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Simultaneous determination of benznidazole and itraconazole using spectrophotometry applied to the analysis of mixture: A tool for quality control in the development of formulations

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ABSTRACT

The aim of this work was the development of an analytical procedure using spectrophotometry for simultaneous determination of benznidazole (BNZ) and itraconazole (ITZ) in a medicine used for the treatment of Chagas disease. In order to achieve this goal, the analysis of mixtures was performed applying the Lambert–Beer law through the absorbances of BNZ and ITZ in the wavelengths 259 and 321 nm, respectively. Diverse tests were carried out for development and validation of the method, which proved to be selective, robust, linear, and precise. The lower limits of detection and quantification demonstrate its sensitivity to quantify small amounts of analytes, enabling its application for various analytical purposes, such as dissolution test and routine assays. In short, the quantification of BNZ and ITZ by analysis of mixtures had shown to be efficient and cost-effective alternative for determination of these drugs in a pharmaceutical dosage form.

1. Introduction

The Chagas disease (also known as barber bug fever) has a major health relevance because of its social and economic impact. Twenty-one countries in Latin America recognize the endemic character of this disease and there are cases of this infection registered in US, Canada, and European Union as a consequence of migration [1]. The World Health Organization estimates that about 7 million people are infected and an additional ten thousand die every year as result of complications from this disease [2].

Discovered one hundred years ago, Chagas disease has two clinical phases and no effective treatment for both [3]. Benznidazole (BNZ) is the drug used for the treatment of this disease in Brazil. Recent studies show a synergic effect of this therapeutic agent with azoles derived as itraconazole (ITZ) [4,5]. The development of a medicine associating both drugs is a challenge and a promising way for the effective treatment of Chagas disease [6,7].

The literature describes diverse methods for the individual determination of BNZ or ITZ (Fig. 1) using spectrophotometry [8,9], voltammetry [10,11], and liquid chromatography [12]. However, simultaneous quantification of these drugs is described only in a study using HPLC-UV for plasma assays [13].

In general, the methods used to analyze multidrug matrix are complex, of high cost and generate significant residues. The spectrophotometry is a low price and widespread technique that does not require a special structure to analyze compounds, and its generation of residues is in agreement with the principles of green analytical chemistry.

According to Lambert–Beer law, the absorbance (**A**) is proportional to the concentration (**C**) and the optical path (**b**) with a proportional constant known as molar absorptivity (**ε**), $\mathbf{A} = \boldsymbol{\varepsilon}\mathbf{.b.C}$ [14]. Additionally, the absorbance is an additive function at any wavelength [15]. When analyzing multiple compounds in a defined wavelength, it is necessary to add the absorbance of pure solutions in order to obtain the absorbance of the final solution. For low concentrations and reproductive analytical conditions the molar absorptivity of these substances is invariable and can be previously calculated with solutions of known concentrations for diverse wavelengths. For the analysis of a sample of unknown concentrations of two or more subjects, it is possible to assemble a system with two or more equations and to calculate the concentrations of all the compounds with the final absorbance and the respective molar absorptivity previously calculated in each wavelength [16].

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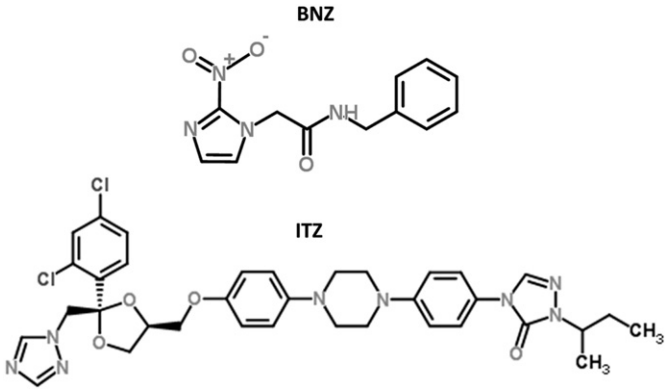

Fig. 1. Molecular structure of BNZ and ITZ.

The aim of this work was the development of a spectrophotometric method for simultaneous quantification of BNZ and ITZ to be used in the quality control of a medicine containing this drug association.

2. Experimental

2.1. Materials and equipment

BNZ (lot 13871, purity of 99.0%) and ITZ (lot 00569488, purity of 99.5%) were obtained for donation from Roche (Basel, Swiss) and Janssen–Cilag (Geel, Belgium), respectively. Microcrystalline cellulose PH102 (Avicel) and hydroxyl propyl methylcellulose (HPMC) were obtained from Colorcon (Cotia, Brazil). All solvents and reagents used in this work were of analytical grade and obtained from Vetec (Duque de Caxias, Brazil). A spectrophotometer UV–VIS (Perkin Elmer, Lambda XLS) with a quartz cuvette of 1 cm of optical path was used for all measurements.

2.2. Method development

The development of the method involved the evaluation of the drugs' solubility in different diluents and the definition of spectrophotometric parameters based on scans. Absorption spectrums were obtained from samples containing ITZ or BNZ or the mixture of both drugs. Analytical curves were made with the selected wavelengths to determinate the molar absorptivity (ε). An equation model system for the analysis of the mixtures was established considering the additive property of absorbance.

2.3. Analytical conditions

The stock solution of 150 mg L^{-1} were prepared using ethanol for BNZ and ethanol:hydrochloric acid 0.2 mol L^{-1} in the proportion 75:25 for ITZ. All work solutions were prepared by dilution with ethanol:hidrochloridric acid (0.2 mol L^{-1}) in the proportion 75:25.

The absorbance of pure compounds and mixtures were measured in two wavelengths — 321 nm and 259 nm. The absorbance for pure standards allowed the construction of a calibration curve and the calculation of the molar absorptivity for ITZ and BNZ in both wavelengths. The concentration of ITZ and BNZ in the sample was determinate with the measure of absorbance of samples in both wavelengths by an equation system.

2.4. Method validation

The nominal concentration solution used for diverse tests of the validation was 17.5 mg L^{-1}. The absorbance of all solutions was measured in 321 and 259 nm. The method was validated in accordance with the current health regulations for quality control of medicines considering the following parameters [17]:

2.5. Robustness

The robustness evaluates the ability of the method to withstand deliberate modifications in their experimental conditions. This test was realized by a 2^3 factorial design considering the variation of three critical experimental parameters in two levels [18]. The factors studied were the concentration of hydrochloric acid (0.15 and 0.25 mol L^{-1}), percentage of water (20 e 30%) and time of analysis (30 and 120 min). The response obtained was the concentration of BNZ and ITZ, and all assays were performed in triplicate. The prediction equation was estimated by the analysis of multiple regressions *step-wise*. The model was validated by the analysis of variance (ANOVA) with a significance level of 0.05. All the statistical calculations were realized using the software *Design-Expert version 9*. Additionally, in order to verify a possible degradation of sample, the spectrophotometric profile of each drug was analyzed.

2.6. Selectivity

The selectivity evaluates the capacity of a method to distinguish and quantify the compounds unequivocally in the presence of interference [17]. In this work, in addition to simultaneous determination of ITZ and BNZ, the capacity of the method to quantify those drugs in the presence of a pharmaceutical matrix typically used in a solid dosage form and present in BNZ commercial product was evaluated [9,19].

Samples containing ITZ and BNZ in the nominal concentration were prepared, with and without the pharmaceutical inactive ingredients — Avicel and HPMC. These assays were performed in quintuplicate, and the results were analyzed by ANOVA one-way with a significance level of 0.05.

2.7. Linearity

This parameter evaluates the linear relationship across the range of the analytical procedure. Three calibration curves were performed in the range of 10 to 25 mg L^{-1} for ITZ and BNZ individually. The linear fit was carried out by linear least square. The test of significance of the angular coefficient and the test of proportionality was evaluated by Student's t-test. The homogeneity of variance and the normality of residues were checked by ANOVA one-way with a significance level of 0.05.

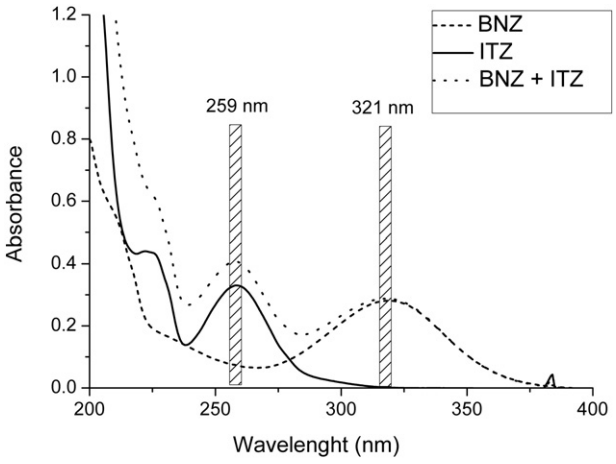

Fig. 2. Absorption spectrum of BNZ, ITZ and its mixture. Selected wavelengths are highlighted.