

Research Article

Spectrophotometric Method for Determination of Primaquine in Pharmaceutical Formulations via Derivatization with 1,2-Naphthoquinone-4-Sulfonate

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Abstract

A rapid, simple and sensitive method for the determination of primaquine (PQ) using sodium 1,2-naphthoquinone-4-sulfonate (NQS) has been developed. The method is based on the formation of a brown color adduct from the reaction between PQ and NQS. The nucleophilic substitution reaction proceeds quantitatively at pH 10 buffer solution with absorption maximum at 485 nm. The calibration curve is linear over the range 10-60 µg/mL and describes by the regression equation $A = 0.005X + 0.055C$ with a regression coefficient 0.9998. The limit of detection and quantity are 3.2 µg/mL and 9.9 µg/mL respectively. The method is simple and can be applied for determination of PQ in pharmaceutical formulation in quality control laboratories.

Keywords: Spectrophotometric; Primaquine; Pharmaceutical formulation; Sodium 1,2-naphthoquinone-4-sulfonate (NQS)

Introduction

The drug primaquine (PQ) chemically known as diphosphate 8-[(4-amino-1-methylbutyl) amino]-6-methoxyquinoline is still the drug of choice for the eradication of the exoerythrocytic liver forms of *Plasmodium vivax* and *Plasmodium ovale* for the prevention of relapse of malaria [1]. The clinical use of PQ as a causal prophylactic and the therapeutic agent is, however, curtailed by its toxic side effect, especially for patients with glucose-6-phosphate dehydrogenase deficiency [2].

Several methods for the determination of PQ have been reported. This includes a reversed-phase HPLC method for the separation and identification of the oxidation products of PQ [3] as well as determination of PQ and its metabolite (carboxyprimaquine) in plasma and blood cell [4] and in calf plasma by LC-EC detection [5]. Other methods such as chemical sensors [6], Capillary electrophoresis [7-9] Spectrophotometric [10-14] and spectrofluorimetry [15] have also been proposed. The British Pharmacopoeia method [16] involves the dissolution of the sample in anhydrous acetic acid with gentle heating. The cooled, sample is titrated against perchloric acid, and the end point is determined potentiometrically.

NQS has been used as a color-developing reagent in spectrophotometric determination of pharmaceutical amines [17-28]. The applications of NQS for determination of NQS of pharmaceutical bearing amine group have recently been reviewed by Elbashir et al., [26]. The reaction between PQ and NQS has not investigated yet, therefore, the present study was devoted to investigate the reaction between NQS and PQ, and use this color reaction in the development of simple, rapid spectrophotometric method for determination of PQ in pharmaceutical formulation.

Experimental

Apparatus

Absorbance was carried out by using a spectrophotometer model

shimadzu 1800. With quartz cells of 1cm optical path length. pH meter was used for pH measurements.

Material and Reagent

All chemicals used were of analytical reagent grade. 1,2-naphthoquinone-4-sulfonate (NQS) and primaquine diphosphate standard (PQ) were obtained from (Aldrich Chemical Co., St. Louis, USA). Commercial primaquine diphosphate pharmaceutical preparations in the form of tablets, (claimed to contain 7.5 mg active ingredient) were purchased from a local drug-store. Doubly distilled water was used to prepare all solutions.

Preparation of standard and sample solution

Stock standard solution of PQ (200 µg/mL)

An accurately 0.02 g of PQ standard was dissolved in distilled water and transferred in 100 mL volumetric flask diluted to mark. The solution was further diluted to obtain working solution.

Sample Solution

Four tablets (PQ 15 mg / tablet) were weighted and finely powdered. A portion of the powder equivalent to 0.09 g of the drug was dissolved in distilled water and transferred into 50 mL volumetric flask the solution was completed to mark, shaken well filtered and then analyzed by following procedure.

NQS (0.4%, w/v)

Was prepared by dissolving 0.4 g in 100 mL volumetric flask and complete the volume with distilled water. The solution was freshly prepared.

Procedure

A 2.0 mL of 200 µg/mL PQ was transferred in 10 mL volumetric flask, 2.0 mL of NQS was added and followed by 1.5 mL of buffer

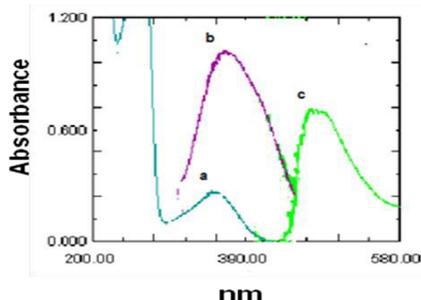


Figure 1: Absorption spectra of (a) PQ (40 mg/ml) against water blank (b) NQS (0.2% (w/v) against water blank (c) the reaction product of PQ with NQS against reagent blank of NQ. λ_{\max} of PQ (351). λ_{\max} of NQS (361) λ_{\max} of complex (485).

solution pH 10, the flask was completed to volume with distilled water and the absorbance was measured at 485 nm against blank.

Job's method

The Job's method of continuous variation [29] was employed master equimolar (1×10^{-3} M). Aqueous solution of PQ and NQS were prepared. Series of master solution of PQ and NQS were made up comprising different complementary proportions (1:9,...:9:1, inclusive) in 10 mL volumetric flask containing 1.5 mL of buffer solution pH=10.

Results and Discussion

Absorption spectra

The absorbance spectrum of PQ were carried out against water it was found that PQ exhibits maximum absorption peak λ_{\max} at 360 nm (Figure 1a). The reaction between PQ and NQS was performed and the absorption spectrum of product was recorded against blank (Figure 1c) it was found that the product is brown colored exhibiting λ_{\max} at 485 nm and the λ_{\max} of NQS at 362 nm (Figure 1b).

Optimization of the reaction conditions

The optimum conditions for the development of method were established by varying the parameters one at a time while keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as, pH, concentration of NQS, time and buffer volume were investigated.

Effect of pH

The effect of pH on the reaction between PQ and NQS was

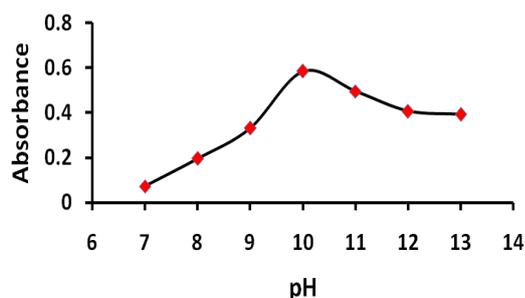


Figure 2: Effect of pH on the reaction of PQ with NQS 2 mL of PQ (40 μ g/ mL) 2mL buffer solution (pH 10) 2.0 mL NQS (0.2 w/v %) temperature: 25 $^{\circ}$ C ; reaction time:15 min.

examined by varying pH from 7 to 13 as shown in Figure 2. The absorbance increased rapidly up to 10 and then decreased. The absorbance reached maximum at pH 10 this often due to degree of nucleophilic substitution is also maximum at pH 10 [25, 26]. However, when pH is higher than 10 the absorbance of the system of PQ–NQS decreases. Presumably it is due to increase of the amount of hydroxide ion. Hydroxide ion has good nucleophilic ability and can hold back the nucleophilic substitution reaction between PQ and NQS, resulting in the descent of the absorbance of each system. Therefore, pH 10 was selected for the optimal experimental conditions.

Effect of standing time

The absorbance of product was determined at different time period at 25 $^{\circ}$ C keeping other conditions unchanged. The results show that the absorbance increased rapidly and reached maximum at 15 min. then decreased until become constant at 25 min. therefore, 15 min. is considered optimum Figure 3.

Effect of NQS concentration

The effect of NQS concentration was studied in the range (0.05-0.6 w/v%) as shown in Figure 4. When the concentration increased more products is formed up to 0.4 after that absorbance decreased therefore, 0.4 w/v% concentration is considered optimum.

Effect of amount of buffer

The effect of amount of buffer on the reaction was studied while keeping other conditions unchanged. The absorbance enhanced with rise of amount of solution and become maximum when the amount

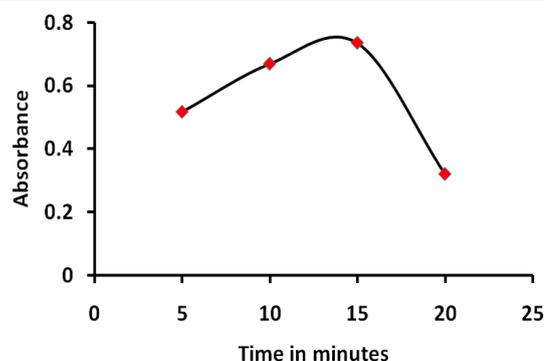


Figure 3: Effect of standing time on the reaction of PQ with NQS 1.5 mL of PQ (40 μ g/ mL) 2.0 mL buffer solution pH 10, 2.0 mL NQS (0.2 w/v %) temperature 25 $^{\circ}$ C.

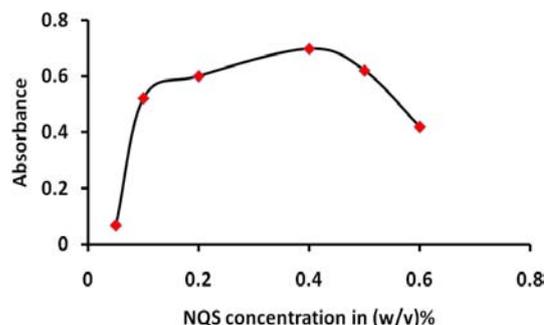


Figure 4: Effect of NQS concentrations on the reaction of PQ with NQS. 2 mL of PQ (40 μ g/mL): 1.5 mL; buffer solution (pH (10): temperature: 25 $^{\circ}$ C; reaction time:15 min.

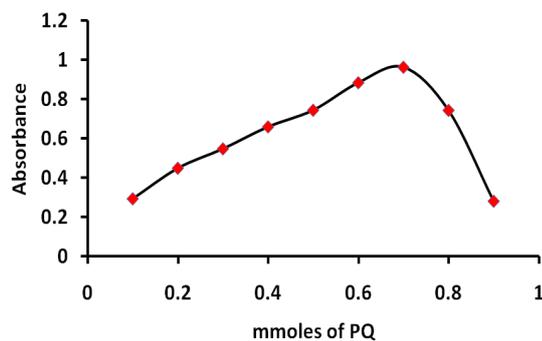
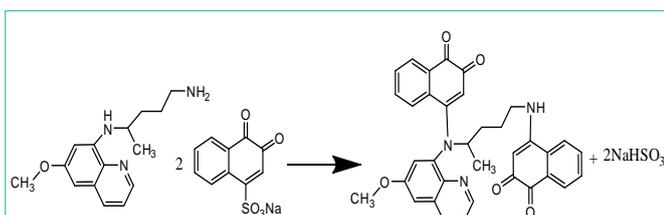


Figure 5: The continuous variation plot for the stoichiometry of the reaction of PQ with NQS.



Scheme 1: Reaction pathway of PQ with NQS.

of buffer 1.5 mL, therefore, 1.5 mL is considered optimum (data not shown).

From the above experiments, the optimized conditions used for the assay were: pH 10.0, NQS concentration 0.4% w/v, volume of the buffer 1.5, reaction time 15 min and temperature 25°C.

Furthermore, the molar ratio of NQS to PQ in the reaction mixture was studied according to Job's method of continuous variation [29]. A 7.50×10^{-4} mol/L standard solution of PQ and solution of NQS were used. The unsymmetrical bell shape of Job's plot confirmed that one molecule of PQ reacts with two molecules of NQS, NQS: PQ 2:1 Figure 5. Based on the observation molar ratio, the reaction pathway was postulated to proceed as shown in Scheme 1.

Validation of Method

Calibration curve

Calibration curve for the determination of PQ by its reaction with NQS was constructed by plotting absorbance as a function of the corresponding concentration. The regression equation for the result was $A = 0.005X + 0.055C$. Where A is absorbance at 485 nm, x concentration of PQ $\mu\text{g/mL}$ (10-60) and R is correlation coefficient. The limit of detection (LOD) and the limit of quantification (LOQ) were determined according to the following formula

$\text{LOD} = 3.3 \text{ SDa} / b$, $\text{LOQ} = 10 \text{ SDa} / b$, SDa is stander deviation of intercept; b is slope [30]. The obtained results are summarized on Table 1.

Recovery of PQ

The standard addition method was applied to check the accuracy of the proposed method is validated by applying stander addition technique in which variable amounts of drug were added to the previously analyzed PQ solution. The result of analysis of pharmaceutical dosage form and recovery study suggests that there is no interference from any excipients that present in the tablet, Table 2.

Table 1: Parameters for the performance of the proposed method.

Parameter	Value
Measurement wavelength nm	485
Linear range ($\mu\text{g/mL}$)	10-60
Intercept	0.0554
Stander deviation of intercept	0.005472
Slope	0.00549
Stander deviation of slope	0.000129
Correlation coefficient(r)	0.9998
Limit of detection($\mu\text{g/mL}$)	3.28
Limit of quantification ($\mu\text{g/mL}$)	9.96

Table 2: Determination of PQ in pharmaceutical preparation, applying standard addition method.

Taken μg (drug) primaquine diphosphate (PQ)	Added μg standard	Found μg	Recovery % \pm RSD ^a
15	10	24.12	96.48 \pm 0.54
"	20	36.6	104.6 \pm 0.54
"	50	62.4	96 \pm 0.77

^aValues are mean of three determination

Table 3: The robustness of the proposed method.

Parameter	Recovery % \pm RSD ^a
Recommended conditions	96.4 \pm 40
NQS concentration (w/v%) 0.38	96.3 \pm 0.82
NQS concentration (w/v%) 0.42	101.5 \pm 1.28
pH 9.8	99.5 \pm 0.23
pH 10.2	100 \pm 0.56
Reaction time (min) 13	100.5 \pm 0.32
Reaction time (min) 17	96.15 \pm 0.52

^aValues are mean of three determination.

Robustness

Robustness was examined by evaluating the influence of small variation of the method variables including the concentration of analytical reagent. pH of buffer solution and time of reaction. In this experiment one parameter was changed where the other kept constant and the recovery percentage was calculated each time it was found that small variation in the method variables did not significantly affect the procedure.

Application of the proposed method to analysis of primaquine dosage form

The proposed method was applied to the pharmaceutical formulations of PQ, indicating the high accuracy of the proposed method for the determination of the studied drug. The proposed method has the advantage of being virtually free from interferences by excipients. The percentage was 96.40 \pm 0.40 (value is means of five determinations), Table 3.

Conclusion

The present paper described the evaluation of NQS as analytical reagent in the development of simple, sensitive, and accurate spectrophotometric method, for the determination of PQ in pharmaceutical formulation. The proposed method is simple,

reliable, specific, accurate, reproducible, and highly sensitive, for the determination of PQ in commercially available dosage forms. The method is also cost effective and environmentally friendly; therefore the proposed method can be used advantageously as a routine method for the determination of PQ in quality control and industry.

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