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Spectrophotometric Determination of Nebivolol Hydrochloride in Bulk and Pharmaceutical Formulations

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Two simple, rapid, sensitive and accurate spectrophotometric methods have been developed and validated for the assay of nebivolol hydrochloride in pure and pharmaceutical formulations. These methods (**A** and **B**) were based on nucleophilic substitution and co-ordination complex formation of nebivolol hydrochloride by 1,2-naphtha quinone-4-sulphonate in alkaline media and cobalt thiocyanate with the maximum absorption at 460 and 630 nm respectively. Reaction conditions were optimized to obtain the maximum colour intensity. Absorbance was found to increase linearly with increase in concentration of nebivolol hydrochloride, which was corroborated by the correlation coefficient values 0.9986 and 0.9984 respectively. The developed methods were validated with respect to linearity, accuracy (recovery), precision, Sandell's sensitivity, molar extinction coefficient and specificity. The system obeyed Beer's law in the range of 10-60 µg/mL and 8-20 µg/mL for nebivolol hydrochloride. Various analytical parameters have been evaluated and the results have been validated by statistical methods.

Key Words: Nebivolol hydrochloride, 1,2-Naphtha quinone-4-sulphonate, Cobalt thiocyanate.

INTRODUCTION

Nebivolol hydrochloride, chemically 1-(6-fluoro chroman-2-yl)-2-([2-(6-fluoro chroman-2-yl)-2-hydroxyl ethyl)] amino) ethanol][1,2]. It is a highly cardio selective vasodilatory β_1 receptor blocker used in treatment of hypertension[3]. The literature survey reveals that several methods are reported for the determination of nebivolol hydrochloride by UV spectrophotometry[3-5], RP-HPLC[6-9], RP-LC[10] and extractive spectrophotometric methods[11,12].

In this paper, we reported two simple and sensitive visible spectrophotometric methods for the assay of nebivolol hydrochloride. These methods are based on nucleophilic substitution and coordination complex formation of drug with reagents such as 1,2-naphtha quinone-4-sulphonate and cobalt thiocyanate giving coloured products.

EXPERIMENTAL

An ELICO, UV-visible digital spectrophotometer with 1 cm matched quartz cells were used for all spectral and absorbance measurements. An ELICO LI- 120 digital pH meter was used for all pH measurements. Pure drug was procured from Hetero Drugs Ltd., Hyderabad as a gift sample. The reagents namely 1,2-naphtha quinone-4-sulphonate, cobalt thiocyanate, NaOH (AR Grade) supplied by SD Fine Chemicals Ltd., Mumbai are used without any further purification. Nitrobenzene was (AR Grade) supplied by SD Fine Chemicals Ltd., Mumbai is used throughout the work.

Standard drug preparation: The working standard solution (1.0 mg/ mL) of nebivolol hydrochloride was prepared by dissolving 100 mg of the drug in 100 mL of distilled water. This stock solution was further diluted with the same solvent to get 200 µg/mL of working standards for both methods respectively.

Pharmaceutical formulations: An accurately weighed portion of tablet content equivalent to about 300 mg of nebivolol hydrochloride was transferred into a 100 mL volumetric flask. About 100 mL of warm isopropyl alcohol were added and shaken well and mixed thoroughly for about 20 min. The solution was filtered. The filtrate was evaporated to dryness. The residue was appropriately diluted for the preparation of formulation solutions for different methods as given under standard solution preparations to fit into the calibration graph.

Preparation of calibration curve: Calibration curves were constructed in accordance with the optimum conditions.

Method-A: Aliquot of standard nebivolol hydrochloride solution, 0.5-3.0 mL (200 µg/mL) were transferred into a series

of calibrated tubes containing 0.2 mL of 0.1 N NaOH and 0.2 mL of 0.5 % 1,2-naphtha quinone-4-sulphonate reagent solution was added in each tube and the contents were heated at 50 ºC for 5 min and cooled. This operation was performed in the dark. Then 0.5 mL of conc. H_2SO_4 was added slowly, mixed and the absorbance (Fig. 1) was measured after 5 min at λ_{max} 460 nm against a reagent blank prepared similarly. The amount of nebivolol hydrochloride was calculated from its calibration curve (Fig. 2).

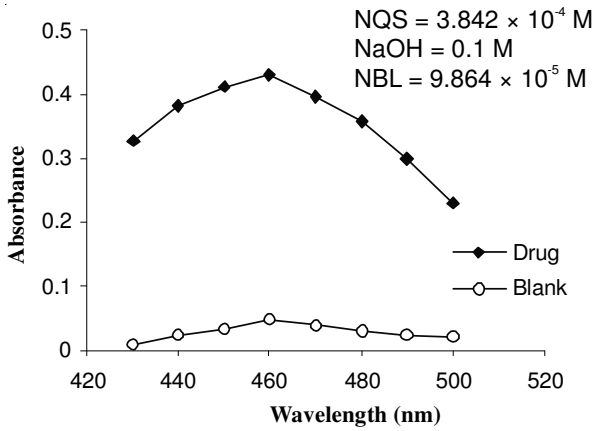


Fig. 1. Absorption spectrum of nebivolol hydrochloride (NBL) with 1,2-naphtha quinone-4-sulphonate (NQS) (Method A)

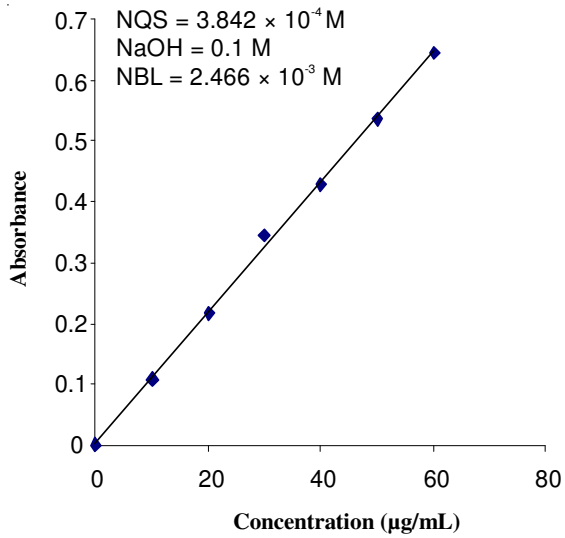


Fig. 2. Beer's law plot of nebivolol hydrochloride (NBL) with 1,2-naphtha quinone-4-sulphonate (NQS) (Method A)

Method-B: Aliquots of standard nebivolol hydrochloride solution, 2.0-5.0 mL (200 µg/mL) were delivered into a series of calibrated tubes. 2.0 mL of buffer of pH 2.0 and 5 mL of cobalt thiocyanate solutions were added and the total volume in each tube was adjusted to 15 mL with distilled water. These solutions in the tubes were transferred to 125 mL separating funnel. To each separating funnel 10.0 mL of nitrobenzene was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured after 20 min absorbance (Fig. 3) was measured at λ_{max} 630 nm against a similar reagent blank. The amount of nebivolol hydrochloride was deduced from its calibration curve (Fig. 4).

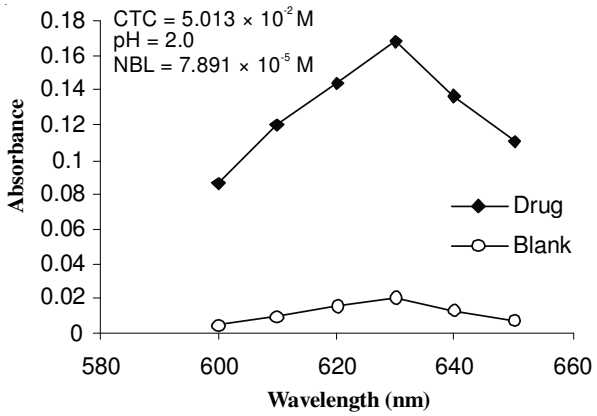


Fig. 3. Absorption spectrum of nebivolol hydrochloride (NBL) with cobalt thiocyanate (CTC) (Method B)

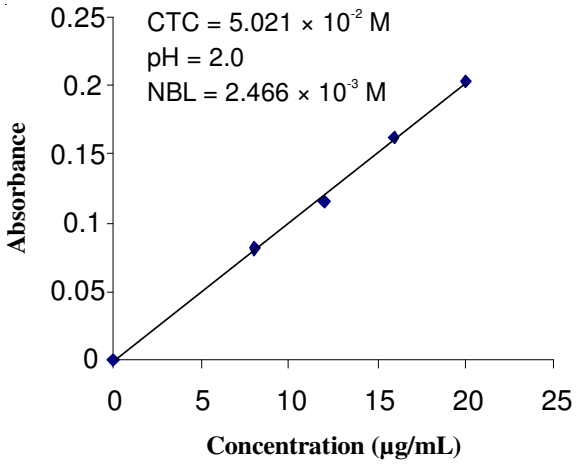


Fig. 4. Beer's law plot of nebivolol hydrochloride (NBL) with cobalt thiocyanate (Method B)

The calibration graphs (Figs. 2 and 4) are the linear over the concentration ranges are within permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in (Table-1). The amount of nebivolol hydrochloride was calculated from the corresponding Beer-Lambert's plot.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR NEBIVOLOL HYDROCHLORIDE

Parameters	Method A	Method B
λ_{max} (nm)	460	630
Beer's law limits (µg/mL)	10 – 60	8-20
Molar absorptivity (L mol^{-1} cm^1)	0.9904×10^3	2.076×10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	9.302×10^{-2}	3.940×10^{-2}
Optimum photometric range (µg/mL)	12.5-50	10 - 18
Regression equation (Y = a+ bc)*:		
Slope (b)	0.0108	0.0008
Intercept (a)	0.0023	0.0101
Correlation coefficient (r)	0.9986	0.9984
Relative standard deviation** (%)	0.3612	0.5264
Range of error (confidence limits)** (%)		
0.05 level	0.1404	0.0440
0.01 level	0.2078	0.0652

*Y = a + bx, where 'Y' is the absorbance and x is the concentration of Nebivolol HCl in µg/mL; **For eight replicates

Optimization of reaction conditions: The reactions were investigated on effect of the reaction time, the reaction temperature, buffer pH, choice of the organic solvent (nitrobenzene) and ratio of the organic phase to the aqueous phase, volume of NaOH solution as well as volume of 1,2-naphtha quinone-4-sulphonate and cobalt thiocyanate solutions. Control experiments are carried out by measuring absorbance at 460 and 630 nm of series of the solutions varying one and fixing the other parameter for method A and B, respectively.

RESULTS AND DISCUSSION

Nebivolol hydrochloride is involved in nucleophilic substitution reaction in basic medium with 1,2-naphtha quinone-4-sulphonate and NaOH as well as co-ordination complex formation with cobalt thiocyanate, which is extracted into nitrobenzene solvent were quantitatively measured.

Optical characteristics: The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, per cent relative standard deviation, per cent range of error (0.05 and 0.01 confidence limits) were calculated for all the methods and results are summarized in Table-1.The values obtained for the determination of nebivolol hydrochloride in pharmaceutical formulations (tablets) by the proposed methods are presented in Table-2. Studies reveal that the common excipients and other additives usually present in the tablets did not interfere in the proposed methods.

TABLE-2
DETERMINATION OF NEBIVOLOL HCl IN PHARMACEUTICAL FORMULATIONS

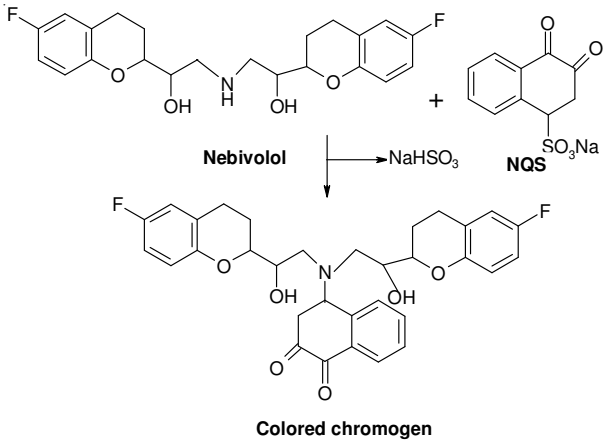
Formulations	Labelled amount (mg/mL)	Amount found* by proposed methods		Recovery** by proposed methods (%)	
		Method-A	Method-B	Method-A	Method-B
Tablet	5	4.95	4.96	99.4	99.8
Tablet	10	9.96	9.95	99.7	99.3

*Average of 8 determinations; **Recovery of amount added to the pharmaceutical formulation (Average of three determinations)

Precision and accuracy: The precision of each methods was ascertained from the absorbance values obtained by the actual determination of 8 replicates of a fixed amount of nebivolol hydrochloride in total solution. The per cent relative standard deviation and percent range error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and results are summarized in Table-1. To determine the accuracy of each proposed method, different amounts of bulk samples of nebivolol hydrochloride within the Beer's law limits were taken any analyzed by the proposed method. The results (per cent recovery) were recorded in Table-2.

Scheme of the coloured products

Method-A: In this method, the secondary amino group of nebivolol hydrochloride permits the colour development with the 1,2-naphtha quinone-4-sulphonate through the nucleophilic substitution reaction. The coloured species is represented as given in the **Scheme-I**.



Scheme-I: Nucleophilic substitution reaction of nebivolol hydrochloride with 1,2-naphtha quinone-4-sulphonate (NQS)

Method-B: In this method the coloured species formed is the coordination complex of drug (electron donor) and the central atom of cobalt thiocyanate, which is extractable into nitrobenzene from aqueous solution. Formation of the coloured complex when nebivolol hydrochloride was treated with cobalt thiocyanate due to the presence of secondary amine group is basis in the present investigation. **Scheme-II** represents the formation of coloured species in the method.

Conclusion

The proposed methods are simple, selective and reproducible and can be used in the routine analysis of nebivolol hydrochloride in bulk drug and formulations with reasonable accuracy and precision.

ACKNOWLEDGEMENTS

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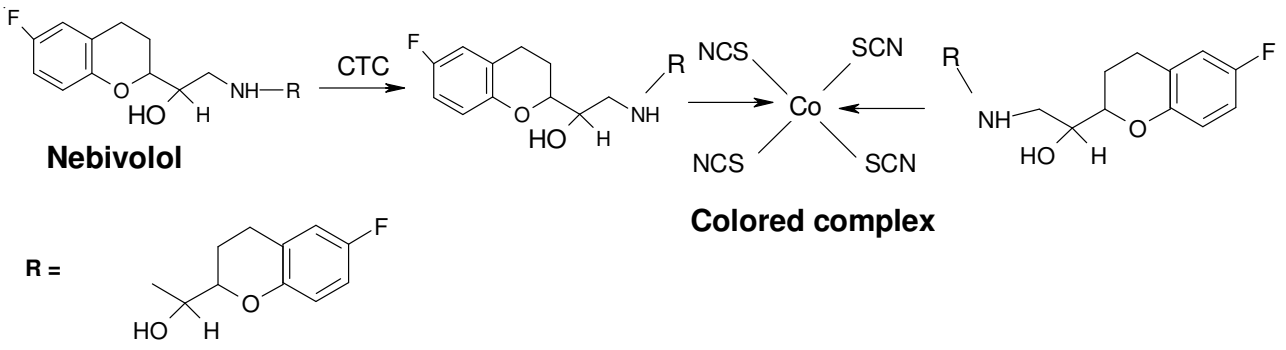

Scheme-II: Co-ordination complex formation of nebivolol hydrochloride with cobalt thiocyanate (CTC)

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