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Simultaneous determination of albendazole metabolites, praziquantel and its metabolite in plasma by high-performance liquid chromatography–electrospray mass spectrometry

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Abstract

The analysis of albendazole sulfoxide, albendazole sulfone, praziquantel and *trans*-4-hydroxypraziquantel in plasma was carried out by high-performance liquid chromatography–mass spectrometry ((LC–MS–MS). The plasma samples were prepared by liquid–liquid extraction using dichloromethane as extracting solvent. The partial HPLC resolution of drug and metabolites was obtained using a cyanopropyl column and a mobile phase consisting of methanol:water (3:7, v/v) plus 0.5% of acetic acid, at a flow rate of 1.0 mL/min. Multi reaction monitoring detection was performed by electrospray ionization in the positive ion mode, conferring additional selectivity to the method. Method validation showed relative standard deviation (precision) and relative errors (accuracy) lower than 15% for all analytes evaluated. The quantification limit was 5 ng/mL and the linear range was 5–2500 ng/mL for all analytes. The method was used for the determination of drug and metabolites in swine plasma samples and proved to be suitable for pharmacokinetic studies.

Keywords: Albendazole sulfoxide; Albendazole sulfone; Praziquantel; *trans*-4-Hydroxypraziquantel; LC–MS–MS; Plasma

1. Introduction

Neurocysticercosis is the most common helminthic disease of the nervous system, being considered a serious public health problem in developing countries of Latin America, Asia, and Africa [1–3]. Although restricted to palliative measures, the treatment of neurocysticercosis has advanced over the last 20 years with the use of praziquantel (PZQ) and albendazole (ABZ), drugs considered effective against the cystic larvae [2,4]. ABZ has been found to be more effective than PZQ, but in some patients there is persistence of cysts even after repeated use of ABZ [2]. For these cases, alternative treatment schedules such as the simultaneous use of PZQ and ABZ has been evaluated [4,5]. PZQ combined with ABZ have also been extensively used in human hydatid disease [6–9].

ABZ is extensively metabolized to its active metabolite albendazole sulfoxide (ASOX) that is further metabolized to the inactive albendazole sulfone (ASON) [10]. Due to this extensive metabolism, plasma concentrations of ABZ are usually low and pharmacokinetic studies are developed using ASOX and ASON concentrations [11–15]. PZQ is metabolized to several hydroxylated metabolites [16–18], mainly *trans*-4-hydroxypraziquantel (TRANS), an active metabolite [19].

To evaluate the kinetic disposition of ABZ and PZQ, selective, sensitive and reproducible analytical methods are required for their quantification in plasma samples as well as their

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metabolites. High-performance liquid chromatography (HPLC) [20–29] and capillary electrophoresis (CE) [30,31] have been used for the development of these methods.

The recent technological advances in coupling mass spectrometry to liquid chromatography (LC–MS and LC–MS–MS) brought new insight into quantitative bioanalysis. The use of this technique for the analysis of ABZ metabolites, PZQ and TRANS has been described only for the isolated drugs. Bonato et al. [32] and Chen et al. [33] reported the use of LC–MS–MS for the development of two methods with quantification limits for ASOX of 5.0 and 4.0 ng/mL, respectively. Further, Bonato et al. [32] reported a quantification limit of 0.5 ng/mL for ASON metabolite. LC–MS–MS was used only for the qualitative analysis of PZQ metabolites [16,34].

This paper describes for the first time a highly sensitive LC–MS–MS assay for the simultaneous determination of ASOX, ASON, PZQ and TRANS in plasma. The validated method was applied for the determination of these drugs and metabolites in swine plasma samples and proved to be suitable for pharmacokinetic studies.

2. Experimental

2.1. Chemicals and reagents

ASOX (99.4%) and ASON (99.8%) were kindly supplied by Robert Young & Co. Ltd. (Glasgow, Scotland, U.K.). PZQ (purity > 99%) and TRANS (purity 85%, confirmed by HPLC) were kindly supplied by Merck (Rio de Janeiro, Brazil) and Dr. G. Blaschke (University of Münster, Germany), respectively. Stock standard solutions were prepared in methanol at the concentration of 0.2 mg/mL. Working solutions in the concentration range of 0.2–100 μg/mL were prepared by appropriate dilution in methanol. The internal standard solution (phenacetin) was prepared in methanol at the concentration of 5 μg/mL. The solutions were stored at −20 °C and were stable for at least 3 months.

All chemicals were of analytical grade or HPLC grade and were purchased from Merck (Darmstadt, Germany) or EM Science (Gibbstown, NJ, USA). The water used to prepare the solutions or mobile phase was purified in 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 (23 ± 2 °C) prior to use.

2.2. Equipment and methods

A Shimadzu (Kyoto, Japan) HPLC system consisting 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 was used. Separations were carried out at 22 °C on a Lichrospher CN column (125 mm × 4.6 mm i.d., 5 μm particle size, Merck, Darmstadt, Germany). A CN guard column (4 mm × 4 mm i.d., Merck) was used to protect the analytical column. The mobile phase for the analysis of PZQ, TRANS, ASOX, ASON and the internal standard was methanol:water (3:7, v/v) plus 0.5% of acetic acid, at a flow rate of 1.0 mL/min.

Table 1
Parents and daughter ions and fragmentation conditions used for multiple reaction monitoring

Drug/metabolite	Reaction monitored	Cone voltage (kV)	Collision energy (eV)
Phenacetin	180 → 110	15	20
ASON	298 → 266	30	20
ASOX	282 → 240	20	13
PZQ	313 → 203	30	20
TRANS	329 → 203	30	20

The MS system was a Quatro LC triple-stage quadrupole (Micromass, United Kingdom), fitted with a Z-electrospray interface operating in the positive ion mode and calibrated with sodium iodide/cesium iodide in the 50–2000 Da range. The source block and desolvatation temperatures were 100 and 250 °C, respectively. Nitrogen was used as both drying and nebulizing gas and argon was used as the collision gas. Cone and collision cell voltages (Table 1) as well as other MS parameters were optimised by direct infusion of the drugs and internal standard solutions prepared in the mobile phase at a flow rate of 20 μL/min. 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.

Quantitation was performed by MRM (dwell time of 0.4 s) of the protonated molecules ($[MH]^+$) 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 quantification were obtained using a Micromass Masslynx 3.0 software.

2.3. Extraction procedure

Before analysis, 1 mL plasma samples was transferred to 15 mL glass tubes and spiked with 25 μL of phenacetin solution (internal standard, 5 μg/mL). After the addition of 200 μL of 4 mg/mL sodium metabisulfite solution and 5 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 injected into the chromatographic system.

2.4. Method validation

To evaluate the linearity of the method, calibration curves were prepared by analyzing 1 mL plasma samples spiked with standard PZQ, TRANS, ASOX and ASON solutions ($n = 2$ for each concentration) resulting in plasma concentrations of 5–2500 ng/mL for each compound (five concentration levels). Sample preparation and chromatographic conditions were as described before. Plots of analyte concentrations versus drug/internal standard peak area ratios were constructed and the linear regression lines were used for the determination of analyte concentration in the samples.

To determine absolute recovery, plasma samples spiked with 10, 25 and 500 ng/mL of each compound were extracted in triplicate by the procedure proposed. The concentrations of the samples were determined on the basis of a calibration curve obtained with the data for the analytes not submitted to extraction. Recovery was expressed as percentage of the amount extracted.

The precision and accuracy of the method were evaluated for 3 days analyzing plasma samples spiked with PZQ, TRANS, ASOX and ASON at the concentrations of 15, 80 (ASOX/ASON) or 100 (PZQ/TRANS) and 400 ng/mL of each analytes ($n = 5$ for each concentration). The results obtained were expressed as relative standard deviations and relative errors.

The quantification limit was assayed by analyzing aliquots of plasma ($n = 5$) spiked at concentrations of 5 ng/mL of each analytes against calibration curves with a concentration range of 5–2500 ng/mL.

Freeze-thaw cycle (three cycles of 12 h, −20 °C) and short-term room temperature (room temperature, 23 ± 2 °C, for 12 h) stability tests were performed for the samples in the concentration of 10 and 500 ng/mL of each drug. The peak areas obtained from both stability tests were compared with the peak areas obtained with freshly prepared samples. Student's *t*-test was applied, with the significance level at $p \leq 0.05$.

2.5. Preliminary pharmacokinetic experiment in swine

In order to evaluate the applicability of the method, several plasma samples collected from a group of swine that received combined treatment with albendazole (5 mg/kg/day) and praziquantel (single dose of 17 mg/kg) as part of a therapeutic study (to be published elsewhere) 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, 8, 10, 12, 14, 16 and 28 h after dosing. After centrifugation for 10 min at 1800 × *g*, plasma samples were transferred to clean tubes and stored at −70 °C until analysis.

3. Results and discussion

3.1. Optimization of the LC–MS–MS method

The selection of precursor and product ions (Fig. 1) to be monitored by the MS–MS procedure, as well as the optimization of the equipment conditions, were carried out by injecting standard solutions of drugs and internal standard in the mobile phase, directly into the ion source using an infusion pump (Table 1). The chromatographic conditions were established using a reversed-phase CN column and methanol:water (3:7, v/v) plus 0.5% acetic acid as the mobile phase. Although matrix peaks were

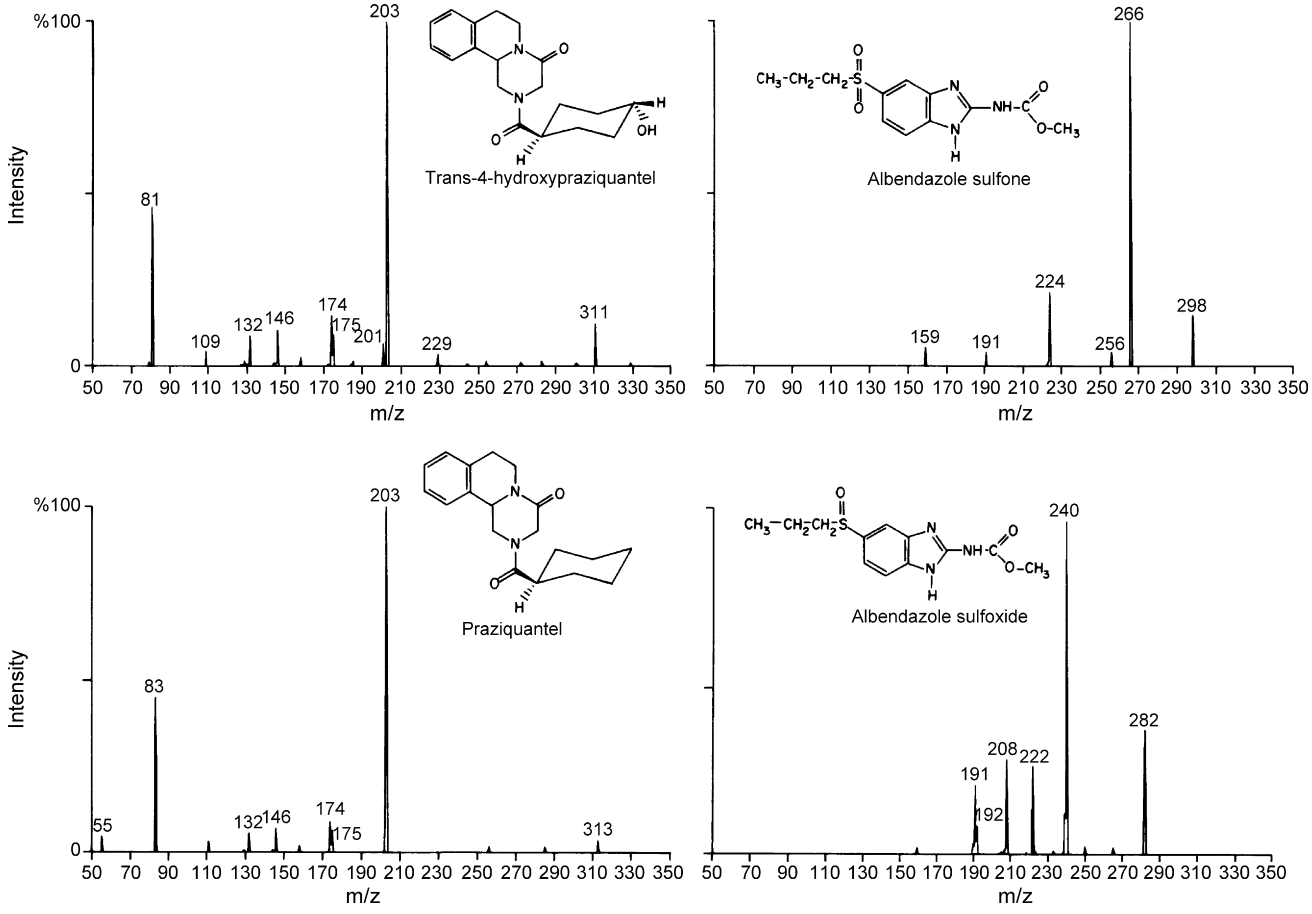

Fig. 1. Product ion mass spectra of the protonated molecules of TRANS, PZQ, ASON, and ASOX.

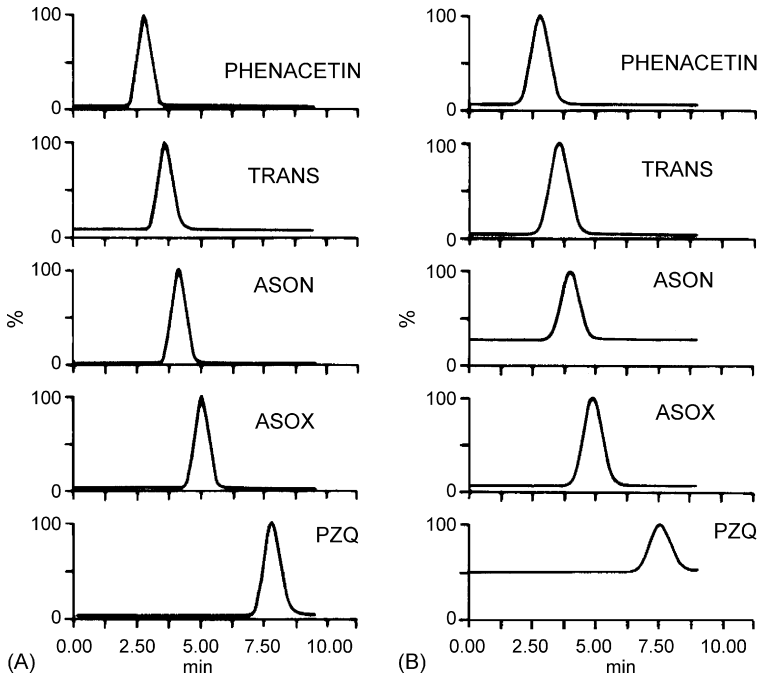

Fig. 2. MRM chromatograms of (A) blank human plasma spiked with PZQ (80 ng/mL), TRANS (80 ng/mL), ASOX (100 ng/mL), ASON (100 ng/mL), and the internal standard; (B) swine plasma sample collected 4 h after administration of ABZ and PZQ.

not detected, ionization suppression effects were evaluated by comparing peak areas obtained in the analysis of water or plasma samples spiked with the drug and metabolites and submitted to extraction procedure. No ionization suppression was observed. The analysis time of less than 10 min is an additional advantage of the method. Fig. 2A shows the selected reaction monitoring chromatograms for PZQ, TRANS, ASOX, ASON and internal standard (phenacetin) under the optimized conditions. Although phenacetin is structurally different from the analytes, it was an acceptable internal standard as proved by the validation parameters.

3.2. Validation of the method

The developed method was validated by evaluating recovery, linearity, precision, accuracy, quantification limit and stability. Coefficients of variation and relative errors of less than 15% were considered acceptable, except for the quantification limit, for which these values were established at 20%, as recommended in the literature [35–37].

Sample preparation was performed by liquid–liquid extraction using dichloromethane as extracting solvent after alkalinization of plasma samples. Table 2 shows mean recoveries

Table 2
Recovery, linearity and quantification limits of the method for the analysis PZQ, TRANS, ASOX and ASON

	Recovery ($n=3$)		Linearity		Quantification limit ($n=5$)		
	(%)	R.S.D. (%)	Range (ng/mL)	r	Conc. (ng/mL)	Precision (R.S.D., %)	Accuracy (E, %)
ASON	64.7	7.5	5–2500	0.9952	4.21	2.6	−15.8
ASOX	54.3	14.6	5–2500	0.9940	4.20	3.1	−16.0
PZQ	63.9	10.9	5–2500	0.9984	4.54	17.2	−9.2
TRANS	73.7	10.7	5–2500	0.9970	4.77	2.7	−4.6

n, Number of determinations; R.S.D., relative standard deviation; r, correlation coefficient; E, relative error.

Table 3
Stability test

Conc. (ng/mL)	p-Value*							
	Freeze-thaw cycles				Short-term room temperature			
	ASOX	ASON	PZQ	TRANS	ASOX	ASON	PZQ	TRANS
10	0.7458	0.6111	0.5122	1.000	0.2524	0.3065	0.0667	0.3739
500	0.7048	0.6392	0.6992	0.2867	0.3432	0.5073	0.4672	0.1010

* Significance level set at $p<0.05$.

Table 4
Precision and accuracy of the method for the analysis of PZQ, TRANS, ASOX and ASON

Drug/nominal concentration (ng/mL)		Day 1			Day 2			Day 3		
		Conc. (ng/mL)	R.S.D. (%)	*E* (%)	Conc. (ng/mL)	R.S.D. (%)	*E* (%)	Conc. (ng/mL)	R.S.D. (%)	*E* (%)
15	ASON	13.75	12.2	−8.3	14.16	11.4	−5.6	14.41	11.6	−3.9
	ASOX	14.17	4.9	−5.5	15.93	12.6	6.2	15.63	9.7	4.2
	PZQ	16.34	9.1	8.9	15.26	12.1	1.7	15.54	12.6	3.6
	TRANS	15.79	8.4	5.3	15.65	3.2	4.3	14.94	9.7	−0.4
80	ASON	71.95	11.2	−10.1	86.21	12.9	7.8	87.29	12.7	9.1
	ASOX	72.81	9.6	−8.9	84.74	6.3	5.9	87.15	6.8	8.9
100	PZQ	87.75	12.7	−12.3	106.45	13.9	6.4	108.14	0.4	8.1
	TRANS	88.66	7.0	−11.3	103.1	0.6	3.1	98.92	7.3	−1.1
400	ASON	363.82	4.7	−9.0	434.79	7.3	8.7	459.35	2.4	14.8
	ASOX	360.99	2.6	−9.7	453.46	9.2	13.4	458.11	8.1	14.5
	PZQ	378.26	6.2	−5.4	460.23	10.0	15.0	380.50	9.6	−4.9
	TRANS	364.64	2.7	−8.8	398.36	6.5	−0.4	441.67	1.1	10.4

n, Number of determinations = 5; R.S.D., relative standard deviation; *E*, systematic error.

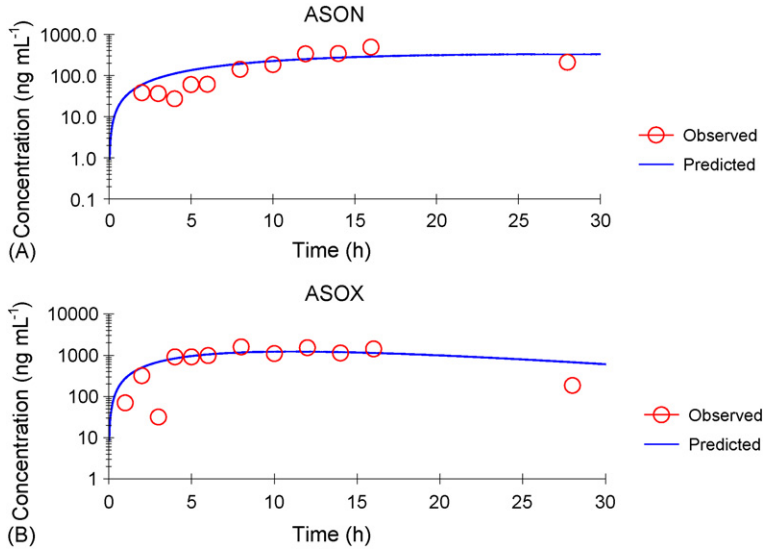

Fig. 3. ASON (A) and ASOX (B) steady-state plasma concentration vs. time obtained after administration of ABZ.

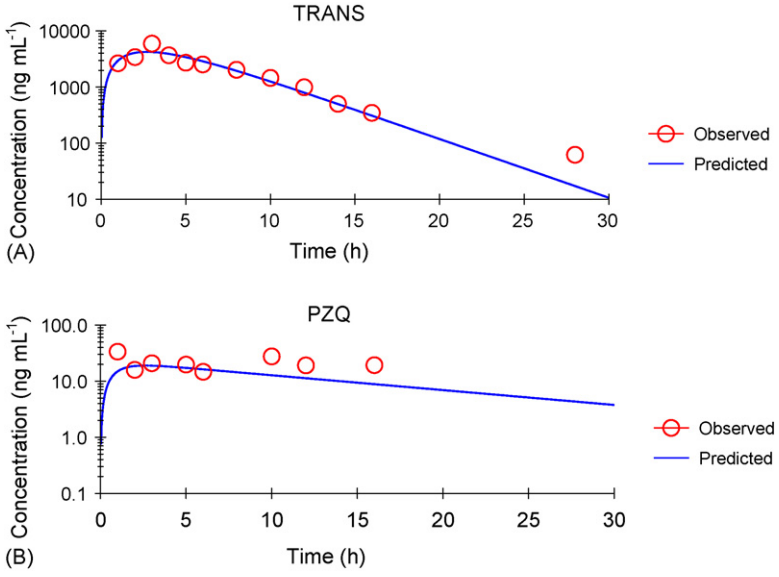

Fig. 4. TRANS (A) and PZQ (B) plasma concentration vs. time obtained after administration of PZQ.

in the range of 64–74%. The relative standard deviations lower than 15% confirm the repeatability of the extraction procedures.

The linearity of the method extended from 5 to 2500 ng/mL, with correlation coefficient higher than 0.99 (Table 2). In addition, 15% deviation from nominal values was considered acceptable for all concentrations except for the concentration of 5 ng/mL, established as the quantification limit.

The freeze thaw cycles and short term room temperature stability tests, evaluated by Student's *t*-test (significance level set at $p < 0.05$), showed acceptable values with $p \geq 0.0667$ (Table 3). The precision and accuracy of the method were performed by replicate analysis of plasma samples spiked with the drugs in three concentrations levels, during 3 consecutive days. Relative standard deviation and relative errors of less than 15% were obtained for all samples analyzed (Table 4).

The chromatograms (Fig. 2B) and plasma concentration-time profiles (Figs. 3 and 4), referring to the analysis of the drugs in swine plasma samples collected after oral administration of PZQ and TRANS illustrated the applicability of the method.

4. Conclusion

This paper reports for the first time the simultaneous analysis of PZQ, TRANS, ASOX and ASON in plasma by LC–MS–MS. The method described here is simple, rapid, and reproducible. The quantification limits obtained using 1 mL plasma were similar to those reported in the literature and were small enough for the method to be used in pharmacokinetic studies. The major advantage of this method when compared to the previously published methods is the high selectivity due to the use of the MS detection system.

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