

High-performance Liquid Chromatography Determination of Praziquantel in Tablets and Raw Materials

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SUMMARY. A specific and sensitive high-performance liquid chromatographic method was developed for the assay of praziquantel in raw materials and tablets. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay in the wavelength selected. The method validation yielded good results and included the range, linearity, precision, accuracy, specificity and recovery.

RESUMEN. "Cromatografía Líquida de Alta Eficiencia para Determinación de Praziquantel en Tabletas y Materias Primas". Un método específico y sensible de cromatografía líquida de alta eficiencia fue desarrollado para el análisis del praziquantel en materias primas y tabletas. También fue encontrado que los excipientes en la preparación comercial de la tableta no interfirieron en el análisis en la longitud de onda seleccionada. La validación del método dio buenos resultados e incluyó la linealidad, la precisión, la exactitud, la especificidad y la recuperación.

INTRODUCTION

Praziquantel (Fig. 1) is the drug of choice in the treatment of schistosomiasis. It is estimated that about 200 million people in the world are currently affected by this tropical disease. Praziquantel has a wide range of activity against trematodes and cestodes, and has been shown to be highly effective against all known species of schistosomes infecting humans ¹⁻⁶.

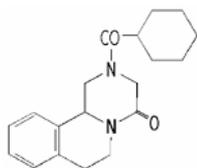


Figure 1.
Chemical structure of praziquantel.

Chemically, the praziquantel (PRZ) is the 2-(ciclohexilcarbonil)-1,2,3,6,7,11b-hexaidro-4H-pirazino [2,1-a] isoquinolin-4-one ⁷. It is well absorbed following oral administration. Approximately 80% of an oral dose of this drug is absorbed from the gastrointestinal tract, however, because of extensive first-pass metabolism, only

a small portion reaches systemic circulation as uncharged praziquantel. Peak serum concentration of PRZ occur approximately 1-3 h after oral administration of usual doses of the drug ⁸.

Several procedures have been developed for the quantitative determination of praziquantel in tablets. These procedures include high performance liquid chromatography, gas chromatography, spectrophotometry and colorimetry ⁸.

The United States Pharmacopeia (USP XXVI) ⁹ describes a HPLC method for determination of praziquantel in tablets using as mobile phase a mixture of acetonitrile and water in the proportion of 60:40 and the UV detector set at 210 nm.

In the development of new dosage forms for drugs, special care must be taken in the selection of the adequate wavelength because other substances present in the formulation used as adjuvants can be absorbed in the same wavelength of the drug, interfering this in the results of the analysis.

In the case of praziquantel, the wavelength of 210 nm shows a high absorptivity, but in some

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cases it is not selective for its quantification because it depends on the excipients being used in the formulation. Some polymers, mainly polyesters, are absorbed in a wavelength of 210 nm, and as these substances are very much used in solid dosage forms, other wavelength must be selected.

The purpose of this study was to validate a new HPLC method for determination of PRZ in tablets, which allow a better selectivity in the presence of excipients, using a UV detector set a 262 nm.

MATERIAL AND METHODS

Samples

The praziquantel tablets were obtained commercially. The tablets were claimed to contain 225 mg of drug, cellulose, corn starch, polyvinylpyrrolidone, sodium laurylsulphate and magnesium stearate as excipients.

Reference substance

Praziquantel reference substance used in this study was purchased in Reiza-Kern, São Paulo, Brazil.

Reagents

All other chemicals used were of analytical grade. The water used was filtered through a Milli-Q apparatus (Milipore).

Instrumentation and conditions

Quantitative HPLC was performed on a Vari-an Pro Star Chromatograph equipped with a Solvent Delivery Module 210; an Ultraviolet-Visible Spectrophotometric Detector 330 (set at 262 nm) and a Rheodine® injection valve with a 100 μ L loop.

Chromatographic separation was accomplished using a LiChrospher® 100 RP-18 (Merck) stainless steel column (25 cm x 4 mm id., 5 μ m particle size). The flow rate was isocratic at 1 mL/min. The mobile phase was prepared by mixing acetonitrile with water 60:40 (% v/v). The UV detector was set at 262 nm. The HPLC system was operated at (30 \pm 1 °C). The mobile phase was filtered through a membrane filter (0.45 μ m X 47 mm) and degassed using an ultrasonic bath for 45 min. The analysis required less than 9 min.

Preparation of standard solutions

Stock solution of praziquantel standard (500 μ g/mL) in acetonitrile:water (60:40) was freshly prepared accurately weighting 50 mg praziquantel reference substance, transferring to a 100 mL volumetric flask followed by addition of mobile

phase to make up the volume. The working solutions were prepared by diluting the stock solution in the mobile phase. The linear regression equation was calculated by plotting the area under the curve and the concentration of the drug in μ g/mL. All determinations were conducted in triplicate.

Simulated sample containing 150 mg of praziquantel, 25.68 mg of cellulose, 25.68 mg of corn starch, 1.125 mg of sodium laurylsulphate, and 22.5 mg of polyvinylpyrrolidone was prepared and analysed using the same HPLC procedure. The samples were homogenised (using a mortar and pestle) and then the corresponding weighted amount of one tablet was submitted to the analytical procedure.

Placebo containing 25.68 mg of cellulose, 25.68 mg of corn starch, 1.125 mg of sodium laurylsulphate, and 22.5 mg of polyvinylpyrrolidone was prepared and analysed by HPLC procedure in the wavelength of 210 and 262 nm in order to verify the possible interference of excipients. The samples were homogenised (using a mortar and pestle) and the corresponding weighted amount of one tablet was submitted to the analytical procedure previous described.

Assay of praziquantel in tablets

The content of ten tablets, each one containing 150 mg of praziquantel, was pulverised using a mortar and pestle. A weighted amount tablet, containing 150 mg was transferred to a 100 ml volumetric flask and the volume was completed with mobile phase. The solution was filtered through Whatman paper and after that through a filter membrane (0.45 μ m). The first 5-mL were discarded. An aliquot of 0.4 ml of the filtrate was adequately diluted with mobile phase in order to yield a solution containing 12 μ g of praziquantel/mL.

Recovery of praziquantel in tablets

The recoveries were determined by adding known amounts of the praziquantel reference substance (6.0, 9.0 and 12.0 μ g/mL) to the samples at the beginning of the process (Table 1). A recovery procedure was then performed.

Reproducibility and validation

The accuracy and precision of the assay, as well as the linearity of the calibration curve were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation of each curve.

Sample concentration (µg/mL)	Amount of standard added (µg/mL)	Amount of standard recovered (µg/mL)	Recovery ^a (%)
10.3	6.0	16.20	98.19
10.3	9.0	20.46	109.72
10.3	12.0	22.48	101.44

Table 1. Experimental values obtained in the recovery test for praziquantel tablets.

^a Mean of three determinations.

RESULTS AND DISCUSSION

Linearity of the standard calibration curve

In this study, was investigated the linearity of method and selected the ideal concentration range to work. The interval showed the desired linearity.

The analytical curve for praziquantel in a concentration range from 1.0 to 14.0 µg/mL was linear presenting correlation coefficient (r) of 0.999. The linear regression equation was found to be

$$y = 38.399 x + 7.8284$$

where y is the area under the curve and x, the concentration of standard in µg/mL.

Specificity

In an attempt to detect interference, simulated and placebo samples were prepared and analysed. Excipients used in these preparations were the most commonly used by the pharmaceutical industry. The chromatogram of simulated sample (Fig. 2) showed the similar profile of chromatogram of praziquantel in tablets (Fig. 3).

The presence of cellulose, corn starch, polyvinylpyrrolidone, sodium laurylsulphate did not interfere in the results of the analysis on the wavelength of 262 nm (Fig. 4). Nevertheless in the wavelength of 210 nm, it can be observed a peak corresponding to one of excipients in the same retention time of praziquantel (Fig. 5).

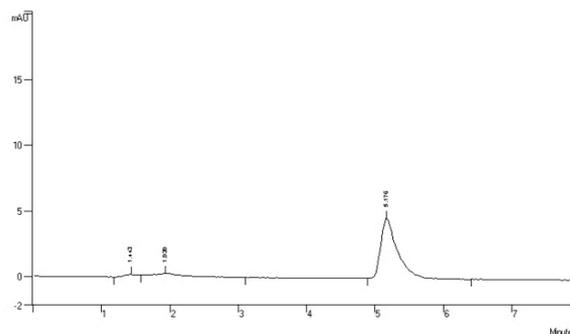


Figure 3. Chromatogram of praziquantel tablets. Conditions: Flow rate: 1 mL/min, mobile phase: acetonitrile: water (60:40), wavelength: 262 nm.

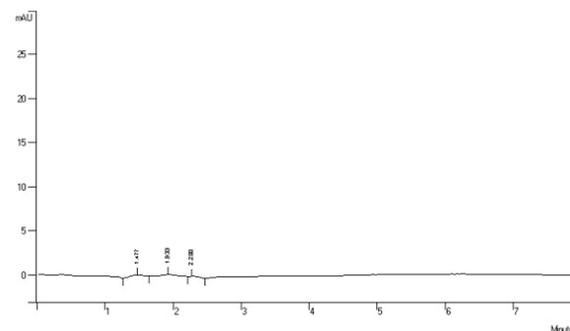


Figure 4. Chromatogram of placebo samples. Conditions: Flow rate: 1 mL/min, mobile phase: acetonitrile: water (60:40), wavelength: 262 nm.

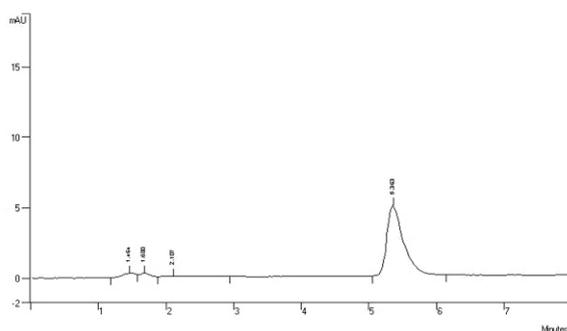


Figure 2. Chromatogram of simulated samples. Conditions: Flow rate: 1 mL/min, mobile phase: acetonitrile: water (60:40), wavelength: 262 nm.

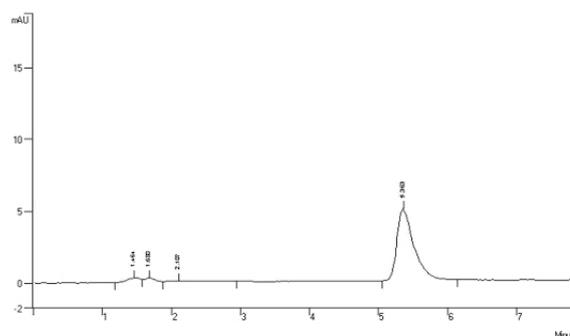


Figure 5. Chromatogram of placebo samples. Conditions: Flow rate: 1 mL/min, mobile phase: acetonitrile: water (60:40), wavelength: 210 nm.

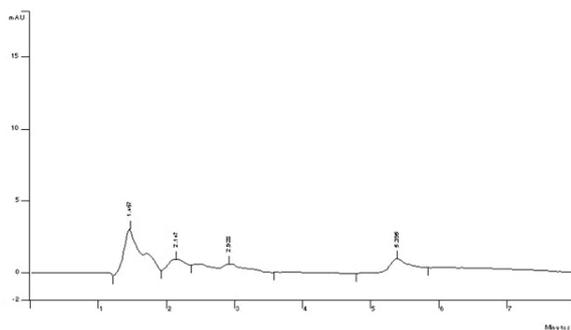


Figure 6. Chromatogram of pure praziquantel. Conditions: Flow rate: 1 mL/min, mobile phase: acetonitrile: water (60:40), wavelength: 262 nm.

Precision

The intra-day assay coefficients of variation (CV) for praziquantel tablets at 12 µg/mL (n=10) in the first day was 1.86%. In the second day was 1.28% and in the third day was 2.16%. The inter-day assay was assessed on 3 individual days, the CV was 1.77% at 12 µg/mL. These data show a remarkable degree of precision and reproducibility of the method, both during within-runs and between-runs.

The retention time repeatability during the precision studies was found to be good to all solutions. The retention times (t_R) of the praziquantel reference substance and the tablets were 5.340 and 5.176 min, respectively. The chromatogram of praziquantel reference standard and tablets shows a peak with the same t_R (Figs. 3 and 6).

Accuracy

The mean absolute recovery values of HPLC method throughout the linear range are presented in Table 1. The results reflected that the method is obviously accurate and ensuring this reliable results.

Limit of detection and limit of quantification

The limit of detection of praziquantel was approximately 53.37 ng/mL. The limit of quantification was set at 161.75 ng/mL being the lowest concentration used in the construction of the standard curve. The assay was considerably simple and sensitive for praziquantel analysis.

CONCLUSIONS

The HPLC method developed in this study proved to be simple, precise and effective to determine praziquantel in tablets. The presence of excipients did not interfere in the results of the analysis when it was performed at 262 nm.

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