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A high performance liquid chromatography method for simultaneous determination of albendazole metabolites in human serum

A. Mirfazaelian [a], S. Dadashzadeh [b], M.R. Rouini [a,*]

[a] *Biopharmaceutics Laboratory, Division of Pharmacokinetics, Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155/6451, Tehran, Iran*

[b] *Department of Pharmaceutics, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, P.O. Box 14155/6153, Tehran, Iran*

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Abstract

A simple assay for albendazole (ABZ) main metabolites—albendazole sulphoxide (ABZ-SO), albendazole sulphone (ABZ-SO_2) and albendazole amino sulphone (ABZ-SO_2-NH_2)—in serum using high performance liquid chromatography was developed. The method involves liquid–liquid extraction of the serum by ethyl acetate, clean up with *n*-hexane and re-extraction with ethyl acetate, followed by separation on RP-C_8 column with a mixture of methanol: acetonitrile: acetic acid: water (40:1:10:49) as the eluting solvent. ABZ-SO and mebendazole—used as internal standard—were detected by UV ($\lambda = 286$ nm), and ABZ-SO_2 and ABZ-SO_2-NH_2 with fluorescence spectrophotometer at (Excitation = 286 nm, Emission = 333 nm) and (Excitation = 286 nm, Emission = 315 nm), respectively. The assay was accurate and reproducible with a detection limit of 10 ng/ml for ABZ-SO, 2 ng/ml for ABZ-SO_2 and 4 ng/ml for ABZ-SO_2-NH_2. Disregarding ABZ determination, which is not of pharmacokinetic importance as it is not found in human plasma after oral administration, the proposed method is appropriate for further pharmacokinetic and metabolism study of this drug.

Keywords: Albendazole metabolites; HPLC; UV; Fluorescence; Albendazole sulphoxide; Albendazole sulphone; Albendazole amino sulphone

1. Introduction

Albendazole (ABZ) is benzimidazole carbamate used as the drug of choice in treatment of echinococcosis [1]. After oral administration, it is quickly oxidized into its pharmacologically active metabolite albendazole sulphoxide (ABZ-SO) [2]. Further liver oxidative and hydrolytic metabolism

* Corresponding author. Tel.: +98-21-6112325; fax: +98-21-6461178
E-mail address: rouini@sina.tums.ac.ir (M.R. Rouini).

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produces albendazole sulphone (ABZ-SO_2) and albendazole amino sulphone (ABZ-SO_2-NH_2), respectively, which are thought to be anthelminitically inactive. Scheme of metabolic pathway is shown in Fig. 1. While ABZ possesses a clear therapeutic effect, pharmacokinetic studies indicate that ABZ-SO is responsible for both anthelmintic and toxic effects [3,4].

Few studies exist on the disposition, pharmacokinetics, and concentration–effect relationship of ABZ perhaps because of the difficulty in analyzing ABZ and its metabolites; the parent compound is undetectable in the serum after administration to man [5,6], rats [7], sheep [2], cattle [8] and other species. Low amounts of ABZ metabolites—especially the 2nd (ABZ-SO_2) and 3rd (ABZ-SO_2-NH_2) are found in biological fluids due to low absorption of the main drug and various routes of ABZ metabolism in human [9], which in turn complicates analysis of ABZ metabolites.

There were few reports on simultaneous determination of ABZ-SO and ABZ-SO_2 in human biological fluids [10–14] and no reports were found for ABZ-SO_2-NH_2 assay in human plasma or serum. We therefore developed a sensitive and specific reversed-phase HPLC method that allowed reliable quantification of the three mainly found metabolites of albendazole—ABZ-SO, ABZ-SO_2 and ABZ-SO_2-NH_2 in human serum. The results indicate the suitability of this method to assess pharmacokinetics of ABZ metabolites in human.

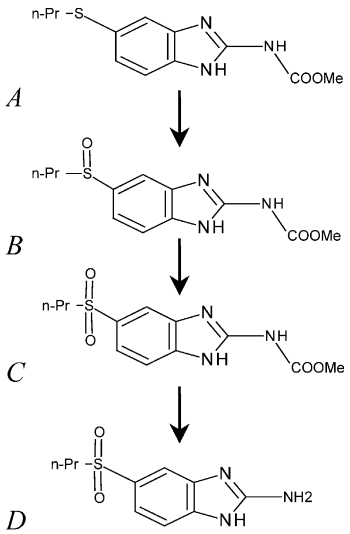

Fig. 1. Proposed main metabolic pathways of ABZ in human. (A) ABZ, (B) ABZ-SO, (C) ABZ-SO_2, (D) ABZ-SO_2-NH_2.

2. Materials and methods

2.1. Chemicals

ABZ-SO, ABZ-SO_2 and ABZ-SO_2-NH_2 were gifts from SmithKline Beecham (Worthing, UK). ABZ and mebendazole (MBZ) were supplied by Daroupakhsh Pharmaceutical Company (Tehran, Iran). Methanol and acetonitrile were of HPLC grade (Riedel-de Haen). Other chemicals were of analytical grade.

2.2. Apparatus and chromatographic conditions

The HPLC system consisted of a Perkin–Elmer solvent delivery system (series 4). The column was RP-C_8 (5 μm, 100×4.6 mm^2 i.d., Grom, Germany) preceded by an RP-C_8 (5 μm, 30×4.6 mm^2 i.d., Pye Unicam, USA) precolumn. Methanol:acetonitrile:acetic acid:water (40:1:10:49) was used as eluent with a flow rate of 0.8 ml/min, filtered through a Schleicher & Schuell RC-58 membrane filter (Dassel, Germany). The sample loop capacity was 50 μl, which was overfilled by 100 μl of the sample. The active metabolite (ABZ-SO) was detected by an ultraviolet spectrophotometer (Pye Unicam 4225, USA) at a wavelength of 286 nm. The other two metabolites (ABZ-SO_2-NH_2 and ABZ-SO_2) were detected by a fluorescence spectrophotometer (Perkin–Elmer LS-4, USA) at (Excitation = 286 nm; Emission = 315 nm, 0–4.2 min), (Excitation = 286 nm; Emission = 330 nm, 4.2–10 min), respectively. The response of the detectors was recorded by a dual pen recorder (Philips-PM 8252, USA) with each channel connected to one of the two detectors.

2.3. Preparation of standard solutions

A stock solution containing either 50 μg/ml ABZ-SO, ABZ-SO_2 and ABZ-SO_2-NH_2 was prepared in methanol. The working standard solution

was prepared by diluting 1.0 ml stock solution up to 10 ml water resulting in a final concentration of 5.0 μg/ml. This working solution was then used for making spiked serum samples.

2.4. Sample preparation

Samples were prepared according to Zeugin et al. [15], with some modifications as follows:

Extraction of serum samples was performed by liquid phase extraction by shaking with ethyl acetate (5 ml, 5 min) after addition of 0.1 ml of internal standard solution (MBZ, 5 μg/ml). The organic phase was then evaporated to dryness at 40 °C under gentle stream of nitrogen. The residue was dissolved in 0.5 ml HCl 0.001 M. The samples were further cleaned by washing up with *n*-hexane (5 ml, 5 min). The organic phase was discarded and the sample was re-extracted with ethylacetate—as above—after alkalination with 1 ml NaOH 0.01 M. The organic phase was evaporated to dryness as described; the residue was re-dissolved in 300 μl of the HPLC solvent and injected onto the HPLC column.

2.5. Calibration curves and limit of detection

The assay for simultaneous determination of the three main metabolites of ABZ was validated over a linear concentration range of 100–2500 ng/ml for ABZ-SO, 5–125 ng/ml for ABZ-SO_2 and 10–250 ng/ml for ABZ-SO_2-NH_2. A calibration curve for each batch was determined using calibration standards at 11 concentration levels for each metabolite; (100, 200, 250, 300, 400, 500, 750, 1000, 1500, 2000, 2500 ng/ml) for ABZ-SO, (5, 10, 12.5, 15, 20, 25, 37.5, 50, 75, 100, 125 ng/ml) for ABZ-SO_2 and (10, 20, 25, 30, 40, 50, 75, 100, 150, 200, 250 ng/ml) for ABZ-SO_2-NH_2. The lines of best fit for calibration standards were determined using linear least-squares regression analysis based on the peak height ratios of the analytes to the internal standard (MBZ) versus concentration of each metabolite. Four concentration levels of QC samples ((250, 500, 1000, 1500 ng/ml) for ABZ-SO, (12.5, 25, 50, 75 ng/ml) for ABZ-SO_2, (25, 50, 100, 150 ng/ml) for ABZ-SO_2-NH_2) were prepared and analyzed on the same day, along with an independent standard curve for quantification. The limit of detection was obtained considering signal to noise ratio of 3.

2.6. Assay recovery and reproducibility

Six replicates were prepared at each concentration for within-day and between-day validations, respectively. The precision was evaluated as the relative standard deviation of the mean expressed as percent (coefficient of variation: CV) for each sample. For within-day and between-day validation, CV of $<15\%$ for each concentration level were acceptable provided that at least 5 out of 6 of the replicates at each level met the criteria. To evaluate assay specificity, six independent lots of human control plasma were extracted without internal standard and analyzed for endogenous co-eluting interference. Triplicates of extracted QC samples and a set of post-extracted spiked QC samples were analyzed in the same assay run to determine the extraction recovery. The extraction efficiency (or recovery) was determined by measuring an extracted sample against a post-extraction spiked sample:

$$\%\ \text{Recovery} = \left(\frac{\text{Response of extracted}}{\text{Response of post-extracted spike}}\right) \times 100 \quad (1)$$

2.7. Human studies

A single oral dose of 800 mg ABZ was administered to 12 healthy volunteers after overnight fasting. Blood samples were drawn at 0, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 8, 10 and 24 h post-dosing. The samples were centrifuged at 3000 rpm for 10 min and the sera were separated and kept frozen at −20 °C until assayed.

3. Results

The chromatograms obtained from blank serum (a); serum spiked with ABZ (500 ng/ml), ABZ-SO

(300 ng/ml), ABZ-SO_2 (15 ng/ml) and ABZ-SO_2-NH_2 (30 ng/ml) (b) and serum of a volunteer after taking 800 mg single oral dose of ABZ (c) are shown in Fig. 2. The retention times for ABZ-SO_2-NH_2, ABZ-SO, ABZ-SO_2, MBZ and ABZ were almost stable at 3.5, 4.2, 4.5, 6.5, 8 min, respectively. As it can be noted in the Fig. 2, blank plasma is clear at the retention times of the compounds of interest. Serum concentration-time profile of the three metabolites in 12 healthy subjects after taking 800 mg single oral dose of ABZ is provided in Fig. 3.

3.1. Calibration curves

The linearity of calibration curves was studied at the concentration ranges of 100–2500 ng/ml for ABZ-SO, 5–125 ng/ml for ABZ-SO_2 and 10–250 ng/ml for ABZ-SO_2-NH_2. Each standard curve showed good linearity over the range of concentrations examined; ABZ-SO: $y = 4.56x - 0.27$, $r^2 = 0.998$, ABZ-SO_2: $y = 0.90x - 0.11$, $r^2 = 0.999$ ABZ-SO_2-NH_2: $y = 0.062x - 0.12$, $r^2 = 0.999$.

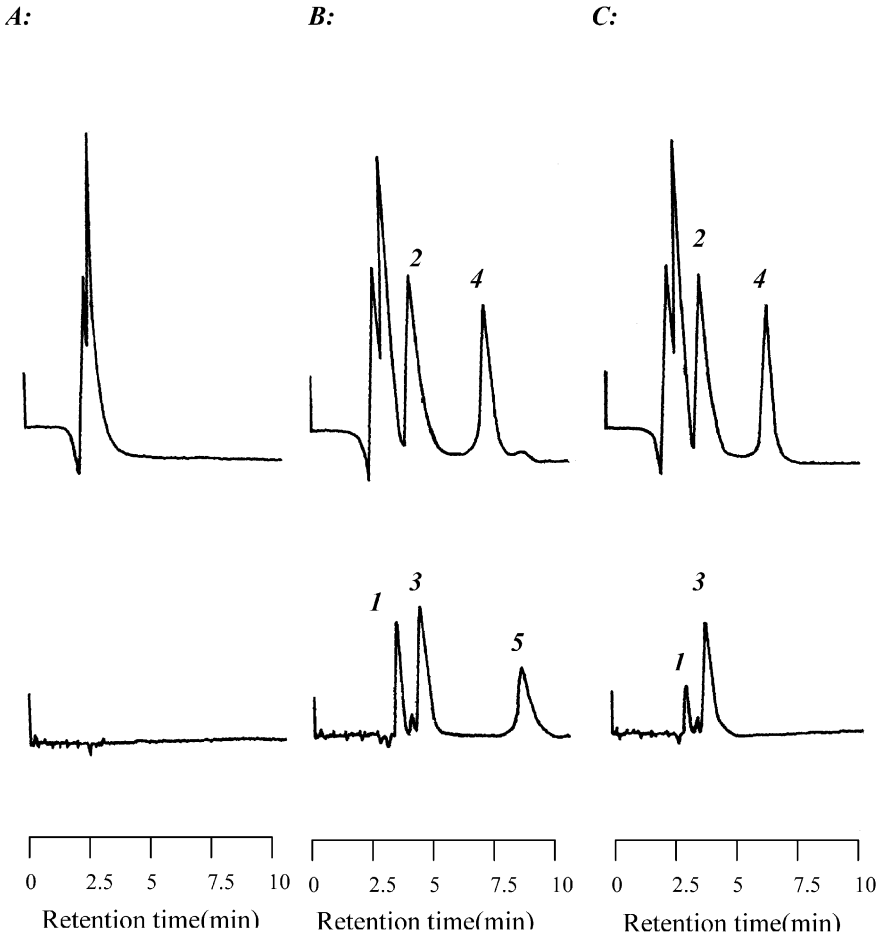

Fig. 2. Sample chromatograms of (A) blank serum (B) serum spiked with 30 ng/ml ABZ-SO_2-NH_2, 300 ng/ml ABZ-SO, 15 ng/ml ABZ-SO_2, and 500 ng/ml ABZ (C) a volunteer 6 h after taking 800 mg single oral dose of the drug (ABZ). Upper and lower chromatograms are detected by UV and fluorescence detectors, respectively. (1) ABZ-SO_2NH_2, (2) ABZ-SO, (3) ABZ-SO_2, (4) MBZ, (5) ABZ.

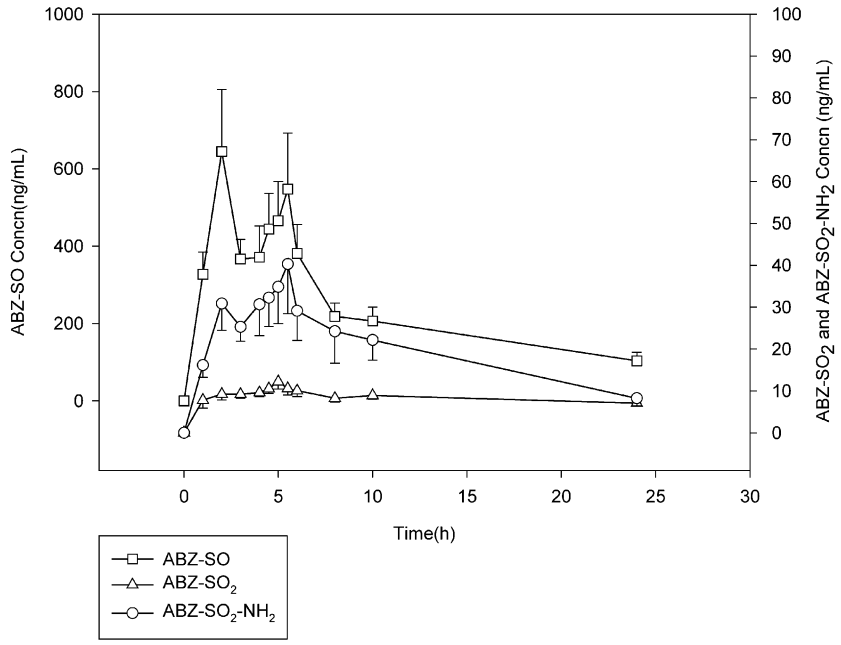

Fig. 3. Serum profile of ABZ-SO, ABZ-SO_2 and ABZ-SO_2-NH_2 in 12 healthy human subjects. Mean ± SE.

3.2. Limit of detection

The limit of detection with considering signal to noise ratio of 3 was 10 ng/ml for ABZ-SO, 2 ng/ml for ABZ-SO_2 and 4 ng/ml for ABZ-SO_2-NH_2.

3.3. Assay recovery and reproducibility

The assay recoveries of ABZ-SO and ABZ-SO_2 and ABZ-SO_2-NH_2 were more than 65, 96 and 70%, respectively for each compound.

Table 1
Analysis validation of ABZ-SO, ABZ-SO_2 and ABZ-SO_2-NH_2 assay ($n = 6$)

	Concentration (ng/ml)	Within-day CV (%)	Between-day CV (%)
ABZ-SO	250	7.256	9.793
	500	9.877	4.520
	750	3.239	8.613
	1000	4.924	3.255
ABZ-SO_2	12.5	5.190	8.421
	25	8.468	7.927
	37.5	9.358	8.243
	50	6.807	7.870
ABZ-SO_2-NH_2	25	8.402	2.875
	50	10.731	2.893
	75	5.191	8.970
	100	7.454	8.316

Within-day and between-day assay coefficients of variation for the compounds are summarized in Table 1. Within- and between-day coefficients of variation were less than 10.7 and 9.8%, respectively.

4. Discussion

Due to low absorption of the main drug and its metabolism to a various metabolites in human [9], low amounts of ABZ metabolites are found in biological fluids—especially ABZ-SO_2 and ABZ-SO_2-NH_2—which complicates analysis of ABZ metabolites. A number of HPLC assay methods have been developed for simultaneous quantification of ABZ metabolites in serum, but no report was found on ABZ-SO_2-NH_2 determination in human. The few articles reporting on simultaneous determination of ABZ-SO and ABZ-SO_2, mostly suffer from lack of sufficient detection limit (especially for ABZ-SO_2) with UV detection due to low serum level of this metabolite [13,14], some show lack of sufficient sensitivity for ABZ-SO determination using fluorescence detector [16], some show short column life [12], difficult system installation [10,17] or long run times [15].

In order to overcome the above shortcomings, we developed a method using both UV (for ABZ-SO quantification) and fluorescence detectors (for ABZ-SO_2 and ABZ-SO_2-NH_2 quantification) simultaneously, which provides very low detection limits. The described sample preparation technique produces clear sample solutions, which in turn lengthens the HPLC column life. As a result of fast metabolism ABZ is not found in serum. Disregarding the main drug (ABZ), therefore detection of this substance is not important from in vivo analytical viewpoint, this method has a short run time of about 8 min. Short run time provides the possibility of running many samples per day. Serum concentration-time profile of the three metabolites in healthy subjects after taking a single oral dose of ABZ (800 mg), provided in Fig. 3, is further proof of the suitability of the developed method for pharmacokinetic studies.

In summary, we have reported an analytical method for the determination of ABZ metabolites, which is sensitive and suitable for human pharmacokinetic studies and possess important advantages, notably determination of ABZ-SO_2-NH_2 (the first time assayed in human serum), low detection limit (especially for ABZ-SO_2 and ABZ-SO_2-NH_2) as a result of simultaneous utilization of UV and fluorescence spectrophotometers and short run time period over currently published methods.

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References

[1] R.S. Goldsmith, in: B.G. Katzung (Ed.), Basic and Clinical Pharmacology, seventh ed., Appleton & Lange, Stamford, 1998, pp. 869–870.
[2] S.E. Marriner, J.A. Bogan, Am. J. Vet. Res. 41 (1980) 1126–1129.
[3] P. Delatour, R.C. Parish, R.J. Gyurik, Ann. Rech. Vet. 12 (1981) 159–167.
[4] C. Villaverde, S. Alvarez, P. Redondo, Xenobiotica 25 (1995) 433–441.
[5] B. Penicaut, H. Maugein, H. Maisonneuve, J.F. Rossignol, Bull. Soc. Pathol. Exot. 76 (1983) 698–708.
[6] S.E. Marriner, D.L. Morris, B. Dickson, J.A. Bogan, Eur. J. Pharmacol. 30 (1986) 705–708.
[7] P. Delatour, F. Garnier, E. Benoit, C. Longin, J. Vet. Pharmacol. Ther. 7 (1984) 139–145.
[8] R.K. Prichard, D.R. Hennessy, J.W. Steel, E. Lacet, Res. Vet. Sci. 39 (1985) 173–178.
[9] WHO Food Additives Series 25, Albendazole, 9/1/2001, 13.
[10] V.L. Lanchote, M.P.C. Marques, O.M. Takayanagui, R. Carvalho, F.O. Paias, P.S. Bonato, J. Chrom. B. 709 (1998) 273–279.
[11] C.E. Lanusse, L.H. Gascon, R.K. Prichard, J. Vet. Pharmacol. Ther. 18 (1995) 196–203.
[12] A. Mirfazaelian, S. Dadashzadeh, M.R. Rouini, J. Pharm. Pharmacol. Commun. 6 (2000) 563–566.
[13] M.C. Valois, O.M. Takayanagui, P.S. Bonato, V.L. Lanchote, D.J. Carvalho, Anal. Toxicol. 18 (1994) 187–190.
[14] A. Prochazkowa, M. Chouki, R. Theurillat, W. Thormann, Electrophoresis 21 (2000) 729–739.