ELSEVIER

Journal of Chromatography B, 723 (1999) 265–271

JOURNAL OF CHROMATOGRAPHY B

Quantitative determination of albendazole and its main metabolites in plasma

Juan José Garcia[a], Francisco Bolás-Fernández[a], Juan José Torrado[b,*]

[a]*Dpto. Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, 28040, Madrid, Spain*
[b]*Dpto. Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, 28040, Madrid, Spain*

Received 3 June 1998; received in revised form 23 October 1998; accepted 26 October 1998

Abstract

Three different and complementary chromatographic methods are described for quantitative determination of albendazole (ABZ) and its two main metabolites: albendazole sulphoxide (SOABZ) and albendazole sulphone (SO_2ABZ). ABZ, SOABZ and SO_2ABZ can be quantified by two RP-HPLC methods with an ODS2 column and two different mobile phases. One of methanol–water (60:40) for ABZ and a second one of phosphoric acid in water–acetonitrile (80:20) for SOABZ and SO_2ABZ. SOABZ bears an asymmetric sulphur centre. Quantitative assay of (+) SOABZ and (−) SOABZ can be performed by HPLC. A chiral AGP column and a mobile phase of sodium phosphate buffer (8 m*M*, pH 7.0) containing different amounts of 2-propanol between 0 to 2% were used. Pharmacokinetic characteristics of ABZ following oral administration of a liquid formulation of ABZ (12 mg/kg) in mice has been studied with these three complementary HPLC methods and the results are reported.

Keywords: Albendazole

1. Introduction

Albendazole (ABZ) is a benzimidazole anthelmintic drug used in the treatment of helminthiases in animals and man. Following oral administration the sulphide (ABZ) is oxidized to sulphoxide (SOABZ), which has an asymmetric sulphur centre. Another metabolite of ABZ is the sulphone (SO_2ABZ). SOABZ is an active metabolite which is known as Ricobendazole. SO_2ABZ is an inactive metabolite. Several other metabolites have been reported previously [1].

Anthelmintic activity of ABZ and SOABZ can be studied in the *Trichinella spiralis* model in mice [2]. By using this model the anthelmintic activity can be determined either in the enteral and/or parenteral stages of this parasite infection. We have recently reported some data about the relationship between anthelmintic activity and pharmacokinetic characteristics [2,3]. Following oral administration ABZ is present at very low concentration in plasma samples. Meanwhile, its main and active metabolite, SOABZ, is present at higher concentrations. For this reason bioavailability of ABZ formulations is frequently studied with regard to SOABZ concentrations. Recently, we have reported a HPLC method to assay SOABZ and SO_2ABZ in plasma [4]. In order to achieve a complete pharmacokinetic study of ABZ

*Corresponding author.

0378-4347/99/$ – see front matter
PII: S0378-4347(98)00497-6

formulations not only do SOABZ and SO_2ABZ have to be quantified, but also ABZ and the two enantiomers (+) and (−) of SOABZ. Quantitative assay of the two enantiomer forms of SOABZ has been studied previously based on chiral-AGP [5] or (*S*)-*N*-(3,5-dinitrobenzoyl)tyrosine [6] columns.

Although there are many analytical methods reported for quantitative assay of ABZ and its main metabolites, most of them require complex processes of sample preparation, such as previous extraction procedures for the samples with organic solvents, followed by evaporation and redissolution [7,8]. Other methods require the use of high volumes of solvents to elute the different components of samples. Thus, the final concentration of these compounds are very low and subsequent evaporation and redissolution steps are also needed [9].

In the present contribution three different and complementary HPLC methods which are useful for the quantitative assay of ABZ and its main metabolites in plasma are described. These analytical methods do not require complex previous treatment of the samples. Special attention has been paid to the determination of quantitation limits for ABZ and its main metabolites. Quantitation limits have been calculated as the lowest concentration of ABZ or its metabolites which gives rise to a signal capable of being quantified by the integrator (signal-to-noise ratio=5).

These methods have been used for the pharmacokinetic study of ABZ after oral administration in mice. Several complete ABZ pharmacokinetic studies have been reported for animals such as goats, cattle, sheep, rats, dogs,... [10–13] but there are not many reported data on pharmacokinetic studies of ABZ in mice.

2. Experimental

2.1. Apparatus

Analytical chromatography was performed with a modular liquid chromatograph equipped with a Gilson 305 isocratic pump, an automatic sampler (Gilson 231 XL) fitted to a 100-μl sampling loop (Rheodyne), a variable wavelength detector (Gilson 116) and an integrator (Spectra-Physics SP-270).

2.2. Chemicals

ABZ and SO_2ABZ were a gift from Smith-Kline Beecham Pharmaceuticals (London, UK). SOABZ was a gift from Chemo Ibérica (Alcala de Henares, Spain). HPLC grade acetonitrile and methanol were obtained from Lab-Scan (Dublin, Ireland). All other chemicals were analytical grade from Panreac (Barcelona, Spain).

2.3. Quantitative assay of ABZ

Analysis was performed on a Spherisorb ODS2 column (200×0.46 mm, 5 μm) from Teknokroma (Barcelona, Spain). A mobile phase of methanol–water (60:40) at a constant flow-rate of 1 ml/min was employed. Samples were measured at 291 nm. The linearity of the method was studied in the range between 0.075–10 μg/ml. The results were analysed by linear regression and the correlation coefficients obtained were always at least 0.99. The quantitation limit for ABZ, defined as the lowest concentration of ABZ which gives rise to a signal capable of being quantified by the integrator (signal-to-noise ratio=5), was found to be 0.07 μg/ml. The specificity of the methods was checked with a blank mouse plasma sample and no significant interference was observed. Inter-day precision was 3.1% during three consecutive days for a plasma sample of concentration 0.5 μg/ml expressed as relative standard deviation (RSD).

2.4. Quantitative assay of SOABZ and SO_2ABZ

A Kromasil ODS2 column (200×0.46 mm, 5 μm) from Teknokroma (Barcelona, Spain) was used as the stationary phase. A mobile phase containing 800 ml of a water solution with 188 μl of phosphoric acid 85% and 200 ml of acetonitrile was used at a constant flow-rate of 1 ml/min. Samples were measured at 290 nm. Validation data of this method can be summarised as follows. Linearity of the method was studied in the range between 0.05–10 μg/ml. The results were analysed by linear regression and the correlation coefficients obtained were always at least 0.99 for SOABZ and 0.98 for SO_2ABZ. The quantitation limits for SOABZ and SO_2ABZ were 0.031 and 0.037 μg/ml, respectively. The specificity

of the methods was checked with a blank mouse plasma sample and no interferences at the retention times of SOABZ and SO_2ABZ were observed. Inter-day precisions were 3.6 and 4.3%, respectively, during three consecutive days for plasma samples of concentration 0.1 μg/ml of SOABZ and 0.1 μg/ml of SO_2ABZ expressed as relative standard deviation (RSD).

2.5. Quantitative assay of (+) SOABZ and (−) SOABZ

A chiral-AGP (100×4 mm, 5 μm) column from ChromTech (Stockholm, Sweden) was used. Samples were measured at 290 nm. A mobile phase of sodium phosphate buffer (8 m*M*, pH 7.0) containing different amounts of 2-propanol between 0 to 2% was used at a constant flow-rate of 0.9 ml/min. Validation data depends on the 2-propanol concentration in the mobile phase. Highest sensitivity was achieved when 2% 2-propanol was used in the mobile phase. Validation data when 2% of 2-propanol is used are as follows. Linearity of the method was studied in the range between 0.05–1.5 μg/ml of (−) and (+) SOABZ. The results were analysed by linear regression and correlation coefficients obtained were always at least 0.99 for (−) SOABZ and 0.98 for (+) SOABZ. Quantitation limits for (−) SOABZ and (+) SOABZ were 0.04 and 0.05 μg/ml, respectively. Specificity of the methods was checked with a blank mouse plasma sample and no interferences at the retention times of (−) SOABZ and (+) SOABZ were observed. Inter-day precisions were 5.3 and 7.2%, respectively, during three consecutive days for a plasma concentration sample of 0.05 μg/ml of (−) SOABZ and 0.05 μg/ml of (+) SOABZ expressed as relative standard deviation (RSD).

2.6. Pharmacokinetic study

ABZ was solubilised in water by using a hydroxypropyl-β-cyclodextrin solution. This solution was orally administered by a single dose of 12 mg/kg to Swiss CD-1 mice (weight 30±3 g). Following drug administration, groups of six mice were sacrificed and blood samples were collected at different times (0.25, 0.5, 0.75, 1.5, 3, 6 and 24 h). Blood samples were heparinized and centrifuged individually after the extraction. Plasma samples were frozen until the HPLC analysis.

2.7. Sample preparation and quantitative assay

Two ml of methanol was added to aliquots of plasma (400 μl) to precipitate proteins. After vortex-mixing for 5 min, samples were centrifuged at 3000 *g* for 10 min and filtered through a nylon 0.45-μm filter (Teknokroma, Spain). In order to quantify ABZ concentration, 100-μl aliquots of the filtered fractions were injected into the HPLC system under the conditions described for the ABZ assay. A second assay under the analytical conditions described for SOABZ and SO_2ABZ quantification was done. Liquid fractions were collected at the retention time of SOABZ. Finally, these samples were assayed in order to quantify proportions of the two enantiomers of SOABZ.

3. Results and discussion

For a complete pharmacokinetic assay of ABZ and its main metabolites, several analytical methods are required. In a previous report we have used only one analytical RP-HPLC method [2]. This method [2] used the same analytical conditions that we have described for the ABZ assay but with a mobile phase containing 50% of methanol. With these conditions we were able to quantify SOABZ, but we could not detect ABZ at the low concentrations which are present after oral administration. Now we have to increase the sensitivity of the method by using a higher proportion of methanol in the mobile phase (60%). A mobile phase with a 60% of methanol mixed with a phosphate buffer is also proposed by USP 23 to quantify ABZ [14]. Fig. 1 shows the results of the assay of a plasma sample containing 0.3 μg/ml of ABZ. The retention time for ABZ is 18.8 min. Unfortunately, this method can not quantify the two main metabolites because under these conditions they have the same retention time (approximately 4.5 min). For this reason a second HPLC assay method is required to separate and quantify the two main metabolites of ABZ. This new method has been validated and reported recently [4]. Fig. 2 shows the assay of a mouse plasma sample

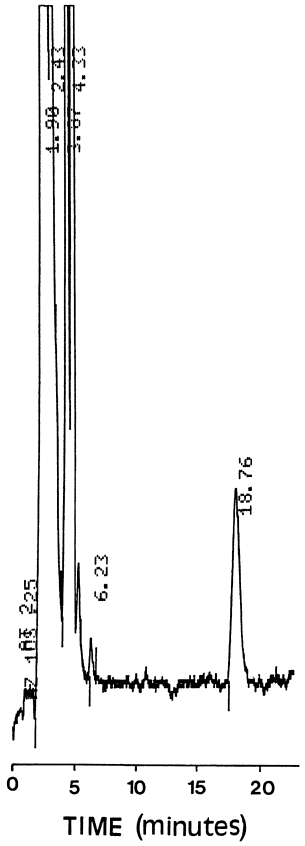

Fig. 1. Chromatogram of a mouse plasma sample following oral administration of ABZ. Retention time for ABZ is 18.8 min.

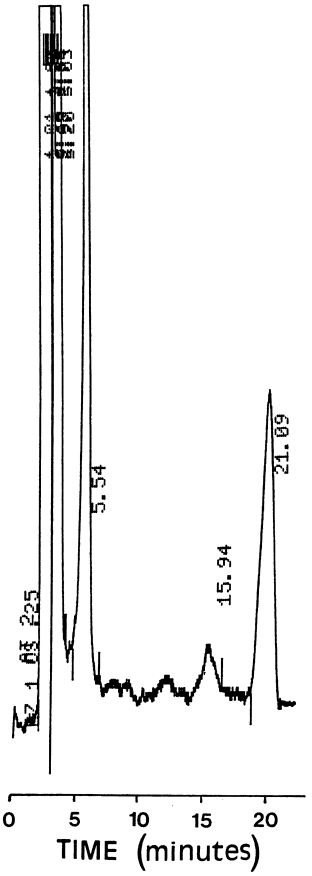

Fig. 2. Chromatogram of a mouse plasma sample showing peaks for SOABZ (5.5 min), SO_2ABZ (15.9 min) and an unknown substance (21.1 min).

after oral administration of ABZ. Retention times for SOABZ and SO_2ABZ are 5.5 and 15.9 min. The retention time with this method for ABZ is too long (more than 40 min) which means that there are practical problems in quantifying the low concentrations of ABZ which are present after oral administration. In fact, in some articles about the pharmacokinetics of ABZ, the analytical methods were not sensitive enough to assay ABZ, and bioavailability of ABZ after oral administration was studied based on its main metabolite SOABZ which is also active [2,15–16]. In order to assay ABZ, SOABZ and SO_2ABZ at least two different HPLC methods are required.

Fig. 3 shows in a semilogarithmic scale the pharmacokinetic profile of ABZ, SOABZ and SO_2ABZ in mice following oral administration of ABZ (12 mg/kg). As was previously reported in other animal species [10–13,15,16] SOABZ is the main metabolite in mouse plasma. Its concentration is higher than ABZ, although it was possible to detect ABZ in this study. C_{max} for ABZ and SOABZ are obtained at 15 min. These t_{max} are probably due to the good bioavailability characteristics of the liquid formulation of ABZ-cyclodextrin. Cyclodextrins are currently used as solubilisers and absorption enhancers and a similar effect on the absorption of mebendazole has been reported previously [17]. Metabolisation of ABZ to SOABZ is a fast process which can be done in the gut prior to absorption or following oral absorption by a first-pass hepatic

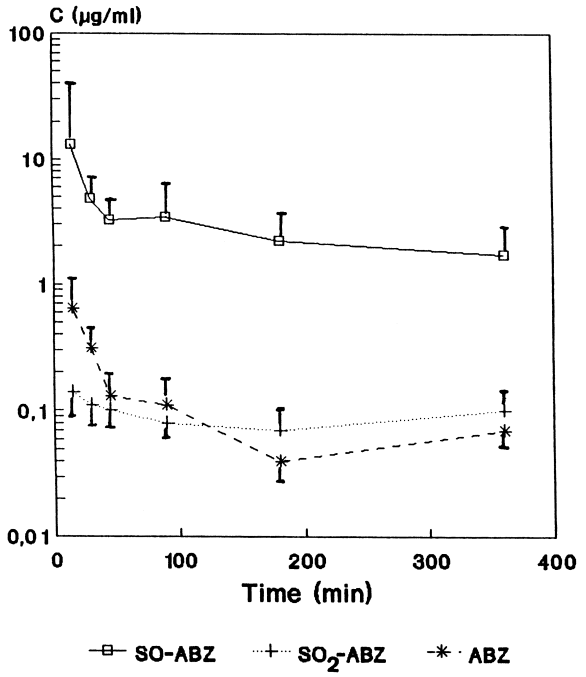

Fig. 3. Semilogarithmic scale of SOABZ, SO_2ABZ and ABZ plasma concentrations versus time following oral administration of ABZ in mice (mean values and standard deviations of six animals).

process as was reported by Galtier et al. [15]. For these two reasons, rapid absorption and fast metabolisation rate, ABZ and SOABZ concentration levels are maximum at the first experimental point available (15 min). SO_2ABZ concentrations following oral administration are low. t_{max} For SO_2ABZ is not clear under our experimental conditions. In other animal studies t_{max} for SO_2ABZ has been reported to be between 6 and 30 h [6,10–13,15].

In order to quantify the two enantiomers of SOABZ, we have used the same method reported by Lienne et al. [5] with a few modifications. Wavelength of detection has been changed from 220 nm to 291 nm to facilitate baseline balance. 2-Propanol at 2% concentration is proposed as a component of the mobile phase to quantify low concentrations of enantiomers. Table 1 shows the characteristics of the chromatographic methods depending on the percentage of 2-propanol in the mobile phase (0, 1 or 2%). Fig. 4 shows chiral resolution of SOABZ of a mouse

Table 1
Influence of the addition of 2-propanol to the aqueous mobile phase on the resolution of SOABZ enantiomers

2-Propanol (%,v/v)	k_2' [a]	α [b]	R_s [c]	Quantitation limit (µg/ml)	
				(+)	(−)
0	13.1	2.4	2.2	0.41	0.21
1	4.6	2.0	2.0	0.13	0.10
2	2.5	1.6	1.1	0.05	0.04

[a] The retention factor k_2' (of the second enantiomer eluted) was calculated as follows: $k_2'=(t_{R2}-t_0)/t_0$, where t_0 was measured by injection of methanol.

[b] Selectivity $\alpha=k_2'/k_1'$.

[c] R_s (Resolution factor)=2 (distance between the two enantiomer peak position/sum of the band widths of the two peaks at their bases); $R_s=2(t_{R2}-t_{R1})/(w_1+w_2)$.

plasma sample after oral ABZ administration when 2% of 2-propanol is used in the mobile phase.

Table 1 shows how when 2-propanol is added, retention factor (k'), selectivity (α), resolution factor (R_s) and quantitation limits change. For high SOABZ concentration, the best chromatographic conditions are obtained when 2-propanol is not added, as was previously described by Lienne et al. [5]. Unfortunately, in the pharmacokinetic studies, very low

Fig. 4. Chromatogram showing chiral resolution of SOABZ in a mouse plasma sample following oral administration of ABZ.

concentrations of SOABZ are collected for the enantiomeric assay. For this reason 2-propanol at 2% was selected as the mobile phase because at this concentration quantitation limits are lower. The experimental quantitation limit was found to be between 0.04 and 0.41 μg/ml depending on the enantiomer and percentage of 2-propanol in the mobile phase.

Fig. 5 shows the enantiomeric proportions of (+) and (−) SOABZ. These proportions are similar to those reported by Delatour and coworkers in rats [6,11] and are different to sheep, goats, dogs, cattle and man [6,10–13]. Enantiomer (−) SOABZ is dominant in rats and mice, while (+) SOABZ is dominant in sheep, goats, dogs, cattle and man. In addition, the plasma concentration ratio (+)/(−) is not a constant and changes with time. The enantiomeric ratio at time 0 is not accessible experimentally, but by extrapolation of the curves it can be considered to be close to a racemate in mice. These are the enantiomeric proportions in the liquid formulation. The plasma concentration ratio (+)/(−) decreases with time and at 24 h is approximately 25/75%. Differences in enantiomer metabolisation can be due to enantioselective enzymatic processes of SOABZ [11].

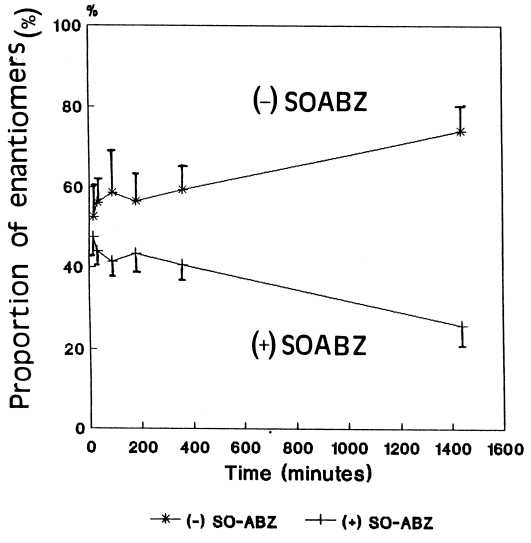

Fig. 5. Evolution versus time of the percentage of the enantiomers of SOABZ in mouse plasma (mean values and standard deviations of six animals).

It can be concluded from the present work that for a complete ABZ pharmacokinetic study several chromatographic methods can be used. We propose the use of three HPLC methods instead of just one or two as has been done in most of the papers already published. First, ABZ should be quantified. Then, SOABZ, the main and active metabolite of ABZ, and SO_2ABZ must also be quantified. Finally, proportions of enantiomers (+) and (−) of SOABZ can also be determined. It can be emphasised that no complex preparation steps are required with the three HPLC analytical methods proposed in this work for pharmacokinetic assay of ABZ and its main metabolites.

Acknowledgements

The authors thank Dr. Torrellas (Smith-Kline Beecham) for ABZ and SO_2ABZ samples and Mr. C. Picornell (Chemo Ibérica) for SOABZ samples.

References

[1] B. Zaber, R.J. Gyurik, A.W. Chow, B. Zaber, E.L. Brunner, J.A. Miller, A.J. Villani, L.A. Petka, R.C. Parish, Drug Metab. Dispos. 9 (1981) 503.
[2] M.L. Lopez-Garcia, S. Torrado-Duran, J. Torrado-Duran, A.R. Martinez Fernandez, F. Bolas-Fernandez, Int. J. Parasitol. 27 (1997) 781.
[3] S. Torrado, M.L. Lopez, G. Torrado, F. Bolas, S. Torrado, R. Cadorniga, Int. J. Pharm. 156 (1997) 181.
[4] S. Rueda-Polo, J. Torrado, F. Bolas, S. Torrado, J. Liq. Chromatogr. Rel.Technol. 21 (1998) 2327.
[5] M. Lienne, M. Caude, R. Rosset, A. Tambute, J. Chromatogr. 472 (1989) 265.
[6] P. Delatour, E. Benoit, M. Caude, A. Tambute, Chirality 2 (1990) 156.
[7] S.E. Marriner, D.L. Morris, B. Dyckson, J.A. Bogan, Eur. J. Clin. Pharmacol. 30 (1986) 705.
[8] G.B.A. Okelo, B. Hagos, J.N. Ngánga, J.O. Ogeto, East African Med. J. 70 (1993) 643.
[9] M. Hurtado, M.T. Medina, J. Sotelo, H. Jung, J. Chromatogr. 494 (1989) 403.
[10] E. Benoit, S. Besse, P. Delatour, Am. J. Vet. Res. 53 (1992) 1663.
[11] P. Delatour, E. Benoit, S. Besse, A. Boukraa, Xenobiotica 21 (1991) 217.
[12] P. Delatour, F. Garnier, E. Benoit, I. Caude, Res. Vet. Sci. 50 (1991) 134.

[13] P. Delatour, E. Benoit, F. Garnier, S. Besse, J. Vet. Pharmacol. Ther. 13 (1990) 361.
[14] United States Pharmacopoeia 23, National Formulary 18, Suppl. 1, 1994, p. 2424.
[15] P. Galtier, M. Alvinerie, J.L. Steimer, P. Francheteau, Y. Plusquellec, G. Houin, J. Pharm. Sci. 80 (1991) 3.
[16] T. Zeugin, T. Zysset, J. Cotting, Ther. Drug Monit. 12 (1990) 187.
[17] Y. Chiba, N. Kohri, K. Iseki, K. Miyazaki, Chem. Pharm. Bull. 39 (1991) 2158.