AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

A Novel Spectrophotometric Method for Determination of Nebivolol Hydrochloride in Tablets Dosage Form With 1,2-Naphthoquinone-4-Sulphonate as a Chromogenic Reagent

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ABSTRACT

Simple, sensitive and specific spectrophotometric method was developed and validated for quantification of nebivolol (NBV) in tablet dosage form. Studies were carried out to investigate the reaction between NBV and 1, 2-naphthoquinone-4-sulphonate (NQS) reagent. In alkaline medium (pH 9), a red-colored product exhibiting maximum absorption peak (λ_{max}) at 521 nm was produced. The stoichiometry of the reaction was investigated and the reaction mechanism was postulated. Under the optimized reaction conditions, Beer's law correlating the absorbance with NBV concentration was obeyed in the range of 2 - 55µg/ml with good correlation coefficient 0.9996. The molar absorptivity was 0.124×10^4l/mol.cm. The limits of detection and quantification were 0.539 and 1.634µg/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. No interference was observed from the excipients that are present in the tablets. The proposed method was applied successfully for the determination of NBV in its pharmaceutical tablets with good accuracy and precisions. The results were compared favorably with those of a reference pre-validated method. The method is practical and valuable in terms of its routine application in quality control laboratories.

Keywords: Nebivolol HCl; 1, 2-Naphthoquinone-4-sulphonate; Spectrophotometry; Pharmaceutical analysis.

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Received 15 September 2015, Accepted 21 September 2015

INTRODUCTION

Please cite this article as: Alhemiary *et al*., A Novel Spectrophotometric Method for Determination of Nebivolol Hydrochloride in Tablets Dosage Form With 1,2-Naphthoquinone-4-Sulphonate as a Chromogenic Reagent. American Journal of PharmTech Research 2015.

Nebivolol [1,2] is chemically 1-(6-flourochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol or 2, 2′-azanediylbis (1-(6-flourochroman-2-yl) ethanol (Figure 1), a new antihypertensive drug, is a racemate of two enantiomers with four chiral centres.. The mechanism action of nebivolol is a competitive and highly selective _1- receptor antagonist and does not show an intrinsic sympathomimetic activity. Nebivolol is endowed with peripheral vaso dilating properties mediated by the modulation of the endogenous production of nitric oxide and thus lowers peripheral resistance. The SRRR- enantiomer (d-nebivolol) is a potent and cardio selective _1-adrenergic blocker. The RSSS- enantiomer (l-nebivolol) has a favourable hemodynamic profile, in that normal energy supply during exercise is nor affected[3].

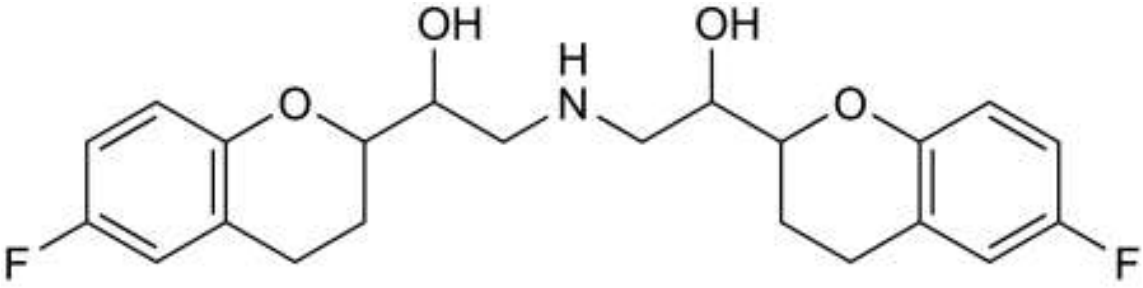


Figure 1: Structure of Nebivolol

Literature survey reveals that few analytical methods were reported which include liquid-chromatography with tandem mass spectrometry [4], RP-HPLC and HPTLC methods [5] and derivative spectrometric determination [6,7], liquid chromatography coupled with electro spray ionization tandem mass spectrometry[8], Stability indicating RP-HPLC estimation[9,10], LC-MS [8,11] UV spectrophotometry[12-15] techniques were reported. 1, 2-naphthoquinone-4-sulphonic sulphonate (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines[16, 17]. However, the reaction between NQS with Nebivolol has not been investigated so far. The present study describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for the determination of Nebivolol in its pharmaceutical dosage forms.

MATERIALS AND METHOD

Apparatus

A GENESYS 10S UV-Vis double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.8nm) connected to an IBM computer loaded with Thermo Spectronic VISION Lite version 4 software and 1-cm quartz cell were used for the registration and treatment of absorption spectra.

Materials and Reagents

All Chemicals used were of analytical reagent grad unless otherwise is mentioned, Nebivolol hydrochloride (Sigma- Aldirch, USA) was obtained and used as received; its purity was > 98%.

1, 2-Naphthoquinone-4-sulphonate (NQS) 0.5 %(w/v):

0.5 g of NQS was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 10ml distilled water, and make up the volume up to the mark with distilled water to obtain a solution of 0.5% (w/v). The solution was freshly prepared and protected from light during the use. Tris buffer was prepared by mixing 100 ml 0.1 M tris (hydroxymethyl) aminomethane with 29.4 ml of 0.1 M HCl[18]. Nebilet® tablets (UNIPHARMA, Syria) and Bivol® (AL-NAHDI INTERNATIONAL MEDICAL CO., LTD , Yemen) were labeled to contain 5 mg NBV per tablet. All solvents and materials used throughout this study were of analytical grade.

Preparation of standard stock solution

An accurately weighed amount 20 mg of NBV was quantitatively transferred into a 100 ml calibrated flask, dissolved in 20 ml distilled water, completed to volume with the same solvent to obtain a stock solution of 200µg/ml. The stock solution was found to be stable for at least two weeks when kept in a refrigerator. The stock solution was further diluted with water to obtain working solutions in the range of 2 - 55µg/ml.

Tablet Sample Solution

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of the active ingredient was transferred into a 100ml calibrated flask, and dissolved in about 40 ml of distilled water. The contents of the flask were swirled, sonicated for 5 minutes, and then completed to volume with water. The contents were mixed well and filtered; the first portion of the filtrate was rejected. The filtered solution was diluted quantitatively with distilled water to obtain suitable concentrations for the analysis by the proposed spectrophotometric method.

General Procedures

NBV solution in the concentration range of2–80µg/ml was transferred into separate 10mlcalibrated flask. A 0.5 ml of tries buffer solution of pH 9 and 1 ml of NQS solution (0.5%, w/v) were added. The reaction solution was allowed to proceed for 10 min at room temperature (25 ± 2°C) and completed to volume with water. The resulting solution was measured at 521 nm against reagent blank treated similarly.

RESULTS AND DISCUSSION

Absorption Spectrum

NBV contains secondary amino group for which many chromogenic reagents are available for color producing reactions. These reactions include formation of colored charge transfer complex with electron acceptor, formation of ion-pair associates with pairing reagents[19], and formation of condensation product with a chromogenic reagent . These methods are usually associated with some major drawbacks such as laborious multiple extraction steps in the analysis by ion-pair based methods[19] or in preparation of the free base of the drug prior to the analysis by charge-transfer-based methods, and long reaction time, thus the procedure is time-consuming. Darwish *et al.* [20, 21] has demonstrated that NQS is a valuable color-developing reagent in the development of simple spectrophotometric methods for the determination of many pharmaceutical amines in the form of their acid salts. For these reasons, the present study was devoted to investigate the reaction between NQS and NBV, and employed this color reaction in the development of a new simple and rapid spectrophotometric method for determination of NBV in its tablets. The reaction between NBV and NQS was performed, and the absorption spectrum of the reaction product was recorded against reagent blank. The product NBV-NQS complex was red colored exhibiting λ_{max} at 521 nm (Figure 2).

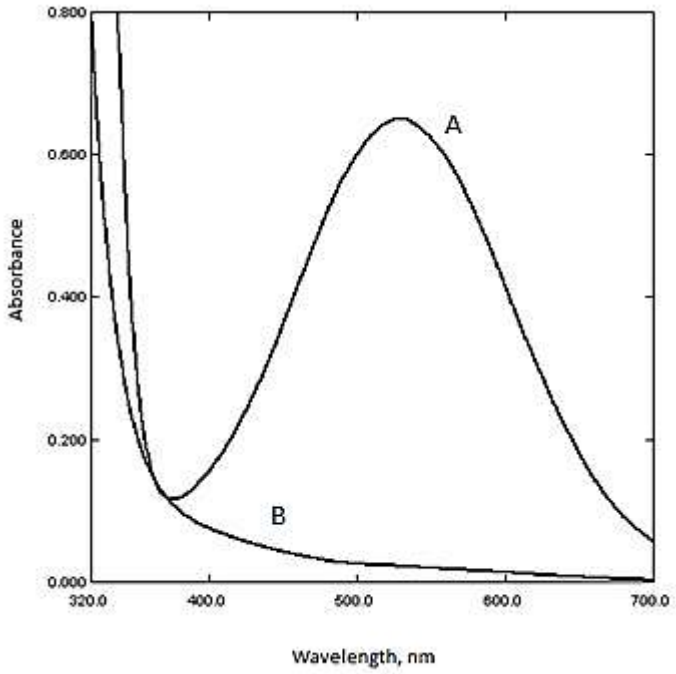


Figure 2: Absorption spectra of A: 25 µg/ml NBV-NQS complex against reagent blank, B: reagent blank against chloroform, under optimum conditions

Effect of Reagent Concentration

Studying the effect of NQS concentration on its reaction with NBV revealed that the reaction was dependent on the NQS concentration as the readings increased with the increase in the reagent concentration (Figure 3). The highest readings were attained at a concentration of 0.5 - 0.6% (w/v) beyond which the readings slightly decreased. A concentration of 0.5% (w/v) was used in all the subsequent experiments.

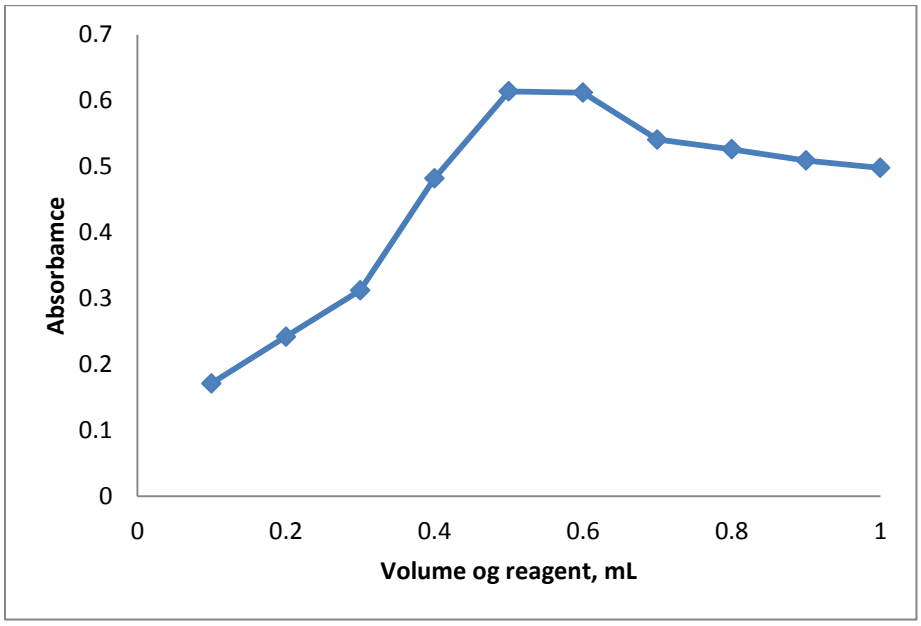


Figure 3: Effect of NQS concentration on the reaction of NBV 25 µg/ml with 0.5 ml NQS (0.5%, w/v).

Effect of pH and Buffer Components

The influence of pH on the reaction of NBV with NQS was investigated by carrying out the reaction in buffer solution of varying pH values. The results revealed that NBV has difficulty to react with NQS in acidic media (Figure.4). This was possibly due to the existence of the amino group of NBV in the form of hydrochloride salt, thus it loses its nucleophilic substitution affinity. As the pH increased, the readings increased rapidly, as the amino group of NBV (in the hydrochloride salt) turns into the free amino group, thus facilitating the nucleophilic substitution. The maximum readings were attained at pH values of 9. At higher pH, sharp decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction of NBV with NQS, and the instability of NQS reagent[22]. In order to investigate the effect of buffer components on the reaction, different buffer solutions of pH 8.5 were tested: Clark, Robinson, phosphate, borate and tris buffers. The highest absorbance's were obtained when tries buffer was used, thus it was used in all the subsequent experiments.

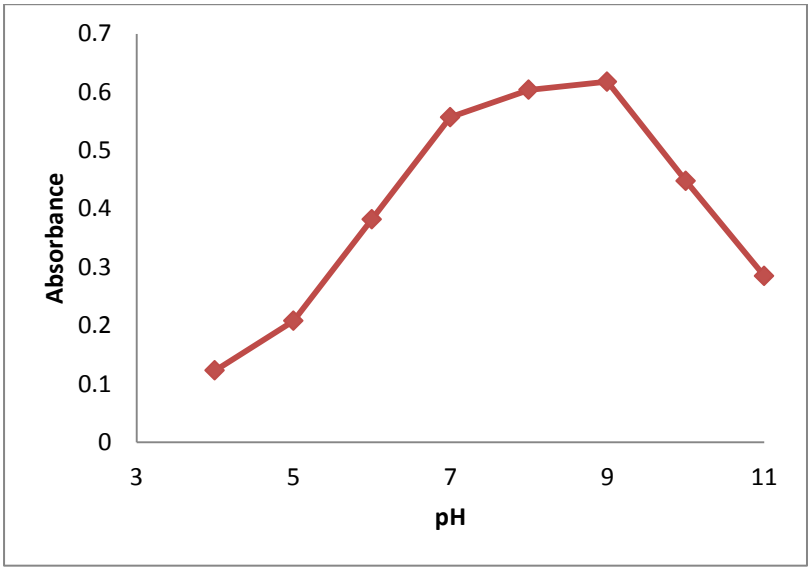


Figure 4: Effect of pH on the reaction of NBV 25 µg/ml with 0.5 ml NQS (0.5%, w/v)

Effect of Temperature and Temperature

The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures (25–90 °C). The results (Figure. 5) revealed that increasing the temperature leads to increase in the absorption values of the reaction solution. The maximum absorbance value was attained at 40°C, and remained stable up to 80°C. At higher temperature, the absorbance value decreased. The decrease in the absorbance values were probably attributed to the instability of the NBV–NQS derivative at high temperature (>70°C). Further experiments were carried out at 50±5°C. The effect of time on the formation of the reaction product was investigated by allowing the reaction to proceed for varying times. The results revealed that the reaction went to completion with 10 min, and longer reaction time up to 25min did not affect the reaction(Figure. 6). Further experiments were carried out at 10 min.

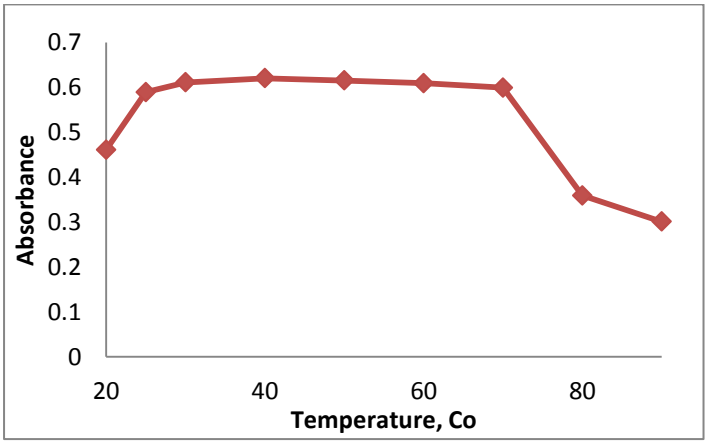


Figure 5: Effect of Temperature on the Reaction of NBV 25 µg/ml with 0.5 ml NQS (0.5%, w/v)

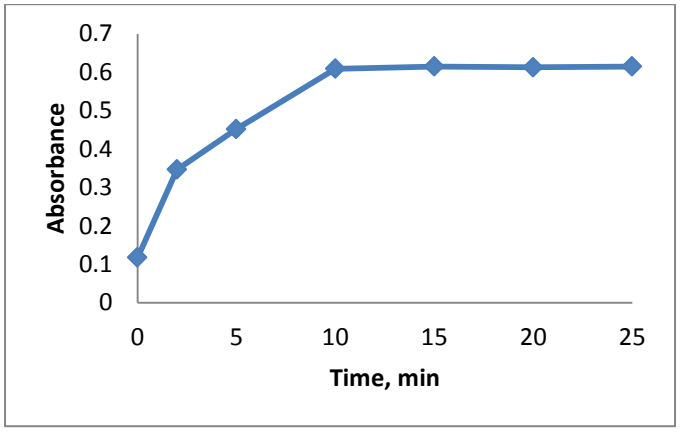


Figure 6: Effect of Time Reaction on the Reaction of NBV 25 µg/ml with 0.5 ml NQS (0.5%, w/v)

Effect of Solvent

Upon diluting the reaction with water, colloids were obtained indicating the incomplete solubility of NBV–NQS in water. Therefore, water could not be used for dilution. In order to select the most appropriate organic solvent for diluting the reaction solution, different solvents were tested: methanol, ethanol, isopropanol, acetone, acetonitrile, dimethylsulphoxide, and 1,4-dioxane. The highest readings were obtained when methanol was used for dilution.

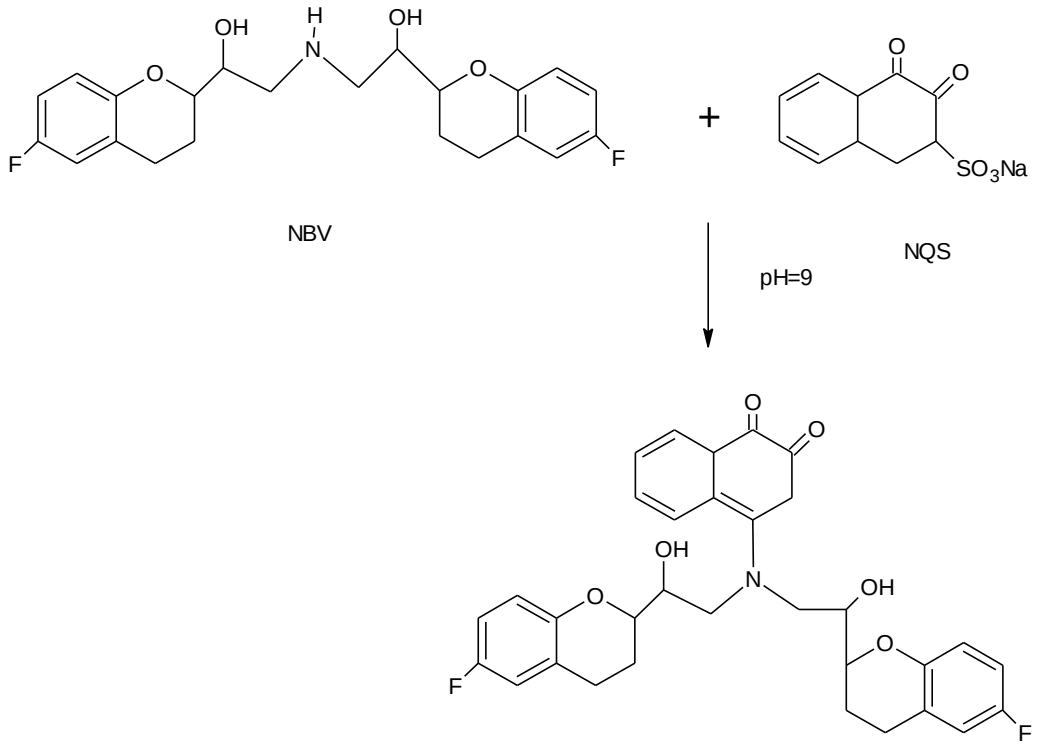
Stability of the Chromogen

Under the aforementioned optimum conditions, the reaction between NBV and NQS was completed within 10 min at 50±5°C, and the absorbance no longer changed after standing for up to25 min. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remains stable for at least 4 h. This allowed the processing of large batches of samples, and their comfortable measurements with convenience. This increased the convenience of the methods as well as made it applicable for large number of samples.

Stoichiometry of the Reaction

Job's method of continuous variation [23] was employed. Master equimolar (5×10^{-3}mol/l) aqueous solutions of NBV and NQS were prepared. Series of 10 mL portions of the master solutions of NBV and NQS were made up comprising different complementary proportions (0 : 10, 1 : 9, 9 : 1, 10 : 0, inclusive) in 10 mL calibrated flasks containing 1 ml of buffer solution (pH 9). The solution was manipulated as described under the general recommended procedures. The results indicated that 1:1 NBV-NQS complex is formed in the method. Five solutions containing NBV and the reagent NQS in various molar ratios. The absorbance of solutions was subsequently measured at 521 nm (Scheme 1). The graphs of the results obtained (Figure. 7) gave a maximum at a molar

ratio of Xmax =0.5 in all the method which indicated the formation of a 1:1 charge transfer complex between NBV and reagent NQS This finding was anticipated by the presence of more basic or electron donating center (–NH) in the NBV.



Scheme 1: Reaction Pathway for the Formation Complex between NBV and NQS

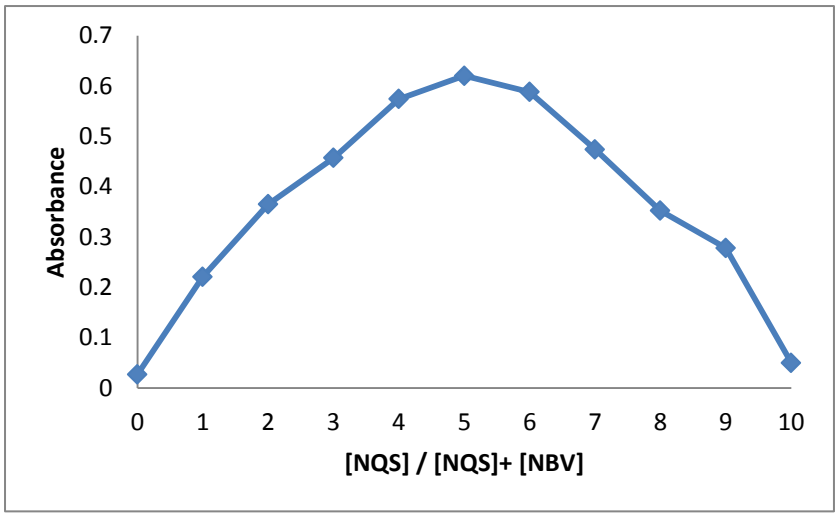


Figure 7: Job's Plot for Determination of Stoichiometry of the Reaction between NBV and NQS.

Method Validation

The proposed methods were validated for linearity, sensitivity, selectivity, accuracy, precision, robustness, ruggedness and recovery according to the current ICH guidelines[24].

Linearity and Sensitivity

At the established experimental conditions, standard calibration curves for NBV with NQS was constructed by plotting absorbance verses concentration. The linear regression curves was obtained in the Beer's law range of 2.0-55µg/ml with correlation coefficient 0.9996. Regression characteristics including slope, intercept, correlation coefficient and also the molar absorptivity values for each proposed method are given in Table 1. The detection limit (LOD) and quantification limit (LOQ) were calculated by using the following equations:

$$LOD = \frac{3.3 \times \sigma}{S} \& LOQ = \frac{10 \times \sigma}{S}$$

Where, σ is the standard deviation of seven replicate determinations under the same conditions as for the sample in the absence of the analyte and S is the slope of the calibration graph. The LOD values were calculated to be 0.539µg/ml (Table 1).

Table 1: Optimum Conditions and Analytical Parameters

Parameters	NBV-NQS complexes
λmax (nm)	521
Linearity range µg/ml	2.0 - 55
Molar absorptivity(l/ mol.cm)	1240
Slope (b)	0.0227
Intercept (a)	0.0051
Correlation coefficient	0.9996
LOD µg/ml	0.539
LOQ µg/ml	1.634

A = b x ± a, where a = intercept, b = slope, and x = concentration (µg/ml)

Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, pure drug NBV solution at three different concentration levels (within the working range) were prepared and analyzed in seven replicates during the same day (intra-day precision) and on five consecutive days (inter-day precision) and the results are presented in Table 2. The percentage relative error (RE %) was ≤ 1.50 which indicates that the accuracy of the methods is satisfactory. Percentage relative standard deviation (RSD%) for intra-day was ≤ 2.88 and for inter-day it was ≤ 1.37 indicating repeatability and usefulness of the proposed methods in the routine analysis. Before proceeding with the analysis of NBV in its tablets, interference liabilities were carried out to explore the effect of common excipients that might be added during tablets formulation. Samples were prepared by mixing known amount (20mg) of PRX with various amounts of the common excipients: starch (50gm), glucose (10gm), lactose (10gm), acacia (1omg), talc (5mg), and magnesium stearate

(10mg). These laboratory-prepared samples were analyzed by the proposed method applying the general recommended procedure. The recovery values were 97.62-101.58 □ ± 0.35− 1.23%, (Table 3). These data confirmed the absence of interference from any of the common excipients with the determination of NBV by the proposed method.

Table 2: Evaluation of Intra-Day and Inter-Day Precision and Accuracy

NBV taken (µg/ml)	Intra-day (n=5)			Inter-day (n=7)		
	Found[a] (µg/ml)	%RSD [b]	% RE [c]	Found µg.mL^{-1}	%RSD	% RE
5	5.184	2.88	3.68	4.99	1.37	0.08
15	14.784	1.93	1.44	15.07	0.75	0.47
25	25.084	0.777	0.336	25.07	0.63	0.26

[a]Mean value of five determinations; [b] Relative standard deviation (%); [c]Relative error (%).

Table 3: Analysis of NBV in Presence of Common Excipients by the Proposed Method

Excipients	Recovery%*± SD
Starch	98.91±0.84
Glucose	101.23±1.23
Lactose	100.62±0.35
Acacia	97.62±0.67
Talc	101.58±1.06
Magnesium stearate	98.01±0.79

*Average of three determinations.

Robustness and Ruggedness

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 97.73–101.23±0.68–1.45% (Table. 4). This indicated the reliability of the proposed method during its routine application for the analysis of NBV. Ruggedness was also tested by applying the proposed methods to the assay of NBV using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the relative standard deviations (RSD) did not exceed 2%.

Applications to Analysis of Tablets

The proposed methods were applied to the determination of NBV in tablets (Table 5). The results obtained were statistically compared with those of the official method[15] by applying the Students t-test for accuracy and F-test for precision. As can be seen from the Table 5, the calculated t-test and F-value at 95 % confidence level did not exceed the tabulated values of 2.78 and 6.39,

respectively, for four degrees of freedom. The results indicated that there is no difference between the proposed methods and the official method with respect to accuracy and precision.

Table 4: Influence of Small Variations in the Assay Conditions on the Analytical Performance of the Proposed Spectrophotometric Method for Determination of Prx Using NQS Reagent

Parameters	Recovery (% ±SD)*
Recommended conditions	99.85 ±0.75
NQS concentration (%, w/v)	
0.25	101.23±0.94
0.75	100.87±1.05
Buffer solution (pH)	
8.5	98.56±0.68
9.5	97.73±1.42
Reaction time (min)	
5	99.68±0.86
10	100.67±1.54

*Values are mean of 3 determinations.

Table 5: Results of Analysis of Tablets by the Proposed Methods

Tablet brand name	Label Claim (mg)	Method	Amount of Drug Estimated* (in mg)	%Recovery ±SD*	T-test**	F-test***
Nabilet	5	Proposed	5.07	101.4± 0.57	1.29	2.18
		Official	4.96	99.20± 1.46	-	-
Bivol	5	Proposed	10.18	101.8± 1.17	1.37	1.96
		Official	9.89	98.90± 0.98	-	-

*Mean value of five determinations.

**Tabulated t-value at the 95% confidence level is 2.78.

***Tabulated F-value at the 95% confidence level is 6.39.

Recovery Study

To further ascertain the accuracy of the proposed methods, recovery experiment was performed via standard addition technique. To a fixed and known amount of NBV in tablet powder (pre analyzed), pure NBV was added at three concentration levels (50, 100, and150% of the level present in the tablet), and the total was measured by the proposed methods. The determination with each concentration was repeated three times, and the results of this study presented in Table 6 indicated that the various excipients present in the formulations did not interfere in the assay, thereby further confirming the accuracy of the methods.

Table 6: Results of Recovery Study

Tablets studied	NBV in tablets (µg/ml)	Pure NBV added µg/ml	Total found µg/ml	%Recovery* ±SD
Nabilet	10	5	15.19	101.27 ± 0.92
		10	20.34	101.70±1.34
		15	24.89	99.56 ± 0.78
Bivol	16	8	24.29	101.21±1.26
		16	32.64	102.00±1.43
		24	39.76	99.40±0.98

*Mean value of three determinations.

CONCLUSION

The present study described the successful evaluation of NQS reagent in the development of simple and rapid spectrophotometric method for the accurate determination of NBV in its dosage forms. In contrast with the previously reported methods for analysis of NBV, the method described herein has many advantages: it does not need expensive sophisticated apparatus, it is simple and rapid, and it has high sensitivity. The proposed method is superior to all the previously reported spectrophotometric methods for determination of NBV in terms of its sensitivity and simplicity. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. Therefore, the method is practical and valuable for its routine application in quality control laboratories for analysis of NBV.

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