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RP-HPLC Method for the Estimation of Nebivolol in Bulk and Pharmaceutical Dosage Form

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A rapid and sensitive high performance liquid chromatographic method was developed for the estimation of nebivolol in bulk and pharamceutical dosage form. Nebivolol was chromatographed on a reverse phase C_{18} column in a mobile phase consisting of acetonitrile and 30 mM KH_2PO_4 buffer (pH 3.1 adjusted with o-H_3PO_4) in the ratio of 55 : 45 (v/v). The mobile phase was pumped at a flow rate of 0.8 mL/min and the eluents were monitored at 286 nm. The calibration curve was linear in the range of 0.5–50 μg/mL. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solution containing 4 μg/mL was 100.81 ± 0.075 indicating high accuracy of the proposed method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining nebivolol in bulk and pharmaceutical dosage forms.

Key Words: Nebivolol, HPLC, Estimation.

INTRODUCTION

Nebivolol hydrochloride[1], chemically, (±)-[2R*[R*[R*(S*)]]]-α,α′[imino-bis-(methylene)]jh-bis-[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride, a new antihypertensive drug, is a racemate of two enantiomers with four chiral centres. The SRRR-enantiomer (d-nebivolol) is a potent and cardioselective β_1-adrenergic blocker. The RSSS-enantiomer (l-nebivolol) has a favourable hemodynamic profile[2–4]. Literature survey reveals that no specific method was reported for the estimation of nebivolol in formulations. So far only one method has been reported for estimation of nebivolol in plasma[5] by HPLC with fluorescence determination and another method has been reported for the location of hydroxyl functions in hydroxylated metabolites of nebivolol in different species[6]. The aim of the present study is to develop simple, precise, rapid, accurate, RP-HPLC method for the determinaton of nebivolol either in bulk drug samples or in pharmaceutical dosage form. The present study describes the determination of nebivolol in bulk drug samples and pharmaceutical dosage form by using RP-C_{18} column with UV detection.

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EXPERIMENTAL

Nebivolol HCl and Tadalafil were obtained from M/s Cadila Pharmaceuticals Ltd., Ahmedabad, India. Acetonitrile, methanol were HPLC grade and supplied by M/s SD Fine Chemicals Ltd., Mumbai, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate and phosphoric acid were AR grade and supplied by Qualigens, Mumbai, India. A gradient HPLC system (Waters) with Waters 1525 binary HPLC pump, Waters 2487 UV dual λ absorbance detector, Waters breeze software and RP C_{18} column (150 × 4.6 mm I.D., W03281S 024, particle size 5 μm) was used.

HPLC Conditions: The mobile phase components acetonitrile and 30 mM KH_2PO_4 buffer (pH adjusted to 3.1 with orthophosphoric acid) were filtered through 0.45 μm membrane filter before use and were pumped from the solvent reservoir at a ratio of 55 : 45 v/v to the column at a flow rate of 0.8 mL/min. The volume of each injection was 20 μL. The column was equilibrated for at least 40 min with the mobile phase flowing through the systems.

Procedure: The solutions were prepared on a weight basis and volumetric flasks were used to minimize solvent evaporation. Stock solution of drug was prepared by dissolving 100 mg of nebivolol equivalent to nebivolol HCl in 100 mL volumetric flask containing 70 mL of methanol, shaken for about 5 min and then made up to volume with methanol. Daily working standard solution of nebivolol HCl was prepared by suitable dilution of the stock solution with triple distilled water. Five sets of nebivolol HCl solution were prepared in mobile phase at concentrations of 0.5, 1.0, 2.0, 4.0, 8.0, 10, 20, 30, 40 and 50 μg/mL with 1 μg/mL Tadalafil solution as internal standard. Each of these samples (20 μL) was injected five times into the column and the peak area of the drug was recorded.

Assay of Nebivolol HCl in Tablets: 20 tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 5 mg of nebivolol was placed in 50 mL volumetric flask and a few mL of methanol was added, shaken well and allowed to stand for 1/2 h with intermittent shaking to ensure complete solubility of the drug. The mixture was then made up to volume with methanol, thoroughly mixed and filtered through a 0.45 μm membrane filter. The filtrate was further diluted with mobile phase to 8 μg/mL solution. All determinations were conducted in triplicate.

Precision: The precision of the assay was determined in terms of intra- and inter-day variation in the peak area ratio for a set of drug solutions on three different days (n = 5). The intra- and inter-day variation in the peak area ratio of the drug solution (8 μg/mL) was calculated in terms of coefficient of variation.

Accuracy: The accuracy of the HPLC assay method was assessed by adding known amount (4 μg/mL) of the drug to a drug solution of known concentration (4 μg/mL) and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (4 μg/mL) was added to the volumetric flask containing the powder sample of the tablet formulation with known amount of the drug. In both the cases the recovery studies were replicated five times. The

accuracy was expressed in terms of the recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the % recovery.

RESULTS AND DISCUSSION

The runtime of the method was set at 5 min and nebivolol appeared on the chromatogram at 2.11 min and the internal standard Tadalafil at 3.3 min (Fig. 1). When the same drug solution was injected 5 times, the retention time of the drug was same. The ratio of peak areas of nebivolol and Tadalafil was calculated and the average values for 5 such determinations were given in Table-1.

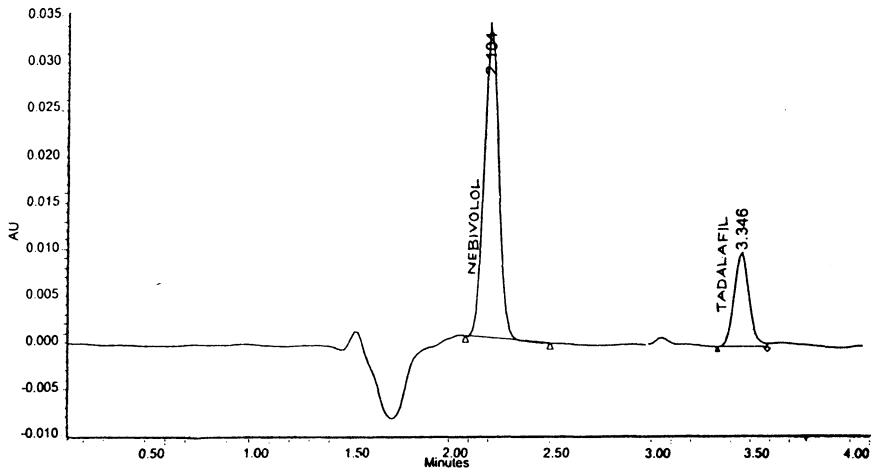

Fig. 1. Typical chromatogram of nebivolol

TABLE-1
CALIBRATION OF THE HPLC METHOD

Concentration of nebivolol (μg/mL)	Peak area ratio*	C.V. (%)
0.5	0.214	0.609
1.0	0.426	0.607
2.0	0.857	0.711
4.0	1.702	0.917
8.0	3.413	0.408
10	4.272	0.152
20	8.539	1.685
30	12.962	1.643
40	17.052	1.027
50	21.358	0.256

*Mean of 5 determinations.

The regression of nebivolol concentration over its peak area ratio was found to be Y = 0.00225 + 0.42764X (r = 0.99998) where 'Y' is the peak area ratio and 'X' is the concentration of nebivolol. This regression equation was used to estimate the amount of nebivolol in tablet formulation. The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 8 μg/mL of nebivolol were repeatedly injected on the same day, the coefficient of variation in the peak area of the drug for five replicate injections was found to be less than 1.25%. Also, the inter-day variation (3 days and five injections) was found to be less than 2.25% (Table-2). Thus, the results show that the proposed HPLC method is highly reproducible. The high % of recovery (100.81 ± 0.075) of nebivolol (Table-3) indicates that the proposed method is highly accurate.

TABLE-2
INTER- AND INTRA-DAY PRECISION FOR NEBIVOLOL ASSAY IN FORMULATION BY THE PROPOSED HPLC METHOD

Nebivolol concentration (μg/mL)	Concentration of nebivolol (μg/mL) found on			
	Intra-day		Inter-day	
	Mean (n = 5)	C.V. (%)	Mean (n = 5)	C.V. (%)
8	7.89	1.2	8.052	2.21

TABLE-3
RECOVERY STUDIES

Amount of drug added to preanalyzed drug solution (μg/mL)	Recovery of nebivolol	
	Amount found (μg/mL) (n = 5)	% recovery (n = 5)
4	4.03	100.81 ± 0.075

The present HPLC method has also been used to quantify nebivolol in tablet dosage form. Nebivolol tablets (containing 5 mg of drug) were analyzed. The average content was found to be 98.75% of the labelled amount (Table-4). No interference peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed HPLC method.

TABLE-4
MEAN AMOUNT OF NEBIVOLOL IN FORMULATION BY PROPOSED HPLC METHOD

Brand of the tablet	Labelled amount (mg)	Observed amount* (mg)	C.V. (%)
Nebicard-5 (Torrent)	5	4.935	0.997

*Mean of 5 determinations.

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REFERENCES

1. The Merck Index (Monograph No. 6519), 12th Edn., p. 1103.
2. A. Vande Water, R. Xhonneux and R. Reneman, *Eur. J. Pharmacol.*, **156**, 95 (1988).
3. P. Pauwels and W. Gommeren, *Mol. Pharmacol.*, **34**, 843 (1988).
4. R. Xhonneux, R. Reneman and L. Wouters, *Eur. J. Pharmacol.*, **181**, 261 (1990).
5. R. Woestenborghs, L. Embrechts and J. Heykants, *Method. Surv. Biochem. Anal.*, **18**, 215 (1988).
6. J. Hendrickx and M. Bockx, *J. Chromatogr. A*, **729**, 341 (1996).

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