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Note

Sensitive high-performance liquid chromatographic assay for albendazole and its main metabolite albendazole sulphoxide in plasma and cerebrospinal fluid

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Albendazole is a potent antihelmintic benzimidazole, widely used for therapy of human gastrointestinal helmintiasis, which has also shown efficacy for the treatment of hydatid cysts [1] Recently, it has been demonstrated that albendazole is highly effective for the treatment of neurocysticercosis, even in those patients who had shown poor therapeutic response to praziquantel [2,3] With this recent evidence of the powerful therapeutic properties of albendazole for parasitic disorders of the brain, determination of the drug in body fluids has become increasingly important in order to settle the doses and length of therapy for its use in the treatment of parasitic disorders of the nervous system

There is little information available on the pharmacokinetics and plasma levels of albendazole in humans Two high-performance liquid chromatography (HPLC) methods for measurement of albendazole in plasma have been reported [4,5], one method [4] requires 4 ml of plasma and several extractions with large volumes of diethyl ether In this report we describe a rapid and sensitive assay for albendazole determination using an analytical wavelength of 295 nm and mebendazole as internal standard The method has also been standardized for determination of albendazole in cerebrospinal fluid

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EXPERIMENTAL

Chemicals and solutions

Albendazole and mebendazole were supplied by Smith, Kline & French (Mexico), albendazole sulphoxide was kindly donated by Smith, Kline & French (Philadelphia, PA, U S.A) Methanol for the mobile phase was chromatographic grade (E. Merck, Darmstadt, F R G) All other reagents were analytical grade (E. Merck) The following aqueous solutions were prepared. 0.05 *M* potassium dihydrogenphosphate, pH adjusted to 5 7 with 0 8 *M* sodium hydroxide, 0 01 *M* potassium dihydrogenphosphate, pH adjusted to 7 4 with 0 8 *M* sodium hydroxide, 0 017 *M* potassium dihydrogenphosphate, pH adjusted to 5 5 with 0 8 *M* sodium hydroxide

Chromatographic conditions

The instruments used were a Beckman high-performance liquid chromatograph (Fullerton, CA, U S A), equipped with two solvent-delivery systems (Model 110 B), automatic gradient controller (Model 240), injection valve fitted with 20-μl sampling loop, variable-wavelength UV detector (Model 164) and data module (Model 427) Extractions were made using Sep-Pak C_{18} cartridges (Waters Assoc) Analysis was performed on an ODS C_{18} column (250×4 6 mm I D., particle size 5 μm) with methanol–0 05 *M* phosphate buffer (pH 5 7) (70 30) as the mobile phase The column was kept at room temperature (20–24°C) The flow-rate was kept constant at 0.8 ml/min The absorbance at 295 nm was recorded at a sensitivity of 0.005 a.u f s

Sample preparation

To 2 ml of plasma or cerebrospinal fluid samples, 100 μl of a methanolic solution of the internal standard mebendazole (5 μg/ml) were added and spiked with various amounts of the compounds in the range 30–1000 ng/ml; then, 2 ml of 0.01 *M* phosphate buffer (pH 7 4) were added, shaken on vortex for 30 s and extracted by passing through a Sep-Pak C_{18} cartridge. The cartridge was prepared by flushing 5 ml of methanol followed by phosphate buffer 0 017 *M* (pH 5 5). After spiked plasma had been passed through the cartridge it was washed with 20 ml of phosphate buffer (pH 7 4) and 1 ml of methanol–water (20 80, v/v) The compounds were then eluted with 3 ml of methanol The 3-ml methanol fractions were evaporated to dryness in pointed glass tubes in a water-bath at 40°C under nitrogen, and the residues were redissolved in 100 μl of methanol Aliquots of 20 μl were injected into the HPLC system

RESULTS AND DISCUSSION

Chromatograms of plasma and cerebrospinal fluid samples are shown in Fig 1 Retention times for albendazole sulphoxide, mebendazole and albendazole

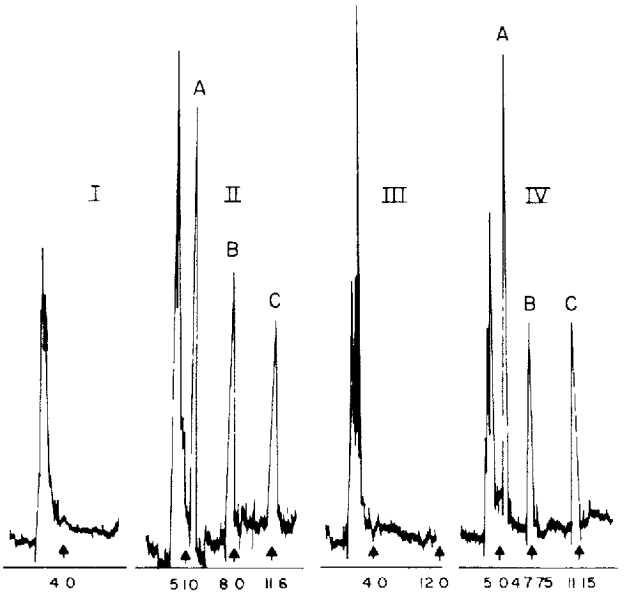

Fig 1 Chromatograms of (I) plasma blank, (II) spiked plasma containing 275 ng/ml albendazole sulphoxide (A), 500 ng/ml mebendazole (B) and 257 ng/ml albendazole (C), (III) cerebrospinal fluid blank and (IV) cerebrospinal fluid containing 500 ng/ml albendazole sulphoxide (A), 500 ng/ml mebendazole (B) and 500 ng/ml albendazole (C) Time in min

TABLE I

REPRODUCIBILITY FOR PLASMA SAMPLES SPIKED WITH ALBENDAZOLE ($n=5$)

Amount added (ng/ml)	Amount found (ng/ml)	Accuracy (%)	Coefficient of variation (%)
952	880 6	92 5	1 34
476	491 7	103 2	2 69
238	249 9	105 0	5 33
119	118 88	99 89	7 0
63 75	61 25	96 07	7 2

were 5 1, 8 0 and 11 6 min, respectively No interfering peaks occurred at these times A linear relationship ($r=0$ 9998) was found when the ratio of the peak height of albendazole and the peak height of the internal standard were plotted against various concentrations of albendazole ranging from 60 to 1000 ng/ml The same relation ($r=0$ 99999) was obtained for albendazole sulphoxide in plasma

The recovery of albendazole and albendazole sulphoxide, assessed by com-

TABLE II

REPRODUCIBILITY FOR PLASMA SAMPLES SPIKED WITH ALBENDAZOLE SULPHOXIDE ($n=5$)

Amount added (ng/ml)	Amount found (ng/ml)	Accuracy (%)	Coefficient of variation (%)
976	930 12	95 29	1 8
488	493 36	101 09	2 64
244	256 20	105 0	4 36
122	119 81	98 20	1 9
29 3	28 50	97 26	4 34

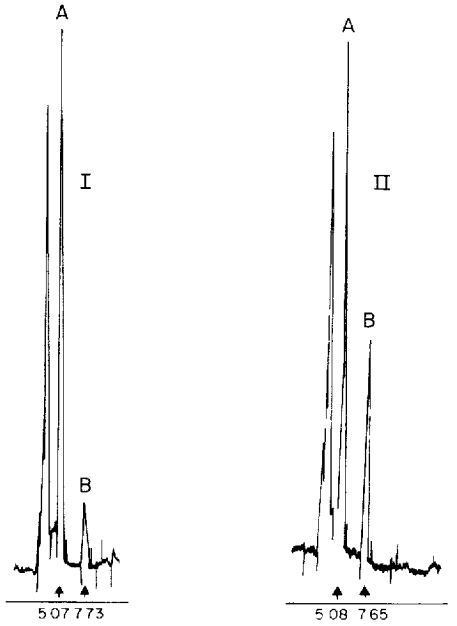

Fig 2 Chromatograms of (I) plasma (1251 ng/ml) and (II) cerebrospinal fluid (313 ng/ml), from a female patient receiving daily 15 mg/kg albendazole Albendazole was not detected, but its metabolite, albendazole sulphoxide (A), was measured Peak B is the internal standard, mebendazole Time in min

parison of peak heights from plasma extracts with those from standard solutions, ranged from 95% to 105% The reproducibility of the assay was determined by replicate analysis of spiked samples Tables I and II show that the maximum within-day coefficients of variation were 7 97% at 60 ng/ml for albendazole and 4.34% for albendazole sulphoxide at 30 ng/ml It was found that samples were stable for at least 4 weeks when stored at $-4°C$ The detection

limit (signal-to-noise ratio = 2) was 15 ng/ml for albendazole sulphoxide and 20 ng/ml for albendazole

This method is being used to estimate plasma levels of the drug in patients with brain cysticercosis We found that determination of albendazole sulphoxide is more reliable than determination of albendazole Fig 2 shows a typical chromatogram from a female patient following the administration of albendazole at 15 mg/kg per day for 7 days It shows that albendazole was not detected, with similar results to those obtained by other authors [6,7], in contrast, albendazole sulphoxide levels were 1251 5 ng/ml in plasma and 313 5 ng/ml in cerebrospinal fluid

This HPLC method is simple, sensitive, and fast (twenty samples can be analysed in a day) It can also be used as a reliable assay in the study of biopharmaceutics and pharmacokinetics of albendazole

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