Clinical & Experimental Pharmacology

Dib et al.,, Clin Exp Pharmacol 2015, 5:6
http://dx.doi.org/10.4172/2161-1459.1000198

Research Open Access

Pharmacokinetic Assessment of Novel Controlled Release Formulations of Ricobendazole Intended for Oral Administration in Dogs

Dib Alicia[1*], Paredes Alejandro[2,3], Eliópulos Natasha[1], Farías Cristina[3,4], Suárez Gonzalo[1], Aldrovandi Ariel[1], Palma Santiago[2,3], Allemandi Daniel[2,3], Lanusse Carlos[3,4] and Sánchez Bruni Sergio[3,4]

[1]*Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universidad de la República, Montevideo, Uruguay*

[2]Department of Pharmacy, Faculty of Chemical Sciences, UNITEFA, Universidad Nacional de Córdoba, Córdoba, Argentina
[3]Consejo Nacional de Investigaciones Científicas y Técnicas-CONICET, Argentina
[4]Laboratory of Pharmacology, Faculty of Veterinary Medicine, CIVETAN-UNCPBA, Tandil, Argentina

[*]**Corresponding author:** Dib Alicia, Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universidad de la República, Montevideo, Uruguay, Tel: 005982 6284215; E-mail: aliciadib2014@gmail.com

Received date: October 31, 2015; **Accepted date:** November 25, 2015; **Published date:** November 30, 2015

Abstract

The aim of this study was to evaluate the influence of different matrices developers on the pharmacokinetic behavior of Ricobendazole (RBZ) controlled release (CR) formulations and test their correlation *in vitro-in vivo*, using one Albendazole (ABZ)-based and one RBZ-based immediate-release formulation as references. The main excipients used for CR formulations were Hydroxypropyl Methyl Cellulose, Cetyl Alcohol, Gelucire 50/02® and Alginic Acid. Pharmacotechnical quality control tests were successfully completed. Twelve parasite-free no pregnant dogs were randomly divided into six groups and received different treatments (single oral doses) using an incomplete block design (two phases) (n=4). Phase I: treatment "A" (ABZ-based immediate-release formulation [25 mg/kg]). Treatment "B" (RBZ-based immediate release formulation [20 mg/kg]) and treatments from "C" to "F" (CR formulations [20 mg/kg]). Phase II was performed after 21 days of washout period. Blood samples were collected over 48 h and analysed by UV High Performance Liquid Chromatography. *In vitro* dissolution profiles showed that matrices agents favored a reservoir effect. Active metabolite Albendazole sulphoxide (ABZSO) or Ricobendazole (RBZ) plasma exposure measured in terms of area under concentration vs time curve (AUC) of all RBZ formulations was greater (p<0,05) when compared with that obtained for ABZ reference formulation. No statistical differences in AUC values were found among all RBZ formulations assayed (p>0.05). Nevertheless, RBZ-Cetyl alcohol formulation showed a statistical difference on its time peak concentration (p<0.05). In conclusion the results obtained *in vitro* do not correlate with those obtained *in vivo*, being this work useful to identify other matrices developers in RBZ-CR formulations.

Keywords: Benzimidazoles; Anthelmintics; Canines; Matrices developers

Introduction

The treatment and control of internal parasitic diseases in small animals are relevant in clinical practice and helminthes parasites epidemiological knowledge is required to understand the pharmacological features of the anthelmintic drugs available in the market. Benzimidazole (BZD) methyl-carbamate compounds have been used to treat parasitic diseases in humans and domestic animals worldwide.

Albendazole (ABZ) is a BZD methyl-carbamate anthelmintic compound effective against lungworms and gastrointestinal nematodes, tapeworms and liver flukes [1,2]. The ABZ active metabolite Albendazole sulphoxide (ABZSO) also known as Ricobendazole (RBZ) intended for oral administration is available in the veterinary market of some South American countries and it is widely used for the treatment of endoparasites in small animals. The active metabolite of ABZ is formed by flavin monooxygenase–mediated sulphonation process (Figure 1) [3].RBZ shows higher bioavailability (+500%) when compared with ABZ [4], being necessary to control its release rate in order to avoid toxicity, because this mentioned higher bioavailability is very close to the toxic level of ABZ itself.

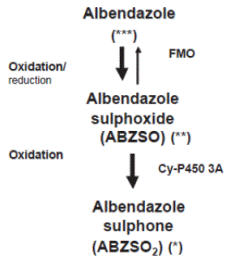

Figure 1: Proposed metabolic pathway for albendazole and metabolites. Oxidation processes are mediated by flavine monooxygenase (FMO) and cytochrome-P450 (CY-P450 3A). The anthelmintic potency of parent drug and metabolites is represented as (*) very poor or no activity, (**) good activity, (***) very good activity.

Oral ingestion in both animals and humans is the traditionally preferred route of drug administration providing a convenient method of effectively achieving both local and systemic effects. In conventional oral drug delivery systems, there is very little control over the drug release. The effective concentration at the target site can be achieved by intermittent administration of grossly excessive doses, which, in most

situations could result in constantly changing, unpredictable, and often subtherapeutic or supratherapeutic plasma concentrations leading to marked side effects. An ideal oral drug delivery system should steadily deliver a measurable and reproducible amount of drug to the target site over a prolonged period.

Controlled-release (CR) delivery systems provide a uniform concentration of drug at the absorption site and thus, after absorption, allow maintenance of plasma concentrations within a therapeutic range, which minimizes side effects and reduces the frequency of administration [5]. Controlled drug delivery occurs when a polymer is combined with a drug or active agent such that the release from the bulk material is pre-designed. The basic rationale of the above-mentioned system is to optimize the biopharmaceutical, pharmacokinetic and pharmacodynamics properties of a drug. Therefore, through reduction in the side effects the cure or control of condition in the shortest possible time by the most suitable route could be possible. It denotes that the system is able to provide some actual therapeutic control, whether this is of a temporal nature, spatial nature, or both [6].

Two hydrophilic polymer matrices based in Hydroxypropyl Methyl Cellulose (HPMC) and Alginic Acid (AA) and two lipid polymer matrices based in Gelucire 50/02® (GE) and Cetyl Alcohol (CA) were designed for this study. HPMC is the most important hydrophilic carrier material used for the preparation of oral controlled drug delivery systems. One of its most important characteristic is the high swellability, which has a significant effect on the release kinetics of an incorporated drug. Upon contact with water or biological fluid, the latter diffused into the device, resulting in polymer chain relaxation with volume expansion [7]. Then, the incorporated drug diffuses out of the system [8]. HPMC is a propylene glycol ether derivative of methylcellulose. The substituent R represents either a $-CH_3$, or a $-CH_2CH(CH_3)OH$ group, or a hydrogen atom (Figure 2).

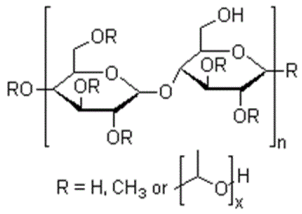

Figure 2: Chemical structure of Hydroxypropyl Methyl Cellulose (HPMC).

Cetyl alcohol, used in pharmaceutical preparations, is a mixture of solid aliphatic alcohols comprising mainly 1-hexadecanol ($C_{16}H_{34}O$). It is widely used in cosmetics and pharmaceutical formulations such as suppositories, modified-release solid dosage forms, emulsions, lotions, creams, and ointments (Figure 3) [7]. Gelucires are a group of inert semisolid waxy amphiphilic excipients, which are surface active in nature and disperse or solubilize in aqueous media forming micelles, microscopic globules or vesicles. Gelucire 50/02® is used as an excipient and has a chemical nature of saturated polyglycolized glycerides. Gelucires have been studied as CR matrices as well as for improvement of physicochemical properties of drugs [9]. The wide varieties of gelucires are characterized by a wide range of melting points from about 33°C to about 64°C and most commonly from about 35°C to about 55°C, by a variety of hydrophilic/lipophilic balance (HLB) values from about 1 to about 14, most commonly from about 7 to about 14. In the designation of gelucire names, (e.g. Gelucire 50/02®) 50 indicates the melting point and 02 its HLB value. As low HLB gelucire can be used to reduce the dissolution rate of drugs, high HLB gelucire can be used for faster release. Gelucire increases the solubility of poorly water-soluble drugs and produces stable slim dispersions on the gastrointestinal walls, giving enhanced drug absorption [10-12]. A study compared the plasma bioavailability in dogs of two oral dosage forms (ABZ formulated as tablets for use in human medicine and ABZ formulated as Gelucire 44/14 capsules). Results did not show and increase bioavailability of Gelucire formulation as expected due to a short gastrointestinal transit in dogs compared with other species including humans that could have limited the absorption process for the drug in this dosage form [13].

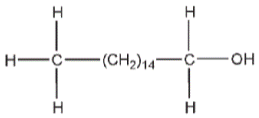

Figure 3: Chemical structure of Cetyl Alcohol.

Alginic acid is a linear glycuronan polymer consisting of a mixture of β-(1-4)-D-mannosyluronic acid and α-(1-4)-L-gulosyluronic acid residues, of general formula $(C_6H_8O_6)n$ (Figure 4). The molecular weight is typically 20.000–240.000. Alginic acid is the main excipient of a variety of oral and topical pharmaceutical formulations. In tablet and capsule formulations, alginic acid is used as both a binder and disintegrating agent at concentrations of 1–5% w/w. In addition, it is widely used as a thickening and suspending agent in a variety of pastes, creams, and gels; and as a stabilizing agent for oil-in-water emulsions [14,15]. The aim of this study was to evaluate the *in vitro-in vivo* influence of different matrices developers on the pharmacokinetic (PK) behavior of RBZ-CR formulations using one ABZ-based and one RBZ-based immediate-release formulations as references.

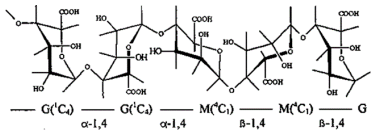

Figure 4: Chemical structure of Alginic Acid.

Material and Methods

Chemicals

Formulation of ABZ (200mg) (Parafarm®, Argentina) used as reference was prepared and pharmacotechnically characterized in a simple solid dispersion (capsules) as reported by Castro et al [16]. RBZ was obtained from *Todo Droga*®, Argentina. All excipients used in formulations development were pharmaceutical grade.

Cetyl alcohol in 5mm pellets was grounded in a mortar and passed through a 30-mesh sieved. Gelucire 50/02® was grounded, deeply frozen and immediately granulated in an oscillating granulator from Erweka®.

Two hundred (500mg) tablets of each formulation based on RBZ (200 mg) were obtained by wet granulation and compressed with a single punch tablet machine DS3® from *Talleres Sánchez*, Argentina. The tablets composition of RBZ formulations are shown in Table 1.

Ingredients (%)	RBZ –IRF (reference)	RBZ-HPMC	RBZ-CA	RBZ-GE	RBZ-AA
RBZ (mg)	200	200	200	200	200
HPMC	-	5	-	-	-
CA	-	-	15	-	-
GE	-	-	-	7.5	-
AA	-	-	-	-	5
SLS					
Lactose	44.5	49.5	32.5	47	44.5
Sodium Chloride	-	-	5	-	5
Explotab®	10	-	-	-	-
Talc	1	1	1	1.5	1
Magnesium Stearate	1.5	1.5	1.5	-	1.5
PVP	3	3	3	3	3

RBZ: Ricobendazole, IRF: immediate release formulation, HPMC: Hydroxypropyl Methyl Cellulose, CA: Cetyl Alcohol, GE: Gelucire 50/02®, AA: Alginic Acid, SLS+ sodium lauryl sulphate, PVP: polyvinylpyrrolidone

Table 1: Tablet composition in percentage (RBZ in mg).

Tableting

Tablet quality control assays

Table 2 illustrates the friability, hardness, *content* uniformity and weight uniformity assays of all RBZ formulations that were carried out under U.S. Pharmacopeial (USP) specifications.

In vitro dissolution assays

Dissolution studies were performed in 900mL of HCl 0.1N at 37°C using a USP XXIV dissolution apparatus 2 (SOTAX AT 7 smart). Basket method at 50rpm was used to avoid flotation. Samples (5mL) were withdrawn at 5, 10, 15, 30, 60, 120, 240, 360 and 480 minutes with replacement of fresh medium, filtered and suitably diluted [17]. Drugs concentration was determined at 289 nm (Evolution 300 UV-Vis Spectrophotometer Thermo Scientific).

In vivo experimental design

Animals: Twelve (n=12) crossbreed dogs (2–8 years old), five males and seven non-pregnant females (30 ± 2.5 kg), were involved in this trial. All animals were gastrointestinal parasites-free demonstrated by two consecutive faecal egg counts reduction test. They had free access to water, were fed with balanced food and were in good health as determined by physical examination before the administration of drugs.

Experimental dogs were randomly allocated into six groups (n=2 each) received six different treatments using an incomplete block design (2 phases separated by 21 days washout period) for obtaining a final experimental n=4 per group. Treatments (ABZ and RBZ immediate-release formulations used as references and CR formulations) were administered in a single oral dose to the experimental animals as illustrated in Table 3.

Groups	G1	G2	G3	G4	G5	G6
Treatments Phase I	(A) ABZ-IRF (reference)	(B) RBZ-IRF (reference)	(C) RBZ-HPMC	(D) RBZ-CA	(E) RBZ-GE	(F) RBZ-AA
Single dose	25 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg
Treatments Phase II	(D) RBZ-CA	(C) RBZ-HPMC	(A) ABZ-IRF (reference)	(B) RBZ-IRF (reference)	(F) RBZ- AA	(E) RBZ-GE
Single dose	20 mg/kg	20 mg/kg	25 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg

ABZ: Albendazole, RBZ: Ricobendazole, IRF: immediate release formulation, HPMC Hydroxypropyl Methyl Cellulose, CA: Cetyl Alcohol. GE: Gelucire 50/02®, AA: Alginic Acid

Table 3: *In vivo* experimental incomplete block design with a final n=4 per group.

Blood samples: Blood samples were taken prior to and following treatments and were collected from the antebrachial vein using a 18 G catheter before administration (time 0) and at 0.25, 0.5, 1, 2, 4, 8, 12, 18, 24, 36 and 48 h after the oral treatments and immediately transferred into heparinized tubes. Plasma was separated by centrifugation at 3000 g for about 15 minutes, placed into plastic tubes and frozen at -20°C until analysis by UV High Performance Liquid Chromatography (HPLC). All animal procedures and management protocols were approved by the Ethics Committee according to the Animals Welfare Policy of the Faculty of Veterinary Medicine, Universidad de la República, Montevideo, Uruguay (www.fvet.edu.uy).

Analytical Procedures

ABZ and ABZSO metabolites analysis

Sample clean up: ABZSO and ABZ-sulphone (ABZSO2) were extracted using disposable C18 columns. Standards of ABZ, ABZSO,

ABZSO2, and Oxibendazole (OBZ) (internal standard) were used for the analytical analysis of blood samples. Ten microliters of OBZ (50 µg/mL) was added to 500 µL of plasma in a glass test tube. Spiked samples were placed into a C18 column preconditioned with 0.5 mL of methanol (Mallinekradt Chemicals) followed by 0.5 mL water, in a vacuum system. Samples were washed (2 mL of water) and then eluted with 2 mL of HPLC-grade methanol. After elution, all samples were concentrated to dryness in a vacuum concentrator and then reconstituted with 200 µL of mobile phase.

HPLC analysis

Experimental and spiked plasma samples (used for validation) were analyzed by HPLC with a UV detector. Fifty microliters of each previously extracted sample was injected and the analytes eluted (flow 1.2 mL/ min) from the analytical column (5 µm, 250 mm × 4.6 mm, C18 column), using a linear gradient method as reported by Sánchez Bruni et al [18]. The compounds were identified by the retention times (minutes) of pure reference standards (ABZSO: 4^{15} min, $ABZSO_2$: 6^{13} min, Oxibendazole: 9^{49} min and ABZ: 12^{20} min). Plasma calibration curves for each analyte were constructed by least squares linear regression analysis giving a correlation coefficient (r) between 0.9987 and 0.9995. The lower limit of quantification of the method was 0.05 µg/mL and the upper quantification limit was 10 µg/mL (ABZSO and $ABZSO_2$). The accuracy of the quality control (QC) samples was within the range of 98 - 101%. The coefficients of correlation or the calibration curves were >0.999.

Pharmacokinetic analysis of the data

The concentration vs time curves for the metabolites ABZSO and $ABZSO_2$ in plasma for each individual animal after the different treatments were fitted with PK Solution 2.0 (Summit Research Services, OH, USA). The equation described by Notari [19], was used to calculate the bioexponential concentration–time curves for ABZSO and $ABZSO_2$ after the oral treatment:

$$Cp = Be^{-\lambda 2t} - Be^{-\lambda 1t}$$

where: Cp = concentration in plasma at time t after administration (µg/ mL); B=concentration at time zero extrapolated from the elimination phase (µg /mL); e=base of the natural logarithm; λ_2= terminal slope (h-1); and λ_1 is the slope obtained by feathering, which represents either the first order absorption rate constant (λ_1) or first order metabolite formation rate constant (λ for) h-1).

The elimination half-life ($t_1/_2\lambda_2$) and absorption ($t_1/_2\lambda_1$) or metabolite formation half-lives ($t_1/_2\lambda$for) were calculated as ln2/λ2 and ln2/λ1, respectively. The peak concentration (C_{max}) and time to peak concentration (T_{max}) were displayed from the plotted concentration–time curve of each analyte. The area under the concentration–time curve (AUC) and area under the first moment curve (AUMC) were calculated by the linear trapezoidal rule [20].

The mean residence time was determined as AUMC/AUC. The mean plasma pharmacokinetic variables for ABZSO y $ABZSO_2$ obtained from the different groups were statistically compared using the non-parametric Kruskal-Wallis test. A value of $p<0.05$ was considered statistically significant.

Results

Pharmacotechnical data

The developed RBZ formulations showed excellent mechanical properties (high hardness, low friability) with very good appearance. In all formulations, a high weight and content were observed (Table 2).

Parameters	RBZ-IRF (reference)	RBZ- HPMC	RBZ- CA	RBZ-GE	RBZ-AA
Weight uniformity (g)	0.51 ± 0.01	0.51 ± 0.03	0.50 ± 0.01	0.50 ±0.04	0.51 ± 0.03
Friability (%)	0.33 ± 0.02	0.23 ± 0.01	0.31 ± 0.02	0.29 ± 0.02	0.19 ± 0.01
Hardness (kg)	5.97 ± 0.91	7.52 ± 1.33	8.31 ± 1.22	6.72 ± 1.13	6.50 ± 1.07
Content uniformity (mg)	187 ± 8.61	183 ± 2.20	202 ± 6.45	186 ± 2.23	191 ± 5.98
RBZ: Ricobendazole, IRF: immediate release formulation, HPMC: Hydroxypropyl Methyl Cellulose, CA: Cetyl Alcohol, GE: Gelucire 50/02®, AA: Alginic Acid.					

Table 2: Quality control data of tested formulations.

In vitro dissolution profiles showed that the presence of matrix agents favored a reservoir effect (Q_{2hs}: RBZ-Alginic Acid: 85%, RBZ-HPMC: 40%, RBZ-Gelucire 50/02®: 30% and RBZ-Cetyl alcohol: 20%), whereas the RBZ-immediate release tablet delivered its full dose rapidly ($Q_{0.5hs}$: 95%) (Figure 5).

Castro et al., using the same method and experimental conditions reported that ABZ formulation presented very low dissolution rate with a maximum of 10% at two hours [16]. Lipid matrices (Gelucire 50/02® and Cetyl Alcohol) showed a slow maximum delivery concentration (8 h) whereas hydrophilic matrices (HPMC and Alginic Acid) showed a delivery profile according to an immediate release behavior. No statistical differences ($p>0.05$) were found when compared between each other.

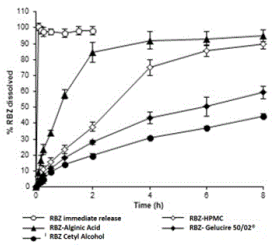

Figure 5: *In vitro* dissolution profiles of RBZ formulations.

Pharmacokinetic data analysis

Plasma PK parameters of ABZSO and inactive metabolite $ABZSO_2$ after oral administration of the six different formulations are shown in Tables 4 and 5 respectively. Albendazole parent drug from ABZ-based immediate-release formulation (treatment A) was not detected in plasma after treatment assayed. The ABZSO plasma exposure after administration was greater when statistically compared with that obtained for treatment A ($p<0,05$), (treatment B: +500% [AUC] and C_{max}+487%; treatment C: [AUC]+600 %, C_{max}+ 500%; treatment D: [AUC] and C_{max}+300%; treatment E: [AUC]+700% and C_{max}+350% and treatment F: [AUC]+350% and C_{max} +250%).

The observed AUC values (0-LOQ) for treatments B, C, D, E and F were 99% and for treatment A was 87% of those AUC values estimated after extrapolation to infinity $_{(0-\infty)}$. The AUC comparative study between RBZ-immediate release formulation and CR different treatments (C, D, E and F) showed minimal statistical differences ($p<= 0.05$).

The comparative plasma PK curves after the oral administration of ABZ and RBZ immediate release formulations with different matrices agents of RBZ CR formulations are shown in Figure 6 (RBZ-Alginic acid), Figure 7 (RBZ-HPMC), Figure 8 (RBZ-Cetyl alcohol) and Figure 9 (RBZ-Gelucire 50/02®).

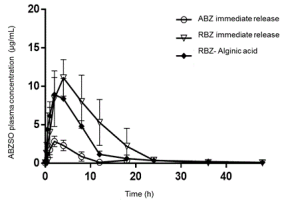

Figure 6: Comparative (mean ± SD) plasma profiles for ABZSO after the oral administration of one ABZ-based immediate release formulation given orally at 25 mg/kg, one RBZ immediate release formulation and RBZ-Alginic acid administered orally at 20 mg/kg.

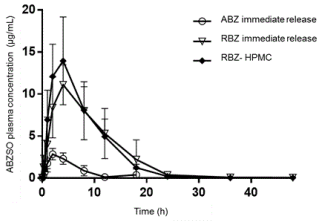

Figure 7: Comparative (mean ± SD) plasma profiles for ABZSO after the oral administration of one ABZ-based immediate release formulation given orally at 25 mg/kg, one RBZ immediate release formulation and RBZ-HPMC (Hydroxypropyl Methyl Cellulose) administered orally at 20 mg/kg.

The time at peak concentration (T_{max}) for RBZ-Cetyl Alcohol showed a statistical difference ($p<0.05$) when compared with the other CR formulations.

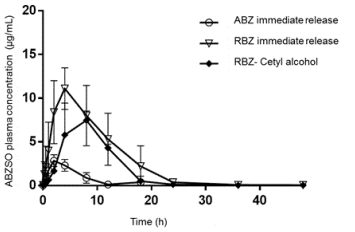

Figure 8: Comparative (mean ± SD) plasma profiles for ABZSO after the oral administration of one ABZ-based immediate release formulation given orally at25 mg/kg, one RBZ immediate release formulation and RBZ-Cetyl alcohol administered orally at 20 mg/kg.

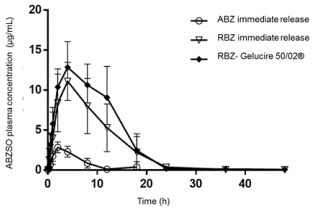

Figure 9: Comparative (mean ± SD) plasma profiles for ABZSO after the oral administration of one ABZ-based immediate release formulation given orally at 25 mg/kg, one RBZ immediate release formulation and RBZ-Gelucire 50/02® administered orally at 20 mg/kg.

Discussion

The pharmacological effects of a drug can generally be observed by a short time after its administration. However, between the administration and the pharmacological effect, the drug must cross rapidly or slowly biological barriers depending of the physical-chemical properties of the molecule given and the nature of the mentioned barrier [21]. The rate of dissolution of Bencimidazoles anthelmintics in the stomach of different animal species is extremely important to achieve an adequate absorption, a consequent bioavailability, a retrograde gastrointestinal (GI) secretion and a high clinical efficacy [21].

The low aqueous solubility of BZD (pKa=7.8) may limit absorption during GI transit [22, 23], and this may be compounded by the short gut transit time of the dog compared to other domestic species [23]. With most conventional drug delivery systems, the drug's level reaches a maximum concentration and then progressively decreases to a subtherapeutic concentration so a new dose administration is necessary. In addition, if the maximum or minimum concentration of drug is located above the level of toxicity or below the minimum effective level, alternately periods of toxicity and ineffectiveness may appear. Albendazole induced toxicosis appeared to be dose related in the dog [24]. The RBZ plasma concentration exhibited a 500% higher bioavailability [4], after a single oral dose (20 mg/kg) [25] when compared with that of ABZ (25 mg/kg). At this point, the therapeutic dose of RBZ can be very close of the ABZ level toxicity.

Controlled drug delivery systems, intended to deliver drugs at predetermined rates for predefined periods, have been used to overcome the shortcomings of conventional drug formulations [26]. In most CR systems, the drug is introduced into a carrier usually known as Polymer. Polymer systems have the advantage to maintain the drug concentration between the toxicity and ineffectiveness levels from a single dose and then release it in a continuous way at a given time. The drug delivery rate is mainly controlled by the physical-chemical properties of the polymer [27,28] but also other factors such as the pH of the medium in which the release of the drug is to be performed may contribute [29].

Both lipids (Gelucire 50/02® and Cetyl Alcohol) and hydrophilic polymers (HPMC and Alginic Acid) matrices effectively modulated the *in vitro* drug release being the lipid formulations, which had the lowest dissolution rate. Even though all CR formulations presented *in vitro* modified-release profiles, the hydrophilic matrices of HPMC and Alginic Acid showed some disintegration, with this being attributable to the low concentration of matrix formers (HPMC and Alginic Acid), which impeded the percolation threshold to be attained in the tablets [30].

All RBZ formulations batches successfully overcame the pharmacotechnical quality control tests, giving great tabletability, high content, weight uniformity and very low friability. Tablets from lipid matrices remained unbroken until the dissolution tests were finished. The presence of a small amount of Sodium Chloride and Sodium Lauryl Sulfate (SLS) (RBZ-Cetyl alcohol and RBZ-Gelucire respectively) as channeling agents in the formulations allowed RBZ to diffuse slowly from the matrix core.

RBZ immediate release tablets delivered rapidly its active ingredient content due to the fast disintegration caused by Explotab® in the formulation [31]. As demonstrated elsewhere [15], ABZ powder showed very low dissolution rate (10% at two hours) due to its extreme hydrophobicity (0.01 mg/mL) [32]. The aqueous solubility of RBZ is approximately six times higher of that of ABZ [33,34].

When ABZ-based immediate release formulation (treatment A) (25 mg/kg) and all the RBZ formulations (treatments B, C, D, E and F) were administered to dogs as a single dose (20 mg/kg) in tablets in this experiment, ABZSO was detected in plasma for 48 h in treatments B (RBZ immediate release formulation), E (RBZ-Gelucire 50/02®) and F (RBZ-Alginic Acid). For treatment C (RBZ-HPMC), the metabolite was detected until 36 h and 24 h for treatment D (RBZ-Cetyl Alcohol) (Table 4).

PK Parameters	**ABZSO Treatment A (ABZ-IRF reference)**	**ABZSO Treatment B (RBZ-IRF reference)**	**ABZSO Treatment C (RBZ-HPMC)**	**ABZSO Treatment D (RBZ-CA)**	**ABZSO Treatment E (RBZ-GE)**	**ABZSO Treatment F (RBZ-AA)**
C_{max} *(µg/mL)*	2.42 ± 1.26[b]	11.1 ± 2.40[a]	14.0 ± 5.16[a]	7.93 ± 1.40[a]	13.41 ± 2.80[a]	9.05 ± 2.30[a]
T_{max} *(h)*	5.34 ± 2.54[b]	4.00 ± 0.00[a]	3.50 ± 1.00[a]	6.50 ± 3.00[b]	4.50 ± 2.50[a]	2.55 ± 1.00[a]
T ½ λ_1 (h)	2.15 ± 1.32[b]	5.25± 0.88[a]	6.15 ± 0.91[a]	5.39 ± 0.77[a]	5.50 ± 0.25[a]	7.12 ± 0.75[a]
$AUC_{(0-\infty)}$ *µg.h/mL*	20.70 ± 7.68[b]	127 ± 60.8[a]	133 ± 45.3[a]	77.4 ± 16.2[a]	167 ± 31.2[a]	79.9 ± 16.1[a]
T ½ λ_2 (h)	2.14 ± 1.38[b]	1.05 ± 0.77[a]	0.56 ± 1.50[a]	2.44 ± 0.46[a]	1.10 ± 0.30[a]	0.20 ± 0.15[a]
MRT (h)	8.24 ± 3.46[a]	9.23 ± 0.35[a]	7.65 ± 1.68[a]	9.33 ± 0.92[a]	9.10 ± 0.86[a]	8.74 ± 1.32[a]
PDP (h)	0.25-18	0.25-48	0.25-36	0.25-24	0.25-48	0.25-48

Albendazole (ABZ), Albendazole sluphoxide (ABZSO), Ricobendazole (RBZ), Hydroxypropyl Methyl Cellulose (HPMC), Cetyl alcohol (CA), Gelucire 50/02® (GE), Alginic acid (AA), Immediate release formulation (IRF). Different superscript letters within each row, indicate statistical differences among groups ($p<0.05$). T½ λ_1: metabolite formation half-life; C_{max}: peak concentration; T_{max}: time at Cmax; $AUC_{(0-\infty)}$: area under the concentration vs. time curve extrapolated to infinity; $AUC_{(0-t)(LOQ)}$: area under the concentration time curve observed from 0 to limit to of quantification (LOQ); T ½ λ_2: elimination half time; MRT: mean residence time; PDP: plasma detection period.

Table 4: Comparative plasma kinetic parameters for Albendazole Sulphoxide after the oral administration of six different formulations in dogs.

The increased systemic concentration of ABZSO is related with its physical and chemical properties when it is compared with its parent drug ABZ [25]. This work showed that the bioavailability of ABZSO after a single oral dose administration was higher not only for the immediate release formulation but also for the CR formulations when compared with that of the parent drug. The AUC comparative study between the different RBZ treatments assayed showed no statistical differences ($p>0.05$). This means that the bioavailability of the active ingredient in the CR formulations is similar despite the presence of matrices developers.

Nevertheless, the peak time concentration (T_{max}) for RBZ-Cetyl Alcohol formulation showed a statistical difference ($p<0.05$) when compared with the other RBZ formulations, which suggested a controlled release *in vivo* performance. Clinical trials in target species to assess efficacy are needed to corroborate this mentioned performance in order to avoid the multidose regimens standardized for 3 to 5 days when using ABZ parent drug in dogs. These clinical trials would also be necessary whether a dose adjustment of RBZ formulations could be thought due to the lower therapeutic index of the drug when it is compared with that of ABZ (Table 5).

PK Parameters	$ABZSO_2$ Treatment A (ABZ-IRF reference)	$ABZSO_2$ Treatment B (RBZ-IRF reference)	$ABZSO_2$ Treatment C (RBZ-HPMC)	$ABZSO_2$ Treatment D (RBZ-CA)	$ABZSO_2$ Treatment E (RBZ-GE)	$ABZSO_2$ Treatment F (RBZ-AA)
C_{max} *(µg/mL)*	0.42 ± 0.32[b]	1.00 ± 0,41[a]	0.93 ± 0.22[a]	0.55 ± 0.41[a]	1.00 ± 3.00[a]	0.50 ± 0.20[a]
T_{max} *(h)*	10.3 ± 3.47[a]	14.0 ± 4.90[a]	9.00 ± 3.83[a]	12.0 ± 0.0[a]	11.0 ± 1.70[a]	1.00 ± 0.70[b]
T ½ λ_1 (h)	2.74 ± 1.98[b]	4.00 ± 1.75[b]	8.46 ± 6.76[a]	5.46 ± 1.23[a]	12.6 ± 3.50[a]	17.4 ± 4.90[a]
$AUC_{(0-\infty)}$ *µg.h/mL*	4.10 ± 1.22[b]	15.63 ± 9.67[a]	16.10 ± 4.40[a]	7.30 ± 3.93[a]	15.48 ± 3.54[a]	8.65 ± 2.98[a]
T ½ λ_2 (h)	2.83 ± 2.05[a]	3.00 ± 0.69[a]	2.23 ± 0.59[a]	10.5 ± 7.56[a]	2.20 ± 0.80[a]	0.40 ± 0.10[b]
MRT (h)	11.7 ± 7.23[a]	13.0 ± 2.94[a]	16.1 ± 8.40[a]	12.2 ± 0.88[a]	18.0 ± 3.40[a]	26.1 ± 7.70[a]
PDP (h)	1.0-18	0.25-48	0.25-36	0.25-24	0.25-48	0.25-48

Albendazole (ABZ), Albendazole Sulphone ($ABZSO_2$), Ricobendazole (RBZ), Hydroxypropyl Methyl Cellulose (HPMC), Cetyl alcohol (CA), Gelucire 50/02® (GE), Alginic acid (AA), Immediate release formulation (IRF). Different superscript letters within each row, indicate statistical differences among groups ($p<0.05$). T½ λ_1: metabolite formation half-life; C_{max}: peak concentration; T_{max}: time at C_{max}; $AUC_{(0-\infty)}$: area under the concentration vs. time curve extrapolated to infinity; $AUC_{(0-t)(LOQ)}$: area under the concentration time curve observed from 0 to limit to of quantification (LOQ); T ½ λ_2: elimination half time; MRT: mean residence time; PDP: plasma detection period.

Table 5: Comparative plasma kinetic parameters for Albendazole Sulphone after the oral administration of six different formulations in dogs.

Authors could not find any previous works that evaluated alternative formulations for this molecule. The outcome of this work would contribute to identify other matrices developers for novel Ricobendazole CR formulations.

Acknowledgement

The authors thank the invaluable support of the people of the Uruguayan Navy Army for providing the animals for this trial.

References

1. Campbell WC (1990) Benzimidazoles: veterinary uses. Parasitol Today 6: 130-133.
2. McKellar QA, Scott EW (1990) The benzimidazole anthelmintic agents--a review. J Vet Pharmacol Ther 13: 223-247.
3. Sanchez Bruni SF, Jones DG, McKellar QA (2006) Pharmacological approaches towards rationalizing the use of endoparasitic drugs in small animals. J Vet Pharmacol Ther 29: 443-457.
4. Dib A, Suarez G, Farias G, Cabrera P, Castro S, et al. (2010) Albendazole sulphoxide kinetic disposition after treatment with different formulations in dogs. J Vet Pharmacol. Therap 34: 136-141.
5. Gupta P, Vermani K, Garg S (2002) Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today 7: 569-579.
6. Srikanth P, Narayana R, Wasim Raja S, Brito Raj S (2013) A review on oral controlled drug delivery. Advanced Pharmaceutics 3: 51-58.
7. Rowe R, Sheskey P, Quinn M (2009) Hydroxypropyl Cellulose, Low-substituted: Handbook of Pharmaceutical Excipients. (6thedn) Pharmaceutical Press,UK.
8. Siepmann J, Peppas NA (2001) Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev 48: 139-157.
9. Sharma D, Singh G, Singh M, Kumar D, Rathore MS (2012) A review on innovative approaches to enhance solubility and dissolution rate of hydrophobic drugs. Novel Science International Journal 7: 486-492.
10. Esquisabel A, San Vicente A, Igartua M, Hernandez M, Gascon A, et al. (1996) Influence of melting point and hydrophilic/lipophilic balance of salbutamol sulfate from lipid matrices. SPT Pharma Sciences 6: 365-369.
11. Ainaoui A, Ouriemchi E, Bidah D, E Amrani M, Vergnaud J (1997) Process of drug release with oral dosage forms with a Gelucire lipid matrix. Journal of Polymer Engineering 17: 245-255.
12. Aungst B, Nguyen N, Rogers N, Rowe S, Hussain M, et al. (1994) Improved oral bioavailability of HIV protease inhibitor using Gelucire 44/04 and labrasol vehicles. Bulletin Techinque Gattefosse 87: 49-54.
13. Sanchez S, Sallovitz J, Savio E, Mckellar Q, Lanusse C (2000) Comparative availability of two oral dosage forms of albendazole in dogs. Vet J 160: 153-156.
14. Rowe R, Sheskey P, Quinn M (2009) Alginic Acid: Handbook of Pharmaceutical Excipients (6thedn), Pharmaceutical Press. London UK.
15. Phillips G, Wedlock D, Williams P (1984) Gums and Stabilizers for the Food Industry 5 IRL Press, Oxford, U.K. 243–246.
16. Castro SG, Dib A, Suarez G, Allemandi D, Lanusse C, et al. (2013) Comparative plasma exposure of albendazole after administration of rapidly disintegrating tablets in dogs. Biomed Res Int 2013: 920305.
17. Veiga M, Gil M, Torrado J (1997) Preformulación. Tecnología Farmacéutica 1: 27-23
18. Sanchez Bruni S, alvarez L, Pis A, Virkel G, Lifchitz A, et al. (1996) Perfil farmacocinético de ricobendazole administrado por via parenteral. Revista de Medicina Veterinaria 77: 145-150.
19. Notari R (1987) Pharmacokinetics: Biopharmaceutics and Clinical Pharmacokinetics. (4thedn), Marcel Dekker Inc. New York.
20. Gibaldi M, Perrier D (1982) Pharmacokinetics. Ed. Marcel Dekker Inc. (2ndedn), New York.
21. Lanusse CE, Prichard RK (1993) Clinical pharmacokinetics and metabolism of benzimidazole anthelmintics in ruminants. Drug Metab Rev 25: 235-279.
22. McKellar QA, Harrison P, Galbraith EA, Inglis H (1990) Pharmacokinetics of fenbendazole in dogs. J Vet Pharmacol Ther 13: 386-392.
23. McKellar QA, Galbraith EA, Baxter P (1993) Oral absorption and bioavailability of fenbendazole in the dog and the effect of concurrent ingestion of food. J Vet Pharmacol Ther 16: 189-198.
24. Stokol T, Randolph JF, Nachbar S, Rodi C, Barr SC (1997) Development of bone marrow toxicosis after albendazole administration in a dog and cat. J Am Vet Med Assoc 210: 1753-1756.

25. Saumell C, Fusé L, Monfrinotti A, Iglesias L, Stefan P, et al. (2006) Evaluación de Ricobendazole vía oral en caninos. Rev. Med. Vet 87: 160-165.
26. Balamurali V, Pramodkumar T, Srujana N, Venkatesh M, Gupta N, et al. (2011) pH sensitive drug delivery systems: A review. American Journal of Drug Discovery and Development 1: 24-48.
27. Juarez D, VillaFuerte L (2011) Gelucire 39/01 as excipient for gastroretentive metronidazole sustained delivery. Int J Pharm Pharm Sci. 3: 86-91.
28. TÃ¸nnesen HH, Karlsen J (2002) Alginate in drug delivery systems. Drug Dev Ind Pharm 28: 621-630.
29. Saez V, Hernáez E, Sanz L, Katime I (2004) Liberación controlada de fármacos. Micropartículas. Revista Iberoamericana de Polímeros 5: 87–101.
30. Miranda A, Millan M, Caraballo I (2006) Study of the critical points of HPMC hydrophilic matrices for controlled drug delivery. Int J Pharm 311: 75-81.
31. Muoz N, Ferrero C, Muoz-Ruiz A, Velasco MV, Jimnez-Castellanos MR (1998) Effect of Explotab on the tabletability of a poorly soluble drug. Drug Dev Ind Pharm 24: 785-791.
32. Galia E, Horton J, Dressman JB (1999) Albendazole generics--a comparative in vitro study. Pharm Res 16: 1871-1875.
33. Zimei W, Razzak M, Tucker I, Medlicott N (2005) Physicochemical characterization of ricobendazole. Solubility, lipophilicity, and ionization characteristics. Journal of Pharmaceutical Sciences 94: 983–993.
34. O'Neil MJ (2006) The Merck index: an encyclopedia of chemicals, drugs and biologicals. (14thedn), RSC Publishing. Merck Inc., Whitehouse Station/Rahway, New Jersey.