A high-performance liquid chromatographic method for determination of praziquantel in rat plasma: Optimization and application to pharmacokinetic study

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Abstract

A simple, sensitive, selective and reproducible method based on a reversed-phase chromatography was developed for the determination of Praziquantel in rat plasma using internal standard as Diazepam. Praziquantel & Diazepam was separated on a C18 column Enable (250mm × 4.6 mm, 5µm), with retention times of 6.4 & 8.5 min. Ultraviolet detection was set at 225 nm. The mobile phase consisted of acetonitrile and distilled water (60:40, v/v), running through the column at a flow rate of 1.0 mL/min. The chromatographic analysis was operated at an ambient temperature. Sample preparation (200 µl plasma) was done by a protein precipitation by using perchloric acid. Calibration curves in plasma at the concentrations 5, 50, 100, 500, 1000, 2000, 3000 & 5000ng/mL were all linear with correlation coefficients (r^2) is 0.9989. The precision of the method based on within-day repeatability and reproducibility (day-to-day variation) was within 15% (relative standard deviation: R.S.D. should be less than 15 according to CDER guidance for Bio-analytical Method Validation). 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 Quantitation (LOQ) was accepted as 5ng/mL using 200µl 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 anti-parasitic drugs. The method appears to be robust and has been applied to a pharmacokinetic study of praziquantel in three groups of rats following a single oral dose of 40 mg/kg body weight of praziquantel.

Keywords: Praziquantel; Pharmacokinetics; HPLC; Rat plasma

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 or

single step liquid–liquid extraction [17]. 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 Praziquantel (PRQ) in plasma using Diazepam as internal standard (IS) and applying the method in pharmacokinetic study to calculate the all the necessary pharmacokinetic parameters which was not mentioned in the previous articles. For the first-time sample preparation step is based only on single step by protein precipitation by using perchloric acid as a protein precipitating agent which is easily available, less costly than solvents used in liquid–liquid extraction. The protein precipitation method is highly effective in giving a clear chromatogram and does not require solvent evaporation by gentle steam of nitrogen or oxygen. More over this method uses less plasma in this study.

2. EXPERIMENTAL

2.1. Chemicals

Praziquantel (Fig 1) was obtained from Micro Labs Ltd., Goa, India & Diazepam (Fig 2) was obtained from Hetro Pharma Ltd. Hyderabad India as a gift samples. Acetonitrile, Methanol and Water (obtained from S. D. Fine Chemicals limited, Worli, Mumbai, India.) of HPLC grade were used. All the other reagents (Perchloric acid) used for the development of liquid chromatographic method for determination of praziquantel in rat plasma was of analytical grade obtained from Merck Specialties private limited, Worli, Mumbai, India.

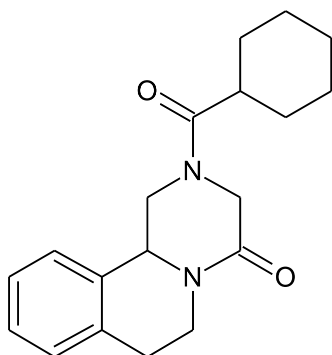

Fig 1. Chemical structures of Praziquantel

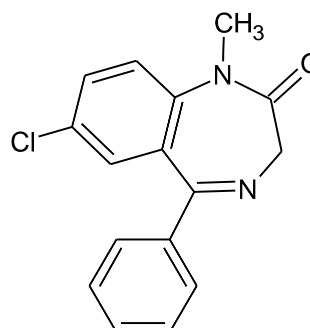

Fig 2. Chemical structures of Diazepam (internal standard)

2.2. Preparation of stock and standard solutions

Stock solution 1 mg/mL of praziquantel & diazepam were prepared in methanol. Standard solution of praziquantel & diazepam was prepared by mixing and diluting the appropriate amounts from the individual stock solution by methanol. The final concentrations of the standard solution were 50000, 30000, 20000, 10000, 1000, 500 & 50 ng/mL and a fixed concentration of the IS (5000 ng/mL). Precision and accuracy standards with concentrations of 50000, 10000, 5000 and 50 ng/mL were also prepared in the same manner and a fixed concentration of the IS (5000 ng/mL). Stock solutions were refrigerated when not in use and replaced on bi-weekly basis. Fresh standard solutions were prepared for each day of analysis or validation.

2.3. Chromatography

A high-performance liquid chromatography (Shimadzu, Kyoto, Japan) was composed of a LC-20AT Prominence solvent delivery module, a manual rheodyne injector with a 20-µl fixed loop and a SPD-20A Prominence UV–visible detector. Separation was performed on Enable C18G column (Column Length: 250mm×4.6mm i.d.; 5µm; particle size, Enable) at an

ambient temperature. The data acquisition was made by Spinchrom Chromatographic Station® CFR Version 2.4.195 (Spinchrom Pvt. Ltd., Chennai, India). The mobile phase consisted of acetonitrile: water in ratio of 60:40 for plasma samples at a flow rate of 1.0 mL/min.

2.4. Sample Collection

The use of animals in this study was approved by GTU (Gujarat Technological University, Ahmadabad, Gujarat, India) and CPCSEA (Committee for the Purpose of Control and Supervision on Experimental Animals). The rats were housed one animal per cage at Animal House of Sigma Institute of Pharmacy, Baroda. The environment was controlled with daily feeding and water.

Healthy albino rats (weighing 150–250 gm) venous blood samples (2 mL) were collected into in 2 mL heparinized-coated micro-centrifuge tube from retro orbital plexus of albino rats. The 2 mL micro-centrifuge containing blood was centrifuged at 15000 rpm for 15 min and the plasma was collected carefully. The collected plasma was stored in -20°C till it was used. Blood sample was collected on regular basis from different rats and plasma was separated till the study is been completed so that the analysis is unbiased in nature.

2.5. Calibration Curves

Blank plasma was collected from untreated anesthetized animals. Plasma calibration point was prepared by spiking 200µl of plasma with 20µl of each PRQ standard solutions (section 2.2) & Diazepam (IS) standard solutions (section 2.2) were vortexed for few min. The calibration curves for rat plasma were in the range of 5-5000 ng/mL & fixed concentration of the IS (500 ng/mL). After plasma was spiked, it was subjected to further sample preparation before analysis.

2.6. Sample Preparation

The plasma samples were treated with protein precipitating agent for the analysis of PRQ. To each 200µl plasma spiked with PRQ (different concentration) and diazepam (fixed concentration) were taken in 1.5 mL micro-centrifuge tubes and simultaneously a blank (200µl of plasma without PRQ & Diazepam) was also taken in 1.5 mL micro-centrifuge tube. Different protein precipitating agents were used such as ammonia sulphate, 15% trichloro acetic acid, 10% sodium tungstate in water, 5-sulphosalicylic acid, zinc sulphate in methanol, different ratios of organic solvents (Acetone, Acetonitrile, Methanol) and different percentages of perchloric acid in water. Lastly it was found that 8.25% of perchloric acid gave a clear chromatogram without any interference with PRQ and Diazepam (IS) so perchloric acid was chosen to be used as a protein precipitating agent to precipitate proteins throughout the whole Bioanalytical study. To each 200 µl of plasma spiked sample was mixed with 45 µl of 8.25% perchloric acid for 30 sec. The samples were centrifuged at 12000 rpm for 15 min this gives a clear supernatant liquid at the top in comparison to above mention protein precipitating agents. 20µl of clear supernatant liquid was transferred in Hamilton Syringe and injected through the rheodyne injector to HPLC column for analysis.

2.7. Validation of the Developed Method

The proposed method was validated as per the CDER guidelines. The developed method was validated by evaluating recovery, linearity, precision, accuracy, quantitation limit and stability. Coefficients of variation and relative errors of less than 15% were considered acceptable, except for the quantitation limit, for which these values were established at 20%, as recommended in the literature. [18-20]

2.7.1. Linearity

The linearity was tested for the concentration range of 5000, 3000, 2000, 1000, 500, 100, 50 & 5 ng/mL for PRQ and a fixed concentration of IS 500 ng/mL and the calibration curve was constructed and evaluated by its coefficient of determination (r^2). The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of an analyte in the sample. Eight different concentrations of PRQ with constant IS concentration were spiked to the blank plasma as described previously and calibration curve was constructed in the specified concentration range. The calibration plot (peak area ratio of PRQ to IS versus PRQ concentration) was generated by replicate analysis (n = 8) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program. The coefficient of determination (r^2) for PRQ in plasma was 0.998 for PRQ and given in Fig 3.

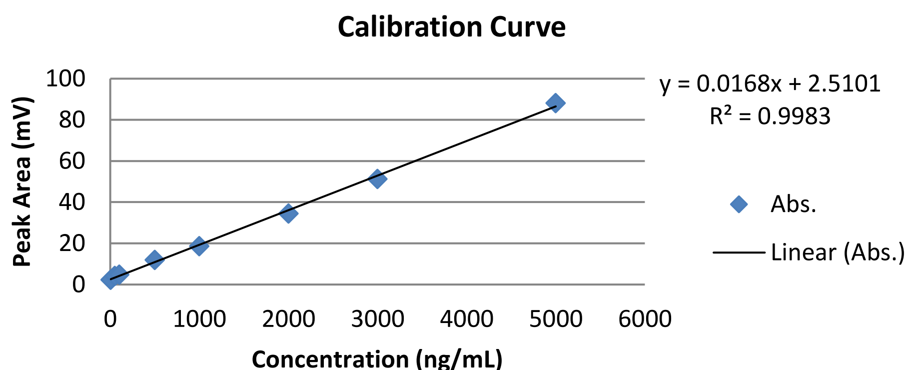

Fig 3. Calibration Curve of Praziquantel in rat plasma

2.7.2. Accuracy

Accuracy of the method was determined by replicate analysis of five sets of samples spiked with four different levels of PRQ (5, 500, 1000 and 5000 ng/mL) with a fixed concentration of Diazepam (500 ng/mL) and comparing the difference between spiked value (theoretical value) and that found value.

2.7.3. Precision

The precision of the method based on within-day repeatability was determined by replicate analysis of five sets of samples spiked with four different concentrations of PRQ (5, 500, 1000 and 5000 ng/mL) with a fixed concentration of Diazepam (500 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 three 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.

2.7.4. Recovery

The analytical recovery of sample preparation procedure for PRQ and the IS (Diazepam) were estimated by comparing the peak heights obtained from samples (plasma) prepared as described in Section 2.5, with those measured with equivalent amounts of PRQ in methanol. Triplicate analysis was performed at concentrations of 5 and 5000 ng/mL for PRQ and at a fixed concentration of 500 ng/mL for IS.

2.7.5. 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.

2.7.6. Limit of Quantitation
The limit of quantitation (LOQ) of the assay procedure was determined from the lowest concentration of PRQ (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 200 µl sample.

2.7.7. Stability
The stability of PRQ was determined by storing spiked plasma samples (at the concentrations of 5, 1000, and 5000 ng/mL with a fixed concentration of Diazepam (500 ng/mL); triplicate analysis for each concentration) in a −20 °C freezer (Sanyo, Japan) for 6 months. Concentrations were measured periodically (1st, 2nd, 4th and 6th month). 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 PRQ were made up in plasma using a stock solution separated from that used to prepare the calibration curve, at the concentrations of 5, 500, 1000 and 5000 ng/mL along with a fixed concentration of Diazepam (500 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 two of the four QC samples had to be within ±20% of their respective nominal value. Two of the four 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 twelve healthy albino rats (weighing 150–250 g) following a single oral dose of 40 mg/kg body weight praziquantel. The study was approved by the CPCSEA (Committee for the Purpose of Control and Supervision on Experimental Animals). Venous blood samples (1.5 mL) were collected into heparinized-coated micro centrifuge plastic tubes (2 mL) from retro orbital plexus of albino rats at the following time points: 0, 0.5, 1, 1.5, 2, 4, 6, 18, 23, 27, 30 and 48 h of dosing.

The blood was collected from the retro orbital plexus and immediately centrifuged at 15000 rpm for 15 min and the plasma was collected carefully. The supernatant plasma layer was separated and stored at −20 °C until analyzed. The plasma samples were analyzed for praziquantel concentrations as described above after sample preparation. The total area under the observed plasma concentration–time curve (AUC) was calculated by using the linear trapezoidal rule. The first order elimination rate constant (k_{el}) was estimated by the least square regression of the points describing the terminal log-linear decaying phase. $T_{1/2}$ was derived from k_{el} ($T_{1/2} = \ln 2/k_{el}$). The absorption rate constant (k_a) was determined by residual method. The maximum observed praziquantel concentration (C_{max}) and the time at which C_{max} was observed (T_{max}) were reported directly from the profile.

3. RESULTS AND DISCUSSION

3.1. Chromatographic separation

A number of HPLC chromatographic systems were investigated to optimize the separation of PRQ. Retention time for PRQ function of stationary phase (Enable C18 reversed-phase column) and the mobile phase using acetonitrile and distilled water at the ratio of 60:40 (v/v). The elution solvent consisting of acetonitrile and distilled water at the ratio of 60:40 (v/v) was chosen as an appropriate elution solvent as it resulted in optimal separation. The retention times for PRQ & Diazepam (IS) were 6.4 & 8.5 min respectively. The chromatograms showed a good baseline separation. Chromatogram of PRQ (2000 ng/mL) & Diazepam (500 ng/mL) (IS) is shown in Fig 4.

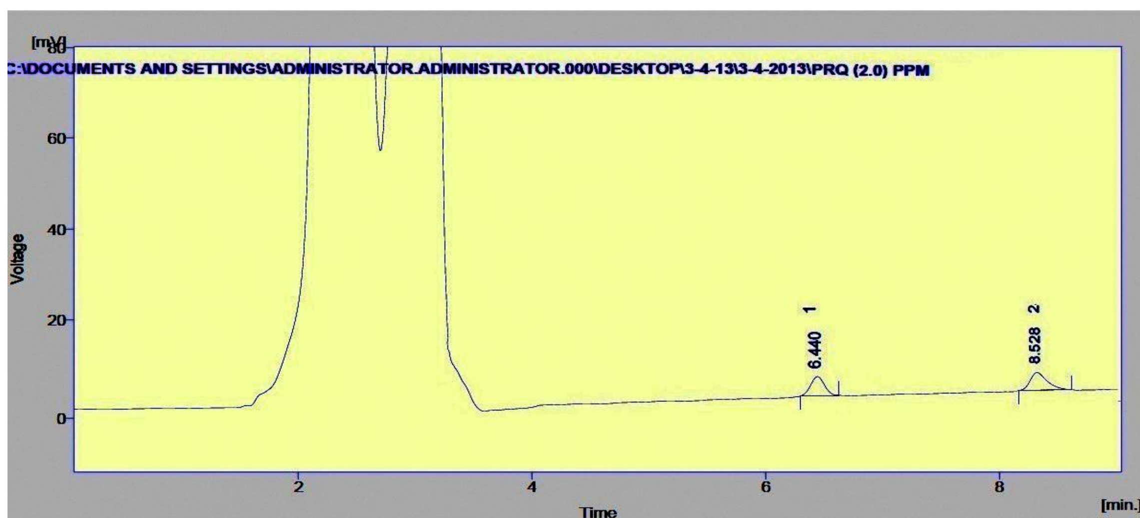

Fig 4. Chromatogram of standard solution of praziquantel (2000 ng/mL) & Diazepam (500 ng/mL) with retention time 6.4 & 8.5 min.

3.2. Sample preparation

The sample preparation step used in this study involved protein precipitation of sample, i.e., perchloric acid (8.25% of perchloric acid) of 45 µl was used to precipitate the proteins from plasma. This condition was found to be the most optimal condition for sample preparation as it resulted in a clean chromatogram.

3.3. Calibration curves

Plasma analysis was calibrated using the concentration range of 5–5000 ng/mL for PRQ with a fixed concentration of Diazepam (500 ng/mL). The calibration curves yielded a linear relationship with correlation coefficients(r) of 0.9989 with equation $Y = 0.016x + 2.510$ where slope was 0.016 and intercept was 2.510 for PRQ.

3.4. Method validation

3.4.1. Linearity

The linearity was tested for the concentration range of 5000, 3000, 2000, 1000, 100, 50 & 5 ng/mL for PRQ with a fixed concentration of Diazepam (500 ng/mL) and the calibration curve was constructed and evaluated by its correlation coefficient. The linear regression equation was calculated by the least squares method using Microsoft Excel® program. The coefficient of determination (r^2) for plasma was 0.998 for PRQ indicating a strong linear relationship between the variable is summarized in Table 1 & 2.

Table 1: Linear regression equations generated from validation of praziquantel in plasma: Slope, Intercept and Coefficient of determination

Analyte	*Matrix*	*Conc.(ng/mL)*	*Area (mV.s)*	*Slope*	*Intercept*	*R^2*
PRQ	Plasma	5	2.357	0.016	2.510	0.998
		50	4.22			
		100	4.77			
		500	11.954			
		1000	18.657			
		2000	34.536			
		3000	51.271			
		5000	88.149			

n=8, for praziquantel in plasma

Table 2: Spectral and statistical data for determination of praziquantel by proposed HPLC method

Parameters	**Value**
Absorption maxima, λmax (nm)	217nm
Linearity range (ng/mL)	5-500
Coefficient of determination (r^2)	0.998
Correlation coefficient (r)	0.9989
Regression equation (Y^a)	Y = 0.016x + 2.510
Slope (b)	0.016
Intercept (a)	2.510
Limit of detection, LOD (ng/mL)	1.515
Limit of quantitation, LOQ (ng/mL)	5

[a] *Y* = mx + c, where *x* is the concentration (ng/mL).

3.4.2. Precision

Inter-day as well as intra-day replicates of PRQ, gave an R.S.D. within 8.05 (should be less than 15 according to CDER guidance for Bio-analytical Method Validation [20]), revealed that the proposed method is highly precise. Little variation of PRQ assays was observed; relative standard deviation (R.S.D.) for all four different concentrations of PRQ observed was all below 15%. The intra-assay (within-day) and inter-assay (day-to-day) variation for PRQ assay at the concentration range 5–5000 ng/mL (5, 500, 1000 and 5000 ng/mL) are summarized in Table 3.

Table 3: Inter-day (n=5) and Intra-day (n=5) precision (%R.S.D.) measured for QC points for praziquantel in plasma.

	T.C.	**Day 1**		**Day 2**		**Day 3**		**Intra-day**	
Plasma	**ng/mL**	**E.C.**	**%R.S.D.**	**E.C.**	**%R.S.D.**	**E.C.**	**%R.S.D.**	**E.C.**	**%R.S.D.**
1	5000	5084.93	1.58	4999.31	1.51	5212.18	2.21	5048.51	1.44
2	1000	1024.81	3.62	987.31	3.09	1018.02	0.98	1017.48	1.14
3	500	509.01	2.36	513.80	5.72	518.55	3.81	524.83	2.82
4	5	5.07	8.05	4.88	8.05	5.32	4.99	5.47	4.50

T.C. denotes theoretical concentration and E.C. denotes experimental concentration.

3.4.3. Accuracy

Accuracy data in the present study ranged from 100.97 to 109.40% for PRQ (Table 4) indicates that there was no interference from endogenous plasma components. Inter-day as well as intra-day range was 5–5000 ng/mL (5, 500, 1000 and 5000 ng/mL) along with a fixed concentration of Diazepam 500 ng/mL.

Table 4: Summary of inter-day (*n* = 5) and intra-day (*n* = 5) precision and accuracy of the method in rat plasma

Nominal concentration (ng/mL)	Mean concentration Found [a] (ng/mL)	S.D.	Precision (%R.S.D.)	Mean accuracy [b] (%)
Inter-day (n=5)				
5000	5098.81	90.61	1.77	101.97
1000	1010.05	25.92	2.56	101.005
500	513.78	20.39	3.96	102.75
5	5.09	0.35	7.03	101.80
Intra-day (n=5)				
5000	5048.51	72.79	1.44	100.97
1000	1017.48	11.66	1.14	101.74
500	524.83	14.81	2.82	104.96
5	5.47	0.24	4.50	109.40

[a] Average of three and six determinations at three concentration levels for inter-day and intra-day respectively.
[b] All the mean accuracies were calculated against their nominal concentrations.

3.4.4. Recovery

The mean recoveries for PRQ in plasma at the concentration range 5–5000 ng/mL were found to be greater than 90%. The results reflect essentially nearly 100% recovery from the spiked plasma and indicate lack of interference from sample preparation procedure.

3.4.5. Selectivity

Selectivity of the chromatographic separation was demonstrated by the absence of interferences from endogenous peaks in plasma. Fig 5 and 6 illustrates typical chromatograms for blank plasma and spiked plasma with PRQ (5000 ng/mL) & Diazepam (500 ng/mL) with retention time of 6.4 & 8.5 min.

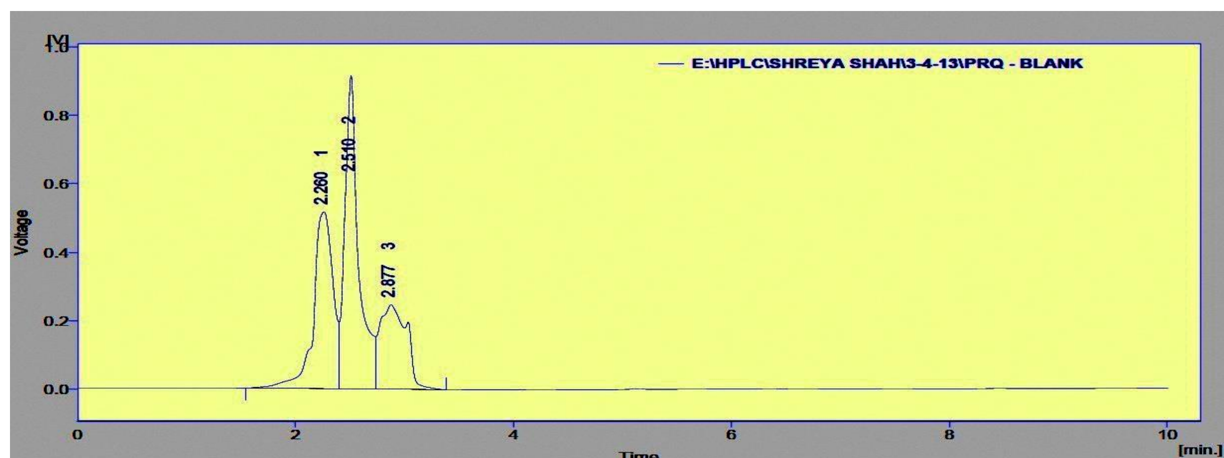

Fig 5. Chromatogram of blank rat plasma without Praziquantel & Diazepam.

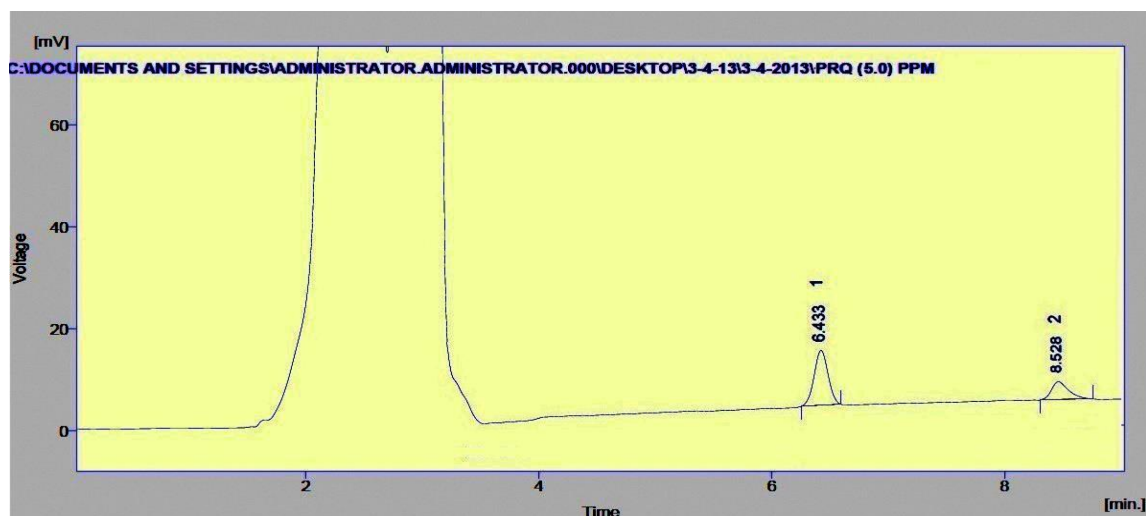

Fig 6. Chromatogram of standard solution of Praziquantel (5000 ng/mL) & Diazepam (500 ng/mL) with retention time 6.4 & 8.5 min.

3.4.6. Specificity

Any potential interference (overlapping peaks) due to plasma endogenous components were within 2–4 min only (Fig 6), later on there was no significant interference from blank plasma that affected the response of PRQ & Diazepam.

3.4.7. Limit of Quantitation

The limit of quantitation (LOQ) in rat plasma for PRQ was accepted as 5ng using 200µl plasma.

3.4.8. Stability

Plasma samples containing PRQ at concentrations of 5, 1000 and 5000 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 quantitation of the analytes. Mean deviation (%) of measured concentrations after storage at the observed periods (1, 2, 4 and 6 months) varied between --4.30 to 8.00% for PRQ (Table 5a). Freezing and thawing for three successive cycles did not affect the measured concentrations. Mean deviation from the theoretical values varied within 0.28 to 3.60 for PRQ (Table 5b).

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 5, 500, 1000 and 5000 ng/mL of PRQ 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).

3.6. System suitability

System suitability tests, an integral part of a chromatographic analysis is used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis [21]. A system suitability test according to USP was performed on the chromatograms obtained from standard and test solutions to check different above-mentioned parameters and the results obtained from six replicate injections of the standard solution are summarized in the Table 6.

Table 5: Storage stability data of Praziquantel in plasma at concentrations 5, 1000, and 5000 ng/mL

a. Long term stability	Concentration added (ng/mL)	Concentration measured (ng/mL)					
		Assay 1	Assay 2	Assay 3	Mean	S.D	%DEV[a]
1st month	5	5.35	5.38	4.51	5.08	0.49	1.60
	1000	1014.18	1028.56	1040.43	1027.72	13.14	2.77
	5000	5002.43	5037.43	5122.43	5054.10	61.71	1.08
2nd month	5	5.58	4.98	5.47	5.35	0.31	7.00
	1000	1019.56	1035.43	1055.43	1036.81	17.97	3.68
	5000	5110.68	5020.56	5072.18	5067.81	45.22	1.35
4th month	5	5.23	5.41	5.55	5.40	0.15	8.00
	1000	1016.68	1010.25	1065.43	1030.79	30.17	3.07
	5000	5075.93	5263.43	5222.18	5187.18	98.52	3.74
6th month	5	4.73	4.83	4.75	4.77	0.05	-4.30
	1000	985.43	1016.68	968.93	990.35	24.25	-0.96
	5000	4995.56	4993.68	4980.31	4989.85	8.31	-0.20
b. Freeze and thaw stability	5	5.35	5.24	4.96	5.18	0.20	3.60
	1000	1025.35	1045.32	1014.23	1028.30	15.75	2.83
	5000	5014.34	5024.36	5004.36	5014.35	10.00	0.28

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

Table 6: System suitability parameters

S. No.	Parameters	Praziquantel
1	Retention time, Rt (min)	6.4
2	Capacity factor (k)	3.07
3	Separation factor (α)	1.42
4	Theoretical plates (USP)	4096
5	HETP (mm)	0.0610
6	Resolution (Rs)	3.48

3.7. Application of assay and analysis of specimens

The developed method was applied to quantify PRQ concentration in pharmacokinetic study carried out on three groups each containing twelve albino rats. HPLC chromatogram of rat plasma after 2 h of oral drug administration (suspension) of PRQ (40mg/kg body weight) with retention times of 6.4 given in Fig 7. Plasma concentration vs time profiles of PRQ given in Fig 8. Various other pharmacokinetic parameters have been summarized in Table 7. The T_{max} and $T_{1/2}$ of PRQ in the present study to demonstrate the clinical applicability of the method, plasma concentrations of PRQ was carried out on three groups each containing twelve albino rats following a single oral dose of 40 mg/kg body weight of PRQ.

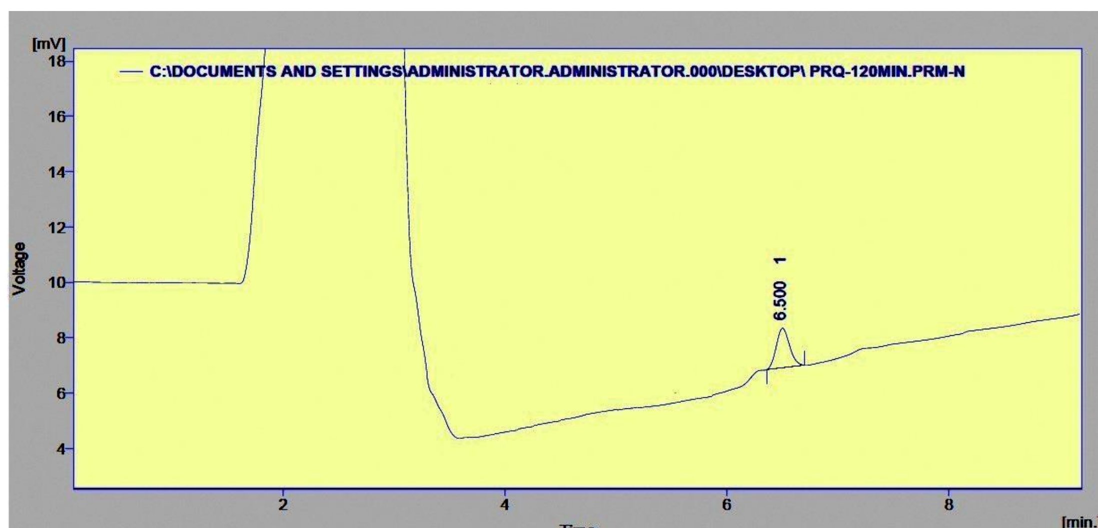

Fig 7. Chromatogram of rat plasma after 2 h of oral administration of praziquantel (40mg/kg body weight) with retention times of 6.5 min.

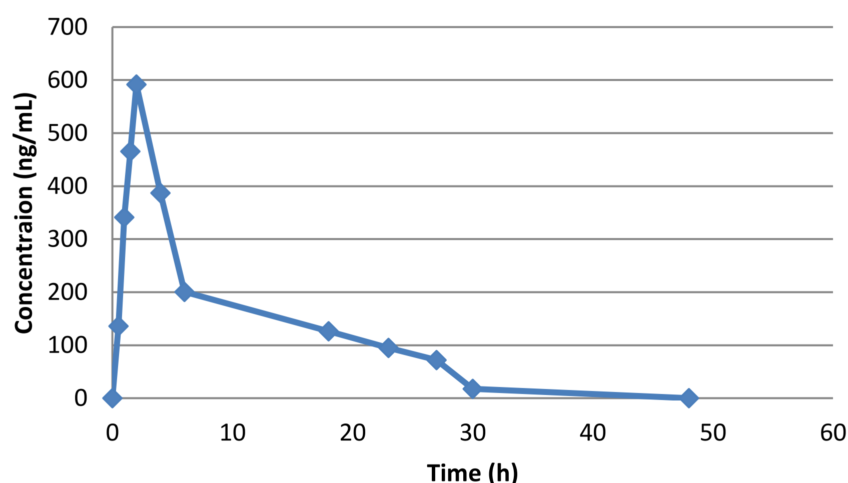

Fig 8. Plasma concentration–time profile of praziquantel up to 48 h in healthy rats following a single oral dose of 40 mg/kg body weight praziquantel

Table 7: Pharmacokinetic parameters of Praziquantel after a single oral dose of 40 mg/kg praziquantel to three groups each containing 12 albino rats

S. No.	Pharmacokinetic parameters	Observed value
1	Absorption rate constant, k_a (h^{-1})	1.059
2	Elimination rate constant, k_{el} (h^{-1})	0.27
3	Time required for maximum plasma concentration, T_{max} (h)	2
4	Maximum plasma concentration, C_{max} (ng/mL)	591.81
5	Plasma half life, $T_{1/2}$ (h)	2.56
6	Area under curve at 30 h, $AUC_{(0\rightarrow30)}$, (ng h/mL)	5128.644
7	Area under curve at infinite time, $AUC_{(0\rightarrow\infty)}$ (ng h/mL)	5192.974
8	Area under momentum curve at 30 h, $AUMC_{(0\rightarrow30)}$ (ng·h^2/mL)	46970.33
9	Volume of distribution, V_d (mL)	0.053
10	Mean residence time, MRT (h)	9.4
11	Total clearance rate, TCR (l/h)	0.0143

4. CONCLUSIONS

The previous HPLC methods described [6,8–15] are all based principally on the method developed by Xio et al. [16]. However, those methods are rather time-consuming as they involve three-step liquid–liquid extraction procedure. Water saturated ethyl acetate was used in sample extraction procedure, which did not produce 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 rat plasma samples.

Total run time was within 10 min. Inter-day as well as intra-day replicates of praziquantel, gave an R.S.D. below 9.00% (should be less than 15 according to CDER guidance for Bio-analytical Method Validation [20]). The major differences with other previously described methods is the sample extraction step, which based on protein precipitation with 8.25% of perchloric acid and U.V. detection which was set at 225 whereas previous methods are at 217 [17] which is a very near U.V. range and can produce interference with methanol absorption. This resulted in clean samples and clear chromatograms (Fig 4 & 5). 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 is basically, its rapidity, simplicity (protein precipitation sample preparation procedure), high sensitivity (LOQ, 5 ng/mL), high selectivity (no interference from endogenous peaks) and for the first time calculating all the pharmacokinetic parameters (Table 7) which was not mentioned in the previous articles [17] where only C_{max} & T_{max} were given. In addition to that sample evaporation does not require which would increase additional cost for analysis.

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