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A simple assay for the simultaneous determination of human plasma albendazole and albendazole sulfoxide levels by high performance liquid chromatography in tandem mass spectrometry with solid-phase extraction

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ABSTRACT

A simple, reproducible and fast (4 min chromatogram) method of liquid chromatography in tandem with mass spectrometry (LC/MS–MS) was developed to determine simultaneously the plasma levels of albendazole (ABZ) and its metabolite albendazole sulfoxide (ABZOX) for pharmacokinetic and clinical analysis. Each plasma sample was extracted by solid phase extraction (SPE) using phenacetin as internal standard (IS). The extracted sample was eluted with a Zorbax XDB-CN column using an isocratic method. The mobile phase consisting of water with 1% acetic acid (40%, A) and MeOH (60%, B), was used at a flow rate of 1 mL/min. ABZ and ABZOX were detected and identified by mass spectrometry with electrospray ionization (ESI) in the positive ion and multiple-reaction monitoring (MRM) mode. The method was linear in the range of 5–1000 ng/mL for ABZ and 10–1500 ng/mL (full validation) or 10–5000 ng/mL (partial validation) for ABZOX, with 5 and 10 ng/mL lower limit of quantification (LLOQ) for ABZ and ABZOX, respectively. The tests of accuracy and precision, matrix effect, extraction recovery and stability of the samples for both ABZ and ABZOX did not deviate more than 20% for the LLOQ and no more than 15% for other quality controls (QCs), according to regulatory agencies.

1. Introduction

ABZ is a benzimidazole derivative used for the treatment of parasitic infections. ABZ concentrations are negligible or undetectable in plasma as it is rapidly converted in the liver to its metabolite ABZOX prior to reaching the systemic circulation. Hence, its systemic anthelmintic activity has been attributed to its primary metabolite, ABZOX. For this reason ABZ is not frequently detected in plasma after oral administration and its pharmacokinetic properties have been studied by determining the plasma concentration of ABZOX [1–3] that is present in plasma concentrations in the order of ng/mL. Additionally, one-fourth of the population absorb as little as 30%, leading to treatment failure. Thus, therapeutic drug monitoring may be warranted to optimize the eradication of parasitic infections in patients resistant to treatment [4]; this monitoring could be useful for an efficient drug therapy of parasitic infections.

To date, several methods have been described for the determination of ABZ metabolites by high performance liquid chromatography (HPLC) with ultra-violet detection (UV) [5–7] and fluorescence detection [2,8], or by using capillary electrophoresis [9]. More recently, LC/MS–MS-based methods have been reported [10–13]. Most of them use protein precipitation [7] and do not remove plasma interference from chromatograms to yield reproducible results or use a liquid–liquid extraction procedure obtaining recovery percentages below 80% [10,12]. Only few reports have used SPE [5,6] that reduces the matrix effect, one of the main problems of ESI sources; however these methods have been performed with HPLC coupled to UV detection. Furthermore, few studies have addressed the pharmacokinetics of ABZ in human plasma because of its rapid metabolism that gives rise to low plasma concentrations [5,12].

Abbreviations: ABZ, albendazole; ABZOX, albendazole sulfoxide; C_{max}, maximal plasma concentration; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; EIC, extraction ion chromatogram; EMA, European Medicine Agency; ESI, electrospray ionization; FDA, regulatory agency Food and Drug Administration; HPLC, high performance liquid chromatography; IS, internal standard; LC/MS–MS, liquid chromatography in tandem with mass spectrometry; LLOQ, lower limit of quantification; MeOH, methanol; MRM, multiple-reaction monitoring; UV, ultra-violet; QC, quality control; RT, retention time; SD, standard deviation; SPE, solid phase extraction; TIC, total ion chromatogram.

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In this context we planned the present study aimed at developing a simple, sensitive, reproducible and fast method of LC/MS–MS to monitor the plasma levels of ABZ and ABZOX for application in clinical trials and in routine clinical practice.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of analytical grade or HPLC grade. ABZ, ABZOX, phenacetin, and formic acid were supplied by SIGMA and acetic acid by Panreac. Methanol (MeOH) and orthophosphoric acid 85% were purchased from SYMTA. The water for preparing the mobile phase was prepared using a Milli-Q system. Blank human plasma samples were kindly donated by the Blood Donation Unit of "Hospital Universitario de la Princesa, Madrid, Spain".

2.2. Stock solutions, calibration standards and QCs

Stock solutions of ABZ/ABZOX were prepared by dissolving an accurately weighted quantity in methanol to obtain a concentration of 1 mg/mL. The IS working solution was prepared by dissolving an exact amount in MeOH to obtain a concentration of 1 mg/mL and was diluted 100 times to give a working solution of 10 μg/mL. Stock solutions of ABZ/ABZOX and its IS were stored at −80 °C until use. Calibration standards were made to obtain concentrations of 5, 10, 20, 30, 50, 100, 500, and 1000 ng/mL for ABZ and 10, 20, 30, 50, 100, 500, 1000, and 1500 ng/mL for ABZOX. The concentrations of quality control (QC) samples were 5 (LLOQ), 10 (QC_{Low}), 450 (QC_{Medium}) and 800 (QC_{High}) ng/mL for ABZ and 10 (LLOQ), 20 (QC_{Low}), 600 (QC_{Medium}) and 1200 (QC_{High}) ng/mL for ABZOX, in plasma or MeOH. The highest concentration of the calibration standard and the four QCs were prepared by adding a certain volume of stock solution to the blank plasma. The other calibration standards were prepared by serial dilution of the highest to the lowest concentration, from 1500 to 5 ng/mL for both molecules; however, calculations were performed in the range 5 to 1000 ng/mL for ABZ and from 10 to 1500 ng/mL for ABZOX, to generate the calibration curve according to the regulatory agency Food and Drug Administration (FDA) [14] and the European Medicine Agency (EMA) [15] on the recommendations for bioanalytical method validation. A sample blank drug-free plasma and sample zero drug-free plasma with IS was included. Both QC calibration standards were stored at −80 °C until analysis, to avoid more than 2 cycles of freezing and no more than 3-month span.

2.3. Chromatographic conditions

The HPLC system consisted of a 1200 Series separation module (Agilent Technologies) controlled by Agilent Mass Hunter Workstation Data Acquisition for programming samples and chromatographic conditions. Separations were carried out at 22 °C on a ZORBAX XDB-CN column (4.6 mm × 150 mm longer and 5 μm particle size, Agilent Technologies). A Zorbax CN guard column (4.6 mm cartridge, Agilent Technologies) was used to preserve the analytical column. The mobile phase consisted of a combination of 1% acetic acid in water (solution A) and MeOH (solution B) allowed us to work in reversed-phase partition chromatography. The chromatographic run was performed under isocratic conditions at the flow rate of 1 mL/min with 40% of solution A and 60% of solution B. The elution times of each sample was 2.879 min for ABZ and 2.126 min for ABZOX, not requiring a post-time to return the column to initial conditions due to the isocratic conditions used. Every time at the end of the day, a washing method of the column was applied consisting of increasing % of MeOH to reach 100% at 1 mL/min flow rate over 40 min, then continued during 10 min more and back to initial conditions within 5 min.

2.4. Mass spectrometry

The mass spectrometry detection system consisted of an Agilent Technologies 6410, triple quadrupole with ESI in positive ion mode. The mass spectrometry was operated in MRM mode.

The settings of the mass spectrometer were as follows: the desolvation gas (N_2) and flow was operated at 250 °C and 9.2 L/min, respectively. Thus, acetic acid of the mobile phase easily volatilized at this temperature. The nebulizer pressure was 40-PSI assuring good nebulization efficiency for the chromatographic conditions and the capillary voltage was 3 kV. The mass spectrometry collision gas was a N_2 of high purity (>99.9995). The fragmentor voltage was 120 V for ABZ, 100 V for ABZOX and 95 V for phenacetin. The collision energy was set at 20 eV for ABZ, 5 eV for ABZOX and 15 eV for phenacetin. After HPLC separation, peak area corresponding to the transition m/z 266 → 234 for ABZ and 282 → 240 for ABZOX was measured relative to that of transition m/z 180 → 110 of its IS (all dwell times were 200 ms) (Table 1).

2.5. Sample preparation

Sample preparation was carried out by solid phase extraction (SPE) using Bond Elut C-18, 50 mg (Agilent Technologies), with a vacuum pressure of about 3–5 mm Hg. The sample was applied, after preconditioning the cartridges with 1 mL MeOH followed by 1 mL Milli-Q water. For this, 200 μL of plasma was spiked with 10 μL of IS of 10 μg/mL and 790 μL of 1% orthophosphoric acid, pH 4.5 for one sample, but the IS has been calculated for more samples and pre-mixed with orthophosphoric acid for the general procedure. Then, a washing step with 1000 μL of 15% MeOH in water followed. Elution was carried out with 1000 μL of 100% MeOH plus 0.1% formic acid collected on a 1 mL 96-well plate. After extraction, samples were transferred to vials or they were directly read from the collection plate. Five microliters of the elute was directly injected into the LC/MS–MS.

2.6. Assay validation procedures

The validation of the method is based on the recommendations published online by the FDA [14] and EMA [15].

2.6.1. Calibration curve and lower limit of quantitation (LLOQ)

Quantitative analysis of ABZ and ABZOX in plasma was performed using phenacetin as IS. Eight calibration standards 5, 10, 20, 30, 50, 100, 500, and 1000 ng/mL for ABZ and eight 10, 20, 30, 50, 100, 500, 100, and 1500 ng/mL for ABZOX were used for full validation. A non-weighted linear regression model adjusted by least squares was used to calculate the equation relating the area ratio of ABZ and ABZOX versus IS, and the concentration of ABZ and ABZOX in the calibration standards. The standard curve was chosen to cover the range of clinically relevant concentrations of patients with parasitic infections. To validate the curve, at least 6 of 8 calibration standards should be less than 15% of the coefficient of variation (CV). For each point of the calibration curve the error of accuracy and CV should be less than 15% for all calibration standards, except for the LLOQ that was less than 20%. LLOQ response of the analyte should be at least 5 times higher than the blank response.

Table 1
Ions and fragmentation conditions used for multiple-reaction monitoring (MRM) mode.

Compound	Reaction monitored	Dwell time (s)	Fragmentor voltage (V)	Collision energy (eV)
ABZ	266 > 234	200	120	20
ABZOX	282 > 240	200	100	5
Phenacetin	180 > 110	200	95	15