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Novel approach for the determination of azithromycin in pharmaceutical formulations by Fourier transform infrared spectroscopy in film-through transmission mode

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ABSTRACT

This work reports the development of a new method for the determination of azithromycin in pharmaceutical formulations employing Fourier transform infrared (FTIR) technique. The measurements were performed using a novel approach based on a film-through transmission mode. Several variables that could influence the analytical performance of the method were evaluated (solvent, nominal resolution, number of scans, mode of measurement and spectral region selected for measurement). Acetonitrile was the best solvent for the determination of azithromycin, employing the absorption band of the C=O group at 1729 cm^{-1}. The extraction of azithromycin from the formulations was made by mechanical shaking of 25 mg of each sample with 1 mL of acetonitrile for less than 5 min. In the optimized conditions (8 cm^{-1} nominal resolution and 25 scans per spectrum), the limit of detection in acetonitrile was 0.07 mg mL^{-1} azithromycin. The method was applied to the determination of azithromycin in six commercial pharmaceutical formulations and recovery test, employed to evaluate the accuracy, provided recovery percentages in the range of 87–108%.

1. Introduction

Antibiotics are specific chemical substances, originally produced by living organisms. Their structural analogs can be obtained through synthetic routes and are able to inhibit, even at low concentrations, vital processes of one or more species of bacteria. Nowadays, the main classes of commercially available antibiotics are penicillins, macrolides, cephalosporins (β-lactam antibiotics), tetracyclines and aminoglycosides [1].

Azithromycin (9-deoxo-9a-methyl-9a-aza-homoerythromycin A) was the first azalide antibiotic, a subclass of the macrolides, used in the treatment of several adult and pediatric infections [2–4]. It was discovered by a group of researchers from Pliva, in 1980, and was firstly commercialized in 1988 as a medicine called Sumamed. In 1991, the Pfizer laboratory launched the Zithromax, which was commercialized in the USA and in the Eastern Europe [5,6]. In Brazil, the legislation allows the production of generic medicines, which resulted in the commercialization of azithromycin by several laboratories, making of a great importance the development of ease and rapid methods for the quality control of such pharmaceutical products.

The specialized literature lists a series of analytical methods for the determination of azithromycin in pharmaceutical formulations. Most of them are based on the UV–vis spectrophotometry [7–10] or spectrofluorimetry [11,12], since no high sensitivities or selectivities are required to perform the analysis. As azithromycin does not absorb UV–vis radiation significantly, in these cases, the analyte must be converted into an absorbing substance through different reactions. So, such methods involve laborious procedures of derivatization that require intense manipulation of the samples, which make them costly and subjected to losses or contamination errors.

The most common analytical technique employed for azithromycin determination is liquid chromatography coupled to different detectors such as mass spectrometry [13–15], fluorescence [16–18] and electrochemical [19,20]. The use of UV–vis detector is not recommended in this case because of the very low absorptivity of azithromycin in these regions of the electromagnetic spectrum. The main drawbacks associated to the liquid chromatography-based methods are the high cost of the instrumentation and the need of a high dilution level and intense cleaning-up treatment of samples before injection into the chromatographic system.

Infrared spectrometry has been largely employed for qualitative and structural analysis and nowadays its use for quantitative purposes is already a reality. There are several works in the current literature reporting the application of infrared spectrometry for the quantification of substances of industrial and environmental interest [21–27]. Even so, only few direct applications of this technique in the determination of active principles of pharmaceuticals are reported [28–32]. Additionally,

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multivariate calibration approaches have also been used in the development of methods for quantification of pharmaceuticals [33–35].

The main goal of this work was to develop a new method for the determination of azithromycin in pharmaceutical formulations, employing Fourier transform infrared spectrometry. This task was carried out through the use of the Dial Path® accessory, which allows to do measurements in transmission mode using a very low volume of sample.

2. Experimental

2.1. Apparatus

Infrared measurements were carried out in the transmission mode with a Fourier transform infrared spectrometer from Agilent Technologies (Palo Alto, CA, USA), model Cary 630. The spectrometer was equipped with the Dial Path® accessory, which was operated using an optical pathlength of 200 μm.

An ultrasonic bath from Unique (São Paulo, Brazil), model 1600 (frequency of 40 MHz), and a roller mixer from Biomixer (São Paulo, Brazil), model MR-II, were used to do the extraction of the AZTR from the samples.

A centrifuge supplied by Sislab (São Paulo, Brazil), model Twister, was used to separate the liquid and solid phases in the extraction of AZTR from the tablets. The centrifugation was performed for 5 min at 3500 rpm.

2.2. Reagents and solutions

Azithromycin (AZTR) standard (pharmaceutical grade) was supplied by Pharma Nostra (Rio de Janeiro, Brazil). Solutions of AZTR were prepared by simple dilution of a convenient mass of the AZTR standard with the desired solvent in 5 mL volumetric flasks. All AZTR solutions were discarded after the use, since they were not stable for more than 12 h.

Solvents employed in this work (acetonitrile, chloroform and dichloromethane) were always of HPLC grade and furnished by Tedia (Fairfield, OH, USA) and deionized water obtained with a Direct Q-3 system (18,2 MΩ cm resistivity), supplied by Millipore (Milford, MD, USA), was employed to wash all glassware and plastic flasks used throughout the experimental work.

2.3. Sample preparation and measurement

Samples analyzed through this work were purchased in drugstores located in the city of Niterói, in the state of Rio de Janeiro, Brazil. Twenty tablets of each sample were powdered employing an agate mortar. Then, 25 mg of the powdered samples was weighed directly into the glass tubes utilized for the extraction and 1 mL of acetonitrile was added to each tube. The obtained suspensions were homogenized using a roller mixer for 2 min and, after that, were centrifuged for 5 min at 3500 rpm. The supernatant solutions were collected and transferred directly to the IR spectrometer.

The same procedure was adopted for the recovery tests. In this case, before addition of acetonitrile, samples were fortified with known amounts of AZTR standard (5, 10 and 20 mg).

Infrared spectra were registered in the range of 4000–650 cm^{-1}, using a nominal resolution of 8 cm^{-1} and 32 scans per spectrum. The area and height of the bands located at 1173 and 1729 cm^{-1} were employed as quantitative variables for the determination of AZTR in tablets. For the band at 1173 cm^{-1}, the baseline was corrected in the range of 1800–820 cm^{-1}. Then, the height at the maximum of the band and the area between 1183 and 1163 cm^{-1} were measured and used to build the analytical curves and for the determination of AZTR in the sample extracts. For the band at 1729 cm^{-1}, the baseline was corrected in the range of 820–1800 cm^{-1}. The height at 1729 cm^{-1} and the area between 1716 and 1742 cm^{-1} were employed in the quantification process. Background was always collected with the empty cell, using the instrumental conditions established for each case.

3. Results and discussion

3.1. Spectra of AZTR in solid-phase

In order to identify possible bands to be used in the development of the method, the spectrum of pure AZTR in solid phase was acquired using the attenuated total reflectance technique (ATR). As it can be seen in Fig. 1, several characteristic bands of AZTR could be observed in the spectrum obtained directly from the solid standard. The main bands identified were: (i) bands related to the axial stretching and bending of C–H of the methyl groups, which were located in the region of 2750–3020 cm^{-1} and 1377 cm^{-1}, respectively; (ii) the sharp and intense band located at 1719 cm^{-1}, which can be assigned to the axial stretching of the C═O group present in the lactone; (iii) other important bands present in the spectrum were those observed in the range of 1134–1221 cm^{-1}, which appeared due to the absorption associated to the axial stretching of C–O [36]. Among these characteristic bands, only two of them could be used for quantitative purposes (1723 and 1173 cm^{-1}), since they were not overlapped with the bands of the solvents tested (acetonitrile, chloroform and dichloromethane). These bands refer to groups not present in the structure of the solvents and, as expected, suffered some displacement in the spectra depending on the solvent used in the preparation of samples.

Only the two bands that were not overlapped with the solvent bands were employed for the development of the method, which was carried out through the evaluation of the effect of the nature of the solvent, the number of scans per spectrum, the nominal resolution and the extraction method. All results were computed taking into account both, peak area and peak height, after an adequate baseline correction.

3.2. Spectra of AZTR in different solvents

The accessory used to measure the spectra of AZTR in solutions was the Dial Path®, from Agilent. In this accessory, a drop (50 μL) of the liquid sample solution is poured on a crystal of ZnSe. Afterwards, the dial selector is rotated to a given position, allowing that another

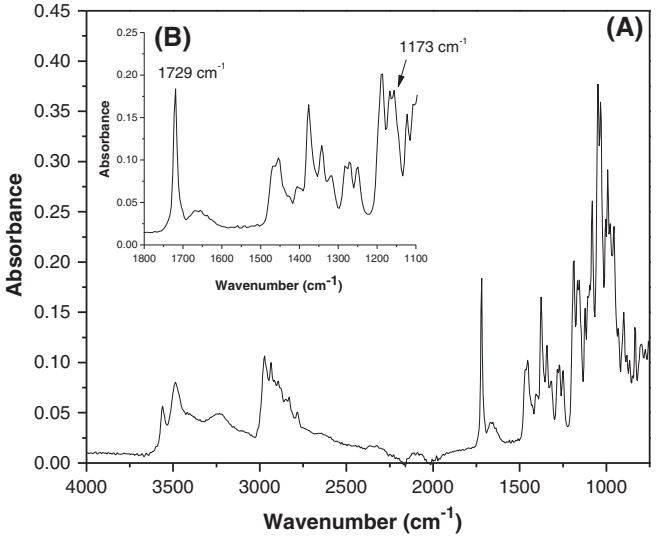

Fig. 1. Infrared spectrum of azithromycin in the solid phase (A) in the whole mid-infrared region and (B) in the range of interest (1800–1100 cm^{-1}).