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Bioavailability and efficacy characteristics of two different oral liquid formulations of albendazole

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Abstract

The oral bioavailability and anthelmintic efficacy in mice of a new formulation of albendazole (ABZ) dissolved in a solution of hydroxypropyl-β-cyclodextrin (HPCD) are compared with a conventional ABZ suspension of carboxymethylcellulose. Plasma concentrations of ABZ and albendazole sulphoxide (ABZ-SO), its active and main metabolite, were assayed by HPLC. The $AUC_{0-\infty}$ and C_{max} values obtained for both ABZ and ABZ-SO, after administration of the ABZ–HPCD solution were significantly higher ($P < 0.01$) than those obtained from the ABZ suspension. Although, the differences between the ABZ and ABZ-SO-T_{max} values were found not to be significant, regardless of the formulation. The anthelmintic activities against enteral (pre-adult) and parenteral (migrating and encysted larvae) stages of *Trichinella spiralis* were studied in mice. The ABZ solution was more efficient against pre-adult and encysted larvae than the ABZ suspension. The efficacy differences between both formulations against the migrating larvae, were found to be not significant ($P < 0.05$). For the migrating parasite stage, there was a linear correlation between the anthelmintic activity and pharmacokinetical parameters with respect to the ABZ-$AUC_{0-\infty}$ value. Meanwhile, for the muscular encysted parasite stage, better relationships were obtained for $AUC_{0-\infty}$ and C_{max} values from ABZ-SO, which had correlation coefficients of 0.996 and 0.987, respectively.

Keywords: Albendazole; Bioavailability; Activity; Efficacy; Cyclodextrin; Pharmacokinetic

1. Introduction

Albendazole (ABZ), methyl [5-(propyl-thio)-1-H-benzimidazole-2yl] carbamate, is a broad spectrum antihelmintic drug with low aqueous solubility, which may limit its oral absorption (Jung et al., 1998). In order to prepare ABZ solutions, different formulation approaches have been ex-

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perimented in the last years. For instance, since ABZ is basic in nature, its solubility could be increased by ionisation in an acid medium, although this increase in solubility is not enough for the preparation of high ABZ concentration doses. Another way of increasing its solubility is by addition of surfactant agents, such as polysorbate and bile salts, or cosolvent agents, like Transcutol® (Torrado et al., 1996a, 1997; Redondo et al., 1998). Some of these excipients may also have some absorption enhancer effects, which can be useful on increasing the oral bioavailability of ABZ formulations. Unfortunately, many of these agents can also be irritant to the digestive system linings and so its use must be restricted whenever possible. Alternatively, the ABZ solubility can be improved by elaboration of solid dispersions with polyvinylpyrrolidone (Torrado et al., 1996b; Lopez et al., 1997); although the elaboration method, rotatory evaporator and the use of organic co-solvents, and the high quantity/concentrations of the complexing agent are the application limiting factors for these products. Recently, the use of hydroxypropyl-β-cyclodextrin (HPCD), has facilitated the formulation of high concentration of ABZ solutions (Castillo et al., 1999; Piel et al., 1999).

The aim of this paper, is to compare the bioavailability and anthelmintic activities/characteristics of HPCD–ABZ solution-formulation to a conventional reference ABZ suspension in sodium carboxymethylcellulose. Moreover, the correlation between the bioavailability and anthelmintic efficacy was studied. Thus, different doses of ABZ (50–100 mg/kg) were orally administered to mice and blood samples were taken for analysis of ABZ concentration and its main metabolites by HPLC (Garcia et al., 1999). Furthermore, the anthelmintic activity of ABZ formulations was studied in the *Trichinella spiralis* model in mice at three different stages (pre-adult, migrating and encysted larvae) of the parasite infection. This in vivo model allows us to study the efficacy at both the enteral stage (pre-adult) and at the parenteral stage (migrating and encysted larvae). Therefore, the model can be used to verify whether a certain formulation might be indicated for various stages of helminthic infections or not (López-García et al., 1997, 1998).

2. Materials and methods

2.1. Chemicals

ABZ and albendazole sulphone (ABZ-SO_2) were kindly supplied by Glaxo-Smithkline (England). Albendazole sulphoxide (ABZ-SO) was a gift from Chemo Ibérica (Madrid, Spain). HPCD was supplied by Cerestar Ibérica (Barcelona, Spain). All the products and materials used in this study comply with the pharmaceutical and analytical standards, respectively.

2.2. ABZ formulations (8 mg/ml) and determination of dispensing error

- ABZ solution was prepared by dissolution of ABZ in a solution of 20% (w/v) HPCD in 0.2 M HCl. The final pH of the solution was 0.92.
- ABZ suspension was prepared in 0.5% (w/v) sodium-carboxymethylcellulose of low viscosity grade (Panreac, Spain).

Dispensing error was determined by injecting/dosing ten samples. In order to achieve an adequate homogeneity the ABZ suspension was stirred by means of a magnetic stirrer while sampling. Then, the samples were assayed by HPLC and the coefficient of variation of the mean value was used as the dispensing error.

2.3. Oral bioavailability study

Swiss CD-1 mice weighing 30–35 g were employed. Food and water were supplied ad libitum. The formulations were administered orally via bucco–gastric tube. Each ABZ dosage form (solution or suspension) was administered at doses of 50 and 100 mg/kg. After drug administration, the mice were divided into groups of at least six, and later the animals were sacrificed and blood samples were collected at the following time intervals: 0.25, 0.5, 0.75, 1.5, 3, 6 and 24 h. Lastly, the blood samples were heparinized and centrifuged individually. After the extraction process, the blood samples were frozen until HPLC analysis.

2.3.1. HPLC analysis

A modular liquid chromatograph equipped with a Gilson 305 and 306 isocratic pump, an automatic sampler (Gilson 231 XL) fitted to a 100 μl sampler loop (Rheodyne), a variable wavelength detector (UV-1575, JASCO) and an integrator (Spectra-Physics SP-270) was used.

In order to precipitate proteins, 2 ml of methanol were added to each aliquot (0.4 ml) of plasma sample. After vortex-mixing for 1 min, samples were centrifuged at $3000 \times g$ for 10 min and filtered through PVDF Durapore® 0.45 μm filter (Millipore). In order to quantify ABZ concentration, 100 μl of the filtered aliquot-fractions were injected into the HPLC system.

The ABZ assay was conducted according to the HPLC methods described in USP 24 (2000), using a 5 μm C_{18} (Hypersil®) 250 × 4.6 mm column and a mobile phase containing 5.5 g of Na_2HPO_4 dissolved in 400 ml of water and mixed with 600 ml of methanol at a flow rate of 1 ml/min. Samples were analysed at 291 nm. Under these conditions, the retention time was 15 min. Validation data for the method can be summarised as follows, the linearity was studied at the range between 0.01–1 μg/ml and the correlation coefficients were approximately 0.99. The obtained ABZ quantitation limit (signal-to-noise ratio = 5) and inter-day precision were 4 ng/ml and 2.9%, respectively.

ABZ-SO and ABZ-SO_2 are the two main metabolites of ABZ and both of them were assayed basing on the method previously described by Garcia et al. (1999). Hypersil® BDS ODS2 column (250 × 4.6mm, 5 μm) and a mobile phase containing 800 ml of water with 188 μl of phosphoric acid 85% and 200 ml of acetonitrile at a flow rate of 1.5 ml/min were used. The obtained samples were measured at 290 nm. Under these conditions, the retention times were 8.5 and 13 min for ABZ-SO and ABZ-SO_2, respectively. Validation data for the method are summarised as following, the linearity was studied in the range between 0.01 and 1 μg/ml and the obtained correlation coefficients were approximately 0.99. The quantitation limits for ABZ-SO and ABZ-SO_2 (signal-to-noise ratio = 5), were 13 and 10 ng/ml, respectively. Whilst, the inter-day precision were 4 and 4.4%, for ABZ-SO AND ABZ-SO_2, respectively.

In order to proceed with the quantitative assay on (+)ABZ-SO and (−)ABZ-SO, the samples were first assayed by the previously described non-chiral procedure and liquid samples were collected in accordance with the ABZ-SO retention time. Then, the samples were concentrated to dryness in vacuum at 70 °C using a Savant Speedvac® concentrator. Then the samples were dissolved in 1 ml of mobile phase and filtered through PVDF Durapore® 0.45 μm filter (Millipore). The chiral assay was performed according to the method previously described by Garcia et al. (1999). A chiral-AGP column (100 × 4 mm, 5 μm) and a mobile phase containing sodium phosphate buffer (8 mM, pH 7.0) with 1.25 ml of 2-propanol at a flow rate of 0.9 ml/min were used. The collected samples were analysed at 290 nm. Under these conditions, retention times for (−)ABZ-SO and (+)ABZ-SO were 2.0 and 3.1 min, respectively. The validation data for the chiral assay method can be summarised as follows: the linearity studied in the range between 0.01 and 1 μg/ml and the obtained correlation coefficients were at least 0.99. On the other hand, the (−)ABZ-SO and (+)ABZ-SO quantitation limit and inter-day precision for the two enantiomers are similar such that 3 ng/ml and 3.5%, respectively.

2.3.2. Bioavailability parameters

T_{max} and C_{max} were estimated as the mean values of time (T_{max}) taken to achieve maximum plasma concentrations (C_{max}) for the six mice with the highest ABZ plasma concentration for each administered formulation. The $AUC_{0-\infty}$ was calculated, for ABZ and ABZ-SO, as the sum of AUC_{0-24} and $AUC_{24-\infty}$. Also, the AUC_{0-24} was calculated by the trapezoidal rule method and $AUC_{24-\infty}$ was estimated as the quotient between C_{24} and K_e. The K_e was calculated as the slope, from the final phase of the log concentration–time curves. Comparative statistical studies on the bioavailability between the two formulations were performed by a one-way analysis of variance (ANOVA) test, and the possible correlation between the bioavailability parameters and the anthelmintic efficiency was studied by linear

correlation analysis and the corresponding correlation coefficients were estimated (Statgraphics, version 4.0).

2.4. Evaluation of the efficacy of ABZ dosage forms on Trichinella spiralis model

The GM-1 isolate of *T. spiralis* was used. The isolate was isoenzymatically identified at *T. spiralis* (Reference Centre for Trichinellosis Istituto Superiore di Santá, Rome) and kept under the code MFEL/ES/S2 GM-1-ISS48 (La Rosa et al., 1992). In order to evaluate the anthelmintic activity of the formulations, groups of ten mice per treatment-dose were orally infected with 300 ± 50 L1 muscle larvae isolated from infected mice carcasses, following an artificial digestion as described by Martínez-Fernández (1978). The same doses of ABZ formulations (suspension or solution) were administered orally by buccogastric tube. Groups of ten mice per formulation and parasite stage were kept as controls, and were administered with vehicle alone. Treatments were applied at three different stages (pre-adult, migrating larvae and encysted larvae) of the parasite. For the pre-adult parasite stage, the formulations were administered 24-h post-infection (p.i.) at 5 and 10 mg/kg.

For treatment of the migrating larvae, first of all, it was necessary to remove the adults remaining in the gut without affecting the migratory new born larvae (Denham and Martinez, 1970). This was achieved by treating both, the control and experimental groups, on day 9 p.i., with trichophon (Neguvon, Bayer S.A., Spain) at 90 mg/kg administered orally plus one intramuscullar injection of atropine sulphate (Bayer S.A.), at 1 mg/kg. Later on, the ABZ formulations were administered at 50 and 100 mg/kg on days 13, 14 and 15 p.i. Treatment against encysted larvae was given at 50 and 100 mg/kg, on days 34, 35 and 36 p.i.

The effectiveness of the treatment against pre-adult stages was assessed on day 6 p.i., after sacrificing the mice (previously anaesthetised with ether) by cervical dislocation. The numbers of adult worms remaining in the gut were collected and counted in accordance with the method described by Denham and Martinez (1970).

The measurement of drug effects on the migrating larvae, was carried out on day 30 p.i. by sacrificing the mice; the animals were skinned, eviscerated and their carcasses processed to free the muscle larvae, as described by Martínez-Fernández (1978). A similar procedure was followed for the estimation of the drug effectiveness against the encysted larvae, except that the animals sacrifice and larval counting were carried out on day 46 p.i.

The comparative statistical anthelmintic efficiency studies between the different drug formulations were performed by paired student t-test. A P value of less than 0.05 was considered as significant.

3. Results and discussion

Fig. 1 shows the mean ABZ drug concentrations after the oral administration of the two different liquid formulations at doses of 50 and 100 mg/kg. It is clear from this figure that the cyclodextrin solution improves ABZ oral bioavailability in relation to the conventional oral suspensions. Due to fast ABZ degradation rate, its plasma concentrations are very low. Under our experimental conditions, its apparent elimination constant was 0.33 ± 0.1 per h. For this reason, in our previous studies, the bioavailability of different ABZ drug formulations has been studied by using the ABZ-SO concentration data, which is its main

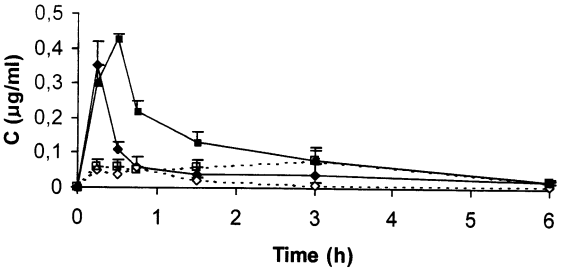

Fig. 1. Mean ABZ plasma concentration–time profiles after oral administration of two ABZ formulations at doses of 50 and 100 mg/kg. Each point represent the average ± S.D. of six experiments. Key: ◆, solution at 50 mg/kg; ◇, suspension at 50 mg/kg; ■, solution at 100 mg/kg and □, suspension at 100 mg/kg.

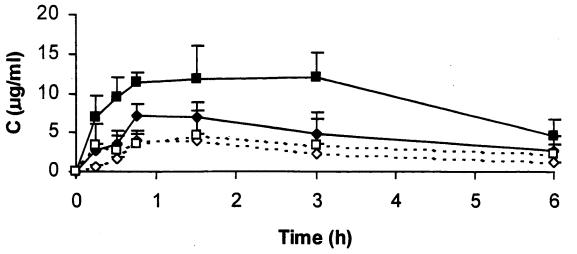

Fig. 2. Mean ABZ-SO plasma concentration–time profiles after oral administration of two ABZ formulations at doses of 50 and 100 mg/kg. Each point represents the average ± S.D. of six experiments. Key: ◆, solution at 50 mg/kg; ◇, suspension at 50 mg/kg; ■, solution at 100 mg/kg and □, suspension at 100 mg/kg.

active metabolite (Torrado et al., 1997). Fig. 2, shows the ABZ-SO plasma levels for the ABZ solution and suspension formulations for the two studied doses. These results are similar to those reported for ABZ, since high drug concentrations were obtained from the administration of cyclodextrin liquid solutions compared with the ABZ suspensions. But ABZ-SO is a chiral molecule with two enantiomers that may have different activity. Therefore, for comparative purposes, it is important to study their proportions at different time-intervals, in order to verify the possible drug formulation effect on the ABZ-SO enantiomerical proportions. Fig. 3 shows how after the oral administration of the different ABZ formulations to mice, the (−)ABZ-SO enantiomer results in a slower drug degradation rate compared with the (+)ABZ-SO enantiomer and hence the (−)ABZ-SO becomes the predominant enantiomerical form in vivo. These proportions are similar to those previously reported in rats (Delatour et al., 1990a, 1991a), and more recently in mice (Garcia et al., 1999). The (−)ABZSO enantiomer is predominant in rats and mice, while (+)ABZSO is predominant in sheep, goats, dogs, cattle and man (Delatour et al., 1990a,b, 1991a,b; Benoit et al., 1992). Under our experimental conditions, no significant differences ($P < 0.05$) in respect to the ABZ-SO enantiomerical proportions, were found among the formulations. Consequently, the mean ABZ-SO results can be used for the correlation studies between bioavailability and efficiency.

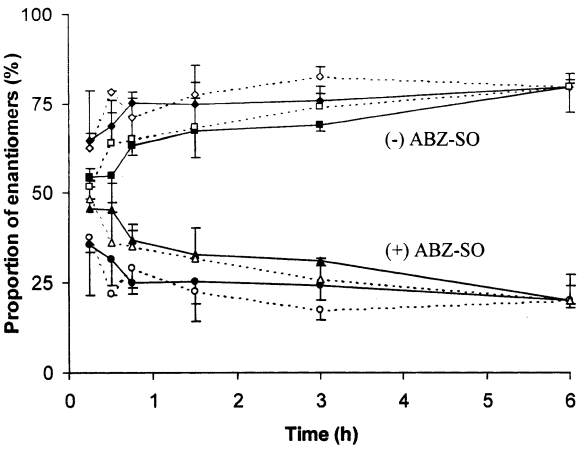

Fig. 3. Evolution vs. time of the enantiomeric proportions (%) of the ABZ-SO enantiomers in mouse plasma (mean values and S.D. of six animals) for the two formulations at the doses of 50 and 100 mg/kg. Key: ◆, (−)ABZ-SO solution at 50 mg/kg; ◇, (−)ABZ-SO suspension at 50 mg/kg; ■, (−)ABZ-SO solution at 100 mg/kg; □, (−)ABZ-SO suspension at 100 mg/kg; ●, (+)ABZ-SO solution at 50 mg/kg; ○, (+)ABZ-SO suspension at 50 mg/kg; ▲, (+)ABZ-SO solution at 100 mg/kg and △, (+)ABZ-SO suspension at 100 mg/kg.

Low $ABZSO_2$ concentrations were obtained after the HPLC assay, usually less than 5% of the ABZSO value. In order to simplify the manuscript the plasma concentrations of the inactive $ABZSO_2$ are not included.

Finally, Fig. 4 shows the anthelmintic efficacy results for both formulations, ABZ solution and suspension, in the three different *T. spiralis* stages: adult worms, migrating and encysted larvae. In terms of efficacy, there is a significant improvement ($P < 0.05$), when ABZ solution was used compared with ABZ suspension at the adult worms and encysted larvae stages. The effect of the ABZ solution against encysted larvae is of great importance, since this formulation can useful for treatment of inoperable or disseminated cases of other systemic helminthic infections such as hydatidosis or neurocysticercosis. The anthelmintic activity of both formulations against the enteral parasite stages could be related to their bioavailability properties.

Tables 1 and 2 show the effectiveness, expressed as the mean percentages of parasites reduction, and some bioavailability parameters, $AUC_{0-\infty}$,

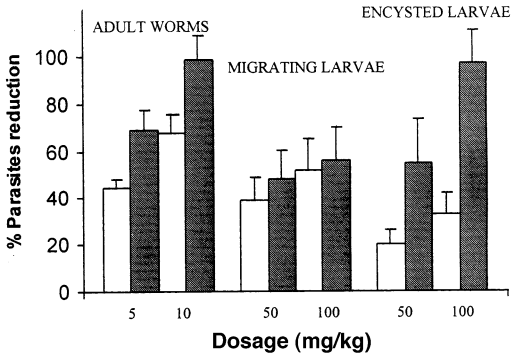

Fig. 4. Antiparasitic efficacy results expressed as the percentages of reduction in parasite loads obtained for the two formulations, ABZ solution (white bars); and ABZ suspension (white/crossed bars), at different doses on the three *T. spiralis* life stages. The values are expressed as the mean and standard deviation for ten animals.

T_{max} and C_{max} for ABZ and ABZ-SO, for the two liquid formulations at the two assayed doses. From these studies, it is evident that the HPCD solution with respect to CMC suspension, significantly ($P < 0.01$) increases the $AUC_{0-\infty}$ and C_{max} values for both ABZ and its main active metabolite ABZ-SO, though the T_{max} values for both ABZ and ABZ-SO were no significantly different ($P < 0.05$). Similar bioavailability characteristics of ABZ liquid formulations has previously been reported (Castillo et al., 1999; Evrard et al., 1998; García-Rodriguez et al., 2001).

The correlation coefficients between the bioavailability parameters and the anthelmintic efficacy for the migratory and encysted parasite stages are also shown in Tables 1 and 2. It is also clear that T_{max} can not be adequately correlated with the activity/drug efficacy tested on the two studied infection stages. However, the $AUC_{0-\infty}$ and C_{max} values, under certain conditions could be well correlated with the anthelmintic activity. Previously, López-García et al. (1997), showed that ABZ has a more potent antihelmintic activity than its active metabolite ABZ-SO. According to this criteria, a better correlation between activity and ABZ bioavailability parameters would be expected than between activity and ABZ-SO pharmacokinetical parameters. For the migrating parasite stage there was neither significant ($P < 0.05$) improvement effect between the two tested liquid formulations nor the ABZ-SO pharmacokinetical characteristics/parameters have good correlation with the activity, but the ABZ–$AUC_{0-\infty}$ could be significantly correlated with activity.

However, for the muscular parasite stage, both the $AUC_{0-\infty}$ and C_{max} values of ABZSO are better linearly correlated with activity than the ABZ bioavailability parameters. The possible explanation for this better correlation relationship

Table 1
Correlation between the anthelmintic efficiency on the *T. spiralis* at the migrating stage and the bioavailability properties, for both ABZ and its main active metabolic ABZ-SO, for two liquid formulations of ABZ: as suspension in CMC and solution of HPCD, at two doses (50 and 100 mg/kg)

	Efficiency reduction (%)	ABZ			ABZ-SO		
		$AUC_{0-\infty}$ ** (μg h/ml)	C_{max} ** (μg/ml)	T_{max} (h)	$AUC_{0-\infty}$ ** (μg h/ml)	C_{max} ** (μg/ml)	T_{max} (h)
CMC-50	39.2	0.20	0.07	0.5	30.9	4.8	1.25
HPCD-50	48.4	0.49	0.35	0.25	57.2	7.9	1.75
CMC100	51.9	0.73	0.09	0.75	42.4	5.1	2
HPCD-100	55.8	0.99	0.39	0.33	100.2	14.4	1.75
Correlation coefficient	–	0.97*	0.61	−0.07	0.79	0.7	0.83

The possible statistical significant differences between the bioavailability parameters (AUC, C_{max} and T_{max}) obtained either from CMC or HPCD formulations were studied by ANOVA test, while the possible correlation between these bioavailability parameters and the anthelmintic efficiency were studied by linear correlation. Key: *, Significant ($P < 0.05$); **, significant ($P < 0.01$).

Table 2
Correlation between the anthelmintic efficiency on the *T. spiralis* encysted larval stage and the bioavailability properties, for both ABZ and its main active metabolite ABZ-SO, for two liquid formulations of ABZ: as suspension in CMC and solution of HPCD, at two doses (50 and 100 mg/kg)

	Efficiency reduction (%)	ABZ			ABZ-SO		
		$AUC_{0-\infty}$ ** (μg h/ml)	C_{max} ** (μg/ml)	T_{max} (h)	$AUC_{0-\infty}$ ** (μg h/ml)	C_{max} ** (μg/ml)	T_{max} (h)
CMC-50	19.9	0.20	0.07	0.5	30.9	4.8	1.25
HPCD-50	55.1	0.49	0.35	0.25	57.2	7.9	1.75
CMC100	33.0	0.73	0.09	0.75	42.4	5.1	2
HPCD-100	97.5	0.99	0.39	0.33	100.2	14.4	1.75
Correlation coefficient	–	0.81	0.90	−0.59	0.99**	0.99*	0.35

The possible statistical significant differences between the bioavailability parameters (AUC, C_{max} and T_{max}) obtained either from CMC or HPCD formulations were studied by ANOVA test, while the possible correlation between these bioavailability parameters and the anthelmintic efficiency were studied by linear correlation. Key: *, Significant ($P < 0.05$); **, significant ($P < 0.01$).

between ABZ-SO anthelmintic activity and pharmacokinetic parameters on the encysted larvae stage with respect to ABZ, may be related to the differences in drug concentration and elimination constants. In Figs. 1 and 2, we can observe that there are higher plasma concentration and lower elimination constant values for ABZ-SO than ABZ. As a result, higher $AUC_{0-\infty}$ values are obtained for the ABZ-SO than for ABZ (see Tables 1 and 2). The AUC higher values (ca. 108 times) may sufficiently compensate the observed differences on activity. Moreover, the longer permanence of the ABZ-SO molecules in the body may facilitate the access to the muscular encysted parasites, and hence justifying its better correlation with anthelmintic activity.

Another important difference between the two liquid formulations, is the significant ($P < 0.01$) low value of the dispensing error for the solution in comparison to the suspension. The syringe volumetric error during the solution dispensing is 1.3%, and the dispensing error for the suspension formulation is 12.4%, since suspension stirring during dispensing process, alone, is not sufficient enough to achieve an uniform solution. Nevertheless, this difference on dispensing process between the formulations does not have a clear cut effect on the in vivo results, due to the high variation coefficients of the drug concentrations (usually around 20%), regardless of the administered formulation. The variation coefficients for the anthelmintic activity between are close to 30%, which are higher than those obtained for the drug concentrations. Even though, the variation coefficients for the anthelmintic activity between both types of liquid formulations, are similar.

In conclusion, the ABZ liquid solution elaborated with HPCD has higher $AUC_{0-\infty}$ and C_{max} values than the ABZ suspension. The liquid solution is significantly ($P < 0.05$) more active against *T. spiralis* infection at the enteral and muscular stages than the conventional CMC suspension. On the other hand, for the migrating parasite infection stage, the best correlation coefficient between activity and bioavailability parameters/characteristics are obtained from the ABZ–$AUC_{0-\infty}$ value. Meanwhile, for the muscular parasite infection stage, better correlation coefficients between ABZ-SO activity and pharmacokinetical parameters/characteristics is obtained for the $AUC_{0-\infty}$ and C_{max} values. Contrary, the T_{max} values seem to have no linear correlation with the anthelmintic effects.

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