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VALIDATED SPECTOPHOTOMETRIC METHODS FOR THE ESTIMATION OF NEBIVOLOL

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ABSTRACT

Two simple, sensitive and rapid spectrophotometric methods (Method A and Method B) in the visible region have been developed for quantitative estimation of nebivolol from bulk drug and its pharmaceutical formulations (Tablets). Method A is based on oxidation followed by coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with nebivolol in presence of ferric chloride to form bluish green color chromogen, which exhibits absorption maximum at 655 nm. The Beer's law was obeyed in the concentration range of 1 - 5 µg/mL. Method B is based on oxidation followed by complex formation with potassium ferricyanide with nebivolol in presence of ferric chloride to form a stable blue colored chromogen with maximum absorption at 728 nm. The Beer's law was obeyed in the concentration range of 5 - 25 µg/mL. The results of the analysis for both the developed methods have been validated statistically by recovery studies. The results obtained with the proposed methods (Method A and Method B) were compared with UV spectrophotometric method in methanol at 282 nm.

Key words: Spectrophotometric, Nebivolol, MBTH, Potassium ferricyanide.

INTRODUCTION

Nebivolol[1,2] is chemically 1-(6-flourochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino}ethanol or 2, 2′-azane-diylbis (1-(6-flourochroman-2-yl) ethanol. Nebivolol is a long-acting, cardio-selective β1-receptor antagonist without partial agonist activity[3] and it used in the treatment of hypertension. Nebivolol is the racemate (dl-nebivolol) of the enantiomers l-nebivolol and d-nebivolol. It is available as nebivolol hydrochloride. It is not official in any pharmacopoeia.

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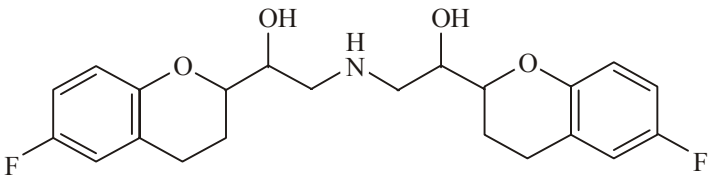

Nebivolol

Literature survey reveals that few analytical methods have been reported, which include liquid-chromatography with tandem mass spectrometry[4], RP-HPLC and HPTLC methods[5] and first order derivative spectrometric determination[6], liquid chromatography coupled with electro spray ionization tandem mass spectrometry[7] of nebivolol in bulk and tablets. There is no analytical method reported for the estimation of nebivolol using visible spectrophotometry in pharmaceutical formulation.

The present work deals with the visible spectrophotometric estimation of nebivolol in pure drug and its formulations. The principle of method A is based on oxidation followed by coupling of MBTH with nebivolol in presence of ferric chloride to form bluish green coloured chromogen. The principle of method B is based on oxidation followed by reduction of potassium ferricyanide with nebivolol in presence of ferric chloride to form green coloured chromogen.

EXPERIMENTAL

Instrument

All spectral measurements were made on Systronics 119 UV/Visible spectrophotometer with 1 cm matched quartz cell.

Materials and reagents

All the purified samples of commercially available analytical grade chemicals are used.

3-Methyl-2-benzothiazolinone hydrazone (MBTH) (0.5% w/v in distilled water)

Potassium ferricyanide (0.2% w/v in distilled water)

Ferric chloride (1% and 1.5% w/v in distilled water)

Methanol

Distilled water

Nebivolol (Hetero Chemicals Ltd.)

Preparation of standard and sample drug solution

Accurately weighed 100 mg of nebivolol (bulk drug or its formulation) was dissolved in 30 mL methanol. It was allowed to stand for sometime to ensure complete solubilisation. The solution was filtered by using Whatmann filter paper. The residue was washed 3 times with 10 mL portions of methanol and the total volume of the filtrate was made upto 100 mL with methanol. The final concentration was made to 1 mL = 1000 µg/mL (stock solution-1). Further dilution was made with methanol to get concentration of 100 µg/mL (stock solution-2).

Assay procedure

Method A: Fresh aliquots of nebivolol ranging from 0.1 - 0.5 mL (1 mL =100 µg/mL) were transferred into a series of 10 mL volumetric flasks. To each of the above aliquots, ferric chloride (1%, 2 mL) and MBTH (0.5%, 1 mL) was added and set aside for 15 min at room temperature. The solution in each volumetric flask was made up to mark with distilled water. The absorbance of bluish green colored chromogen was measured at 655 nm against the reagent blank. The amount of nebivolol present in the sample solution was computed from its calibration curve.

Method B: Fresh aliquots of nebivolol ranging from 0.5 – 2.5 mL (1 mL = 100 µg/mL) were transferred into a series of 10 mL volumetric flasks. To each of the above aliquots ferric chloride (1.5%, 1 mL) and potassium ferricyanide (0.2%, 0.5 mL) was added and set aside for 10 min at room temperature. The solution in each volumetric flask was made up to the mark with distilled water. The absorbance of blue colored chromogen was measured at 728 nm against the reagent blank. The amount of nebivolol present in the sample solution was computed from its calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity and Sandell's sensitivity were calculated and are reported in Table 1. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r) for different concentrations and the results are summarized in Table 1.

The percent relative standard deviation and percent range of errors (0.05 and 0.01 level of confidence limits) were calculated from the eight measurements, 3/4th of the amount of upper Beer's law limits in each method are summarized in Table 1. The results showed that the methods have reasonable precision. The results obtained with the proposed

methods are compared with the results obtained with UV-spectrophotometric method. The results obtained with proposed methods confirmed the suitability of these methods for pharmaceutical dosage forms. In both the methods, the optimum concentration for the estimation of nebivolol was established by varying one parameter at a time and keeping the other fixed. The effect of product on the absorbance of the coloured species was observed and it was incorporated in the procedures. The optimum concentration for the estimation of nebivolol was established by varying drug concentration by keeping the reagent concentration fixed. After establishing the optimum concentration for drug, the reagent concentration was varied. The above ranges of drug and reagent concentrations were chosen so that the coloured species formed gave better absorbance and obeyed Beer's law satisfactorily.

Table 1: Optical characteristics and precision

	Method A	**Method B**
λmax (nm)	655	728
Beer's Law limit (µg/mL)	1-5	5-25
Color	Bluish green	Blue
Molar absorptivity ($L/mol^{-1}cm^{-1}$)	5.2409×10^4	1.447×10^4
% RSD	0.282	0.0379
Sandell's sensitivity (µg/mL/0.001 abs units)	0.041	0.056
Regression equation (Y^*)		
Slope (b)	0.370	0.5208
Intercept (a)	0.7476	7.3254
Correlation coefficient (r)	0.9991	0.9997
Range of errors**		
Confidence limits with 0.05 level	± 0.001437	± 0.0002612
Confidence limits with 0.01 level	± 0.002127	± 0.0003864

*Y= bc +a, Y is the absorbance unit and c is the concentration in µg/mL.

**Eight measurements.

The other active ingredients and excipients like starch, gelatin, talc, magnesium sterate, lactose and steric acid present in pharmaceutical dosage forms did not interfere in the

estimation. When some commercial dosage forms (T1 and T2) were analyzed by this method, the accuracy of the method was confirmed by the recovery studies, by adding a known amount of pure drug to the formulation already analyzed by these methods and the analytical data are presented in Table 2.

Table 2: Estimation of nebivolol in pharmaceutical preparations

Sample	Labeled amount (mg)	Amount obtained (mg)			Percentage recovery*	
		Proposed methods		Reference method (UV method)		
		A	B		A	B
T1	5	4.97	4.96	99.98	99.40	99.20
T2	5	4.93	4.91	99.90	98.60	98.20

*Mean and standard deviation of eight determinations.

T1 and T2 are tablets from Torrent and Lupin Pharmaceuticals, respectively.

The methods reported were found to be simple, sensitive, accurate, precise and economical and can be used in the determination of nebivolol from pharmaceutical dosage forms in a routine manner.

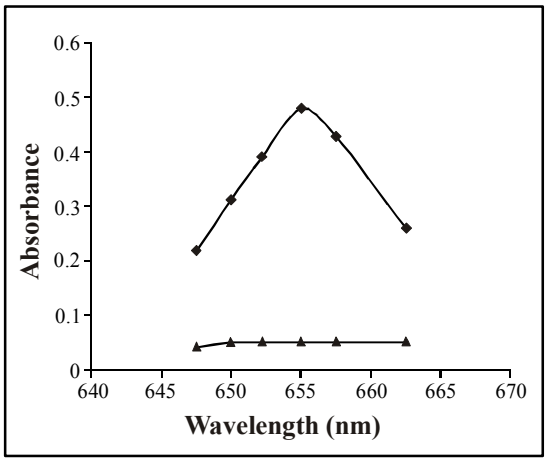

Fig. 1: (♦) Chromogen Vs 3-methyl-2-benzothiazolinone hydrazone (MBTH) Nevbivolol 4 µg/mL (▲) 3-Methyl-2-benzothiazolinone hydrazone (MBTH) Vs solvent

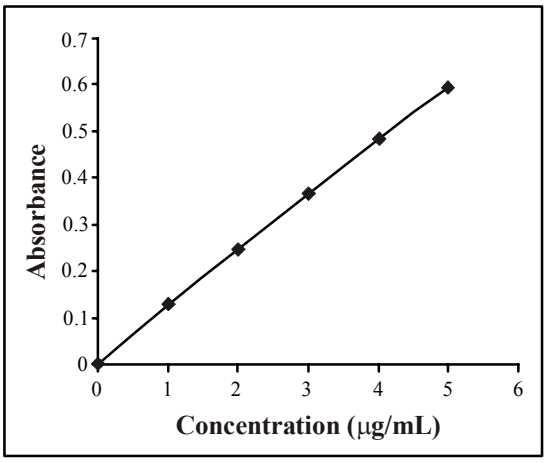

Fig. 2: Beer's law curve for nebivolol with MBTH

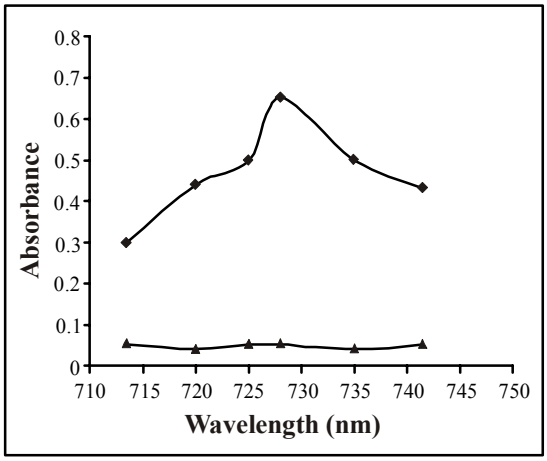

Fig. 3: (♦) Chromogen Vs Potassium ferricyanide nevbivolol 15 µg/mL (▲) Potassium ferricyanide Vs solvent

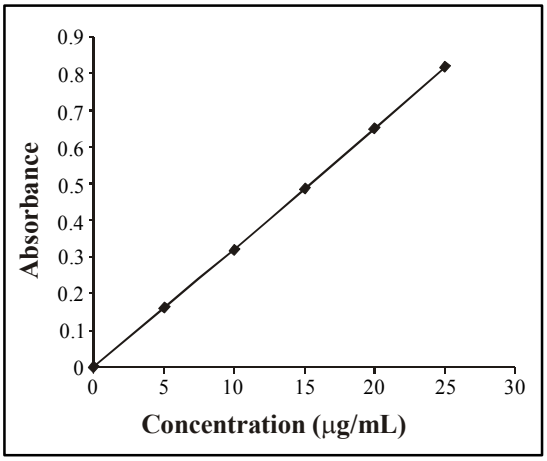

Fig. 4: Beer's law curve for nebivolol with potassium ferricyanide

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REFERENCES

1. M. J. O'Neil (Ed.), The Merck Index-An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co., Inc., 14th Edition, (2006) p. 6432.
2. S. C. Sweetman (Ed.). Martindale-The Complete Drug Reference, Pharmaceutical Press, London (U.K.), 35th Edition, (2007) p. 121.
3. G. H. Joel and E. L. Lee (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, New York, 10th Edition, (2001) p. 257.
4. M. Thevis, G. Opferman and W. Schanzer, Biomed. Chromatogram., **15**, 393-402, (2001).
5. L. J. Patel, B. N. Suhagia and P. B. Shah. Indian J. Pharm. Sci., **69(4)**, 594-596, (2007).

6. A. Atul, Shrikhedkar, M. Prasad Bugdane and J. Sanjay Surana, Pharma Review, 141 (2008).

7. N. V. S. Rama Krishna. J. Pharma. Bio. Anal., **39**, 1006-1013 (2005).

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