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Original Article

Liquid chromatography–tandem mass spectrometry method for simultaneous determination of albendazole and albendazole sulfoxide in human plasma for bioequivalence studies ☆

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ABSTRACT

An improved high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed for sensitive and rapid determination of albendazole (ABZ) and its active metabolite, albendazole sulfoxide (ABZSO), in the positive ionization mode. The method utilized solid phase extraction (SPE) for sample preparation of the analytes and their deuterated internal standards (ISs) from 100 μL human plasma. The chromatography was carried out on Hypurity C_{18} column using acetonitrile-2.0 mM ammonium acetate, pH 5.0 (80:20, v/v) as the mobile phase. The assay exhibited a linear response over the concentration range of 0.200–50.0 ng/mL for ABZ and 3.00–600 ng/mL for ABZSO. The recoveries of the analytes and ISs ranged from 86.03%–89.66% and 89.85%–98.94%, respectively. Matrix effect, expressed as IS-normalized matrix factors, ranged from 0.985 to 1.042 for the both analytes. The method was successfully applied for two separate studies in healthy subjects using single dose of 400 mg conventional tablets and 400 mg chewable ABZ tablets, respectively.

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1. Introduction

Albendazole (ABZ), a benzimidazole derivative, is characterized as a broad spectrum anthelmintic agent and has shown good efficacy in the treatment of echinococcosis, hydatid cysts and neurocysticercosis [1]. It is a hydrophobic drug; therefore, it is poorly absorbed from the gastrointestinal tract. Further, ABZ is inconsistently absorbed from the intestine, which is dependent on the type of food and pH of the stomach [2]. Due to this reason, several clinical studies have reported significant inter-individual variability to it as a result of low aqueous solubility [3]. After oral administration, ABZ undergoes rapid hepatic oxidation by liver microsomal enzymes to its major pharmacologically active metabolite, albendazole sulfoxide (ABZSO) which is responsible for anthelmintic as well as toxic effects. ABZSO is approximately 70% bound with plasma protein and is widely distributed throughout the body. It can be detected in urine, cerebrospinal fluid, liver, bile, cyst wall and cyst fluid. ABZSO is further transformed into albendazole sulfone ($ABZSO_2$), which does not possess any anthelmintic activity [4]. Due to extensive metabolism, the plasma concentration of ABZ is found to be very low. Nevertheless, the pharmacokinetic properties of ABZ have been determined by measuring its plasma concentration in healthy subjects, patients and different animal species. Moreover, the plasma concentration of ABZSO is observed to increase in a dose-dependent manner following ingestion of fatty food [4,5].

As evident from literature, the techniques of choice for the analysis of ABZ and/or its metabolites in biological samples include high performance liquid chromatography with UV [6–10], fluorescence [11–16] and mass spectrometry detection [17–23] apart from capillary electrophoresis [24]. Due to rapid conversion of ABZ into its metabolites, several methods report the determination of ABZSO and inactive metabolites like $ABZSO_2$ and albendazole 2-aminosulphone ($ABZASO_2$) as racemate or enantiomers in different biological matrices like human plasma [11,17], sheep plasma [9,14], human serum [12], sheep spermatozoa and seminal plasma [13], and bovine plasma [15]. Simultaneous analysis of ABZ and its metabolites is also addressed in different biological samples such as human plasma [7, 8, 18, 21, 24], ovine plasma [6], mouse plasma [10], muscle tissues [19], turkey plasma [16], rat

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plasma [20], silk worm *bombyx mori* hemolymph [22] and rabbit plasma [23]. Only two methods are reported for the simultaneous determination of ABZ and ABZSO in human plasma by liquid chromatography–tandem mass spectrometry (LC–MS/MS) method [18,21]. Chen et al. [18] developed an assay with the linear concentration range of 0.4–200 ng/mL for ABZ and 4.0–2000 ng/mL for ABZSO using liquid–liquid extraction (LLE). The method required 500 μL plasma samples and the analysis time was 5.0 min. The other reported method was more rapid (4.0 min) and required lower plasma volume (200 mL) for processing, but it was less sensitive (ABZ: 5 ng/mL and ABZSO: 10 ng/mL) [21]. Both the methods were applied to a pharmacokinetic study in healthy subjects using 400 mg ABZ.

The aim of the present study was to develop a sensitive, selective and rapid LC–MS/MS method for the simultaneous determination of ABZ and ABZSO in human plasma for clinical studies. The proposed method is practically free from matrix interference and is successfully applied for bioequivalence studies in healthy subjects with 400 mg conventional tablets and 400 mg (2 × 200 mg) chewable tablets, respectively.

2. Experimental

2.1. Chemicals and materials

Albendazole (ABZ, purity: 99.6%) and albendazole sulfoxide (ABZSO, purity: 99.99%) were purchased from Vivan Life Sciences Pvt. Ltd. (Mumbai, India). Albendazole-d3 (ABZ-d3, purity: 99.30%) was procured from Clearsynth Labs Limited (Mumbai, India) while albendazole sulfoxide-d5 (ABZSO-d5, purity: 99.30%) was obtained from Syncom (Mumbai, India). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were procured from J.T. Baker Inc. (Phillipsburg, NJ, USA). Bioultra grade ammonium acetate (AA) and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solid phase extraction (SPE) cartridges, Strata™-X (30 mg/1.0 mL), were purchased from Phenomenex India (Hyderabad, India). Water was purified using Milli-Q water purification system from Millipore (Bangalore, India). Blank human blood was collected with Na heparin as anticoagulant from healthy and drug free volunteers. Plasma was separated by centrifugation at 2061 *g* at 10 °C and stored at −70 °C.

2.2. Liquid chromatography (LC) and mass spectrometry (MS) operating conditions

A Shimadzu HPLC system (Kyoto, Japan) with a Hypurity C_{18} (50 mm × 4.6 mm, 5 μm) column from Thermo Scientific (Cheshire, UK) was used for chromatographic separation of the analytes. The column temperature was maintained at 40 °C. The mobile phase consisted of ACN and 2.0 mM AA in water (pH 5.0, adjusted with acetic acid in 80:20 (v/v)). For isocratic elution, the flow rate of the mobile phase was set at 0.5 mL/min. The autosampler temperature was maintained at 5 °C, injection volume was kept at 2 μL, and the pressure of the system was maintained at 450 psi. The LC system was connected to a triple quadrupole mass spectrometer MDS SCIEX API-5500 (Toronto, Canada), equipped with electrospray ionization (ESI) and operated in positive ionization mode. The optimized source parameters for the analytes and internal standards (ISs, including ABZ-d3 and ABZSO-d5) were set as follows: ion spray voltage, 2500 V; curtain gas, 43 psi; Gas 1, 50 psi; Gas 2, 60 psi; turbo heater temperature, 450 °C; collision activation dissociation, 7 psi. The compound dependent mass parameters and multiple reaction monitoring (MRM) transitions used for quantitation of analytes and ISs are summarized in Table 1. Analyst classic software version 1.5.2 was used to control all parameters of LC and MS.

2.3. Calibration standards and quality control samples

Stock solutions of ABZ (0.2 mg/mL) and ABZSO (1.0 mg/mL) were prepared by dissolving accurately weighed amounts in methanol. Calibration standards (CSs) and quality control (QC) samples were made by spiking blank plasma with appropriate volumes of working solutions prepared from intermediate stock solutions for both the analytes. The final CS concentrations were 0.200, 0.400, 1.20, 5.00, 10.00, 20.0, 30.0, 42.5 and 50.0 ng/mL for ABZ; 3.00, 6.00, 18.0, 60.0, 120, 240, 360, 510 and 600 ng/mL for ABZSO, respectively. The QC samples were prepared at five levels, i.e., 0.200/3.00 ng/mL (lower limit of quantification quality control (LLOQ QC)), 0.600/9.00 ng/mL (low quality control (LQC)), 6.50/78.0 ng/mL (medium quality control-1 (MQC-1)) , 16.0/192 ng/mL (medium quality control-2 (MQC-2)) and 40.0/480 ng/mL (high quality control (HQC)) for ABZ/ABZSO, respectively.

Separate stock solutions (20.0 μg/mL for ABZ-d3 and 100.0 μg/mL for ABZSO-d5) of ISs were prepared by dissolving accurately weighed amounts in methanol. Their combined working solution (ABZ-d3: 10.00 ng/mL and ABZSO-d5: 500 ng/mL) was prepared from their stock solutions in methanol:water (60:40, v/v). Standard stock and working solutions used for spiking were stored at 2–8 °C until use, while CSs and QC samples in plasma were kept at −70 °C.

2.4. Extraction procedure

Prior to extraction, all frozen subject samples, CSs and QC samples were thawed and allowed to equilibrate at room temperature. An aliquot of 100 μL of spiked plasma sample/ subject sample was fortified with 25 μL of combined working solution of ISs and vortexed for 10 s. Further, 100 μL of 2 mM AA solution in water was added, vortexed for another 60 s and centrifuged at 13148 *g* for 5 min at 10 °C. Extraction of analytes and ISs was performed on Phenomenex Strata™-X (30 mg/mL) cartridges which were pre-treated sequentially with 1.0 mL of methanol (MeOH) and 1.0 mL of water. The plasma matrix was drained from the cartridges by applying positive nitrogen pressure. The cartridges were washed twice with 1.0 mL of 10% (v/v) MeOH in water, and the analytes and ISs were eluted with 1 mL of mobile phase. The samples were briefly vortexed and 2 μL of the eluent was used for injection into the chromatographic system.

Table 1
Optimized mass parameters and MRM transitions for albendazole, albendazole sulfoxide and their internal standards.

Analytes	Quadrupole 1 mass (amu)	Quadrupole 2 mass (amu)	Dwell time (ms)	Declustering potential (V)	Entrance potential (V)	Collision energy (eV)	Collision cell exit potential (V)
Albendazole	266.1	234.1	200	70.00	10.00	27.00	16.00
Albendazole sulfoxide	282.1	240.0	200	85.00	10.00	18.00	17.00
Albendazole-d3	269.1	234.1	200	70.00	10.00	27.00	16.00
Albendazole sulfoxide-d5	287.1	241.1	200	80.00	10.00	18.00	15.00

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