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METHOD DEVELOPMENT AND VALIDATION OF UV – SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF NEBIVOLOL IN BULK AND TABLET DOSAGE FORM

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Abstract: A simple, precise and accurate UV Spectrophotometric method has been developed and validated for estimation of Nebivolol in bulk and tablet dosage form. In this method Nebivolol shows λmax at 284nm using DMSO as a solvent and calibration graphs were plotted over the concentrations ranging from 20 to 80µg/ml of Nebivolol with correlation coefficient 0.9998. The proposed method was validated as per ICH Q2 (R1) guidelines for precision, linearity, accuracy and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.330µg/ml and 1.000µg/ml respectively by simple UV spectroscopy. The proposed method was validated.

Keywords: Nebivolol, DMSO, UV-Spectroscopy, Validation.

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INTRODUCTION

Nebivolol (NEB) is chemically known as 1-(6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-yl)-2-{[2-(6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2-hydroxyethyl]amino}ethan-1-ol, It is a highly cardioselective vasodilatory beta1 receptor blocker used in treatment of hypertension. Nebivolol is a selective β1-receptor antagonist. Activation of β1-receptors by epinephrine increases the heart rate and the blood pressure, and the heart consumes more oxygen. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. In addition, beta blockers prevent the release of renin, which is a hormone produced by the kidneys which leads to constriction of blood vessels. At high enough concentrations, this drug may also bind beta 2 receptors[1].

Different analytical methods have been reported in the literature for the assay of nebivolol in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC, LC-MS[2–13]. The present investigation reports a simple UV spectrophotometric method for the analysis of nebivolol in bulk as well as in tablet dosage form. The developed method was validated as per ICH guidelines[14].

EXPERIMENTAL

Materials & Methods:

The spectrophotometric measurements were carried out using a Shimadzu UV-1700 UV/Vis spectrophotometer with 1cm matched quartz cell and Shimadzu ELB 300 analytical balance, Nebivolol pure drug (99.91%) was obtained as a gift sample from Torrent Pharma (Baddi, India). All chemicals and reagents used were of analytical grade. Formulation used for studies was developed by Torrent Pharmaceutical Industries Ltd. Nebivolol tablets, Nebicard (Torrent Pharmaceutical Industries Ltd) and Nebinex (Glenmark) were procured from local drug stores.

Preparation of Standard solution:

Standard drug of Nebivolol was proposed by dissolving 25mg pure Nebivolol in DMSO and transferred into 250ml volumetric flask to obtain 100µg/ml of stock solution. The standard solution of Nebivolol having concentration of 50µg/ml was scanned in UV range (200-400nm) in 1.0 cm cell against in solvent as blank and spectrum was obtained.

Determination of λmax:

50µg/ml of Nebivolol was prepared and scanned in UV range of 200-400nm and spectrum was obtained. The λmax was found to be at 284nm wavelength where absorbance was found

maximum at this wavelength. Hence it is considered as absorbance maxima (λmax) shown in Figure-1.

Preparation of calibration curve:

Standard stock solution was suitably diluted with DMSO to obtain concentrations ranging from 20-80µg/ml. Absorbance of these solutions was measured at 284nm. Calibration curve was obtained by plotting graph between concentration and absorbance shown in Figure-2.

Preparation of test solution:

20 Tablets were weighed and its average weight was determined. An accurately weighed tablet powder equivalent to 25mg of Nebivolol transferred into 250ml volumetric flask dissolved in DMSO, sonicated for 10min and volume was made up to the mark. Solution was filtered using whattman filter paper (No.41) to obtain 100µg/ml stock solution.

METHOD VALIDATION:

Linearity:

The absorbances were observed from 20 to 80µg/ml and were shown in Table-1. Linearity was obtained between 20 to 80µg/ml. Concentration graph was plotted for concentration and absorbance. The equation of calibration curve obtained was y = 0.013x + 0.003. The correlation coefficient (r) was 0.9998 shown in Figure-2.

Accuracy:

To determine the accuracy of the method recovery was performed by standard addition method. To pre-analyzed sample known amount of standard Nebivolol was spiked in different concentrations. The recovery was performed at three levels 50%, 100% and 150% of standard Nebivolol. Solutions were analyzed and percentage recovery was calculated from calibration curve shown in Table-2

Precision: It was ascertained by replicate analysis of the homogenous sample of tablet powder and the concurrent values of estimation are shown in Table-3 for two different brands of the sample by proposed method.

Interday & Intraday Precision

The concentration of 20, 40 and 60µg/ml of Nebivolol (on label claim basis) was taken. The absorbance of the final solution was read after 0hr, 12hr and 24hr in 1.0 cm cell at selected wavelength. The results were recorded in Table-5

Similarly the absorbance of the 20, 40 and 60µg/ml solutions were read on 1st, 2nd and 3rd day. The amount of Nebivolol was estimated by comparison with the standard and taking A(1%, 1cm) at 284nm. The results were recorded in Table-6

Ruggedness:

It was carried out by analyzing the sample by different days and estimation of drug by proposed methods. Results of studies are shown in Table-7

RESULTS AND DISCUSSIONS:

Attempt has been made to develop rapid, sensitive, economic, precise and accurate analytical method for Nebivolol in pure and pharmaceutical dosage form. The proposed method is based on UV Spectrophotometric absorption in UV region using DMSO as solvent. Maximum absorbance was found to be at 284nm. LOD and LOQ were found to be 0.330µg/ml and 1.000µg/ml. Beer's law was obeyed in concentrations ranging from 20 to 80µg/ml. The correlation coefficient values were above 0.9998 which shows that absorbance was linear with concentration. The optical characteristics such as Beer's law limit, correlation coefficient, slope, intercept, molar absorptivity, scandell's sensitivity were calculated and validated (Table-8). Precision of the method was confirmed by Intraday and Interday analysis, %RSD values were found to be less than 2.0. The percent recovery was found to be nearly 100% indicating reproducibility and accuracy of the methods. Hence the proposed method could be effectively adopted for routine quality control of Nebivolol in bulk and formulated tablet dosage form.

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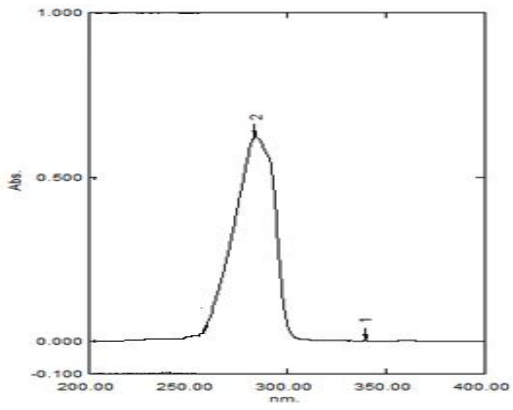

Fig - 1: UV Spectrum of Nebivolol with DMSO

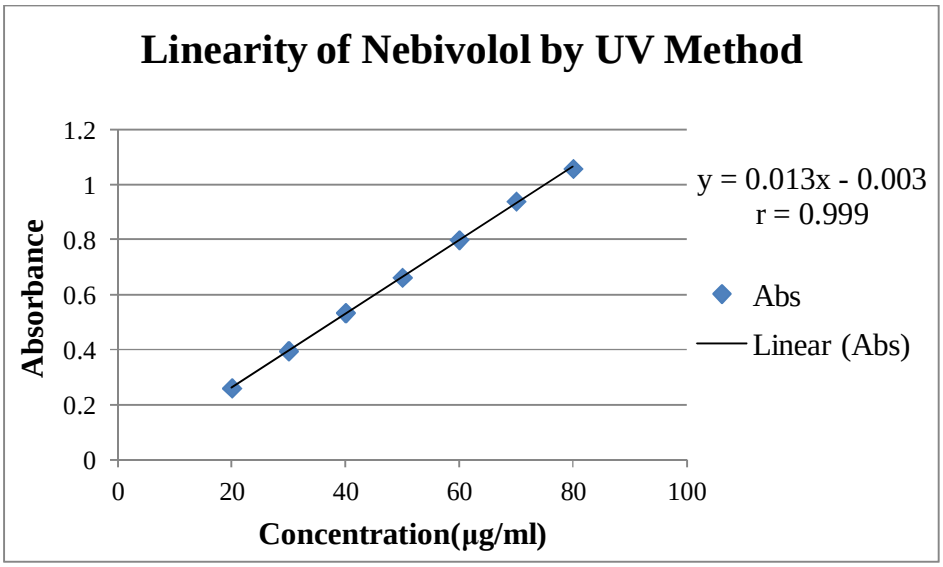

Fig - 2: Calibration curve of Nebivolol with DMSO showing linearity relationship

Table 1: Calibration data for analysis of Nebivolol in DMSO at λmax = 284nm

S.No.	Concentration (µg/ml)	Mean Absorbance (± SD)
1	20	0.261(0.002)
2	30	0.396(0.0015)
3	40	0.534(0.001)
4	50	0.663(0.0006)
5	60	0.799(0.0015)
6	70	0.940(0.0012)
7	80	1.058(0.001)

Table-2: Recovery data of Nebivolol in DMSO

Ingredient	Amount of drug from formulation	Amount of standard added	Percentage added	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery (Mean ± RSD)*
Nebivolol	10µg	5µg	50%	19.93	19.81	99.36 ± 0.52
Nebivolol	10µg	10µg	100%	39.85	39.91	100.15 ± 0.29
Nebivolol	10µg	15µg	150%	59.78	59.82	100.08 ± 0.18

*n=3 (Average of 3 determinations)

Table-3: Results of analysis of laboratory samples (Assay)

Sample	Label claim	Amount found	% Purity ± RSD*
NEBICARD(Torrent)	5mg	5.01	100.03 ± 0.12
NEBINEX(Glenmark)	2.5mg	2.50mg	99.84 ± 0.23

*n=3 (Average of 3 determinations)

Table-4: Lowest Limit of detection and Lowest Limit of quantification

LOD (µg/ml)	LOQ (µg/ml)
0.330	1.000

Table-5: Results of Intraday Precision of Nebivolol in DMSO

Parameter	% Recovery Estimated (Mean + RSD)*		
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)
At 0 hr	99.91 ± 0.38	99.91 ± 0.19	99.91 ± 0.13
At 12 hr	100.29 ± 0.38	99.84 ± 0.20	100.04 ± 0.33
At 24 hr	99.74 ± 0.42	100.14 ± 0.36	99.95 ± 0.19

*n=3 (Average of 3 determinations)

Table-6: Results of Inter-day Precision of Nebivolol in DMSO

Parameter	% Recovery Estimated (Mean + RSD)*		
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)
Day-1	100.01 ± 0.24	99.90 ± 0.17	99.85 ± 0.18
Day-2	99.87 ± 0.35	100.05 ± 0.24	99.95 ± 0.19
Day-3	100.14 ± 0.20	99.96 ± 0.21	100.04 ± 0.13

*n=3 (Average of 3 determinations)

Table-7: Results of Ruggedness of Nebivolol in DMSO

Ruggedness	% RSD*
Analyst – 1	0.10
Analyst – 2	0.12

*n=3 (Average of 3 determinations)

Table-8: Validation Parameters

Parameters	Results
Absorption maxima λmax (nm)	284
Beer's law limit (µg/ml)	20-80
Absorptivity (1mole^{-1}, cms^{-1})	0.5376 x 10^4
Sandell's sensitivity (µg/cm2/0.001)	0.0754
Correlation coefficient	0.9998
Regression equation	y = 0.013x - 0.003
Limit of detection	0.330
Limit of quantification	1.000
Precision(% RSD)	0.26

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