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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF NEBIVOLOL HYDROCHLORIDE FOLLOWING ICH GUIDLINES AND STUDY OF ITS DEGRADATION PROFILE

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ABSTRACT

A UV-Spectrophotometric method is described for the determination of Nebivolol hydrochloride (NEB). The method is based on the measurement of the absorbance of NEB solution in methanol: water (10:90) at 281 nm. The system obeyed Beer's law over the concentration range of 5.0- 50.0 µg/mL. The degradation behaviour of NEB was investigated under dry heat treatment, UV-degradation, acid hydrolysis, alkali hydrolysis and oxidation; and found to degrade under acid hydrolysis, alkali hydrolysis and oxidation. The method was applied to the determination of NEB in tablets.

Keywords: Nebivolol hydrochloride; UV-Spectrophotometric determination; degradation study.

INTRODUCTION

Nebivolol hydrochloride (NEB) is chemically known as α, α'-[iminobis(methylene)]bis[6-flouro-3,4-dihydro-2H-1-benzopyran-2-methanol]hydrochloride [1], it is a highly selectiveβ_1-blocker with nitric oxide-mediated vasodilatory actions and beneficial effects on vascular endothelial function. It has been clinically used for the treatment of hypertension andchronic heart failure [2].

It is official in martindale [3] the extra pharmacopoeia. Different analytical methods have been reported in the literature for the assay of NEB in pharmaceuticals and include spectrophotometry, TLC, LC, HPTLC, LC-MS [4–10].

On the other hand, UV-spectrophotometry is still the technique of choice since it is simple, sensitive, economical, rapid and more easily manageable. Literature survey revealed that no stability indicating UV-spectrophotometric method has ever been reported for the quantification of NEB.

The present investigation reports the development and validation of a UV spectrophotometric method for quantification of NEB in tablet dosage form and study of its degradation profile. Stress testing of NEB was carried out according to International Conference on Harmonization (ICH) guidelines[11] entitled 'stability testing of new drug substances and products' and investigates the degradation studies in thermal degradation, UV degradation, acid hydrolysis, alkali hydrolysis and oxidation. The proposed method was demonstrated to be simple, selective and cost-effective compared to many reported methods.

EXPERIMENTAL

Instrument

The spectrophotometric measurements were carried out using JASCO V360 Double beam UV- VIS Spectrophotometer with 1cm U.V. matched quartz cells.

Sample

Pharmaceutical grade Nebivolol Hydrochloride (NEB) was received from Cipla Ltd, Mumbai, India. Two brands of tablets namely, NEBICARD (Torrent Ltd) and NEBINEX (Glenmark Pharmaceutical Ltd) were procured from the local commercial sources.

Reagents and chemicals

All reagents and chemicals used were of analytical reagent grade whereas methanol was of HPLC grade. Doubly-distilled water was used to prepare solutions wherever required. Aqueous solutions of 0.01,0.1,1 M hydrochloric acid, 0.01,0.1,1 M sodium hydroxide (Merck, Mumbai, India) and 3 and 30% H_2O_2 (Loba Chemie Pvt. Ltd., Mumbai, India; 30% w/v) were prepared in the usual way.

Standard drug solution

5mg of NEB was accurately weighed and dissolved in 10ml Methanol in 50ml volumetric flask. The volume was made up to the mark with distilled water to give 100µg/ml stock solution.

Procedures

Construction of calibration curve

Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 mL of 100 µg/mL NEB standard solution were accurately transferred into a series of 10 mL calibrated flask and made up to the mark with distilled water. The absorbance of the resulting solution was measured at 281 nm against blank.

Calibration curve was prepared by plotting the absorbance *versus* concentration of drug. The concentration of the unknown was read from the calibration curve or computed from the regression equation derived using the Beer's law data.

Procedure for tablets

Twenty tablets each of two brands were weighed and ground into a fine powder. Powder equivalent to 10 mg of NEB was transferred into a 50 mL calibrated flask and 10 mL of methanol was added to the flask and the content was shaken thoroughly for 15-20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with distilled water, mixed well and filtered using a Whatman No. 42 filter paper. An aliquot of the filtrate (200 µg/mL in NEB) was diluted to get 50 µg/mL NEB and analysed for NEB following the procedure described above.

Procedure for placebo blank analysis

Placebo blank is a mixture of normally added excipients in formulations. Based on the amount of excipients present in a NEB tablet , a placebo blank of the composition: Lactose monohydrate, crospovidone Type A, poloxamer 188, povidone K 30, microcrystalline cellulose, magnesium stearate was made and its solution was prepared as described under *"Procedure for tablets"*, and then analysed using the procedure described under *"Construction of calibration curve".*

Procedure for the determination of NEB in synthetic mixture

To the placebo blank of the composition described above, 10 mg of NEB was added and homogenized, transferred to a 50 mL calibrated flask and the solution was prepared as described under "*Procedure for tablets*", and then subjected to analysis by the procedure described under *"Construction of calibration curve".* This analysis was performed to study the interference by excipients such as

Lactose monohydrate, crospovidone Type A, poloxamer 188, povidone K 30, microcrystalline cellulose and magnesium stearate.

Conduct of stress studies

The stress studies were carried out under the conditions of dry heat, UV-degradation, hydrolysis and oxidation. For dry heat stress testing, solid drug was kept in Petri dish in an oven at 60 °C for 4 h and after cooling to room temperature, 5 mg of NEB was weighed and transferred to a 100 mL calibrated flask, dissolved in methanol and diluted up to the mark with distilled water. The absorption spectrum was recorded from 240-350 nm. The UV degradation study was carried out by exposing the stock solution of NEB (50 µg/mL) to UV radiation in a UV chamber for 12000 lux h. The same solution was diluted to obtain 10 µg/mL NEB and the absorption spectrum was recorded. For acid degradation studies, 2 mL of 50 µg/mL NEB was taken separately in three 10 mL calibrated flasks and mixed with 5 mL of 0.01,0.1 and 1M HCl (acid hydrolysis), and kept on hot water bath set at 80 °C for 2 h. Then, the solution was cooled to room temperature and diluted to the mark with methanol: water (10;90) and the absorption spectra of the resulting solutions were recorded. For alkali degradation studies, 2 mL of 50 µg/mL NEB was taken separately in three 10 mL calibrated flasks and mixed with 5 mL of 0.01,0.1and 1M NaOH (alkaline hydrolysis) , and kept on hot water bath set at 80 °C for 2 h. Then, the solution was cooled to room temperature and diluted to the mark with methanol: water (10;90) and the absorption spectra of the resulting solutions were recorded. For oxidative degradation, 2 mL of 50 µg/mL NEB was taken separately in two 10 mL calibrated flasks and mixed with 5 mL 3% and 30% H_2O_2 (oxidative degradation) and kept on hot water bath set at 80 °C for 2 h. Then, the solution was cooled to room temperature and diluted to the mark with methanol: water (10;90) and the absorption spectra of the resulting solutions were recorded. The absorbance values obtained in stress studies were compared with the data obtained in calibration curve i.e. in the absence of forced degradation.

RESULTS AND DISCUSSION

Spectral characteristics

The absorption spectra of 5-50 µg/mL NEB solution in Methanol: water (10:90) was recorded between 200-400 nm and showed an absorption maximum at 281 nm, and at this wavelength methanol : water had insignificant absorbance. Therefore, 281 nm was used as analytical wavelength (λmax). Figure 1 represents the absorption spectra of NEB in Methanol: water (10:90) along with blank.

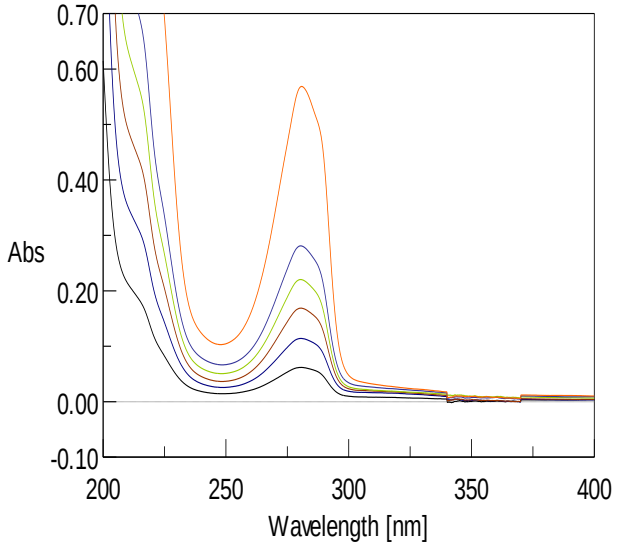

Figure 1: Absorption spectrum for neb in* methanol:water (10:90) *(5-50 µg/ml neb)

Forced degradation of NEB

Forced degradation studies provide an indication of the stability-indicating property of the drug. The study was carried out after subjecting NEB to dry heat treatment, UV-degradation, acid and alkali hydrolysis; and oxidation. The UV spectra of stress NEB samples which were subjected to dry heat treatment and UV-degradation (Figures 2a and 2b) were similar to that of the standard NEB sample (Figure 1) and it showed that NEB did not undergo degradation under these conditions.

NEB subjected to acid and alkali hydrolysis showed degradation, since the absorbance values obtained under these stressed conditions (Figures 2c and 2d) were smaller than the original value of standard NEB sample (Figure 1). As shown in Figures 2a and 2b, the degradation of NEB is much lesser under acidic and basic environment in comparison to oxidative condition.

The absorption spectrum (Figure 2e) obtained under oxidation condition with H_2O_2 shows complete degradation of NEB.

Method validation

The proposed method was validated for linearity, sensitivity, precision, accuracy, robustness, ruggedness, selectivity, interference and recovery.

Linearity and sensitivity

Linear correlation was obtained between the absorbance and concentration of NEB in the range of 5.0-50 µg/mL. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection and quantification are calculated as per the current ICH guidelines [12] are compiled in the same table, speak of the excellent sensitivity of the proposed method.

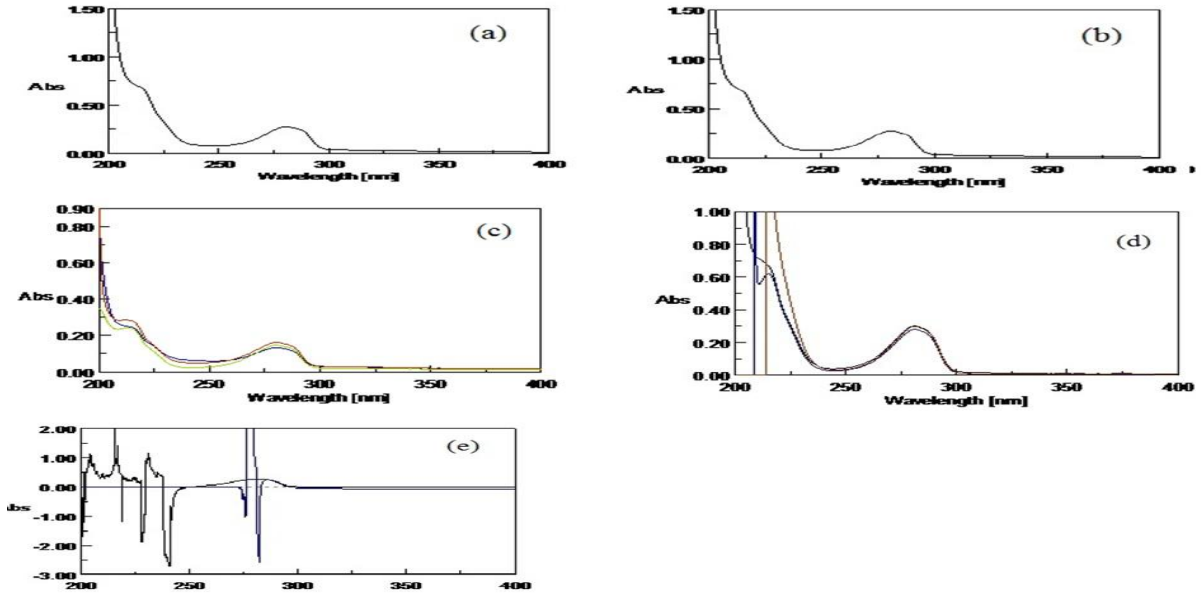

Figure 2: Degradation study of neb solution treated with (a). dry heat at 60 °c for 4 h, (b). uv radiation for 12000 lux h, (c). 0.01, 0.1 and 1m hcl (acid hydrolysis), (d). 0.01, 0.1 and 1m naoh (alkaline hydrolysis) and (e). 3% and 30% w/v h_2o_2

Table 1: Sensitivity and regression parameters

Parameter	Result
λmax(nm)	281nm
Beer's law limit(µg/mL)	5-50
Molar absorptivity (ε), L/mol cm	4.87×10^4
Sendell's sensitivity,µg/cm²	0.090
Limit of detection LOD µg/mL	0.0064
Limit of quantification LOQ µg/mL	0.0196
Regression equation(y = m x + c)	Y=0.011x-0.001
Slope(b)	0.011
Intercept(a)	0.001
Correlation coefficient(r)	0.999

Precision and accuracy

Intra-day precision and accuracy of the proposed method were evaluated by replicate analysis (n = 7) of calibration standards at three different concentration levels on the same day. Inter-day precision and accuracy were determined by assaying the calibration standards at the same concentration levels on 5 consecutive days. Percentage relative standard deviation (RSD, %) as precision and percentage relative error (RE, %) as accuracy of the proposed method were calculated. These results of accuracy and precision showed that the proposed methods have good repeatability and reproducibility (Table 2).

TABLE 2: Evaluation of intra-day and inter-day accuracy and precision

NEB taken, µg/mL	Intra-day accuracy and precision (n = 5)			Inter-day accuracy and precision (n =5)		
	NEB found, µg/mL	%RE	%RSD	NEB found, µg/mL	%RE	%RSD
5	4.92	0.64	1.52	4.88	2.36	0.77
15	14.96	0.25	0.59	15.07	0.49	0.23
25	24.98	0.06	0.31	24.84	0.65	0.21

RE: Relative error and RSD: Relative standard deviation.

Robustness and ruggedness

Method robustness and ruggedness were demonstrated by determination of NEB at 3 different concentrations. Method robustness was tested by measuring the absorbance at 280, 281 and 282 nm whereas the method ruggedness was performed by four different analysts, and also with three different instruments by a single analyst. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in the Table 3.

TABLE 1 : Robustness and ruggedness expressed as intermediate precision (% rsd)

NEB taken, µg/mL	Method robustness	Method ruggedness	
		Parameter altered	
	Wavelength*, nm, RSD % (n = 3)	Inter-analysts' RSD, % (n = 3)	Inter-instruments' RSD, % (n = 3)
5	0.8	1.8	2.67
15	0.69	0.43	1.41
25	0.72	0.29	1.15

Selectivity and interference

The proposed method was tested for selectivity by placebo blank and synthetic mixture analyses. The analysis of placebo blank solution was subjected to analysis according to the recommended procedure described under "*construction of calibration curve*" and found that there was no interference from the inactive ingredients as indicated by the near blank absorbance. This result shows the selectivity of the method.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution yielded percent recoveries which ranged from 102.16-105.23 with standard deviation of 0.57-0.91. The results of this study are presented in Table 4 indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the selectivity of the proposed method.

TABLE 4 : Recovery of the drug from synthetic mixture

NEB in synthetic mixture taken	NEB recovered[a] (Percent ± SD)
5	105.23 ± 0.63
15	102.16 ± 0.57
25	104.54 ± 0.91

Application to analysis of commercial samples

In order to check the validity of the proposed method, NEB was determined in some commercial formulations. Table 5 presents the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the labelled claim.

TABLE 5 : Rresults of analysis of tablets by the proposed method

Brand name	Label claim, mg/tablets	Found[a] (Percent of label claim ± SD)
Nebicard	5mg	100.33 ± 0.31
Nebinex	5mg	100.81 ± 0.16

Recovery studies

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed capsule powder was spiked with pure NEB at 3 concentration levels (50, 100 and 150% of that in Tablets) and the total was found by the proposed method. The added NEB recovery percentage values ranged from 101.16-102.08% with standard deviation of 0.55-0.91 (Table 6) indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination.

TABLE 6 : Accuracy assessment by recovery experiments

Brand studied	NEB in Tablets µg/mL	Pure NEB added, µg/mL	Total found, µg/mL	Pure NEB recovered[a] Percent ± SD
Nebicard	10	5	15.30	101.97 ± 0.91
	10	10	20.23	101.16 ±0.55
	10	15	25.52	102.08 ±0.84

[a]Mean value of 3 measurements.

CONCLUSIONS

Stress testing is an important aspect of the drug development process. The present study reports a simple method for quantification of NEB in tablets as well as stress testing study. The previously reported HPLC and LC-MS require judicious control of pH of the medium besides requiring expensive and sophisticated instruments. In contrast, the present method requires simple and inexpensive instrument and involves minimal manipulation producing relatively more and accurate results, and thus can be used for the routine determination of NEB in its available dosage forms.

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