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Albendazole sulphoxide concentrations in plasma of endemic normals from a lymphatic filariasis endemic region using liquid chromatography

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Abstract

A simple and sensitive reversed-phase isocratic HPLC method for the determination of albendazole and its metabolites has been developed. The mobile phase consisting of acetonitrile–water–perchloric acid (70%) (30:110:0.06 (v/v/v)) was pumped at a flow rate of 0.80 ml/min on a 5 μm, reverse phase, Discovery® RPamide C16 column with UV detection at 290 nm. The calibration graphs were linear in the range of 0.05–1 μg/ml for albendazole, albendazole sulphoxide and albendazole sulphone. The limit of quantification was 50 ng/ml for albendazole, 25 ng/ml for albendazole sulphoxide and 30 ng/ml for albendazole sulphone. The within-day and day-to-day coefficient of variation averaged 4.98 and 6.95% for albendazole, 3.83 and 6.83% for albendazole sulphoxide and 3.44 and 5.51% for albendazole sulphone, respectively. The mean extraction recoveries of albendazole, albendazole sulphoxide and albendazole sulphone were 79.25, 93.03 and 88.78%, respectively. The method was applied to determine the plasma levels of albendazole sulphoxide in endemic normals administered with albendazole during pharmacokinetic studies.

Keyword: Albendazole sulphoxide

1. Introduction

Bancroftian filariasis is an important cause of morbidity, deformity and disability in the developing world with over 100 million people affected in more than 70 countries [1]. Most programs for control of filariasis include mosquito control measures and drug therapy for suppression of microfilaraemia. Recent studies have shown that the benzimidazole derivative albendazole has significant antifilarial activity in human onchocerciasis [2,3], loiasis [4] and bancroftian filariasis [5].

Albendazole [5-(propylthio)-1H-benzimidazole-2-yl]carbamate (ABZ; cf. Fig. 1), is a broad-spectrum anthelminthic widely used in the treatment of helminthiasis in animals and man. Following oral administration ABZ, undergoes an extensive metabolism by liver microsomal enzymes to its major active metabolite, albendazole sulphoxide (ABZSO, cf. Fig. 1) [6,7]. Some of the sulphoxide is further metabolized to albendazole sulphone ($ABZSO_2$, cf. Fig. 1) which does not appear to have any anthelminthic activity [6,7]. ABZ is present at very low concentrations in plasma samples while ABZSO is present at higher concentrations. The metabolism and bioavailability of ABZ is studied with regard to ABZSO concentrations for this reason.

Determination of ABZ and its metabolites in biological fluids has become increasingly important in order to settle the doses, length and frequency of therapy for its use in the treatment of lymphatic filariasis. Very little information is available on the pharmacokinetics and plasma levels of albendazole and its metabolites in endemic normals from lymphatic filariasis endemic regions administered with albendazole. Several liquid chromatographic (LC) methods have been described for the determination of ABZ and/or its metabolites in biological fluids in onchocerciasis patients [8], brain cysticercosis patients [7,9], ovine plasma [10,11] and mouse plasma [12,13] using different columns and mobile phases for the drug and its metabolites [13] or the use of a gradient elution procedure [14]. The simultaneous determination of ABZ, ABZSO and $ABZSO_2$ has been described in one paper [10] using normal phase HPLC and in a recent one [15] simultaneous measurement of albenda-

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zole metabolites is described using a three step extraction procedure with UV and fluorescence detection.

In the present study, we describe a simple and sensitive reverse phase HPLC method which permits simultaneous measurement of ABZ, ABZSO and $ABZSO_2$ using a single mobile phase in isocratic mode and report the plasma levels of ABZSO in endemic normals from a lymphatic filariasis endemic area during pharmacokinetic studies of albendazole.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile and dichloromethane were purchased from Qualigens fine chemicals (Bombay, India). Water was deionized and triple distilled. All other chemicals were of analytical grade and were used without further purification. Albendazole and mebendazole (used as the internal standard, cf. Fig. 1) were purchased from Sigma–Aldrich (Hyderabad, India). Albendazole sulphoxide and albendazole sulphone were kindly supplied by Smithkline Beecham (London, UK). Albendazole tablets (trade name: Vermitel) were purchased from AstraZeneca Pharma (Bangalore, India).

2.2. Standard solutions

The stock solutions of ABZ, ABZSO and $ABZSO_2$ were prepared in methanol at a concentration of 1.0 mg/ml. Intermediate and working standard solutions covering the concentration range of 0.025–1 μg/ml were prepared by diluting the stock solution with methanol. A stock solution of the internal standard (mebendazole) was prepared in methanol (1 mg/ml). A working standard was prepared by diluting the stock with methanol to yield a concentration of 10 μg/ml. An amount of 25 μl of this working solution (equivalent to a 0.25 μg mebendazole) was used.

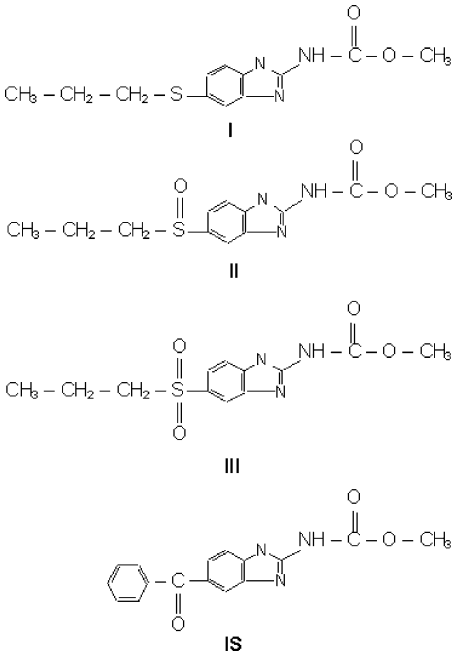

Fig. 1. Structures of: albendazole (I), albendazole sulphoxide (II), albendazole sulphone (III) and mebendazole (internal standard (IS)).

2.3. Chromatography

Chromatography was performed on System Gold™ chromatographic system (Beckman Instruments, Inc., San Ramon, CA, USA) equipped with two 110B solvent delivery modules, a 7125 Rheodyne injector and a 406 analog interface module. Analysis was performed on a 5 μm (15 cm × 4.6 mm) reverse phase Discovery® RPamide C16 column (Supelco, Bellefonte, PA, USA). The eluate was monitored UV-spectrophotometrically at 290 nm using a 166 programmable detector module (Beckman). The chromatographic data was analyzed using Beckman system Gold Chromatography software package.

The mobile phase comprised acetonitrile–water–perchloric acid (70%) (30:110:0.06 (v/v/v)) pumped at a flow rate of 0.80 ml/min. The mobile phase was filtered and degassed under vacuum before use. Chromatography was performed at ambient temperature.

2.4. Pharmacokinetic study

The subjects in the study were 10 healthy volunteers (age group: 23–42 years, average body weight: 59.8 kg) from the village Baghamari, district Khurda, Orissa, India, endemic for lymphatic filariasis. Prior consent was obtained before drug administration. After ascertaining that they were negative for lymphatic filariasis (confirmed through slide examination and CFA test) and no other antifilarial drug had been taken before starting the treatment, all patients were given 600 mg of albendazole after a simple fatty breakfast.

Intravenous blood (2 ml) was collected prior to dosage and at 1, 2, 4, 8, 24, 48 and 72 h there after. Heparin was used as an anticoagulant. The heparinised blood was centrifuged at 1000 × *g* for 15 min to separate plasma and stored at −20 °C until the HPLC analysis.

2.5. Extraction

The extraction was performed in glass culture tubes (15 ml capacity) pretreated with dichlorodimethyl silane in 5% (v/v) chloroform to minimize drug adsorption as described [8]. Briefly, to 0.5 ml (0.2–1 ml) sample of plasma (standard or analyze), 25 μl internal standard was added. Acetonitrile (0.2–1 ml) was added to each sample (0.2–1 ml) to precipitate plasma proteins. Following vortex-mixing (5 s) the samples were centrifuged (1000 × *g* for 10 min) before being transferred to clean culture tubes to

which 1 ml distilled water was added. This mixture was extracted with dichloromethane (10 ml) by vortex-mixing (10 s). After further centrifugation (1000 × *g* for 10 min) the aqueous phase was discarded and the organic layer transferred to a clean tube and evaporated to dryness under nitrogen at 37 °C. Samples were reconstituted with 0.1 ml mobile phase and an aliquot (0.01–0.05 ml) was used for chromatography.

2.6. Calibration

Calibration curves were prepared with pooled blank plasma samples spiked with known amounts of ABZ, ABZSO and $ABZSO_2$ to a fixed amount (25 μl) of the internal standard (mebendazole: equivalent to 0.25 μg). The concentration ranges of ABZ, ABZSO and $ABZSO_2$ were 0.05 μg to 1 μg/ml covering the therapeutic range of ABZ. The plasma standards were run through the procedure and calibration curves were constructed by plotting the peak height ratio for each compound with respect to the internal standard, against the amount of compound added to each blank plasma sample. Several blank plasma samples (with and without internal standard) were also run along with plasma standards as quality control samples.

2.7. Accuracy and intra and inter assay precision

The within-day reproducibility (coefficient of variation (C.V.)) was evaluated by the repeated ($n = 5$) analysis of three plasma standards, containing 0.50, 0.25 and 0.05 μg/ml of ABZ, ABZSO and $ABZSO_2$ each. The day-to-day reproducibility was determined by assaying plasma standards of three different concentrations containing 0.50, 0.25 and 0.05 μg/ml of ABZ, ABZSO and $ABZSO_2$ each on different days ($n = 5$). Accuracy of the method was determined by analyzing samples of known concentrations ($n = 5$).

2.8. Recovery

The recovery (extraction yield) of the benzimidazoles was determined by comparing the chromatographic peak height for spiked plasma standards with that obtained by direct injection of standards in methanol. The concentrations used for ABZ, ABZSO and $ABZSO_2$ were 1.0, 0.50, 0.10 and 0.05 μg/ml.

2.9. Results and discussion

The mobile phase consisting of acetonitrile–water–perchloric acid (70%) (30:110:0.06 (v/v/v)) at a flow rate of 0.80 ml/min was found to be the most suitable for achieving the baseline separation of ABZ, ABZSO, $ABZSO_2$ and mebendazole (internal standard) in a single chromatographic run within 15 min. The retention times of ABZ, ABZSO, $ABZSO_2$ and mebendazole were 12.3, 4.66, 5.77 and 10.07 min (Fig. 2).

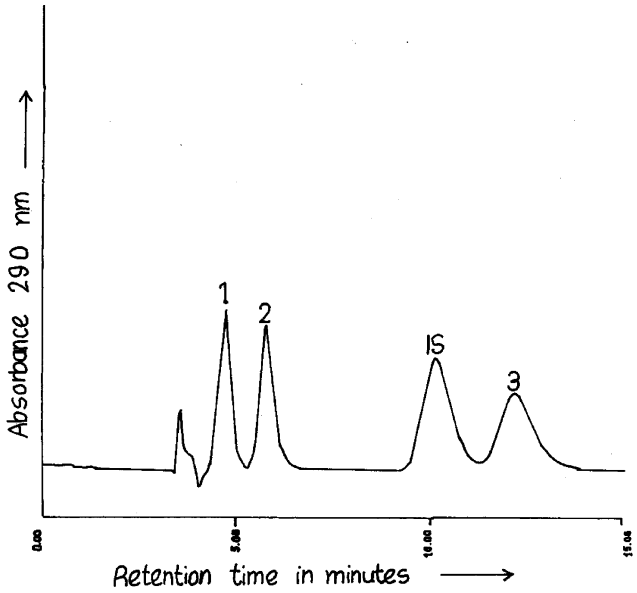

Fig. 2. Chromatogram showing the separation of albendazole and its metabolites on μRPamide C16 column using acetonitrile–water–perchloric acid (70%) (30:110:0.06 (v/v/v)) as the mobile phase at a flow rate of 0.80 ml/min with UV detection at 290 nm. Peaks: (1) albendazole sulphoxide (0.1 μg/ml); (2) albendazole sulphone (0.14 μg/ml); (IS) mebendazole (internal standard, 0.25 μg/ml); (3) albendazole (0.18 μg/ml).

An increase in the proportion of acetonitrile in the mobile phase decreases the retention, whereas an increase in water increases the retention of ABZ, ABZSO, $ABZSO_2$ and mebendazole. The perchloric acid content affects the separation of the two metabolites following a reversed phase ion-pair mechanism as stated earlier [16]. During the study, a large number of calibration curves were obtained for each compound and all were linear with correlation coefficients above 0.998. The limit of quantification was 50 ng/ml for ABZ, 25 ng/ml for ABZSO and 30 ng/ml for $ABZSO_2$. The signal-to-noise ratio was equal to 5. The within-day and day-to-day coefficient of variation (C.V.) were 4.98 and 6.95% for ABZ, 3.83 and 6.83% for ABZSO and 3.44 and 5.51% for $ABZSO_2$, respectively (Table 1). The average recoveries of ABZ, ABZSO and $ABZSO_2$ were 79.25, 93.03 and 88.78%, respectively (Table 2).

Fig. 3 shows the chromatographic behavior of: (A) a blank plasma extract; (B) a blank plasma with mebendazole (internal standard) from an endemic normal before administration of the drug; and (C) a plasma extract of a endemic normal at 4 h after oral administration of 600 mg of albendazole. Some endogenous peaks from plasma appeared in the chromatogram but they did not interfere in the determination of ABZSO.

The mean ABZSO concentrations in plasma of endemic normals following oral administration of 600 mg albendazole as a single dose are given in Table 3. ABZ was not detected in any sample, confirming the findings of earlier studies [8,9]. The ABZSO attains peak plasma concentrations of 362.50 ng/ml but with a wide inter-individual varia-

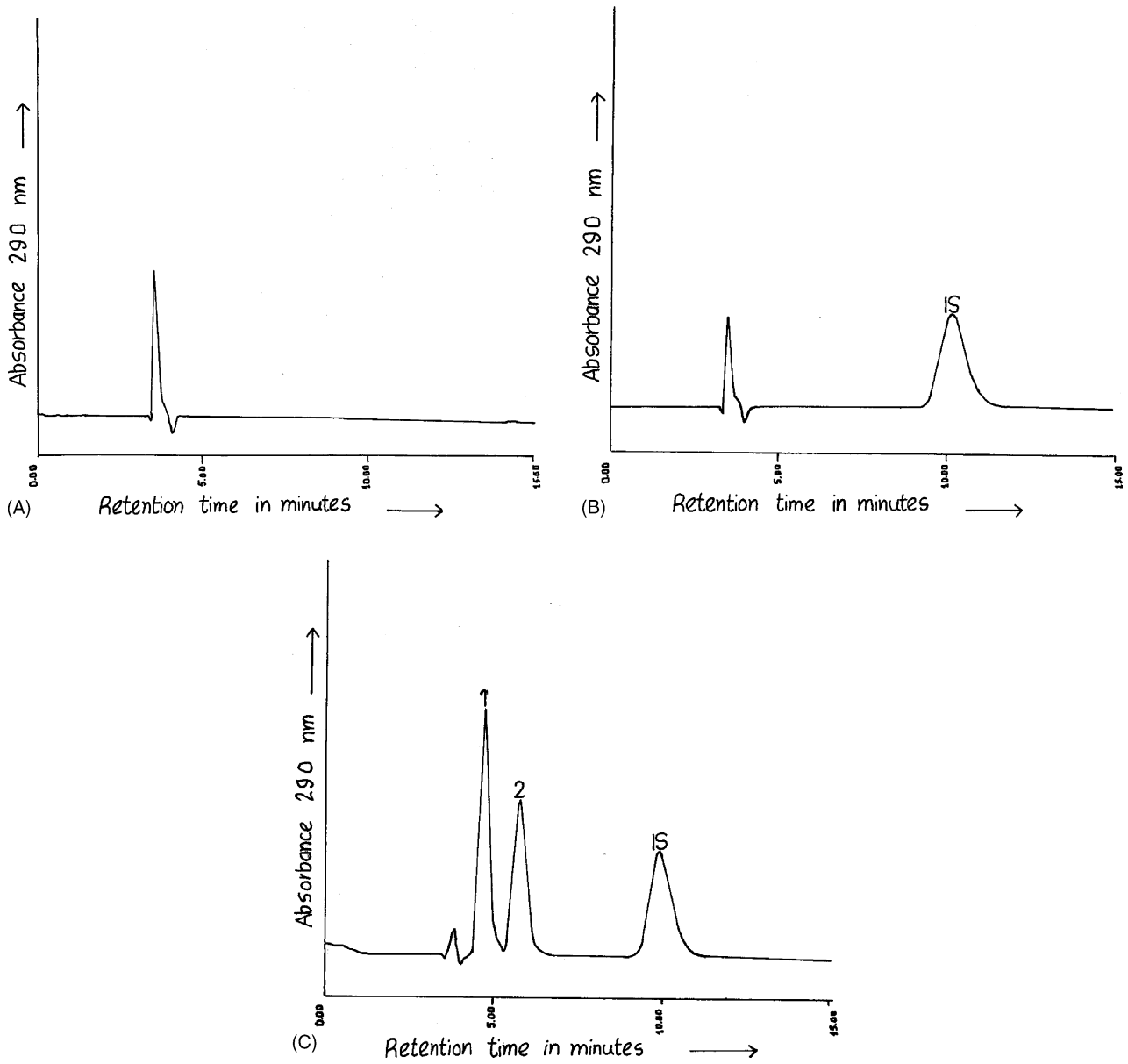

Fig. 3. Chromatograms showing the separation of albendazole sulphoxide and albendazole sulphone on μRPamide C16 column with acetonitrile–water–perchloric acid (70%) (30:110:0.06 (v/v/v)) as the mobile phase at a flow rate of 0.80 ml/min with UV detection at 290 nm. (A) Chromatogram of a blank plasma extract obtained from an endemic normal before drug administration. (B) Chromatogram of a blank plasma extract with (IS) mebendazole. (C) Chromatogram of a plasma extract taken 4 h post-dose after oral administration of 600 mg of albendazole to an endemic normal. Peaks: (1) albendazole sulphoxide (0.34 μg/ml); (2) albendazole sulphone; (IS) mebendazole.

tion. Peak ABZSO plasma concentrations of 300 ng/ml have been reported following a 400 mg oral dose with a wide inter-individual variation [17]. Mean peak ABZSO plasma concentrations were reached within 2–4 h though variations were observed in the time taken for peak plasma concentrations. Peak plasma concentrations of ABZSO were attained within 2 h for four cases while six cases attained within 4 h for the 600 mg dose. Hoaksey et al. [8] have reported the peak concentration of ABZSO within 4 h for the 800 mg dose and within 12 h for the 1200 mg dose, respectively. Our results too, indicate approximate proportionality between dose and peak plasma concentrations of ABZSO. The mean plasma concentrations–time profiles for ABZSO are shown in Fig. 4.

The chromatographic system described here is suitable for the separation and determination of ABZ, ABZSO and $ABZSO_2$ within 15 min in a single chromatographic run. Good baseline separation between the two metabolites is achieved using perchloric acid as the separation follows a reversed-phase ion-pair mechanism. The method satisfies the criteria required for an assay required for human pharmacokinetic studies and is relatively easier and simpler for

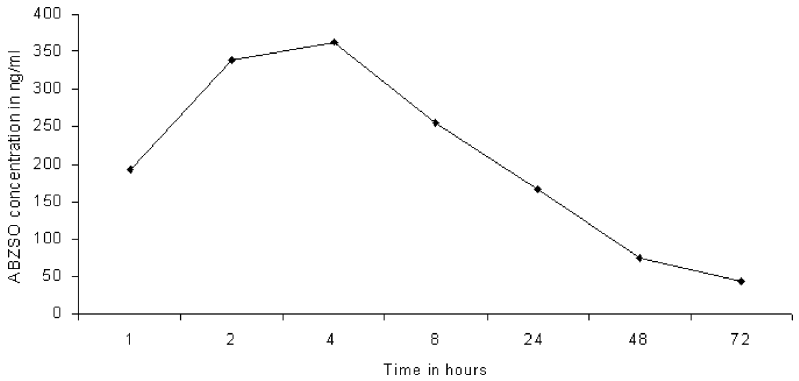

Fig. 4. Mean (±S.D.) plasma concentrations–time profile of albendazole sulphoxide following administration of 600 mg albendazole to endemic normals ($n = 10$).

Table 1
Accuracy and precision of the HPLC method for albendazole, albendazole sulphoxide and albendazole sulphone (spiked plasma samples; $n = 5$)

Concentration (μg/ml)	C.V. (%)		Accuracy (%)
	Within-day	Day-to-day	
Albendazole			
0.50	4.10	5.42	6.22
0.25	4.98	8.00	7.00
0.05	5.87	7.44	7.44
Mean ± S.D.	4.98 ± 0.89	6.95 ± 1.36	
Albendazole sulphoxide			
0.50	3.87	5.59	3.00
0.25	2.95	6.76	5.45
0.05	4.68	8.15	6.11
Mean ± S.D.	3.83 ± 0.87	6.83 ± 1.28	
Albendazole sulphone			
0.50	2.54	6.87	4.35
0.25	3.87	4.45	5.21
0.05	3.92	5.22	6.35
Mean ± S.D.	3.44 ± 0.78	5.51 ± 1.24	

the analysis of large number of samples required for such studies. The study has shown good relation between dose, plasma concentrations of ABZSO and time, which may have therapeutic significance for the treatment of lymphatic filariasis patients.

Table 2
Recovery (%) of the HPLC method for albendazole, albendazole sulphoxide and albendazole sulphone in plasma

Concentration (μg/ml)	Percentage recovery (mean ± S.D. ($n = 5$))		
	Albendazole	Albendazole sulphoxide	Albendazole sulphone
1.0	78.1 ± 4.5	89.7 ± 2.50	82.3 ± 3.10
0.5	80.4 ± 3.5	95.5 ± 2.80	91.0 ± 2.80
0.1	84.6 ± 6.3	94.6 ± 3.90	92.2 ± 3.5
0.05	73.9 ± 6.7	92.3 ± 4.80	89.6 ± 4.8
Mean ± S.D.	79.25 ± 4.47	93.03 ± 2.59	88.78 ± 4.45

Table 3
Mean (±S.D.) plasma concentrations[a] of albendazole sulphoxide (ABZSO) in endemic normals following administration of 600 mg albendazole as a single dose

Time after drug administration (h)	Concentration[b] (ng/ml) ABZSO
1	192.50 ± 124.20 (60.0–360.0)
2	312.5 ± 152.62 (220.0–540.0)
4	362.50 ± 233.86 (200.0–700.0)
8	255.00 ± 110.91 (180.0–420.0)
24	166.60 ± 82.19 (60.0–260.0)
48	74.66 ± 6.19 (66.0–80.0)
72	44.00 ± 0.82 (43.0–45.0)

[a] Mean concentrations of 10 endemic normals.
[b] Values in parentheses indicate concentration range.

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