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Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 871–876
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

A high-performance liquid chromatographic method for determination of praziquantel in plasma

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Received 5 June 2004; received in revised form 26 July 2004; accepted 31 July 2004
Available online 25 September 2004

Abstract

A simple, sensitive, selective and reproducible method based on a reversed-phase chromatography was developed for the determination of praziquantel in human plasma. Praziquantel was separated from the internal standard (diazepam) on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size), with retention times of 4.8 and 6.2 min, respectively. Ultraviolet detection was set at 217 nm. The mobile phase consisted of acetonitrile and distilled water (70:30, v/v), running through the column at a flow rate of 1.0 ml/min. The chromatographic analysis was operated at 25 °C. Sample preparation (1 ml plasma) was done by a single step liquid–liquid extraction with the mixture of methyl-*tert*-butylether and dichloromethane at the ratio of 2:1 (v/v). Calibration curves in plasma at the concentrations 0, 50, 100, 200, 400, 800 and 1600 ng/ml were all linear with correlation coefficients better than 0.999. The precision of the method based on within-day repeatability and reproducibility (day-to-day variation) was below 15% (relative standard deviation: R.S.D.). Good accuracy was observed for both the intra-day or inter-day assays, as indicated by the minimal deviation of mean values found with measured samples from that of the theoretical values (below ±15%). Limit of quantification (LOQ) was accepted as 5 ng using 1 ml samples. The mean recovery for praziquantel and the internal standard were greater than 90% for both praziquantel and internal standard. The method was free from interference from the commonly used antibiotic and antiparasitic drugs. The method appears to be robust and has been applied to a pharmacokinetic study of praziquantel in three healthy Thai volunteers following a single oral dose of 40 mg/kg body weight praziquantel.

Keywords: Praziquantel; Anthelminthics; High-performance liquid chromatography

1. Introduction

Praziquantel is a pyrazinoisoquinoline derivative [2-(cyclohexyl-carbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino [2,1-a]isoquinoline-4-one], which is the treatment of choice for most human trematode and cestode infections, and is widely used in schistosomiasis, as well as other fluke infections pathogenic to human [1]. Advantages of this drug include high efficacy after oral administration, low toxicity and a single day therapeutic regimen.

A number of analytical methods have been reported for determination of praziquantel in human and animal biological fluids and tissue organ extracts. These methods involve radiometric assay [2], fluorometric assay [3], enzyme-linked immunosorbent assay [4], thin-layer chromatography (TLC), gas chromatography [5,7] and high-performance liquid chromatography (HPLC) [6,8–15]. Most of the HPLC with UV detection methods described previously are based principally on the method developed by Xio et al. [16]. Sample preparation methods in these methods are rather time-consuming as they involve three-step liquid–liquid extraction. Furthermore, the procedures do not produce clean samples and clear chromatograms. We have described in this paper, a simple, sensitive, and selective HPLC method for determination of

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doi:10.1016/j.jpba.2004.07.043

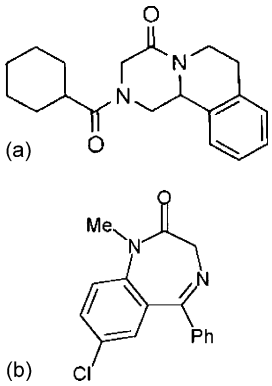

Fig. 1. Chemical structures of (a) praziquantel and (b) internal standard (diazepam).

praziquantel in plasma. Sample preparation step is based only on single step liquid–liquid extraction.

2. Experimental

2.1. Chemicals

All solvents (methanol, acetonitrile, dichloromethane, methyl-*tert*-butylether) were HPLC grade. Organic solvents were purchased from Fison Scientific Equipment (Bishop Meadow Road, Loughborough, UK). Praziquantel (Fig. 1a), diazepam (Fig. 1b) were obtained from Sigma (St. Louis, MO, USA).

2.2. Standard stock solutions

Stock solutions were made with praziquantel and the internal standard (diazepam). Appropriate amounts of chemicals were dissolved in methanol in volumetric flasks. Stock solutions for praziquantel and internal standard were prepared at the concentration of 1000 ng/μl. The stock solutions were further diluted to make working solutions at concentrations of 50 ng/μl for praziquantel, and 100 ng/μl for the internal standard. Standard solutions were stored at −20 °C until use.

2.3. Chromatography

The method was developed on a chromatographic system consisting of Spectra System P4000 HPLC solvent Delivery/Controller, equipped with a Rheodye 7125 injector with a 50 μl loop (Rheodyne, Berkeley, CA, USA) and an ultraviolet detector (Spetra System UV 1000). The wavelength was set at 217 nm. The separation was carried out on a reversed phase column Luna C18 (250 mm × 4.6 mm, 5 μm particle size: Phenomenex^{TM}, USA). The elution solvent consisted of acetonitrile and distilled water (70:30, v/v). The chromatographic analysis was operated at 25 °C. Aliquots of 100 μl samples or standard solutions were injected onto the column with a mobile phase at flow rate of 1.0 ml/min. Distilled water was vacuum filtered and degassed through 0.2 μm pore size polymeric PTFE filters.

2.4. Sample preparation

This procedure was validated on specimens using 1 ml of spiked human plasma. Outdated human plasma was obtained from the Blood Bank of Thammasat Chalermprakiet Hospital, Thammasat University and stored frozen in aliquots at −20 °C. To 1 ml plasma, was added 600 ng (100 ng/μl) internal standard working solution (diazepam 100 ng/μl). The samples were vortex mixed for 2–3 s and extracted with 6 ml methyl-*tert*-butylether/dichloromethane mixture (2:1, v/v). After being subjected to mechanical tumbling for 30 min (speed 6), the organic layer was separated through centrifugation at 1500 × *g* for 30 min. The upper organic layer was transferred to a clean tube and evaporated to dryness under a stream of oxygen at 45 °C. The residue was reconstituted in 200 μl of mobile phase and 100 μl were injected onto the HPLC column.

2.5. Calibration curves

Calibration curves were prepared by replicate analysis of seven plasma samples (1 ml each) spiked with varying concentrations of praziquantel (0, 50, 100, 200, 400, 800 and 1600 ng/ml) and a fixed concentration of the internal standard (600 ng/ml). Samples were analyzed as described in Section 2.4.

2.6. Data analysis

The internal standard corrected for variation in the sample preparation step used. Peak detection, peak height integration, peak height ratio calculation, calibration curve fitting (least square regression without weighting) and calculation of sample concentrations were performed by the ChromQuest® software version 4.

2.7. Method validation

2.7.1. Precision

The precision of the method based on *within-day repeatability* was determined by replicate analysis of six sets of samples spiked with six different concentrations of praziquantel (50, 100, 200, 400, 800, and 1600 ng/ml). The *reproducibility* (*day-to-day variation*) of the method was validated using the same concentration range of plasma as described above, but only a single determination of each concentration was made on six different days. Relative standard deviation (R.S.D.) were calculated from the ratios of standard deviation (S.D.) to the mean and expressed as percentage.

2.7.2. Accuracy

Accuracy of the method was determined by replicate analysis of six sets of samples spiked with six different levels of

praziquantel (50, 100, 200, 400, 800, and 1600 ng/ml) and comparing the difference between spiked value and that actually found (theoretical value).

2.7.3. Recovery

The analytical recovery of sample preparation procedure for praziquantel and the internal standard (diazepam) was estimated by comparing the peak heights obtained from samples (plasma) prepared as described in Section 2.4, with those measured with equivalent amounts of praziquantel in methanol. Triplicate analysis was performed at concentrations of 50 and 400 ng/ml for praziquantel and at concentration of 600 ng/ml for internal standard.

2.7.4. Selectivity

The selectivity of the method was verified by checking for interference by albendazole, albendazole sulphoxide (active metabolite of albendazole), ivermectin, including the commonly used antibiotics ampicillin, penicillin and gentamycin after subjecting them to sample preparation procedures. Albendazole and ivermectin are antiparasitic drugs which are used in combination with praziquantel in the control of filariasis and geohelminths.

2.7.5. Limit of quantification

The limit of quantification (LOQ) of the assay procedure was determined from the lowest concentration of praziquantel (in spiked plasma sample) that produced a peak height three times the baseline noise at a sensitivity of 0.005 aufs (absorbance unit full scale) in a 1 ml sample.

2.7.6. Stability

The stability of praziquantel was determined by storing spiked plasma samples (at the concentrations of 100, 400, and 1600 ng/ml; triplicate analysis for each concentration) in a −20 °C freezer (Sanyo, Japan) for 6 months. Concentrations were measured periodically (1, 2, 3 and 6 months). For freeze and thaw stability, samples were frozen at −20 °C for at least 24 h and thawed unassisted at room temperature (25 °C). When completely thawed, the samples were transferred back to the original freezer and refrozen for at least 24 h. The process was repeated for three cycles.

2.8. Quality control

Quality control (QC) samples for praziquantel were made up in plasma using a stock solution separated from that used to prepare the calibration curve, at the concentrations of 100, 400, and 1600 ng/ml. Samples were aliquoted into cryovials, and stored frozen at −20 °C for use with each analytical run. The results of the QC samples provided the basis of accepting or rejecting the run. At least four of the six QC samples had to be within ±20% of their respective nominal value. Two of the six QC samples could be outside the ±20% of their respective nominal value, but not at the same concentration.

2.9. Application of the method to biological samples

The method was applied to the investigation of the pharmacokinetics of praziquantel in three healthy Thai volunteers (aged 20–23 years, weighing 50–52.5 kg) following a single oral dose of 40 mg/kg body weight praziquantel. Informed consents were obtained from all volunteers prior to the study. The study was approved by the Ethics Committee of the Faculty of Medicine, Thammasat University. Venous blood samples (3 ml) were collected into heparinized-coated plastic tubes at the following time points: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60 and 72 h of dosing.

3. Results and discussion

3.1. Chromatographic separation

A number of HPLC chromatographic systems were investigated to optimise the separation of praziquantel and the internal standard (diazepam). Retention maps were generated for both compounds as a function of stationary phase (Luna C18 reversed-phase column) and mobile phase. The elution solvent consisting of acetonitrile and distilled water at the ratio of 70:30 (v/v) was chosen as an appropriate elution solvent as it resulted in optimal separation. The retention times of praziquantel, and the internal standard were approximately 4.8 and 6.2 min, respectively. The chromatograms showed a good baseline separation. Chromatograms of standard solution of praziquantel and internal standard are shown in Fig. 2.

3.2. Sample preparation

The sample preparation step used in this study involved only a single step of sample preparation, i.e., liquid–liquid extraction with a mixture of organic solvents (methyl-*tert*-butylether and dichloromethane). This condition was found to be the most optimal condition for sample preparation as it resulted in a clean chromatogram. The internal standard corrected for variation in the sample preparation step used.

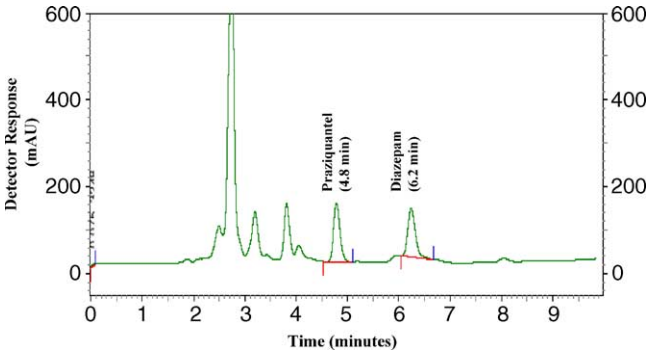

Fig. 2. Chromatogram of standard solution of praziquantel (2500 ng) and internal standard (diazepam: 5000 ng), with retention times of 4.8 and 6.2 min, respectively.

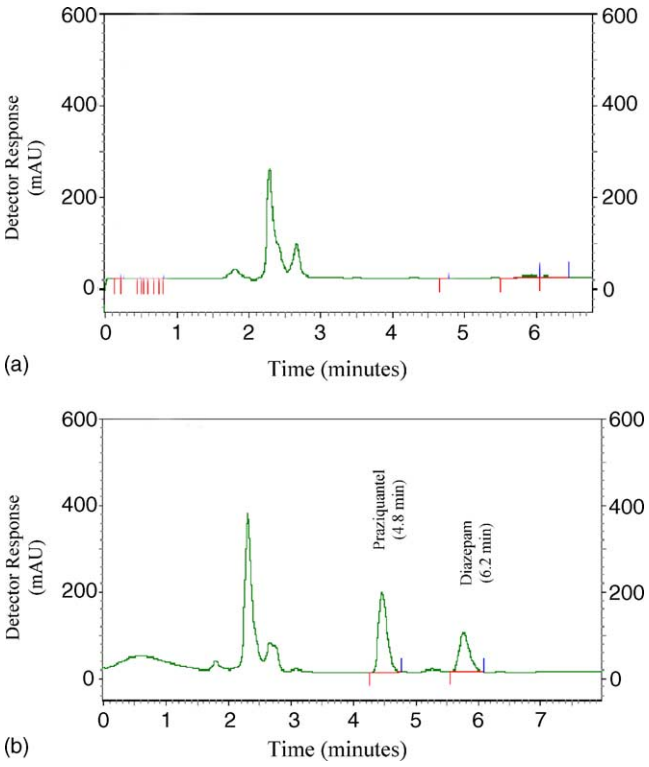

Fig. 3. Chromatogram of (a) blank plasma, (b) plasma spiked with 1600 ng/ml praziquantel and 600 ng/ml internal standard (retention times of 4.8 and 6.2 min, respectively).

Chromatograms of blank plasma and plasma spiked with praziquantel at the concentration of 1600 ng/ml (with a fixed concentration of internal standard of 600 ng/ml) are shown in Fig. 3a and b, respectively.

3.3. Calibration curves

Plasma analysis was calibrated using the concentration range of 0–1600 ng/ml. All calibration curves yielded linear relationships with correlation coefficients of 0.999 or better.

3.4. Method validation

3.4.1. Precision

Little variation of praziquantel assays was observed; relative standard deviation (R.S.D.) for six analyses at the concentration range observed were all below 15%. The intra-assay (within-day) and inter-assay (day-to-day) variation for praziquantel assay at the concentration range 0–1600 ng/ml are summarized in Table 1. The intra- and inter-day assay variation varied between +0.99 and +3.70%, and +0.66 and +8.73%, respectively.

3.4.2. Accuracy

Good accuracy was observed from both the intra-day or inter-day assays, as indicated by the minimal deviation of mean values found with measured samples from that of the theoretical values (actual amount added). The intra-assay (within-day) and inter-assay (day-to-day) accuracy for praziquantel assay at the concentration range 0–1600 ng/ml are summarized in Table 1. The intra- and inter-day assay accuracy expressed as the mean deviation from the theoretical values varied between −1 and +0.25%, and −0.35 and +0.33%, respectively.

3.4.3. Recovery

The mean recoveries for praziquantel in plasma at the concentration range 0–1600 ng/ml including the internal standard in all cases were greater than 90%. The results reflect essentially 100% recovery from the spiked plasma and indicate lack of interference from sample preparation procedure.

3.4.4. Selectivity

Selectivity of the chromatographic separation was demonstrated by the absence of interferences from endogenous peaks in plasma. Fig. 3a and b illustrates typical chromatograms for blank plasma, spiked plasma with praziquantel and internal standard.

3.4.5. Limit of quantification

The limit of quantification (LOQ) in human plasma for praziquantel were accepted as 5 ng using 1 ml plasma.

3.4.6. Stability

Plasma samples containing praziquantel at concentrations of 100, 400, and 1600 ng/ml were found to be stable when stored in a −20 °C freezer for a minimum of 6 months without significant decomposition of the drug. Long-term storage of the spiked samples for up to 6 months did not appear to affect the quantification of the analytes. Mean deviation (%)

Table 1
Summary of assay precision and accuracy (intra-assay and inter-assay) for praziquantel assay in plasma

Concentration added (ng/ml)	Precision (%R.S.D.)		Accuracy (%DMV)[a]	
	Intra-assay (N = 6)	Inter-assay (N = 6)	Intra-assay (N = 6)	Inter-assay (N = 6)
50	3.70	8.73	−1	+0.33
100	3.48	5.69	−0.5	−0.33
200	2.47	4.67	−0.08	−0.16
400	1.41	1.60	+0.25	+0.16
800	1.05	0.66	−0.10	−0.35
1600	0.99	0.64	+0.06	−0.07

[a] %DMV = deviation of mean value from theoretical value (%).

Table 2
Storage stability data of praziquantel in plasma at concentrations 100, 400, and 1600 ng/ml

Time period (month)	Concentration added (ng/ml)	Concentration measured (ng/ml)				
		Assay 1	Assay 2	Assay 3	Mean (S.D.)	%DEV[a]
(a) Long-term stability at 1, 2, 3 and 6 months						
1	100	105	98	100	101 (3.6)	+1.0
	400	402	405	389	398 (8.5)	−0.33
	1600	1590	1605	1582	1592 (11.6)	−0.47
2	100	102	98	98	99 (2.3)	−0.66
	400	410	390	385	395 (13.2)	−1.25
	1600	1590	1591	1600	1593 (5.5)	−0.39
3	100	95	98	101	98 (3.0)	−2.0
	400	401	410	385	398 (12.6)	−0.33
	1600	1610	1580	1592	1594 (15.0)	−0.37
6	100	198	95	101	98 (3.0)	−1.25
	400	410	390	385	395 (13.2)	−0.25
	1600	1590	1598	1600	1596 (5.2)	−0.10
(b) Freeze and thaw stability						
	100	95	98	95	96 (1.7)	−4
	400	401	410	380	397 (15.3)	−0.74
	1600	1580	1585	1606	1590 (13.7)	−0.6

[a] %DEV = deviation of single value from theoretical value (%).

of measured concentrations after storage at the observed periods (1, 2, 3 and 6 months) varied between −2.0 and +1.0% (Table 2a). Freezing and thawing for three successive cycles did not affect the measured concentrations. Mean deviation from the theoretical values varied between −4.0 and −0.6% (Table 2b).

3.5. Quality control

Three validated analysts, conducted the plasma analysis. A standard curve and quality control specimens were included with each analysis. Control samples with nominal concentration of 100, 400 and 1600 ng/ml praziquantel were analyzed at the beginning and the end of the analytical run. Results were all within the acceptable limit (±20% of their respective nominal values).

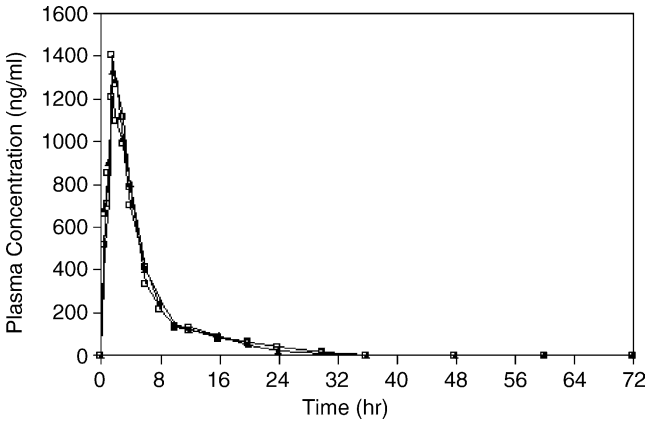

Fig. 4. Plasma concentration–time profile of praziquantel in three healthy Thai volunteers following a single oral dose of 40 mg/kg body weight praziquantel.

3.6. Application of assay and analysis of specimens

To demonstrate the clinical applicability of the method, plasma concentrations of praziquantel were determined in three healthy Thai volunteers following a single oral dose of 40 mg/kg body weight praziquantel. Plasma concentration–time profiles of praziquantel are shown in Fig. 4.

4. Conclusions

The previous HPLC methods described [6,8–15] are all based principally on the method developed by Xio et al. [16]. However, these methods are rather time-consuming as they involve three-step liquid–liquid extraction procedure. Water saturated ethylacetate was used in sample extraction procedure, which did not produce clean samples and clear chromatograms. We describe a HPLC assay procedure based on a reversed-phase C18 chromatography with ultraviolet detection, for the selective, sensitive, accurate and reproducible quantitative analysis of praziquantel in human plasma samples. Total run time was within 7 min. The major difference with other previously described methods is the sample extraction step, which based on only a single step extraction with a mixture of methyl-*tert*-butylether and dichloromethane mixture (2:1, v/v). This resulted in clean samples and clear chromatograms (Fig. 3a). The analytical method for the determination of praziquantel in plasma established in this study meets the criteria for application to routine clinical drug level monitoring or pharmacokinetic study. The advantage of the method over previously reported methods are basically, its rapidity, simplicity (one-step sample preparation procedure),

high sensitivity (LOQ, 5 ng/ml), and high selectivity (no interference from endogenous peaks). In addition, sample evaporation does not require nitrogen stream which would increase additional cost of analysis.

Acknowledgements

This investigation received financial support from the UNDP/World/Bank/WHO Special Programme for Research and Training in Tropical Diseases. We thank Dr. Janis Lazdins, WHO/TDR for his support during the study.

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