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Journal of Chromatography B, 709 (1998) 273–279

JOURNAL OF CHROMATOGRAPHY B

Simultaneous determination of albendazole sulfoxide enantiomers and albendazole sulfone in plasma

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Received 18 November 1997; received in revised form 26 January 1998; accepted 10 February 1998

Abstract

A high-performance liquid chromatographic method has been developed for the simultaneous determination of albendazole sulfoxide (ABZSO) enantiomers and albendazole sulfone ($ABZSO_2$) in human plasma. The resolution of ABZSO enantiomers and $ABZSO_2$ was obtained on a Chiralpak® AD column using hexane–isopropanol–ethanol (81:14.25:4.75, v/v/v) as the mobile phase. The drugs were detected by fluorescence (λ_{exc}=280 nm, λ_{em}=320 nm). The drugs were extracted from 500 μl plasma with ethyl acetate, and after solvent evaporation, the residues were dissolved in the mobile phase and chromatographed. The method was precise and accurate for the three compounds, as judged by the coefficients of variation and relative errors observed. Linear standard curves were obtained in the concentration range of 5–2500 ng/ml for ABZSO enantiomers and 1–500 ng/ml for $ABZSO_2$. A typical plasma concentration–time profile is presented for one patient under treatment for neurocysticercosis.

Keywords: Enantiomer separation; Cysticercosis; Albendazole

1. Introduction

Albendazole (ABZ) has proved to be effective in the treatment of neurocysticercosis, the most common parasitic disease of the central nervous system, affecting thousands of people in developing countries in Latin America, Asia and Africa [1–5]. In addition, albendazole is a potent benzimidazole anthelminthic agent and widely used in veterinary and human medicine [6].

ABZ undergoes extensive metabolism by liver microsomal enzymes, and probably in the gastrointestinal tract [7], to its major active metabolite albendazole sulfoxide (ABZSO). This metabolite is further metabolized to albendazole sulfone ($ABZSO_2$), which does not appear to have any activity (Fig. 1).

ABZ is a prochiral drug and after metabolization, two enantiomeric antipodes of ABZSO may occur: (+)-ABZSO and (−)-ABZSO. Although the enantioselective pharmacokinetics of ABZSO has been studied in animals [8–12] and in healthy human volunteers [9], the biological effect of individual enantiomers has not been described.

The enantioselective analysis of ABZSO in the plasma of animals and humans has been done by

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0378-4347/98/$19.00
PII: S0378-4347(98)00082-6

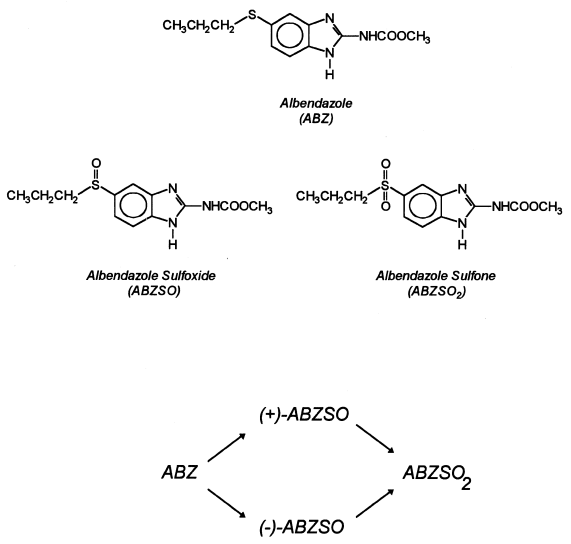

Fig. 1. Schematic metabolic pathway of ABZ.

high-performance liquid chromatography using chiral stationary phases based on (S)-*N*-(3,5-dinitrobenzoyl)tyrosine-O-(2-propen-1-yl)methyl ester [13] or α_1-acid glycoprotein covalently bonded to silica [10,14]. Recently, we reported a new procedure using a cellulose tribenzoate derivative as chiral stationary phase (Chiralcel® OB-H column) [15]. Although these methods have been applied in pharmacokinetic studies carried out on animals or humans with emphasis on the plasma concentration ratio (+)-ABZSO/(−)-ABZSO [8–12], the confidence limits are missing, except for the study recently reported by our group. Other disadvantages include the use of large volumes of plasma and the difficulties in measuring the (−)-ABZSO enantiomer present in human plasma at concentrations approximately ten times lower than those of the other enantiomer. In addition, these methods do not allow the determination of $ABZSO_2$. In order to avoid the interference of this metabolite, Delatour et al. reported the use of a clean-up step based on reversed-phase high-performance liquid chromatography [10]. Although $ABZSO_2$ did not interfere with the determination of ABZSO enantiomers in the methods proposed by our group [15] and by Lienne et al. [13], the quantification of this metabolite was also impossible due to the longer retention time and because it elutes as a broader peak.

The method presented here is suitable for the simultaneous determination of ABZSO enantiomers and $ABZSO_2$, permitting the evaluation of the second step in the metabolism of ABZ. In addition, the method proposed is more sensitive than the previous one.

2. Experimental

2.1. Chemicals and reagents

Rac-ABZSO (99.4%) and $ABZSO_2$ (99.8%) were kindly supplied by Robert Young and Co. (Glasgow, UK). Standard solutions were prepared in methanol at the concentration range of 0.2 to 20.0 μg/ml for ABZSO enantiomers and 0.02 to 2.0 μg/ml for $ABZSO_2$. The solutions were stored at −20°C. Sodium metabisulfite was of analytical grade and purchased from Merck (São Paulo, Brazil). Hexane, isopropanol and ethanol were HPLC grade and obtained from Merck. Ethyl acetate was of analytical grade and purchased from Grupo Química (Rio de Janeiro, Brazil).

2.2. Equipment and chromatographic conditions

The HPLC system consisted of a Shimadzu instrument (Kyoto, Japan): LC-10AS solvent pump, 7125 Rheodyne injector with a 20 μl loop, a RF 551 fluorescence detector (λ_{exc}=280 nm, λ_{em}=320 nm) and a CR6-A integrator.

The chiral column was based on amylose tris(3,5-dimethylphenyl carbamate) coated on a 10 μm silica-gel substrate (Chiralpak® AD column, 250 mm×4.6 mm, Chiral Technologies Inc., Exton, USA) and was protected with a 4 mm×4 mm CN precolumn (Merck, Darmstadt, Germany). Elution was carried out at a flow-rate of 1.2 ml/min using hexane–isopropanol–ethanol (81:14.25:4.75, v/v/v) as the mobile phase.

2.3. Extraction procedure

Plasma samples (500 μl) were supplemented with 200 μl of a 4 mg/ml sodium metabisulfite solution (to prevent ABZSO oxidation) and 3 ml of ethyl acetate. The tubes were capped and submitted to mechanical shaking at 200 cycles/min for 20 min,

and then centrifuged at 1800 *g* for 5 min. The tubes were frozen and 2 ml of the upper organic phase was transferred to clean glass centrifuge tubes. After evaporation of the organic phase to dryness under a stream of nitrogen gas, the residues were dissolved in 50 μl of the mobile phase for injection.

2.4. Preparation of the calibration curve

Calibration curves based on peak height were constructed for each assay by adding 25 μl of standard ABZSO and $ABZSO_2$ solutions to 500 μl of drug-free human plasma, followed by the extraction procedure. The concentration range was 5 to 500 ng/ml for both ABZSO enantiomers and 1 to 100 ng/ml for $ABZSO_2$.

2.5. Method validation

The absolute recovery of each compound was assessed at three concentration levels (10, 100 and 500 ng/ml for ABZSO enantiomers and 2, 20 and 100 ng/ml for $ABZSO_2$) by comparing the peak height after extraction with the peak height obtained from direct injection of equivalent quantities of standard solutions. Linearity was determined by extending the range of plasma concentration to 2500 ng/ml for ABZSO enantiomers and to 500 ng/ml for $ABZSO_2$.

The precision and accuracy of the method were determined by replicate analysis of drug-free plasma samples spiked with (+)-ABZSO and (−)-ABZSO at concentrations of 15 and 250 ng/ml and with $ABZSO_2$ at concentrations of 3 and 50 ng/ml.

Selectivity was evaluated by analyzing several drugs frequently used in combination with albendazole for the treatment of neurocysticercosis and other common drugs.

The method was evaluated in a pilot pharmacokinetic study carried out on a patient admitted to the University Hospital of the Faculty of Medicine of Ribeirão Preto-USP for treatment of neurocysticercosis. The patient (DR, male, 64.9 kg and 27 years old) freely consented to participate in the study, which was approved by the Ethics Committee (No. 13.130/94, 02/13/95). The patient had normal hepatic, cardiac and renal functions as confirmed by clinical examination and laboratory tests. Therapy was started with the administration of 5.0 mg/kg albendazole (ZENTEL®, 400 mg, chewing tablets, Smithkline Beechman Farmacêutica, São Paulo, Brazil) three times daily, for 8 days. The patient was simultaneously treated with dexamethasone and phenytoin. On day 8, the last dose of albendazole was administered. Serial blood samples were collected up to 12 h after administration of the drug. The blood was collected into tubes containing EDTA–Na and centrifuged and the separated plasma was stored at −20°C until analysis.

3. Results and discussion

The polysaccharide-derived chiral stationary phases are among the most widely used in the resolution of racemic compounds by high-performance liquid chromatography. Several arylcarbamates, acetates and benzoates of cellulose or amylose coated on silica gel substrate are commercially available. The column prepared with the tris(3,5-dimethylphenyl carbamate) derivative of amylose (Chiralpak® AD) has shown higher chiral recognition than the similar derivative of cellulose (Chiralcel® OD), probably due to different higher-order structures of the two derivatives [16,17].

In a previous paper we reported a method for the resolution of ABZSO enantiomers using the Chiralcel® OB-H column, a chiral stationary phase based on a cellulose tribenzoate derivative [15]. Although the resolution of ABZSO enantiomers was obtained within 15 min, the disadvantage of the method was the elution of $ABZSO_2$ after 45 min as a broader peak which impairs the quantification of this metabolite and prolongs the analysis time. Using the Chiralpak® AD column it was possible to obtain the complete resolution of ABZSO enantiomers. In addition, the $ABZSO_2$ peak eluted within 30 min as a symmetrical peak. The resolution of ABZSO enantiomers, $ABZSO_2$ and the unchanged drug ABZ was highly influenced by the mobile phase composition. Using hexane–isopropanol mixtures, $ABZSO_2$ eluted with a retention time similar to that of the (−)-ABZSO enantiomer. On the other hand, using mixtures of hexane and ethanol the (+)-enantiomer of ABZSO eluted closer to the ABZ peak.

It was not possible to resolve the four compounds by changing the percentage of isopropanol or ethanol. Using a ternary mobile phase with a correct proportion between hexane, isopropanol and ethanol the enantiomers of ABZSO and $ABZSO_2$ could be resolved with no interference from the unchanged drug ABZ which elutes with a retention time of 8.5 min. The influence of alcohol of different structures on retention and stereoselectivity on polysaccharide-based stationary phases has been studied for several compounds [18–23]. It was suggested that alcohol in the mobile phase alters the steric environment of the chiral cavities in the chiral stationary phase by binding to chiral and achiral sites at or near the chiral cavity.

The elution order was established by the injection of the individual enantiomers previously separated using the Chiralcel® OB-H column and hexane–ethanol (93:7, v/v) as the mobile phase [15]. An inversion of the elution order of ABZSO enantiomers was observed in the Chiralpak® AD column when compared to the Chiralcel® OB-H column, i.e. (+)-ABZSO elutes first in the amylose derived stationary phase and after (−)-ABZSO in the cellulose derived stationary phase. This result can be explained by different interactions of ABZSO enantiomers with the chiral adsorbing sites of the polysaccharides derivatives (carbonyl groups of ester in the Chiralcel® OB-H column and carbamate groups in the Chiralpak® AD column) and by the different higher-order structures of cellulose and amylose [16,17].

The procedure employed for the extraction of ABZSO enantiomers and $ABZSO_2$ from plasma samples was simple and efficient in removing endogenous interferents. Representative chromatograms obtained from the drug-free plasma, plasma spiked with the drugs and plasma from a patient under treatment with ABZ are shown in Fig. 2. The mean recoveries of (+)-ABZSO and (−)-ABZSO from plasma were 91.3 and 92.4%, respectively, whereas the $ABZSO_2$ metabolite was completely recovered from plasma samples.

The linearity of the method was evaluated in the concentration range 5–2500 ng/ml for ABZSO enantiomers and 1–500 ng/ml for $ABZSO_2$. The regression lines were linear over the concentrations examined and the correlation coefficients of the

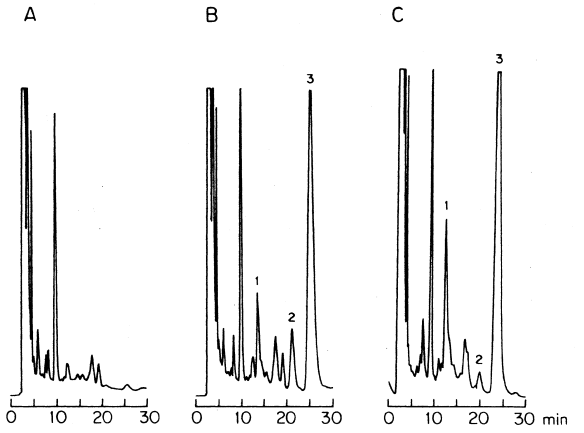

Fig. 2. Chromatograms obtained from (A) drug-free plasma; (B) plasma spiked with (+)-ABZSO (1), (−)-ABZSO (2) and $ABZSO_2$ (3), and (C) a plasma sample from a patient treated with albendazole. Chromatographic conditions: Chiralpak® AD column (10 μm particle size, 250 mm×4.6 mm); hexane–isopropanol–ethanol (81:14.25:4.75, v/v/v) as mobile phase at the flow-rate of 1.2 ml/min; fluorescence detection (λ_{exc}=280 nm, λ_{em}=320 nm).

calibration curves ranged from 0.996 to 0.998. The lower concentrations in the calibration curves were considered as a quantification limit of the method, i.e. 5 ng/ml for both ABZSO enantiomers and 1 ng/ml for $ABZSO_2$. These values were low enough for the application of the method to clinical pharmacokinetic studies. A higher quantification limit was obtained in our previous study using the Chiralcel® OB-H column.

To assess the precision and accuracy of the method, plasma samples containing ABZSO and $ABZSO_2$ at different concentrations were analyzed repeatedly. The results summarized in Table 1 show that ABZSO enantiomers and $ABZSO_2$ can be accurately and precisely determined in 500 μl of plasma by the present method.

Several drugs are used in combination with albendazole in the treatment of neurocysticercosis in order to control epileptic crises (carbamazepine, phenobarbital, phenytoin) or to prevent the acute inflammatory reaction due to cysticercal death (dexamethasone). These and other common drugs were evaluated for interference with the analytical method by injecting a solution prepared in the mobile phase at concentrations within the therapeutic range. None of the drugs interfered with the determination of ABZSO enantiomers or $ABZSO_2$ (Table 2). The high selec-

Table 1
Analytical precision and accuracy of the determination of (+)-ABZSO, (−)-ABZSO and $ABZSO_2$ from spiked plasma samples

	Concentration added (ng/ml)					
	(+)-ABZSO		(−)-ABZSO		$ABZSO_2$	
	15	250	15	250	3	50
Within-day precision						
n	10	10	10	10	10	10
C.V.	8.1	4.4	8.6	4.8	5.8	5.1
Between-day precision						
n	5	5	5	5	5	5
C.V.	8.3	4.9	4.4	4.5	7.9	2.8
Within-day accuracy						
Concentration obtained (ng/ml)	16.14	264.22	14.05	254.66	3.23	54.9
Relative error (%)	+7.6	+5.7	−6.3	+1.9	+7.7	+9.8
Between-day accuracy						
Concentration obtained (ng/ml)	15.45	256.80	13.59	251.90	2.87	53.74
Relative error (%)	+3.0	+2.7	−9.4	+0.8	−4.3	+7.5

tivity observed in the present study was mainly determined by the use of fluorescence detection.

The present method was used to determine the plasma levels of ABZSO enantiomers and $ABZSO_2$ in a patient treated with albendazole. Serial samples were collected after the last dose of the medication. The results obtained in the analysis of these samples are presented in Fig. 3. The method described in this paper allows the determination of ABZSO enantiomers and $ABZSO_2$ up to 12 h after administration of the medication. In addition, the decay curves for the plasma concentrations of (+)-ABZSO and (−)-ABZSO show that the (+)-enantiomer is accumulated in agreement with data reported in the literature [9].

Table 2
Retention time of the drugs studied as interferents

Drug	Concentration (μg/ml)	Retention time (min)
Albendazole	0.20	8.5
(+)-ABZSO	0.20	13.7
(−)-ABZSO	0.20	21.6
$ABZSO_2$	0.10	25.7
Amitryptiline	0.20	ND
Antipyrine	20.00	ND
Carbamazepine	12.00	ND
Cimetidine	1.50	ND
Dexamethasone	0.10	11.5
Diazepam	2.50	ND
Haloperidol	0.05	ND
Lorazepam	0.20	ND
Metoprolol	0.20	ND
Nifedipine	0.10	ND
Phenobarbital	40.00	ND
Phenytoin	20.00	8.4
Ranitidine	0.50	10.1
Salicylic acid	100.00	ND

ND, not detected up to 30 min.

4. Conclusions

The enantioselective method described is simple, rapid, selective, reproducible and sensitive, and allows simultaneous determination of ABZSO enantiomers and $ABZSO_2$ in plasma. The method, which requires a small volume of plasma (500 μl), is sufficiently sensitive for clinical pharmacokinetic studies.

Acknowledgements

The authors are grateful to FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) for financial support and to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pesso-

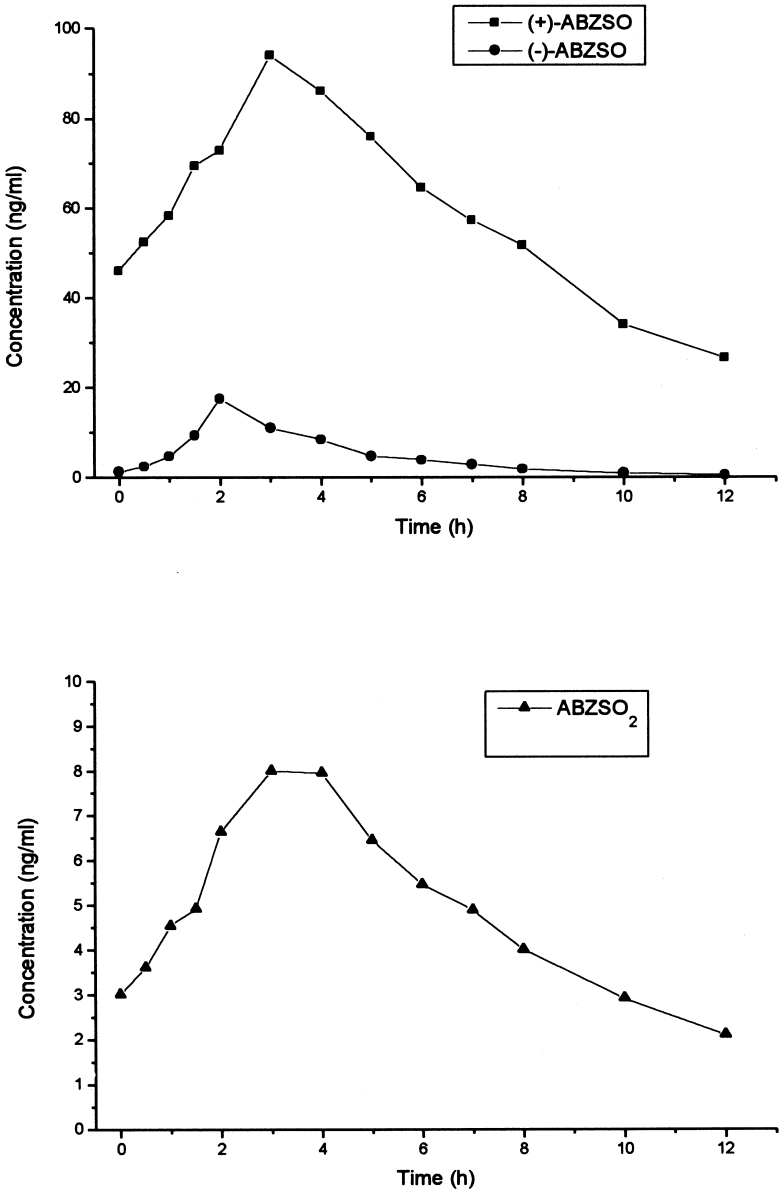

Fig. 3. Graphic representation of the steady-state plasma concentration versus time curve.

al de Nível Superior) for granting research fellowships.

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