9997
ENR	Y = 22036 X+92211	0.9997
SAR	Y = 7358.9 X+8332.2	0.9961

3.2 Physicochemical Parameters

Physicochemical parameters analyzed are shown in Table 2. Dissolved oxygen (OD), pH, electrical conductivity (EC), total dissolved solids (TDS), temperature and total suspended solids were measured in accordance with CONAMA 357/2005 and 430/2011 resolutions [8, 9].

The pH values measured are not in accordance with CONAMA 357/2005 resolution, which define a range from 6 to 9. The lowest value obtained was 5.63 and the highest was 6.10.

Oxygen dissolved in water is associated with several variables such as temperature, salinity, human interference and microorganisms activity [10]. The values expected for freshwater class I, II and III have not be less than 6 mg.L^{-1}, 5 mg.L^{-1} and 4 mg.L^{-1}, respectively [8, 9]. However, some results obtained from Anil River are not in accordance with resolution values, because they varied from 2.44 to 4.11.

Electrical conductivity is a parameter that can be influenced by factors such as ion types and temperature. Since there is no upper limit, it is necessary to observe the variations that are associated with occurrence of industrial wastes. Samples that are highly contaminated by sewage show electrical conductivity values in a range from 100 to 10,000 (μS.cm^{-1}) [11]. The values obtained from Anil River varied between 249.17 and 608.50, which testify the contamination.

Solids in water can have natural or human origins. For class II freshwater the maximum limit is 500 mg.L^{-1} [8, 9]. The highest value for total dissolved solids obtained in Anil River was 302.83.

Table 2   Physicochemical parameters in Anil River.

Collection Time	pH	OD (mg.L^{-1})	E.C (µS.cm^{-1})	TDS (ppm)	T (ºC)	TSS (mg.L^{-1})
09:00 am-02:00 pm	5.71	3.01	268.67	133.75	28.72	9.90
03:00 pm-08:00 pm	5.95	2.44	295.33	147.33	28.70	7.73
09:00 pm-02:00 am	6.05	3.17	249.17	124.67	28.07	3.20
03:00 am-08:00 am	6.10	3.79	608.50	302.83	28.30	2.53
09:00 am-02:00 pm	5.63	4.11	392.33	196.50	28.92	1.07
03:00 pm-08:00 pm	5.85	3.37	516.50	250.67	29.08	3.00

CONAMA 357/2005 resolution does not define values for water temperature but this parameter affects the solubility and toxicity of many other parameters.

The occurrence of sewage in water may futher increase the temperature. This situation was not observed in Anil River because the average temperature was 28.63°C. The results for total suspended solids (TSS) presented average values from 1.07 to 9.90 contemplated by climatic conditions.

3.3 Occurrence of Fluoroquinolones

The results for FQ's analyses are presented in Table 3. LEV. NOR and CIP were found in concentrations of ng.L^{-1}. ENR and SAR were not detected.

Table 3   Fluoroquinolones in Anil River water.

Collection Time	Fluoroquinolone (ng.L^{-1})				
	LEV	NOR	CIP	ENR	SAR
09:00 am-02:00 pm	*	40	48.8	*	*
03:00 pm-08:00 pm	*	10	*	*	*
09:00 pm-02:00 am	*	67.7	112.6	*	*
03:00 am-08:00 am	*	10.6	*	*	*
09:00 am-02:00 pm	21.6	*	11.4	*	*
03:00 pm-08:00 pm	100.6	*	49.9	*	*

* Undetected concentrations

4. Conclusion

The results obtained from analyses in Anil River confirm the contamination by domestic wastes. This is proved by pH, oxygen dissolved and electrical conductivity values and occurrence of levofloxacin. ciprofloxacin and norfloxacin, because these pharmaceuticals are widely used in human applications. The absence of enrofloxacin and sarafloxacin antibiotics probably is due to they are exclusively used in veterinary medicine and these activities are not be so prominent in the collection region. The results demonstrate the importance of effective actions against contamination in environment by sewage. Since they could have compounds such as fluoroquinolones that may cause a strong negative impact to aquatic organisms.

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