Research article

Biomedical Chromatography

Received 27 September 2010, Accepted 09 November 2010 Published online in Wiley Online Library: 04 February 2011

(wileyonlinelibrary.com) DOI 10.1002/bmc.1588

Application of ultra-performance columns in high-performance liquid chromatography for determination of albendazole and its metabolites in turkeys

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ABSTRACT: Methods for determination of albendazole (ALB), albendazole sulfoxide (SOX) and albendazole sulfone (SON) in turkey blood plasma, using high-performance liquid chromatography (HPLC) with fluorescence detection, were developed. Moreover, comparison of HPLC columns with ultra-performance liquid chromatography (UPLC) columns was performed. Albendazol was administered orally in 5-week-old birds (n=18) at a dose of 25 mg/kg b.w. Accuracy and precision of the developed method were satisfactory and stability studies showed acceptable variation (below 15%) in ALB, SOX and SON concentrations when the samples were stored at –75°C for 15 days. UPLC® columns gave higher peaks from typical HPLC columns retaining high quality of analysis. Pharmacokinetic analysis indicated quick elimination of ALB from turkey blood plasma. The mean residence time of SON was at least two times longer than that of SOX and four times longer than that of ALB. The elimination half-lives for ALB, SOX and SON were 0.7 ± 0.27, 5.37 ± 6.03, 9.17 ± 5.12 h, respectively. The obtained results indicate that the described method allows for precise determination of albendazole and its metabolites in turkey plasma. Moreover, using UPLC columns in HPLC apparatus results in higher sensitivity as compared with the classical HPLC columns. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: albendazole; pharmacokinetics; ricobendazole; HPLC; UPLC; turkeys

Introduction

Albendazole (ALB) is a broad spectrum methylcarbamate benzimidazole anthelmintic drug with structural formula closely related to fenbendazole. Currently it is extensively used in veterinary and human medicine as an antiparasitic agent (Dayan, 2003; Vuitton, 2009).

Albendazole is metabolized intestinally to albendazole sulfoxide (SOX) metabolite by cytochrome P450 (CYP450) and flavin-containing monooxygenase (Molina *et al*., 2007). In mammals (humans, calves, sheep, goats, pigs, rabbits, ewes, dogs and mice) and turkey liver, it is metabolized by CYP3A4 to the SOX – with pharmacological activity (ricobendazole) and albendazole sulfone (SON) – without pharmacological activity. A native drug is highly (89–92%) bound to plasma proteins whereas active SOX is bound only in 62–67% (Jung *et al*., 1998). In human pharmacokinetics studies, plasma levels of SOX at the steady state showed high intraindividual variability (Jung *et al*., 1992). In veterinary practice, ALB is used in mammals but not in poultry. Callait *et al*. (2002) showed that ALB is ineffective in the treatment of *Histomonas meleagridis* (turkey blackhead), one of the most important diseases of turkeys. However, another study indicated that ALB can be effective in protection of turkeys against *Heterakis gallinarum* or flagellated form of *Histomonas* (Hegngi *et al*., 1999). Clinical effectiveness in the treatment of turkeys infected by *Ascaridia dissimilis* is described for homologous drug fenbendazole (Yazwinski *et al*., 1993, 2002). Unfortunately, both ALB studies were performed without any knowledge of the pharmacokinetics of ALB and its active metabolite SOX. Because of possible high coefficients of variation, pharmacokinetic parameters of SOX and rapid metabolism of ALB, the bioanalytical method for the ALB and SOX determination must be accurate, precise and stable in large sets of analysis. UV detectors have been extensively used for the determination of ALB and SOX, and solid-phase extraction technique has often been used for ALB and SOX analysis (Valois *et al*., 1994; Kitzman *et al*., 2002; Wu *et al*., 2005). The employment of high performance liquid chromatography (HPLC) with mass spectrometry HPLC-MS^n methods has provided selective and sensitive bioanalytical

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Abbreviations used: ALB, albendazole; CYP450, cytochromes P450; MRT, mean residence time; SON, albendazole sulfone; SOX, albendazole sulfoxide.

methods for ALB metabolism and pharmacokinetics analysis (Bonato *et al*., 2007). However, strategies of ALB, SOX and SON analysis using ultra-performance liquid chromatography (UPLC®) columns with HPLC equipment are unknown. Therefore, in the present study, the development and validation of a new method (using two different internal standards, different gradient elution and one-step liquid–liquid extraction) for determination of ALB and its metabolites (SOX and SON) using HPLC apparatus with fluorescence detection have been described. Moreover, comparison of HPLC columns with UPLC columns was performed and concentrations of ALB and its main metabolites in turkey plasma were determined.

Materials and methods

Chemicals and reagents

The following reference materials were used: ALB (CAS no. 54965-21-8; PI Drug and Pharmaceuticals Ltd), SOX (CAS no. 54029-12-8) and SON (CAS no. 75184-71-3; Witega Laboratorien GmbH), propranolol as internal standard 1 (IS1; CAS no. 525-66-6; LGC Promochem), thiabendazole as internal standard 2 (IS2; CAS no. 148-79-8) and sodium dodecyl sulfate (Fluka, Sigma-Aldrich), methanol, acetonitrile and HPLC-grade water, acetic acid (glacial), ammonium hydroxide, ammonium acetate and ethyl acetate (J.T. Baker, Mallinckrodt Baker Inc.).

Equipment

Separation module Alliance Waters 2695 was equipped and operated under Empower Pro software (Waters Corporation). The fluorescence detector was a Waters 2475 (Waters Corporation). The group of HPLC columns included XBridge™ Phenyl 3.5 µm, 2.1/100 mm (Waters Corporation), Hypersil Gold aQ 3 µm, 3.0/100 mm (Thermo Scientific), Synergi Fusion-RP 80 4 µm, 4.6/150 mm (Phenomenex®), Allure Basix 3 µm 4.6/100 mm (Restek USA) and LiChrosorb® RP_8, 10 µm, 4.0/250 mm (Merck KGa). The group of UPLC® columns included Acquity UPLC® HSS T3 1.8 µm, 2.1/50 mm and Acquity UPLC® BEH C_{18} 1.7 µm, 2.1/100 mm (Waters Corporation), Pinnacle DB C_{18} 1.9 µm, 2.1/50 mm (Restek USA), VisionHT C_{18}-P 1.5 µm, 2.0/50 mm (Grace) and Hypersil GOLD 1.9 µm, 2.1/50 mm (Thermo Scientific). Analytical balance was a WAX- 40/160 (RADWAG). The centrifuge was an MPW 350 R (MPW Medical Instruments). Freezer temperature was −80°C (Forma Scientific). The evaporator was a Zymark® TurboVap LV® (Caliper Life Sciences). Nitrogen was in accordance with PN-C-84920-97, 99.995% (Amco Gaz). The pipette controller was a Pipetus® (Hirschmann® Laborgeräte GmbH). Automatic volumetric pipettes of 10–100 and 100–1000 µL (Socorex Isba S.A.) were used. pH was measured using a 302 pH meter (Hanna Instruments®). The Vortex TTS-2 was from IKA® Werke GmbH. Magnetic stirrers (Big Squid, IKA® Werke GmbH), a syringe filter (UniFlo® RC, 13 mm, 0.45 µm, Whatman®), total recovery vials (Waters Corporation), 13/100 mm glass tubes (Pyrex®) and 2 mL Eppendorf microfuge tubes (BrandTech Scientific Inc) were used.

Animals and experimental procedure

The animal procedure was approved by the Local Ethics Committee on Animal Care and Supply in Olsztyn (agreement no. 44/2009) in accordance with the guidelines provided by the Polish Council and Animal Care. The study was harmonized with the ethical principles that have their origins in the Declaration of Helsinki. The bioanalytical part of the study was performed in accordance with the Good Laboratory Practice (GLP) requirements. Twenty 4-week-old female turkeys, type BIG-6, were obtained from a commercial farm in Kieźliny, near Olsztyn, and transported to the animal house of the Faculty of Veterinary Medicine, University of Warmia and Mazury. At the new place, birds were fed with the same standard turkey grower diet and water *ad libitum*. After one week of acclimatization, 18 birds were not fed for 10 h (at night), and thereafter birds were treated with albendazole (Valbazen 10%, Pfizer Animal Hellth, Belgium) at a dose of 25 mg/kg b.w. A single dose of albendazole was diluted in distilled water to a final volume of 3 mL and administered orally (directly into the crop, using a thin plastic tube). After drug administration, the birds were not fed for 2 h but had access to water, and their behavior was observed. Blood samples (1 mL) were collected into heparinized tubes from the brachial vein at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h after drug administration. After centrifugation (2000 ***g*** for 10 min), plasma samples were collected and stored in the −75°C ultrafreezer until albendazole and its metabolites were determined. The remaining two birds were bled for the collection of blank samples. All birds were euthanized by intraperitoneal injection of sodium pentobarbital (Morbital, Biowet Puławy, Poland) at the end of experiment.

Chromatographic conditions

The gradient HPLC elution mobile phase was composed of acetonitrile and 0.09 M ammonium acetate buffer (v/v) at pH 4.5. Compositions of acetonitrile:buffer (v/v) in mobile phase A and mobile phase B were 1:9 and 6:4, respectively. For ALB and IS1 the gradient was (min; %A; %B): (0; 75; 25), (3.5; 75; 25), (4; 50; 50), (15; 5; 95), (15.1; 75; 25), (23; 75; 25); for SOX, SON and IS2 analysis (min; %A; %B): (0; 90; 10), (3.5; 90; 10), (4.0; 50; 50), (15; 5; 95), (15.1; 90; 10), (23; 90; 10). The flow rate was 0.20 mL/min. Two Acquity® UPLC HSS T3 1.8 µm, 50 × 2.1 mm columns (Waters Corporation, Milford, USA) were used for albendazole and its metabolites analysis. The fluorescence detector was working at 288 nm (excitation energy) and 310 nm (emission). The column was maintained at an ambient temperature (25°C), while the autosampler temperature was set at 10°C. The maximal pressure of the system during the analysis was 2350–2400 psi. Under these conditions, the retention time of ALB and IS1 was 12.5 and 9.9 min, respectively, and for SOX, SON and IS2 was about 5.9, 9.6 and 7.1 min, respectively. The injection volume was 10 µL and the total run time was set for 23 min.

Calibration curves and quality control

Preparation of the ALB and IS1 standard quality and control samples. Stock solutions of ALB (1.0 mg/mL and intermediate standard solution 1.0 µg/mL), and IS1 (1.0 mg/mL and intermediate standard solution 1.0 µg/mL) were prepared by dissolving each of the accurately weighed reference compounds in MeOH. The calibration curve consisted of eight standards based on working solutions of ALB: 10.5, 20.4, 29.9, 39.9, 49.8, 99.7, 247.0 and 548.0 ng/mL. Working solution of IS1 was 5.0 µg/mL.

Stock and working solutions of ALB were used to prepare three levels of quality control (QC) working solutions; at the lowest concentrations (lowest quality control, LQC), 20.0 ng/mL;

at the medium concentration level (medium quality control, MQC), 100.0 ng/mL; and at the high concentration level (high quality control, HQC), 250.0 ng/mL.

All working solutions were prepared in 25 mL volumetric flask and were stored along with the stock solutions at 4°C. Calibration standards, LQC, MQC, HQC and IS1 samples were prepared in the same matrix (turkey plasma).

Preparation of the SOX, SON and IS2 standard quality and control samples. Stock solutions of SOX (1.0 mg/mL), SON (0.2 mg/mL) and intermediate standard solution (25.0 µg/mL) and IS2 (10.0 µg/mL) were prepared by dissolving each of the accurately weighed reference compounds in MeOH. Working solutions of SOX were 51.6, 109.0, 254.0, 509.0, 763.0, 1527.0, 2181.0 and 2726.00 ng/mL. Working solutions of SON were 9.99, 20.0, 30.6, 40.0, 50.6, 99.9, 246.0 and 502.0 ng/mL. Working solution of IS2 was 10.0 µg/mL. Working stock solutions were used to prepare three levels of QC working solutions; LQC, MQC and HQC for SOX and SON were 100.0 and 20.0 ng/mL, 750.0 and 100.0 ng/mL and 2100.0 and 250.0 ng/mL, respectively.

Sample extraction and preparation

All plasma samples for ALB determination were thawed at room temperature and vortexed. The volume of 0.250 mL of plasma was transferred into glass tubes and 20 µL of IS1 (5.0 µg/mL) working standard solution was added. During mixing on vortex at 1400 rpm, 0.05 mL of sodium dodecyl sulfate solution and 1.0 mL of ethyl acetate were added followed by centrifugation of the glass tubes for 20 min at 4075 ***g*** at 21°C. After centrifugation the glass tube was frozen at −75°C for 10 min, the upper layer was transferred into a clean glass tube and 0.05 mL of sodium dodecyl sulfate solution was added. The sample was evaporated at 45°C under a stream of nitrogen. The dry residue was re-constituted in 200 µL of mixture of acetonitrile with 0.09M ammonium acetate buffer at pH 4.5, 1:1 (v/v), and filtered by syringe filter RC 0.45 µm into an autosampler vial.

All plasma samples for SOX and SON determination were thawed at room temperature and vortexed. The volume of 0.500 mL of plasma was transferred into glass tubes and 20 µL of IS2 (10.0 µg/mL) working standard solution was added. During mixing on vortex at 1400 rpm, 0.1 mL of sodium dodecyl sulfate solution and 2.0 mL of ethyl acetate were added. The next step was the centrifugation of the glass tubes for 20 min at 4075 ***g*** at 21°C. After centrifugation the glass tube was frozen at −75°C for 10 min and upper layer was transferred into clean glass tube. The sample was evaporated at 45°C under a stream of nitrogen. Dry residue was re-constituted in 200 µL of mobile phase A, and filtered by syringe filter RC 0.45 µm into autosampler vial.

Validation

A calibration curve, containing eight non-zero standards for each analytical run, was prepared. Linearity in both the validation procedures was assessed for eight calibration points for ALB, SOX and SON. Study of linearity was conducted during the subsequent 3 days. A zero and a blank plasma sample were also prepared for each analytical run during the study to confirm the absence of interferences. A least square regression analysis of the data was performed to determinate the calibration curve parameters $y = a \times x + b$. Response−peak area ratio analyte/internal standard×concentration of internal standard was the basis for calculation of concentration in plasma samples. The calculated concentrations were determined from the linear regression with $1/y$ weighting method for both analytical methods. Accuracy was established over the range of linearity based on the data from the linearity study performed in six runs during 3 days. Data from nine runs were used in order to assess the extraction efficiency of the analytical method. Samples from all the standard curve points, extracted according to the developed method, were compared with the standard curve samples for each concentration. Stability experiments were performed to evaluate the autosampler stability, freeze and thaw stability and working standard solution stability. To evaluate the whole stability study, plasma samples containing LQC, MQC and HQC concentrations of ALB, SOX and SON were used.

UPLC and HPLC columns comparison

Comparison of two groups of chromatography columns was performed based on validated method for ALB determination in plasma. Comparison was made using spiked plasma samples and standard solution for all columns. Four UPLC® columns were run with flow rate 0.20 mL/min, except the Acquity UPLC® BEH C_{18} 1.7 µm, 2.1/100 mm where the flow rate 0.15 mL/min was used. Three HPLC columns were run with flow rate 1.00 mL/min, except XBridge™ Phenyl 3.5 µm, 2.1/100 mm where 0.50 mL/min was used and LiChrosorb® RP-8, 10 µm, 4.0/250 mm, where 1.50 mL/min was used. Two peaks, ALB and IS1, were measured and compared. Inclusion criteria (based on the system suitability, SST, of the validated method) for both groups of columns were retention time for ALB and IS1 between 5.0 and 14.0 min, $K' \geq 3.5$, USP tailing factor ≤ 1.4, maximal backpressure $\leq$ 3600 psi without any changes in validated method except the flow rate.

Data analysis

Validation of the bioanalytical method was confirmed for all concentration levels obtained over three subsequent days. All calculations were processed by Validation Manager software version: 2.18l (VWR International SAS). Pharmacokinetic analysis was performed using WinNonLin 6.0 Professional, software (Pharsight Corporation).

Results

Method validation

The current analytical method was fully validated in our laboratory, according to the United States Food and Drug Administration (FDA) and European Medicines Agency (EMEA), bioanalytical method validation requirements (ICH, 1994; FDA and CDER, 2000, 2001; EMEA, 2001). The extracts provided by the fast liquid–liquid extraction without shaking were very clean and the column back-pressure measured during the analysis of the last samples increased *ca* 5% compared with the initial values. The matrix effect on the analysis was of minor significance. Typical chromatograms of plasma samples spiked with ALB, SOX, SON and IS are presented in Figs 1 and 2. Back-

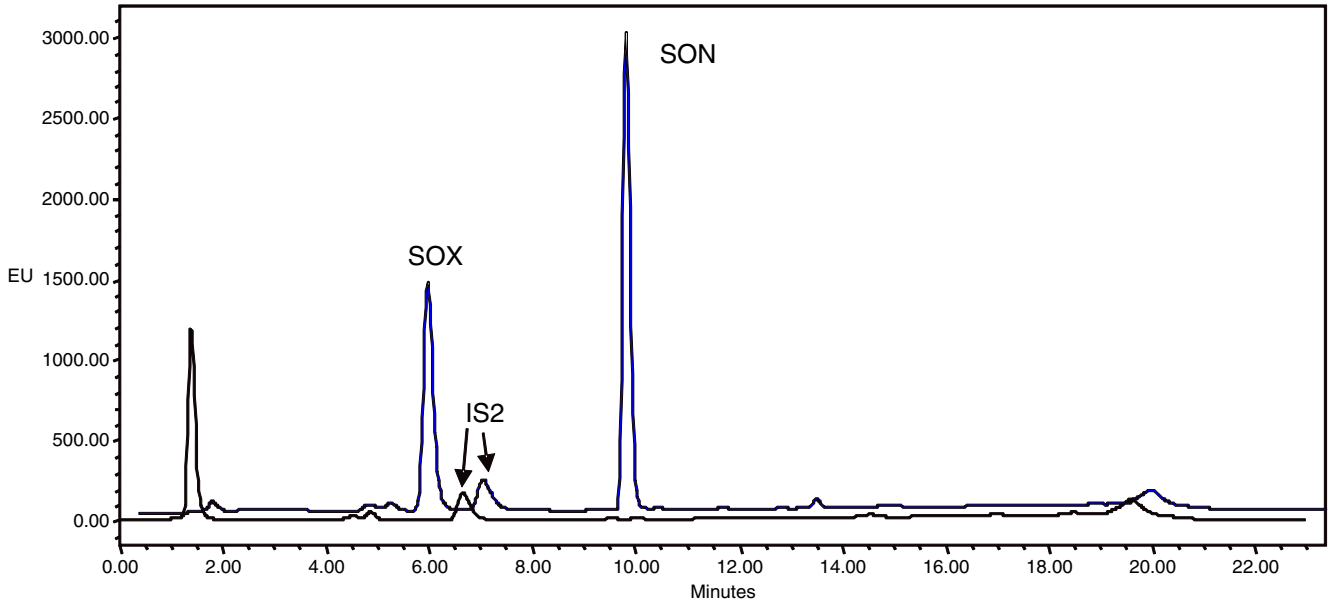

Figure 1. Representative chromatograms of albendazole sulfoxide (SOX) and albendazole sulfone (SON) in spiked blood plasma samples using thiabendazole (IS2) as an internal standard.

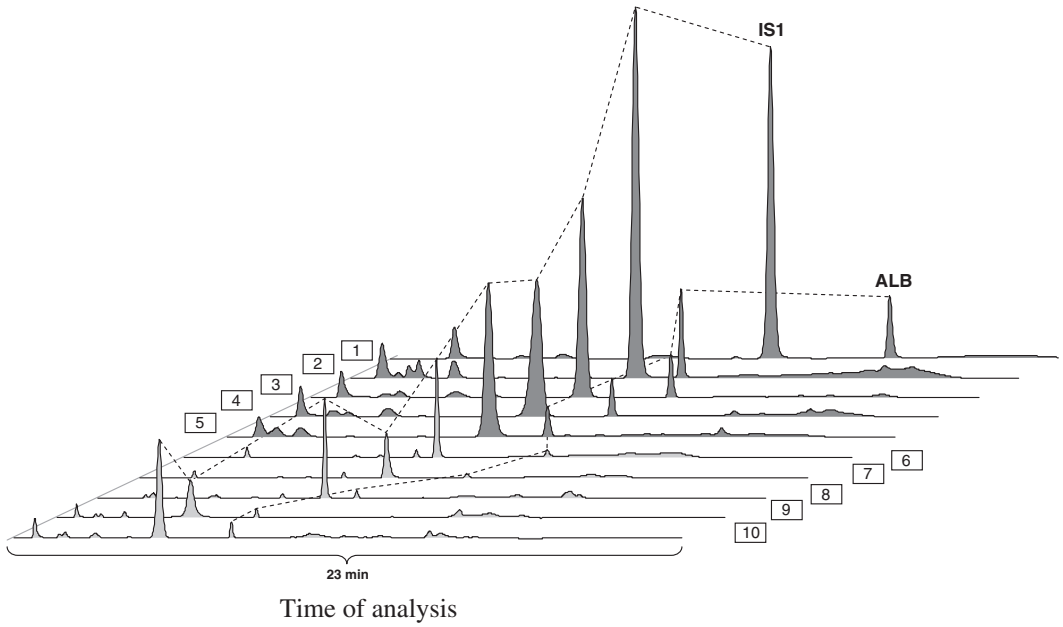

Figure 2. Representative albendazole (ALB) and propranolol (as an internal standard, IS1) chromatograms with different UPLC® and HPLC columns and mobile phase flow rate. Type of columns: 1, Acquity UPLC BEH C_{18}, 1.7 µm, 2.1 × 100 mm; flow 0.15 mL/min; 2, Pinnacle DB C_{18}, 1.9 µm, 2.1 × 50 mm; flow 0.20 mL/min; 3, Acquity UPLC HSS T3, 1.8 µm, 2.1 × 50 mm; flow 0.20 mL/min; 4, Hypersil Gold, 1.9 µm, 2.1 × 50 mm; flow 0.20 mL/min; 5, Vision HT C_{18}-P, 1.5 µm, 2.0 × 50 mm; flow 0.20 mL/min; 6, Synergi Fusion-RP 80 4u, 4.6 × 150 mm; flow 1.0 mL/min; 7, LiChrosorb RP_8 10 µm, 4 × 250 mm; flow 1.5 mL/min; 8, Allure Basix 3 µm 4.6 × 100 mm; flow 1.0 mL/min; 9, Hypersil Gold aQ 3 µm, 3 × 100 mm; flow 1.0 mL/min; 10, XBridge Phenyl 3.5 µm, 3 × 150 mm; flow 0.5 mL/min.

calculated concentrations of ALB, SOX and SON were obtained from calibration standards with the equation of the relevant calibration curve for 3 days (Table 1). In all cases, the values of mean recovery were within the acceptable range. The method obtained the precision acceptance criteria: repeatability coefficient of the variation ≤10% and at the lowest limit of quantitation (LLOQ)≤15%. The intermediate precision coefficient of variation was ≤15% (Table 2) and at the LOQ≤20%. For all concentrations the total recoveries (efficiency of extraction) for ALB, SOX and SON were 64.90±8.70, 55.10±13.00 and

68.90 ± 10.60%, respectively. The method meets the accuracy acceptance criteria (Table 3) according to FDA guidelines (FDA and CDER, 2001).

Autosampler stability for ALB and IS1 was established for the time period of 16 h. Autosampler stability for SOX, SON and IS2 was established for the time period of 28 h. The working standard solutions of analytes and internal standards were stable for storage at 0–8°C for a period of 15 days. All stability data are presented in Table 4.

Column comparison

For both groups of columns, mean values of peak area were calculated. Peak values obtained from spiked plasma samples and standards solution are presented in Table 5; peak area, peak height and peak resolution (Kprime) for ALB and IS1 in UPLC® group were significantly higher ($P < 0.05$) as compared with the HPLC group. Insignificant differences were found in tailing factor and USP plate count number. Standard solution

Table 1. The results of calibration curves from linearity analysis for albendazole (ALB), albendazole sulfoxide (SOX) and albendazole sulfone (SON) during the subsequent 3 days

Curves parameters	Analytes/day of study								
	ALB			SOX			SON		
	1	2	3	1	2	3	1	2	3
Slope	0.12	0.02	0.12	0.89	1.01	1.08	6.99	7.71	7.44
Intercept	−1.00	−0.80	−0.84	−36.00	−22.30	−77.40	7.90	7.71	7.88
R	0.997	0.998	0.998	0.997	0.996	0.997	0.998	0.995	0.997

Table 2. The results of calibration curves from precision analysis for albendazole (ALB), albendazole sulfoxide (SOX) and albendazole sulfone (SON)

Curve point	Analytes					
	ALB		SOX		SON	
	Rep.	Inter.	Rep.	Inter.	Rep.	Inter.
1	1.47	2.44	3.40	10.10	10.60	10.60
2	5.38	5.38	7.60	8.60	9.20	9.20
3	5.57	5.79	3.60	8.50	5.50	5.60
4	6.05	7.70	7.00	8.40	7.20	7.60
5	3.78	4.69	3.50	6.10	5.70	6.80
6	6.44	6.44	3.10	3.10	4.50	5.30
7	2.62	2.86	8.30	8.30	8.60	8.60
8	3.87	3.87	6.30	6.30	6.60	6.60

Rep., repeatability precision relative standard deviation (%). Inter., intermediate precision relative standard deviation (%). Curve point, from lowest to highest concentrations of the respective curve.

Table 3. The results of calibration curves from accuracy analysis for albendazole (ALB), albendazole sulfoxide (SOX) and albendazole sulfone (SON)

Quality control point	Analytes					
	ALB		SOX		SON	
	BIAS (%)	RSD (%)	BIAS (%)	RSD (%)	BIAS (%)	RSD (%)
LQC	−1.00	5.20	−5.50	8.70	−1.00	8.30
MQC	−6.80	6.00	4.00	5.10	6.10	5.50
HQC	1.80	2.80	−0.70	8.20	−3.50	8.10

LQC, lower concentration quality point; MQC, middle concentration quality point; HQC, higher concentration quality point; RSD, relative standard deviation.

Table 4. Stability data of the albendazole (ALB), albendazole sulfoxide (SOX) and albendazole sulfone (SON) spiked samples

Stability analysis (RSD %)	Analyte	LQC	MQC	HQC
Freeze–thaw cycle–short term stability	ALB	0.80	3.40	−5.30
	SOX	−1.00	13.50	12.40
	SON	1.40	10.50	12.90
Third freeze–thaw cycle	ALB	−9.50	2.20	−7.30
	SOX	1.50	−1.70	−1.40
	SON	−2.80	2.00	2.70
Autosampler	ALB	4.30	11.10	10.10
	SOX	8.60	−1.30	−1.40
	SON	7.30	−3.80	−3.30
Working standard	ALB	−11.20	−8.00	−0.90
	SOX	8.10	10.70	−6.80
	SON	−12.40	−9.10	−9.50

Table 5. The UPLC® column and HPLC column peak values comparison based on albendazole determination method in spiked blood plasma and standard solution samples

Peak parameters	Propranolol			Albendazole		
	UPLC/HPLC	RSD (%)	*p*-Value	UPLC/HPLC	RSD (%)	*p*-Value
Spiked blood plasma						
Area	5.21*	33.29	0.0002	8.62*	41.85	0.0007
Height	3.09*	43.01	0.0054	6.08*	49.29	0.0017
K'	1.54*	25.95	0.0133	1.40*	25.11	0.0327
USP tailing	0.91	10.98	0.1040	1.06	6.69	0.3198
USP plate count	0.55	79.45	0.1625	1.02	25.69	0.4628
Standards solutions						
Area	5.07*	29.13	0.00002	8.67*	42.44	0.0007
Height	2.78*	40.11	0.0043	6.18*	47.21	0.0007
K'	1.53*	27.25	0.0174	1.41*	25.05	0.0312
USP tailing	0.88	13.62	0.0837	1.40	25.05	0.0800
USP plate count	0.48	86.77	0.1310	1.07	22.12	0.3331

RSD (%), relative standard deviation; USP, *United States Pharmacopoeia*.

peak value to spiked blood plasma value ratio for albendazole was 1.48 (peak area) and 1.48 (peak height), for UPLC® columns and 1.49 (peak area) and 1.50 (peak height) for HPLC columns ($P < 0.05$). Mean maximal and minimal backpressure in UPLC® columns were 2792.00 ± 15.82 and 2296.00 ± 16.57 psi, respectively (Table 6).

Pharmacokinetics

The curves of changes in ALB and its metabolite concentrations are shown in Fig. 3, and calculated pharmacokinetic parameters are presented in Table 7. The pharmacokinetics of the parent drug indicates its quick elimination as most of the drug was eliminated within 10 h. However, its maximal concentration (C_{max}) was high and similar to C_{max} of SOX. Most of the SOX was eliminated within 24 h, while SON was eliminated within 48 h after drug administration. The area under the curve (AUC) relationship of SON/SOX was 1.60, while the relationship of C_{max} was the opposite. The C_{max} for SON/ALB was 0.64 and the C_{max} for SOX/ALB was 0.94.

The mean residence time (MRT) indicated very slow elimination of SON. This parameter for SON was 4.22 times longer than for ALB, while the MRT of SOX was only 2.06 times longer than for ALB.

Discusion

In the present study, liquid–liquid extraction (LLE) HPLC-FL methods for the quantification of ALB and its metabolites (SOX and SON) in turkey plasma using an internal standard were developed. The method described needs a small volume of blood plasma and a small number of steps in sample preparation. Moreover, applying the LLE method does not need the shaking phase.

The available literature indicates that, for determination of ALB and its metabolites, HPLC columns with particle size packings >2 µm can be used (Kitzman *et al*., 2002; Mirfazaelian *et al*., 2002). However, recent data indicate that UPLC columns can be used in the analysis of residues of veterinary drugs (Kaufmann *et al*., 2007; Ortelli *et al*., 2009; Garrido *et al*., 2010). In

Table 6. The UPLC® column back-pressure values using HPLC equipment on albendazole determination method with changed flow rate

Column	UPLC columns backpressure trough gradient separation on HPLC (psi)			
	Maximum	Minimum	Δ psi (% max)	Flow (mL/min)
Hypersil Gold 1.9 µm, 2.1 × 50 mm	1964.00	1608.00	18.13	0.20
Acquity UPLC® HSS T3 1.8 µm, 2.1 × 50 mm	2472.00	2017.00	18.41	0.20
VisionHT C_{18}-P 1.5 µm, 2.0 × 50 mm	2751.00	2261.00	17.81	0.20
Pinnacle DB C_{18} 1.9 µm, 2.1 × 50 mm	3253.00	2660.00	18.23	0.20
Acquity UPLC® BEH C_{18} 1.7 µm, 2.1 × 100 mm	3520.00	2936.00	16.59	0.15
M	2792.00	2296.40	17.83	—
RSD (%)	15.82	16.57	4.61	—

Δ psi (% max), difference between maximal and minimal backpressure as a percentage of maximal pressure value; psi, pounds per square inch; *M*, mean, RSD (%), relative standard deviation.

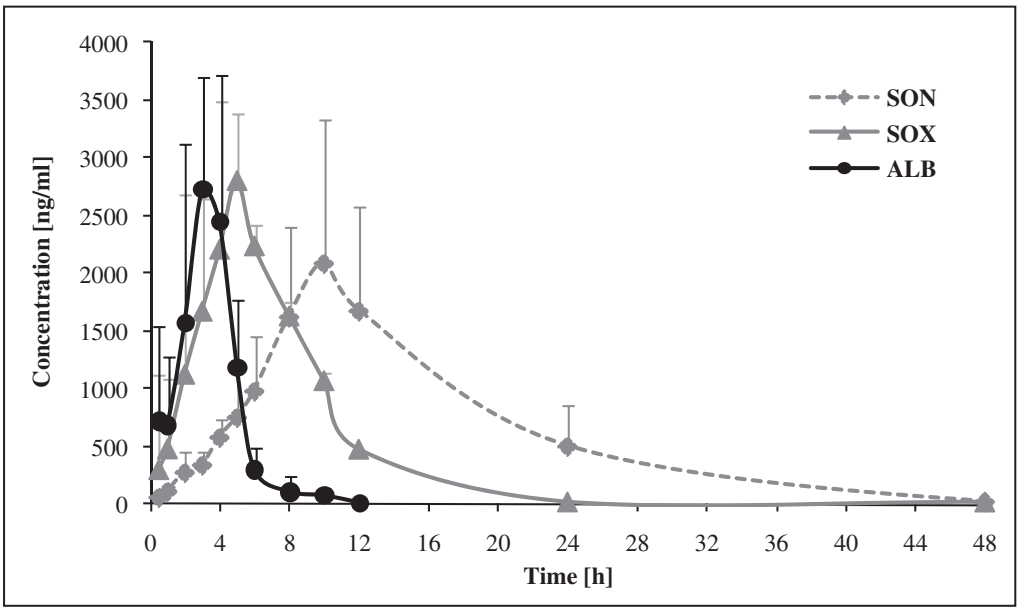

Figure 3. Concentrations (mean ± SD; $n = 18$) of albendazole (ALB) and its metabolites albendazole sulfoxide (SOX) and albendazole sulfone (SON) in turkey plasma after oral administration of drug at a dose of 25 mg/kg b.w.

Table 7. Pharmacokinetics (mean ± SD; $n = 18$) of albendazole and its metabolites: albendazole sulfoxide and albendazole sulfone in turkey plasma after oral drug administration at a dose of 20 mg/kg b.w.

Pharmacokinetic parameters	Albendazole		Albendazole sulphone		Albendazole sulphoxide	
	M	SD	*M*	SD	*M*	SD
AUMC (h^2ng/mL)	29632.90	11783.20	444059.98	259051.18	139698.35	101585.30
AUC (h ng/mL)	9002.91	2946.66	31370.63	15627.82	19604.29	9533.37
C_{max} (ng/mL)	3413.69	1123.01	2196.30	1218.09	3219.45	1753.89
t_{max} (h)	2.90	0.88	9.60	1.26	5.30	1.83
$t_{1/2k01}$ (h)	1.61	0.40	7.61	1.44	3.08	1.30
$t_{1/2kel}$ (h)	0.70	0.27	9.17	5.12	5.37	6.03
MRT (h)	3.23	0.59	13.64	2.23	6.67	2.11
V_c (mL/kg)	9.13	7.06	0.58	0.33	2.68	1.54

AUMC, area under first moment of the curve; AUC, area under the curve; C_{max}, maximal concentration; t_{max}, time to reach maximal concentration; $t_{1/2\,k01}$, drug half-life in absorption phase (albendazole), metabolite hal-life in formation phas; $t_{1/2kel}$, elimination phase half-life; MRT, mean residence time; V_c, volume of distribution.

the present study, comparison of peak values of ALB from two groups of columns clearly indicates the possibility of utilizing advanced UPLC columns for HPLC analysis using low flow rate (≤ 0.20 mL/min). Adaptation of UPLC columns for bioanalytical methods results in higher peaks from typical HPLC columns while retaining high quality of analysis. Moreover, using UPLC columns reduced 5–10 times the mobile phase used. The presented comparison also shows that significant matrix effect depended on the substance used for both HPLC and UPLC® columns.

In the available literature there is no data about pharmacokinetics of ALB and its metabolites in poultry and exotic birds. There is also a lack of data about the pharmacokinetics of ricobendazole. Some available data describe only the distribution of SOX in eggs of laying hens after drug administration directly into the eggs (Navarro *et al*., 1997). At present, in the pharmacotherapy of birds, only flubendazole from the benzimidazole compounds is used. The level of MRL for this drug was determined for hen eggs and hen and turkey tissues (EMEA, 2006; Danaher *et al*., 2007). In the present study, pharmacokinetic analysis of albendazole indicated a significant variability of its concentrations in turkey plasma. For ALB, variability of its concentrations in the case of absorption, including C_{max}, was higher than the variability observed in the distribution and elimination phase. In turn, variability for concentrations of ALB metabolites in the beginning phase of their development including C_{max} was lower than the variability observed in the phase of distribution and elimination.

Between-subject variability was higher than 30% for most pharmacokinetic parameters calculated. The analysis of AUC for metabolites indicated the predominance in production of SON rather than SOX. In the present study, the AUC index for SOX/ALB was 2.17, similar to the level described in sheep (Moreno *et al*., 2004), about three times lower than in dogs (Gokbulut *et al*., 2007) and about seven times lower than in humans (Bonato *et al*., 2003; Chen *et al*., 2004). In turn, the AUC index for SON/ALB was 3.48 and it was insignificantly higher than in sheep (Moreno *et al*., 2004) and humans (Bonato *et al*., 2003; Chen *et al*. 2004) and almost seven times higher than in dogs (Gokbulut *et al*. 2007). The AUC index for SOX/SON was 0.62 and it was lower than in sheep (1.01), humans (7.43) and dogs (12.46). These values indicated different ALB metabolism in turkey as compared with mammalian species and presumed the involvement mostly of CYP1A, CYP3A4 and flavin-monooxygenase in the metabolism of this drug in birds (Molina *et al*., 2007; Bettencourt *et al*., 2009). It is documented that MRT for SOX in rats, sheep, goats and dogs ranged between 6 and 17 h, while the MRT for SON in these species was determined as between 8 and 22 h (Merino *et al*., 2003; Gokbulut *et al*., 2007; Capece *et al*., 2009). In the present study, the MRT was similar for both SOX and SON. The difference between the MRT for the mentioned mammalian species and ALB metabolites in turkey is illustrated by the MRT index SOX/SON. This value for rats, sheep, goats and dogs is equal to 1.4, while in turkey, it is only 0.5, which indicates significant differences in distribution of ALB metabolites in mammals and birds.

Ricobendazole/SOX, similar to SON, is an active metabolite of albendazole and netobimin. In the present study, the half-life time in the elimination phase for SOX was significantly longer than for ALB. Moreover, elimination of this metabolite from the blood was relatively long, despite the fact that it had a three-times lower volume of distribution and only a two-times lower value of clearance as compared with ALB. In turn SON had almost 15-times lower value of volume of distribution as compared with the parent drug and the lowest value of clearance as compared with ALB and SOX.

In summary, the analytical method described in the present study allows for determination of ALB, SOX and SON concentrations at the level of LOQ equal to 10.5, 51.6 and 9.99 ng/mL, respectively. Moreover, the obtained data confirmed the possibility of usage of UPLC columns in HPLC determination of albendazole. Furthermore, pharmacokinetic data indicated significant differences in ALB metabolism in turkey as compared with those described in mammalian species.

Acknowledgments

The authors thank Dr hab Wieslaw Raszewski, for financial support and Professor Witold Gumułka for valuable comments and critical reading of the manuscript.

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