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Aspects of albendazole metabolism in western lowland gorillas (*Gorilla gorilla gorilla*) compared to humans and other species assessed by HPLC, LC-MS, and chiral electrokinetic chromatography

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The benzimidazole anthelmintic drug albendazole becomes metabolized to chiral albendazole sulfoxide, albendazole sulfone, albendazole 2-aminosulfone, and other metabolites. High-performance liquid chromatography with UV absorbance and fluorescence detection, liquid chromatography with mass spectrometry, and chiral electrokinetic chromatography were used to analyze albendazole metabolites in extracts of western lowland gorilla (*Gorilla gorilla gorilla*) plasma of patients with alveolar echinococcosis and the data are compared to those obtained with human patient samples and to those reported for other species in the literature. The data revealed that the albendazole sulfoxide to albendazole sulfone concentration ratio in gorilla plasma is significantly smaller compared to that in samples of men, rats, dogs, and cattle. It is, however, similar to what was observed in sheep and goats. The (+)-albendazole sulfoxide to (−)-albendazole sulfoxide enantiomeric ratio in gorilla blood determined by chiral electrokinetic chromatography was found to be >1 which is comparable to what was observed in humans, dogs, sheep, goats, and cattle but different to mice and rats. Furthermore, the electrokinetic chromatography data suggest that small amounts of albendazole 2-aminosulfone may be present in plasma of gorillas and humans. The data gathered with the various separation-based assays illustrate the value of technology featuring different analyte separation and detection principles. This is the first account describing aspects of the albendazole metabolism in gorillas.

KEYWORDS
albendazole 2-aminosulfone, albendazole sulfone, albendazole sulfoxide, enantioselectivity of sulfoxidation, gorilla

Abbreviations: ABZ, albendazole; ABZSO, albendazole sulfoxide; $ABZSO_2$, albendazole sulfone; $ABZSO_2$-NH_2, albendazole 2-aminosulfone; CBZ, cyclobendazole; OxBZ, oxfenbendazole; S-β-CD, sulfated β-cyclodextrin; TDM, therapeutic drug monitoring

1 | INTRODUCTION

Albendazole (ABZ) is a benzimidazole anthelmintic drug widely used in veterinary and human pharmacotherapy. It has a broad spectrum of activity against intestinal and tissue parasites and was found to be effective in the treatment of echinococcosis and neurocysticercosis. ABZ is a prochiral sulfide drug that undergoes rapid hepatic oxidation to albendazole sulfoxide (ABZSO), a chiral compound with a sulfur stereogenic center. ABZSO has anthelmintic activity and is further oxidized to achiral albendazole sulfone ($ABZSO_2$), albendazole 2-aminosulfone ($ABZSO_2$-NH_2), and other metabolites. $ABZSO_2$ and $ABZSO_2$-NH_2 are assumed to be anthelmintically inactive [1–3]. The chemical structures of ABZ and its three main metabolites are presented in Figure 1. Furthermore, the sulfoxidation of ABZ in man and different animals has been reported to be enantioselective [4]. After oral administration, ABZ is usually undetectable in plasma, whereas ABZSO reaches its maximum concentration about 2-3 h after dosing. Due to the high hydrophobicity of the drug, ABZ has low absorption which varies strongly in different individuals. Bioavailability is enhanced if ABZ is taken with a fatty meal. For optimized therapy against tissue parasites, ABZSO plasma levels should be >1 μM and therapeutic drug monitoring (TDM) is recommended [2,3,5–9].

For analysis of ABZ and/or its metabolites in body fluids, methods based upon thin-layer chromatography [1], HPLC with UV absorbance detection [2,5,9–12], HPLC with UV and/or fluorescence detection [13–15], LC-MS [16–19], and nonaqueous capillary electrophoresis with UV detection [20] were developed. Furthermore, assays based on chiral HPLC [4,12,21–23] and EKC with UV absorbance and/or fluorescence detection [24–27] were used to assess the enantioselectivity of ABZSO formation and elimination.

For more than 30 years, ABZSO plasma levels of patients were routinely measured by HPLC in our departmental drug assay laboratory to provide TDM service to the medical community in Switzerland [9,20]. ABZSO and $ABZSO_2$ were also determined in research samples, including vesicle fluids from in vitro treatment of cultivated *Echinococcus multilocularis* metacestodes with the two compounds [28], mouse serum to determine the pharmacokinetics of ABZSO in mice following infection with *E. multilocularis* metacestodes and uptake of a single dose of ABZ and a combination of nitazoxanide and ABZ [29], and mouse serum to measure ABZSO and $ABZSO_2$ levels after administration of ABZ emulsified in honey to mice by voluntary feeding or gavage [30]. In the three projects, monitoring was used to demonstrate that both compounds were taken up rapidly by the parasite, that combined ABZ/nitazoxanide chemotherapy resulted in increased ABZSO serum levels for the period of 4-8 h following drug uptake compared to application of ABZ alone, and that honey presents a viable vehicle for drug delivery, respectively. Moreover, nonaqueous capillary electrophoresis with on-column UV absorbance detection was investigated for TDM of ABZ in human plasma [20] and EKC with UV and fluorescence detection was explored to assess the stereoselectivity of ABZ sulfoxidation in man via analysis of ABZSO in plasma and saliva of patients under ABZ pharmacotherapy [25–27].

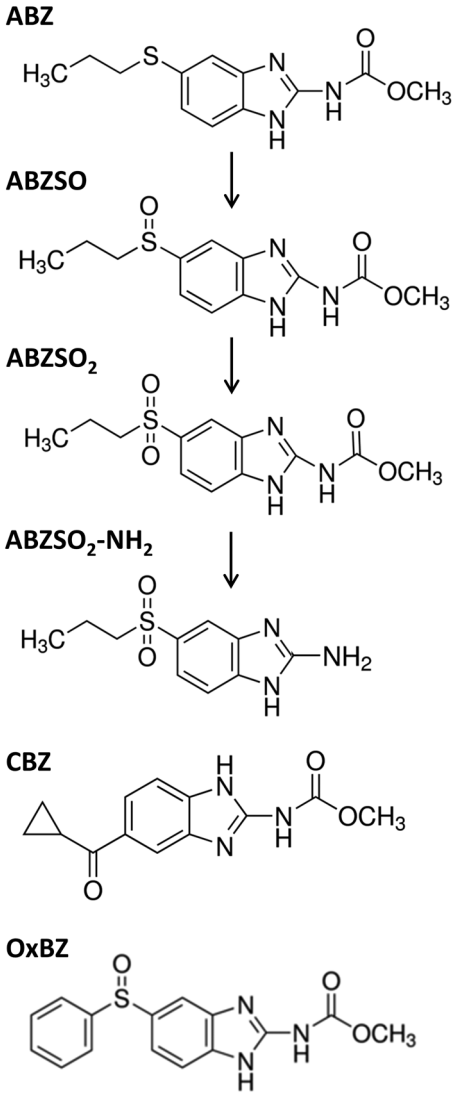

FIGURE 1 Chemical structures of ABZ, its metabolites ABZSO, $ABZSO_2$ and $ABZSO_2$-NH_2, and the two internal standards CBZ and OxBZ. ABZSO and OxBZ are chiral compounds with a sulfur stereogenic center

Our TDM laboratory received plasma samples from western lowland gorillas (*Gorilla gorilla gorilla*) diagnosed with alveolar echinococcosis and treated with ABZ in a Swiss zoo [31]. They were received to monitor ABZSO levels with the routine assay in order to ensure plasma levels above the therapeutic value determined for humans

(>1 μM). The data obtained indicated the presence of a second ABZ metabolite with a chromatographic behavior comparable to that of $ABZSO_2$. Its magnitude, however, was noted to be much larger than $ABZSO_2$ observed in humans. A similar HPLC pattern was monitored in sera of Japanese monkeys (*Macaca fuscata*) with alveolar echinococcosis that were treated with ABZ. In this work, the two ABZ metabolite peaks were assigned to ABZSO and $ABZSO_2$-NH_2 [32]. No scientific article about the metabolism of ABZ in gorillas was found in the literature.

The goals of the work reported in this article were to characterize the metabolic pattern of ABZ in extracts of gorilla plasma that stemmed from animals that were treated with ABZ. Plasma extracts were analyzed using (i) HPLC with UV absorbance and fluorescence detection, (ii) LC-MS, and (iii) chiral EKC. Data obtained with gorilla plasma were compared to those monitored in plasma of patients treated with ABZ. Differences and similarities of the ABZ metabolism in gorillas and humans could thereby be elucidated and is reported here for the first time. Furthermore, the gorilla data are compared to those reported for other species in the literature.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All chemicals were of analytical grade. ABZ was purchased from Sigma (St. Louis, USA), cyclobendazole (CBZ) from Janssen Research Foundation (Beerse, Belgium), and $ABZSO_2$-NH_2 was from Sigma-Aldrich (Schnelldorf, Germany). ABZSO, $ABZSO_2$, and oxfenbendazole (OxBZ) were a gift from SmithKline Beecham Pharmaceuticals (Brantfort, UK). Multiple isomer preparations of sulfated β-cyclodextrin (S-β-CD) with 12-15 mol sulfate/mol β-CD (lot 05112LH, Sigma-Aldrich) and with 4-7 mol sulfate/mol β-CD (SC1605, lot 1123, Michigan Diagnostics [Royal Oak, MI, USA]) were used. Bovine serum (adult) was purchased from Sigma and was used for the preparation of calibration and control samples.

2.2 | Origin of samples and sample preparation

Human patient samples were received for TDM service from medical facilities in Switzerland. Six gorilla samples were obtained from the Zoo Basel (Basel, Switzerland) and stemmed from gorillas with alveolar echinococcosis that were treated with ABZ (10 mg/kg PO two times a day) in a suspended formulation for cattle (Valbazen 10%, Zoetis, Zürich, Switzerland) [31]. The gorilla samples were obtained during general anesthesia for clinical examinations and animal transfers. The recommended time point of blood sampling for human therapy is about 4 h after drug administration [33] and blood collection for the gorillas varied between 2.5 and 10 h after oral application of ABZ [31]. All samples were stored in polypropylene vials at –20°C until analysis. For liquid/liquid extraction, 0.50 mL (HPLC, EKC) or 0.25 mL (LC-MS) of sample was combined with 500 μL of 0.25 M carbonate buffer (pH 10.3) and 4-5 mL of dichloromethane in a 10 mL glass tube. After gentle shaking of the closed tube for 10 min using a horizontal shaker and centrifugation at about 1750 *g* (3500 rpm) for 10 min, the aqueous (upper) phase was removed and the organic phase was evaporated to dryness at 40°C employing a gentle stream of air. The residue was reconstituted in 200 μL methanol (HPLC), 150 μL methanol (LC-MS), or 50 μL of fivefold diluted pH 8.7 Tris buffer that did not contain any chiral selector (EKC). Extraction recovery for ABZSO, $ABZSO_2$, CBZ, and OxBZ were in the range of 65-80% [20,26].

2.3 | HPLC routine assay with UV detection

ABZSO serum levels were determined by HPLC on a Waters Alliance System with a model 2695 separations module and a model 2487 absorbance detector (Waters, Milford, MA, USA) and is a modification of the method described elsewhere [20,29]. Briefly, the assay is based upon liquid/liquid extraction at alkaline pH using dichloromethane, a reversed phase EC 250/4 Nucleosil 100-5 C18 column (Macherey-Nagel, Oensingen, Switzerland), addition of CBZ as internal standard and solute detection at 230 nm. The mobile phase comprised a mixture of a 5 mM aqueous potassium dihydrogenphosphate solution (pH adjusted to 6.5 with a few drops of 20 % KOH) and acetonitrile (68:32, v/v). The flow rate was 0.7 mL/min and the temperature was ambient. A six-point internal calibration between 0.40 and 8 μM ABZSO was used. The calibration graphs for ABZSO were linear ($r^2 \geq 0.998$) with F values > 1000 ($p < 0.001$) and the limit for quantitation was taken as 0.4 μM. Interday drug level repeatability was less than 8%.

2.4 | LC-MS and HPLC with absorbance and fluorescence detection

ABZSO and $ABZSO_2$ serum levels were determined by LC-MS using an EC 125/4 Nucleodur C18 Isis 5 μm HPLC column (Macherey - Nagel) together with a HP series

1100 (Hewlett Packard, Waldbronn, Germany) coupled to an Agilent 6120 Single Quadrupole LC-MS System with an APCI/ESI interface (Agilent Technologies, Santa Clara, CA). This setup with gradient elution was previously employed to monitor the antibiotic drug cefepime in extracts of human serum and plasma [34] and in urine [35]. For analysis of ABZSO and $ABZSO_2$, isocratic elution was used with a mobile phase composed of 20 mM formic acid (63 %) and methanol (37 %) and the flow rate was 0.7 mL/min. The column oven temperature was set to 25°C and the injection volume was 10 µL. Nitrogen, provided by a nitrogen generator N2-Mistral-4 (Schmidlin Labor, Neuheim, Switzerland), was used as nebulizing and desolvation gas. The flow rate of nitrogen was 9 L/min, drying temperature was 250°C, and nebulizing pressure was 50 psig. The vaporizer temperature was 225°C, the capillary voltage was set to 2000 V, and the charging voltage was 1800 V. ABZ, ABZSO, $ABZSO_2$, $ABZSO_2$-NH_2, and CBZ (internal standard) were detected in the positive SIM mode with *m/z* mass values of 266.2, 282.1, 298.2, 240.2, and 260.2, respectively. These values correspond to the $[M+H]^+$ ions of the molecules. Quantitation was based upon six-point internal calibration in the range of 0.4 and 8 µM for ABZSO and $ABZSO_2$ using peak areas for data evaluation. The calibration graphs were linear ($r^2 \geq 0.991$) with F values ≥ 333 ($p < 0.001$), the limit for quantitation was taken as 0.30 µM for each compound, and interday drug level repeatability was less than 4.5%. The same setup without MS was also employed for experiments with UV absorbance (230 nm; G1315A DAD detector of Hewlett Packard) and fluorescence (280 nm excitation/320 nm emission; G1321A FLD detector of Hewlett Packard) detection. This approach was used for analyte identification and not for quantitation.

2.5 | Chiral electrokinetic chromatography

The chiral assay used was similar to that reported previously [26]. A Proteome Lab PA 800 instrument (Beckman Coulter, Fullerton, CA, USA) was equipped with a photodiode array detector set to 230 nm and a 50 µm id fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 55 cm total length (effective length 45 cm). The background electrolyte consisted of 20 mM Tris buffer (adjusted to pH 8.7 with phosphoric acid) containing 20 mg/mL (2% w/v) S-β-CD of Michigan Diagnostics. The samples were injected hydrodynamically (1 psi, 7 s). All temperatures were set to 20°C. A separation voltage of 18 kV was applied and the current was about 30 µA. Corrected peak areas based on an internal calibration with OxBZ (sum of both enantiomers) as internal standard were used for quantification. The calibration ranges were from 0.25 to 4 µM for the ABZSO enantiomers and from 0.5 to 8 µM for $ABZSO_2$. The calibration graphs for the ABZSO enantiomers and $ABZSO_2$ were linear ($r^2 \geq 0.991$) with F values ≥ 423 ($p < 0.001$) and the limit for quantitation was taken as 0.15 µM for each ABZSO enantiomer and 0.25 µM for $ABZSO_2$. Interday drug level repeatability was less than 7%.

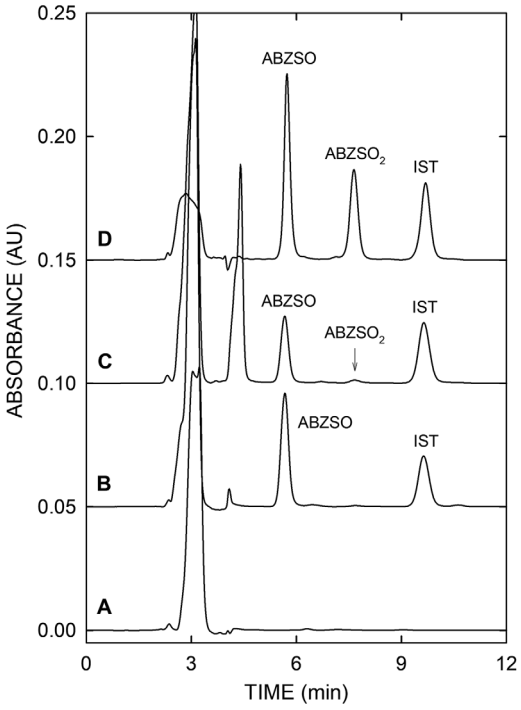

FIGURE 2 HPLC chromatograms with absorbance detection (230 nm) obtained with (A) bovine serum blank, (B) a calibrator sample comprising 6.0 µM ABZSO, (C) a human sample whose ABZSO level was determined to be 3.09 µM, and (D) a gorilla sample with an ABZSO level of 6.51 µM. IST refers to CBZ used as the internal standard

3 | RESULTS AND DISCUSSION

3.1 | Metabolite pattern monitored with the routine HPLC assay

Chromatograms from the routine TDM assay of ABZSO, the pharmacological active metabolite of ABZ, are presented in Figure 2. The HPLC assay with analyte extraction at alkaline pH using dichloromethane and UV absorbance detection was employed to provide TDM service to the medical community in Switzerland. More than 5000 patient samples were analyzed during the past 30 years to enable the control of the therapy with ABZ with the goal of having an ABZSO plasma level >1 µM [9,33]. A typical chromatogram of a patient sample is depicted in Figure 2C. This sample was determined to contain 3.09 µM

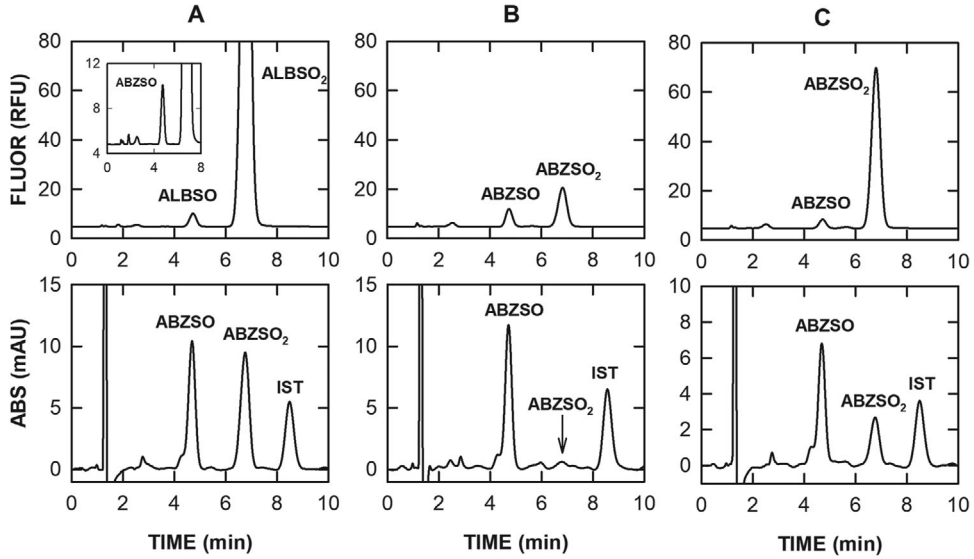

FIGURE 3 HPLC chromatograms with absorbance detection (230 nm, lower graphs) and fluorescence detection (280 nm excitation/320 nm emission, upper graphs) obtained with (A) a calibrator sample comprising 6.0 μM of ABZSO and 6.0 μM $ABZSO_2$, (B) a human sample whose ABZSO and $ABZSO_2$ levels were determined to be 5.58 and 0.32 μM, respectively, and (C) a gorilla sample whose ABZSO and $ABZSO_2$ levels were determined to be 5.99 and 2.69 μM, respectively. The insert in the upper graph of panel A depicts the ABZSO fluorescence peak at an expanded *y*-scale. IST refers to CBZ used as the internal standard

ABZSO. The chromatogram also exhibits a small peak of $ABZSO_2$ which was not quantitated in routine applications as it is assumed to be inactive. No ABZ can be detected with this assay as the parent drug is eluting much later than the internal standard [9].

The amount of $ABZSO_2$ in human plasma is reported to be much smaller than that of ABZSO in samples drawn about 4 h after drug ingestion of patients under pharmacotherapy with ABZ (Figure 2C) and for the following of the temporal behavior of the ABZSO and $ABZSO_2$ plasma levels after single dose administration of ABZ [9,12,13,16–20,26]. In the analyses of gorilla plasma, however, a strong peak eluting at the same time as $ABZSO_2$ was detected in all samples that were collected 2.5 to 10 h after oral application of ABZ. A typical chromatogram is depicted in Figure 2D. The magnitude of this peak was observed to be somewhat smaller than that of ABZSO (Figure 2D). In other sera, about equal or even larger amounts than those of ABZSO were monitored. These data suggest that a large amount of $ABZSO_2$ is present in these samples. HPLC with fluorescent detection was used to monitor ABZ metabolites in serum of Japanese monkeys (*Macata fuscata*) with alveolar echinococcosis that were treated with ABZ. In that work with samples collected 21-23 h after drug administration, the authors claim to have found significant amounts of $ABZSO_2$-NH_2 in addition to ABZSO whereas $ABZSO_2$ is not mentioned [32]. This is different to our result and prompted us to undertake further investigations using HPLC with absorbance and fluorescence detection, LC-MS, and EKC with absorbance detection.

3.2 | Identification of albendazole metabolites by HPLC with fluorescence detection and LC-MS

In the configuration used for LC-MS, chromatograms with UV absorbance detection were not as clean as those monitored with the routine assay. This is shown with the data presented as lower graphs in Figure 3 and is best illustrated with the unresolved interference at the front of the ABZSO peak. Thus, these assay conditions with UV absorbance detection cannot be employed for the accurate determination of ABZSO and $ABZSO_2$ levels in the plasma extracts of humans and gorillas. Nevertheless, the suspected difference in the $ABZSO_2$ plasma concentrations between human and gorilla samples (Figure 2C and D) is still visible. Using fluorescence detection, however, clear chromatograms are obtained (upper graphs of Figure 3). These data indicate that the fluorescence of $ABZSO_2$ is much stronger than that of ABZSO, and that CBZ used as internal standard is not fluorescing at all (Figure 3A). The former aspect was previously noted in EKC with lamp-based fluorescence detection [26] and LIF detection [27]. The fluorescence data also suggest that the amount of $ABZSO_2$ in the gorilla plasma is indeed much larger than that in the human sample (Figure 3B and C). Furthermore, this detection format could be used for quantification of the analytes provided that a fluorescing internal standard is included. Its use for quantitation of ABZSO and $ABZSO_2$ in the plasma extracts, however, was not further explored. Moreover, ABZ and $ABZSO_2$-NH_2 applied as standards

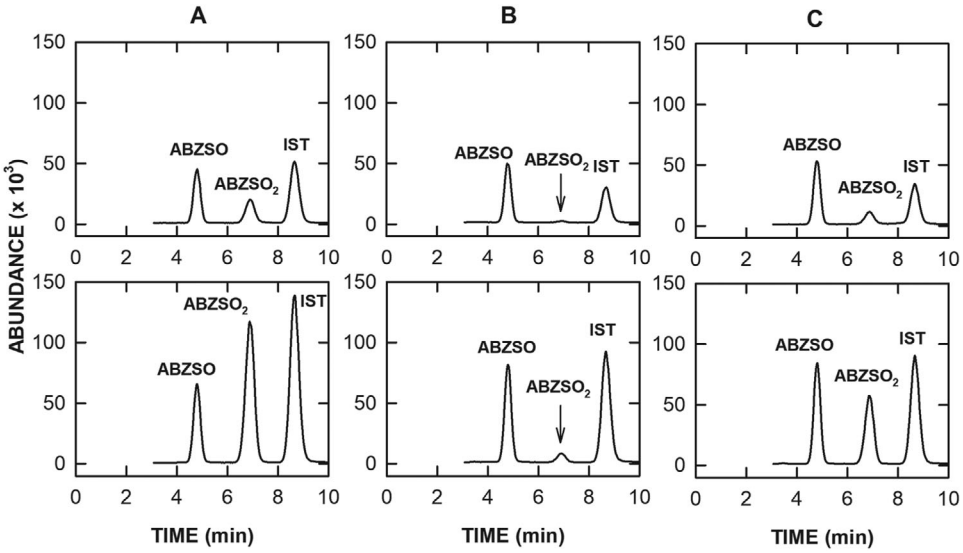

FIGURE 4 LC-MS chromatograms of $[M+H]^+$ ions (lower graphs; combined mass traces of 282.1, 298.2, and 260.2 for ABZSO, $ABZSO_2$, and CBZ, respectively) and of fragments (upper graphs; combined mass traces of 240.0, 266.0, and 228.2 for ABZSO, $ABZSO_2$, and CBZ, respectively) obtained with (A) a calibrator sample comprising 4.0 μM of ABZSO and $ABZSO_2$, (B) a human sample whose ABZSO and $ABZSO_2$ levels were determined to be 5.58 and 0.32 μM, respectively, and (C) a gorilla sample whose ABZSO and $ABZSO_2$ levels were determined to be 5.99 and 2.69 μM, respectively. IST refers to CBZ used as the internal standard

TABLE 1 Concentrations of albendazole metabolites and their concentration ratio

	Human samples (n = 6)[a]			Gorilla samples (n = 6)		
	ABZSO (μM)	$ABZSO_2$ (μM)	Ratio[b),c)]	ABZSO (μM)	$ABZSO_2$ (μM)	Ratio[b),c)]
Mean	6.79	0.51	13.97	3.68	2.77	1.59
SD	2.84	0.23	4.49	1.36	1.05	1.04
Median	6.61	0.45	14.93	3.20	2.86	1.04
Lowest value	2.98	0.32	8.28	2.35	0.97	0.91
Highest value	11.10	0.93	19.30	5.99	4.20	3.43

[a)] Samples with $ABZSO_2$ levels >0.3 μM.
[b)] ABZSO/$ABZSO_2$ concentration ratio.
[c)] Human versus gorilla comparison of ABZSO/$ABZSO_2$ ratio: significant difference ($p = 0.002$; Mann–Whitney Rank Sum Test).

were found to elute after 24 min and within the void peak at about 1.4 min, respectively (data not shown). This reveals that the parent drug and the additional metabolite do not interfere with the analysis of ABZSO and $ABZSO_2$ under the investigated conditions.

LC-MS was used to unambiguously identify and quantitate the two eluting ABZ metabolites ABZSO and $ABZSO_2$ in plasma of gorillas and humans that were treated with ABZ. LC-MS chromatograms obtained with a calibrator serum comprising 4.0 μM ABZSO and 4.0 μM $ABZSO_2$ (panel A), a patient sample containing 5.58 μM ABZSO and 0.32 μM $ABZSO_2$ (panel B) and a gorilla sample with 5.99 μM ABZSO and 2.69 μM $ABZSO_2$ (panel C) are presented in Figure 4. ABZSO, $ABZSO_2$, and CBZ (internal standard) are detected as nicely separated peaks. The lower graphs represent SIM data for the $[M+H]^+$ ions whereas the upper graphs depict SIM data for the protonated ions of the most abundant fragments of the three compounds formed during the electrospray ionization process and/or shortly thereafter. The three ions represent *m/z* values of 240.0 (ABZSO, $[M\text{-}C_3H_6+H]^+$), 266.0 ($ABZSO_2$, $[M\text{-}CH_3OH+H]^+$), and 228.2 (CBZ, $[M\text{-}CH_3OH+H]^+$) and provided comparable data with lower abundance than those of the $[M+H]^+$ ions. The fragments of the metabolites correspond to those reported in Refs. [1,16,17].

The mass traces for the $[M+H]^+$ ions were used for quantitation of ABZSO and $ABZSO_2$ levels in samples of human and gorilla patients. For data comparison (Table 1), the data of six human patients with $ABZSO_2$ levels >0.3 μM were selected and compared to those of the

six gorilla samples. The selection of the human samples enabled proper determination of both metabolites in all samples. The data presented in Table 1 confirm the assumption, that the ABZSO/$ABZSO_2$ concentration ratio in humans is significantly higher compared to that measured for the gorilla samples ($p = 0.002$; assessed with the Mann–Whitney Rank Sum Test). For ABZSO, comparative data analysis of the ABZSO plasma levels with LC-MS and the routine HPLC assay revealed a linear relationship with a coefficient of determination r of 0.985 (y-intercept: 0.25 μM; slope 0.901 with UV and LC-MS data taken along y- and x-axis, respectively). This indicates that there is agreement between HPLC and LC-MS data.

3.3 | Albendazole sulfoxide enantiomer and metabolite analysis with electrokinetic chromatography

Because of the observed difference in the ABZSO/$ABZSO_2$ concentration ratio, it was of interest to assess whether there is also a difference in the enantiomeric ratio of the ABZSO stereoisomers. The chiral assay used was similar to that reported previously in which the enantiomers of ABZSO and OxBZ were resolved and separated from $ABZSO_2$ as anionically migrating analytes in a pH 7 Tris buffer containing S-β-CD as complexing agent [26,27]. Figure 5 depicts typical electropherograms for bovine serum blank with internal standard (graph A), a calibrator serum containing 2 μM of each ABZSO enantiomer and 4 μM $ABZSO_2$ (graph B), a patient plasma (graph C) and two gorilla samples (graphs D and E). OxBZ was used as internal standard (as in [26]). In the EKC assay, CBZ employed as internal standard in HPLC and LC-MS was detected between the two ABZSO enantiomers. The samples of panels B to D were the same as those analyzed in Figure 4. The data reveal that (−)-ABZSO is detected prior to (+)-ABZSO, that $ABZSO_2$ is detected shortly before the enantiomers of OxBZ (internal standard) and that the OxBZ enantiomers are incompletely resolved. The overall pattern is somewhat different to that reported previously [26]. The reason lies in the nature of the chiral selector used. At the time of our previous publication, S-β-CD of Aldrich had 7-11 mol sulfate/mol β-CD. This product became no longer available and was substituted with S-β-CD that contains 12-15 mol sulfate/mol β-CD [36]. With the new formulation, however, the enantiomers of ABZSO and OxBZ could not be resolved (data not shown). Thus, the data presented in Figure 5 were measured with a commercial product from Michigan Diagnostics which features 4-7 mol sulfate/mol β-CD. This selector provided nice resolution of the ABZSO enantiomers and an incomplete resolution of the stereoisomers of OxBZ. As the latter fact was unimportant for the present study, no investigations were executed to improve this resolution. For quantitation, the sum of the unresolved peaks was used. Furthermore, the buffer pH was raised from 7.0 to 8.7 and thereby remains in the pH range in which ABZSO is neutral [8].

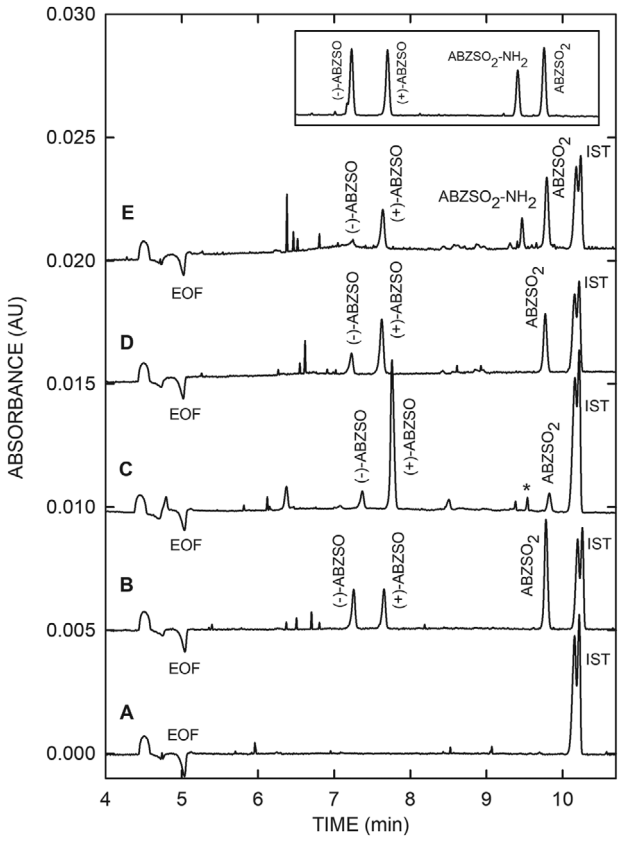

FIGURE 5 EKC electropherograms with absorbance detection at 230 nm obtained with (A) bovine serum blank, (B) a calibrator sample comprising 2.0 μM of each ABZSO enantiomer and 4 μM $ABZSO_2$, (C) a human sample whose ABZSO and $ABZSO_2$ levels were determined to be 5.58 and 0.32 μM, respectively, (D) a gorilla sample whose ABZSO and $ABZSO_2$ levels were determined to be 5.99 and 2.69 μM, respectively, and (E) a gorilla sample whose ABZSO and $ABZSO_2$ levels were determined to be 3.08 and 3.14 μM, respectively. The insert depicts an electropherogram obtained with the standards of ABZSO, $ABZSO_2$, and $ABZSO_2$-NH_2. The peak marked with an asterisk in graph C could represent $ABZSO_2$-NH_2. IST refers to OxBZ used as the internal standard and EOF marks the electroosmotic flow

The EKC data obtained with the six gorilla samples and the six patient samples (same samples as for the ABZSO/$ABZSO_2$ concentration ratio of Table 1) are summarized in Table 2. ABZSO (sum of the two enantiomers) and $ABZSO_2$ levels in the 12 samples determined by EKC compared well with those monitored by LC-MS. Comparative analysis of the concentration levels of these compounds revealed linear relationships with regression coefficients r of 0.972 and 0.996, respectively. In all six gorilla

TABLE 2 Concentrations of albendazole sulfoxide enantiomers and their enantiomeric ratio

	Human samples (n = 6)[a]			Gorilla samples (n = 6)		
	(−)-ABZSO (μM)	(+)-ABZSO (μM)	Ratio[b),c)]	(−)-ABZSO (μM)	(+)-ABZSO (μM)	Ratio[b),c)]
Mean	1.39	5.73	5.41	0.64	2.90	6.50
SD	0.84	1.33	2.94	0.47	0.80	3.93
Median	1.33	6.01	4.21	0.53	2.57	4.56
Lowest value	0.46	4.00	2.73	0.18	2.14	2.86
Highest value	2.64	7.20	9.54	1.45	4.14	11.89

[a)]Samples with $ABZSO_2$ levels > 0.3 μM.
[b)](+)-ABZSO/(−)-ABZSO enantiomeric ratio.
[c)]Human versus gorilla comparison of enantiomeric ratio: no significant difference ($p = 0.485$; Mann–Whitney Rank Sum Test).

samples, the concentration of the (−)-ABZSO enantiomer was smaller compared to that of (+)-ABZSO. This is also the case in the human patient samples analyzed in this study, previously [26] and by others [4,22,23,37,38]. Analysis of the ratio data of the two groups with the Mann–Whitney Rank Sum Test revealed no statistical difference ($p = 0.485$) between the two input groups. These data suggest that sulfoxidation of ABZ in western lowland gorillas is stereoselective and the stereoselectivity is the same as in humans, that is, with the predominance of the (+)-ABZSO enantiomer. The same was observed in blood of dog [38], goat [39], sheep [4,21,39], and cattle [4,39], whereas the opposite was found in the blood of rats [4,21,38] and mice [11]. It is worth to note that i.v. application of racemic ABZSO to sheep [40] and cattle [41] revealed that the (−)-ABZSO enantiomer was depleted significantly faster from plasma compared with the (+)-ABZSO antipode whereas the opposite was observed in rats [42].

$ABZSO_2$-NH_2 cannot be analyzed under the HPLC and LC-MS conditions described in this article. Mirfazaelian et al. [13] have reported an HPLC assay for all three ABZ metabolites in human serum which requires both UV absorbance (for ABZSO) and fluorescence (for $ABZSO_2$ and $ABZSO_2$-NH_2) detection. This approach provided a poor chromatographic separation of the compounds of interest. The three analytes can be precisely determined on a reversed-phase column in presence of positively and negatively charged pairing ions in the mobile phase [14]. The same was found to be true under the EKC conditions used in our laboratory. An electropherogram obtained with the standards of ABZSO, $ABZSO_2$, and $ABZSO_2$-NH_2 is presented as insert in Figure 5. $ABZSO_2$-NH_2 was detected before $ABZSO_2$ and appears to be present in one of the two depicted electropherograms obtained with gorilla samples (graph E of Figure 5). The same could be the case with the peak marked with an asterisk in graph C of Figure 5 as well as the additional peak observed in the electropherogram of the human patient samples monitored with fluorescence detection in Refs. [26,27]. More work is required to investigate the analysis of $ABZSO_2$-NH_2 in human and gorilla samples. These data suggest that EKC would be suitable to simultaneously monitor the metabolites of ABZ, including the enantiomers of ABZSO, in extracts of body fluids. Fluorescence would thereby provide highest detection selectivity and sensitivity and a mean to recognize also minor metabolites of ABZ, particularly the sulfone metabolites [1].

4 | CONCLUDING REMARKS

The data obtained with HPLC, LC-MS, and chiral EKC revealed that the ABZSO/$ABZSO_2$ concentration ratio in gorilla plasma is significantly smaller compared to that in human samples. $ABZSO_2$ plasma levels in the samples of gorillas were found to be smaller, equal, or even larger than those of ABZSO. This is in contrast to human samples of patients under ABZ pharmacotherapy [9,18,20,26] and found in pharmacokinetic studies of men, rats, dogs, and cattle [12,13,19,21,38]. It is, however, similar to what was observed in sheep [14,21,39,40] and goats [39]. In the blood of gorillas, the (+)-ABZSO/(−)-ABZSO enantiomeric ratio determined by chiral EKC was found to be >1 (the concentration of the (−)-ABZSO enantiomer was smaller compared to that of (+)-ABZSO) which is comparable to what was observed in humans, dogs, sheep, goats, and cattle [4,21–23,26,37–39] but different to mice and rats, species with a predominance of the (−)-ABZSO enantiomer and thus an (+)-ABZSO/(−)-ABZSO enantiomeric ratio <1 [4,11,21,38]. Furthermore, the gathered EKC data suggest that small amounts of $ABZSO_2$-NH_2 may be present in plasma of gorillas. This, however, was not validated, but would make sense if the pharmacokinetics is similar to what was described for sheep [14]. For Japanese monkeys, no $ABZSO_2$ but significant plasma levels of ABZSO and $ABZSO_2$-NH_2 were reported [32]. This finding is difficult to comprehend because $ABZSO_2$-NH_2 is a metabolite of $ABZSO_2$ (Figure 1). It may, however, also

be formed by sulfoxidation of ABZSO-NH_2, a minor metabolite of ABZSO [1]. More samples, particularly those to assess the metabolic pattern over 3 to 12 h after drug administration, would be required to elucidate a more detailed picture of the metabolism of ABZ in western lowland gorillas.

The data gathered with the various separation-based assays illustrate the value of technology featuring different analyte separation and detection principles. EKC with UV absorbance detection is thereby shown to provide a simple mean to monitor all main metabolites of ABZ including the enantiomers of ABZSO. The same assay format with combined absorbance and fluorescence detection would have the potential to monitor also minor metabolites of ABZ in extracts of body fluids and tissues. More work is required to elucidate the suitability of this approach.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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