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A validated UV spectrophotometric method for estimation of nebivolol hydrochloride in bulk and pharmaceutical formulation

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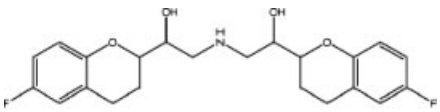
A simple, sensitive and accurate UV spectrophotometric method was developed for the assay of nebivolol hydrochloride in raw material and tablets. Validation of the method yielded good results concerning range, linearity, precision and accuracy. The absorbance was measured at 282 nm for nebivolol hydrochloride tablet solution. The linearity range was found to be 5–50 μg/mL for the drug. It was found that the excipients present in the commercial formulation did not interfere with the method.

1. Introduction

Nebivolol hydrochloride is a new selective beta-receptor antagonist (Kalus et al. 2004). It is chemically α,α′-[iminobis(methylene)bis[6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol] hydrochloride (Budavari 2001) and is not yet official in any pharmacopoeia. A survey of the literature has not revealed any UV spectrophotometric method for the determination of the drug, whereas reports are available for the estimation of the drug by a spectrofluorimetric method in tablets and human serum (Sankar et al. 2005), a RP-HPLC method in bulk and pharmaceutical dosage form (Rajeswari et al. 2005), a HPLC-fluorescence method (Woestenborghs et al. 1988) and a liquid chromatographic method coupled with electrospray ionization tandem mass spectrometry (Ramakrishna et al. 2005) in human plasma.

In the present study, a simple, economical, precise and accurate analytical method for the estimation of nebivolol hydrochloride in pure form and in solid dosage form was developed. The results of the analysis were validated by statistical methods and recovery studies.



Nebivolol (CAS number 9920-09-6)

2. Investigations, results and discussion

Nebivolol hydrochloride was analyzed by UV spectrophotometric method both as a raw material and in a pharmaceutical tablet formulation. The linear regression equation was calculated to be $y = 0.014x + 0.004$ where x and y are concentration in μg/mL and absorbance respectively.

A standard calibration curve of the drug was drawn by plotting absorbance versus concentration. The UV absorption spectrum (Fig.) was monitored at 282 nm. Agreement with Beer's law was evident from the concentration range of the final dilution of 5–50 μg/mL. The correlation coefficient was obtained as 0.9995 indicating very good linearity. The experimental results obtained for the determination in tablets are shown in Table 1. The method had excellent reproducibility for a standard solution of 100 μg/mL. The average purity reached 99.12%.

The detailed accuracy is shown in Table 2. In this test the observed concentrations of nebivolol hydrochloride reference substance in the capsule were not significantly different from the stated concentrations by Student's t test, $P = 0.05$ (100.95%, $n = 6$).

No interfering intensity was found in the UV spectra due to the tablet excipients. Nebivolol hydrochloride was shown to be stable during all the procedure.

Table 1: Analysis of nebivolol hydrochloride tablets (5 mg)

Sl. No	A*	% Analysis ± S.D.	SEM	% C.V.
1	0.289	100.952 ± 0.976	0.398	0.967
2	0.283			
3	0.286			
4	0.289			
5	0.289			
6	0.284			

* Absorbance – Average of three determinations, S.D.: Standard deviation, SEM: Standard error of mean, C.V.: Coefficient of variance

Table 2: Recovery studies of nebivolol hydrochloride tablets

Sl. No.	Spiked amount (μg/mL)	Recovery amount (μg)	Recovery (%)	Recovery (%) ± S.D.
1	20	20.357	101.786	100.784 ± 0.714
2	25	25.214	100.857	
3	30	30.000	100.000	
4	35	35.071	100.204	
5	40	40.428	101.071	

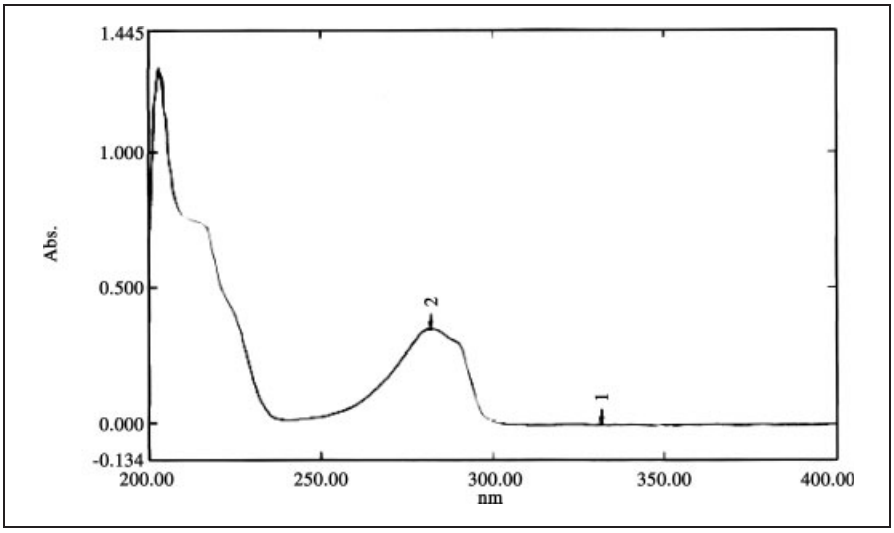


Fig.:
UV spectrum of nebivolol hydrochloride measured in methanol

Table 3: ANOVA of intra- and inter-day assay of nebivolol hydrochloride tablets

Source of variation	SS	df	MS	F_{stat}*	F at level 1%	F at level 5%
Rows	5.527211	5	1.105442	1.11588	5.64	3.33
Columns	2.423469	2	1.211735	1.223176	7.56	4.10
Error	9.906463	10	0.990646			
Total	17.85714	17				

* F_{stat} < F at level 1% and 5% in both cases

3. Experimental

3.1. Chemicals

Nebivolol hydrochloride reference substance was the kind gift from M/S Glenmark Pharmaceuticals, (Mumbai, India). Tablets of brand Nebistar®5 (Hetero Drugs Ltd, Hyderabad, India) containing 5 mg of nebivolol hydrochloride were procured from a local pharmacy. The solvent used for the experiment was methanol (AR grade, Merck, India).

3.2. Equipment

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with a spectra manager software UV Probe was employed with a spectral bandwidth of 1 nm and a wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on an electronic balance (Precisa 310M, Switzerland).

3.3. Standard solution

The standard solution of nebivolol hydrochloride was prepared by accurately weighing 10 mg of the drug. It was diluted in a 100 mL volumetric flask with methanol to give a range of solutions with final concentrations of 5–50 μg/mL. The absorbance of each solution was determined at 282 nm.

3.4. Sample preparation

For the analysis of the dosage form, twenty tablets of nebivolol hydrochloride (5 mg) were ground to fine powder and mixed thoroughly. Powder equivalent to 10 mg of the drug was transferred to a 100 ml volumetric flask and dissolved in about 40 ml methanol by shaking on a rotary flask shaker for 2 h. The solution was filtered through Whatman filter paper (No. 41). The filter paper was washed with the blank. The washings were added to the filtrate and the final volume was made up to 100 mL with the blank. After suitable dilution, the absorbance of the final sample corresponding to 20 μg/mL was recorded against the blank at 282 nm. All the determination was conducted in triplicate.

The data were analyzed by linear simple regression by the least-squares method. The recoveries were determined by adding known amounts of nebivolol hydrochloride reference substance (0, 50, 100, 150 and 200 μg) to the samples at beginning of the process. A recovery test was then performed.

The precision and accuracy of the assay as well as linearity of the calibration curve were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation of each curve. The statistical data were calculated by ANOVA (Table 3).

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