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Research Article

REVERSE PHASE HPLC METHOD FOR THE ANALYSIS OF NEBIVOLOL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Nebivolol[1]chemically [1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol)] is a long acting cardio selective beta-blocker currently used for the treatment of hypertension. A sensitive and precise RP-HPLC method has been developed and validated for the determination of Nebivolol in dosage forms. The drug was chromatographed on a C-18 column using a mixture of water and Methanol in the ratio (40:60) as mobile phase at a flow rate of 1.0 ml/min. Chlorzoxazone was used as an internal standard and detection was done at 282 nm. Linearity range is found to be 5-100 µg/ml with a correlation coefficient 0.9999 .The mean recoveries obtained for Nebivolol range from 99.6-100.8%. Due to its simplicity, rapidness, high precision and accuracy the proposed method may be used for determining Nebivolol in bulk and dosage forms.

Key words: Nebivolol, RP-HPLC

INTRODUCTION

Nebivolol[1] chemically [1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol)] is a long acting cardio selective beta blocker currently used for the treatment of hypertension. A survey of literature revealed that very few methods for the separation and estimation of Nebivolol have been reported. Simultaneous determination of fixed dose combination of Nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to pharmacokinetic study have been reported[2], Rapid quantification of Nebivolol in human plasma by liquid chromatography coupled with electro spray ionization tandem mass spectrometry [3].and a stability–indicating column high-performance liquid chromatographic assay method for determination of Nebivolol in tablet formulation[4]have been reported. The authors now propose a precise and accurate HPLC method for the determination of Nebivolol in this communication.

EXPERIMENTAL PROCEDURE

Instrumentation:

An isocratic HPLC system Shimadzu LC10AT VP series HPLC pumps, and SPD 10A VP UV-Visible absorbance detector, Hamilton injecting syringe and Shimadzu CLASS-VP Version 6.12 SP1software, Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 micron) column were used for the separation.

Drugs and Chemicals:

Pure samples of Nebivolol and Chlorzoxazone (internal standard) were gifted by Cadila Pharmaceuticals Pvt.ltd (Ahmedabad, India) and Elder pharmaceuticals Limited (Mumbai, India) respectively. And Nebistar tablets (Lupin, pharma) containing 2.5 mg 5 mg, and 10mg of Nebivolol were purchased from the local market. Purified water was prepared using a Millipore Milli-Q (HPLC grade) water purification system. Acetonitrile used as HPLC grade.

Chromatographic conditions:

The mobile phase used in this study was a mixture of water and Methanol in the ratio of 40:60 v/v. The mobile phase was filtered before use through a 0.45 µ membrane and degassed for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 25 ± 1^0C.The eluents were monitored at 282 nm.

Procedure:

Stock solution of the drug and internal standard were prepared by dissolving 10mg of Nebivolol and 10 mg of internal standard (chlorzoxazone) separately in 10 ml volumetric flasks to get 1mg/ml solution and sonicated for about 10 min to ensure complete solubility of drug. Subsequent dilutions of this solution ranging from 5-100 µg/ml were made in 10ml volumetric flasks after addition of 5ml Chlorzoxazone (5µg/ml) as internal standard to each dilution. Before injection of the drug solutions, the column was equilibrated at least for 30 min with the mobile phase flowing through the system.20µl of each solution was injected in to the HPLC system to obtain the chromatogram. The area under the peaks of the drug and the internal standard were noted. Using these values the mean peak area ratio of the drug to that of internal standard for each dilution was calculated. Regression of the drug concentration over these ratios was computed. Calibration curve was constructed by plotting mean peak area against the corresponding drug concentrations. Model chromatogram was shown in fig-1.

Estimation of Nebivolol in Tablet dosage forms:

Ten tablets were weighed to get the average weight and pulverized and the powder equivalent to 10mg of Nebivolol was extracted with methanol and sonicated for about 15 min and made-up the volume to 10 ml to get a stock solution of 1 mg/ml. This solution was filtered through a 0.45 µ membrane filter. This solution was further diluted stepwise with mobile phase and spiked with required amount of internal standard. These solutions were injected into the

column .The mean peak area ratio of the drug to the internal standard was calculated. The drug content in the tablets was quantified using the regression equation. The results are given in table-3.

METHOD VALIDATION

Linearity:

The standard curve was obtained in the concentration range of 5-100 µg/mL. The linearity of the method was evaluated by regression analysis using the least squares method.

Precision:

The precision of the assay was determined in terms of repeatability and intra and inter-day variations in the peak areas for a set of drug solutions on three different days. The intra and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of %R.S.D.

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of bulk sample Nebivolol and internal standard to the pre-analyzed formulation.

System suitability:

System suitability studies were carried out as specified in U.S.P. These parameters include column efficiency (N), resolution (R), capacity factor ($K^{'}$), Selectivity factor (α) and Peak asymmetry factor (A_S).

RESULTS AND DISCUSSION

To achieve sharp peaks with good resolution under isocratic conditions several mobile phase compositions were tried. Peak symmetry was obtained with mobile phase consisting of Water: Methanol (40:60). Quantification was achieved with UV detection at 282nm.Under the above mentioned chromatographic conditions the retention time obtained for Nebivolol and internal standard were 3.150and 4.125 min. A model chromatogram is shown in fig-1. The linear regression data showed a good linear relationship over a concentration range 5-100µg/ml The regression curve was constructed by linear regression fitting into mathematical expression Y=0.0407+0.0174X(r=0.9999) where Y is the peak area ratio and X is the concentration. These values are given in (Table-1).The intra-day and inter-day precision were determined by analyzing Nebivolol solutions containing 20 and 80µg/ml by the proposed method .A low coefficient of variation was observed. This shows that the present HPLC method is highly precise.

Accuracy and recovery studies were carried out using standard addition method All solutions were prepared and analyzed in triplicate .There was a high recovery 100.8±1.38 of Nebivolol indicating that the proposed methods is highly accurate. %recovery values were given in (Table-2). The drug content in tablets was quantified using the proposed analytical method. Results were shown in table-3.The system suitability parameters are theoretical plates(n) -1600,Tailing factor (T)-1.33, Resolution (R)-0.98, also revealed that the values are with in the specified limits.

Table-1

CALIBRATION OF THE HPLC METHOD FOR THE ESTIMATION OF NEBIVOLOL

Concentration (µg/ml)	Peak area ratio
5	0.245
10	0.410
20	0.8193
30	1.2389
40	1.6437
50	2.0626
80	3.275
100	4.093

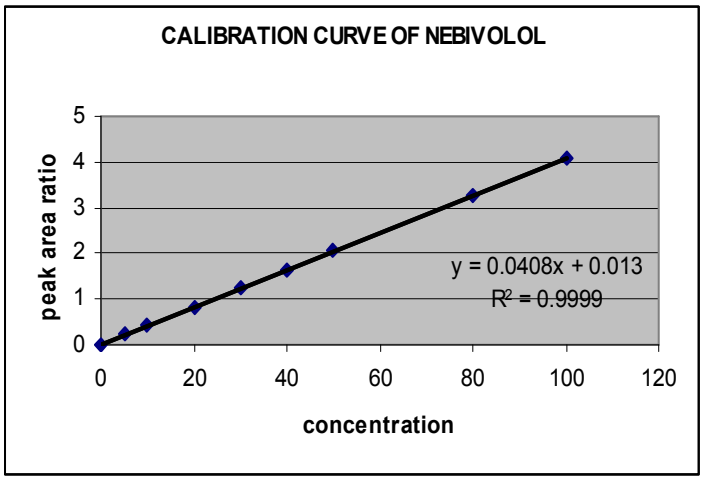

Fig-1 HPLC Chromatogram of Nebivolol

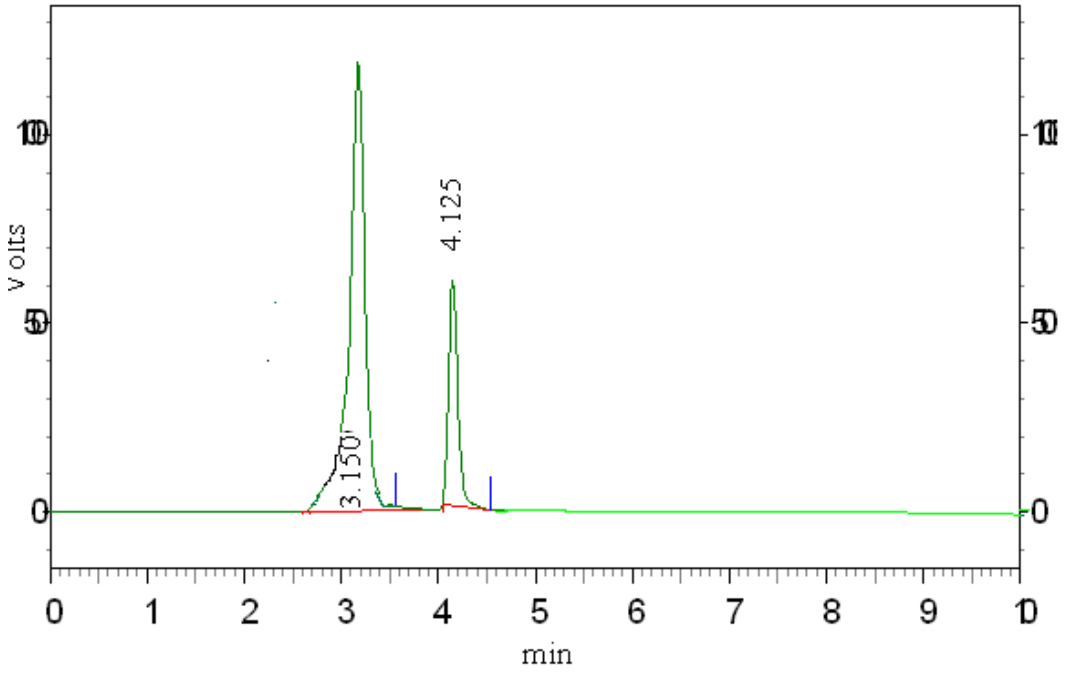

Fig-2 UV-Spectra of nebivolol

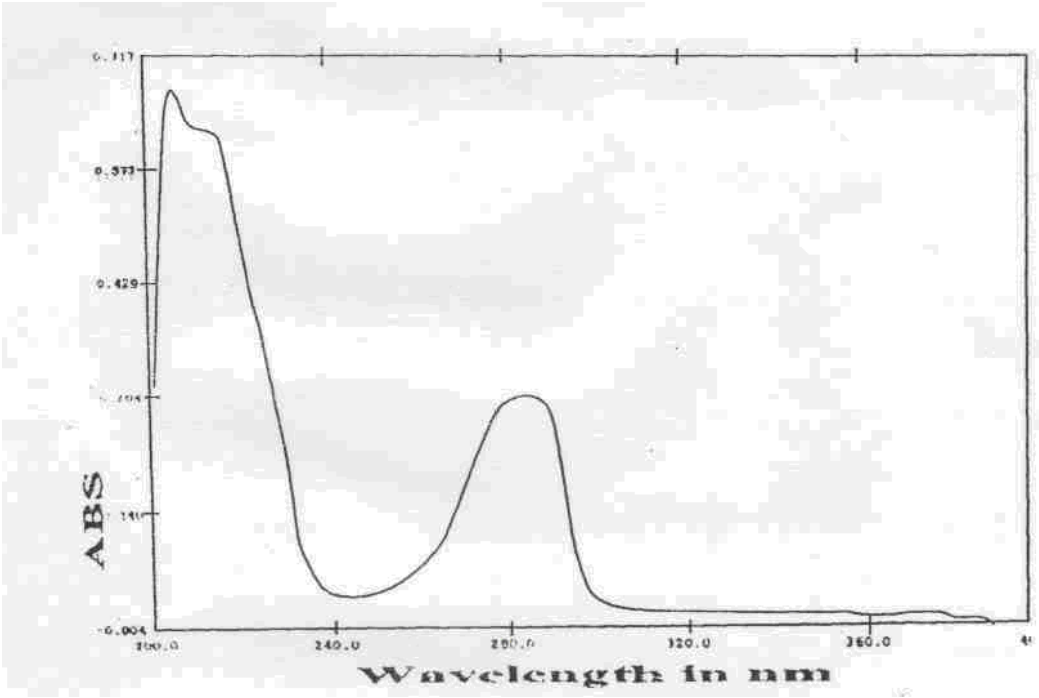

TABLE- 2

ACCURACY STUDIES

Amount of drug added (mg) N=3	Amount found (Mean±sd) N=3	%recovery(Mean± sd) N=3
2.5	2.49±0.02	100.08±1.02
5	4.98±0.06	99.6±1.19
7.5	7.56±0.10	100.8±1.38

TABLE-3

AMOUNT OF NEBIVOLOL IN DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Formulation	Labeled Claim(mg)	Amount found mg±sd	%recovery(±sd)
Brand I	5.0	5.068±0.02	101.36±0.69
Brand II	5.0	5.077±0.01	101.54±0.16

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for determination of Nebivolol in pure state and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non- interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of nebivolol in pure form and its dosage form.

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