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Optimization methodology for detection of antimicrobial ciprofloxacin by HPLC-FLD

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Abstract:- Antimicrobial of quinolonas group, ciprofloxacin is used to treat respiratory tract infections, pneumonia, acute bronchitis and gastrointestinal infections. Ciprofloxacin can be found in water bodies and wastewater (between µg/L to ng/L) and be associated with increase of resistance of some microorganisms. Therefore, the aim of this work was optimize the process of ciprofloxacin analysis by high performance liquid chromatography coupled to fluorescence detector (HPLC-FLD). The best conditions for ciprofloxacin detection were: retention time of 6.23 min and the wavelengths of emission and excitation were 250 and 410 nm, respectively.

Keywords:- ciprofloxacin, high performance liquid chromatography, fluorescence detector.

I. INTRODUCTION

Antibiotics are a family of drugs most commonly used because it is designed to contribute to human health. These are widely prescribed drugs for therapeutic and prophylactic against microbial infections and often so erroneous, patients are concurrently treated with antibiotics of different groups. Belonging to the family of antibiotics called fluoroquinolones (FQS), have received increasing attention as the environmental concern, they are present in wastewater.

Quinolone antibiotics (QAs), can be pipemidic acid, ofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, difloxacin, and sarafloxacin tosufloxacina, which comprise an important class of drugs that have been widely used in the last 20 years in Europe and the United States. The QAs are active against many gram-negative and gram-positive and act by inhibition of DNA gyrase, a key enzyme in DNA replication [1].

Ciprofloxacin is a quinolone licensed for use in human medicine but also are used in applications in livestock and agriculture [2]. In livestock, in several animal species, including pigs, ciprofloxacin (CIP) is the major metabolite of enrofloxacin and both are found in bile and urine of animals that received enrofloxacin.

Investigations on the occurrence of FQS in wastewaters, effluents and natural waters have been conducted in several European countries such as Switzerland [3], France, Italy, Sweden and Greece 4]. Several authors [3,5] analyzed the FQS raw sludge, effluent, wastewater and river water samples in Switzerland. However, many studies have been developed in European countries and there is little information available about the occurrence of ciprofloxacin in the effluent wastewater and natural waters in Brazil. The residues of antibiotics eliminated by the human body are found in waters of both rivers and in municipal wastewater, making it necessary to study its effect on the environment and human health.

Current methods of analysis in environmental matrices FQS are based on liquid chromatography (LC), coupled with fluorescence detection [6,7], ultraviolet (UV) [8,9,10] or mass spectrometer detector (MS) [1,11,12]. Various types of stationary phase (reverse phase [8.13], among others) and mobile phases (changes in ionic strength, acidic and / or the presence of modifiers such as acids) have been used.

Due to the scarcity of data and methods developed in Brazil, the objective of this work, such as innovation, was to optimize the methodology review process of the antibiotic ciprofloxacin (CIP) by high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) in order to make it effective for the analysis of samples real scan with regard to contamination of the drug.

II. MATERIALS AND METHODS

Solvents used were methanol (MTedia Company, USA), acetonitrile (MTedia Company, USA), ultrapure water (Pure-Q), formic acid (Merck), glacial acetic acid (Merck) and an analytical standard ciprofloxacin (purity 99.0% , Fluka, Sigma-Aldrich, Steinheim, Germany).

Following chromatographic conditions were employed in this study: high-efficiency liquid chromatography coupled to fluorescence detection (HPLC-FLD, model 1200, Agilent Technologies) software for data acquisition (Agilent ChemStation version B.03.02, January 17, 2008) , Kromasil 100 C18 column (5µm x 4.6 x 250 mm), flow 0.5, 1.5 and 2.0 mL min -1; wavelengths (λ) of 250 and 410 nm, excitation and emission , respectively, and different solvent mixtures as mobile phases. In this step, we used a standard solution of ciprofloxacin at 5 ppm, diluted with methanol.

Mobile phases used for the tests of ciprofloxacin by liquid chromatography coupled to fluorescence detection, cited in the literature is acetonitrile were water [1], acetonitrile and acidified water [10] and acetonitrile, methanol and

acidified water [14]. Starting from the elution conditions reported in the literature [14,15] they were optimized to obtain the best possible resolution for ciprofloxacin. For the tests, we used the standard diluted in the ciprofloxacin concentration of 5 ppm. The tests were performed so that the analyte present peak with high resolution in accordance with the streams, mobile phase and injection volumes tested.

III. RESULTS AND DISCUSSION

For optimization of the mobile phase, nine different mobile phases were evaluated for analysis of ciprofloxacin: a) water, 0.1% formic acid / methanol (MeOH) (78% isocratic mode of water and 22% MeOH), b) water 0.1% formic acid / MeOH (78% isocratic mode of water and 22% MeOH), c) water, 0.1% formic acid / MeOH (78% isocratic mode of water and 22% MeOH); d) water, 0.1% formic acid / MeOH (0-6 min - 78% water and 22% MeOH; 6-30 min - 90% water and 10% MeOH), e) water 0.1% formic acid / MeOH (0-6 min - 100% MeOH, 6-30 min - 78% water and 22% MeOH), f) water, 0.1% formic acid / acetonitrile (ACN) (0-3 min - 90% water and 10% ACN, 3-8 min - 80% water and 20% ACN; 8-17 min - 65% water and 35% ACN; 17-22 min, 50% water and 50% ACN) g) solution of ultrapure water, acetonitrile and glacial acetic acid (100% isocratic mode (87% water, 12% ACN and 1% glacial acetic acid), h) water 0.01M oxalic acid / MeOH (isocratic 72% water and 28% MeOH) and i) solution ultrapure water, ACN and glacial acetic acid (100% isocratic mode (87% water, 12% ACN and 1% glacial acetic acid).

Tests carried out to optimize chromatographic method, were used in common, a Kromasil 100 C18 column (4.6 x 250 mm 5µmx) and the wavelengths of 250 and 410 nm wavelength excitation and emission respectively. The mobile phase, injection volume and time of chromatographic run were variable in the nine tests performed.

According to the results of the optimized conditions reported here, was confirmed by analyzing the ciprofloxacin, the best spectral resolution was found at a retention time of approximately 6.23 minutes (Figure 1), unlike [1], which obtained the retention time of 19.27 minutes for the same compound using the mobile phase a mixture of ACN and water (98:2, pH: 3.0, phase A), and ACN (phase B), used in this study, which was a solution of water, ACN and glacial acetic acid (87:12:1). Figure 1 shows two chromatograms that illustrate the worst and the best conditions for the chromatographic resolution.

Chromatogram of Figure 1 showed that the use of the mobile phase containing 87% water, 12% acetonitrile and 1% glacial acetic acid isocratic mode (100% of the solution mentioned) and flow of 1.5 mL min^{-1}, that provided better separation of the analyte under study and is considered suitable for analysis. Figure 1 is the chromatogram shown in the worst condition of ciprofloxacin analysis under the same conditions of the mobile phase chromatogram of the best condition, only with larger flow of 2.0 mL min^{-1}. The optimized conditions for analysis of ciprofloxacin were, mobile phase, water: acetonitrile: acetic acid (87:12:1), isocratic mode, the run time was 15 min, injection volume was 10 mL and the flow was 1.5 mL min^{-1}.

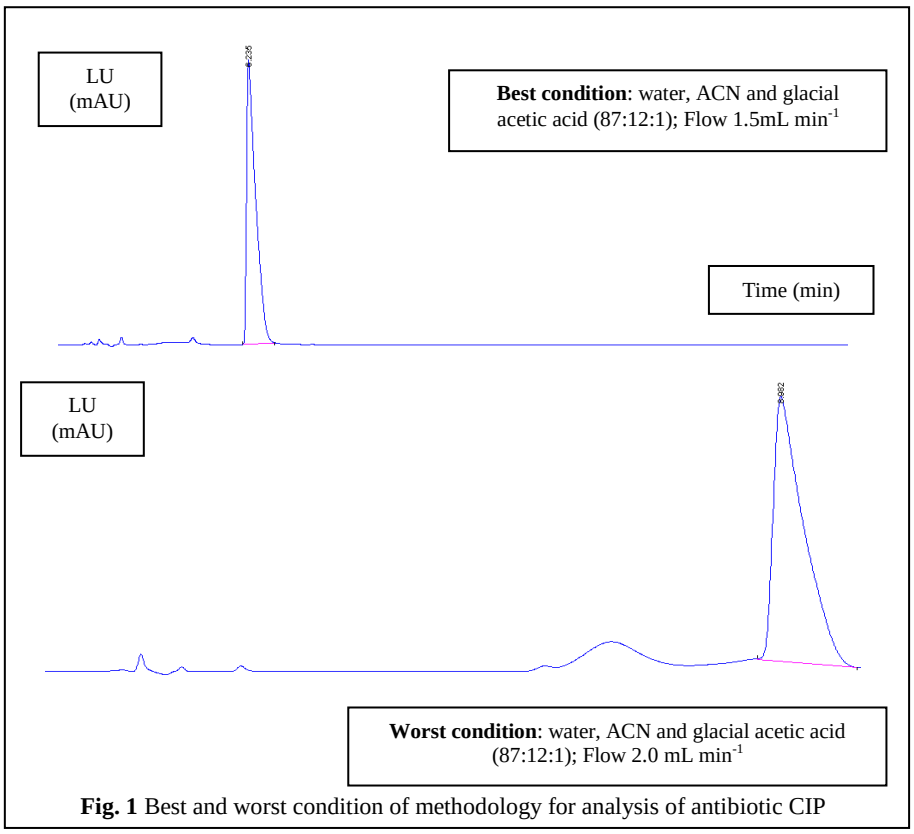

Fig. 1 Best and worst condition of methodology for analysis of antibiotic CIP

Nakata et al. [1] used excitation wavelength of 278 nm and emission at 445 nm and, unlike the wavelengths used in this work, which were 250 and 410 nm excitation and emission, respectively, a good sensitivity was observed. However, [10] used as mobile phase acidified solution of 25 mM orthophosphoric acid and acetonitrile, unlike the one used in this work. Marazuela and Moreno-Bondi (2004) [10] obtained for the retention time of 9 min IPC, higher than obtained in this work for 6.2 min, as [16] had a retention time of 2.9 min for CIP. Separation of C18 column using CIP due to partition of the

compounds of the aqueous (polar) and the solid phase (nonpolar). This column has functional groups which allow a better separation of polar compounds in aqueous solution, enabling rapid equilibrium of the gradient column analysis.

Also using a high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) [14] used the same flow of 1.5 ml min-1, also in isocratic conditions using acetonitrile, methanol and water as the mobile phase. The authors [16], using water as mobile phase acidified to 0.02 M ortho-phosphoric acid and acetonitrile (80:20) at a rate of 0.8 ml min-1. In this work, methanol was tested, but was not observable for the CIP and, therefore, was not used for analyzes.

The mobile phases water, acetonitrile and acetic acid (87:12:1) isocratic mode showed a peak well defined, free from interference. The other stages of development showed no peaks at a retention time of the pattern and the other mobile phases were not identified peaks related to the pattern so the mobile phase showed good sensitivity chosen isocratic mode. The solution used as the mobile phase was acidified with glacial acetic acid since, according to Kemper [17], antibiotics consist of polar and nonpolar groups, which may be separated or protonated (ionized not) depending on the pH.

Examining the chromatograms, retention time 6.23 min CIP and wavelengths of excitation and emission were 250 and 410 nm respectively, which showed improved response for this compound. The HPLC-FLD method under the conditions tested reached the proposed aim, it was shown that represents a valuable tool for use in monitoring of ciprofloxacin in real matrices, the method was easy, reliable and sensitive.

IV. CONCLUSIONS

This study suggests that the analysis of ciprofloxacin should be monitored using appropriate methodologies, adapted to the conditions of the laboratory and applied studies of QAs in wastewater, sewage effluents in Sewage Treatment Plants (WWTP) in sediments and in samples of soil sites near agricultural facilities. This is necessary for a correct assessment of the environmental distribution and risk caused by this antibiotic in the various environmental compartments.

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