Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 536 (2005) 91–99
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Comparison of milk residue profiles after oral and subcutaneous administration of benzimidazole anthelmintics to dairy cows

L. Moreno [a, b, *], F. Imperiale [a, b], L. Mottier [a, b], L. Alvarez [a, b], C. Lanusse [a, b]

[a] *Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario, 7000 Tandil, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

Received 2 November 2004; received in revised form 22 December 2004; accepted 22 December 2004
Available online 2 February 2005

Abstract

The current experimental work reports on the comparison of the milk residue profile of the benzimidazole (BZD) anthelmintics after their administration by the oral and subcutaneous (SC) routes to dairy cows. The cows were distributed in four groups and treated as follows—Group 1: oxfendazole (OFZ) by oral route (5 mg kg^{-1}); Group 2: albendazole (ABZ) by oral route (5 mg kg^{-1}); Group 3: albendazole sulphoxide (ABZSO) by SC administration (3 mg kg^{-1}); Group 4: OFZ by SC route (3 mg kg^{-1}). After drug administrations milk samples were collected and frozen at −20 °C until analyzed by liquid chromatography (LC). A complete validation of the analytical methodology was accomplished. Regression curves were linear over the concentrations examined and the correlation coefficients (r) ranged between 0.994 and 0.999. The mean extraction recovery range between 77 and 97%. Residual concentrations of OFZ, fenbendazole sulphone ($FBZSO_2$) and FBZ were recovered in milk after OFZ oral administration. OFZ reached the highest concentration in milk (0.39 ± 0.10 μg ml^{-1}) at 12 h post-treatment, being detected up to 72 h post-treatment. In contrast, FBZ was not detected in cow milk and $FBZSO_2$ was the main analyte recovered from the milk with the maximum milk residues (0.042 ± 0.003 μg ml^{-1}) achieved at after 36 h following the SC injection of OFZ. ABZSO and $ABZSO_2$ were the metabolites recovered in milk following oral (ABZ) and SC (ABZSO) treatments in dairy cows. $ABZSO_2$ was the analyte recovered at the highest residual concentration (0.86 ± 0.33 μg ml^{-1}) at 12 h after oral administration of ABZ. However, ABZSO was the main compound measured in cow milk following its SC injection (0.18 μg ml^{-1}) at 12 h post-treatment. Overall, the total milk residue levels (sum of parent drug and metabolites) were higher after oral compared to parenteral treatments in dairy cows. These results reported here are discussed according to the acceptable maximum residue limits (MRLs) established for BZD compounds in cow milk.

Keywords: Milk residues; Albendazole; Albendazole sulphoxide; Oxfendazole; Oral administration; Subcutaneous administration

1. Introduction

It has been shown that cattle may develop protective immunity against parasites in the first or the second grazing seasons. However, adult cows can still be infected with several gastrointestinal (GI) nematodes [1]. These infections are normally sub-clinical but they have been associated with decreased levels of milk production [2]. In addition to this, several reports have demonstrated that treatment of infected dairy cows can advantageously influence milk production. Enhancement of milk production of ≈0.35–0.63 kg day^{-1} after anthelmintic treatment of naturally infected lactating dairy cows has been shown [3,4].

Anthelmintic drugs are widely used in veterinary medicine for prevention and treatment of animals mainly against gastrointestinal nematodes and lungworms. Benzimidazole (BZD) compounds currently marketed as broad-spectrum anthelmintics for use in ruminants, include albendazole (ABZ), albendazole sulphoxide (ABZSO), fenbendazole (FBZ), and oxfendazole (OFZ). It is well known that BZD anthelmintics

* Corresponding author. Tel.: +54 2293426667.
E-mail address: lmoreno@vet.unicen.edu.ar (L. Moreno).

0003-2670/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2004.12.069

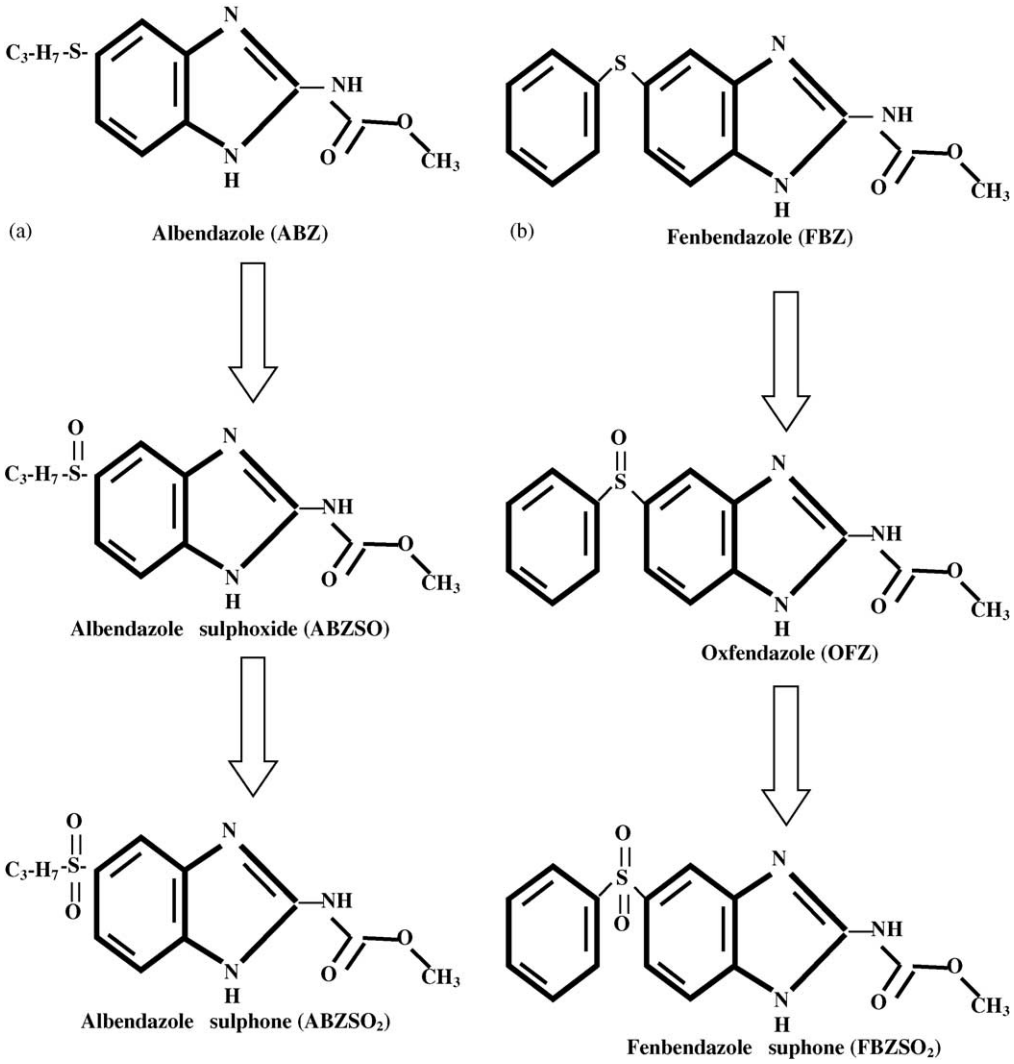

Fig. 1. Chemical structures of the benzimidazole compounds assayed in the current work: (a) albendazole (ABZ) and its metabolites, albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$); (b) fenbendazole (FBZ) and its metabolites, oxfendazole (OFZ) and fenbendazole sulphone ($FBZSO_2$).

are extensively metabolized in all mammalian species studied [5,6]. Parent drugs are rapidly metabolized by two different microsomal enzymatic systems in the liver of sheep and cattle: flavin-containing monooxygenase (FMO) and cytochrome P-450 system. The sulphoxide (ABZSO) (active) and the sulphone ($ABZSO_2$) (inactive) metabolites are the main molecules recovered in plasma of sheep and cattle after treatment with ABZ parent drug (Fig. 1a). On the other hand, after FBZ administration to sheep and cattle, in addition to the sulphoxide (OFZ) (active) and the sulphone ($FBZSO_2$) (inactive) metabolites, FBZ parent drug was recovered from plasma [7,8] (Fig. 1b). Although these BZD anthelmintic have a wide safety margin in treated animals, a teratogenic effect has been described for ABZ in some animal species [9]. Currently BZD compounds can be used in dairy animals but required withdrawal times after treatment must be respected to avoid residual concentration above the defined maximum residues (MRL). MRLs for BZD anthelmintics have now been defined by regulatory agencies [10,11].

Low water solubility is an important limitation for the formulation of the most potent BZD methylcarbamate anthelmintics (ABZ, FBZ, etc.), restricting their formulation to suspensions for oral/intraruminal administration [12]. Poor and/or erratic gastrointestinal absorption is an inconvenience for the systemic availability and resultant efficacy of enterally administered BZD compounds. In an attempt to overcome this problem, injectable formulations (aqueous solutions) of ABZSO and OFZ have been developed to control helmint parasites in cattle. The milk residues of some orally given BZD compounds in dairy animals have been reported [13–16]. However, the pattern of milk excretion for parenterally administered BZD anthelmintic in dairy cows has not been described.

The goals of the work reported here were: (1) To develop and validate an alternative analytical method to quantify

residual concentrations of BZD-related molecules in bovine milk. (2) To apply the developed methodology to compare the milk residual concentrations following oral (OFZ and ABZ) and parenteral (OFZ and ABZSO) administrations of BZD methylcarbamate anthelmintics at therapeutic dose rates to lactating dairy cows.

2. Experimental

2.1. Animal trial

Twenty-four healthy Holstein cows, during the second lactation period, with an average body weight of 583 ± 25 kg were used in this experiment. The cows were kept on pasture over the duration of the study. Animals were randomly allocated in four groups of six and were treated as follows—Group 1: animals received oxfendazole (OFZ) (aqueous suspension) by the oral route (5 mg kg^{-1}); Group 2: cows received albendazole (ABZ) (aqueous suspension) by the oral route (5 mg kg^{-1}); Group 3: animals were treated with albendazole sulphoxide (ABZSO) (aqueous injectable solution) by SC administration (3 mg kg^{-1}); Group 4: animals were treated with OFZ (aqueous solution) given by the SC route (3 mg kg^{-1}). The anthelmintic doses were calculated individually according to body weight. The cows were milked twice a day using milking machine. Milk samples collected before treatments were used as blank controls for development of the analytical method. Milk samples (approximately 50 ml) were collected at the morning and afternoon milkings over 5 days after drug administration. The samples were well homogenized, aliquoted for analysis into plastic tubes and frozen at −20 °C until analyzed by liquid chromatography (LC).

2.2. Materials and reagents

Pure reference standards of FBZ, OFZ, $FBZSO_2$ (Rhone Merieux, Lyon, France), ABZ, ABZSO, $ABZSO_2$, and the internal standard (IS) oxibendazole (OBZ) (Schering Plough, Kenilworth, USA) were used for the validation of the analytical methodology. Acetonitrile and methanol solvents used during the extraction and drug analysis were HPLC grade and purchased from Sintorgan® S.A. (Buenos Aires, Argentina). Ammonium acetate (HPLC grade) was from Merck (Haar, Germany). Water was double distilled and deionized using a water purification system (Simplicity®, Millipore, Sao Paulo, Brazil). The following commercially available formulations were used in the current experiments: (1) OFZ suspension for oral administration (Vermox®, 50 mg ml^{-1}, Over, Argentina); (2) OFZ injectable solution (Endovet®, 150 mg ml^{-1}, Novartis, Argentina); (3) ABZ micronized suspension for oral administration (Valbazen®, 100 mg ml^{-1}, Pfizer, Argentina); (4) ABZSO injectable solution (Bayverm P.I.®, 150 mg ml^{-1}, Bayer, Argentina).

2.3. Milk samples analysis

2.3.1. Drug extraction

Milk samples (0.5 ml) containing FBZ, OFZ, $FBZSO_2$ or ABZ, ABZSO, $ABZSO_2$ were placed into a 5 ml plastic tube and spiked with the IS (1 μg ml^{-1}). Drug molecules were extracted from milk by addition of 0.5 ml acetonitrile under a high speed vortexing shaker (Multi-tube Vortexer, VWR Scientific Products, West Chester, PA, USA). After centrifugation (BR 4i Centrifuge, Jouan®, Saint Herblain, France) at $2000 \times g$ for 15 min at 10 °C to allow phase separation, the clear supernatant was transferred to a 10 ml plastic tube, and then 6 ml of pure water were added. The mixture was vortexed for 5 s and transferred to C_{18} cartridges (100 mg, 1 ml, Lichrolut®, Merck, Darmstadt, Germany) using a manifold vacuum (Baker spe-24G, Phillipsburg, USA). The cartridges were previously conditioned with 0.5 ml of methanol, followed by 0.5 ml of water. All samples were applied and then sequentially washed with 0.5 ml of water, dried with air for 1 min and eluted with 2 ml of methanol. The eluted volume was evaporated (40 °C) to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, USA), and then reconstituted with 300 μl of mobile phase.

2.3.2. LC conditions

Experimental and fortified milk samples were analyzed for FBZ, OFZ, $FBZSO_2$ and ABZ, ABZSO, $ABZSO_2$ by LC. Fifty microliters of each sample were injected in a Shimadzu Chromatography system (Shimadzu Corporation, Kyoto, Japan), with two LC-10AS solvent pumps, an automatic sample injector (SIL-10A), an ultraviolet visible spectrophotometric detector (UV) (SPD-10A), a column oven (Eppendorf TC-45, Eppendorf, Madison, WI, USA) set at 30 °C, and a CBM-10A data integrator. Data and chromatograms were collected and analyzed using the Class LC10 software (SPD-10A, Shimadzu Corporation, Kyoto, Japan). A C_{18} reversed-phase column (Luna®, Phenomenex, Torrance, CA, USA) of 250 mm × 4.6 mm with 5 μm particle size was used for separation. The method used was adapted from a procedure previously developed in our lab [17]. Elution from the stationary phase was carried out at a flow rate of 1.2 ml min^{-1} using acetonitrile and ammonium acetate buffer (0.025 M, pH 6.6) as the mobile phase. The gradient changed linearly from 30:70 (acetonitrile:ammonium acetate buffer) to 50:50 in 5 min, then maintained for 8 min and modified to 30:70 in 1 min, in which was maintained over 3 min. The detection of drugs/metabolites was done at a wavelength of 292 nm.

2.3.3. Validation procedure

A complete validation of the analytical procedures for the extraction and quantification of the BZD anthelmintic residues in milk was performed before the analysis of the experimental samples. Stock and working solutions of two different mix of standards FBZ + OFZ + $FBZSO_2$ and ABZ + ABZSO + $ABZSO_2$ in methanol were prepared. The linearity of the method was tested after elaboration of analyti-

cal calibration curves for each compound in milk. Blank milk samples were fortified with each analyte in a range between 0.01 and 2 mg ml^{-1}, plus the IS (1 μg ml^{-1}). The analytical calibration curves for each parent drug and its metabolites in milk were obtained using the linear least squares regression procedure, using the *run test* and ANOVA to determine if the data differed from a straight line. The extraction efficiency of the six analytes was determined by comparison of the peak areas from fortified blank milk samples (0.1, 0.5, 1 mg ml^{-1}, $n = 5$) with the peak areas resulting from direct injections of equivalent quantities of standards in mobile phase. Precision and accuracy (intra- and inter-assay) of the method were determined by evaluation of replicates of drug-free milk ($n = 5$) with each compound at three different concentrations (0.1, 0.5, 1 mg ml^{-1}). Precision was expressed as coefficient of variation (%CV). The accuracy of a measurement is defined as the closeness of the measured value to the true value. Accuracy of the method was defined by the differences between measured and nominal concentration obtained by intra- and inter-day assays (5 consecutive working days), and expressed as the relative error (%RE). The theoretical limit of detection (LOD) was estimated integrating the baseline noise of the system in the area covering the mean retention time of each compound in blank milk samples ($n = 5$) spiked with IS. The theoretical LOD was defined as the mean baseline noise/IS peak area ratio plus three standard deviations (SD). The limit of quantification (LOQ) was calculated as the lowest drug concentration ($n = 5$) on the standard curve that could be quantitated with precision not exceeding 20% and accuracy within 20% of nominal.

3. Results and discussion

The analytical procedures, including chemical extraction and LC analysis of BZD anthelmintics in cow milk were validated appropriately. Representative chromatograms are shown in Fig. 2. Those chromatograms were obtained after the analysis of milk blank samples spiked with either the IS alone (Fig. 2a), OFZ, $FBZSO_2$ and FBZ at a concentration of 0.5 mg ml^{-1} (Fig. 2b) or ABZSO, $ABZSO_2$ and ABZ at the same concentration (0.5 mg ml^{-1}) (Fig. 2c). No major endogenous chromatographic peaks, which could interfere with the resolution of drugs were observed. Peaks of interest were well separated from other sample components and also from neighboring peaks; besides, good peak shape was ob-

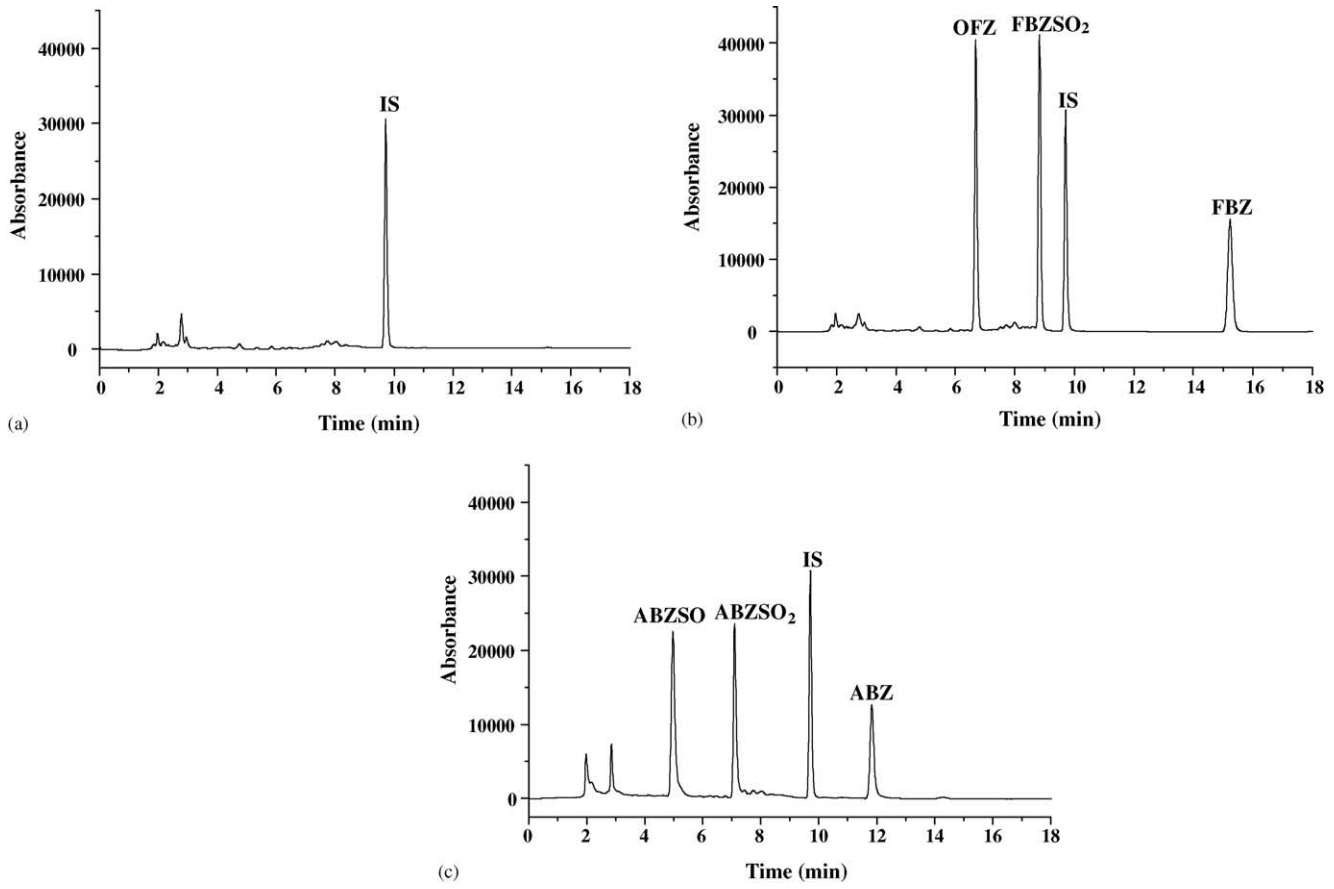

Fig. 2. Chromatographic separation of fenbendazole (FBZ), oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$) and albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in cow milk samples. Chromatograms include blank milk samples spiked with: (a) the internal standard (IS): oxibendazole (OBZ); (b) fenbendazole (FBZ), oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$) (0.5 μg ml^{-1}) plus the IS; (c) albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) (0.5 μg ml^{-1}) plus the IS.

Table 1
Analytical recovery (range and CV) ($n = 5$), limit of detection (LOD) ($n = 5$), limit of quantification (LOQ) ($n = 5$) and linearity ($n = 3$) of the methodology developed to measure fenbendazole (FBZ), oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$), albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in bovine milk

Drug compounds	Recovery (%)	LOD ($\mu g\,ml^{-1}$)	LOQ ($\mu g\,ml^{-1}$)	Linearity, r
FBZ	77–80 (1.6)	0.014	0.025	0.999
OFZ	88–89 (0.9)	0.004	0.01	0.999
$FBZSO_2$	90–92 (1.7)	0.014	0.025	0.999
ABZ	83–91 (6.4)	0.026	0.05	0.995
ABZSO	79–95 (8.0)	0.020	0.025	0.995
$ABZSO_2$	81–97 (8.9)	0.009	0.025	0.994

tained for the compound under analysis. Under the described chromatographic conditions, the retention times (min) were 4.9 (ABZSO), 6.6 (OFZ), 7.1 ($ABZSO_2$), 8.8 ($FBZSO_2$), 9.7 (OBZ), 11.8 (ABZ), and 15.2 (FBZ). The results of the complete validation of the analytical methodology are summarized in Tables 1 and 2. The regression analyses were linear over the concentrations examined and the correlation coefficients (r) of the calibration curves ranged between 0.994 and 0.999. The mean extraction recovery values obtained for the milk samples spiked separately with a mixture of FBZ and its metabolites and ABZ and its metabolites ranged between 77 and 97%. These high recoveries were obtained using this simple method based on liquid-to-liquid extraction with only 0.5 ml of acetonitrile combined with solid phase extraction. The analysis of low ($0.1\,mg\,ml^{-1}$), middle ($0.5\,mg\,ml^{-1}$), and high ($1\,mg\,ml^{-1}$) drug concentration values was used to determine intra- and inter-day precision and accuracy. As shown in Table 2, the method exhibited a high degree of intra- and inter-day precision and accuracy, which is evident by the low CV ($\leq$16%) and RE (from −15.0 to 23%). The LOD values in milk were between 0.004 and $0.026\,mg\,ml^{-1}$. LOQ values shown in Table 1 were low enough to make the procedure applicable to the routine use in the analysis of residual levels of these BZD compounds in cow milk.

The validated method was successfully applied to quantify anthelmintic drug residues in milk from cows treated with different BZD formulations administered by the oral and SC routes. Milk residual concentration profiles (mean ± S.D.) of OFZ, $FBZSO_2$ and FBZ after OFZ administration by the oral route ($5\,mg\,kg^{-1}$) are shown in Fig. 3a. OFZ was the analyte that reached the highest concentration in milk, with the maximum measured concentration ($0.39 \pm 0.10\,mg\,ml^{-1}$) at 12 h after dosing, being detected until 72 h post-treatment. $FBZSO_2$ reached its maximum concentration at 36 h ($0.17 \pm 0.04\,mg\,ml^{-1}$) after administration and it was measured in milk up to 72 h post-treatment. The lowest drug concentration corresponded to FBZ, the thioether compound produced by ruminal sulphoreduction of OFZ [18]. This compound attained its highest level at 24 h post-treatment ($0.10 \pm 0.02\,mg\,ml^{-1}$), being detectable in milk up to 48 h post-treatment.

OFZ and $FBZSO_2$ milk residual concentrations obtained after OFZ administration by the SC route at $3\,mg\,kg^{-1}$ are shown in Fig. 3b. In contrast to the oral administration of OFZ, FBZ was not detected in cow milk. $FBZSO_2$ was not quantified in the milk from the first-milking. However, this molecule was the main analyte recovered from the milk of OFZ treated cows by the SC route, reaching the maximum milk residue level ($0.042 \pm 0.003\,mg\,ml^{-1}$) after 36 h post-administration. The highest OFZ milk residue ($0.03 \pm 0.01\,mg\,ml^{-1}$) was measured at the first milking followed by a fast disposition which only allowed its measurement in milk up to 48–60 h post-treatment.

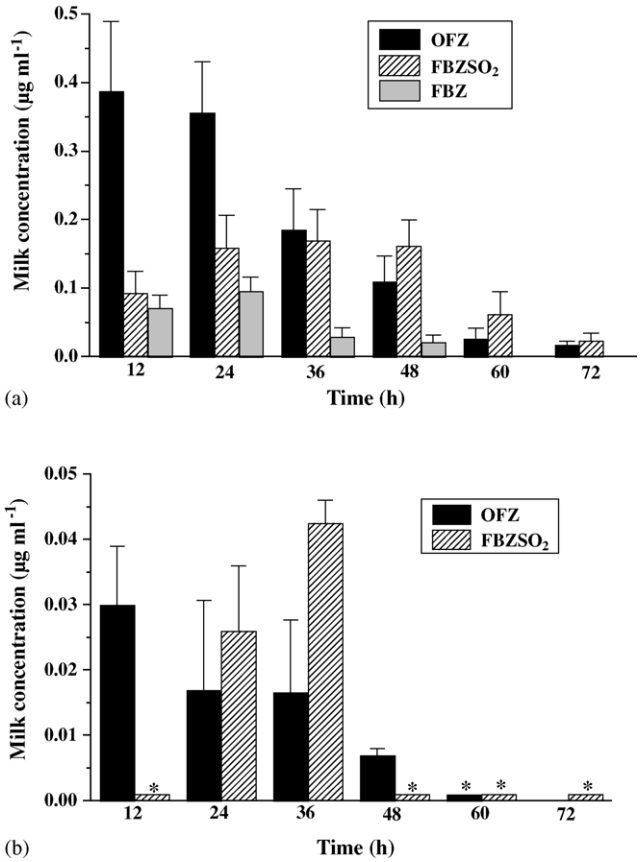

Fig. 3. Milk concentration profiles (mean ± S.D.) ($n = 6$) of oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$) and fenbendazole (FBZ) measured after oral ($5\,mg\,kg^{-1}$) (a) and subcutaneous ($3\,mg\,kg^{-1}$) (b) administration of OFZ to dairy cows. * Peak detected under the limit of quantification (LOQ).

Table 2
Analytical precision and accuracy ($n = 5$) for the determination of fenbendazole (FBZ), oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$), albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in spiked cow milk samples

	Added concentration ($\mu g\,ml^{-1}$)																	
	FBZ			OFZ			$FBZSO_2$			ABZ			ABZSO			$ABZSO_2$		
	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1
Intra-day precision (%CV)	2	5	2	2	4	1	5	4	1	7	4	7	5	6	5	6	6	7
Inter-day precision (%CV)	3	9	10	2	16	7	10	13	15	6	5	9	7	9	3	3	1	4
Accuracy																		
Measured concentration ($\mu g\,ml^{-1}$)	0.10	0.53	0.90	0.08	0.51	1.23	0.10	0.48	1.06	0.10	0.48	0.94	0.09	0.49	0.90	0.09	0.46	0.90
%RE	3	6	−10	−15	2	23	4	−5	6	2	−4	−6	−6	−2	−10	−14	−8	−10

Relative error (%RE) = 100 × [(measured concentration − nominal concentration)/nominal concentration].

Several earlier reports have described anthelmintic drug residues in whole milk after FBZ oral administration to dairy cattle. In a pilot study ^{14}C-labelled FBZ was administered to dairy cow as an oral suspension, and it was shown that this drug was transferred into the milk, peaking at 24–36 h post-treatment at a mean total residue level of $0.53 \pm 0.11\,\mu g$ FBZ equivalents ml^{-1} of whole milk [19]. These authors reported similar residue profiles after the administration of FBZ as three different formulations: paste preparation produced the highest total residue level ($0.32 \pm 0.05\,mg\,ml^{-1}$), followed by feed top dressing ($0.26 \pm 0.16\,mg\,ml^{-1}$) and oral drench ($0.16 \pm 0.06\,mg\,ml^{-1}$) [13]. In a more recent study, FBZ residues in milk were analyzed by ELISA, these results agreed with those previously described, reporting peak levels of FBZ in milk at 12–24 h, being the metabolites detected at much higher levels than the parent compound, peaking at 24–36 h, and declined gradually [16]. Although OFZ (a FBZ-related anthelmintic) was the drug administered by the oral route in the present study, milk residue profiles showed a similar trend to that found after FBZ administration in dairy cattle. The highest residue level corresponded to OFZ, followed by the sulphone metabolite, which is consistent with the results obtained after FBZ treatment [13]. FBZ was the analyte recovered at the lowest concentration. The main difference obtained in the present experimental work compared to previous reports (where FBZ was orally administered at the same dose) was related to the higher concentrations of all the analytes measured in the milk of OFZ treated cows. As a consequence, the total residue peak in milk after OFZ oral administration (Fig. 5a) at 24 h post-treatment was also higher ($0.61 \pm 0.14\,mg\,ml^{-1}$) being the double from that reported in this previous work [13]. However, a higher value of total residue peak ($0.53 \pm 0.11\,mg\,ml^{-1}$) has also been reported [19] after oral administration of FBZ, probably due to a difference in the administered formulation.

The FBZ sulphide was detected in milk after oral treatment with OFZ. Reductive metabolism of BZD sulphoxide can occur in the gastrointestinal tract [20]. Therefore, OFZ can be reduced back to its respective thioether by the ruminal and intestinal microflora [18,20,21] and may act as a source of FBZ in the digestive tract. This gastrointestinal metabolic reduction may be of primary importance for the antiparasitic efficacy of BZD thioethers. Since the thioethers have a greater affinity for parasite tubulin than the sulphoxides [22,23], this bacteria-mediated reduction may have a significant importance for the efficacy against gastrointestinal parasites. This metabolic sulphoreduction may also account for the detection of FBZ in milk at residual concentrations for up to 48 h after oral treatment of OFZ.

After ABZ oral administration ($5\,mg\,kg^{-1}$) to dairy cows, milk residues of both ABZSO and $ABZSO_2$ metabolites were measured, meanwhile ABZ parent drug was not detected in milk (Fig. 4a). Milk residues were measured at first and

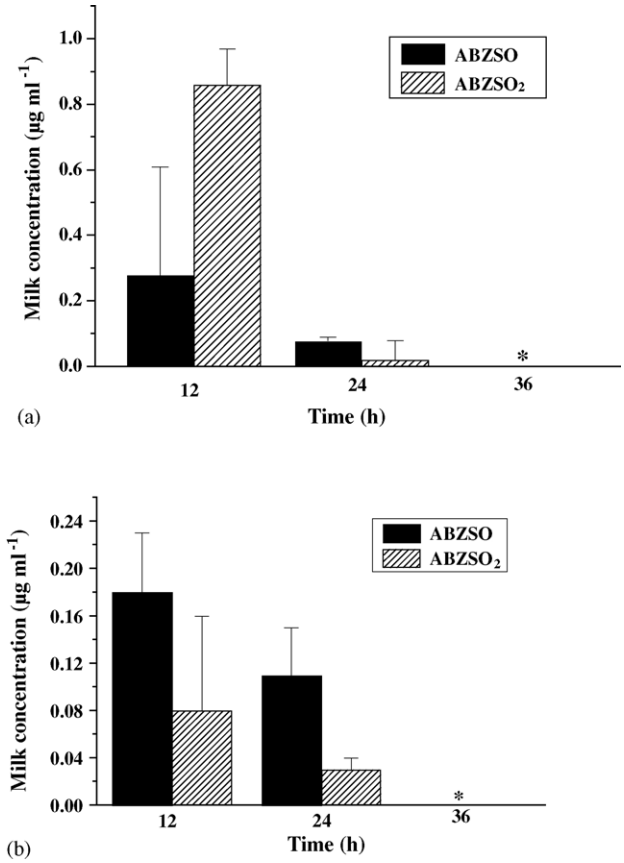

Fig. 4. Milk residual concentration profiles (mean ± S.D.) ($n = 6$) of albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) measured after oral administration of ABZ (5 mg kg^{-1}) (a) and subcutaneous administration of ABZSO (3 mg kg^{-1}) (b) to dairy cows. * Residual concentrations not detected.

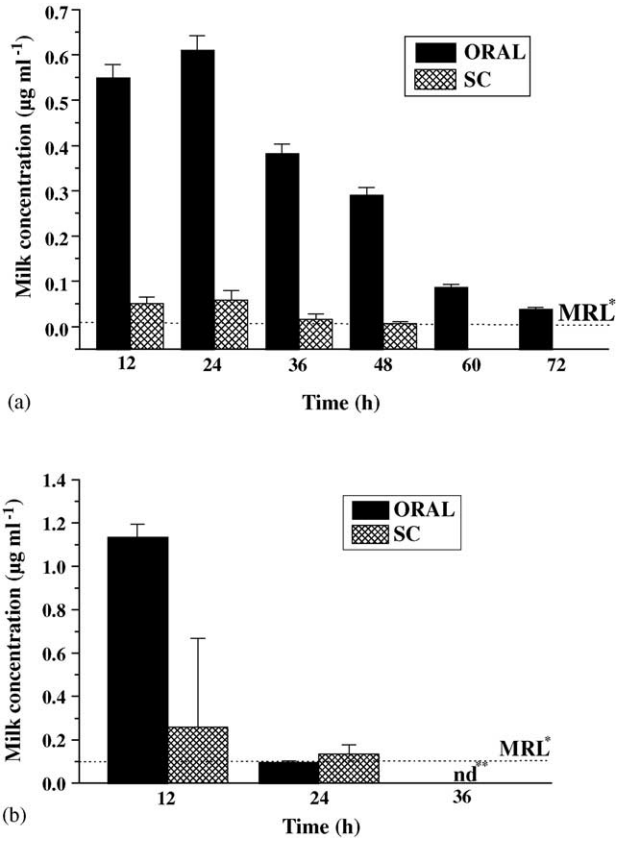

Fig. 5. Comparison of the total residual levels measured in milk following: oral (5 mg kg^{-1}) and parenteral (3 mg kg^{-1}) treatments with oxfendazole (OFZ) (a) and oral treatment (5 mg kg^{-1}) with albendazole (ABZ) and parenteral treatment (3 mg kg^{-1}) with albendazole sulphoxide (ABZSO) (b) in dairy cows. Values express the sum of the analytes recovered in milk: OFZ, $FBZSO_2$ and FBZ in panel (a) and ABZSO and $ABZSO_2$ in panel (b). MRL^*: maximum residue limits according to Council Regulation of the European Union No. 2377/90 and Commission Regulation Nos. 508/1999, 2385/1999, 2393/1999 and 807/2001; nd^{**}: residue concentrations not detected.

second milking, with maximum concentrations of ABZSO (0.28 ± 0.11 mg ml^{-1}) and $ABZSO_2$ (0.86 ± 0.33 mg ml^{-1}) detected at 12 h post-treatment. These residual concentrations decreased markedly at the time of the second milking post-treatment (24 h). Besides, $ABZSO_2$ milk concentrations were lower than those measured for ABZSO. Therefore, the milk residue profile after ABZ oral administration was different to that found after OFZ oral treatment. These results are consistent with the faster metabolism of ABZ parent drug compared to FBZ [5]. It suffers a rapid first-pass metabolism and it is not detected in bloodstream, being only the sulphone (at higher concentrations) and the sulphoxide metabolites found in plasma after ABZ administration to calves [5]. However, after both FBZ or OFZ oral administration, slower metabolic processes have been described [24], with the sulphide parent drug besides the metabolites detected in plasma, and as a consequence, in milk.

Milk residues after ABZ administration to lactating dairy cows have been previously described [14]. Similar to the results obtained in the current experimental work, the highest residue level corresponded to $ABZSO_2$, however, this metabolite was measured for a longer period of detection, probably due to the higher dose administered. ABZSO and $ABZSO_2$ milk residues were also found in milk after subcutaneous injection of ABZSO at 3 mg kg^{-1} (Fig. 4b). The milk residue levels of ABZSO and $ABZSO_2$ were lower than those obtained after the oral administration of ABZ. ABZSO was the analyte recovered at the highest residue level (0.18 mg ml^{-1}) obtained at 12 h post-administration. Milk residual concentrations markedly decreased at the second milking, and ABZSO continued being the highest milk residue.

The differences between milk residue levels of ABZ-related compounds found after oral and SC administrations were similar to those described for OFZ administered by oral and the SC routes. Similarly, the total drug residues in milk after SC administration (Fig. 5b) were lower than those obtained after the oral administration. Poor drug absorption in the gastrointestinal tract and the low water solubility are im-

portant limitations for BZD formulation, bioavailability and efficacy. The oral/intraruminal administrations of BZD anthelmintics formulated as aqueous suspensions have been the most commonly used in ruminants. Drug absorption in the digestive tract depends on lipid solubility and degree of ionization at the different pH levels [25]. Additionally, dissolution of drug particles is a crucial step that precedes the gastrointestinal absorption of a drug formulated as a suspension [26]. BZD aqueous solubility is markedly higher at low pH values [12], therefore, the dissolution of BZD particles given as drug suspension is greater at the acid abomasal pH. From this point of view, the greater hydrosolubility of ABZSO compared with ABZ, was the starting point in the search of a pharmacotechnical strategy to achieve a stable aqueous solution of ABZSO for injectable administration to cattle [25]. Effectively, a more complete and rapid absorption of the soluble drug from the site of injection accounts for the greater plasma availability obtained following SC injection of ABZSO compared with the GI absorption of ABZ administered at the same dose as an oral suspension to calves [25]. Additionally, BZD anthelmintic drugs suffer an important presystemic first-pass effect after their enteral administration to ruminants, and lower concentrations of the parent drug/metabolites in plasma, and consequently lower milk concentrations could be expected after the oral compared with the parenteral administration. However, the total milk residue levels (Fig. 5a and b) obtained following the oral administration of OFZ or ABZ tend to be higher than those obtained following SC administration of OFZ or ABZSO, respectively, at the recommended doses. This differential milk excretion pattern may also be related to the higher doses used by the oral (5 mg kg^{-1}) compared to the SC (3 mg kg^{-1}) route of administration.

Different MRLs for anthelmintic drugs have been set by several regulatory agencies. In the United States, for fenbendazole a tolerance level of 0.6 $\mu g\,ml^{-1}$ (OFZ marker residue) in bovine milk has been established by the food and drug administration (FDA) [10]. The residual concentration profiles measured in cow milk after OFZ administration by both the oral and subcutaneous routes were below the FDA subjected MRL values. Following these results a withdrawal time for milk consumption after OFZ treatment may not be required. There is no data available about ABZ tolerance level in milk by the FDA. On the other hand, the European Union has set MRLs in cow milk for OFZ (as sum of extractable residues that may be oxidized to $FBZSO_2$) of 0.01 $\mu g\,ml^{-1}$, and for ABZ or ABZSO (as sum of all metabolites) of 0.1 $\mu g\,ml^{-1}$ [11]. According to this, our results confirmed the appropriateness of currently prescribed withdrawal times for bovine milk (3 days for ABZ and ABZSO and 5 days for OFZ) after the oral or SC administrations of the commercially available formulations used in the present study. Overall, the work reported here provides useful information of the application of an alternative analytical methodology to quantify BZD residual concentrations in milk from treated cows. The characterization of the milk residue disposition of BZD-related anthelmintic compounds in dairy cattle reported here is useful to establish suitable withdrawal times to ensure the quality of milk-derived products from treated animals, which contribute to the safety of the consumers. The comparison of the milk residue excretion patterns after BZD anthelmintic administered by oral and parenteral routes in dairy cattle contribute to the available knowledge on the subject. This is practically relevant and useful for the BZD parenteral formulations now available in some countries for use in cattle.

Acknowledgments

Laura Moreno is a recipient of a post-doctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. This research was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 08-07277) (Argentina), Universidad Nacional del Centro de la Pcia de Buenos Aires (Argentina) and CONICET (Argentina).

References

[1] F. Borgsteede, J. Tibben, J. Cornelissen, J. Agneessens, C. Gaasenbeek, Vet. Parasitol. 89 (2000) 287.
[2] J. Vercruysse, E. Claerebout, Vet. Parasitol. 98 (2001) 195.
[3] S. Gross, W. Ryan, H. Ploeger, Vet. Rec. 144 (1999) 581.
[4] J. Sanchez, I. Dohoo, J. Carrier, L. DesCôteaux, Prev. Vet. Med. 63 (2004) 237.
[5] C. Lanusse, R. Prichard, Drug Metab. Rev. 25 (1993) 235.
[6] P. Galtier, M. Alvinerie, P. Delatour, Am. J. Vet. Res. 46 (1986) 447.
[7] S. Marriner, J. Bogan, Am. J. Vet. Res. 41 (1980) 1126.
[8] R. Prichard, D. Hennessy, J. Steel, E. Lacey, Res. Vet. Sci. 39 (1985) 113.
[9] P. Delatour, R. Parish, in: A.G. Rico (Ed.), Drug Residues in Animals, Academic Press, Orlando, FL, 1986, p. 175.
[10] Tolerances for Residues of New Animal Drugs in Food, Code of Federal Regulations, Part 556.275, Title 21, U.S. Food and Drug Administration, 2001, revised as of April 1, 2004.
[11] Council Regulation of the European Union No. 2377/90 and Commission Regulation Nos. 508/1999, 2385/1999, 2393/1999 and 807/2001.
[12] Q. McKellar, E. Scott, J. Vet. Pharmacol. Ther. 13 (1990) 223.
[13] S. Barker, L. Kappel, J. Vet. Pharmacol. Ther. 20 (1997) 160.
[14] D. Fletouris, N. Botsoglou, I. Psomas, A. Mantis, Anal. Chim. Acta 345 (1997) 111.
[15] K. Takeba, K. Fujinuma, M. Sakamoto, T. Miyazaki, H. Oka, Y. Itoh, H. Nakazawa, J. Chromatogr. A 882 (2000) 99.
[16] D. Brandon, A. Bates, R. Binder, W. Montangue Jr., L. Whitehand, S. Barker, J. Agric. Food. Chem. 50 (2002) 5791.
[17] L. Mottier, L. Moreno, L. Alvarez, G. Virkel, G. Lanusse, J. Pharm. Biomed. Anal. 35 (2004) 21.
[18] G. Virkel, A. Lifschitz, A. Pis, C. Lanusse, J. Vet. Pharmacol. Ther. 25 (2002) 15.
[19] L. Kappel, S. Barker, J. Vet. Pharmacol. Ther. 19 (1996) 416.
[20] C. Lanusse, B. Nare, L. Gascon, R. Prichard, Xenobiotica 22 (1992) 419.
[21] C. Beretta, L. Fadini, J. Stracciari, C. Montesissa, Biochem. Pharmacol. 36 (1987) 3107.

[22] E. Lacey, Parasitol. Today 6 (1990) 112.
[23] G. Lubega, R. Princhard, Exp. Parasitol. 73 (1991) 203.
[24] D. Hennessy, Parasitol. Today 9 (1993) 329.
[25] C. Lanusse, G. Virkel, S. Sanchez, L. Alvarez, A. Lifschitz, F. Imperiale, Res. Vet. Sci. 65 (1998) 5.
[26] G. Koritz, Influence of ruminant gastrointestinal physiology on the pharmacokinetics of drugs in dosage forms administered orally, in: Y. Ruckebusch, P. Toutain, G. Koritz (Eds.), Veterinary Pharmacology and Toxicology, 1st ed., AVI Publishing Company Inc., Westport, USA, 1982, p. 151.