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HPLC assay for albendazole and metabolites in human plasma for clinical pharmacokinetic studies

Dennis Kitzman, Kuei-Ju Cheng, Lawrence Fleckenstein *

College of Pharmacy, The University of Iowa, S-427 Pharmacy Building, Iowa City, IA 52242, USA

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Abstract

A sensitive and selective HPLC chromatography method using UV detection (295 nm) was developed for the determination of albendazole, albendazole sulfoxide (ABZSO), and albendazole sulfone (ABZSO2) in human plasma. Albendazole, ABZSO, ABZSO2, and the internal standard, oxibendazole, were extracted from human plasma by loading onto a conditioned C_{18} SPE cartridge, rinsing with 15% methanol, and eluting with 90% methanol. Samples were evaporated under a stream of nitrogen, reconstituted with mobile phase, 1.25% triethylamine in water–methanol–acetonitrile (72:15:13, v/v/v) (pH* 3.1), and injected onto a Waters μBondapak™ Phenyl 3.9 × 300 mm HPLC column. Mobile phase flow rate was 1.0 ml/min. The retention times of albendazole, ABZSO, ABZSO2, and the internal standard were approximately 24.4, 7.9, 13.4, and 11.3 min, respectively. Total run time was 30 min. The assay was linear for concentration ranges in human plasma of 20–600 ng/ml for albendazole, 20–1000 ng/ml for ABZSO, and 20–300 ng/ml for ABZSO2. The analysis of quality control samples demonstrated excellent precision. Coefficients of variation for albendazole (20, 400, 600 ng/ml) were 6.7, 8.1 and 7.0%; ABZSO (20, 400, 800 ng/ml) were 6.0, 8.5 and 5.9%; ABZSO2 (20, 150, 300 ng/ml) were 3.1, 3.9 and 2.3%, respectively. The method appears to be robust and has been applied to a pharmacokinetic study of albendazole in healthy volunteers.

Keywords: Albendazole; Albendazole sulfoxide; Albendazole sulfone; HPLC

1. Introduction

Albendazole is a benzimidazole derivative used for treatment of intestinal helminthiasis and echinococcosis. Albendazole is an effective drug for treatment of these diseases but the therapeutic response in echinococcosis is unpredictable due to poor bioavailability [1,2]. A single dose of albendazole in combination with diethylcarbamazine has been found safe and efficacious for the treatment of *Brugia malayi* infection and is used in treatment programs for the elimination of lymphatic filariasis [3,4]. Albendazole undergoes first-pass metabolism and during the absorption process it is likely that the albendazole is metabolized by intestinal mucosal cells and the liver into the active metabolite ABZSO, a mixture of $R(+)$ and $S(-)$ enantiomers. In animals, formation of

* Corresponding author. Tel.: +1-319-335-8804; fax: +1-319-353-5646
E-mail address: l-fleckenstein@uiowa.edu (L. Fleckenstein).

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$R(+)$ ABZSO is catalyzed by microsomal flavin monooxidase (FMO) and of $S(-)$ ABZSO by cytochrome P450 enzymes (CYP3A) [5,6]. The $R(+)$ enantiomer seems to predominant, but the contribution of both enzyme systems to the metabolic process is variable and not well characterized in humans. This first-pass metabolism is extensive and thus, albendazole plasma levels are relatively low after a normal therapeutic dose (400 mg). Subsequently, ABZSO is metabolized by another cytochrome P450 enzyme (CYP2C) to the inactive metabolite, ABZSO2 [7].

Bogan and Marriner reported an HPLC assay of albendazole for plasma and gastrointestinal (GI) fluid [8]. The procedure involved liquid–liquid extraction and had a recovery of 83–100%, and had a sensitivity of 20 ng/ml with a 4 ml sample of plasma or GI fluid. The assay only quantitated albendazole and not the sulfoxide or sulfone metabolites. Another HPLC assay method for albendazole and its principal metabolites in sheep plasma has been reported by Alvinerie and Galtier [9]. The method has been reported to be sensitive, specific and reproducible using normal phase chromatography with mebendazole as the internal standard and UV detection at 225 nm. Only 100 μl of the sample was used and extracted using ethyl acetate as extraction solvent. The standard curves in plasma were linear for albendazole and its metabolites at concentrations of 0.1–10 μg/ml. The extraction recovery of albendazole, ABZSO and ABZSO2 were reported to be 78.2, 84.2 and 81.2%, respectively.

Hoaksey et al. reported a sensitive and selective reversed-phase HPLC method for the determination of albendazole and its active metabolite, ABZSO in human plasma [10]. They carried out a single step extraction of plasma with dichloromethane and separation on a μBondapak phenyl column. The assay was characterized down to 50 ng/ml for albendazole and 20 ng/ml for ABZSO. Hurtado et al., have described a rapid and sensitive assay for albendazole and ABZSO determination in plasma and CSF using an analytical wavelength of 295 nm, mebendazole as internal standard, and a ODS C_{18} column for chromatographic separation [11]. The calibration curve was validated over a concentration range of 30–1000 ng/ml. Extractions were made using Sep-Pak C_{18} cartridges. Recovery of albendazole and ABZSO from plasma extracts ranged from 95 to 100%. Valois et al., reported a method for the determination of ABZSO and ABZSO2. They use a liquid extraction with chloroform-isopropanol, separation on an RP-18 column and detection at 290 nm [12]. Garcia and coworkers have reported chromatographic conditions for quantitative determination of albendazole and another for ABZSO and ABZSO2 by using a reversed phase HPLC method with an ODS2 column and two different mobile phases [13]. Lanchote developed a HPLC method for the simultaneous determination of ABZSO enantiomers and ABZSO2 in human plasma [14]. The compounds were extracted from plasma with ethyl acetate, separated on a Chiralpak AD column and detected by fluorescence. The limits of quantification were 5 ng/ml for both ABZSO enantiomers and 1 ng/ml for ABZSO2. Chiap et al. reported a method for albendazole, ABZSO and ABZSO2 in ovine plasma by liquid chromatography [15]. The method uses dialysis as a purification step, followed by enrichment of the dialysate on a precolumn and liquid chromatography. A gradient elution was applied for separation of the analytes. UV detection at 295 nm was used and the limits of quantification for albendazole and metabolites were 10 and 7.5 ng/ml, respectively.

A nonaqueous capillary electrophoresis method for the determination of plasma albendazole, ABZSO and ABZSO2 was described by Prochazkova and coworkers [16]. The assay uses liquid–liquid extraction using dichloromethane with recovery between 63 and 98%. The limit of detection for the three compounds was 8×10^{-7} M, which is less sensitive than most HPLC methods. Albendazole was undetectable in all patient samples and ABZSO2 was below or close to the limit of detection.

An inexpensive, robust method for the quantitization of plasma levels of albendazole and ABZSO after 400 mg doses was needed to study pharmacokinetic effects when used in combination with diethylcarbamazine for lymphatic filariasis elimination. Separation of ABZSO enantiomers was deemed unnecessary and deter-

Table 1
Calibration curve parameters and statistics in human plasma

Curve	Albendazole			ABZSO			ABZSO2		
	Slope	*y*-Intercept	Correlation coefficient	Slope	*y*-Intercept	Correlation coefficient	Slope	*y*-Intercept	Correlation coefficient
1	0.001712	−0.003077	0.9823	0.004635	0.007059	0.9989	0.002382	−0.001120	0.9972
2	0.001761	0.000311	0.9955	0.004334	0.001399	0.9986	0.002212	−0.004680	0.9903
3	0.001720	−0.002928	0.9919	0.004312	0.000207	0.9979	0.002235	0.006005	0.9894
4	0.001777	−0.001683	0.9928	0.004264	0.000483	0.9883	0.002253	0.002973	0.9982
5	0.001675	0.004692	0.9990	0.004350	−0.007300	0.9956	0.001937	0.006782	0.9863
6	0.001471	−0.005110	0.9973	0.004546	0.000049	0.9981	0.001937	0.006782	0.9926
Mean	0.001686	−0.001299	0.9931	0.004407	0.000316	0.9962	0.002221	0.000659	0.9923
S.D.	0.000111	0.003434		0.000148	0.004577		0.000152	0.005431	
CV (%)	6.61			3.36			6.86		

CV, coefficient of variation; S.D., standard deviation.

mination of albendazole bioavailability was the primary objective of this project. For the analysis of albendazole and its metabolites in body fluids, most methods are unable to efficiently determine albendazole and its metabolites simultaneously. Other methods lack the sensitivity required for clinical pharmacokinetic studies [15]. Some of the published liquid–liquid extraction procedures do not adequately remove plasma interference from the chromatograms to yield reproducible results. Initially, in the development of this methodology, only albendazole and ABZSO were validated. When actual subject samples were analyzed it became evident that the ABZSO2 metabolite was clearly present in some specimens and this methodology was also capable of quantitating ABZSO2. At this point validation work was done for ABZSO2 and it was added to the assay. This study reports a sensitive, selective, accurate, and reproducible HPLC assay using UV detection for the simultaneous determination of albendazole, ABZSO and ABZSO2 in human plasma using oxibendazole as an internal standard. With the use of an autoinjector, this method is suitable for processing the large number of albendazole plasma samples withdrawn during clinical pharmacokinetic studies.

2. Experimental

2.1. Solvents and chemicals

All solvents were HPLC grade. Organic solvents, triethylamine, and *o*-phosphoric acid 85% were purchased from Fisher Scientific (Fair Lawn, NJ, USA). A Milli-Q Plus water system (Millipore Corporation, Bedford, MA) produced ultra pure analytical grade Type I water. A solution of 1.25% triethylamine in water–methanol–acetonitrile (72:15:13, v/v/v) was adjusted to pH* 3.10 using 85% *o*-phosphoric acid and used as the mobile phase. A 15% solution of methanol in water, and a 90% solution of methanol in water were prepared as wash and elution solvents. Albendazole and oxibendazole were obtained from Sigma (St. Louis, MO). ABZSO and ABZSO2 were obtained from SmithKline Beecham (West Sussex, UK).

2.2. Standard stock solutions

Stock solutions were made with albendazole, oxibendazole, ABZSO, and ABZSO2. The chemicals were weighed on a Mettler-Toledo AG104 analytical balance (Mettler-Toledo, Inc., Hightstown, NJ, USA). Appropriate amounts of chemical were dissolved in methanol in volumetric flasks. The concentrations of stock solutions were: albendazole (0.2 mg/ml), oxibendazole (0.05 mg/ml), ABZSO (0.5 mg/ml), and ABZSO2 (0.1 mg/ml). Working standards were prepared for each concentration in the standard curve. The working standards contained 20, 80, 200, 400, 500, and 600 ng of ABZ, 20, 80, 200, 400, 600, 800, and 1000 ng of ABZSO, and 20, 40, 100, 200, and 300 ng of ABZSO2 per 25 μl of methanol. A 5 μg/ml solution of oxibendazole was prepared as working solution for the internal standard. This concentration was chosen to give a peak height ratio of 1.0 near the midpoint of the ABZSO standard curve. Standard solutions were stored at 4 °C.

2.3. Instrumentation

Chromatography was preformed using a Waters 501 HPLC pump, a Waters 717 plus Autosampler, a Waters 486 Tunable Absorbance Detector, and a Waters μBondapak™ Phenyl 125 Å 10 μm 3.9×300 mm column. Results were plotted and processed using a Shimadzu CR501 Chromatopac. The mobile phase (see Section 2.1) flow rate was 1.0 ml/min.

2.4. Extraction procedure

This procedure was validated using 1.0 ml of spiked human plasma. Human plasma was obtained from the Blood Bank at the University of Iowa Hospitals and Clinics and stored frozen in aliquots at -20 °C. Extraction of albendazole, ABZSO, ABZSO2, and oxibendazole was preformed by solid phase extraction (SPE). Varian Bond Elut C_{18}, 50 mg, 1 ml reservoir cartridges (Varian, Harbor City, CA, USA) and an SPE vacuum manifold (Alltech, Deerfield, IL, USA) were used for the procedure. Pipetman® precision microliter pipettes (Woburn, MA, USA) were used throughout the assay.

Table 2
Albendazole, ABZSO, and ABZSO2 in human plasma, back calculated calibration standard values

	Albendazole		ABZSO		ABZSO2	
	Concentration (ng/ml)	Calculated concentration (ng/ml)	Concentration (ng/ml)	Calculated concentration (ng/ml)	Concentration (ng/ml)	Calculated concentration (ng/ml)
	20	19.9	20	19.8	20	20.9
	80	87.9	80	86.4	40	35.5
	200	190	200	182	100	99.9
	400	389	400	381	200	212
	500	495	600	612	300	302
	600	604	800	827		
			1000	1010		
Slope		0.00155		0.00455		0.00238
Intercept		−0.0054		0.00005		−0.0011
R		0.9994		0.9991		0.9986

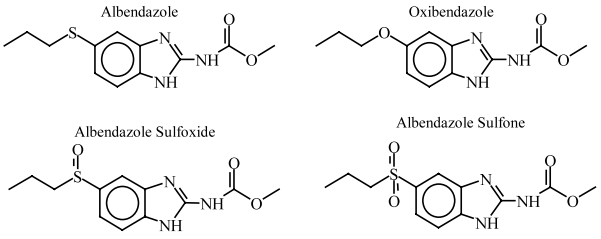

Fig. 1. Structures of albendazole, ABZSO, ABZSO2, and the internal standard, oxibendazole.

Calibration curves were prepared daily with each set of samples. Calibration curves consisted of a plasma blank and spiked plasma. Spiked plasma was prepared by adding 25 μl of the working solution for each point in the standard curve and 25 μl of internal standard to 1.0 ml of human plasma in a 10 ml borosilicate glass test tube. For each sample, a SPE cartridge was activated by aspirating with 1 ml of methanol followed by 1 ml of HPLC grade water. Samples were transferred to the activated cartridges and aspirated at a vacuum of 3–5 mm of Hg. Each cartridge was washed with 15% methanol in water. After washing, samples were eluted with 1.0 ml of 90% methanol in water under a vacuum of 3–5 mm of Hg into clean borosilicate glass 10 ml test tubes. The elutant was evaporated in a 37 °C N-EVAP® (Berlin, MA, USA), under a gentle stream of nitrogen. Dried samples were reconstituted with 100 μl of mobile phase, vortex mixed for 15 s and placed in an ultrasonic bath (Branson, Danbury, CT, USA) for 2 min. The samples were ultrasonicated to consistently redissolve albendazole. The reconstituted samples were transferred to vials containing 250 μl polypropylene conical inserts and placed on the autosampler where 5–25 μl (25 μl [ABZSO] < 400; 10 μl 400 ≤ [ABZSO] ≤ 800; 5 μl [ABZSO] > 800) was injected. Varying the injection volume was necessary to avoid reaching the UV detector's detection limit. In practice, plasma levels of ABZSO are highly variable, but most clinical samples have concentrations of less than 400 ng/ml. Samples with ABZSO concentration greater than 400 ng/ml were reinjected at lower volume.

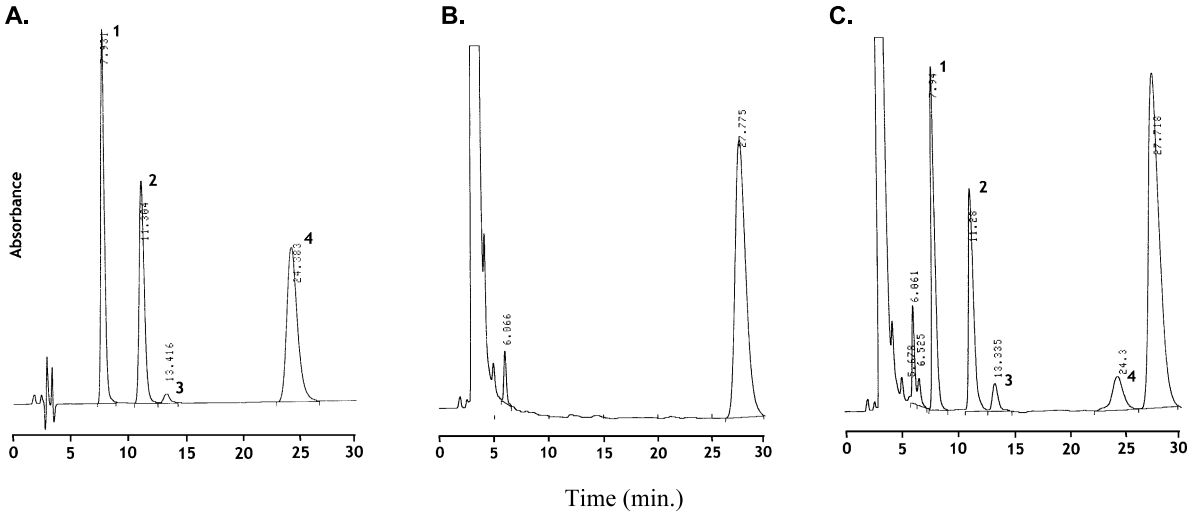

Fig. 2. Chromatograms of (A) albendazole (peak 4, r.t. = 24.383) 400 ng/ml, ABZSO (peak 1, r.t. = 7.931) 400 ng/ml, ABZSO2 (peak 3, r.t. = 13.416) 17 ng/ml, and oxibendazole (peak 2, r.t. = 11.304) 125 ng/ml in methanol, (B) blank (analyte-free) human plasma, (C) plasma sample from a patient given a 400 mg oral dose of albendazole. Calculated levels are, albendazole 92 ng/ml (peak 4, r.t. = 24.3 min), ABZSO 355 ng/ml (peak 1, r.t. = 7.94 min) and ABZSO2 44 ng/ml (peak 3, r.t. = 13.335 min) (r.t., retention time).

Table 3
Intra- and inter-day precision and accuracy for albendazole, ABZSO, and ABZSO2 in human plasma

Theoretical concentration (ng/ml)	Albendazole			ABZSO			ABZSO2		
	20	400	600	20	400	800	20	150	300
Intra-day run									
Overall mean ($n = 6$)	18	399	615	21	416	818	20	156	318
S.D.	1.2	32.2	42.7	1.2	35.4	48.1	0.6	6.1	7.2
CV (%)	6.7	8.1	7.0	6.0	8.5	5.9	3.1	3.9	2.3
Accuracy (%)	89.8	99.8	102.5	104.5	104.1	102.3	102.1	104.0	105.9
Inter-day run									
Overall mean ($n = 12$)	18	393	604	21	391	779	20	143	301
S.D.	1.3	25.9	53.6	1.4	36.3	60.4	1.1	14.8	19.8
CV (%)	7.3	6.6	8.9	6.8	9.3	7.8	5.6	10.3	6.6
Accuracy (%)	91.4	98.3	100.7	103.2	97.8	97.4	98.4	95.4	100.3

CV, coefficient of variation; DMT, deviation of mean value from nominal; S.D., standard deviation.

Table 4
Recovery of albendazole, ABZSO, ABZSO2, and oxibendazole (internal standard) from human plasma

	Concentration (ng/ml)	Mean extract height	n	CV (%)	Mean pure height	n	CV (%)	Mean % recovery
Albendazole	40	306	6	10.1	284	6	5.6	108
	400	2820	6	5.4	3297	6	3.8	86
	600	1916	6	3.3	2073	6	1.9	92
	Overall							95
ABZSO	40	970	6	7.3	984	6	2.7	99
	400	9783	6	2.4	10009	6	0.5	98
	800	8358	6	6.1	8691	6	2.9	96
	Overall							98
ABZSO2	40	399	5	5.6	489	5	5.4	82
	300	3609	25	6.3	4477	6	6.4	81
	Overall							81
Oxibendazole	125	6034	25	4.2	7150	6	6.1	84

CV, coefficient of variation.

2.5. Quality controls

Quality control (QC) samples for autosampler stability and for intra- and inter-day assays were individually prepared by spiking 1.0 ml of plasma with albendazole and ABZSO or with ABZSO2. QC samples were prepared at concentrations of 20/20, 400/400, and 600/800 ng/ml of albendazole/ABZSO, and at concentrations of 20, 150 and 300 ng/ml of ABZSO2. QC samples for freeze–thaw stability and subject analysis were prepared in bulk. Bulk QCs were prepared at concentrations of 40/40, 400/400, and 600/800 ng/ml of albendazole/ABZSO and at concentrations of 40, 150, and 300 ng/ml of ABZSO2. Bulk QCs were divided into 2.5 ml nalgene cryovials and stored at -20 °C.

2.6. Data regression

Chromatographic data were collected using a Shimadzu CR501 Chromatopac. Peak height ratios of albendazole/oxibendazole, ABZSO/oxibendazole, and ABZSO2/oxibendazole were calculated. Equations for the calibration curves were obtained by 1/concentration squared ($1/c^2$)

weighted linear regression analysis. The equations were used to calculate the concentrations of albendazole, ABZSO, and ABZSO2 in samples and QC samples by peak height ratios.

2.7. Precision, accuracy and recovery

Precision, accuracy, and recovery were evaluated by analyzing six spiked plasma samples at each concentration level for each substance; albendazole: 20, 400, 600 ng/ml; ABZSO: 20, 400, 800 ng/ml; ABZSO2: 20, 150, 300 ng/ml. The coefficient of variation at each concentration was calculated to determine the precision of the method. The accuracy of the method was determined by comparing the measured concentrations of the extracted plasma samples to the true concentration spiked into the sample. Recovery was assessed by comparing the peak height obtained for extracted spiked samples to the peak height obtained for unextracted standard mixtures representing 100% recovery.

2.8. Stability

Freeze and thaw stability, storage stability at −20 °C and autosampler stability experiments were conducted. QC samples containing 40/40 and 600/800 ng/ml of albendazole/ABZSO in plasma and QC samples containing 40 and 300 ng/ml of ABZSO2 in plasma were subjected to three freeze–thaw cycles. Samples were frozen at −20 °C for at least 24 h and thawed unassisted at room temperature. When completely thawed, the samples were transferred back to the original freezer and refrozen for at least 24 h. Autosampler stability was conducted by repeated injection of extracted spiked ABZSO (800 ng/ml)/albendazole (600 ng/ml) plasma samples and extracted spiked ABZSO2 (300 ng/ml) plasma samples.

Table 5
Freeze and thaw stability of albendazole, ABZSO, and ABZSO2 in plasma specimens during three freeze–thaw cycles

Theoretical concentration (ng/ml)	Albendazole		ABZSO		ABZSO2	
	40	600	40	800	40	300
Cycle 1	$n=6$	$n=6$	$n=6$	$n=6$	$n=5$	$n=5$
Mean	40.5	646	41.0	828	38.7	301
S.D.	1.56	25.4	2.11	33.2	1.48	5.69
CV (%)	3.9	3.9	5.1	4.0	3.8	1.9
Accuracy (%)	101.2	107.7	102.5	103.5	96.8	100.3
Cycle 2	$n=6$	$n=6$	$n=6$	$n=6$	$n=5$	$n=5$
Mean	44.8	637	40.3	866	40.5	309
S.D.	3.51	14.9	2.18	46.2	0.96	6.69
CV (%)	7.8	2.3	5.4	5.3	2.4	2.2
Accuracy (%)	112.0	106.2	100.8	108.2	101.2	103.0
Cycle 3	$n=6$	$n=6$	$n=6$	$n=6$	$n=5$	$n=5$
Mean	36.7	635	42.6	880	37.4	252
S.D.	3.06	74.9	3.16	18.7	1.84	20.5
CV (%)	8.3	11.8	7.4	2.1	4.9	8.2
Accuracy (%)	91.8	106.8	106.5	110.0	93.5	84.0
Cycle 4					$n=5$	$n=5$
Mean					40.0	314
S.D.					1.52	11.0
CV (%)					3.8	3.5
Accuracy (%)					100.0	104.7

CV, coefficient of variation; S.D., standard deviation.

Table 6
Autosampler stability of an ABZSO (800 ng/ml), ABZSO2 (300 ng/ml), and albendazole (600 ng/ml) extracted human plasma sample

Time (h)	Calculated concentration of albendazole (ng/ml)	Calculated concentration of ABZSO (ng/ml)	Calculated concentration of ABZSO2 (ng/ml)
0	589	787	321
1	498	856	321
2	596	805	290
3	623	810	309
4	493	875	313
5	580	800	323
6	574	838	299
7	589	808	296
8	617	812	322
9	608	850	284
10	600	813	313
11	600	778	289
12	554	787	306
13	593	776	314
14	528	839	315
15	535	831	310
16	573	779	310
17	597	803	314
18	682	865	303
19	616	802	304
20	564	816	278
21	568	994	278
22	512	855	315
23	597	784	288
24	611	802	316
Mean	580	823	305
S.D.	42.6	45.7	13.8
CV(%)	7.3	5.6	4.5

CV, coefficient of variation; S.D., standard deviation.

3. Results and discussion

3.1. Separation

The molecular structures of albendazole, ABZSO (major metabolite), ABZSO2 (minor metabolite), and oxibendazole (internal standard) are shown in Fig. 1. Fig. 2(A) shows an injection standard containing 400 ng/ml albendazole, 400 ng/ml ABZSO, 17 ng/ml ABZSO2 and 125 ng/ml oxibendazole. A representative chromatogram of plasma blank is shown in Fig. 2(B). Fig. 2(C) shows a representative chromatogram from a subject who took oral albendazole. The retention times of albendazole, ABZSO, ABZSO2, and the internal standard are approximately 24.4, 7.9, 13.4 and 11.3 min, respectively. The chromatograms show a good baseline separation. The blank is free from interfering peaks at the retention times of albendazole, ABZSO, ABZSO2, and oxibendazole. A number of C_{18} columns were tested, but because of the differences of hydrophobic character between albendazole and its two metabolites, the simultaneous separation of the three analytes gave rise to excessive run times. This was a particular problem for the more hydrophobic albendazole, which eluted very slowly and had a broadened peak and reduced detection. Use of the μBondapak™ Phenyl had the appropriate selectivity to achieve the desired separation with a reasonable run time. The autoinjector and integrator allow unattended injection of multiple samples, with a run time of 30 min per sample.

Table 7
Storage stability of albendazole, ABZSO, and ABZSO2 in human plasma at −20 °C

Time (days)	0	5	15	30	40	60
Albendazole						
318 *ng/ml*						
Mean ($n = 4$)	283	286	274	278	278	275
% Baseline	100	101	97	98	98	97
S.D.	2.1	5.2	3.0	5.4	5.9	4.1
CV (%)	0.7	1.8	1.1	2.0	2.1	1.5
636 *ng/ml*						
Mean ($n = 4$)	563	576	554	558	564	546
Baseline (%)	100	102	98	99	100	97
S.D.	4.1	4.6	3.9	3.6	3.0	4.8
CV (%)	0.7	0.8	0.7	0.6	0.5	0.9
1166 *ng/ml*						
Mean ($n = 4$)	960	982	939	954	951	952
Baseline (%)	100	102	98	99	99	99
S.D.	1.7	2.2	0.8	1.9	1.5	6.6
CV (%)	0.2	0.2	0.1	0.2	0.2	0.7
ABZSO						
294 *ng/ml*						
Mean ($n = 4$)	264	275	260	254	263	257
Baseline (%)	100	104	98	96	99	97
S.D.	6.3	1.7	7.5	11.5	11.2	5.9
CV (%)	2.4	0.6	2.9	4.5	4.2	2.3
589 *ng/ml*						
Mean ($n = 4$)	524	537	513	512	503	517
Baseline (%)	100	102	98	98	96	99
S.D.	11.0	3.8	8.8	8.0	2.6	2.2
CV (%)	2.1	0.7	1.7	1.6	0.5	0.4
1079 *ng/ml*						
Mean ($n = 4$)	971	937	961	934	912	931
Baseline (%)	100	97	99	96	94	96
S.D.	11.2	4.9	7.5	4.5	6.7	3.0
CV (%)	1.1	0.5	2.9	0.5	0.7	0.3
ABZSO2						
295 *ng/ml*						
Mean ($n = 4$)	265	273	262	254	262	254
Baseline (%)	100	103	99	96	99	96
S.D.	5.3	4.8	7.8	9.1	2.9	8.8
CV (%)	2.0	1.8	3.0	3.6	1.1	3.4
590 *ng/ml*						
Mean ($n = 4$)	537	519	534	518	523	517
Baseline (%)	100	97	99	96	97	96
S.D.	4.1	6.5	4.2	8.1	26.8	6.3
CV (%)	0.8	1.2	0.8	1.6	5.1	1.2
1081 *ng/ml*						
Mean ($n = 4$)	984	955	973	942	948	928
Baseline (%)	100	97	99	96	96	94
S.D.	3.7	3.9	2.8	2.5	6.1	2.5
CV (%)	0.4	0.4	0.3	0.3	0.6	0.3

CV, coefficient of variation; Baseline (%), percent detected in sample relative to day 0; S.D., standard deviation.

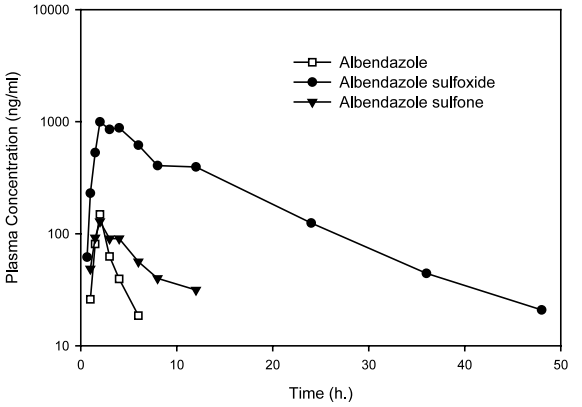

Fig. 3. A plasma concentration–time profile for a subject who received an oral dose of 400 mg albendazole.

3.2. Linearity

Calibration curve parameters for albendazole, ABZSO, and ABZSO2 are shown in Table 1. Results were calculated using peak height ratios. Calibration curves were linear using $1/c^2$ weighted linear regression in the concentration ranges of 20–600 ng/ml for albendazole, 20–1000 ng/ml for ABZSO, and 20–300 ng/ml for ABZSO2. The calibration curves cover the range of expected concentrations for analyzing the plasma of subjects given a 400 mg oral dose of albendazole.

The limit of quantification (LOQ) in human plasma for albendazole, ABZSO, and ABZSO2 was accepted as 20 ng/ml. This was greater than five times the baseline noise seen at the retention times of the analytes. Plasma samples were spiked to a nominal concentration of 20 ng/ml with working solutions and internal standard and carried through the extraction procedure. At the LOQ, the coefficient of variation ($n = 6$) of the measured concentrations were 6.6, 6.0, and 3.1%, and the deviation of the mean of the measured concentrations from the nominal values were -10.2, 4.5 and 2.1% for albendazole, ABZSO and ABZSO2, respectively (Table 2).

3.3. Precision and accuracy

Data on precision and accuracy are shown in Table 3. Precision values varied from 2.3 to 10.3% for intra- and inter-day analyses. Accuracy varied from -10.2 to 5.9%. The results indicate there is good reproducibility and accuracy for the determination of albendazole and its metabolites for samples determined on the same or different days.

3.4. Determination of the extraction efficiency

Recovery was tested at low and high concentrations of albendazole, ABZSO, ABZSO2, and oxibendazole. Absolute recoveries were determined by comparing the peak areas of extracted QC samples with the peak areas of recovery standards (unextracted equivalents of extracted QC samples). The mean recoveries for albendazole, ABZSO, ABZSO2, and oxibendazole were 95, 98, 81 and 84%, respectively (Table 4). The results demonstrate that the extraction efficiency is relatively constant over the range considered.

3.5. Stability

Freezing and thawing of the QC samples did not appear to effect the quantitation of the analytes (Table 5). A fourth freeze–thaw cycle was done with ABZSO2 to check that the 300 ng value from the third cycle did not represent loss of ABZSO2.

Autoinjector stability was carried out over 24 h by repeated injections of extracted plasma samples containing albendazole, ABZSO and ABZSO2 at room temperature. The results presented in Table 6 show the extracted specimens remained stable for at least 24 h.

Storage stability of albendazole, ABZSO, and ABZSO2 were determined prior to collection of clinical specimens and concentrations were selected based upon anticipated drug and metabolite levels. Samples containing 318, 636, and 1166 ng/ml of albendazole in plasma, 294, 589 and 1079 ng/ml of ABZSO in plasma, and 295, 590 and 1081 ng/ml of ABZSO2 in plasma were subjected to storage at -20 °C for 60 days. Plasma samples ($n = 4$) were taken for analysis at 0, 5, 15, 30, 40 and 60 days (Table 7). Samples stored at -20 °C remained stable over this period.

3.6. Interference by other drugs

Ethylenediaminetetraacetic acid (EDTA), diethylcarbamazine (DEC), mebendazole, thiabendazole, and fenbendazole have been tested for potential interferences with the assay. No interference were found.

3.7. Application of the method

This method was successfully applied to a clinical pharmacokinetic study of 28 healthy subjects who received an oral dose of albendazole (400 mg). The method appears to be robust as more than 400 plasma samples have been successfully analyzed. A plasma concentration–time profile for a subject who received an oral dose of 400 mg albendazole and had relatively high ABZSO concentrations is presented Fig. 3.

Some methods do not have sufficient sensitivity to detect albendazole after usual 400 mg clinical doses [16]. After oral administration of 400 mg, albendazole concentrations were relatively low, but could be quantitated (≥ 20 ng/ml) in 24 of 28 subjects receiving the 400 mg dose.

4. Conclusions

A HPLC assay procedure using SPE for the specific and quantitative analysis of albendazole, ABZSO, and ABZSO2 in human plasma samples is described. The assay uses oxibendazole as an internal standard and has a run time of approximately 30 min. All three analytes are quantitated in a single injection. The chromatographs provide clear separation with no interfering peaks. The assay has been validated and the results of validation demonstrate that the standard curve is linear over the following concentration ranges: albendazole -20 to 600 ng/ml, ABZSO -20 to 1000 ng/ml, ABZSO2 -20 to 300 ng/ml. The assay is reproducible and accurate. The analysis requires 1.0 ml of plasma and has a detection limit of 20 ng/ml for albendazole, ABZSO and ABZSO2. Autoinjector stability has been demonstrated for up to 24 h. The extraction procedure and the chromatographic set-up are simple, reliable and consistent. The assay meets the guidelines for bioanalytical methods validation for human studies [17,18].

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