RESEARCH ARTICLE

UV Spectrophotometric method for the estimation of nebivolol HCL in bulk and pharmaceutical formulations

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ABSTRACT

In this study, a simple, sensitive and highly accurate ultraviolet pectrophotometric method has been developed and validated for determination of nebivolol hydrochloride in bulk and pharmaceutical formulations. The method is based on the measurement of the absorbance of nebivolol hydrochloride solution in methanol: phosphate buffer pH 6.8 (10:90) at 282 nm in the wavelength range of 200 - 400 nm. Beer's law was obeyed in the concentration range of 10-50 µg/mL. The slope, intercept and correlation coefficient were also calculated. Results of percentage recovery shows that the method was not affected by the presence of common excipients in tablets. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification which proves suitability of proposed method for routine estimation of nebivolol HCL in bulk and pharmaceutical formulations.

Keywords: Nebivolol hydrochloride, Spectrophotometry, Estimation, Tablets.

INTRODUCTION

Nebivolol is a third-generation β (beta) 1 selective works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure [1]. Nebivolol hydrochloride (NEB-H) chemically α, α'- [Iminobis (methylene)] bis [6-fluoro-3, 4-dihydro-2H-1-benzopyran-2- methanol] hydrochloride is a white odourless powder used for the treatment of hypertension and heart failure [3-5]. Its mode of action is lowering blood pressure by reducing peripheral vascular resistance, and significantly increasesstroke volume with preservation of cardiac output. The net hemodynamic effect of nebivolol is the result of a balance between the depressant effects of beta-blockade and an action that maintains the cardiac output. Nebivolol is the racemate (dl-nebivolol and d-nebivolol. It is a competitive and highly selective beta-1 receptor antagonist with mild vasodilating properties, possibly due to an interaction with the L-arginine/nitric oxide pathway. In animal models nebivolol has been shown to induce endothelium – dependent arterial relaxation in a dose dependent manner, by stimulation of the release of endothelial nitric oxide. Nitric oxide is produced in artery walls and acts to relax vascular smooth muscle cells. It also inhibits platelet aggregation and adhesion and may protect against vascular damage as it inhibits leukocyte activation and vascular smooth muscle cell proliferation [6-7].

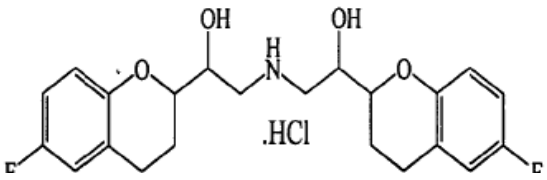

Fig. 1: Structure of Nebivolol hydrochloride

Literature survey reveals that several spectrophotometry [8], LC [9], HPTLC [10], HPLC [11], RP-HPLC [12-13], LC-MS [14] methods have been reported for the estimation of nebivolol hydrochloride in pure and tablet dosage form. The scope of present investigation was to develop and

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validate UV spectroscopy method for quantification of nebivolol hydrochloride in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Materials

Nebivolol hydrochloride was obtained as a gift sample from Cipla Ltd., Mumbai, India. All analytical grade chemicals and solvents were supplied by S.D. Fine Chemicals, Mumbai, India. Distilled water was used to prepare all solutions. Freshly prepared solutions were always employed. Two brands of tablets namely, NEBICARD (Torrent Ltd) and NEBINEX (Glenmark Pharmaceutical Ltd) were purchased from the local market.

Equipment

The UV-Visible Spectrophotometer (Jasco-V630) with data processing system was used. The sample solution was recorded in 1cm quartz cells against solvent blank over the range 200-400nm. A Citizen electronic analytical balance was used for weighing the sample. An ultrasonicator bath (PCI Analytics Pvt. Ltd.) was used for sonicating the tablet powder.

Development of method

Accurately weighed 10 mg of nebivolol hydrochloride was solubilized by 10 ml of methanol in a 100 ml volumetric flask, and phosphate buffer pH 6.8 was added to make up the volume so as to give stock solution of concentration 100 µg/ml. The standard solutions were diluted with phosphate buffer pH 6.8 to obtain various dilutions (10, 20, 30, 40, 50 µg/ml) in standard volumetric flasks (10 ml). The dilutions were scanned in the wavelength range of 200-400 nm. The _max of Nebivolol hydrochloride was found at 282 nm. The linear relationship was observed over the range of 10-50 µg/ml. Absorbances were noted at 282 nm against pH 6.8 phosphate buffer as a blank. A calibration graph of the absorbance versus the concentration of the drug was plotted and represented in figure 1.

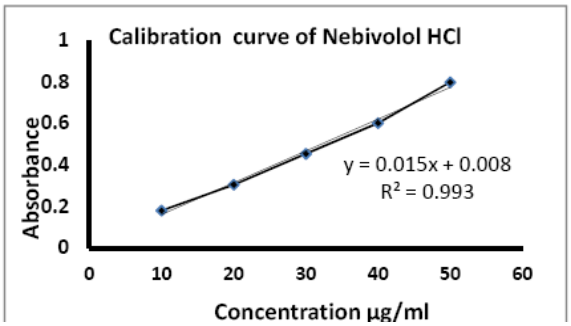

Fig.1 Calibration curve of Nebivolol hydrochloride in pH 6.8 phosphate buffer

Procedure for dosage forms

For analysis of commercial formulations, twenty tablets were taken and powdered. Tablet powder equivalent to 10 mg of Nebivolol hydrochloride was dissolved in small quantity of methanol into a 100 mL volumetric flask and final volume was made up to 100 ml with pH 6.8 phosphate buffer and sonicated for 30 minutes. Then the absorbance of the solution (after suitable dilution) was measured at 282 nm using UV/visible spectrophotometer (Jasco-V630) against pH 6.8 phosphate buffer as a blank. The percentage drug content was calculated with the help of calibration curve (n=3).

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [15-16].

Linearity (Calibration curve)

The developed method validates as per ICH guidelines. The plot of absorbance verses concentration is shown in figure 1. It can be seen that plot is linear in the concentration range of 10-50 µg/ml with correlation coefficient (r^2) of 0.993.

Precision (repeatability)

Intraday and interday precision was determined by measurement of the absorbance for three times on same day and on three different days. The relative standard deviation for replicates of sample solution was less than 2 % which meet the acceptance criteria for established method. The obtained results are presented in table 1.

Table 1: Precision study for proposed method

Concentration (µg/ml)	Absorbance Mean	Standard deviation	% Relative standard deviation
Intraday precision (n=3)			
10	0.150	0.0030	2
20	0.312	0.0026	0.83
30	0.433	0.0031	0.71
Interday precision (n=3)			
10	0.148	0.0035	2.02
20	0.312	0.0030	0.96
30	0.430	0.0042	0.97

Accuracy (recovery study)

Recovery studies were carried out by adding a known quantity of pure drug to the preanalysed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated as per ICH guidelines. The data were presented in table 2.

Table 2: Recovery study

Sr. No.	Label claim, mg/tablet	Amount of standard added, mg	Total amount recovered, mg	% Recovery	Standard deviation	% Relative standard deviation
1	5.00	5	9.93	99.3	0.0020	1.34
2	5.00	10	15.06	100.4	0.0021	0.88
3	5.00	15	19.73	98.65	0.0020	0.67

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were separately determined based on method of the intercept and the average value of slope. (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline.

$LOD = 3.3\ \sigma/S$ $LOQ = 10\ \sigma\ /S$

Where, σ = the standard deviation of the response.

S = slope of the calibration curve.

RESULTS AND DISCUSSION

Beers law is obeyed over the concentration range of 10 - 50 µg/ml, using regression analysis the linear equation y = 0.015x + 0.008 with a correlation coefficient of 0.993. The limit of detection was found to be 0.677 µg/mL. The limit of quantification was found to be 2.05 µg/mL. The percentage purity of Nebivolol hydrochloride in Brand I (NEBICARD), Brand II (NEBINEX) was found to be 99 %, 100.2 % respectively. Precision was calculated with intra and interday variation. Recovery study was performed on formulations and % RSD was found. The optical parameters such as Beer's law limit, slope, and intercept values were calculated and given in table 3. Method was validated for accuracy and precision. The accuracy of method was proved by performing recovery studies in commercially available formulations. The results were given in table 2 and shows relative standard deviation of less than 2 % was observed for analysis of three replicate samples, indicating precision and reproducibility. The percentage recovery value indicates that there is no interference from the excipients present in the formulation. The applicability of the proposed method for the assay of Nebivolol hydrochloride in tablet formulation was examined by analyzing commercial formulations and the results are tabulated in table 4. The result obtained were good agreement with the label claims of marketed products. The results of analysis of the commercial tablets and the recovery study of the drug suggested that there is no interference from any excipients such as starch, lactose, magnesium stearate etc. which are commonly present in tablets.

Table 3: Optical parameters for determination of Nebivolol hydrochloride

Sr. No.	Parameters	Data
1	λ-Max	282 nm
2	Beer's law limit	10 – 50 µg/mL
3	Regression equation	y = 0.015x + 0.008
4	Correlation coefficient	R^2 = 0.993
5	Slope	0.015
6	Intercept	0.008
7	Limit of detection	7 0.677 µg/mL
8	Limit of quantification	8 2.05 µg/mL

Table 4: Results of assay

Formulation	Label claim, mg/tablet	Amount found*, mg/tablet	% Amount found	% C.V.
Brand I (NEBICARD)	5.00	4.95	99	1.52
Brand II (NEBINEX)	5.00	5.01	100.2	2.01

*Mean of three determinations.

CONCLUSION

The simple spectrophotometric method for determination of Nebivolol hydrochloride have been developed and validated as per ICH guidelines. The developed method is found to be sensitive, accurate and reproducible and can be used for the routine quality control analysis of Nebivolol hydrochloride in bulk and pharmaceutical formulations.

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