International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s,2025
https://theaspd.com/index.php

A Hplc Method Development And Validation For The Quantification Of A Potential Anthelmintic Drug Praziquantel

Dr. Kiran C. Mahajan[1*], Dr. Sunil J. Aher[2], Dr. Suvarnalata S. Mahajan[3], Archana S. More[4], Madhuri M. Desai[5], Mr. Sarang S. Mahamuni[6], Mr. Anand M. Vitthaldas[7]

[1]Department of Pharmaceutics, SGMSPM's Sharadchandra Pawar of College of Pharmacy, Dumberwadi (Otur), Tal- Junnar, Dist.- Pune, Maharashtra, 410504

[2]Department of Pharmaceutics, SERS's Sanjivani College of Pharmaceutical Education & Research, Kopargaon, Maharashtra, 423603

[3]Department of Pharmaceutics, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Jalgaon, Maharashtra, 425107

[4]Department of Pharmaceutical Chemistry, JSPM'S Rajashri Shahu College of Pharmacy & Research, Tathawade, Pune, Maharashtra, 411033

[5]Department of Pharmaceutics, Krishna Vishwa Vidyapeeth (Deemed to be University), Krishna Institute of Pharmacy, Malkapur, Karad, Maharashtra, 415539

[6]Department of Pharmaceutical Chemistry, KCT's Krishna College of Pharmacy, Karad, Maharashtra, 415539[7]

[7]Department of Pharmaceutics, Anekant Education Society's College of Pharmacy, Religious Jain Minority Institute, Baramati, Maharashtra, 413102

kirancmahajan@gmail.com*

Abstract: *A simple, rapid, sensitive high-performance liquid chromatography method was developed and validated for simultaneous measurement of praziquantel with an internal standard, Diazepam, at single wavelength of 217 nm. Chromatographic separation was performed on an enable C18 column (250 mm × 4.6 mm, 5mm and a mobile phase consisting of acetonitrile:methanol:water (50:10:40, v/v), at a flow rate of 1.0 ml/min. The calibration curve was linear ($r^2 \geq 0.999$) over the concentration range 10–1500 ng/ml. No interference was found by the excipients in the mixture.*

Keywords*: Praziquantel, Diazepam, HPLC, UV detection, Validation*

INTRODUCTION

Praziquantel is a prescription drug used as anti-worm medication. It prevents newly hatched insect larvae (worms) from growing or multiplying in your body. Praziquantel is used to treat infections caused by Schistosoma worms, which enter the body through skin that has come into contact with contaminated water. Praziquantel is a pyrazinoisoquinoline derivative with anthelminthic property. Praziquantel increases the permeability of the tegument of susceptible worms, resulting in an influx and increase in intra-tegumental calcium leading to rapid contractions and paralysis of the worm's musculature through a subsequent increase in levels of calcium in the sarcoplasmic reticulum. In addition, vacuolization of the tegumental syncytium and blebbing results in tegument disintegration, leads to antigen exposure and elicit host defense responses to the worm. The result is the formation of granulomas and phagocytosis.[1,2]

Instruments

Jasco PU-2085 Plus with quaternary gradient pump having UV/VIS detector was used for method development. The HPLC system was built with chromatopro software. HPLC analysis was performed using a Hypersil ODS C18 (average particle size 5 mm) column (250 mm, 4.6 mm).

Mobile Phase

The mobile phase consisted of Acetonitrile- Methanol-Water (50:10:40 v/v). The eluent was monitored with the UV detector at 217 nm with a flow rate of 1 mL/min and sample size of 20 µl was carried out. For filtration 0.45 mm membrane filter was used.[3]

Table 1: HPLC System Parameters

Column	C18
Mobile Phase Ratio	50:10:40 v/v
mobile phase	Acetonitrile- Methanol-Water
flow rate	1ml/min
Wavelength	217nm
Drug	Praziquantel
Sample Size	20µl
Retention Time	6.41 min

Selection of Mobile Phase

For selecting mobile extraction with different solvents were performed to find suitable mobile phase for Bioanalytical method. Extraction was performed with Methanol, acetonitrile and water.

Preparation of standard stock solutions

1. A stock solution of 1mg/ml praziquantel and diazepam were prepared in methanol.
2. The standard solution of praziquantel and diazepam were prepared by mixing and diluting it with an exact amount of an individual stock solution of methanol.
3. The final concentration of the standard solution was prepared in the concentration range of 10, 50, 100, 500, 1000, 1300 and 1500 ng/ml.
4. And the fixed concentration diazepam (Internal Standard) was taken as 500 ng/ml.
5. Different concentration of 100, 500, 1000 ng/ml were also developed for its precision and accuracy by the same method with a fixed concentration of the IS (500 ng/ml).
6. The fresh standard solution was prepared every time before analysis and validation.

Preparation of Sample solution

1. 40 mg Praziquantel was accurately weighed and transferred to 100 ml volumetric flask.
2. To this 80 mL of mobile phase was added and sonicated for 15 min.
3. The final volume was made up to 100 mL with mobile phase and the solution was filtered through the membrane filter 0.22µ.
4. This filtrate was further diluted to yield concentration of 100 µg/ml Praziquantel.
5. The total area under Curve (AUC) was calculated verses time by using linear trapezoidal rule.
6. The data was used to calculate $R^2$and linear regression equation.

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 100 ng/ml - 1500 ng/ml of Praziquantel. These were injected and peak areas used to plot calibration curves against the concentration.[4]

Method validation [5-8]

Method validation was carried out as per the guidelines given by International Conference on Harmonization and United States Pharmacopeia. The proposed method was validated by studying the fallowing parameters. The developed Bioanalytical HPLC method was validated as per the International Conference on Harmonization (ICH) guidelines for system suitability, specificity, precision, linearity and robustness.

1. Linearity

The ratio of peak areas of Praziquantel to Diazepam (Internal Standard) at various concentrations of Praziquantel. The chromatographic responses (ratio of peak areas of Praziquantel to Diazepam IS versus Praziquantel concentration) were checked for linearity over an analytical range of 100.0–1500.0 ng/ml.

2. System Suitability

The standard solutions of Praziquantel having concentrations 100 ng/ml in six replicates were injected into the HPLC system. The chromatographic parameters like Rt values and peak areas were calculated for the standard solutions and the values acquired established the suitability of the system for the analysis.

3. Specificity

The blank, standard and sample solutions of Praziquantel having concentration 100 µg /ml were injected in duplicates. The chromatograms obtained for all the solutions were compared to determine the specificity of the method.

4. Method precision

The samples solutions of Praziquantel having concentrations 100 µg /ml were injected in 6 replicates and analyzed for drug content. The % assay for both the analytes were calculated and results were expressed in terms of % Relative Standard Deviation.

5. Robustness

The effects of changes in chromatographic conditions were determined conferring to ICH guidelines to establish robustness of the Bioanalytical method. The change in flow rate, composition of mobile phase was considered. The retention time of Praziquantel was determined and % RSD for each changing chromatographic condition was calculated.

Results and Discussion:

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 100 ng/ml - 1500 ng/ml of Praziquantel. These were injected and peak areas used to plot calibration curves against the concentration. The correlation coefficient values of these seven analytes were 0.999. The results are shown in Table 2.

Table 2: Linearity concentration Vs Area under Curve

Concentration ng/ml	AUC
10	4.78
50	23.44
100	74.21
500	418.55
1000	892.34
1300	1134.23
1500	1345.67
r^2	0.999

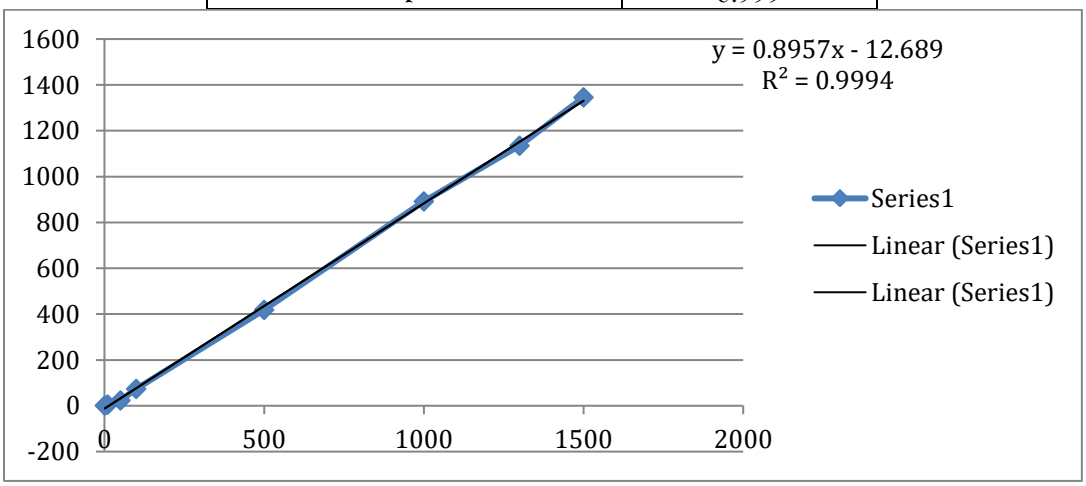

Figure 1: Linearity graph

Validation of method

The developed bioanalytical HPLC method for Praziquantel was validated as per the International Conference on Harmonization (ICH) guidelines for system suitability, specificity, precision, linearity, accuracy and robustness.

1. **Linearity range**

The ratio of peak areas of Praziquantel to Diazepam (Internal Standard) at various concentrations of Praziquantel in plasma is shown in Table 3. The chromatographic responses (ratio of peak areas of Praziquantel to Diazepam IS versus Praziquantel concentration) were found to be linear over an analytical range of 100.0–1500.0 ng/mL with regression coefficient (r^2) value 0.999, which showed reproducibility of the method. The linear regression equation obtained was y = 0.895x - 12.68.

Table 3: The ratio of peak areas of Praziquantel to Praziquantel IS at various concentrations

Concentration of Praziquantel (ng/mL)	Peak area		Peak area of the chromatogram Ratio
	Praziquantel	Praziquantel IS	
10	4.78	4.33	1.103926097
50	23.44	22.98	1.020017406
100	74.21	74.53	0.995706427
500	418.55	416.22	2.725073279
1000	892.34	895.09	1.50339072
1300	1134.23	1124.67	1.008500271
1500	1345.67	1347.81	0.998412239

2. **System Suitability**

The standard solutions of Praziquantel having concentrations 100 ng/mL in six replicates were injected into the HPLC system. The chromatographic parameters like Rt values and peak areas were calculated for the standard solutions and the values acquired established the suitability of the system for the analysis.

Table 4: System suitability Parameters

Drug	Retention Time (Rt)	Peak Area
Praziquantel	6.41	2245311

3. **Precision and accuracy**

Precision and accuracy of the method for the estimation of Praziquantel in plasma. y = 0.895x - 12.68.

Table 5: Intra-day precision (Day 1)

Concentration (ng/mL)	Intra-day precision			RSD %	Accuracy %
100	79.24	91.92	102.7039	2.13	102.7039106
500	448.91	461.59	515.743	1.0916	103.1486034
1000	891.56	904.24	1010.324	1.1166	101.0324022

Table 6: Intra-day precision (Day 2)

Concentration (ng/mL)	Intra-day precision			RSD %	Accuracy %
100	81.39	94.07	105.1061	2.13	105.1061453
500	449.04	461.72	515.8883	1.0916	103.1776536
1000	892.33	905.01	1011.184	1.1166	101.1184358

Table 7: Intra-day precision (Day 3)

Concentration (ng/mL)	Intra-day precision			RSD %	Accuracy %
100	78.99	91.67	102.4246	2.13	102.424581
500	447.21	459.89	513.8436	1.0916	102.7687151

1000	891.11	903.79	1009.821	1.1166	100.9821229

Table 8: Inter-day precision

Concentration (ng/mL)	Inter-day precision			RSD %	Accuracy %
100	78.68	91.36	102.0782	2.705	102.0782123
500	449.91	462.59	516.8603	1.1748	103.372067
1000	891.44	904.12	1010.19	1.212	101.0189944

Table 9: Intra-day Accuracy

Concentration (ng/mL)	Concentration (ng/mL)	Concentration (ng/mL)	Average	Standard Deviation	Relative standard Deviation
100.56	100.11	100.24	112	0.2315887	0.206775639
500.87	500.31	500.9	559.6648	0.3323151	0.059377525
1000.81	1000.78	1000.25	1117.598	0.3150132	0.028186637

Table 10: Inter-day Accuracy

Concentration (ng/mL)	Concentration (ng/mL)	Concentration (ng/mL)	Average	Standard Deviation	Relative standard Deviation
100.39	100.22	100.11	111.8547	0.1410674	0.126116559
500.91	500.8	500.17	558.8492	0.399291	0.071448803
1000.51	1000.09	1000.23	1117.575	0.2138535	0.01913549

4. **Specificity:** The blank, standard and sample solutions of Praziquantel having concentration 100ng/mL were injected in duplicates. The chromatograms obtained for all the solutions were compared to determine the specificity of the method.

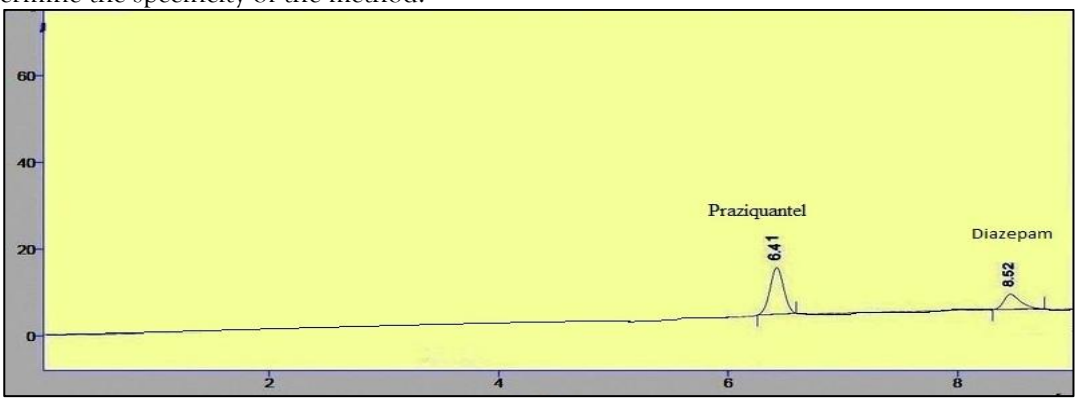

Figure 2: Chromatogram of STD Praziquantel and Diazepam IS solution

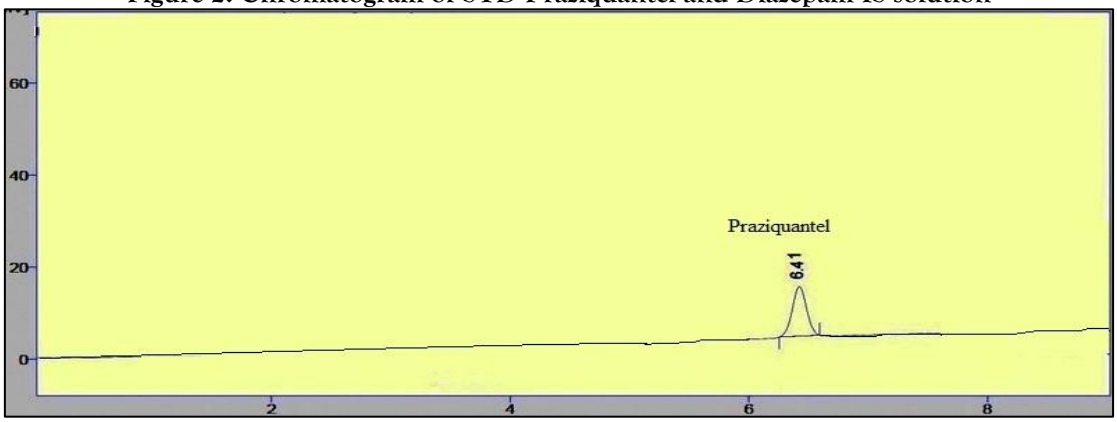

Figure 3: Chromatogram of Praziquantel

After comparing the chromatograms of the blank, standard and sample solutions were obtained and compared. The chromatograms obtained shows that there is no any co-eluting peaks at the respective retention time of Praziquantel (Rt- 6.41). Therefore, the method was found to be specific.

5. Method precision

The samples solutions of Praziquantel having concentrations 100 µg /ml were injected in 6 replicates and analyzed for drug content. The % assay for both the analytes were calculated and results were expressed in terms of % Relative Standard Deviation.

Table 11: Method Precision

Sampling Sr. Number	Praziquantel % Assay
1	91.23
2	92.01
3	91.11
4	91.06
5	92.44
6	91.05
Mean % Assay	91.48
Standard Deviation	0.78
% Relative Standard Deviation	0.65

The Average % assay for Praziquantel was determined and the %RSD values were found to be within the acceptable criteria <2.0%.

6. Robustness

The effects of changes in chromatographic conditions were determined conferring to ICH guidelines to establish robustness of the bioanalytical method. The change in flow rate, composition of mobile phase was considered. The retention time of Praziquantel was determined and % RSD for each changing chromatographic condition was calculated.

Table 12: Robustness parameters

Sr. No	Parameters	Established Parameters	Changes in the parameters
1	Flow	1.0 ml/min	1.1 ml/min
2	Mobile phase composition (acetonitrile: Methanol: Water)	50:10:40%	60:10:30%

Table 13: Robustness for Praziquantel

Robustness parameter		% RSD for Praziquantel
Flow (mL/min)	1.0	0.27
	1.1	0.28
Mobile phase composition (acetonitrile: Methanol: Water)	50:10:40%	0.22
	60:10:30%	0.39

The changes in the flow rate and mobile phase composition had no effect chromatographic analysis of as Praziquantel (Table 13). The % RSD of peak area responses for Praziquantel standard solutions analyzed in replicates were found to be less than 2.0 %. Thus, the method was found to be robust.

CONCLUSION

The proposed methods were validated as per International Conference on Harmonization guidelines for linearity, accuracy, precision and robustness for estimation of praziquantel in a mixture, and the results were found to be satisfactory. An HPLC method for praziquantel has been developed, which is simple, precise and selective for simultaneous determination of the drug. The result obtained were within the acceptance criteria for the respective parameters.

REFERENCES:

1. S.R. Shah et al. Method development and validation for simulataneous estimation of albendazole and praziquantel in bulk and in a synthetic mixture, Journal of Taibah University for Science,2014, 8 :54–63
2. Bhoomi Dineshkumar Patel, Anamika Mishra, Ankit Chaudhary. RP - HPLC Method Development and Validation for Simultaneous Estimation of Pyrantel Pamoate, Praziquantel, Febantel in Tablet. Research Journal of Pharmacy and Technology. 2022, 15(8):3535-9.
3. Rubiana M. Mainardes, Priscila O. Cinto & Maria P.D. Gremião, High-performance Liquid Chromatography Determination of Praziquantel in Tablets and Raw Materials, Acta Farm. Bonaerense, 2006, 25 (4), 67-70.
4. Naga Venkata Suresh Kumar Devaka, Vallabhaneni Madhusudhan Rao, Chromatographic Quantification of Ivermectin and Pranziquantel in the Tablets Using Stability Indicating RP-HPLC Method, Pharmaceutical Sciences, 2019, 25(3), 254-261
5. Kumudini SR, Sunil RD, Vidhya KB, Amruta LS. Validated HPTLC method for simultaneous estimation of ivermectin and albendazole in formulation. Asian J Pharm Biol Res. 2011;1(3), 330- 6.
6. Mayur Atmaram Mahajan, Vaishali D. Rakibe, Sandip J. Sonawane, Nilesh K. Pathade, Amit B. Chaudhari, Development and validation of an RP-HPLC method for the simultaneous estimation of praziquantel and ivermectin in bulk and combined tablet dosage form. International Journal Of Food And Nutritional Sciences, 2024, 13(4),253-262.
7. Hu, C., Wang, D., Chen, K., Liu, X., Chen, Q., & Sun, D. Development and Validation of Process for Resolution of Praziquantel Amine for Preparation of Chiral Praziquantel. Asian Journal of Chemistry, 2014, 26(23), 8158–8162.
8. Gültekin Y, Öztürk N, Filazi A, Deniz A, Korkmaz Ç, Pezik E, Barın G, Vural İ. Simultaneous determination of albendazole sulfoxide and praziquantel from PLGA nanoparticles and validation of new HPLC method. J Res Pharm. 2022; 26(6), 1608-1618.