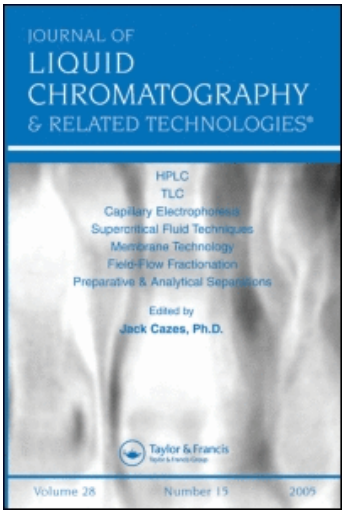

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF ALBENDAZOLE AND ITS SULFOXIDE METABOLITE IN HUMAN ORGANS AND FLUIDS DURING HYDATATIDOSIS.

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ABSTRACT

Albendazole and albendazole sulfoxide were quantified in serum, bile, liver, lungs and hydatid cyst fluid and walls of man receiving albendazole before surgical procedure during therapy of hydatidosis. The frozen tissue specimens were crushed and suspended in phosphate buffer, then the suspension was extracted with ethylacetate as for liquid specimens. The parent drug and its metabolite were extracted at the same time. Albendazole was quantified on a bonded hydrophobic stationnary phase and albendazole sulfoxide on a silica column at 254 nm. This assay was designed for monitoring albendazole and albendazole sulfoxide in serum of patients on long course treatment. The limit of sensitivity in serum was 10 $ng.ml^{-1}$ for the parent drug and its metabolite. An accumulation of these two drugs was shown in organs and bile, sometimes in the liquid and membrane of the cyst.

INTRODUCTION

Albendazole (methyl (5-propylthio) - 1 H - benzimidazol 2-yl) carbamate is a broad spectrum anthelmintic agent generaly used in animals. Recently benzimidazole anthelmintics were used in man to test their efficacy in E. Granulosus

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human echinococcosis (1) (2) (3) ; however the results of clinical trials required reevaluation as well as studies on the pharmacokinetics of these drugs in man. The determination of albendazole and its metabolite in man could serve to standardize the use of this drug in therapy of hydatidosis.

Nine metabolites of albendazole were identified in urine of different species (cattle, sheep, rats and mice) using radiolabeled product (4) ; the synthetic pathway to obtain metabolites and the thin-layer chomatographic technique corresponding to their identification were given by the authors (4). Despite this fact only albendazole and albendazole sulfoxyde were present in human samples tested in these thin-larger chromatographic systems.

Benzimidazoles were also investigated by high performance liquid chromatography : mebendazole and its metabolites in human plasma (5) (6), thiabendazole for industrial hygiene measurement (7), and all different commercial benzimidazoles in sheep plasma (8) ; but in this later paper the limit of sensitivity was approximately 80 $ng.ml^{-1}$ for albendazole on one ml of plasma ; albendazole was not detectable in any plasma of sheep ; all separations in these papers were obtained after an organic extraction on a reverse phase system, but none indicated metabolites of parent drug.

In this study a bonded phase system was used for quantitation of albendazole and a silica column was used for quantitation of a albendazole sulfoxyde ; but for the two products the same extraction procedure was performed.

A first group of patients had liver hydatidosis and lung hydatidosis. A surgical operation was scheduled for all patients from this group. There were volunteers to receive albendazole prior to surgery. This group was designed to evaluate the drug concentration in the target organs for hydatid cysts and finally in the cysts themselves as well as the monitoring of the blood drug levels during the course of a potential therapeutic treatment.

A second group of patients received the test drug therapeutically and/or prophylactically following cyst rup-

tures during surgical procedures or for residual or recurrent single or multiple cysts.

MATERIAL AND METHODS

a) Chromatographic equipment

Analyses were performed with a Waters Associates liquid Chromatograph (Waters Assoc., Paris, France) equipped with a model 440 absorbance detector, a model 6,000 A pump, a Wisp 710 B injector and 10 mv recorder.

b) Solvents and standards

Freshly distilled deionized water was used throughout the procedure. Methanol, acetonitril and ethylacetate were analytical grade (Merck, Darmstadt, Germany). Albendazole and albendazole sulfoxide were kindly given by Smith, Kline French (France).

c) Chromatographic eluent

For the determination of albendazole sulfoxide the mobile phase consisted of a mixture of acetonitrile : methanol : water (250/12/5). For the determination of albendazole it consisted of a mixture of methanol : water (65 :35) Each mobile phase was filtered through a 0,6 µm filter (Millipore corp., Paris, France) and degassed using ultrasonics.

d) In vitro samples

Plasma Albendazole and albendazole sulfoxide were directly prepared in pooled human plasma and congealed in aliquots at - 80°C. Each day of analysis an aliquot of each point of the standard curve was decongealed for standardization.

Bile Albendazole and albendazole sulfoxide were prepared in pooled human bile and congealed in aliquots at - 80°C

Organs Albendazole and albendazole sulfoxide were prepared in phosphate buffer saline (pH 7.4) and congealed in aliquots at - 80°C.

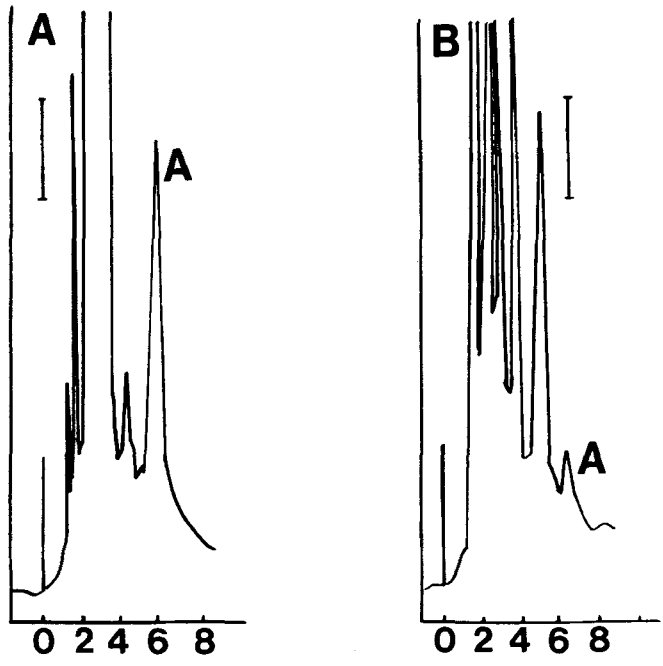

FIGURE 1

Chromatographic separation of albendazole in extracts of bile (A) and serum (B). Albendazole's peak is indicated by the letter A.
The vertical lines correspond to 0.002 absorbance units.

e) In vivo samples

Organs of patients (liver, lungs and cyst) or liquid (serum, bile or hydatic liquid) were sampled during surgery. They were immediately congealed at - 80°C.

f) Chemical assay

Extraction One ml of phosphate buffer (pH 7.4) and one ml of ethylacetate was added to one ml of serum, bile or hydatic liquid. The extraction was performed during 15 minutes on a rotor type agitator.

Organs (a few milligrammes depending on surgery) were weighted after decongealation, put in a few milliliters of

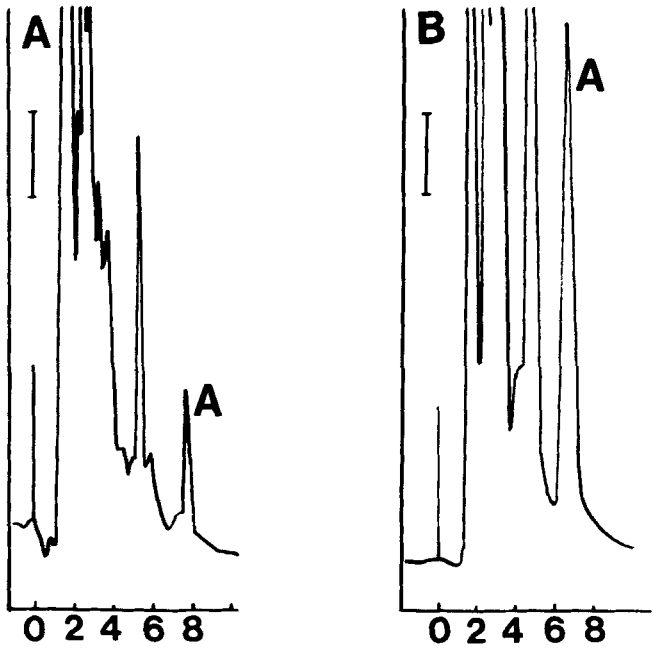

FIGURE 2

Chromatographic separation of albendazole in extracts of lungs (A) and liver (B). Albendazole's peak is indicated by the letter A. The vertical lines correspond to 0.002 absorbance units.

phosphate buffer (pH 7.4) and crushed with an ultrathurax apparatus. On ml of the supernatant was added to one ml of ethyl acetate and the extraction was performed during 15 minutes on a rotor type agitation. Standards were treated in the same manner. In each case 700 μl of the organic phase was evaporated and 100 μl of the mobile phase was added to the residue and passed through 0.22μ m filters and 35 μl of this liquid injected into the chromatograph.

Chromatography A reverse phase system was chosen to quantitate albendazole and a normal phase system for albendazole sulfoxide. In each cas 35 μl of the extract was injected onto the columns : a μBondapack C18 (Waters) for

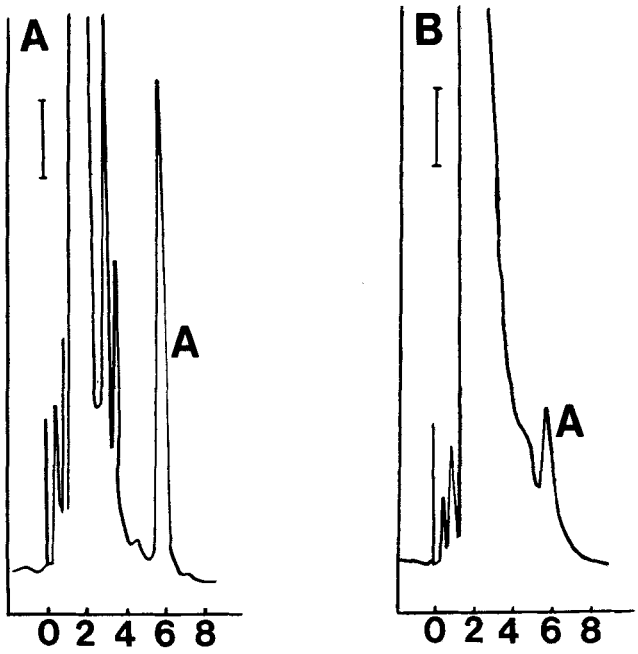

FIGURE 3

Chromatographic separation of albendazole sufoxide in extracts of serum (A) and bile (B). The peak of albendazole sufoxide is indicated by the letter A. The vertical lines correspond to 0.002 absorbance units.

albendazole and a micro-Porasil (Waters) for albendazole sulfoxide. Eluent pumped through at 1.5 ml/min. The absorbance detector was set at 254 nm at a sensitivity of 0.005 absorbance units full scale. Quantitation was based on recorded peak heights. Three standard curves were used : one for bile and another for organs.

RESULTS

Chromatographic separation

The retention time of albendazole was 6.6. minutes in the described conditions and 6 minutes for albendazole sufoxide ; They were constant in serum, bile or supernatant of organs. In the system of albendazole, albendazole sulfoxide

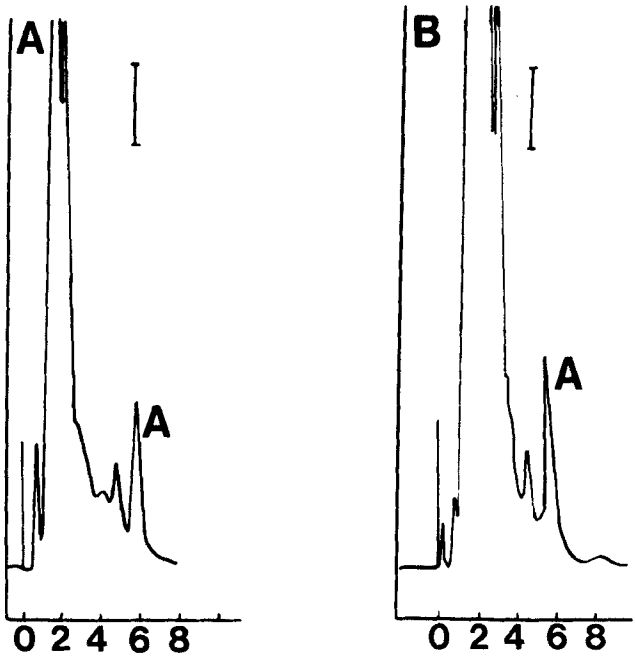

FIGURE 4

Chromatographic separation of albendazole sulfoxide in extracts of cyst wall (A) and cyst liquid (B). The peak of albendazole sulfoxide is indicated by the letter A. The vertical lines correspond to 0.002 absorbance units.

is undetectable : its time of retention is too near the dead volume and it is the same for albendazole in the system of albendazole sulfoxide. The chromatograms were shown in Figures (1-2) for albendazole and Figures (3-4-5) for albendazole sulfoxide in the different extracts.

Recovery

The three standard curves showed good linearity in the range studied : 10 - 100 $ng.ml^{-1}$ for albendazole and 10 - 800 $ng.ml^{-1}$ for albendazole sulfoxide . The recovery of these assays was tested using the dilution test and by adding known amounts of drug to known extracts. For albendazole the range of the recovery was between 94 % to 102 %

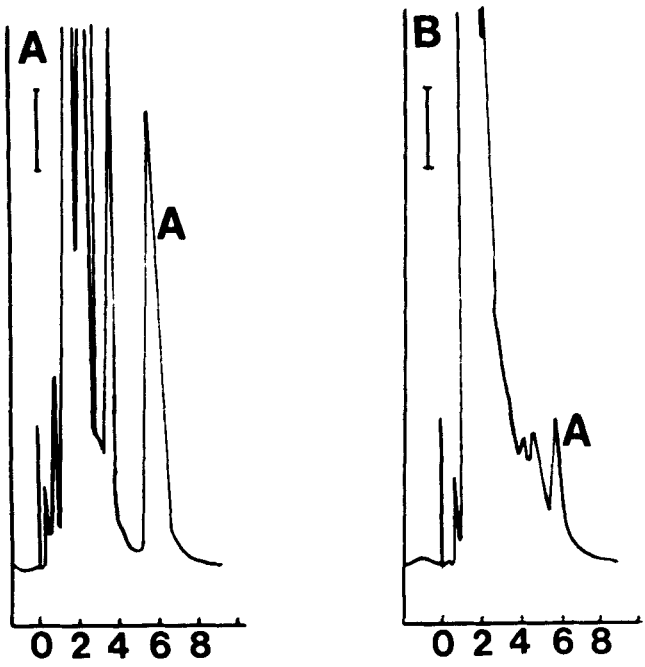

FIGURE 5

Chromatographic separation of albendazole sulfoxide in extracts of lung (A) and liver (B). The peak ol albendazole sulfoxide is indicated by the letter A. The vertical lines correspond to 0.002 absorbance units.

and for albendazole sulfoxide from 93 % to 101 % in the series of two tests.

Sensitivity

The limit of sensitivity of the method was determined with pooled serum, bile or phosphate buffer and by dilution of known human samples. The limit of detection could be evaluated to 10 $ng.ml^{-1}$ for albendazole and 10 $ng.ml^{-1}$ for albendazole sulfoxide in the described conditions. This limit is largely sufficient for clinical monitoring.

Precision

. Intra-assay variation was studied on three sera and biles which were determined tenfold on the same day with

TABLE I

PATIENTS	Serum ng.ml^{-1}		Bile ng.ml^{-1}		Cyst wall ng.g^{-1} wet tissue		Cyst liquid ng.ml^{-1}		Liver ng.g-1 wet tissue		Lungs ng.g^{-1} wet tissue	
	*A	*B	A	B	A	B	A	B	A	B	A	B
1	6	133	5	350	< 4	83	< 1	180		1 730		
2	< 5	130	< 5	248	< 5	138	8	504	121	272		
3	< 5	480			< 5	53	< 10	94			< 5	712
4	< 5	350			< 5	68	< 5	98			< 5	718
5	< 5	180			< 5	92	< 5	158			< 5	816
6	< 5	50									10	720
7	24	65	200	90							406	554
8	38	90	60	350							139	97
9	10	160			< 10	< 10	< 5	< 10	70	732		
10	45	350	30	570	15	83	8	180	40	1 210		

*A Albendazole *B Albendazole sulfoxide

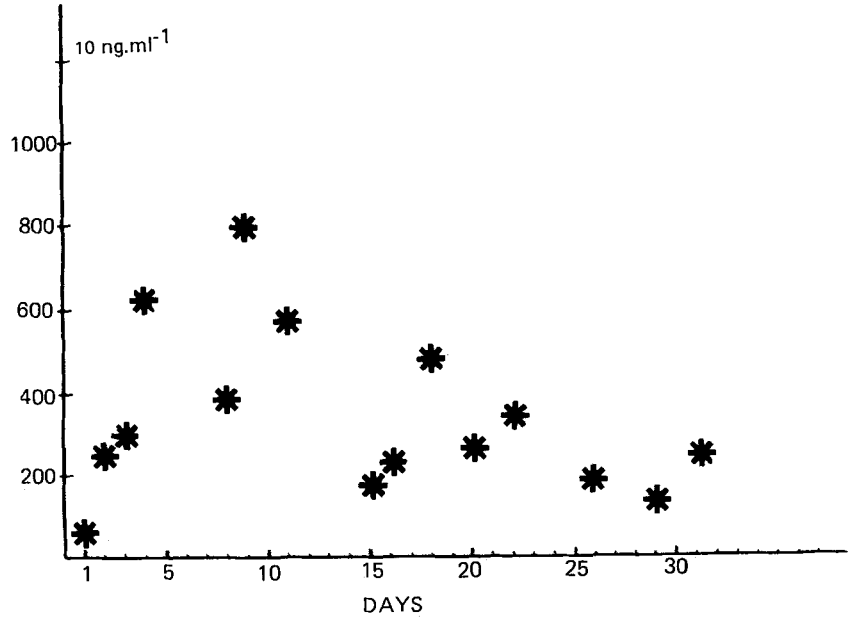

FIGURE 6

Concentration of albendazole sulfoxide in serum for a patient of the second group plotted against days of treatment.

the two techniques. For albendazole sulfoxide, the maximum variation was 8 % and 7.5 % for albendazole on the range of the standard curve.

. Inter-assay variation was calculated on the same sera and biles which were stored at - 80°C and assayed ten times on different days ; the variation was 10 % for albendazole sulfoxide and 9.5 % for albendazole (the coefficient of variation was defined with one standard deviation).

. Patient values

The first group received 5 to 7 mg. Kg^{-1} of albendazole for two 39 consecutive days. The last dose of albendazole was administred 10 to 12 hours before surgical operation. The drug was administered morning and evening before meals as 200 mg tables (see table I).

The second group received 5 to 7 $mg.Kg^{-1}$ of albendazole for 30 consecutive days the treatment being renewed after an interval of two weeks. The observed values were shown in Figure 6 for one patient ; the same type of curve was obtained for other patients. The mean value for albendazole was below 10 $ng.ml^{-1}$ and most of time albendazole was undectectable in patient's serum. Albendazole sulfoxide had very high values 275 $ng.ml^{-1} \pm 210$ $ng.ml^{-1}$.

DISCUSSION

The procedure described above permits one to monitor chemotherapy of human hydatidosis with albendazole . Alben dazole and albendazole sulfoxide needed to be determined in serum ; albendazole had a too low concentration in serum, except in a few patients (cirrhosis). The two different ways to quantitate albendazole and its metabolite were needed because of the difficulty of having an isocratic separation on a column in extracts. Tissue penetration was better for the metabolite than for the parent drug. Albendazole was undetectable in the cyst and cystic liquid. It is clear that the determination of albendazole sulfoxide in serum could serve to monitor albendazole therapy and permit controlled clinical trials. This HPLC analysis of albendazole sulfoxide is a simple, sensitive and reliable method for such studies.

REFERENCES

1. Heath D.D., Christie M.J., Chevis R.A.R., Parasitology, 70, 273-285 (1975).
2. Braithwaite P.A., Aust. NZ J. Surg., 51 : 23-27 (1981)
3. Saimot A.G., Meulemans A., Hay J.M., Mohler J., Manuel C. Coulaud J.P., Nouv. Pr Med., 38, 3121-3126 (1981)
4. Gyurik R.J., Show A.W., Zaber B., Brunner E.L., Miller J.A., Willani A.J., Petka L.A., Parish R.C., Drug Metab. Disp., 9, 6, 503-508 (1981).

5. Alton K.B., Patrick J.E., Mc Guire J.L., J. of Pharm.Sci 68, 7, 880-882 (1979).
6. Allan R.J., Goodman H.T. Watson T.R., J. of Chrom., 1983 311-319 (1980).
7. Belinky B.R., J. of Chrom., 238, 506-508 (1982).
8. Bogan J.A., Marriner S., J. of Pharm. Sci, 69, 4, 421-423 (1980).