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Simultaneous determination of albendazole and its major active metabolite in human plasma using a sensitive and specific liquid chromatographic–tandem mass spectrometric method

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Abstract

A method for the simultaneous determination of albendazole (ABZ) and its major active metabolite albendazole sulfoxide (ABZ-SO) was developed and validated. The analytes were extracted from plasma samples by liquid–liquid extraction and analyzed using liquid chromatography−tandem mass spectrometry with an electrospray ionization interface. Estazolam was used as the internal standard. The assay was linear in the concentration range 0.4–200 ng/ml for ABZ and 4.0–2000 ng/ml for ABZ-SO. The intra- and inter-run precision (R.S.D.), calculated from quality control (QC) samples was less than 7.1 and 9.4% for ABZ and ABZ-SO, respectively. The accuracy as determined from QC samples was within ±3% for the analytes. Recoveries of ABZ and ABZ-SO were greater than 77 and 53%, respectively, over the calibration curve range. The method developed was successfully applied to pharmacokinetic studies of ABZ and ABZ-SO after an oral dose of 400 mg albendazole to healthy volunteers.

Keywords: Albendazole; Albendazole sulfoxide; Electrospray; Liquid chromatography−tandem mass spectrometry; Plasma sample; Pharmacokinetics

1. Introduction

Albendazole (ABZ, Fig. 1) is a benzimidazole carbamate anthelmintic active against most nematodes and some cestodes. It is used in the treatment of intestinal and tissue nematode infections and in higher doses in the treatment of echinococcosis [1,2]. Following oral administration, ABZ undergoes extensive metabolism to its major active metabolite, albendazole sulfoxide (ABZ-SO, Fig. 1). This metabolite is further transformed to an inactive metabolite, albendazole sulfone (ABZ-SO_2) [3]. Due to extensive metabolism, plasma concentration of ABZ was very low after oral administration. Therefore, pharmacokinetics of ABZ and the bioavailability of ABZ formulations have been studied with regard to plasma concentrations of ABZ-SO and ABZ-SO_2 [4–8].

Many analytical methods including HPLC with UV detection or fluorescence detection, and LC−MS−MS [7–14] have been reported in the literature for the

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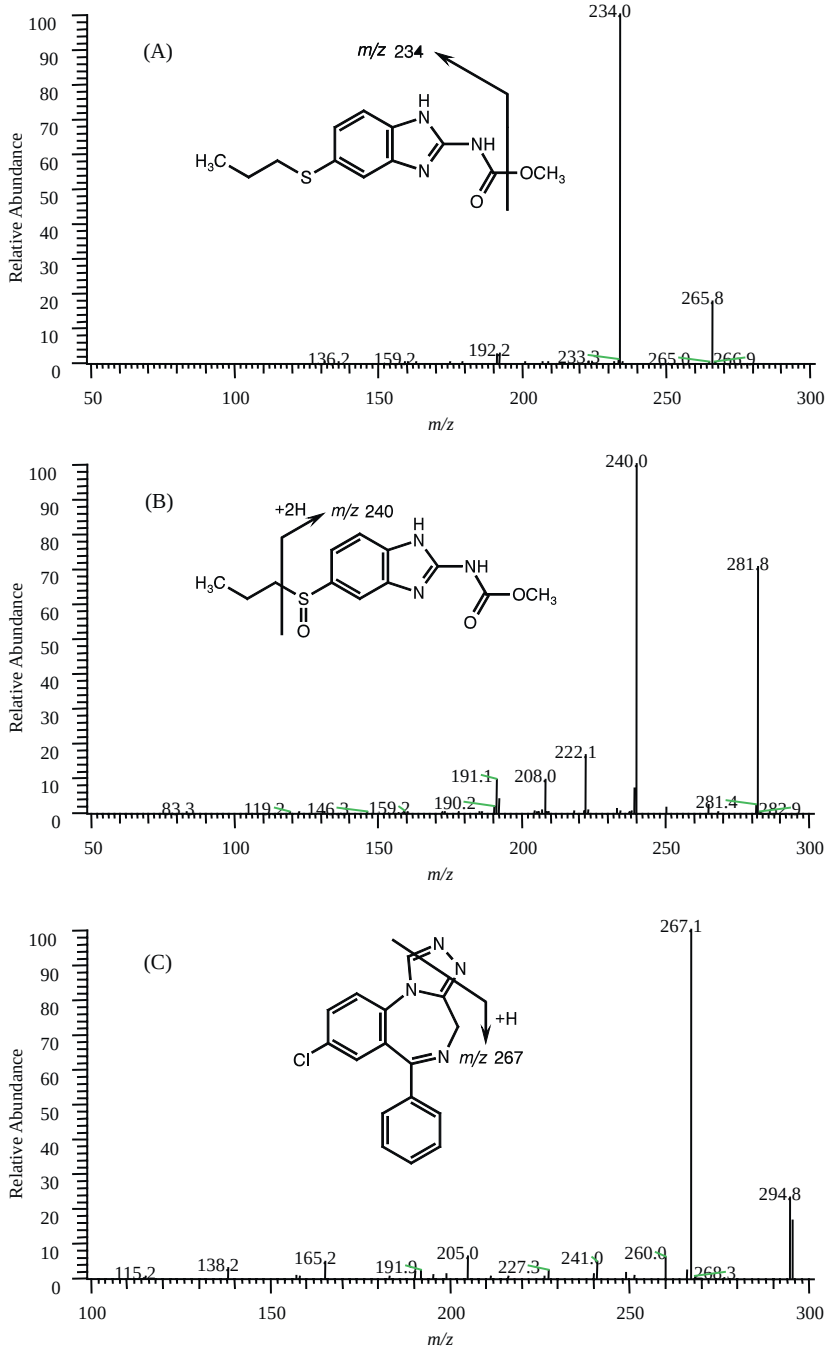

Fig. 1. Product ion mass spectra of $[M + H]^+$ of ABZ (A), ABZ-SO (B) and estazolam (C).

quantitative analysis of ABZ-SO and ABZ-SO_2 in plasma. Several methods [15–17] have been employed for the determination of ABZ and its major metabolite in plasma. But the sensitivity of these methods was not sufficient for the parent drug: the lower limit of quantitation was more than 10 ng/ml. Until now, no complete pharmacokinetic study has been reported for ABZ in humans. So a sensitive and reliable method for assay of ABZ seemed to be necessary.

In the present paper, a sensitive and specific LC–MS–MS method was developed and validated for the simultaneous determination of ABZ and its major active metabolite ABZ-SO in human plasma. This assay method was successfully applied to complete pharmacokinetic studies of ABZ and ABZ-SO after a single oral administration of 400 mg ABZ to 20 healthy volunteers.

2. Experimental

2.1. Materials

Albendazole was kindly supplied by Hanjiang Pharmaceutical Ltd. (Xinjiang, China) and purity was greater than 99.6%. Albendazole sulfoxide was synthesized in our laboratory with a purity of more than 99.1%. Estazolam (internal standard, IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile were of HPLC grade, and all other chemicals used were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. LC–MS–MS instrumentation and analytical conditions

A Shimadzu LC-10AD pump (Kyoto, Japan) was used for solvent delivery. Chromatography was performed on a Kromasil C_8 column (150 mm × 4.6 mm i.d., 5 μm, Aote, Tianjin, China) and a SecurityGuard C_{18} guard column (4 mm × 3.0 mm i.d., Phenomenex, Torrance, CA, USA). The column was maintained at 20 °C. A mobile phase of methanol–water–formic acid (80:20:1 (v/v/v)) at a flow-rate of 0.5 ml/min was employed.

A Finnigan TSQ (API II) triple quadrupole mass spectrometer (San Jose, CA, USA) was interfaced to the HPLC system using an electrospray ionization (ESI) source. The mass spectrometer was operated in the positive ion detection mode with the spray voltage set at 4.5 kV. Nitrogen was used as the sheath gas (80 p.s.i.) and auxiliary gas (3 l/min). The heated capillary temperature was set at 280 °C. For collision-induced dissociation (CID), argon was used as a collision gas at a pressure of 1.4 mTorr. Quantitation was performed using selected reaction monitoring (SRM) of the transitions of m/z 266 → 234 for ABZ, m/z 282 → 240 for ABZ-SO and m/z 295 → 267 for the IS, with a scan time of 0.3 s per transition. The collision energies of 20, 25, and 30 eV were used for ABZ, ABZ-SO, and IS, respectively. The peak widths of precursor and product ions were maintained at ∼0.8 U at half-height in the SRM mode.

2.3. Data acquisition

Data acquisition and analysis were performed using the Xcalibr 1.1 software (Finnigan, San Jose, CA, USA). Post-acquisition quantitative analyses were performed using LCQuan software (Finnigan, San Jose, CA, USA). The calibration curves were constructed from the peak area ratios of each analyte to the IS versus plasma concentrations using a $1/x^2$ weighted linear least-squares regression model. Concentrations of each analyte in quality control samples (QCs) or unknown samples were subsequently interpolated from these calibration curves.

2.4. Preparation of standard and quality control samples

The stock standard solutions of ABZ and ABZ-SO were prepared in methanol, resulting in final concentrations of 400 μg/ml for each analyte. The stock solutions were further diluted with the mobile phase to achieve standard working solutions at concentrations of 1000/10 000, 800/8000, 500/5000, 250/2500, 100/1000, 40/400, 15/150, 5/50, and 2/20 ng/ml for ABZ/ABZ-SO. Internal standard working solution (5000 ng/ml) was prepared by diluting the 400 μg/ml stock solution of estazolam with the mobile phase. All the working solutions were kept at 4 °C.

The standard solutions (100 μl) were used to spike blank plasma samples (0.5 ml), either for calibration curves of both analytes or for QCs in the prestudy validation and during the pharmacokinetic study.

2.5. Sample preparation

To a 0.5 ml aliquot of plasma sample, 100 μl of the mobile phase, 100 μl of the IS (5000 ng/ml estazolam in the mobile phase) and 0.5 ml of phosphate buffer (10 mM, pH 7.0) were added. The mixed samples were extracted with 3 ml of *n*-hexane−dichloromethane−isopropanol (100:50:5 (v/v/v)) by vortex mixing for 1 min and shaking for 10 min. After centrifugation at 2000 × *g* for 5 min, the upper organic layer was removed and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase. A 20 μl aliquot of the solution was injected onto the LC−MS−MS system.

2.6. Application to pharmacokinetic study

The LC−MS−MS procedure developed was used to investigate the plasma profile of ABZ and its active metabolite ABZ-SO after an oral administration of 400 mg ABZ tablet. A clinical study on 20 healthy male volunteers was approved by the Ethics Committee of the People's Hospital of Liaoning Province. Blood (4 ml) was removed by venepuncture prior to dosage and at 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, and 36 h thereafter. Following centrifugation (2000 × *g* for 10 min) the plasma was removed and stored at −20 °C until analysis.

The pharmacokinetic parameters of ABZ and ABZ-SO were calculated by non-compartmental analysis. The maximum plasma concentrations (C_{max}) and their time of occurrence (T_{max}) were both obtained directly from the measured data. The area under the plasma concentration−time curve from time zero to the time of the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method, the terminal elimination rate constant (k_e) was estimated by linear least-squares regression of the last four points of the plasma concentration−time curve and the corresponding elimination half-life ($T_{1/2}$) was then calculated as $0.693/k_e$.

3. Results and discussion

3.1. Sample preparation

Due to the differences of hydrophobic character between ABZ and ABZ-SO, it is difficult to obtain the identical extraction recoveries for ABZ and ABZ-SO. Owing to extensive metabolism, plasma concentrations of ABZ were very low compared with those of the active metabolite ABZ-SO after oral administration. Therefore, to improve the ABZ recovery from the plasma, many kinds of organic solvents including diethyl ether, ethyl acetate, dichloromethane, *n*-hexane, etc. were attempted in this experiment. It was found that ethyl ether and ethyl acetate yielded higher recovery only for ABZ-SO. However, the mixed solvent of *n*-hexane−dichloromethane−isopropanol (100:50:5 (v/v/v)) used in the experiment could provide not only high recovery for ABZ, but also reproducible recovery for ABZ-SO. The extraction yield was determined by comparing the peak area ratios of the analyte to the internal standard between the pre-extraction spike and the post-extraction spike of the analytes in human plasma, with the IS spiked post-extraction in both cases. The extraction recoveries of ABZ were 77.0 ± 4.1, 77.3 ± 3.3, and 78.6 ± 2.6% at the concentrations of 1, 20, and 160 ng/ml, respectively; and those of ABZ-SO were 53.2 ± 3.9, 56.7 ± 1.1, and 58.5 ± 2.3% at the concentrations of 10, 200, and 1600 ng/ml, respectively. The recovery at each concentration level was assessed on the six replicates. The average extraction recovery of IS was 81.7 ± 3.5%.

3.2. LC−MS−MS optimization

ABZ, ABZ-SO and estazolam (IS) were at first characterized by MS and MS−MS by flow injection analysis to ascertain their precursor ions and to select product ions for use in SRM, respectively. Full-scan Q1 mass spectra of ABZ and ABZ-SO in the positive-ion mode both formed protonated molecule $[M + H]^+$ at *m/z* 266 and 282, respectively, as the base peak ions under the reported chromatographic conditions. The $[M + H]^+$ ions were therefore, selected as the precursor ions to obtain their major fragment ions for SRM analysis. The most suitable collision energy for the analytes and IS was determined by observing the response of the obtained fragment ion peaks. Fig. 1

displays the product ion spectra of ABZ, ABZ-SO and the IS. A predominant fragment ion at *m/z* 234 for ABZ was formed when the collision energy was 20 eV and $[M + H]^+$ ion for ABZ was reduced by more than 90%. The fragment ion at *m/z* 234 was formed by loss of a neutral methanol from $[M + H]^+$ ion. The transition of *m/z* 266 → 234 was chosen in the SRM acquisition for ABZ. Whereas the collision behavior of $[M + H]^+$ of ABZ-SO is strongly dependent on the collision energy. A major fragment ion at *m/z* 240 was formed by addition of 20−25 eV of the collision energy (Fig. 1B), while $[M + H]^+$ ion was reduced by less than 50%. When higher collision energy was used, the intensity of $[M + H]^+$ ion was obviously reduced and the more fragment ions were formed, resulting in the weaker MS response. Thus, SRM was performed by monitoring the transition of $[M + H]^+ \rightarrow$ *m/z* 240 for ABZ-SO using 25 eV collision energy. The fragment ion at *m/z* 240 was attributed to neutral loss of propylene by breaking the single carbon–sulfur bond.

Using similar procedures the precursor ion of the internal standard was determined to be the base peak $[M + H]^+$ ion at *m/z* 295. The SRM transition was determined to be *m/z* 295 → 267 (Fig. 1C).

3.3. Method validation

3.3.1. Assay selectivity

The LC−MS−MS method has high specificity because only ions derived from the analytes of interest are monitored. Comparison the chromatograms of six independent plasma samples and spiked human plasma matrices indicated no significant interferences at the retention times of the analytes and IS (Fig. 2). The nominal retention times for ABZ, ABZ-SO, and IS were 3.6, 3.3, and 4.6 min, respectively.

Under the present LC−MS−MS conditions, another major metabolite, ABZ-SO_2 could be found in plasma samples from the volunteers receiving 400 mg ABZ using SRM of the transition *m/z* 298 → *m/z* 266. Because different SRM transitions were monitored for ABZ, ABZ-SO, and ABZ-SO_2, respectively, no interference was found for each other.

3.3.2. Linearity of calibration curves and lower limit of quantitation

During prestudy validation, the calibration curves were defined in three runs based on triplicate assays of the spiked plasma samples. Excellent linearity was obtained over the concentration range of 0.4−200 ng/ml for ABZ and 4.0−2000 ng/ml for ABZ-SO in human plasma. Typical equation of calibration curves were as follows:

ABZ	$y = -0.731 \times 10^{-4} + 7.945 \times 10^{-3}x$	$r = 0.9985$
ABZ-SO	$y = 1.889 \times 10^{-4} + 4.904 \times 10^{-3}x$	$r = 0.9979$

The lower limit of quantitation (LLOQ) for determination of ABZ and ABZ-SO in plasma, defined as the lowest concentration analyzed with accuracy within ±15% and a precision ≤15%, were 0.4 and 4.0 ng/ml, respectively. These limits are sufficient for clinical pharmacokinetic studies of ABZ and its major metabolite following an oral administration of ABZ.

3.3.3. Assay precision and accuracy

Intra- and inter-run precision was assessed from the results of QCs by using a one-way analysis of variance (ANOVA). The mean values and R.S.D. for QCs at three concentration levels were calculated over three validation runs. Six replicates of each QC level were determined in each run.

The accuracy of the method was determined by calculating the percentage deviation observed in the analysis of QCs and expressed as the relative error (R.E.).

Table 1 summarizes the intra- and inter-run precision and accuracy for ABZ and ABZ-SO from the QC samples. The intra-run precision was less than 7% for each QC level of ABZ and less than 8.5% for each QC level of ABZ-SO. The inter-run precision was less than 10% for ABZ and ABZ-SO. The accuracy derived from QC samples was within ±3% for each QC level of ABZ and ABZ-SO.

3.3.4. Analyte stability

The stability of ABZ and ABZ-SO in plasma and mobile phase were investigated. The analytes was found to be stable after three freeze-thaw cycles in plasma. The accuracy calculated from QC samples ranged from −6.8 to 9.5%. Analyte was also shown to be stable in human plasma at room temperature for at least 0.5 h (R.E. <8%) and in the reconstitution mobile phase for 24 h (RE <6.7%). Standard stock

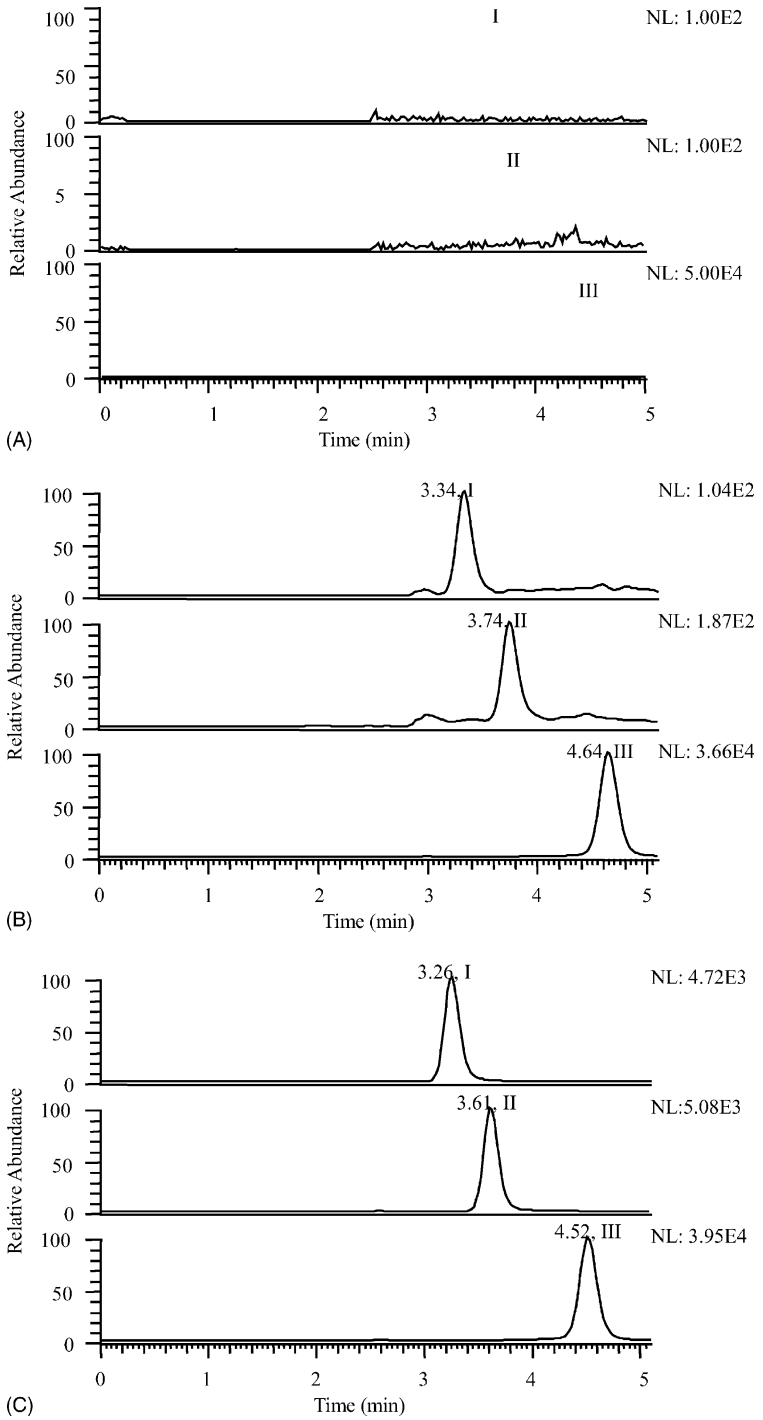

Fig. 2. Representative SRM chromatograms of ABZ-SO (I), ABZ (II) and IS (III) in human plasma. (A) A blank plasma sample, (B) a blank plasma sample spiked with ABZ and ABZ-SO at the lower limit of quantitation (0.4 and 4.0 ng/ml, respectively), and IS (1000 ng/ml), and (C) plasma sample from a volunteer 2 h after administration of 400 mg ABZ.

Table 1
Summary of precision and accuracy from QC samples of human plasma extracts ($n = 3$ days and six replicates per day)

Analyte	Added C (ng/ml)	Found C (ng/ml)	Intra-run R.S.D. (%)	Inter-run R.S.D. (%)	Relative error (%)
ABZ	1.00	0.99	6.7	7.1	−0.9
	20.0	20.2	5.5	5.2	0.8
	160.0	163.3	5.0	2.1	2.1
ABZ-SO	10.0	10.1	8.0	2.6	1.2
	200.0	204.4	6.9	9.4	2.2
	1600	1560	5.3	3.7	−2.5

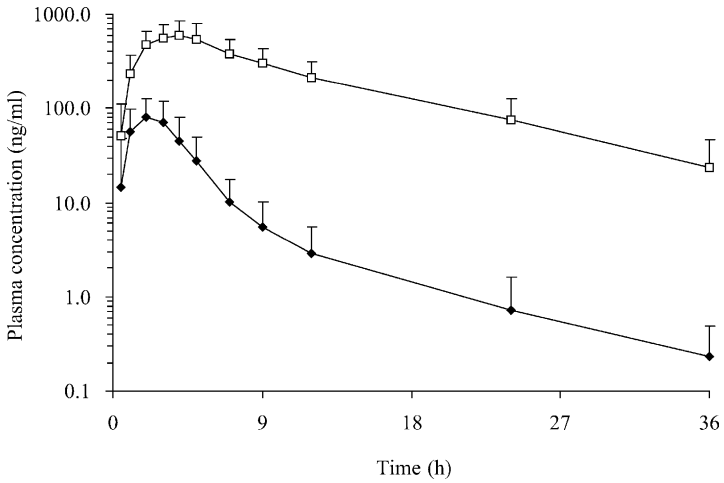

Fig. 3. Mean plasma concentration−time profile of ABZ (◆) and ABZ-SO (□) after an oral dose of 400 mg ABZ to 20 healthy volunteers.

solutions of ABZ and ABZ-SO were shown to remain stable for at least 20 days at 4 °C.

3.4. Application of the method to pharmacokinetic study

The LC−MS−MS method developed was used to investigate the pharmacokinetics of ABZ and its active metabolite ABZ-SO after an oral administration of 400 mg ABZ tablet to 20 healthy volunteers. The method with the LLOQ of 0.4 ng/ml for ABZ is more sensitive than the reported ones (LLOQ > 10 ng/ml) [15–17]. Therefore, it allows the determination of ABZ up to 36 h after an oral administration.

The mean plasma concentration−time curves of ABZ and ABZ-SO are presented in Fig. 3. The corresponding pharmacokinetic parameters (C_{max}, T_{max}, $T_{1/2}$, k_e, AUC_{0-36}, and $AUC_{0-\infty}$) are presented in Table 2.

Table 2
The main pharmacokinetic parameters of ABZ and ABZ-SO after an oral administrsation of 400 mg ABZ to 20 healthy volunteers (mean ± S.D.)

	ABZ	ABZ-SO
C_{max} (ng/ml)	96.4 ± 50.1	635 ± 229
T_{max} (h)	2.3 ± 1.1	3.6 ± 1.0
$T_{1/2}$ (h)	5.4 ± 2.6	7.2 ± 2.1
k_e (1/h)	0.18 ± 0.15	0.10 ± 0.03
AUC_{0-t} (ng × h/ml)	426 ± 203	6773 ± 2878
$AUC_{0-\infty}$ (ng × h/ml)	429 ± 204	7088 ± 3115

4. Conclusions

A sensitive, accurate and precise procedure based on LC−MS−MS has been developed and validated for the simultaneous determination of ABZ and ABZ-SO in human plasma with the LLOQ of 0.4 ng/ml for ABZ

and 4.0 ng/ml for ABZ-SO. It was proved superior in sensitivity than the previously reported HPLC methods. This method was used successfully to evaluate the pharmacokinetics of ABZ and ABZ-SO after an oral administration of ABZ.

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