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Short Communication

LC determination of praziquantel in human plasma

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Abstract

A simple high-performance liquid chromatographic (HPLC) method for the determination of praziquantel in human plasma was developed and validated. The present method was described by adding drop-wise 0.2 M Zinc sulfate and acetonitrile to plasma sample for deproteinization. This method used a reversed-phase Spherisorb ODS 2 column (5 μm), 250 × 4.6 mm i.d. as a stationary phase with a mobile phase consisting of acetonitrile-methanol-water (36:10:54, v/v/v), a flow rate of 1.5 ml/min and UV detection wavelength of 217 nm. Diazepam was used as internal standard. The standard calibration curve was linear over the concentration range of 100–2000 ng/ml ($r = 0.999$). The equation of a linear regression line was $y = 8.05\text{E-}04 + 7.25\text{E-}04x$ with slope and intercept values of 0.0007 and 0.0008, respectively. The limit of detection was 12.25 ng/ml and the limit of quantification was set at 100 ng/ml. The intra- and inter-day assay coefficients of variation (CV) were 3.0 ± 1.7 and $6.3 \pm 1.9\%$, respectively. The percentage of recovery was 102.1 ± 5.6. Therefore, the HPLC method described here was simple, rapid and reproducible since it did not require extraction and evaporation processes in sample preparation, which will reduce time-consuming or expensive sample preparation.

Keywords: Praziquantel; High-performance liquid chromatography; Reversed-phase HPLC

1. Introduction

Praziquantel, a pyrazinoisoquinoline derivative, has a wide range of activity against trematodes and cestodes, and is widely used in schistosomiasis, as well as other fluke infections pathogenic to humans [1]. Praziquantel is readily absorbed after oral administration, even when taken with a meal, so that peak plasma levels in humans occur in 1–2 h. The drug is about 80% bound to plasma proteins. Its plasma half-life is 0.8–2 h, compared with 4–6 h for its metabolites. About 70% of an oral dose of praziquantel is recovered as metabolites in the urine within 24 h [2]. However, more than 80% of the dose is absorbed with 80% of an oral dose excreted as hydroxylated metabolites in urine within 4 days [1]. Advantages of this agent include high efficacy after oral administration, low toxicity and a single day therapeutic regimen.

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A number of studies have been reported on the pharmacokinetics of praziquantel, probably lack of suitable and simple high-performance liquid chromatographic (HPLC) methods for praziquantel analysis in the plasma sample. Presently, there are various methods used in the analysis of praziquantel in human plasma and body fluids including radiometric [3], fluorometric [4], gas–liquid chromatographic [5], and HPLC method [6]. Later, a number of HPLC methods for praziquantel analysis were slightly modified [7–10] on the basis of HPLC method previously described by Xiao et al. [6]. Among these, an HPLC method for determination of praziquantel in plasma seems to be more simple than other methods. However, the HPLC method presently used in praziquantel analysis previously reported [6] was not simple because it required more times and cost of consuming in sample preparation. Briefly, the 1-ml serum sample was extracted three times with 2 ml of water-saturated ethyl acetate. Each sample was mixed for 1 min after addition of organic solvent and phase separation was achieved by centrifugation at $1600 \times g$ for 5 min. Then, the three ethyl acetate phases from each sample were combined and transferred to a 10-ml pear-shape flask for removal of solvent by rotoevaporation. The sample residue was resuspended in a mobile phase and aliquots of volume were injected into the HPLC apparatus.

Therefore, in this paper we developed the simple, rapid and sensitive HPLC assay method using deproteinization without organic solvent extraction and evaporating processes to dryness by rotoevaporation or under a nitrogen stream in sample preparation as previously described [6–10] to simultaneously determine praziquantel in human plasma. The assay method was also validated for linearity, precision, accuracy, limit of detection and limit of quantification. In addition, this HPLC method had more advantages than those previously reported in which it reduced time-consuming and cost of praziquantel analysis in human plasma and other body fluids. Thus, it is appropriate for pharmacokinetic studies.

2. Materials and methods

2.1. Standard chemicals and solvents

The standard praziquantel and diazepam were purchased from Sigma Chemical Co (St. Louis, Missouri, USA). A HPLC grade of acetonitrile and methanol were bought from J.T. Baker (Phillipsburg, NJ, USA). Other chemicals and reagents were of analytical grade. Water was purified for HPLC by the Milli Q water purification system (Millipore, Milford, MA, USA).

2.2. Instruments and chromatographic conditions

The HPLC system consisted of a Waters 515 pump and a Waters 717 plus autosampler (Waters Associates, Milford, MA, USA). The column was reverse-phase Spherisorb ODS 2 (5 μm, 250×4.6 mm i.d., Waters Associates, Milford, MA, USA). The mobile phase consisted of a mixture of acetonitrile–methanol–water (36:54:10, v/v/v), which was filtered through a 0.45 μm membrane filter (Nyron 66, Millipore, Milford, MA, USA) and degased by ultrasonification (Tru-Sweep, ETL Testing Laboratories, Cortland, NY, USA) for 10 min before using. The mobile phase was freshly prepared in each day. The flow rate was 1.5 ml/min. The praziquantel was detected by the UV detector (SpectroMonitor Model 3100, Milton Roy, Riviera Beach, FL, USA) at 217 nm. All analyses were performed at room temperature.

2.3. Preparation of standard solutions

The stock standard solution of praziquantel at a concentration of 400 μg/ml was prepared in methanol and stored at 4 °C. Working standard solutions of praziquantel in blank human plasma (100, 200, 400, 800, 1600 and 2000 ng/ml) for the calibration curve were prepared each day by serial dilutions of the stock standard solution. Intra-day, inter-day assay coefficients of variation (CV) and the percentage of recovery were also determined.

2.4. Sample preparation

To 200 μl of plasma sample in a 2-ml stoppered microcentrifuge tube was added with a 50 μl of diazepam internal standard solution (840 ng/ml) and 50 μl of 0.2 M Zinc sulphate solution drop-wise, and mixed for 30 s on a vortex mixer (Vortex Genie-2, Scientific Industries, Bohemia, NY, USA). Four-hundred microliters of acetonitrile was then added drop-wise and shaken thoroughly on a vortex mixer for 30 s. The final concentration of diazepam internal standard in the mixture used in this study was 60 ng/ml. After 15 min the mixture was centrifuged at $10\,000 \times g$ for 10 min. A 100 μl of supernatant was injected into HPLC system using a reverse-phase Spherisorb ODS 2 column with acetonitrile–methanol–water (36:54:10, v/v/v) as a mobile phase, and detected by the UV detector at 217 nm.

2.5. Study protocol in a healthy subject

The plasma concentration-time profile after oral administration of a single dose of 40 mg/kg praziquantel was studied in one normal healthy subject. The study protocol in a human subject was approved by the Human Ethics Committee of the Faculty of Science, Prince of Songkla University, and the subject must give a written consent before participating in the study. Blood samples (5 ml) were collected to heparinized tubes via a sterile catheter cannulated in a forearm vein before praziquantel ingestion and at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h post dosing. All blood samples were centrifuged at $2500 \times g$ for 15 min. The plasma was separated and kept at -70 °C for praziquantel analysis within 4 weeks.

Table 1
The intra- and inter-day assay coefficients of variation of the present HPLC method for the determination of praziquantel concentration in human plasma

Concentration (ng/ml)	% Coefficients of variation (CV)	
	Intra-day assay[a]	Inter-day assay[b]
100	3.8	4.9
200	6.1	8.1
400	2.2	5.4
800	2.0	4.0
1600	1.5	8.8
2000	2.6	6.8
Mean (S.D.)	3.0 (1.7)	6.3 (1.9)

[a] $n = 5$.
[b] $n = 10$.

3. Results and discussion

3.1. Method validation tests

3.1.1. Linearity of the standard calibration curve

The standard calibration curve for praziquantel at concentrations of 100, 200, 400, 800, 1600, and 2000 ng/ml was linear with the correlation coefficient (r) of 0.999. The equation of linear regression line was $y = 8.0487\text{E-}04 + 7.2484\text{E-}04x$ with slope and intercept values of 0.0007 and 0.0008, respectively.

3.1.2. Precision

The intra-day assay coefficients of variation (CV) for praziquantel were 3.8% at 100 ng/ml, 6.1% at 200 ng/ml, 2.2% at 400 ng/ml, 2.0% at 800 ng/ml, 1.5% at 1600 ng/ml and 2.6% at 2000 ng/ml ($n = 5$), whereas the inter-day assay was assessed on 10 individual days; the CV were 4.9% at 100 ng/ml, 8.1% at 200 ng/ml, 5.4% at 400 ng/ml, 4.0% at 800 ng/ml, 8.8% at 1600 ng/ml and 6.8% at 2000 ng/ml ($n = 10$). All results are shown in Table 1. These data show a remarkably degree of precision and reproducibility for the method both during within-runs and between-runs.

3.1.3. Accuracy

The mean absolute recovery values of the present HPLC method throughout the linear range are presented in Table 2. The results in Table 2 reflect that the method is obviously accurate and this ensures reliable results.

3.1.4. Limit of detection and limit of quantification

The limit of detection (LOD) of praziquantel in plasma was approximately 12.25 ng/ml by consid-

Table 2
The absolute recovery of the present HPLC method for the determination of praziquantel concentration in human plasma

Added concentration (ng/ml)	Measured concentration (ng/ml)	% Recovery
100	110.0	110.0
200	205.8	102.9
400	418.4	104.6
800	827.2	103.4
1600	1542.4	96.4
2000	1900.0	95.0
Mean (S.D.)		102.1 (5.6)

$n = 5$.

ering a signal-to-noise ratio of 3:1. The LOQ was set at 100 ng/ml being the lowest concentration used in the construction of the standard curve. Although this assay method produced less sensitivity than that of proposed method by Xiao et al. (12.25 vs. 5.0 ng/ml) [6], the assay was considerably simple and sensitive in praziquantel analysis for pharmacokinetic studies.

3.1.5. Sample stability

Previous study [6] has been reported that the stability of praziquantel in one serum sample analyzed immediately compared with those of analyzed after 1, 2, 3, and 4 weeks of storage at -20 °C was not altered. The same concentrations were found after storage up to 4 weeks. Thus, in our experiments the plasma samples were separated and kept at -70 °C for praziquantel analysis within 4 weeks after storage.

3.2. Chromatograms

The representative chromatograms recorded from blank human plasma, plasma spiked with working standard solutions of praziquantel, and plasma of one normal healthy subject after ingestion a single dose of 40 mg/kg praziquantel were shown in Fig. 1. The retention times for praziquantel and diazepam (internal standard) were approximately 14 and 17 min, respectively. The HPLC method described here showed excellent separation of praziquantel from internal standard diazepam. In addition, no interference peaks from plasma components were observed in chromatograms.

3.3. The plasma concentration-time profile of praziquantel in human plasma

The plasma concentration-time profile of one normal healthy volunteer after administration of a single oral dose of 40 mg/kg praziquantel was shown in Fig. 2. The elimination half-life ($t_{1/2}$, 0–12 h) of praziquantel determined from the plasma concentration-time profile was 2.7 h. This parameter was comparable with that reported by Mandour et al. [7]. The present developed HPLC method was sufficiently sensitive to permit pharmacokinetic studies of praziquantel in human plasma although it had less sensitivity than that of previously described [6] (limit of detection; 12.25 vs. 5.0 ng/ml). Moreover, this method was developed on the basis of plasma protein precipitation by 0.2 M Zinc sulphate and acetonitrile without extraction and evaporating processes in sample preparation. Therefore, the method is simple, rapid and inexpensive and suitable for praziquantel analysis in pharmacokinetic studies.

4. Conclusion

A number of HPLC methods have been reported for simultaneous quantitation of praziquantel in human plasma, serum and body fluids [6–10]. However, these methods required extraction and evaporating processes in sample preparation, which were more time-consuming and costly in praziquantel analysis. Therefore, we developed a simple HPLC assay method for praziquantel in human plasma, which was sensitive, rapid and inexpensive without extraction and evaporating processes in steps of sample preparation on the basis of protein precipitation using 0.2 M zinc-sulphate and acetonitrile. Moreover, the present method could be applied to simultaneous determination of hydroxypraziquantel (a major metabolite) in human plasma, however, it needs further investigation in this purpose.

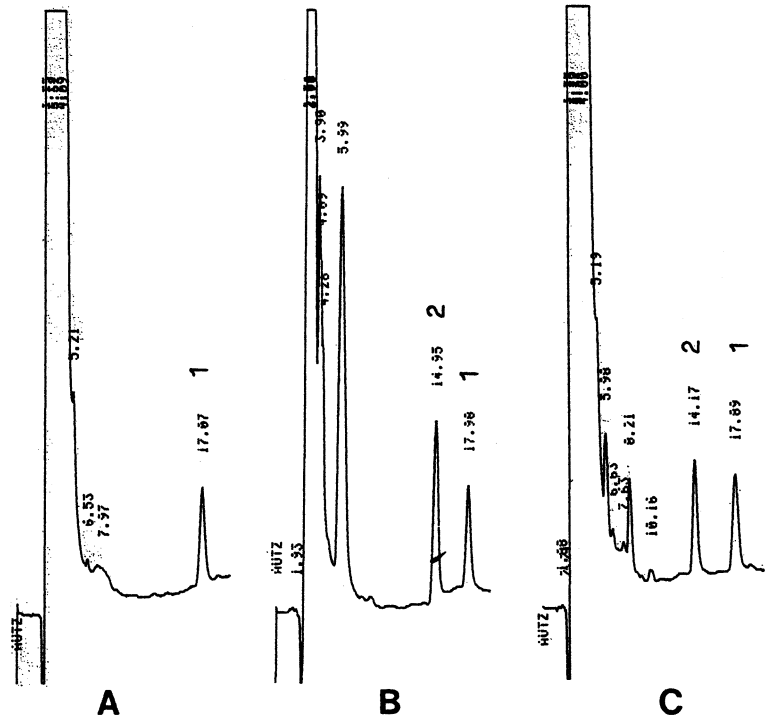

Fig. 1. Representative chromatograms obtained from blank human plasma spiked with diazepam internal standard (A), blank human plasma spiked with 2000 ng/ml standard praziquantel and diazepam internal standard (B), and plasma from one normal healthy subject containing 1650 ng/ml praziquantel collected 2 h after ingestion of a single dose of 40 mg/kg praziquantel (C). Attenuation = 4 (*Y*-axis); chart speed = 3 mm/min (*X*-axis); 1, diazepam internal standard, 2, praziquantel.

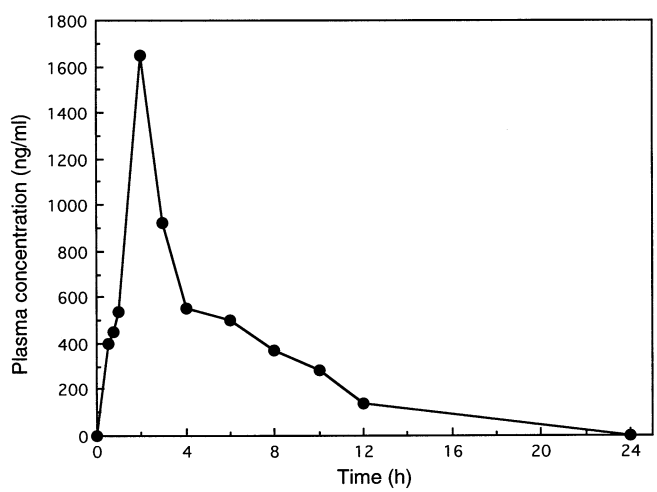

Fig. 2. The plasma concentration-time profile of praziquantel in one normal healthy subject after a single oral dose of 40 mg/kg praziquantel.

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