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Bioavailability of Albendazole and its Metabolities in Plasma of *Pangasianodon hypophthalmus* with High Performance Liquid Chromatography

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A B S T R A C T

In the present experiment the depletion profile study of Albendazole and its metabolites (ABZSO and $ABZSO_2$) were carried out in the plasma of *Pangasianodon hypophthalmus.* The experiment consists of three treatment groups and control. A 20mg/kg body weight dose of ABZ was given through intraperitoneal, intramuscular and orally respectively to the three treatment groups while no drug was given to control group. The blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, and 48h intervals to determine the ABZ, ABZSO and $ABZSO_2$ using High Performance Liquid Chromatography (HPLC) having C-18 reversed–phase analytical column. The samples were processed by mobile phase of Methanol-Water (60:40) at a constant flow rate of 1 ml/ min and detected at 295 nm using a UV/Visible detector. The drug was detected in the plasma of orally given treatment group only. The Maximum concentration (C_{max}) of ABZ 3.461± 0.06 μgml^{-1} found at 8 h (T_{max}) and retention time (RT) of 23.88 minutes. For ABZSO Maximum concentration (C_{max}) detected was 0.037 ±0.009 μgml^{-1} at 8 h (T_{max}) with RT of 5.61minutes and $ABZSO_2$ C_{max} found was 0.023 ± 0.007 μgml^{-1} at 12 h (T_{max}) with RT of 19.4 minutes. The concentration of metabolites reached to the levels of negligible after 48 h. The present study reveals that giving ABZ through orally is efficient method for treatment of helminth infections in fishes.

Introduction

Albendazole (ABZ, [5-(propylthio)-1H-benzimidazol-2-yl]-carbamate), is potent broad spectrum anthelmintic widely used for the treatment of Tapeworms, liver flukes, lung and gastrointestinal round worms in mammals (McKellar and Scott 1990).

The albendazole (ABZ) given is readily absorbed from the gut and through oxidation converted into its different metabolites, albendazole sulfoxide (ABZSO) major active metabolite, albendazole sulfone ($ABZSO_2$), and albendazole 2-aminosulfone (ABZ-2-NH_2SO_2) (Gottschall *et al.*, 1990). Albendazole biotransformation metabolites have been studied in many animals dogs (Delatour *et al.*, 1990), rabbits (Li *et al.*, 1995), sheep (Chiap *et al.*, 2000), goats (Delatour *et al.*, 1991) and humans (Rawden *et al.*, 2000). Albendazole acts either by disruption of energy metabolism in helminths (inhibition of fumarate reductase), or

disruption of the polymerization of tubulin in cellular microtubules (Manger B, 1991).

In fishes, the Nematodes (Roundworms) are the most common parasites found in marine fishes (Hilderbrand *et al.,* 1985). Helminths infections are quite common in both feral and cultured fish (Petrushevski and Shulman, 1961). Freshwater and brackish water fishes are affected with huge infections in predatory fish, particularly those fish species which are utilizing fish as intermediate or transient hosts (Paperna *et al.,* 1996). Jadhav (2010) reported that most host belonging to catfish families Schilbeidae, Bagridae, Heteropneustidae, Siluridae, Mastacembelidae, and Clariidae have been reported as definitive hosts of cestodes. Catfishes are significant fish fauna of wetlands and are economically vital as a food source of high nutritive value. Catfish of the family Pangasiidae especially *Pangasianodon hypophthalmus* is one of the promising species for aquaculture because of its omnivorous feeding habit, adaptability to crowded conditions, hardiness and a good market. *P. hypophthalmus* has immense economic importance in many countries of South and Southeast Asia, including India, Bangladesh, Thailand, Vietnam, and Malaysia.

Albendazole is extensively used in ruminants to treat gastrointestinal helminths (McKellar *et al.,* 2002; Gokbulut *et al.,* 2000) but there are reports that ABZ is also used in fish antiparasitic infections (Schmahl and Benini, 1998; Tojo *et al.,* 1992). The pharmacokinetic study of ABZ was carried out in humans (Mirfazaelian *et al.,* 2000) and sheep (Cristòfol *et al.,* 1998). While in fishes the depletion of ABZ and its metabolites after oral administration in rainbow trout, and Atlantic salmon has been studied by (Shaikh *et al.,* 2006). Reverse-phase high-performance liquid chromatography (HPLC) is one of the important analytical methods for determination of ABZ and its derivatives in the plasma. Albendazoleas widely used in veterinary medicine, so the literature also reports methods for the determination of residues of ABZ and its metabolites in milk (De Ruyck *et al.,* 2000), ovine plasma (Chiap *et al.,* 2000) and in human plasma (Garcia *et al.,* 1999). But in the case of fishes, limited research is carried out for determination of ABZ in fish plasma, so the aim of the present study was to know the efficient method of giving ABZ to fish, further knowing the concentration of ABZ and its metabolites in fish plasma.

Materials and Methods

Experimental design and Culture Conditions

The experiment was conducted at wet laboratory of the Central Institute of Fisheries Education. About 120 fishes of *P. hypophthalmus* (average weight 100 + 30 g) were obtained from mahad fish centre, Maharashtra. The fish were allowed to acclimate for two methods by feeding a drug-free commercial diet. Mean water temperature, pH and dissolved oxygen were around 26 ± 2.00^{0} C, 7.4 and 6.4±0.01 ppm, respectively during the experimental period. On the day before drug administration, the fish were not fed. The experiment was carried out on 12 tanks with 10 fishes in each tank.

The tanks were kept in four groups with three tanks in each group. Among these, first group received intra peritoneal injection of ABZ of 20 mg/kg body weight, second group received intra muscular injection of ABZ of 20 mg/ kg body weight, whereas, third group was given an oral dose of ABZ of 20 mg/kg body weight and fourth group taken as control group. For injection, ABZ was dissolved in DMSO and for oral dose, ABZ suspension was made in 0.5% carboxy methyl cellulose (CMC).

Sampling

Blood was collected from each group through caudal vein at ½ h, 1h, 2h, 4h, 8h, 12h, 24h, and 48h. Blood collected into EDTA coating tubes and centrifuged at 2000 rpm at 4º C for 20 minutes. After centrifugation supernatant was collected and freezed immediately at -20º C until further analysis. Each plasma sample collected was analysed for ABZ, ABZSO and $ABZSO_2$ concentrations which was measured by high performance liquid chromatography (HPLC) technique.

Sample preparation and HPLC analysis

Two ml methanol was added to aliquots of plasma (400 µl) to precipitate protein. After vortex mixing for five minutes, sample were centrifuged at 3000 g for 10 min and filtered through a nylon 0.22-µm filter. 30µl aliquots of the filtered fractions were injected into the HPLC for further analysis

Instrumentation and chromatographic conditions

An HPLC system consisting of binary HPLC pump (Waters 1525), automatic sampler (Waters 2707). A mobile phase of Methanol-Water (60:40) at a constant flow rate of 1 ml/minute was employed. Analysis was performed on a sunfire^{TM} prep silica column (250 mm x 4.6 mm, 5.0 µ).

The samples were measured at 291 nm using a UV/Visible detector (Water 2489) and data were analyzed with Water Breeze^{TM} 2 integrator system. ABZ, ABZSO and $ABZSO_2$ were purchased from sigma-Aldrich, India. All chemicals were of HPLC grade.

Standard solution preparation

Standards of different concentration were prepared in methanol. Working standard solution was in the range of 10 µg/ml-0.05 µg/ml for each ABZ, ABZSO and $ABZSO_2$.

Results and Discussion

Calibration curves

The linearity of the detector response for the compounds was evaluated by injecting a total of 10 working standards solution of various concentrations. The calibration curves are given in of ABZ, ABZ-SO and ABZ-SO_2 in figure 1.

Concentration of ABZ, ABZSO and $ABZSO_2$ in plasma

For oral treatment the parent drug ABZ was detected in plasma up to 48 h. The retention time for ABZ was found at 23.507whereas for ABZSO and $ABZSO_2$ the retention times are 5.545 and 16.952 respectively shown in figure 2. The depletion profile of ABZ, ABZ-SO and ABZ-SO_2 from the drug plasma is shown in figure 3 (A&B). The highest concentration of plasma ABZ 3.46 µg/ml (C_{max}) was found at 8 h (T_{max}). ABZSO and ABZ-SO_2 was also detected upto 48 h. For ABZSOC_{max} was 0.037 µg/ml at 8 h ($T_{max)}$ whereas for $ABZSO_2$, C_{max} was 0.023 µg/ml at 12 h (T_{max}).

The ABZSO is active metabolite of ABZ in *P. hypophthalmus*. Concentration of ABZ-SO in plasma was higher than $ABZSO_2$ concentration. For intramuscular and intraperitoneal treatments, ABZ in DMSO was administered to fish but neither ABZ nor its metabolites were detected in any of these two treatments. Moreover, high mortality was reported in the fishes given intramuscular and intraperitoneal treatments with DMSO. Hence, it can be concluded that the antihelminthic drug ABZ shows best results when it is given as oral treatment to the target animal.

Fig.1 The calibration curve of of ABZ, ABZ-SO and ABZ-SO_2 standards solution of various concentration range from10 µg/ml to 0.05 µg/ml

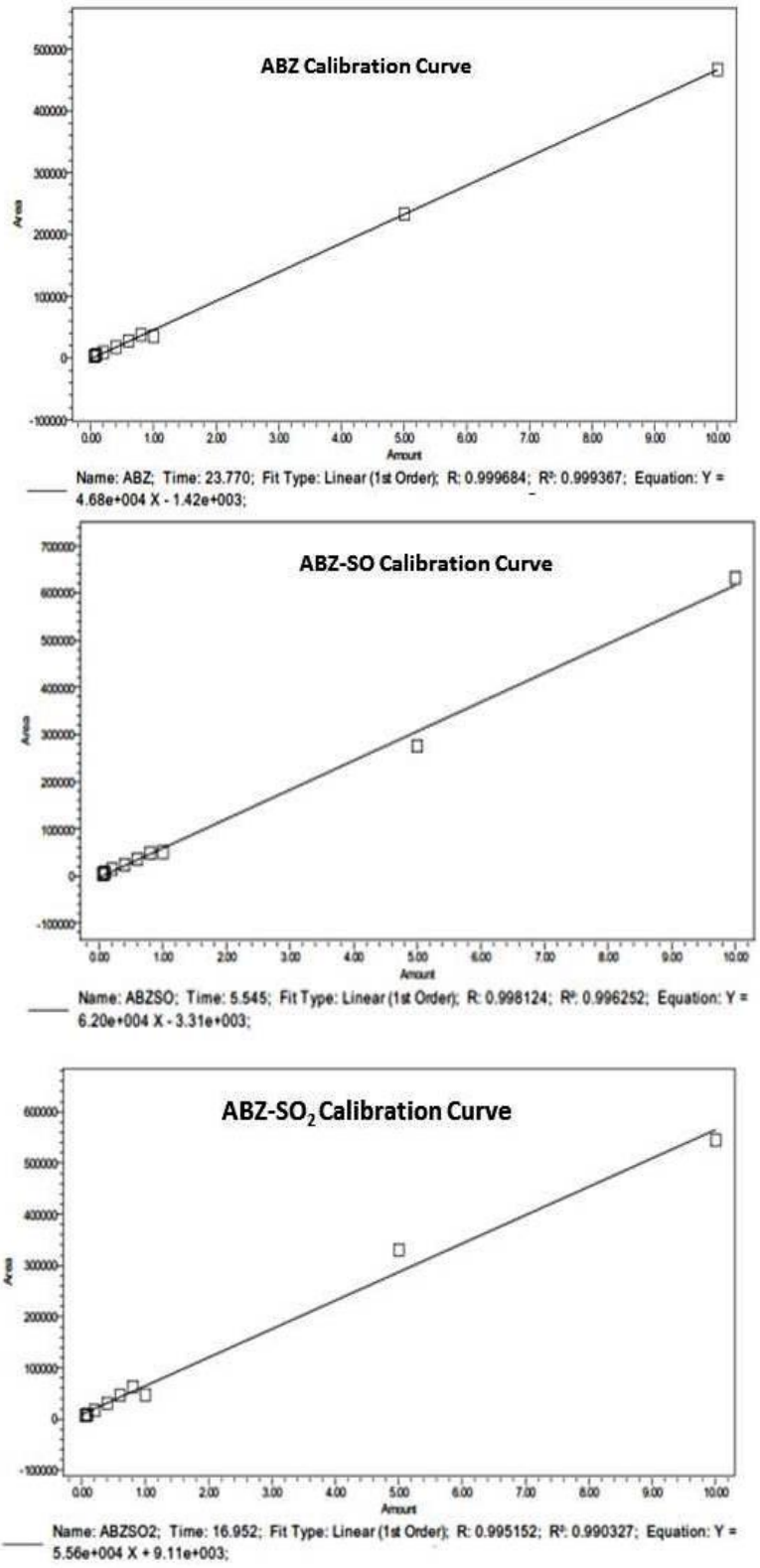


Fig.2 Chromatographs of ABZ, ABZ-SO and ABZ-SO_2 detected in plasma sample collected at with different Retention times

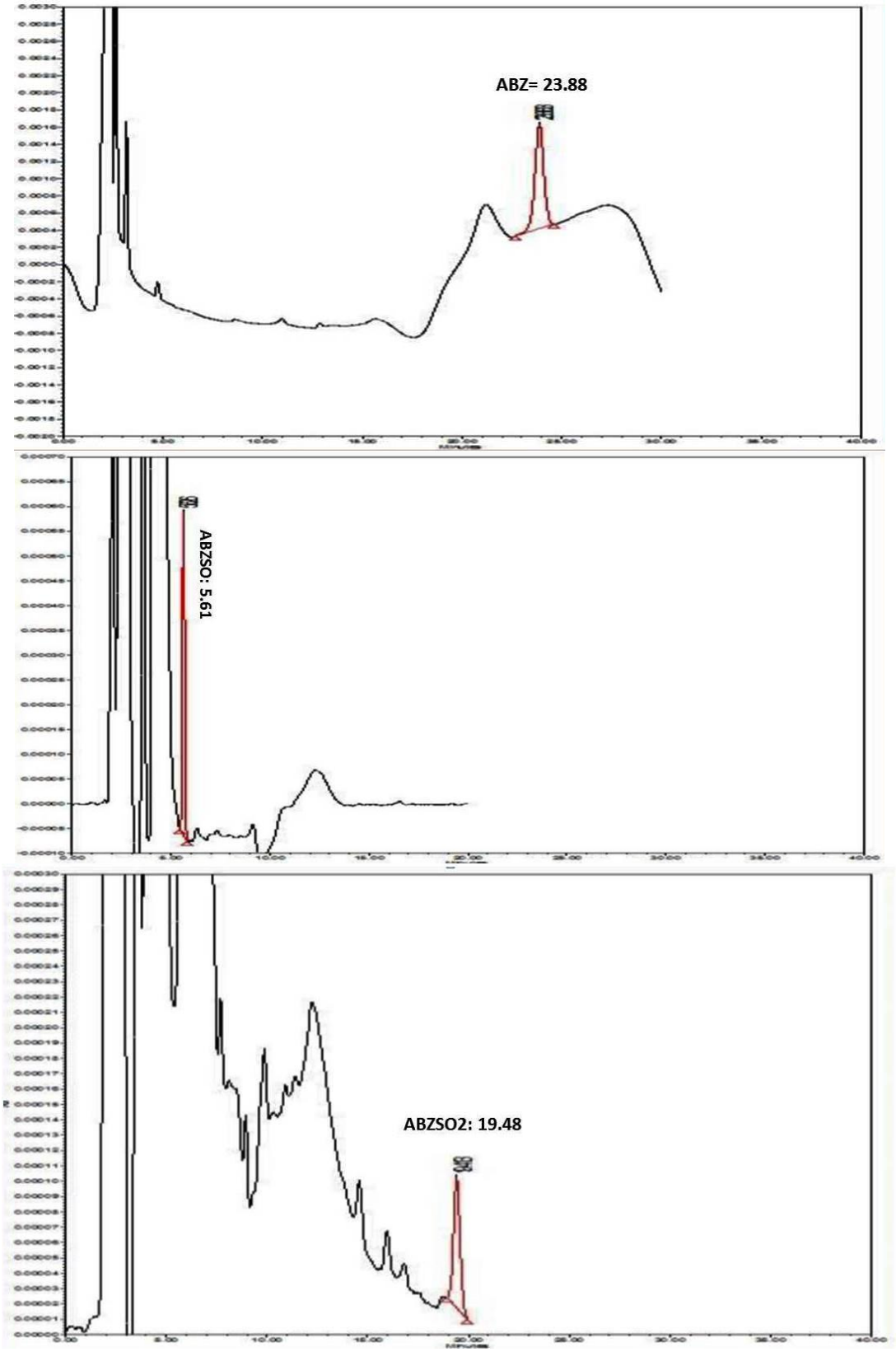


Fig.3A Plasma depletion concentration of ABZ vs time period following oral ABZ administration. Each time point (n= 3) represent the mean ± SE, with significant difference ($p<0.05$) at different time intervals

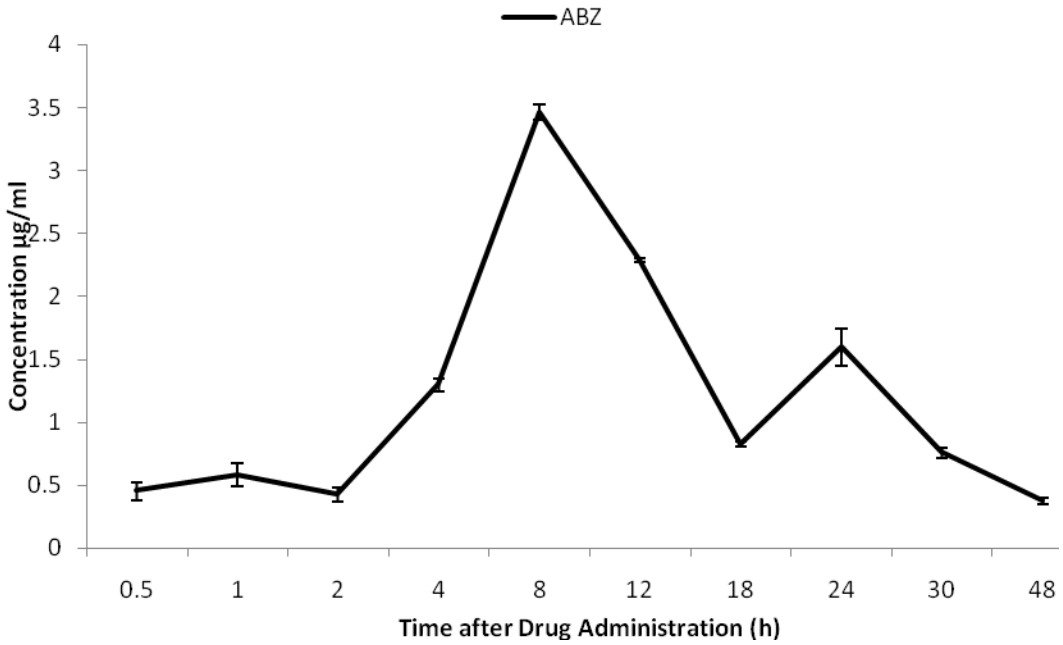
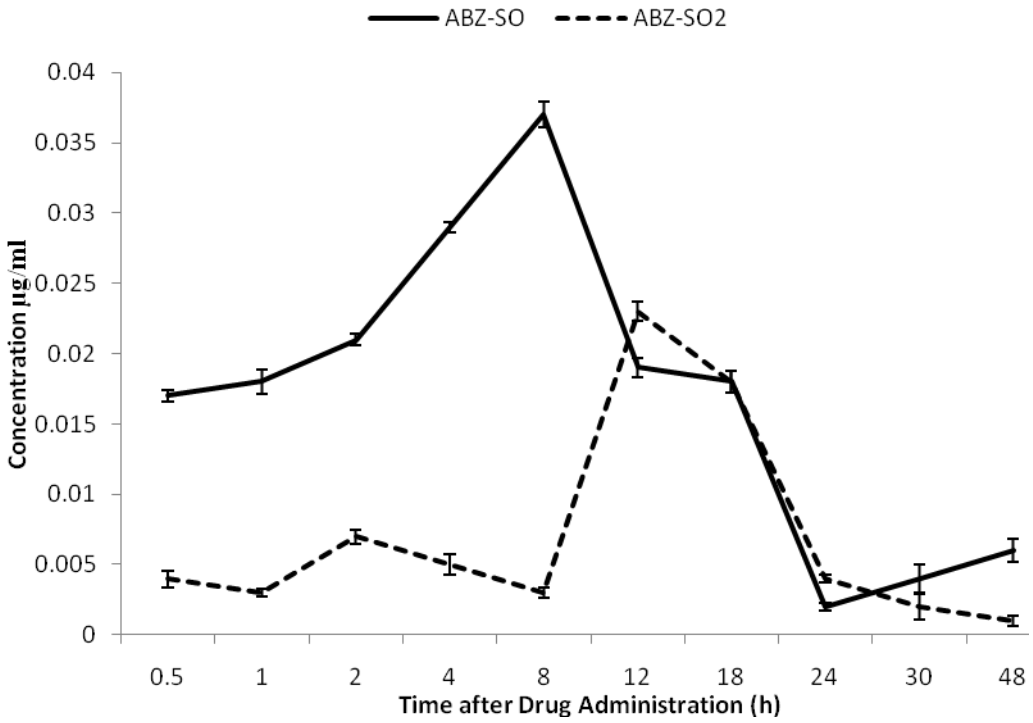


Fig.3B Plasma depletion concentration of ABZSO and $ABZSO_2$ vs time period concentration after oral ABZ administration. Each time point (n= 3) represent the mean ±SE, with significant difference ($p<0.05$) at different time intervals



In the present study, the pharmacokinetics of ABZ and its metabolites was done using HLPC to detect the concentration in plasma. Our study was comparable with the earlier study of Polo *et al.,* (2013) who have performed the HPLC assay for the quantification of Albendazole and metabolites,] ABZSO and ABZSO2 in plasma of mice. In this study calibration graphs containing in the range of 0.05 to 10 ugml-1 of ABZ, ABZSO and ABZSO2 with calibration curves were linear (r2 ≥ 0.990) which is similarly reported by (Shah *et al.,* 2014; Shaikh *et al.,* 2003). A 20 mg single dosage was given through oral intubation, intramuscular and intraperitoneal, the drug was found in oral dose only. The results are comparable with Cai *et al.,* (2007) who after giving the ABZ dose to rabbits oral as well as intraperitoneally found ABZ level were significantly higher after oral administration. The possibility of ABZ in rabbits may be due to a higher dosage of 150 mg given by Cai *et al.,* (2007) comparable to our study giving only 20mg/ body weight of fish. Further no reports of ABZ through intramuscular and intraperitoneally are available in fishes. In the present study, the concentration of ABZ and its metabolites were investigated up to 48 hours. These results are comparable with the of Shaikh *et al.,* (2003) who found that ABZ was depleted by 24 h in rainbow trout and tilapia by 48 h in salmon, respectively. The maximum concentration (Cmax) of ABZ 3.4614 µg/ml was found at 8h which is comparable with the earlier work of Mckellar *et al.,* (1995) who found maximum concentration (Cmax) 1.27± 0. 27 µg/ml at 8 h (Tmax) in 8 month old lambs.For ABZ-SO maximum concentration (Cmax) of 0.037 µg/ml was found at 8 h (Tmax) which is comparable with results of Goudah A (2003) who also found highest concentration ABZ-SO at 7.7 h after treatment in sheep. Further, the maximum concentration (Cmax) 0.023 µg/ml of ABZ-SO2 at 12 h (Tmax) which is very much similar to Gokbulut *et al.,* (2006) who found maximum concentration (Cmax) 0.04 ± 0.00 µg/ml at 8 h in donkeys. The concentration of parent compound ABZ and its metabolites ABZ-SO and ABZ-SO2 decline as the time increase from the point of administration. The concentration of parent compound was highest comparable to ABZSO and ABZSO2 in fish plasma.

In conclusion, the present study reveals that oral treatment is the best method for administration of Albendazole dosage for the helminthic diseases. Further, the depletion of Albendazole in fish takes near about 48 h for the effective removal of the drug from the plasma of fish. Hence, providing sufficient time for the removal of parasites from the fishes.

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