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RP-HPLC Method for the Estimation of Nebivolol in Tablet Dosage Form

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Abstract: A reverse phase HPLC method is described for the determination of nebivolol in tablet dosage form. Chromatography was carried on a Hypersil ODS C_{18} column using a mixture of methanol and water (80:20 v/v) as the mobile phase at a flow rate of 1.0 mL/min with detection at 282 nm. Chlorzoxazone was used as the internal standard. The retention times were 3.175 min and 4.158 min for nebivolol and chlorzoxazone respectively. The detector response was linear in the concentration of 1-400 µg/mL. The limit of detection and limit of quantification was 0.0779 and 0.2361 µg/mL respectively. The percentage assay of nebivolol was 99.974%. The method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, fast, accurate and precise and hence can be applied for routine quality control of nebivolol in bulk and tablet dosage form.

Keywords: Nebivolol, RP-HPLC, Estimation, Tablets.

Introduction

Nebivolol with a chemical name [α-α'-{Iminobis (methylene)} *bis* {6-fluoro-3, 4-dihydro-2*H*-1-benzopyran-2-methanol}] is a long acting, cardio selective beta-blocker currently licensed for the treatment of hypertension. Nebivolol is a racemate of two enantiomers, SRRR- nebivolol (or *d*- Nebivolol) and RSSS- nebivolol (or *L*- Nebivolol). It combines two pharmacological activities: (i) it is a competitive and selective B1-recepter antagonist which is attributable to the *d*-enantiomer, (ii) it has mild vasodilating properties, possible due to an interaction with the *L*-arginine/nitric oxide pathway. It is reported to have vasodilating activity

but to lack intrinsic sympathomimetic and membrane stabilizing activity. Nebivolol is used in the management of hypertension. It is given by mouth as the hydrochloride although doses are expressed in terms of base. The usual dose is 5 mg daily. An initial dose of 2.5 mg daily is employed in the elderly and in patients with renal impairment[1-2].

It is official in martindale[2]-the extra pharmacopoeia. A few analytical methods have been reported in pharmaceutical formulation, which include UV method[3-4], Liquid chromatography-mass spectroscopic (LC-MS) methods[5-7] for analysis of nebivolol in biological fluids, HPLC[8-11] and flourimetric methods[12]. In view of the above fact, some simple analytical methods are in need for its quantitative estimation. In the present work, one simple, sensitive, economical and accurate RP-HPLC method has been developed for the quantitative estimation of nebivolol in bulk and pharmaceutical formulations (tablets).

Experimental

Nebivolol was obtained as a gift sample from Mepro Pharma Ltd, Gujurat. Methanol HPLC grade (MERCK Ltd.), Water HPLC grade (triple distilled water) were used.

Instrument

High Performance Liquid Chromatograph with Shimadzu LC-10AT and LC-10AT VP series HPLC pumps, with a 20 µL sample loop (manual), and SPD 10A VP UV-Visible absorbance detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software.

Chromatographic conditions

Chromatographic separations were achieved using a Hypersil ODS C_{18} (250 x 4.6 mm, 5µ) analytical column. The mobile phase consisting of methanol and water (80:20 v/v) was passed through 0.45 µm membrane filter and degassed by ultrasonication. The flow rate was maintained at 1.0 mL/min and the measurements were made at 282 nm. The column and the HPLC system were kept in ambient temperature.

Preparation of mobile phase

The mobile phase was prepared by mixing of triple distilled water and methanol in the ratio of 20:80 and sonicated for degassing followed by filtration.

Preparation of standard stock solution

Accurately weighed 50 mg of nebivolol standard was taken in 50 mL volumetric flask. This was dissolved in 25 mL of mobile phase and sonicated for 5 minutes and then diluted to 50 mL with the mobile phase to get 1 mg/mL standard stock solution. Then the stock solution of internal standard (Chlorzoxazone) was prepared by dissolving 50 mg of chlorzoxazone in 50 mL volumetric flasks containing 25 mL of mobile phase and then the final volume was made up to 50 mL with the mobile phase.

Working standard solution

5 mL of the nebivolol stock solution was taken in 50 mL volumetric flask and thereafter made up to 50 mL with mobile phase to get a concentration of 100 µg/mL.

Preparation of sample solution

Twenty tablets (Nebicard, Torrent Pharma) were weighed accurately and finely powdered. The powder equivalent to 50 mg was taken in 50 mL volumetric flask. This was dissolved in

25 mL mobile phase and sonicated for 15 minutes with internal shaking. Then the volume was finally made to 100 mL. The above solution was centrifuged at 3000 rpm for five minutes to get a clear solution. Then pippetted out 5 mL of clear supernatant liquid into 50 mL volumetric flask and made up the volume with mobile phase to get a concentration of 100 µg/mL.

Linearity

Several aliquots of standard stock solutions (0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0) mL (1 mL = 100 µg) and (1.0, 2.0, 3.0 and 4.0) mL (1 mL = 1000 µg) of nebivolol were taken in different 10 mL volumetric flask along with internal standard (Chlorzoxazone) 1 mg/mL (0.2 mL) and diluted up to the mark with mobile phase. Evaluation was performed with SPD 10A VP Ultra-Violet Visible absorbance detector at 282 nm. Peak area was recorded for all the peaks and a Calibration graph was obtained by plotting peak area ratio (drug/internal standard) *versus* concentration of nebivolol (Figure 2). The plot of peak area ratio of each sample against respective concentration of Nebivolol was found to be linear in the range of 1.0- 400.0 µg/mL with correlation co-efficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective slope (m), intercept (b), standard deviation and correlation coefficient are given in Table 1.

Table 1. Linear regression data for calibration curves.

Drug	Nebivolol
Internal standard	Chlorzoxazone
Concentration range, µg/mL	1 - 400
Slope, m	0.0407
Intercept, b	0.021
Correlation coefficient	0.9999
Variance	0.0021
% RSD	0.0415

Assay

20 µL of sample solution with required amount of internal standard solution was injected into the injector of liquid chromatograph. The retention time were found to be 3.175 and 4.150 minutes for nebivolol and internal standard respectively. The amount of drug present per tablet was calculated by comparing the peak area ratio of the sample solution with that of the standard solution. The data are presented in Table 2.

Table 2. Results of HPLC assay and recovery studies.

Sample	Amount claim, mg/tablet	Amount found, mg/tablet	% Recovery*
1	5	4.983	99.666
2	5	5.003	100.052
3	5	5.008	100.165
4	5	4.992	99.833
5	5	4.990	99.799
	Mean	4.995	99.904

**Average of three different concentration levels.*

Recovery studies

Accuracy was determined by recovery studies of nebivolol , known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

Results and Discussion

As per the USP-XXIV system suitability tests were carried out on freshly prepared standard stock solution of nebivolol. Parameters that were studied to evaluate the suitability of the system are given in Table 3.

Table 3. Validation summary

Validation parameter (System suitability)	Results
Theoretical Plates (N)	2309
Linearity range, mcg/mL	1-400
Tailing factor	1.333
Retention time in minutes	
Nebivolol	3.175
Internal standard	4.158
LOD, µg /mL	0.0779
LOQ, µg /mL	0.2361

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for nebivolol were found to be 0.0779 and 0.2361 µg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram as shown in Figure 1, it was found that the retention time of nebivolol and internal standard were 3.175 and 4.158 minutes respectively. A mixture of methanol and water in a ratio of 80:20 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.9999) was observed between the concentration range of 1.0-400.0 µg/mL. The assay of nebivolol tablets was found to be 99.974%. From the recovery, studies it was found that about 99.904 % of nebivolol was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the Tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of nebivolol within a short analysis time.

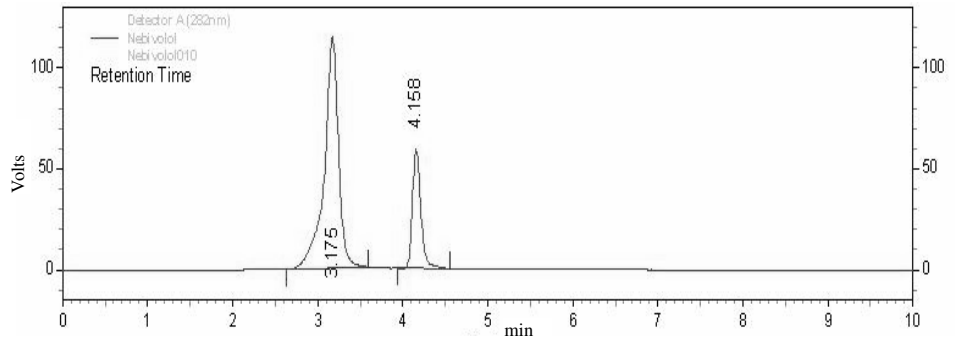

Figure 1. Typical chromatogram of nebivolol by HPLC.

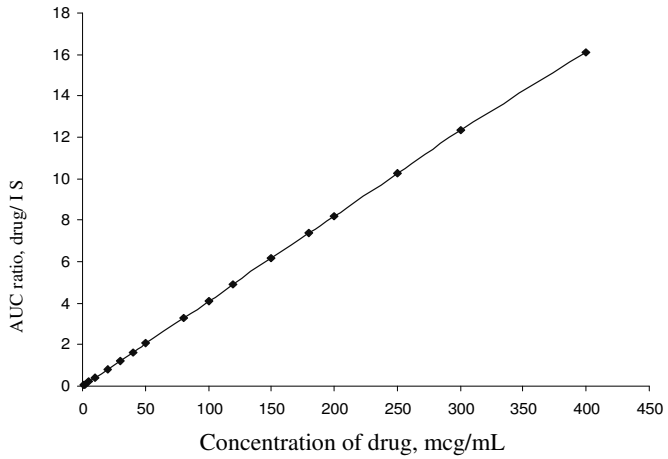

Figure 2. Calibration curve of nebivolol by HPLC.

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