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Development and Validation of RP- HPLC Method for Determination of Primaquine in Extended Release Tablets

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SUMMARY. A RP-HPLC method was developed and validated to quantify primaquine diphosphate in extended release tablets. The validation parameters yielded good results and included the range, linearity, precision, accuracy, specificity, detection and quantification limits. Isocratic chromatography was performed on a C_{18} column with a mobile phase composed by acetonitrile, methanol, 1 M perchloric acid and water, at flow rate of 1.0 mL/min using UV detection at 254 nm. The proposed technique demonstrated to be appropriate for routine analysis and quality control assays of PQ in extended release tablets.

RESUMEN. "Desarrollo y Validación de Método Analítico Cromatográfico para la Determinación De Primaquina En Tabletas de Liberación Prolongada". Un método de cromatografía líquida de alta eficiencia (HPLC) fue desarrollado y validado para la determinación de difosfato de primaquina (PQ) en tabletas de liberación prolongada. Los parámetros de la validación dieron buenos resultados e incluyen: rango, linealidad, precisión, exactitud, especificidad y límites de detección y cuantificación. La separación en HPLC fue llevada a cabo con una columna C_{18} y una fase móvil compuesta de acetonitrilo, metanol, ácido perclórico 1M y agua, bombeados isocráticamente a un flujo de 1,0 ml/min, detección a 254 nm. La técnica propuesta demuestra ser apropiada para el análisis de rutina y ensayos de control de calidad de PQ en tabletas de liberación prolongada.

INTRODUCTION

Primaquine diphosphate (PQ), chemically know as 1,4-pentanediamine,N4-(6-methoxy- 8-quinolinyl) diphosphate, is a clinical drug for treatment of malaria to produce radical cure and prevent relapse of vivax and ovale malarias [1]. Despite its activity, primaquine shows side-effect such as gastrointestinal disturbs [2,3]. In addition, this drug has short plasma elimination halftime about 5-6 h and suffers first-pass metabolism [4,5]. As result of its pharmacokinetic profile and toxicological behaviour, primaquine seems to be a good candidate for extended release tablets. For the proper design and formulation of this new pharmaceutical form it is required appropriate measures of quality control such as detection and quantification of the drug in pharmaceutical formulation.

A survey of literature has revealed several analytical methods for the determination of primaquine in pharmaceutical preparation and biological fluids including conductometric [6], spectrophotometric [7], colorimetric [8], polarographic titration [9] and chromatographic methods [10-12]. In fact, a HPLC assay for determination of primaquine in extended release tablets have not been reported in the scientific literature.

Most of pharmacopoeias use titration methods for the determination of primaquine [13,14]. This methodology is impractical for routine of pharmaceutical samples due to exhausted time consuming. On the order hand, the high performance liquid chromatography method could be easily adapted for routine and quality control analysis due to their sensitivity, repeatability and specificity, for the determination of active con-

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tent in pharmaceutical formulations with sufficient reliability [15]. The mentioned liquid chromatographic method has not been described in official pharmacopoeia.

Since our research involves the development and evaluation of primaquine extended release tablets, the major purpose of this work was developed and validated a simple, accurate and stability indicating HPLC method.

MATERIALS AND METHODS

Chemicals and Reagents

Primaquine reference standard was purchased from the U.S. Pharmacopoeia-Rockville. The sample of primaquine phosphate (PQ) was donated by Institute of Technology in Drugs - Fundação Oswaldo Cruz / Far-Manguinhos - Brazil (description number 32482; 98,5% purity). Ultrapure water was obtained from Milli-Q® Plus apparatus (Millipore®) and distilled water from Baumer S/A water purification unit. Methanol and acetonitrile HPLC grade were purchased from Vetec (Brazil) and perchloric acid was analytical reagent grade. Polyethylene oxide (PEO) of different molecular weight (4.10^6 Da and 8.10^6 Da) from Sigma-Aldrich was used as matrix hydrophilic material. The others excipients were microcrystalline cellulose (MC; Avicel® Ph-102), colloidal silicon dioxide (Aerosil®, Galena), talc and sodium stearyl fumarate.

Instrumentation and analytical conditions

The HPLC analysis was performed on a Shimadzu LC-10A chromatographic system equipped with an LC-10AD pump, UV-Vis detector, and a SCL-10AVP system controller. The data were acquired and processed using CLASS-VP 6.14 software program. The column used was an RP-C18A Merck (150 x 4,6 mm particle size of 5 µm). The mobile phase composition was acetonitrile, methanol, 1 M perchloric acid and water (33:6:1:87). The flow rate was of 1.0 mL/min and the injection volume was 20 µL for all standards and samples. The detection was investigated at 254 nm.

Preparation of standard solutions

A 200 μgmL^{-1} stock solution of primaquine phosphate was obtained by dissolving 20 mg of reference standard in water to a 100 mL volumetric flask. Appropriate amounts (5-15 mL) of the stock solution were transferred to a set of 100 mL volumetric flasks and diluted with water, yielding concentrations of 10, 16, 20, 24 and 30 μgmL^{-1}.

Preparation of extended release tablets

Two formulations (F60P4 and F60P8) were prepared by direct compression of the physical mixtures of the drug, hydrophilic polymer and excipients. Each tablet contains 30% of primaquine phosphate and 60% polyethylene oxide (POE 4.10^6 for F60P4 and POE 8.10^6 for F60P8) maintaining a total mass of 175 mg with different loadings of others excipients.

Preparation of assay sample

Ten tablets (F60P4 or F60P8) were weighed, finely powdered and portions equivalent to 20 mg of primaquine phosphate was transferred to 100 mL volumetric flask and dissolved in ultrapure water, obtaining a final concentration of 200 μgmL^{-1}. An aliquot of this solution was diluted in ultrapure water at concentration of 20 μgmL^{-1}.

Method Validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [16]. The parameters used were: linearity, range, accuracy, specificity, precision and robustness.

Linearity and range

The linearity an interval were determinate using a calibration curve obtained from standard solution of primaquine phosphate. Standard solutions of different concentration of 10, 16, 20, 24 and 30 μgmL^{-1} were used. The solutions were prepared in triplicate. Calibration curve were constructed by plotting the concentration of PQ versus corresponding mean peak area. The linearity was expressed as a correlation coefficient by linear regression analysis.

Accuracy

The accuracy of the method was evaluated by a recovery test. PQ samples were fortified with known concentrations of reference standard at 3 different levels. Aliquots of 3 mL of sample (200 μgmL^{-1}) were transferred into 50 mL volumetric flasks containing 1, 2 and 3 mL of PQ standard solutions. The volume was brought to 100 mL with ultrapure water, obtaining final concentrations of 16, 20 and 24 μgmL^{-1}. The percent recovery was determined in triplicate analysis and calculated by using the formula proposed by the AOAC [17].

Precision

The precision of the method was investigated by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying six samples with equal concentration of 20 μgmL^{-1}, during the same day. The

intermediate precision was evaluated comparing the repeatability assay in 3 different days. The results were expressed as relative standard deviation (R.S.D.) of the measurements.

Specificity/selectivity

The specificity was evaluated by analyzing solutions containing the excipients employed for the preparation of primaquine extended release tablets. The ability to measure specifically the analyte was examined for the absence of interference or overlaps with the PQ responses. Moreover, the specificity was determined by photolytic degradation. Standard solution (20 μgmL^{-1}) was exposed to UV light for 1 week. The ability to separate the analyte from degradation products these solutions was evaluated by performing the experiment and looking for the change in the chromatographic pattern compared with freshly prepared solutions.

Detection (DL) and quantitation limits (QL)

The *DL* and *QL* for HPLC method were calculated on the basis of response and slope of the regression equation. The *DL* may be expressed as:

$$DL = \frac{3.3\ \sigma}{S}$$

and *QL* as:

$$QL = \frac{10\ \sigma}{S}$$

where σ is the standard deviation of the response and *S* is the slope of calibration curve.

RESULTS AND DISCUSSION

The proposed HPLC method is simple, fast, inexpensive and effective to quantify PQ in extended release tablets.

The chromatography conditions were chosen as a function of primaquine's physical chemical parameters as well on existing literature. The mobile phase and detector used, was based on Dua *et al.* [18]. These authors's purposed a methodology for determination of oxidation products of primaquine, whose method was modified and adapted to quantify primaquine in extended release tablets. To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity in a short separation time. The best peak symmetry was achieved with a flow rate of 1.0 mL min^{-1} at 40 °C column temperature. The 1M perchloride acid ensures the primaquine stability, increasing its solubility due to formation of ionized groups. A unique symmetrical peak was observed in a typical chromatograms obtained from analysis of PQ standard and sample (Fig. 1B and 1C). The retention time for PQ was about 4.0 min, which allows a rapid determination for routine sample analysis.

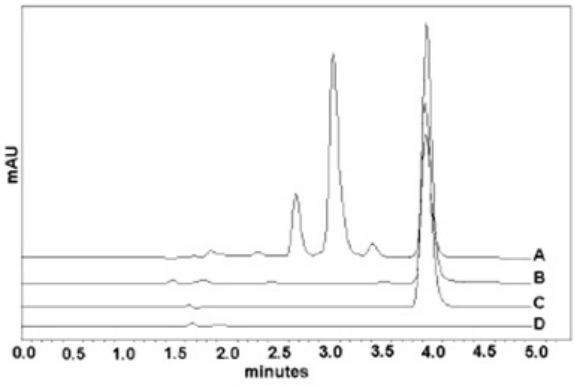

Figure 1. Typical chromatograms obtained under de experimental conditions: (**A**) sample solution (20 μgmL^{-1}) submitted to photolytic degradation; (**B**) standard solution (20 μgmL^{-1}); (**C**) sample solution of 20 μgmL^{-1}; (**D**) placebo solution.

	HPLC method
Correlation coefficient (R^2)	0.9989
Regression equation	slope: 34227 ± 1.46 intercept: 50552 ± 0.83
Detection limit (DL)	1.36 μgmL^{-1}
Quantification limit (QL)	4.13 μgmL^{-1}
Precision Intra-day (n = 6)*	19.86 ± 0.04; 0.18
Precision Inter-day (n = 3)*	20.23 ± 0.42; 2.05

Table 1. Calculated linear regression parameters with 95% confidence limits, detection limits, quantification limits and precision data evaluated by intra-day and inter-day studies.* Mean concentration ± S.D. ($\mu g\ mL^{-1}$); R.S. D. (%).

The specificity of the method was evaluated by analyzing a sample solution without PQ (placebo), and the chromatograms showed that there is no interference or overlaps of the excipients with primaquine response at 254 nm detection wavelengths (Fig. 1D). Additionally, specificity was confirmed through the photolytic studies, and the degradation products showed a lower detector response compared to primaquine active substance, indicating that the analytical method was capable to differentiate and separate its degradation products (Fig. 1A).

Appropriate linearity data was observed in the 10.0-30.0 (μgmL^{-1} range (Table 1). The regression lines had a correlation coefficient value

Method	Formulation	Amount (μgmL^{-1})		Recovery (%)*
		Added	Found	
HPLC	F60P4	4.0	3.88	96.90 ± 1.33
		8.0	7.95	99.41 ± 1.15
		12.0	12.00	100.85 ± 1.70
	F60P8	4.0	3.92	97.90 ± 1.67
		8.0	7.66	95.75 ± 0.9
		12.0	12.10	100.85 ± 1.43

Table 2. Experimental values obtained in the recovery test for primaquine in tablets by HPLC method.

(R2) > 0.99 and y-intercepts were not significantly different from zero at 95% confidence level. The calculated detection and quantification limits indicate a high sensitivity of the method. The precision intra- and inter-day run are showed in Table 1. The relative standard deviation (R.S.D) values were < 2.1%, confirming the precision of the method.

The accuracy of the method was determined by a recovery test. It was assessed from three replicate determinations of three different fortified solutions. No significant differences were observed between amounts of PQ added and the amounts found. The obtained values were within the range of 95.5 - 101.0% (Table 2), satisfying the acceptance criteria for the study.

At last, the robustness was also investigated. Slightly variations on the method conditions had no significant effect on assayed data or on chromatographic performance, indicating the robustness of method and its suitability for other laboratories.

CONCLUSIONS

A simple, precise, accuracy and convenience HPLC method was developed and validated for quantitative determination of PQ in extended release tablets. The results demonstrated that technique have advantages of easy sample preparation and shorter time of analysis. The proposed method proved to be suitable for routine of quality control of primaquine extended release tablets, such as assay, uniformity of content and stability studies.

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