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Research Article

DEVELOPMENT AND VALIDATION METHOD FOR QUANTIFICATION OF NEBIVOLOL IN FORMULATION ANALYSIS BY USING RP-HPLC

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ABSTRACT

A simple, rapid, precise and accurate RP-HPLC method was developed and validated for rapid assay of Nebivolol in tablet dosage form. Chromatographic separation of Nebivolol was performed by using a C_{18} column as stationary phase with a mobile phase comprising of 0.05 M of potassium dihydrogen phosphate, methyl alcohol (30:70 V/V) at flow rate of 1.0 ml/min and U.V detection at 225 nm. The linearity of Nebivolol is in the range of 10-50 ppm/ml. The limits of detection (LOD) and quantification (LOQ) were found to be 0.15 and 0.5ppm respectively. The recovery was calculated by standard addition method. The proposed method was found to be accurate, precise and rapid for the analysis of Nebivolol in formulation.

INTRODUCTION

Nebivolol is a lipophilic β-blocker and is generally used to treat high blood pressure. The molecular formula of nebivolol is $C_{22}H_{25}F_2NO_4$ and molecular weight is 405.4. IUPAC name is 1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol. The half life is 10 hrs. Nebivolol lowers blood pressure by, and significantly increases stroke reducing peripheral vascular resistance volume with preservation of cardiac output. It is available under the trade name Nebilet and is stored at room temperature away from moisture and heat. The drug is highly cardio selective at 5mg. However at doses above 10mg nebivolol loses its cardio selectivity and block both β1 and β2 receptors.

MATERIALS AND METHODS

Methanol, potassium dihydrogen phosphate used were of analytical grade.

Chromatographic separation was performed with PEAK High performance Liquid Chromatography having isocratic pump equipped with PEAK LC-UV7000 variable wavelength detector. Chromatograms and data were recorded by means of PEAK Chromatographic software version 1.06.

PREPARATION OF STANDARD SOLUTION

10mg of nebivolol was taken in a 10ml volumetric flask and 10ml of mobile phase was added to obtain 1000ppm of nebivolol. One ml of the stock solution was pipetted out into a 100ml volumetric flask and made up to the mark. The resulting solution was filtered through nylon filter paper. The calibration curve was plotted with the five concentrations of 10-50ppm of working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

CHROMATOGRAPHIC CONDITIONS

Mobile phase	: 0.05M potassium dihydrogen phosphate and methanol (30:70)
P^H	: 3.6
Analytical Column	: C18
U.V. detection	: 225nm
Flow rate	: 1.0ml/min
Injection volume	: 20µl
Temperature	: ambient
Runtime	: 8min
Retention time	: 4.637min

METHOD VALIDATION PROCEDURE

The method is validated for linearity, precision and accuracy. Standard plots were constructed with concentrations 10-50ppm prepared in triplicate to test linearity. The peak area of nebivolol was plotted against the concentration and a linear graph was obtained. The precision of assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from 6 replicate injections of freshly prepared nebivolol test solution in the same equipment on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine the intermediate precision. Peak area of the nebivolol was determined and precision was reported as %RSD (Relative Standard deviation)

LINEARITY

Linearity for the developed method was checked by preparing five solutions of different concentrations ranging from 10-50ppm. The chromatograms were developed and the peak areas are given in Table-1. A linear relationship between area vs. concentration was observed in the range of study.

PRECISION

The Intra-day and Inter-day precisions were determined by taking sample solution of 30ppm concentration. The RSD values for Intra-day and inter-day were found to be 0.510% and 0.212% respectively. The values were given in the Table-2.

ACCURACY AND RECOVERY

To study the accuracy of the proposed method recovery studies were carried out at different spiked levels. A fixed amount of pre analysed samples were taken and standard drug was added at 50%, 100% and 150% levels. Each level is repeated three times. The lower value of RSD of assay indicated that the method is accurate. The results are given in Table-3.

LOQ AND LOD

Limit of Quantification and Limit of Detection were calculated at 0.5ppm and 0.15ppm respectively as per ICH guidelines.

ROBUSTNESS

Robustness was carried by varying two parameters from the optimized chromatographic conditions. No significant change was observed.

SPECIFICITY

The specificity was determined by comparing test results obtained from analysis of sample solution containing exclusive ingredients with that of test results obtained from standard drugs.

FORMULATION

The commercially available Nebilet-5mg tablet was powdered and a solution of 30ppm was prepared. The solution was filtered and injected into the chromatographic system and chromatogram was recorded. The percentage of Nebivolol in tablet was found to be about 0.88.

RESULTS AND DISCUSSIONS

RP-HPLC method developed for determination of drugs has great importance in the quality control analysis. The chromatograms for pure drug were obtained by using different mobile phases like methanol, acetonitrile, THF and different buffers like potassium dihydrogen phosphate in different volume ratios. Different columns like C8, C18, phenyl, cyano with different dimension were used. The retention time and tailing factor were calculated. Finally 0.05M potassium dihydrogen phosphate and methanol (30:70 v/v) and C18 analyzed column were selected which gave a sharp and symmetrical peak with 0.96 tailing. Calibration

graph was found to be linear in the range 10-50ppm. Five different concentrations of nebivolol in the given range were prepared and injected into HPLC. The slope (m) and intercept(c) obtained were found to be 22624.79 and 10494.73 respectively.

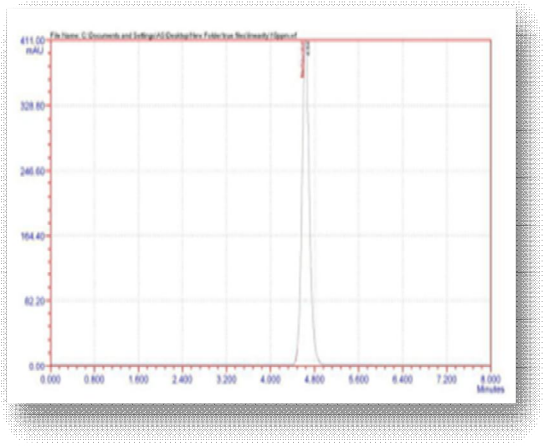
A plot drawn between peak area and concentration of drug solution in the range studied was found to have excellent linear correlation with a correlation coefficient of 0.999.

The LOQ and LOD of nebivolol were found to be 0.15ppm and 0.5ppm respectively indicating the the sensitivity of the method.

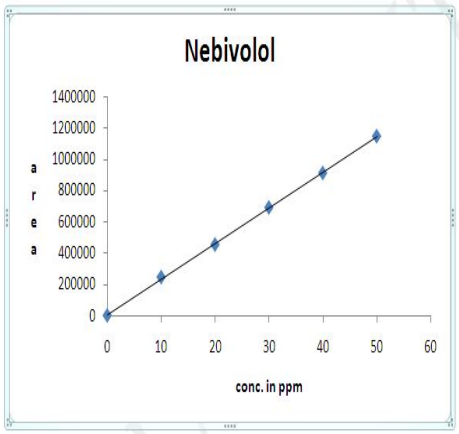
The percentage assay or average amount of nebivolol in formulation was found to be 0.88. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicated high precision of the method.

CONCLUSION

RP-HPLC method for the determination of nebivolol from their formulation was found to be accurate and precise. Thus the proposed HPLC method can be successfully applied for the routine quality control analysis of nebivolol formulations.



A Typical RP-HPLC Chromatogram for Nebivolol



Linearity graph for Nebivolol

Table 1

Linearity level	Concentration ppm	Area
1	10	244672.1
2	20	452938.4
3	30	690648.7
4	40	911103.3
5	50	1146828.9

Table 2

No. of Injections	Intra day	Inter day
1	710926	705473.8
2	718770.3	702836.3
3	717463.8	702503.7
4	717409.9	701658.1
5	710223.1	701753.3
6	713762.2	701509.8

Table 3

concentration	% recovery	Mean recovery
50 %	99.36	98.98 %
100 %	99.16	
150 %	98.42	

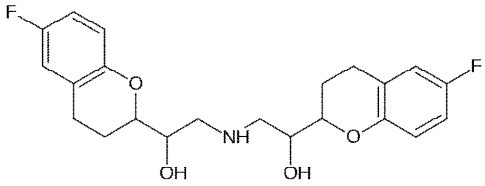


Fig. 1: Structure of Nebivolol

REFERENCES

1. Bilal Yilmaz. RP-HPLC method for determination of Nebivolol in pharmaceutical preparations; International Journal of Pharmaceutical Science Review and Research. 2010;1.
2. Cheymol G, Woestenborghs R, Snoeck E, Ianucci R, Le Moing JP, Naditch L, Levron JC and Poirier JM. Pharmacokinetic Study and Cardiovascular Monitoring of Nebivolol in Normal and Obese Subjects. Eur J Clin Pharmacol. 1997;51:493-498.
3. Kachhadia PK, Doshi AS and Joshi HS. Development and validation of a stability-indicating column high performance liquid chromatographic assay method for determination of nebivolol in tablet formulation. JAOAC Int. 2008;91:557-561.
4. Kamila MM, Mondal N, Ghosh LK and Gupta BK. A validated UV spectrophotometric method for estimation of nebivolol hydrochloride in bulk and pharmaceutical formulation. Pharmazie. 2007;62:486-487.
5. Maurer HH, Tenberken O, Kratzch C, Weber AA and Peters FT. Screening for library-assisted identification and fully validated quantification of 22 beta-blockers in blood plasma by liquid chromatography-mass spectrometry with atmospheric pressure chemicalionization. J Chromatogr A. 2004;1058:169-181.
6. Ramakrishna NVS, Vishwottam KN, Koteshwara MS, Santosh MM and Varma DP. Rapid quantification of nebivolol in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal. 2005;39:1006-1013.