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Simultaneous liquid chromatography–tandem mass spectrometric determination of albendazole sulfoxide and albendazole sulfone in plasma

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Abstract

This paper describes a simple, fast, sensitive and reliable method for the simultaneous determination of albendazole sulfoxide (ASOX) and albendazole sulfone (ASON), the two most important metabolites of the drug albendazole (ABZ), in plasma samples using liquid chromatography and tandem mass spectrometry. After liquid–liquid extraction with dichloromethane, the two albendazole metabolites and the internal standard phenacetin were resolved in a CN column using the mobile phase methanol–water (4:6, v/v) acidified with 1% acetic acid. Detection by electrospray mass spectrometry was carried out in the positive ion mode. The method was linear up to 2500 and 250 ng/ml for ASOX and ASON, respectively, with mean recoveries of more than 85%. The precision and accuracy data, based on within- and between-day variations over 5 days, were lower than 15%. The quantitation limits of 0.5 and 5.0 ng/ml for ASON and ASOX are low enough for the method to be suitable for pharmacokinetic studies. Pharmacokinetic data obtained with the proposed method following oral administration of ABZ to a patient with neurocysticercosis are also reported.

Keywords: Albendazole sulfoxide; Albendazole sulfone

1. Introduction

The great advantages of LC–MS–MS over other conventional analytical techniques [1–5] make it the first choice technique for the analysis of drugs and their metabolites in biological samples. On this basis, we are reporting here the development of a simple LC–MS–MS method for the analysis of two albendazole metabolites in plasma samples suitable for pharmacokinetic studies.

Albendazole, methyl[5-(propylthio)-1H-benzimidazol-2-yl]carbamate (ABZ), has proved to be effective in the treatment of neurocysticercosis, the most common parasitic disease of the central nervous system, affecting thousands of people in developing countries in Latin America, Asia and Africa [6–8]. ABZ undergoes extensive metabolism by liver microsomal enzymes, and probably in the gastrointestinal tract [9], to its major active metabolite albendazole sulfoxide (ASOX). This metabolite is further metabolized to albendazole sulfone (ASON),

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which does not appear to have any activity. ABZ is not frequently detected in plasma after oral administration due to its low systemic availability, a consequence of poor gastrointestinal absorption and extensive metabolism. As a result, the pharmacokinetic properties of ABZ have been studied by determining the plasma concentration of ASOX and ASON [10–13]. For this purpose, efficient, fast and reliable methods are required, since ASOX and ASON are present in plasma samples at concentrations of the order of ng/ml.

Many analytical methods have been reported in the literature for the quantitative analysis of the main ABZ metabolites in human plasma, almost all of them based on reversed-phase high-performance liquid chromatography [14–18]. Capillary electrophoresis was also recently used for the determination of ABZ and its two metabolites in human plasma [19]. ABZ is widely used in veterinary medicine, so the literature also reports methods for the determination of residues of ABZ and its metabolites in milk [20–22] and in ovine plasma [23].

ABZ metabolites are frequently determined by UV detection. Fluorescence detection was also used by our group in two enantioselective methods for the determination of ASOX [24,25]. Recently BALIZS [26] reported the first method for the determination of benzimidazole residues in muscle, liver and egg samples using liquid chromatography and tandem mass spectrometry (LC–MS–MS). ABZ, ASOX and ASON were among the several benzimidazoles studied. In spite of this, there is no method described in the literature for the simultaneous analysis of ASOX and ASON in human plasma using this technique and suitable for pharmacokinetic studies.

2. Experimental

Drugs and chemicals

ASOX (99.4%) and ASON (99.8%) were kindly supplied by Robert Young (Glasgow, Scotland, UK). Stock standard solutions were prepared in methanol at the concentration of 0.2 mg/ml. Working solutions of both compounds (0.2–100 and 0.02–10 μg/ml, for ASOX and ASON, respectively) were prepared by appropriate dilution in methanol. The internal standard solution (phenacetin) was prepared in methanol at the concentration of 4 μg/ml. The solutions were stored at −20 °C and were stable for at least 3 months.

Methanol and dichloromethane (EM Science, Gibbstown, USA) were of HPLC grade. All other chemicals were analytical-reagent grade and were used without further purification. The water used for the mobile phase preparation was purified with a Milli-Q Plus System (Millipore, Bedford, MA, USA).

Pooled drug-free human plasma was obtained from healthy volunteers, stored at −20 °C and allowed to thaw at ambient temperature prior to use.

2.1. Instruments and conditions

The HPLC system consisted of two LC10AD solvent pumps, an SLC 10A system controller, a CTO-10AS column oven and a 7125 Rheodyne injector with a 20-μl loop (Shimadzu, Kyoto, Japan). A UV detector (SPD 10A, Shimadzu) set at 260 nm was also used in some experiments. Separations were carried out at 22 °C on a Lichrospher CN column (125×4.6 mm I.D., 5-μm particle size, Merck, Darmstadt, Germany). A CN guard column (4×4 mm I.D., Merck) was used to protect the analytical column. The mobile phase consisted of methanol–water (4:6, v/v) acidified with 1% acetic acid, at a flow-rate of 1 ml/min.

The MS system was a Quatro LC triple-stage quadrupole (Micromass, Manchester, UK), fitted with a Z-electrospray interface operated in the positive ion mode and calibrated with sodium iodide–cesium iodide in the range of 50–2000 Da. The source block and desolvation temperatures were 100 and 250 °C, respectively. Nitrogen was used as both drying and nebulizing gas at 44 and 555 l/h, respectively. Argon was used as collision gas at a pressure of approximately 3.5×10^{-3} mbar. The HPLC eluent was split by a Valco valve and a flow-rate of approximately 0.1 ml/min was introduced into the stainless steel capillary probe held at 3.0 kV. The cone and collision cell voltage were optimised for each compound (Table 1) and resolutions were in the range of 0.6–0.8 for all compounds. Optimization of MS conditions was obtained by direct infusion of standard solutions diluted with

Table 1
Ions and fragmentation conditions used for multiple reaction monitoring

Drug/ metabolite	Reaction monitored	Cone voltage (kV)	Collision energy (kV)
Phenacetin	180>110	15.0	25.0
ASON	298>266	30.0	20.0
ASOX	282>240	20.0	15.0
ABZ	266>234	30.0	20.0

the mobile phase in the concentration of 10 μg/ml into the ion spray at a flow-rate of 20 μl/min.

Quantitation was performed by MRM (dwell time of 0.4 s) of the protonated molecular (precursor) ions ($[MH]^+$ and their corresponding product ion (Table 1) using an internal standard calibration method with peak area ratios and $1/x$ weighting. The peak area ratios for calibration curves and quantitation were obtained using a Micromass Masslynx 3.0 software. Although ABZ was not introduced in the method for quantitation, its fragmentation reaction was also monitored.

2.2. Extraction procedure

Plasma samples of 1 ml were transferred to 15-ml glass tubes and spiked with 25 μl of phenacetin solution (internal standard, 4 μg/ml). After the addition of 200 μl of a 4 mg/l sodium metabisulfite solution and 4 ml dichloromethane, the tubes were capped, shaken horizontally for 20 min and then centrifuged for 5 min at 1800 *g*. The organic phases were transferred to clean tubes and the solvent was evaporated to dryness. The residues were dissolved in 50 μl mobile phase and immediately chromatographed.

2.3. Recovery and linearity

The analytical recovery of ASOX and AXON was determined at plasma concentrations of 50, 500 and 2500 ng/ml of ASOX and 5, 50 and 250 ng/ml of ASON ($n=4$). Drug-free plasma (1 ml) was spiked with known amounts of the two ABZ metabolites to achieve the concentration previously specified. These samples were submitted to the extraction procedure and 3 ml of the organic phases were transferred to tubes containing the internal standard solution. Peak area ratios were compared with those obtained by the direct injection of the metabolites and internal standard into the mobile phase.

The linearity of the method was evaluated using calibration curves prepared by adding 25 μl of the working solutions to 1 ml of drug-free plasma in order to obtain the concentration range of 1.25 to 250 ng/ml of ASON and 12.5 to 2500 ng/ml of ASOX. The samples were then assayed in triplicate by the described procedure.

2.4. Precision and accuracy

The precision and accuracy of the assay were determined by analyzing aliquots of two spiked plasma samples. Within-day precision and accuracy were determined by analyzing 10 aliquots of spiked human plasma and between-day precision and accuracy were determined over a 1-week period ($n=5$). The precision and accuracy of the method were calculated as the relative standard deviation (coefficient of variation, C.V.) and the percent deviation of observed concentration from theoretical concentration, respectively.

2.5. Sensitivity and selectivity

Sensitivity was evaluated by determining the quantitation limit. Plasma samples ($n=5$) were spiked with ASOX and ASON at the concentrations of 5.0 and 0.5 ng/ml, respectively, and analyzed under the conditions previously established. Criteria for the limits of quantitation were established as the lowest concentration for which the variation in precision and accuracy were less than 20% [27]. In spite of the selectivity of LC–MS–MS, undetected drugs and metabolites could affect the ionization process [28], reducing the signal for the compounds of interest. Thus, spectral interference of drugs commonly used in combination with albendazole for the treatment of neurocysticercosis (carbamazepine, primidone, phenytoin, phenobarbital, sodium valproate, dexamethasone and cimetidine) was evaluated by injecting a laboratory-made solution of ASOX, ASON and phenacetin, at concentrations of 5, 0.5 and 2 μg/ml, respectively, spiked or not with the drugs evaluated for interference (125 μg/ml).

UV detection was also carried out in order to detect the interferent drugs. Sodium valproate was detected by MS–MS, monitoring the fragmentation reaction m/z 145>71, because it is not a UV absorbing species.

2.6. Preliminary human experiment

In order to evaluate the applicability of the method, several plasma samples collected from a patient submitted to treatment with albendazole

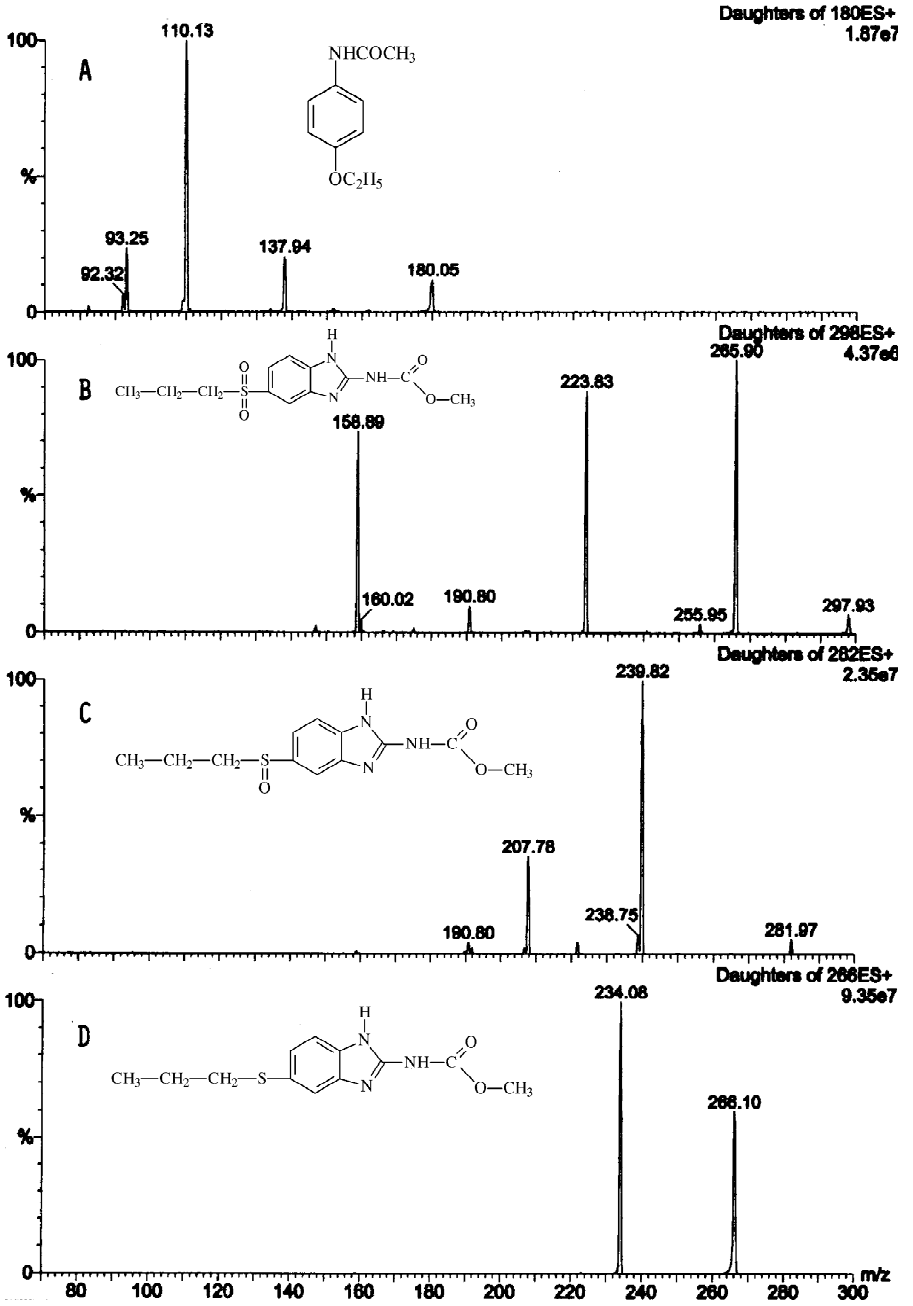

Fig. 1. Product ion mass spectra of the protonated molecule of phenacetin (A), ASON (B), ASOX (C) and ABZ (D).

(Zentel, 15 mg/kg per day) were analyzed under the conditions established in the present study. Blood samples were taken at timed intervals, i.e., 0, 1, 2, 3, 4, 5, 6, 7, 8 and 12 h after dosing. Blood samples were collected into heparinized tubes and centrifuged at 1800 *g* for 10 min and the plasmas were transferred to clean tubes and stored at −20 °C until analysis.

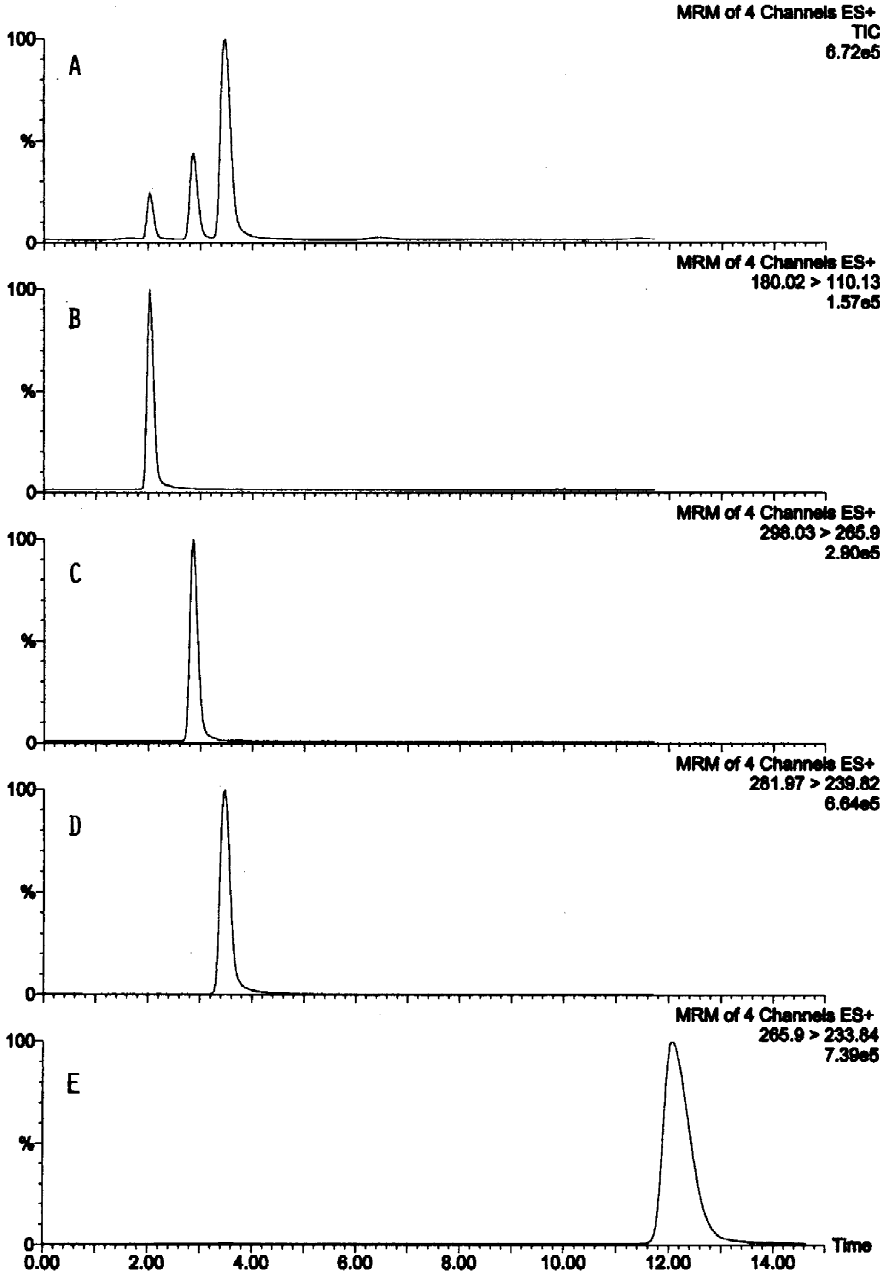

Fig. 2. Total ion chromatogram (A) and ion chromatograms of phenacetin (B), ASON (C), ASOX (D) and ABZ (E), acquired by multiple reaction monitoring.

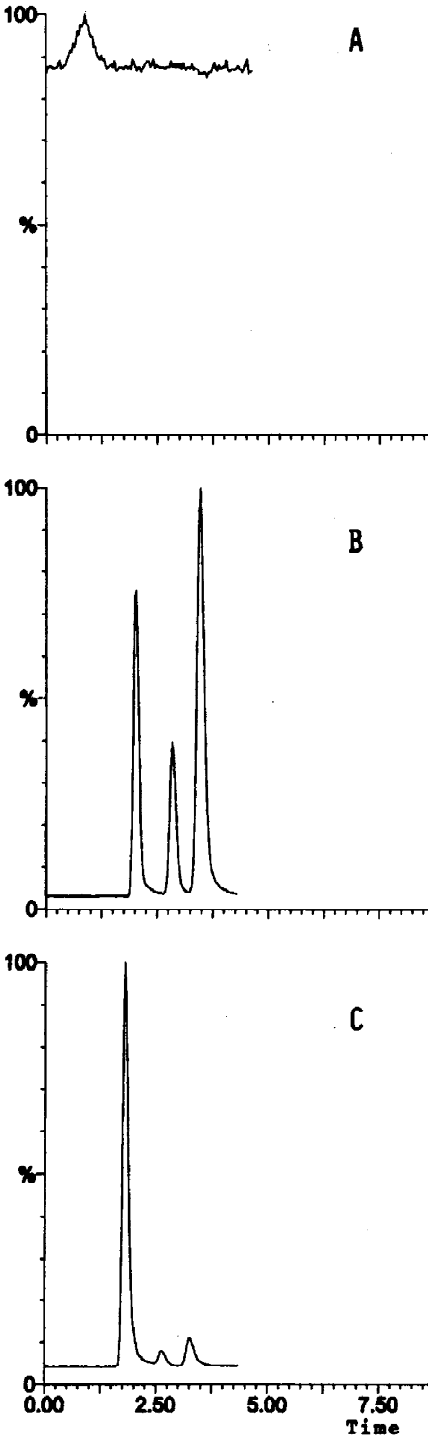

Fig. 3. Total ion chromatogram of (A) blank human plasma; (B) blank human plasma spiked with ASOX, ASON and the internal standard; (C) plasma sample collected from a patient 4 h after administration of ABZ.

3. Results and discussion

The selection of precursor and product ions to be monitored by the MS–MS procedure, as well as optimization of the equipment conditions were carried out by injecting standard solutions of ASOX, ASON and phenacetin into the mobile phase, directly into the ion source using an infusion pump. The ESI^+ full-scan spectra of all compounds indicated that the protonated molecules ($[MH]^+$) were the most abundant ions, so these ions were selected to detect the most abundant product ions (Table 1 and Fig. 1). Similar product ions for ABZ and ASON were monitored by Balizs [26]. The product ion monitored for ASOX was at m/z 240 due to its higher intensity in the mass spectra. Sodium adducts were also observed in the full scan spectra of all compounds due to the presence of sodium in plasma samples as well as due to the addition of sodium metabisulfite in the extraction procedure. This process reduced the intensities of the protonated molecules but did not affect sensitivity and linearity significantly.

The chromatographic conditions were optimized in order to obtain the complete resolution of ASOX, ASON and phenacetin along with a run time as short as possible but avoiding the elution at the void volume to minimize the spectral interference by

Table 2
Recovery, linearity, and quantitation limit of the method for the analysis of ASON and ASOX in plasma

Parameters	ASON	ASOX
Recovery (ASON/ASOX, %):		
5/50 ng/ml	90.5 (6.3)	87.2 (7.3)
50/500 ng/ml	87.3 (9.1)	89.6 (8.8)
250/2500 ng/ml	91.1 (7.3)	86.6 (3.3)
Linearity:		
Range (ng/ml)	1.25–250	12.5–2500
Determination coefficient (r^2)	0.9876	0.9922
Quantitation limit:		
Concentration (ng/ml)	0.59	5.56
Within-day precision (C.V., %), $n=5$	20.1	16.8
Within-day accuracy (%), $n=5$	18.0	11.2

Recovery: coefficient of variation reported in parenthesis; $n=4$ for each concentration.

Table 3
Analytical precision and accuracy of the determination of ASOX and ASON from spiked plasma samples

Concentration added (ng/ml)	ASOX		ASON	
	90	1000	9	100
Within-day precision				
n	10	10	10	10
C.V.	6.9	9.2	8.7	11.8
Between-day precision				
n	5	5	5	5
C.V.	9.8	6.7	9.0	9.8
Within-day accuracy				
Concentration obtained (ng/ml)	97.54	1101.26	9.59	100.60
Relative error (%)	8.4	10.1	6.5	0.6
Between-day accuracy				
Concentration obtained (ng/ml)	95.50	1116.29	8.49	105.16
Relative error (%)	6.1	11.6	−5.6	5.2

endogenous compounds not retained in the column. Although phenacetin is not the ideal internal standard for ABZ metabolites it was selected due to satisfactory results obtained with a previous method developed by our group [24] and because it is not used as a medicine in our country. Representative ion chromatograms of ASOX, ASON and phenacetin, as well as the ion chromatogram for ABZ, are shown in Fig. 2.

The method was developed using liquid–liquid extraction for sample preparation. During extraction procedure optimization, several solvents and mixture of solvents were evaluated. Ethyl ether or diisopropyl ether proved to be good extractors for ASON but not for ASOX. Among all solvents evaluated dichloromethane showed the best compromise between the extraction of ASOX and ASON and was selected for this method. Sodium metabisulfite was added [18,24,25] to avoid ASOX oxidation during sample workup. Typical total ion chromatograms of drug-free human plasma, standard calibration plasma spiked with ASOX, ASON and the internal standard, and treated subject plasma are shown in Fig. 3.

Tables 2 and 3 summarize the data obtained in the validation of the method. The calibration curves (Table 2), obtained by least-squares linear regres-

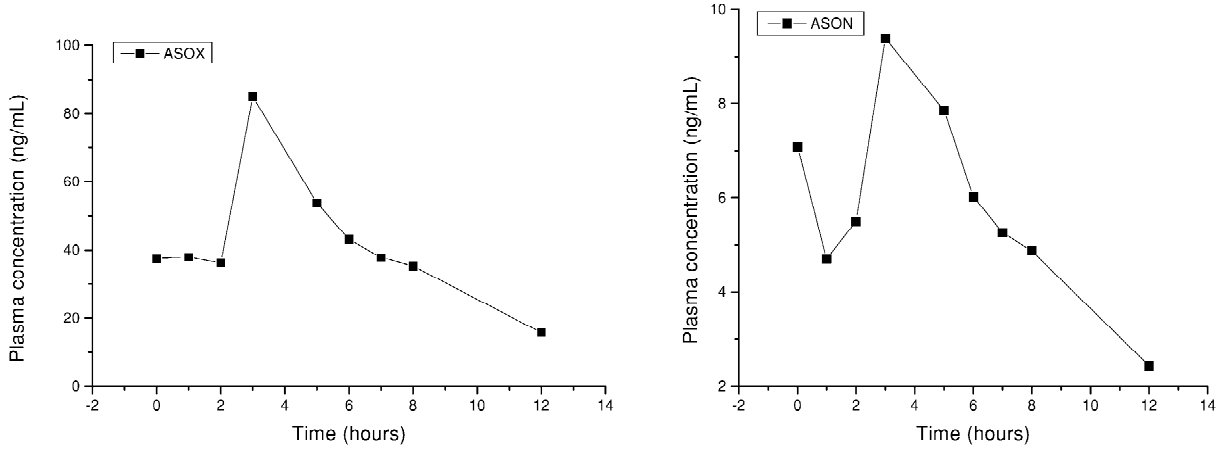

Fig. 4. ASOX and ASON steady-state plasma concentration versus time obtained after administration of ABZ.

Table 4
Kinetic parameters of ASOX and ASON

Parameter	ASOX	ASON
C^{ss}_{max} (ng ml^{-1})	85.26	9.38
C^{ss}_{min} (ng ml^{-1})	15.73	2.43
C^{ss}_{av} (ng ml^{-1})	41.31	5.55
t_{max} (h)	3.0	3.0
$t_{1/2}$ (h)	3.9	4.3
k_{el} (h^{-1})	0.18	0.16
AUC$^{0-12}$ (ng h ml^{-1})	495.70	66.65

C^{ss}_{max}, maximum plasma concentration at steady state; C^{ss}_{min}, minimum plasma concentration at steady state; C^{ss}_{av}, average plasma concentration at steady state; t_{max}, time to reach maximum concentration; $t_{1/2}$, elimination half-life; k_{el}, elimination rate constant; AUC, area under the plasma concentration–time curve.

sion, were linear up to 250 ng/ml of plasma for ASON and 2500 ng/ml for ASOX. Table 2 also shows reproducible recovery of the drugs using the proposed extraction procedure.

Precision and accuracy were assessed by performing replicate analysis of spiked samples against calibration curves. Within-day and between-day precision are reported in Table 3. Coefficients of variation of less than 15% were obtained for all samples analyzed. In addition, the method was accurate since the deviation from the theoretical value was also in the 15% range (Table 3).

In the present study we used the quantitation limit as a parameter for the measurement of the sensitivity of the method, defined as the lowest concentration which can be determined with acceptable accuracy and precision (coefficient of variation and systematic error lower than 20%) [27]. The quantitation limit determined from extracted spiked plasma was 0.5 ng/ml and 5.0 ng/ml for ASON and ASOX, respectively (Table 2).

As expected the proposed method proved to be highly selective. The drugs frequently used in combination with ABZ for the treatment of neurocysticercosis were analyzed using a UV detector and the retention times were compared with the retention times for ABZ metabolites and the internal standard. Among the drugs studied, phenytoin and carbamazepine eluted close to ASOX and phenobarbital close to the internal standard. In spite of this, they did not reduce the intensity of the signal significantly ($<1\%$) and could be considered as non-interferent.

Fig. 4 shows the plasma concentration–time curves of ASOX and ASON obtained from a healthy subject treated with multiple doses of ABZ. Although our data were obtained from only one subject, our results (Table 4) are in agreement with the literature [10,13]. The preliminary results of this pharmacokinetic investigation suggest that the assay is sensitive enough to be used in pharmacokinetic studies of ABZ.

4. Conclusion

The method described in the present study is simple, sensitive and rapid allowing the analysis of the two metabolites of ABZ in less than 4 min. In the validation step, the method proved to be highly reproducible and accurate, with coefficients of variation and systematic errors within the 15% range. The quantitation limit of 0.5 and 5.0 ng/ml for ASON and ASOX, respectively, permits the use of the method in studies of kinetic disposition.

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