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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 ☆

Dhiraj M. Rathod [a,b], Keyur R. Patel [b], Hiren N. Mistri [b], Arvind G. Jangid [b], Pranav S. Shrivastav [c,*], Mallika Sanyal [d]

[a] Department of Chemistry, Kadi Sarva Vishwavidyalaya, Gandhinagar, Ahmedabad 382015, India
[b] Bioanalytical Research Department, Accutest Research Lab, Bodakdev, Ahmedabad 380059, India
[c] Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad 380009, India
[d] Department of Chemistry, St. Xavier's College, Navrangpura, Ahmedabad 380009, India

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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.

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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* Corresponding author.
E-mail address: pranav_shrivastav@yahoo.com (P.S. Shrivastav).

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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

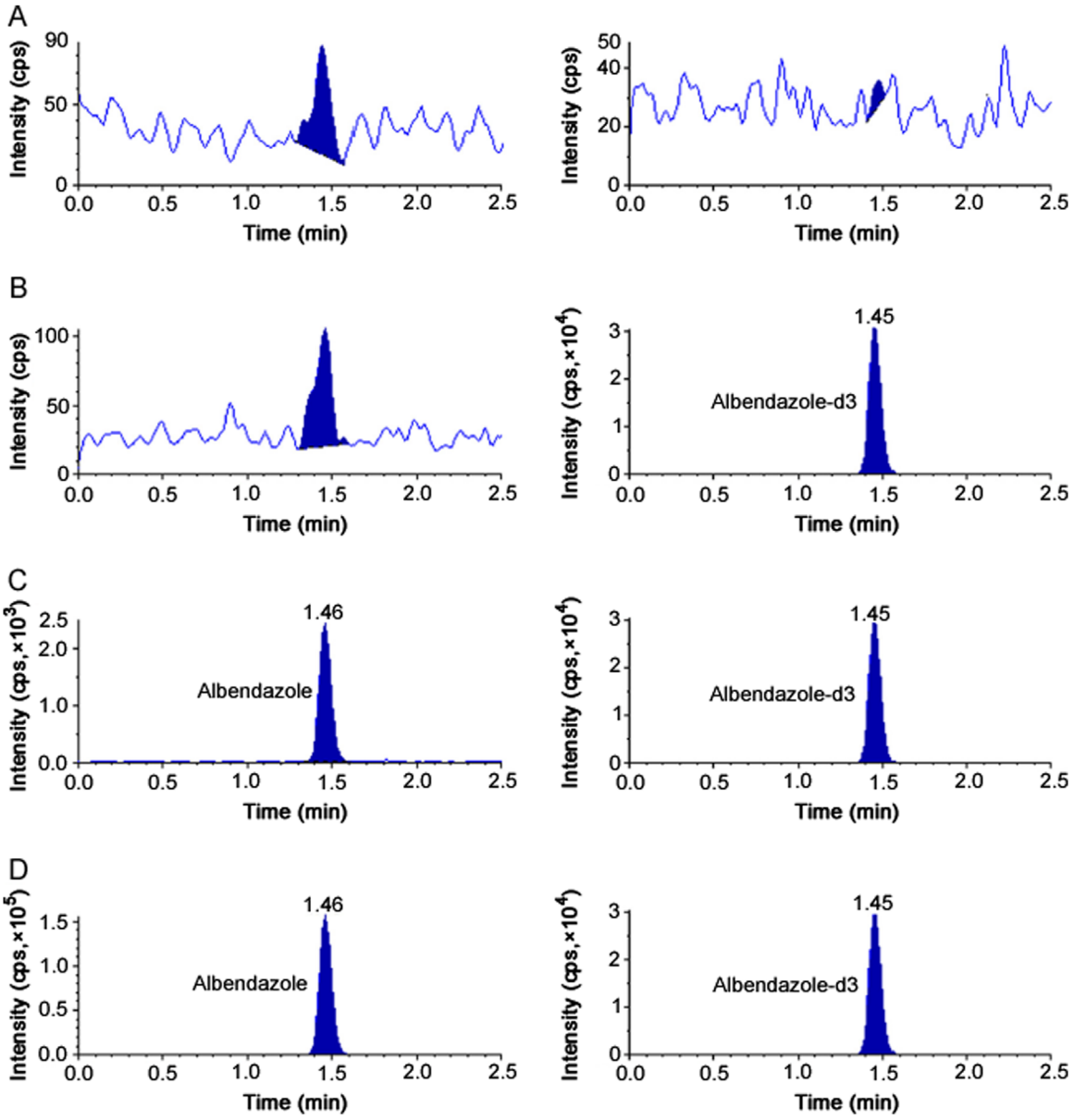

Fig. 1. Representative MRM chromatograms of albendazole in (A) double blank plasma (without analyte and IS), (B) blank plasma with working solution of IS, (C) LLOQ sample and IS, and (D) subject sample at C_{max} and IS after oral administration of 400 mg of conventional albendazole tablet.

2.5. Validation procedures

The validation of the method was in accordance with the United States Food and Drug Administration (USFDA) guidelines [25] and was performed as discussed in our previous report [26]. The detailed procedures and their acceptance criteria are briefed in Supplementary material.

2.6. Bioequivalence study and incurred sample reanalysis (ISR)

The study design was an open label, randomized, balanced, crossover, two-treatment, two-period and two-sequence bioequivalence design between a single dose of (i) 400 mg of albendazole conventional tablets (Generic Company, India) with a reference tablet formulation, ALBENZA® (Albendazole, 2×200 mg) from Amedra Pharmaceuticals LLC, USA and (ii) 2×200 mg of albendazole chewable tablets (Generic Company, India) with a corresponding reference product, ALBENZA® (Albendazole, 2×200 mg) chewable tablet from GlaxoSmithKline, Mississauga, Ontario, Canada in 51 healthy adult Indian subjects under fasting. The subjects were informed about the objectives and a written consent was given. The work was subject to review by an independent ethics committee constituted as per Indian Council of Medical Research (ICMR), India, which approved the study protocol. Both the studies were accomplished following International Conference on Harmonization, E6 Good Clinical Practice guidelines [27]. The subjects were orally administered with a single dose of test and reference formulation with 240 mL of water after a recommended wash out period of 7 days. Blood samples were collected at 0.00 (pre-dose), 0.50, 1.00, 1.50, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.50, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00 and 72.00 h after oral administration of test and reference formulations in labeled Na heparin-vacuettes. Plasma was separated by centrifugation and kept frozen at -70 °C until analysis. During study, subjects had a standard diet while water intake was unmonitored. The pharmacokinetic parameters of ABZ and ABZSO were estimated using SAS software version 9.2.

The assay reproducibility was checked by reanalyzing of 168

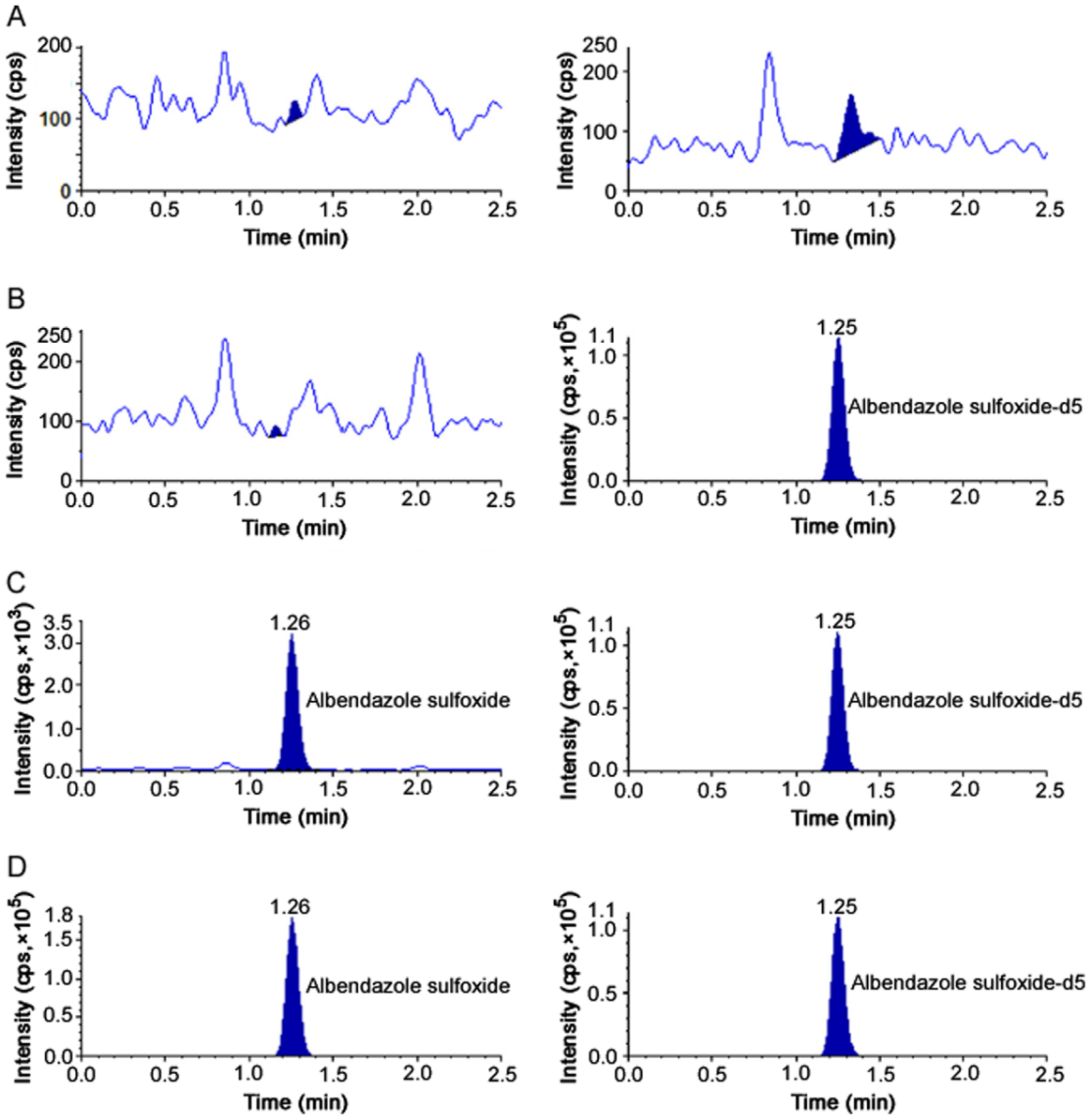

Fig. 2. Representative MRM chromatograms of albendazole sulfoxide in (A) double blank plasma (without analyte and IS), (B) blank plasma with working solution of IS (C) LLOQ sample and IS, and (D) subject sample at C_{max} and IS after oral administration of 400 mg of conventional albendazole tablet.

incurred samples near the maximum plasma concentration (C_{max}) and the elimination phase in the pharmacokinetic profile of the drug from each study. The results were compared with initial pharmacokinetic analysis using the same procedure. As per the acceptance criteria at least two-thirds of the original results and repeat results should be within 20% of each other [28].

3. Results and discussion

3.1. LC–MS/MS method development

During method development, the ionization of the both analytes and ISs was accomplished in the positive ESI mode as the both drugs are basic in nature due to the presence of secondary amino groups. The full scan Q1 MS spectra for ABZ, ABZSO, ABZ-d3 and ABZSO-d5 predominantly contained protonated precursor $[M+H]^+$ ions at *m/z* 266.1, 282.1, 269.1 and 287.1, respectively under the optimized chromatographic conditions. Fragmentation was initiated using sufficient nitrogen for collision activated dissociation to obtain major product ions at *m/z* 234.1, 240.0, 234.1 and 241.1, respectively by applying 27 eV collision energy (CE) to break the precursor ions of ABZ and ABZ-d3, and 18 eV CE for ABZSO and ABZSO-d5 ions, respectively. The identical product ion (*m/z* 234.1) formed for ABZ and ABZ-d3 can be ascribed to the neutral loss of MeOH and deuterated MeOH, respectively. However, the fragment ion at *m/z* 240.0 for ABZSO was different from that at *m/z* 241.1 for ABZSO-d5 by one mass unit (Supplementary Fig. 1). Nonetheless the both the fragments were attributed to the neutral loss of propylene from the precursor ions via cleavage of carbon-sulfur bond. Similar observation was also reported by Zhang et al. [19] using ABZSO-d3 as the IS. Further, in addition to the quantifier ion, one qualifier ion was also selected for unambiguous proof of the analytes. For mass spectral identity, a qualifying transition of *m/z* 266.1 → 191.1 for ABZ and *m/z* 282.1 → 222.0 for ABZSO was also monitored. Cross talk, a memory effect of

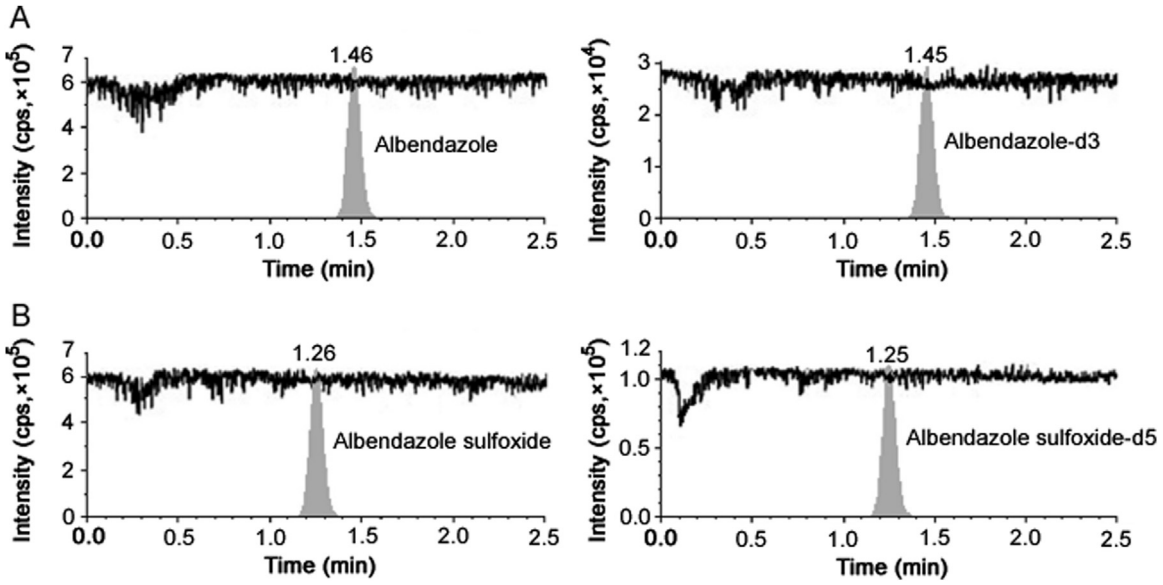

Fig. 3. Post column analyte infusion MRM chromatograms of (A) albendazole and albendazole-d3 and (B) albendazole sulfoxide and albendazole sulfoxide-d5 at ULOQ concentration.

the collision cell, due to contributions of ions of identical mass to other precursor-product ion channels, was also studied. The results of the cross talk experiment showed no measurable change in the peak area of ABZ and its IS which share an identical product ion at their respective MRMs.

Chromatographic conditions were optimized to achieve a short runtime, adequate retention, acceptable peak shapes and baseline separation of the drugs. Due to difference in the hydrophobic character of ABZ and ABZSO, three different columns, namely Hypurity C_{18}, Hypurity C_8 and Hypurity Advance, having identical dimensions (50 mm × 4.6 mm, 5 μm) and pore size (190 Å) but different carbon loading of 13%, 8% and 10%, respectively, were tested. Mobile phase composition of MeOH/ACN and AA buffer (1.0–8.0 mM) was optimized by varying the flow rate from 0.30 to 1.0 mL/min and changing the pH of the buffer from 3.0 to 6.0. Although the baseline separation of analytes was possible on all three columns, the peak shape was not acceptable on Hypurity C_8 and Hypurity Advance columns. Moreover, the response of ABZSO was not adequate on Hypurity Advance column, while a small tailing was observed for ABZ. Thus, different composition of the organic diluents and the aqueous part (90:10, 85:15, 80:20, 70:30 and 60:40, v/v) were tested on Hypurity C_{18} column. Finally, the best separation for the analytes with adequate response and better peak shape was achieved with ACN and 2.0 mM ammonium acetate (pH 5.0, adjusted with acetic acid) in 80:20 (v/v) as the mobile phase at a flow rate of 0.5 mL/min. Under the optimized conditions, the analytes were baseline-resolved with a resolution factor of 1.1 within 2.5 min. The representative chromatograms in Figs. 1 and 2 show no interference of endogenous compounds at the retention time of ABZ and ABZSO for samples spiked at LLOQ concentration and subject samples at C_{max}. Further, the reproducibility (%CV) in the measurement of retention time for the analytes was ≤0.5% for 90 injections. There was no interference of commonly used medications by subjects at the retention time of the analytes. Post column infusion chromatograms further substantiate the absence of matrix effects with no signal enhancement or suppression at the retention time of ABZ, ABZSO and their deuterated analogs (Fig. 3).

Several methods have utilized LLE for the simultaneous determination of ABZ and its metabolites [18,19,22]. Mainly mixed solvent systems have been used due to the difference in the ionization constants (basic nitrogen, pKa ~2.8 (ABZ) and ~3.5 (ABZSO) and acidic nitrogen, pKa ~10.3 (ABZ) and ~9.8 (ABZSO)) and lipophilicity of ABZ (log *P* 3.14) and ABZSO (log *P* 0.97) [9]. Initially, protein precipitation was tried using 10% tetrahydrofuran in ACN as reported by Sharma et al. [20]. However, the recovery was inconsistent, mainly at the LLOQ and LQC concentration of the analytes. Thereafter, LLE was initiated using binary and ternary combination of *n*-hexane, methyl *tert*-butyl ether, ethyl acetate and dichloromethane. In all the solvent mixtures, the recovery found was in the range of 62%–78% for both the analytes. Noticeably, in few of the samples, the extracts became slightly turbid after about 30 min at room temperature. Thus, SPE was tested on different extraction cartridges like Phenomenex Strata-X, Oasis HLB and Lichrosep DVB HL. Under identical conditions of washing and analyte elution, Phenomenex Strata-X provided superior recovery for both the analytes in comparison to the other two cartridges and hence was selected in the present work. Further, it was observed that initial dilution of the plasma sample (100 μL) with 100 μL of 2 mM AA solution was necessary to get adequate response and consistency in the recovery of ABZSO. In the absence of AA solution, there was inconsistent retention of ABZSO, mainly at higher concentration. The role of washing solution (10%, 20%, 30% and 40% MeOH in water) was also critically examined and it was found that 10% MeOH gave highly consistent and quantitative recoveries for both the analytes at five QC levels. With higher proportion of methanol, there was some loss in the recovery of ABZSO. The extracts thus obtained were directly used for injection into the chromatographic system without drying and reconstitution unlike previous study employing SPE [7].

A comparative assessment of all LC–MS/MS based methods for the simultaneous determination of ABZ and ABZSO is summarized in Table 2. The present method is highly sensitive and rapid compared to all existing methods for ABZ and ABZSO in different biological fluids [18,20–22] except one method [19]. However, that method was focused on the analysis of ABZ and its metabolites from fish tissue samples (2.0 *g*). Another important aspect of the newly developed method was the use of very low human plasma volume for processing and much shorter analysis time compared to these methods.

Table 2
Comparative assessment summary of liquid chromatography–tandem mass spectrometry methods developed for the simultaneous determination of albendazole and its metabolites in biological matrices.

Serial No.	Extraction procedure; sample volume; internal standard	Mean analyte recovery (%)	Linear range (ng/mL)	Retention time (min); run time (min)	Application	Ref.
1	LLE with 3 mL *n*-hexane-DCM-isopropanol; 500 μL human plasma; estazolam	77.6 for ABZ and 56.1 for ABZSO	0.4–200 for ABZ and 4.0–2000 for ABZSO	3.61 for ABZ and 3.26 for ABZSO; 5.0	Pharmacokinetic studies with 400 mg ABZ tablet to 20 healthy subjects	[18]
2[a]	LLE with 15 mL ethyl acetate; 2.0 g fish muscle tissue; deuterated analogs for all the analytes	100.1 for ABZ and 106.9 for ABZSO	0.1–20 for ABZ and ABZSO	2.29 for ABZ and 1.72 for ABZSO; 4.0	Analysis of ABZ and its three metabolites in 60 fish tissue samples	[19]
3	PP with 400 μL 10% THF in acetonitrile; 50 μL rat plasma; phenacetin	85.3 for ABZ and 85.4 for ABZSO	2.01–2007 for ABZ and 6.02–6020 for ABZSO	1.66 for ABZ and 1.50 for ABZSO; 3.5	Pharmacokinetic analysis with 30 mg/kg ABZ to 4 male Sprague-Dawley rats	[20]
4	SPE with Bond Elut C-18, (50 mg) cartridges; 200 μL human plasma; phenacetin	109.3 for ABZ and 93.5 for ABZSO	5.0–1000 for ABZ and 10–1500 for ABZSO	2.87 for ABZ and 2.16 for ABZSO; 4.0	Pharmacokinetic studies with 400 mg ABZ tablet to 12 healthy subjects	[21]
5[a]	LLE with 5 mL ethyl acetate-acetonitrile followed by ultra-filtration; 1.0 mL silkworm hemolymph; sample homogenate	98.7 for ABZ and 90.4 for ABZSO	LOQ of 1.32 for ABZ and 16.67 for ABZSO	2.45 for ABZ and 2.01 for ABZSO; 8.0	Analysis of ABZ and its three metabolites in silkworm hemolymph	[22]
6	SPE with Strata-X (30 mg/1.0 mL) cartridges; 100 μL human plasma; deuterated analogs for both the analytes	87.9 for ABZ and 88.2 for ABZSO	0.200–50 for ABZ and 3.0-600 for ABZSO	1.46 for ABZ and 1.26 for ABZSO; 2.5	Bioequivalence study with 400 mg ABZ conventional tablet and 400 mg ABZ chewable tablet to 51 healthy subjects	PM

[a] Along with albendazole sulfone and albendazole 2-aminosulfone; LLE: liquid–liquid extraction; SPE: solid phase extraction; PP: protein precipitation; ABZ: albendazole; ABZSO: albendazole sulfoxide; DCM: dichloromethane; THF: tetrahydrofuran; LOQ: limit of quantitation; PM: present method

Table 3
Extraction recovery for albendazole and albendazole sulfoxide.

Quality control level	Area response ($n=6$)		Extraction recovery (%)
	Pre-extraction spiking	Post-extraction spiking	
Albendazole			
LQC	33840	38328	88.29
MQC-1	361753	415092	87.15
MQC-2	909848	1021957	89.03
HQC	2226462	2543946	87.52
Albendazole sulfoxide			
LQC	26465	30762	86.03
MQC-1	236036	266707	88.50
MQC-2	582621	656099	88.80
HQC	1620905	1807835	89.66
Albendazole-d3			
LQC	275014	277960	98.94
MQC-1	259290	269897	96.07
MQC-2	271688	276274	98.34
HQC	256789	265251	96.81
Albendazole sulfoxide-d5			
LQC	302504	322396	93.83
MQC-1	284407	312604	90.98
MQC-2	288423	314803	91.62
HQC	310593	345679	89.85

Extraction recovery: pre-extraction spiking/post-extraction spiking.

3.2. Method validation results

The column and autosampler carryover experiment showed minimal carryover in blank plasma ($\leq 0.56\%$ of LLOQ sample for ABZ and ABZSO) after subsequent injection of upper limit of quantitation (ULOQ) sample under the optimized conditions. ABZ and ABZSO gave a linear response ($r^2 \geq 0.9976$) for the established concentration range of 0.200-50 ng/mL and 3.00–600 ng/mL, respectively. The mean linear equations for calibration curve concentrations were $y=(0.40100 \pm 0.01229)x+(0.00247 \pm 0.00227)$ and $y=(0.00940 \pm 0.00010)x+(0.00233 \pm 0.00134)$ for ABZ and ABZSO respectively. According to the linear regression model used with $1/x^2$ weighting, the accuracy of the back calculated concentration ranged from 91.93% to 104.87% and the precision (%CV) varied from 0.11% to 3.52% for both the analytes. The signal-to-noise ratio was ≥ 28 at the LLOQ concentration for ABZ and ABZSO.

The intra and inter-day precision ranged from 1.11% to 6.64% and the accuracy varied from 95.40% to 105.59% for the analytes (Supplementary Table 1). The mean relative recoveries determined by comparing the absolute signal of blank plasma samples spiked before and after extraction at four QC levels were 88.00% and 88.25% for ABZ and ABZSO, respectively. Similarly, the mean recoveries for ABZ-d3 and ABZSO-d5 were 97.54% and 91.57%, respectively (Table 3). Absolute matrix effects expressed as matrix factors at LQC and HQC levels are given in Table 4. The IS-normalized matrix factors ranged from 0.985 to 1.042 for the both analytes. Relative matrix effect was also evaluated at LQC and HQC levels in Na-heparin plasma, lipemic and haemolysed plasma sources. The precision (%CV) in the measurement of ABZ and ABZSO concentration at these levels ranged from 1.15% to 2.17% for the both analytes as shown in Supplementary Table 2.

The stock solutions of the analytes and ISs in MeOH kept for short-term (at 25 °C) and long-term (at 5 °C) stability tests were found unchanged up to 17 h and at least 13 days, respectively. The bench-top stability of the analytes in plasma was established up to 16 h. Both ABZ and ABZSO were stable (within $\pm 6\%$ of initial concentration) during five freeze-thaw cycles at −70 °C and for at least 50 h in the autosampler. Processed sample stability of the

Table 4
Matrix factors for albendazole and albendazole sulfoxide in human plasma ($n=6$).

Analyte	Mean area response				Matrix factor		IS-normalized matrix factor	
	Post-extraction spiking		Neat samples in mobile phase					
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
Albendazole	38328	2543946	41235	2775184	0.930	0.917	1.009	0.986
Albendazole-d3	277960	265251	301444	285178	0.922	0.930	–	–
Albendazole sulfoxide	30762	1807835	32077	1843428	0.959	0.981	1.042	0.985
Albendazole sulfoxide-d5	322396	345679	350468	347008	0.920	0.996	–	–

Matrix factor: post-extraction spiking/neat samples in mobile phase.

Table 5
Stability results for albendazole and albendazole sulfoxide under different conditions ($n=6$).

Storage condition	QC level	Albendazole		Albendazole sulfoxide	
		Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
Bench-top stability; 16 h, 25 °C	LQC	103.21	1.04	104.23	2.44
	HQC	96.21	1.08	96.23	1.63
Freeze and thaw stability; 5 cycles, −70 °C	LQC	99.46	5.03	104.79	5.43
	HQC	93.94	3.92	94.01	3.20
Auto-sampler stability; 50 h, 5 °C	LQC	103.57	1.72	104.75	2.61
	HQC	96.31	1.54	95.34	2.05
Processed sample stability; 17 h, 25 °C	LQC	103.39	3.11	101.55	2.27
	HQC	97.17	1.07	96.01	1.11
Long-term stability in plasma; 139 days, −70 °C	LQC	105.00	4.76	102.45	3.11
	HQC	103.88	5.78	99.06	2.16

analytes was established up to 17 h at 25 °C. Spiked plasma samples stored at −70 °C for long-term stability experiment were found stable for a minimum period of 139 days. The detailed stability results are presented in Table 5.

The precision (%CV) and accuracy values to prove the ruggedness of the method with different columns and analysts ranged from 3.75% to 6.12% and 95.60% to 104.81%, respectively, for ABZ and ABZSO across five QC levels. Similarly, the %CV and accuracy in establishing the dilution reliability of 1/2th and 1/4th dilution varied from 2.86% to 4.19% and 92.8% to 102.7%, respectively, for the both analytes.

3.3. Application of the method and ISR results

To the best of our knowledge, there have been no reports on the pharmacokinetics of ABZ in healthy Indian subjects. The present study was undertaken to evaluate the pharmacokinetics of single dose of 400 mg of conventional ABZ tablet formulation and 400 mg (2 × 200 mg) of chewable ABZ tablet formulation. The mean profiles obtained for ABZ and ABZSO after oral administration of test and

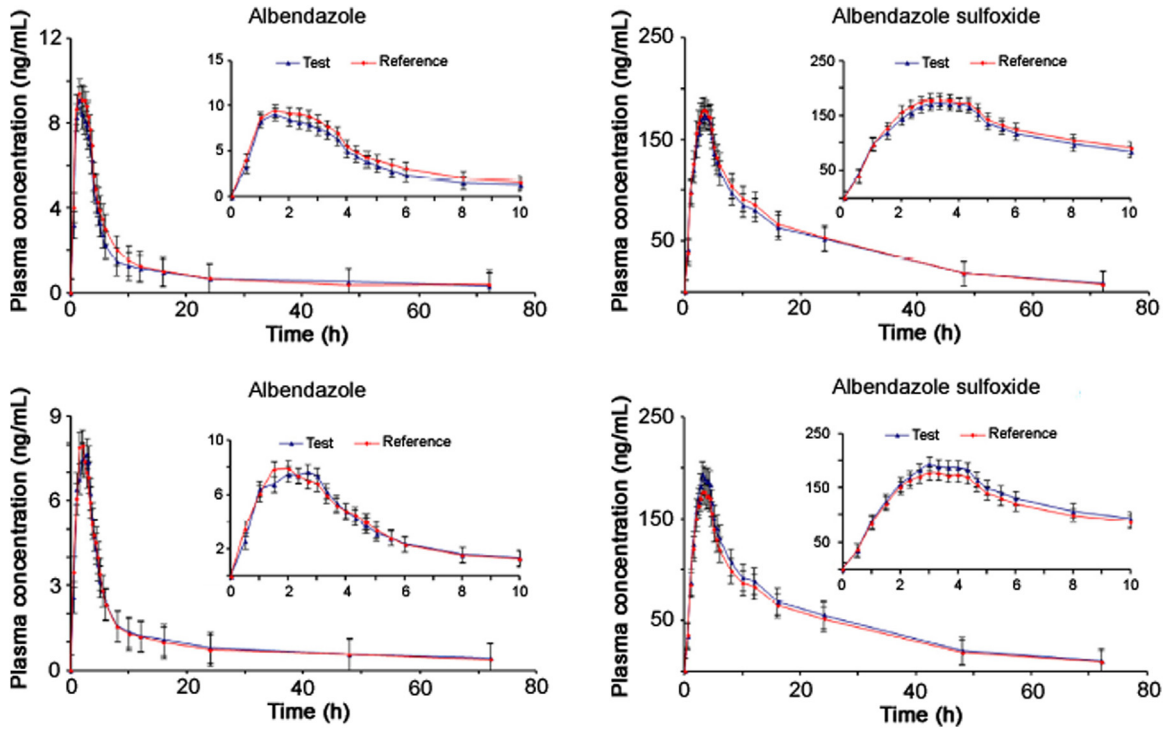

Fig. 4. Mean plasma concentration–time profiles of albendazole and albendazole sulfoxide after oral administration of (A) 400 mg of conventional albendazole tablet formulation and (B) 400 mg (2 × 200 mg) of chewable albendazole tablet formulations to 51 healthy Indian subjects.

Table 6
Pharmacokinetic parameters (mean ± SD) following oral administration of different albendazole tablet formulations to 51 healthy Indian subjects under fasting.

Parameter	Albendazole		Albendazole sulfoxide	
	Test	Reference	Test	Reference
Single 400 mg conventional tablet				
C_{max} (ng/mL)	11.14 ± 5.01	11.49 ± 4.43	193.50 ± 52.87	207.06 ± 59.55
T_{max} (h)	1.94 ± 0.82	2.07 ± 1.60	3.18 ± 1.45	3.13 ± 2.22
$t_{1/2}$ (h)	4.53 ± 1.24	5.13 ± 1.26	15.81 ± 4.63	17.03 ± 3.34
$AUC_{0\text{-}72h}$ (h ng/mL)	51.97 ± 38.44	53.43 ± 36.00	3049.71 ± 1721.07	3306.32 ± 1771.49
$AUC_{0\text{-}inf}$ (h ng/mL)	64.68 ± 41.84	67.61 ± 39.68	3474.95 ± 1776.46	3805.68 ± 2236.19
K_{el} (1/h)	0.149 ± 0.026	0.137 ± 0.043	0.049 ± 0.018	0.045 ± 0.015
2 × 200 mg chewable tablet formulation				
C_{max} (ng/mL)	9.55 ± 4.76	10.06 ± 5.04	198.19 ± 30.17	192.53 ± 27.87
T_{max} (h)	2.23 ± 1.26	2.19 ± 1.22	2.89 ± 0.99	3.00 ± 0.67
$t_{1/2}$ (h)	5.83 ± 2.07	5.68 ± 2.58	16.05 ± 5.01	15.98 ± 4.65
$AUC_{0\text{-}72h}$ (h ng/mL)	53.02 ± 25.01	60.22 ± 28.11	3203.80 ± 1647.21	3068.32 ± 1671.05
$AUC_{0\text{-}inf}$ (h ng/mL)	66.49 ± 6.46	75.38 ± 41.97	3501.30 ± 1662.43	3386.80 ± 1713.15
K_{el} (1/h)	0.116 ± 0.053	0.119 ± 0.034	0.050 ± 0.018	0.052 ± 0.020

reference formulations to 51 healthy Indian subjects are shown in Fig. 4. It was evident from the mean pharmacokinetic parameters that there was no significant difference between the conventional and chewable tablet formulations for the both analytes in any parameter (Table 6). However, except for the time point of maximum plasma concentration (T_{max}) values, there was little correlation between other parameters from the studies conducted in Chinese [18] or Caucasian healthy subjects [21] with identical dose strength. The C_{max} values obtained for ABZ and ABZSO were 96.4 and 635 ng/mL in Chinese subjects and 57.49 and 829.95 ng/mL in Caucasian subjects, respectively. It was observed that C_{max} for the both analytes in the Indian subjects was much lower than these values. Similar observation was noted for the area under the plasma concentration vs time curve from zero hour to 72 h ($AUC_{0–72h}$) values for ABZ and ABZSO. These significant differences in the pharmacokinetic parameters can be attributed to age, gender (body size and muscle mass) and ethnicity, which have clinically relevant effects. Nevertheless, the large inter-individual variation in the pharmacokinetic parameters observed is consistent with both these studies [18, 21]. Further, the reproducibility of the developed method was confirmed by reanalysis of 168 incurred samples for each study (total 336). The % change in the concentration of both the analytes was within ± 17% from the initial pharmacokinetic results for both the studies.

4. Conclusions

A new sensitive and rapid method has been developed for the simultaneous determination of ABZ and its active metabolite ABZSO in human plasma. Compared to the existing methods in plasma samples, the present method has the advantages of high throughput, sensitivity, and small plasma volume for processing. The method has shown adequate consistency to analyze ABZ and ABZSO in clinical samples with acceptable accuracy and precision. The method was extensively validated according to FDA guidelines and showed good reproducibility as evident from the results of incurred sample reanalysis. The optimized extraction procedure ensured clear samples for direct injection without drying and reconstitution steps. The recovery of the analytes was good with minimal matrix interference.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2016.02.002.

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