



Quantification study of Azithromycin drugs in soil, by the infrared technique with Fourier Transform (IFTR)

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ABSTRACT

Some classes of drugs represent greater criticality as environmental contamination; antibiotics in general represent the most potent contaminants and cause greater damage to the environment. The purpose of this work is to quantify the percentage of soil contamination by Azithromycin in presentations of 500 mg, through the application of Infrared Spectroscopy with Fourier Transform. It was verified that the drug exerts significant impacts to the environment, even in the characteristic of micropollutant, and that its extraction when performed with Pure Acetonitrile allows its residues in soil samples to be satisfactorily quantified.

Keywords: antibiotic discard, environment, Infrared Fourier Transform.

Estudo da quantificação do medicamento Azitromicina no solo, pela técnica de Infravermelho com o Transformada de Fourier (IFTR)

RESUMO

Algumas classes de drogas representam maior criticidade como contaminação ambiental; os antibióticos em geral representam os contaminantes mais potentes e que causam maiores danos ao meio ambiente. O objetivo deste trabalho é quantificar a porcentagem de contaminação do solo por Azitromicina em apresentações de 500 mg, através da aplicação da Espectroscopia no Infravermelho com Transformada de Fourier. Verificou-se que o fármaco, mesmo na característica de micropolvente, exerce impactos significativos ao meio ambiente e que sua extração, quando realizada com o Acetonitrila pura, permite quantificar satisfatoriamente seus resíduos em amostras de solo.

Palavras-chave: contaminação de solo por medicamento, descarte de antibióticos, meio ambiente.



1. INTRODUCTION

The presence of drug residues in aquatic and terrestrial environments has been observed since the 1970s, and studies have shown that these emerging contaminants are in several countries around the world.

Studies over the last two decades have shown that many antibiotics are present in wastewater, animal manure and biosolids, which are mostly biologically active, and hence create potential risks to the environment (Chen et al., 2011; Papadopoulos et al., 2009; Yan et al., 2013)

Some groups of drugs deserve special attention, antibiotics and hormones among them. Antibiotics can provide resistant bacteria development and hormones can affect characteristics of aquatic organisms' reproductive systems, such as the feminization of male fish in contaminated rivers (Melo et al., 2009).

The potent antibiotic commonly used called "Azithromycin" will be treated in this work, due to its high toxicity and contamination capacity; it is produced in significant amounts of about 10,000 tons per year (Febrafarma, 2016).

As an easily accessible drug to the population, at the end of a treatment there is the disposal of unused pills as a medicine residue directly into the environment, through direct discharges into the treated or non-treated domestic sewage network in waterways. Effluents from pharmaceutical industries, rural effluents, the presence of drugs in animal manure used for soil fertilization and inadequate disposal of drugs after the shelf life expiration must also be considered (Melo et al., 2009).

Aspects related to this disposal are very important, since it is estimated that 20% of purchased drugs are destined for disposal as trash.³ This situation is aggravated by the lack of programs to collect expired drugs in the residences. It is worth mentioning that Brazil ranks among the ten largest consumers of medicines in the world, despite the high costs of these products (Febrafarma, 2016).

Antibiotics are frequently detected in different environmental matrices, with concentrations between ng/L to low mg/L in wastewater (Awad et al., 2014; Watkinson et al., 2009), ng/g to mg/g (dry weight, dw) in animal manure (Hu et al., 2010; Li et al., 2015), ng/g to low µg/g dw in soil (Li et al., 2015; 2011; Pan et al., 2016) and ng/g dw in plant tissues (Hu et al., 2010; Pan et al., 2016; Wu et al., 2015).

The majority of antibiotics present in soil are polar compounds with ionizable functional groups, and root uptake is expected to be an important route of exposure for plants when they are grown in antibiotic-contaminated soil (Pan and Chu, 2017).

Some articles such as Melo et al. (2009) and Zuccato et al. (2006) reported that there is a concern in relation to the development of sufficiently sensitive analytical methods for the residual drugs determination in aquatic environments with detection limits in the order of µg/L and ng/L.

Studies like Santos et al. (2007) showed the development of an analytical methodology for the trace element antibiotics quantification as pollutants in the aqueous medium, using the liquid chromatography with spectrophotometric detector in the ultraviolet region (HPLC-UV).

Studies by Bila and Dezotti (2003) and Almeida and Weber (2005) stated that the detection of low concentrations of different drug groups in the aquatic environment showed that solid phase extraction is the best medium for analysis, and often requires specific phases associated with high-performance liquid chromatography coupled to mass spectrometry (HPLC/MS), or high-performance liquid chromatography coupled to two mass spectrometers in series (HPLCMS/MS).

The analytical methodology elucidated in this work aims to quantify the Azithromycin antibiotic, through the simulation of contaminated soil and its recovery; applying the FT-IR (Infrared with Fourier transform) technique. This methodology is justified by its potentialities, which translate into the performance of an analysis without destruction and often without previous sample treatment, in the device robustness, the speed and ease of execution as well as the technique selectivity.

2. MATERIAL AND METHODS

2.1. Experimental Part: Sample Preparation and Soil Contamination Study

For the accomplishment of the experiment, the soil contamination was simulated by an antibiotic that was discarded incorrectly. In order to perform the same, a container was filled with 10 mL of soil. In this same container, 1 tablet of Azithromycin 500 mg was added and daily the sample was watered with 2 mL of water, the objective of which was to simulate the exposure of soil to rainwater. The determination of the volume of 2 mL of water to irrigate the samples was based on the pluviometric index of July 2016, in the city of São Paulo. Thus, the greater the contact with rainwater, the greater the spread of the antibiotic in the soil. The samples were exposed in the open air and were watered with 2 mL of water for 20 consecutive days. The soil samples were collected every 5 days, and the antibiotic azithromycin was extracted by washing the soil samples with pure acetonitrile solvent extractor, after which the samples were analyzed in the FT equipment. The infrared spectra with diffuse reflectance of all the mixtures of pure substances were carried out in the spectral range from 1600 to 1700 cm^{-1} using a resolution of 32 scans.

2.2. Reagents

The standard substance used in analyses to perform the calibration curve was Azithromycin PA, with 95.2% purity, supplied by Jubilant[®], Batch: AZDO/1008053.

Soil samples were contaminated with Azithromycin 500 mg pills, supplied by the Brazilian National Pharmaceutical Company.

2.3. Equipaments

The equipment involved in the analysis was: Infrared Spectrometer with Fourier Transform, Thermo Scientific[®], Model Nicolet IS5 and Shimadzu[®] Scale, Model AUY220; in addition to Acetonitrile organic solvent, Merck[®] Batch: I623030, validity 02/31/2015.

2.4. Extracting Solvent

For the sample extraction, the best extracting solvent was evaluated by means of an extraction test, for which approximately 0.9 g of soil sample were weighed in triplicate and each was contaminated with 0.1 g of Azithromycin.

From this preparation, three extracting solvents were used in differentiated compositions to evaluate which would have the best removal potential of Azithromycin in the soil.

Three different concentrations of this extracting solvent were prepared: 50% Acetonitrile + 50% Water, 80% Acetonitrile + 20% Water and pure Acetonitrile; the samples were submitted to equal amounts of 20 ml of these solvents.

The next step to ensure the material extracting was the agitation process and constant mixing of the soil sample contaminated with its respective extracting solvents for 10 minutes with a magnetic bar. To obtain the samples spectra, they were filtered in a paper filter and the recovered samples were obtained.

Extraction performance (% R) was calculated from Equation 1:

$$\%R = \left(\frac{C_{AZ} \cdot V_e}{M_{AZ}} \right) \cdot 100\% \quad (1)$$

Where: M_{AZ} is the Azithromycin mass inserted into the soil, C_{AZ} is the Azithromycin concentration analyzed in the extracting solvent and V_e is the extracting solvent volume.

2.5. Obtaining the Standard Solution and Calibration Curve

The spectral region choice to construct the calibration curve model was based on the elaboration of the pure Azithromycin substance spectrum in ten different concentrations. The model used for the calibration curve was based on the Lambert-Beer Law.

A stock solution was prepared after weight mass concentration correction as a function of the power purity of the Azithromycin standard used (950.2 $\mu\text{g}/\text{mg}$).

The 1.0524 g mass of pure Azithromycin was then weighed into a 100 ml volumetric flask; the dilution was performed with a 50% mixture of Ultra Purified Water and 50% Acetonitrile organic solvent. This solution presented a 9.9999 g/L concentration of Azithromycin stock solution from which standard solutions diluted at concentrations of 0.5 g/L to 7.0 g/L were prepared. The solution's pH remained around 7.0.

Each of the standard solutions diluted by FT-IR with 32 scans of resolution was individually read and thus the curves were obtained to determine the ratio of the concentrations in relation to their areas, which were evaluated from Microcal Origin[®] Software. The choice band to perform the calibration curve was determined in the 1600-1700 cm^{-1} vibrational energy region.

After establishing and quantifying the concentration x area ratio, the data were treated with scatter plots in Excel[®].

3. RESULTS AND DISCUSSION

The spectral region choice was based on a study by Mallah et al. (2011), who developed a technique for the determination of Azithromycin via FT-IR by compacting the solid substance with KBr pellets. In this work, the spectral range of 1744 to 1709 cm^{-1} was used to analyze the concentration of the substance due to the linearity presented with the areas generated by the vibration band of the C = O group present in the chemical structure of Azithromycin, according to Figure 1. This model allowed comparisons of analytical techniques and provided a guidance on the spectral region of the best evidence for Azithromycin substance. Even without KBr pellet compaction, it was possible to determine the Azithromycin recovery only with the use of Acetonitrile organic solvent.

After determining the best spectral band to work, it was possible to construct the calibration curve. The spectra reproduced in the overlapping concentrations, according to Figure 2, show that the spectral region in the range of 1600 to 1700 cm^{-1} provided better reproducibility for calibration curve execution, without compaction in KBr pellets, only with extraction in Acetonitrile.

3.1. Calibration Curve

Obtaining the calibration curve that relates the concentration of the samples with their respective areas proves the linearity of the method. To confirm the validity of the analytical method, the calibration was performed in triplicate on different dates and the results and the standard deviation obtained were compared in relation to the three curves.

It was observed that as the concentrations increased, the area obtained was proportional, in the 1600-1700 cm^{-1} spectral region.

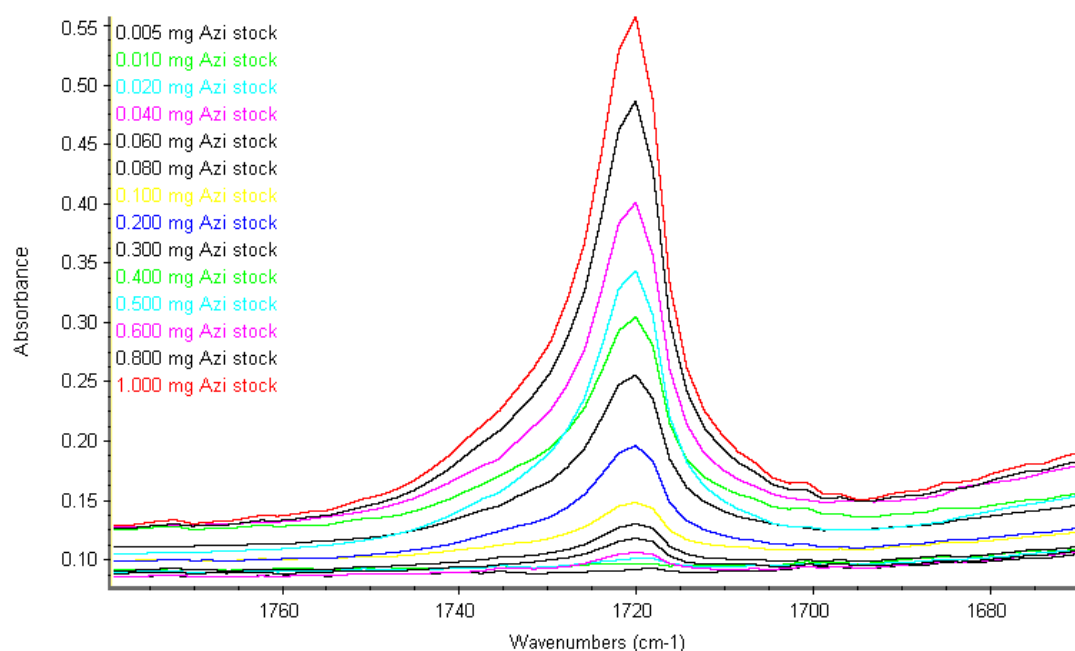


Figure 1. Results obtained by band C = O Carbonyl's determination, in Azithromycin compacted with KBr.

Source: Adapted from Mallah (2011).

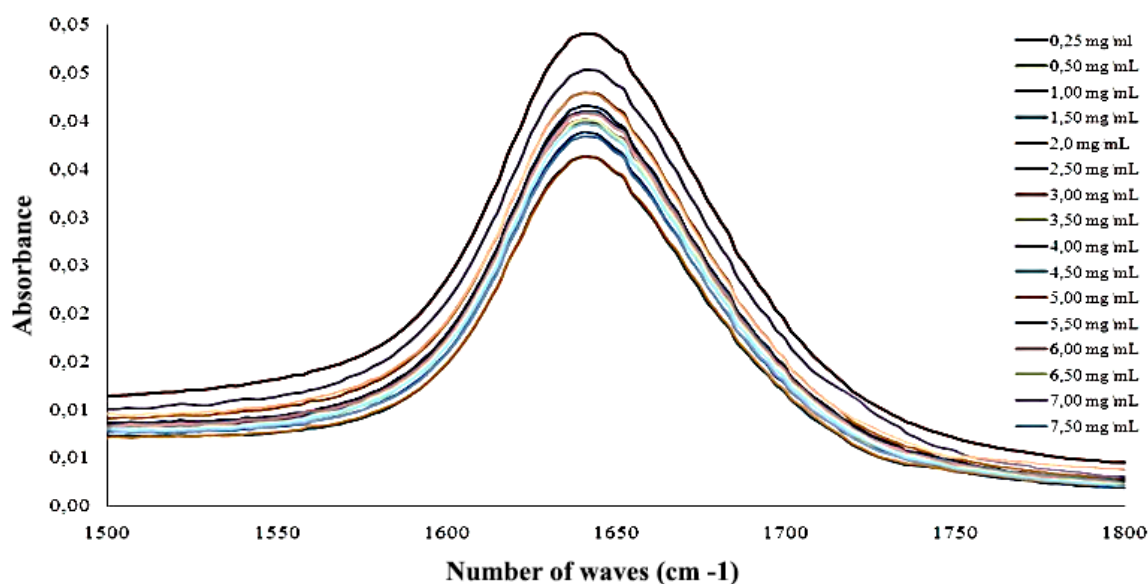


Figure 2. Peak of interest for calibration at 1600-1700 range.

Figure 3 presents the best response obtained for the data dispersion, which can be easily presented by a straight line, which coefficient of co-relation (R) obtained was 0.999, according to the calibration curve 1, represented by Equation 2.

$$C = 0,082 \cdot A + 4,0963 \quad R = 0.9991 \quad (2)$$

After application of the t Student test, the following results were obtained: the value of t calculated was 0.04199 and the value of the standard deviation grouped, S_G , was 2.17527. Thus, the limit of confidence could be expressed by Equation 3:

$$LC = \mu \pm 0,091352 \quad (3)$$

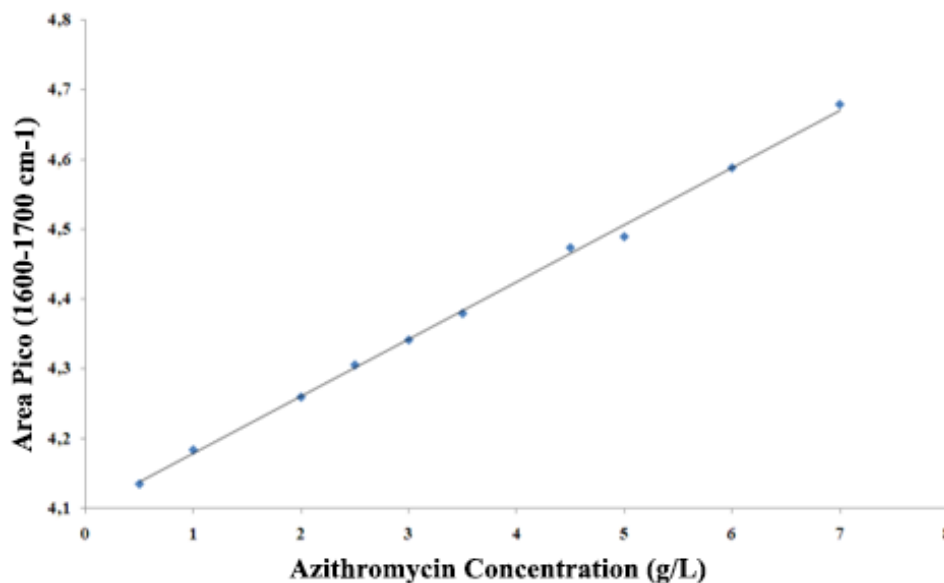


Figure 3. Linearity obtained for the first calibration curve.

3.2. Choosing the Extracting Solution

Extraction test was efficient according to Table 1, where it was possible to prove that pure Acetonitrile was more efficient in the extraction process of the Azithromycin drug from the soil.

Table 1. Extraction efficiency of the solvents tested.

Test	% Extracting Solvent	C_{Az} (g/L)	$C_{AzCalc.}$ (g/L)	% Recovering	Deviation
1	ACN:H ₂ O (50:50)	4.5950	2.3255	49.5840	0.1479
2	ACN:H ₂ O (80:20)	4.6900	3.6253	77.8643	0.1263
3	ACN	4.7150	4.9331	104.625	0.2556

Where: C_{Az} = expected azithromycin concentration, $C_{AzCalc.}$ = recovered concentration.

Comparing to a study by Eickhoff et al. (2009), the residual drug detection in the aquatic environment and in the soil, in the range of $\mu\text{g.L}^{-1}$ and ng.L^{-1} , the methods described in the literature are based on solid phase extraction, in some cases, derivatization of the acidic substance and subsequent determination of the derivative by gas chromatography coupled to mass spectrometry (GC-MS) or high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS).

Thus, it was found that it is possible to extract Azithromycin from soil with an organic solvent, without the need for a sample derivatization test or acid digestion.

Using the extraction deviations that obtained the best recovery, we will have the limits of detection (LD) and quantification (LQ), as follows Equations 4 and 5:

$$LD = 3 \cdot 0,25563 = 0,76689\text{g/L} \quad (4)$$

$$LQ = 10 \cdot 0,25563 = 2,55630\text{g/L} \quad (5)$$

3.3. Samples results

Figure 4 represents the spectra overlap of Azithromycin 50 mg samples in soil, where the work spectral range of the 1600-1700 cm^{-1} vibrational energy bands was considered, representing the $\text{C}=\text{O}$ Carbonyl's stretching, used for the quantification of the sample in relation to the soil.

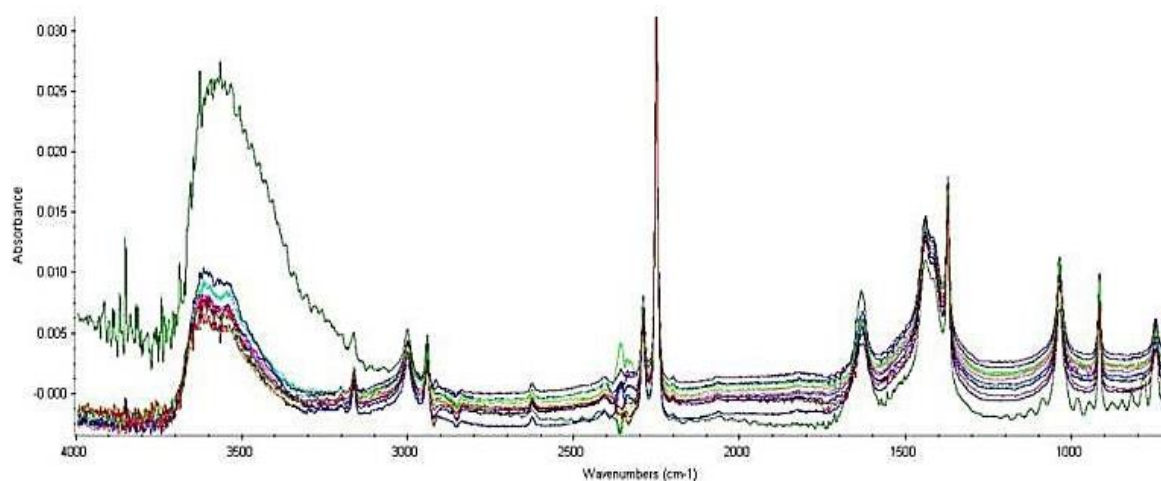


Figure 4. Spectra overlap of contaminated soil samples with 500 mg Azithromycin.

Table 2 represents the %'s mean recovery obtained in the soil samples' triplicates when exposed to the 500 mg Azithromycin concentration for a period of 0 to 20 days.

Table 2. Results for soil contamination with 500 mg Azithromycin.

Exposure Days	% drug recovery
5 days – 500 mg concentration	21.35% Azithromycin in soil recovery
10 days – 500 mg concentration	13.48% Azithromycin in soil recovery
15 days – 500 mg concentration	11.40% Azithromycin in soil recovery
20 days – 500 mg concentration	10.14% Azithromycin in soil recovery

According to the presented results, it was possible to verify that the longer the exposure time of the Azithromycin drug in contact with the soil, being influenced by the dragging degree of the 2 ml of water projected daily on the sample, the greater its recovery. That is, compared to everyday situations, when discarding the drug-containing packaging incorrectly, when it is exposed to the environment and undergoes influences from external factors such as temperature and rainfall drag, these drugs can contaminate soil.

Rabølle and Spliid (2000) conducted soil column studies of four antibiotics under saturated steady-state conditions and found that most of them remained in the top few centimetres of the soil columns. Surface soil has a higher concentration of organic matter than subsurface layers, which promotes the adsorption of antibiotics that decreases their downward leaching. The application of animal manure in soil may decrease the leachability of tetracycline-hydrochloride (Jones et al., 2005). Soil pH also affects the fraction of ionized organic compounds, and most antibiotics are fairly water soluble and able to ionize according to the soil Ph. (Figueroa-Diva et al., 2010; Srinivasan et al., 2013).

4. CONCLUSION

This work can be affirmed as an application proposal of an analytical methodology FT-IR for Azithromycin antibiotic characterization and quantification in soil.

The Pure Acetonitrile extraction method evaluated proved to be effective for removal of the antibiotic from soil, and the relation of the sample exposure time, the time conditions, temperature and rainfall index also proved to simulate what occurs in the environment.

As the days passed, with constant exposure the samples became increasingly “diluted” and interacted with the soil; it was also found that the drug's 500 mg concentration provided significant recoveries in percentages.

The study also highlights the need to collect and dispose of medicines correctly, because when in contact with soil they may bioaccumulate damage organisms in the food chain.

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