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# Development and Validation of Spectrophotometric Method for Determination of Thiamine (VB1) in Pharmaceutical Formulations using 1,2-Naphthoquin-4-Sulphonate (NQS)

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## Abstract

Simple and sensitive spectrophotometric method for the determination of thiamine (VB1) in pharmaceutical formulations has been reported. The proposed method is based on the reaction between the (VB1) and sodium 1,2-naphthoquin-4-sulphonate (NQS) at alkaline medium (pH 11) to form deep brown product. Beer's law is obeyed in the range 10-40 µg/ml of thiamine at maximum wavelength of 487 nm. Under optimized reaction conditions, linear regression equation of the calibration curve is:  $A = 0.022x + 0.171$  (µg/ml) with a linear correlation coefficient of 0.997.

The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.71 µg/mL and 5.18 µg/ml respectively. The method has been successfully applied to the determination of thiamine (VB1) in pharmaceutical formulations, and can be used as a replacement of the existing sophisticated method used in quality control laboratories.

**Keywords:** Thiamine (VB1); Spectrophotometric; Pharmaceutical formulation; Method validation; Sodium 1,2-naphthoquin-4-sulfonate (NQS)

## Introduction

Thiamine hydrochloride (vitamin B1, a water-soluble vitamin), is a natural nutrient present in food and also added as an essential nutrient. It has been used for the prevention and treatment of beriberi, neuralgia, etc. in medical doses or as vitamin B1-enriched food or drinks [1]. It is necessary for carbohydrate metabolism and for the maintenance of neural activity [2]. Thiamine is one of the most important natural compounds of the B complex. It is named B1 because it was the first B vitamin discovered. All B vitamins help the body to convert food into glucose, which is "burned" to produce energy. These B vitamins help the body metabolize fats and protein. Thiamine is found in both plants and animals and plays a crucial role in certain metabolic reactions [3,4].

Since its discovery and isolation, there have been numerous reports on the determination of thiamine by capillary electrophoresis [5], chromatographic [6], spectrophotometric [1,7], spectrofluorimetric [8,9], chemiluminescence [10], potentiometric [11] and UV-irradiation methods [12]. These methods, however, have the disadvantages of high cost and require expensive sophisticated instrumentation, besides tedious experimental steps before determination.

Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories. Some spectrophotometric methods were reported for determination of thiamine in pharmaceutical formulations [1,7]. However these methods were associated with some major drawbacks such as pre-treatment oxidation by expensive chemicals, pre-concentration or a sequence of multiple reactions is required before color development.

NQS has been used as a color-developing reagent in the spectrophotometric determination of many pharmaceutical amines [13-27]. In depth review on the applications of NQS for determination of pharmaceutical bearing amine group have been reported by Elbashir et al. [24]. The reaction between NQS and thiamine has not been investigated yet. Therefore, the present study was devoted to investigate the reaction between NQS and thiamine, using this color reaction in the development of simple rapid spectrophotometric method for determination of thiamine in its dosage form.

## Experimental

### Apparatus

Absorbance was carried out by using UV-Visible spectrophotometer model Shimadzu mini 1240 with quartz cells of 1cm optical path length, pH meter was used for pH measurements, Analytical Balance and ultrasonic bath.

### Material and Reagent

Thiamine hydrochloride was obtained from Autama Development company Ltd, Khartoum, Sudan (agent of Sigma-Aldrich), and used as received; its purity was 99.15%. A solution of sodium-1,2-naphthoquinone-4-sulfonate (NQS) 0.6% (w/v) was prepared by dissolving 0.6 g in distilled water, transferred into a 100 mL volumetric flask and diluted to the mark with distilled water and mixed well. The solution was freshly prepared and protected from light during use; buffer solution of pH 11.0 was prepared by mixing 100 ml of 0.1M aqueous solution of sodium bicarbonate with 50 ml of 0.1M solution of sodium carbonate and adjusted to pH 11.0 with 1M sodium hydroxide. All other chemicals were of analytical grade.

### Preparation of Standard and Sample Solution

#### Stock standard solution of thiamine (1000 µg/mL)

An accurately 250 mg of thiamine hydrochloride standard was dissolved in distilled water, transferred into 250 ml volumetric flask, diluted to the mark with same solvent and mixed well. This stock solution was further diluted to obtain working solutions in the range of 10-40 µg/mL.

#### Sample solution

Five capsules (Thiamine 100 mg/capsule) were weighted and finely-grinded. A portion of the powder equivalent to 25 mg of the drug was weighed and dissolved in distilled water, filtered and then transferred into 250 volumetric flasks, completed to the mark with distilled water to give a solution of 100 µg/mL. The solution was then analyzed by the following procedure.

## Procedure

A 1.0 mL of 100 µg/mL of thiamine was transferred into 10 ml volumetric flask, 2.0 mL of 0.6% (NQS) was added and followed by 2.0 mL pH 11.0 ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ ) buffer solution. The reaction was completed to volume with distilled water, and the resulting solution was measured at 487 nm against reagent blank treated similarly.

### Job's method

The Job's method of continuous variation was employed to determine the stoichiometric ratio of the reaction. Master equimolar ( $5 \times 10^{-3}$  M) aqueous solution of thiamine hydrochloride and NQS were prepared. Series of 10 mL portions of the master solution of thiamine and NQS were made up comprising different complementary proportions (1:9,...9:1, inclusive) in 10 mL volumetric flask containing 2 mL of buffer solution (pH=11.0). The solution was further manipulated as described under the general recommended procedures.

## Results and Discussion

### Absorption Spectra

The absorption spectrum of thiamine was recorded against water (Figure 1), it was found that thiamine exhibits a maximum absorption peak ( $\lambda_{\text{max}}$ ) at 232 nm. Because of highly blue shifted  $\lambda_{\text{max}}$  of thiamine, its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of thiamine red-shifted light-absorbing derivative was necessary. The reaction between thiamine and NQS was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting  $\lambda_{\text{max}}$  at 487 nm, and the  $\lambda_{\text{max}}$  of NQS was 361 nm. The  $\lambda_{\text{max}}$  of thiamine-NQS derivative was red-shifted, eliminating any potential interference. Therefore, the measurements were carried out at 487 nm.

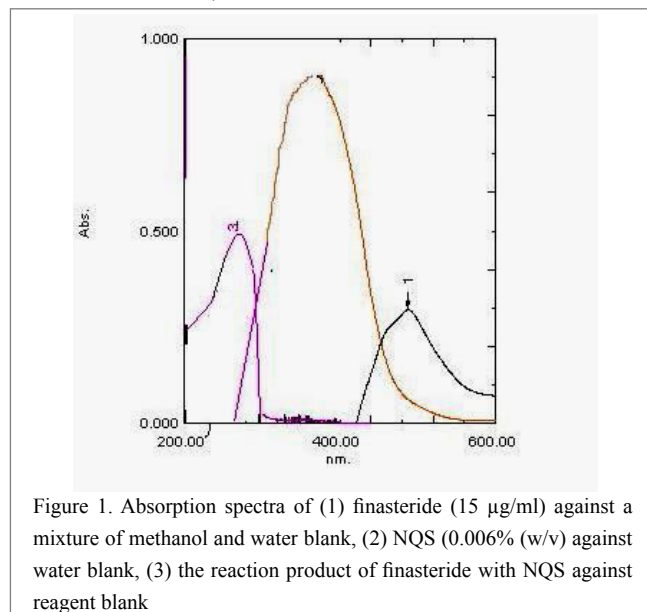


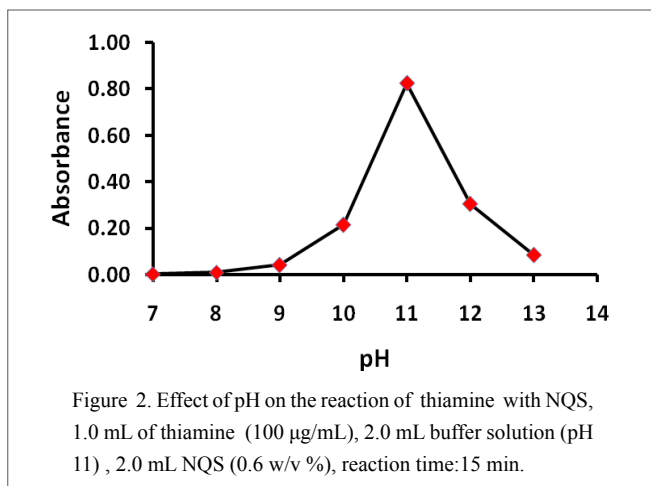
Figure 1. Absorption spectra of (1) finasteride (15 µg/ml) against a mixture of methanol and water blank, (2) NQS (0.006% (w/v) against water blank, (3) the reaction product of finasteride with NQS against reagent blank

## 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, time, buffer volume and concentration of NQS were investigated.

### Effect of pH

The effect of pH on the reaction between thiamine and NQS was examined by varying pH from 7.0 to 13.0. As shown in Figure 2, the absorbance of the product is low at pH 7.0, indicating that thiamine has difficulty to react with (NQS) in neutral media. This was possibly due to the existence of the amino group of thiamine in the form of hydrochloride salt, thus it loses its nucleophilic substitution capability. As the pH increased from 7 to 13, the readings increased rapidly, and the amino group of thiamine turns into the free amino group, thus facilitating the nucleophilic substitution. The maximum readings were attained at pH value of 11.0. At pH values more than 11.0 decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction of thiamine with NQS. Reaction of NQS with compound bearing primary amines at pH 11.0 was reported [28].



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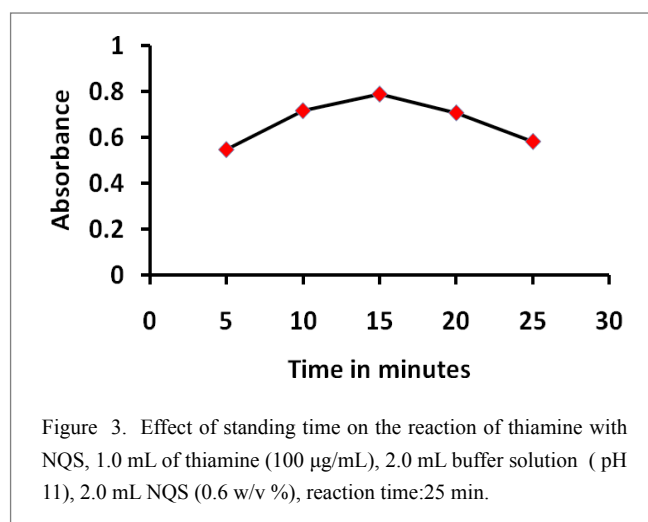
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### Effect of Time

The absorbance of the reaction product was determined at different time (Figure 3). Keeping other conditions unchanged, the absorbance of the reaction product was measured after standing for different time periods at 25°C. The results show that thiamine react with NQS at 25°C and the absorbance begins to increase instantly and becomes constant after 15 min. Furthermore, it is also observed that the absorbance remain constant for 30 min.



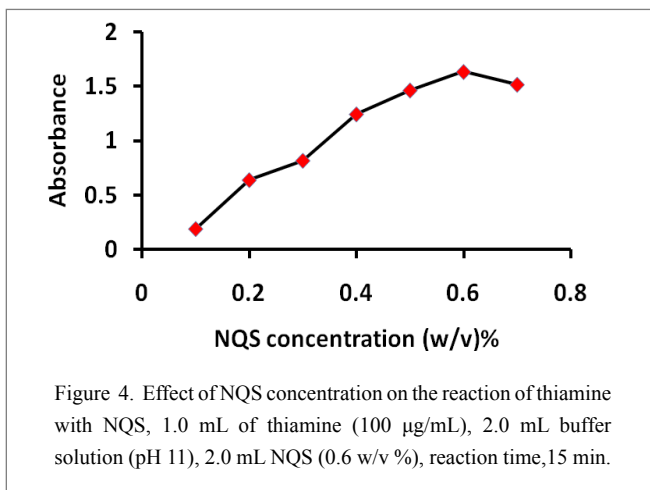
### Effect of Amount of the Buffer

Keeping pH at 11.0, the effect of amount of buffer solution on the absorbance of reaction product was also studied (data not shown). It shows that the absorbance of the reaction product enhances rapidly with the rise of amount of buffer solution, and becomes maximal when the amount of buffer solution is 2.0 mL. Therefore, the amount of 2.0 mL buffer solution was selected to ensure the highest absorbance.

### Effect of NQS Concentration

The studying of NQS concentrations revealed that the reaction was dependent on NQS reagent. The highest absorption intensity was attained at NQS concentration of 0.6% (w/v), and higher concentration of NQS (1.0%, w/v) had no effect on the absorption values, as shown in Figure 4.

From the above experiments, the optimized conditions used for the assay were: pH 11.0, NQS concentration 0.6% w/v, volume of the buffer 2.0 mL, reaction time 15 min and temperature 25°C.



### Validation of Method

The method was validated for the following parameters: specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness according to the International Conference on Harmonization (ICH) guidelines [28].

### Linearity, Limit of Detection (LOD) Limit of Quantification (LOQ)

The linearity was evaluated by linear regression analysis determined by constructing seven concentrations of thiamine, in the range of 10–40 µg/mL, which was calculated by the least square regression method to calculate the calibration equation and the correlation coefficient. The calibration curves were constructed by plotting concentration versus absorbance, using linear regression analysis. The regression equation for the results was  $A=0.022x + 0.171$  ( $r^2=0.997$ ), where A is the absorbance at 487 nm, x is the concentration of thiamine in µg/mL in the range of 10-40 µg/mL, and r is correlation coefficient (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the following formula  $LOD=3.3 \times SDA/b$ , and  $LOQ=10 \times SDA/b$ , SDA is the standard deviation of the blank, b is the slope under the ICH guidelines. The LOD and LOQ were found to be 1.71 and 5.18 µg/mL, respectively (Table 1).

### Accuracy

The accuracy of the proposed method was carried out by applying 3 different concentrations 10, 20, and 30 µg/mL of thiamine drug within linear range calculated as the percentage of the drug recovered from the samples (Table 2).

Table 1. Parameters for the performance of the proposed method

Parameter	value
Measurement wavelength (nm)	487
Linear range ( µg/mL)	10-40
Intercept	0.171643
Standard deviation of the Blank	0.000455
Slope	0.022155
Correlation coefficient (r)	0.997
Limit of detection, LOD ( µg/mL)	1.71
Limit of quant., LOQ ( µg/mL)	5.18

Table 2. Recovery studies for the determination of thiamine by the proposed method.

Sample No.	Sample content (µg/mL)	Thiamine standard amount		
(µg/mL)	Amount found (Total) (µg/mL)	Recovery ± SD*		
1	5	10	14.95	99.0% ± 0.01
2	5	20	25.05	100.2% ± 0.01
3	5	30	35.00	100.0% ± 0.01

\*Values are mean of three determinations.

### Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were recorded in Table 3. This indicated the reliability of the proposed method to routine application for the analysis of thiamine.

### Reaction Mechanism

It has been reported that NQS could react with amino group of secondary amine derivative. Similarly, amino group of thiamine, taking on nucleophilicity due lone electron pair of nitrogen atom, trend to attack on the electron-deficient center in NQS, namely no.4 carbon atom (3,4-C=C carbon bond conjugate with 2-C=O, as a result 4-C of NQS becomes electron lacking center). At the same time, it has been proved that the composition of product is 1:2 of thiamine and NQS (Figure 5). So it is concluded that amino group of thiamine react with 4-sodium sulphonate of NQS molecule respectively, to form brown N-alkyl-amino-naphthoquinone. The reaction equation is shown in Figure 6.

Table 3. Influence of small variation in the assay condition on the analytical performance of the proposed spectrophotometric method for determination of Thiamine using NQS reagent

Parameter	Recovery (% ± SD)
Recommended conditions	95.33±0.006
NQS concentration (% w/v) 0.58	102.37±0.016
NQS concentration (% w/v) 0.62	97.82±0.013
Buffer solution (pH) 10.8	96.01±0.029
Buffer solution (pH) 11.2	93.63±0.044
Reaction time (min) 13	98.63±0.009
Reaction time (min) 17	96.56±0.055

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

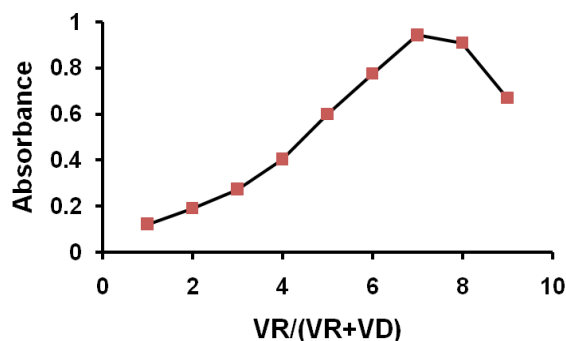
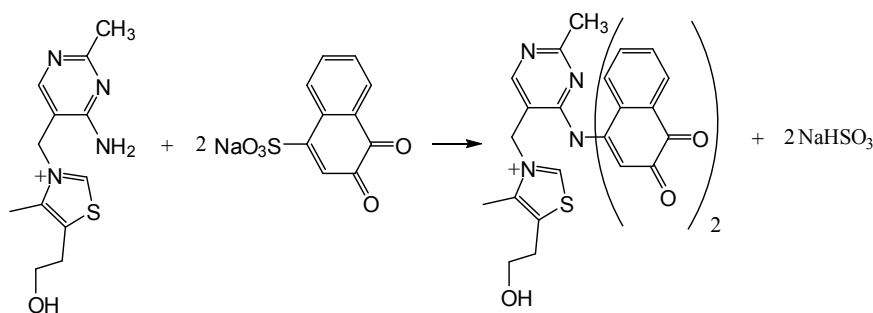


Figure 6. Scheme for the reaction pathway of thiamine with NQS



### Application of the Proposed Method to Analysis of Thiamine Dosage Form

Thiamine tablets were subjected to the analysis by the proposed and the label claim agrees well with our new method as shown in Table 4. The proposed method has the advantage of being virtually free from interferences by excipients.

Table 4. Analysis of Thiamine -containing dosage form by the proposed method.

Brand name of label claim (mg)	Amount found (mg)	(% found ± SD)a
Thiamine tablets (100 mg)	95.55	95.33±0.006

a: values are mean of five determinations

### Conclusion

The present paper described the evaluation of NQS as analytical reagents in the development of simple, sensitive, and accurate spectrophotometric methods, for the determination of thiamine in pharmaceutical formulation. The proposed method is simple, reliable, specific, accurate, reproducible, and highly sensitive, for the determination of thiamine in commercially available dosage forms. The procedure presented here does not need necessitate any expensive apparatus; therefore the proposed method can be used advantageously as a routine method for the determination of thiamine in quality control laboratories, our method can be applied to the determination of other secondary amine derivatives as well.

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