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UV Spectrophotometric Method Development and Validation for Estimation of Nebivolol hydrochloride as API and in Pharmaceutical Dosage Form.

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ABSTRACT

UV Spectrophotometric method is a common, delicate and very precise method used to be developed and validated for estimation of Nebivolol hydrochloride as API and pharmaceutical dosage form. The method is primarily depends on the measurement of the absorbance of Nebivolol hydrochloride concentrations in methanol**:** 0.01N HCl (10:90) at 281nm in the wavelength reach about 200-400nm. The concentration range is 10-60µg/ml and obeyed the Beers law. The critical parameters of the calibration curve had been also calculated. The presence of frequent excipients in tablets does not affect the percentage recoveries results. The developed method was validated as per as ICH Q2 (R1) guidelines in terms of accuracy, precision, linearity, LOD and LOQ were found to be 1.955µg/ml and 5.925µg/ml respectively and the percentage of drug release shows the satisfactory result at 75 rpm with temperature 37±0.5ºC. This proposed method has been utilized effectively for the evaluation of the drug as API and its pharmaceutical formulations.

Keywords: Nebivolol hydrochloride, UV- Spectrophotometer, Estimation, Validation.

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INTRODUCTION

Nebivolol hydrochloride is a cardio -selective beta 1 receptor blocker. The main functions of the Nebivolol hydrochloride is blood vessel relaxation & lowering heart rate with increasing blood flow as well as reduce blood pressure [1-2]. It is an antihypertensive agents used for the treatment of hypertension and heart failure. The physical appearance of the Nebivolol hydrochloride is white odourless powder and chemically known as α, α1–(imino bis (methylene)) bis (6-fluoro-3, 4-dihydro-2 *H* -1-benzopyran-2-methanol) hydrochloride (figure 1). And the chemical formula of Nebivolol hydrochloride is $C_{22}H_{26}ClF_2NO_4$. Molecular weight of the Nebivolol hydrochloride is 441.9g/mol [3-4].

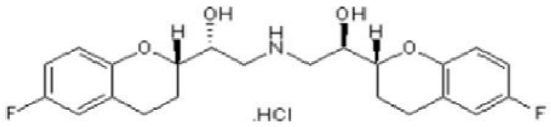

Figure 1: Chemical structure of Nebivolol hydrochloride

In physiologically, the activation of beta 1 receptor through the epinephrine as result as increases heart rate as the same time increases the blood pressure. Nebivolol hydrochloride shows to the reverse effect on the epinephrine and stopped the work on the beta 1 receptors. Otherwise, the rennin hormones mainly produce from the kidney. The principle function of the rennin is constriction of blood vessel and the beta blocker antagonist shows to the adverse effect on this rennin release process from kidney [5].

The estimation of Nebivolol hydrochloride in bulk and pharmaceuticals formulation performed in numerous spectrometry have been included in the research paper as LC, TLC, HPTLC, HPLC, RP-HPLC, LC-MS [3-13]. The aims and objectives of this present research to develop and validate of UV method for estimation of Nebivolol hydrochloride in bulk and pharmaceutical formulations.

MATERIALS AND METHOD

Materials

Pure API of Nebivolol Hydrochloride (NEB) was received as a gift sample from Cadila Pharmaceuticals Ltd. Ankleshwar, Gujarat. Analytical graded chemicals and reagents were used in this project. Different brands of Nebivolol hydrochloride tablets such as NEBINEX (Glenmark Pharmaceutical Ltd) and NEBICARD (Torrent Pharmaceutical Ltd) were used in this project and these tablets gained from the topical pharmacy shops.

Equipments

Double beam UV visible spectrophotometer (Shimadzu UV 1800) was used with 1cm quartz cells. An electrical balance (Shimadzu ELB 300), an ultrasonicator bath (PCI analytics Pvt. Ltd) and Electrolab EDT dissolution tester (model EDT-08Lx) was used in this project for weighing the pure sample and sonicating the powder of pharmaceutical formulation.

Preparation of standard drug solution

10 mg of Nebivolol hydrochloride was weighed and transferred into 100ml volumetric flask. And dissolved with the 10 ml methanol and was sonicated. That the 100ml volumetric flask upto 100 ml with 0.01N HCl to get final concentrations 100µg/ml.

Determination of absorbance maxima

The standard stock solution was further dilution with 0.01N HCl and the diluted solution scanned in UV spectrophotometer with the range of 200-400nm. The absorbance maxima of Nebivolol hydrochloride was measured at 281nm (figure 2). At this wavelength the absorption was maximum level.

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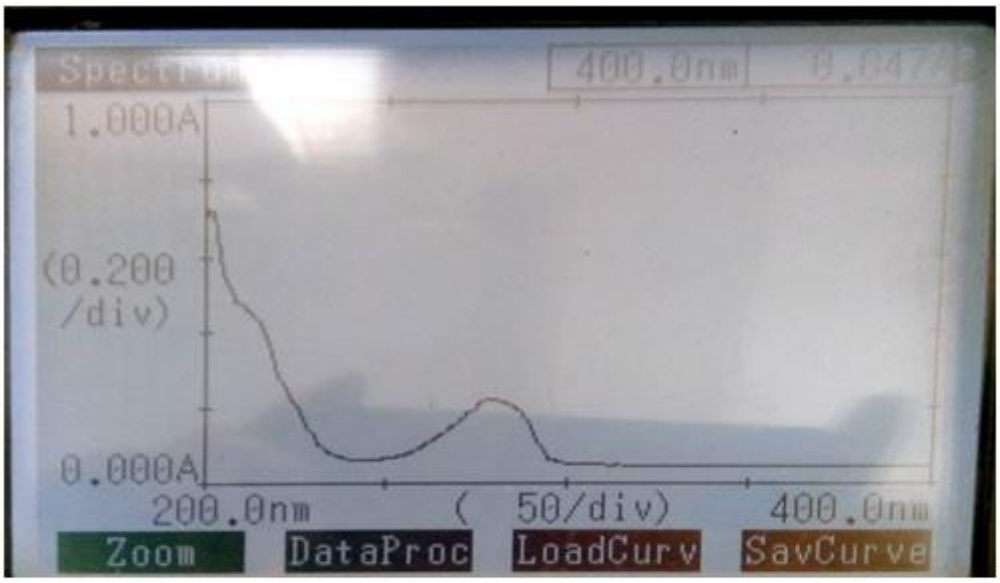

Figure 2: UV spectrum of Nebivolol hydrochloride with 0.01N HCl

Preparation of calibration curve:

The standard stock solutions further dilution with 0.01 N HCl and to get the final concentration ranging from 10-60µg/ml. The final concentrations scanned with the UV spectrophotometer at 281 nm against as a blank solution 0.01 N HCl. The concentration v/s absorbance of the Nebivolol hydrochloride was plotted and the calibration curve represented in figure 3.

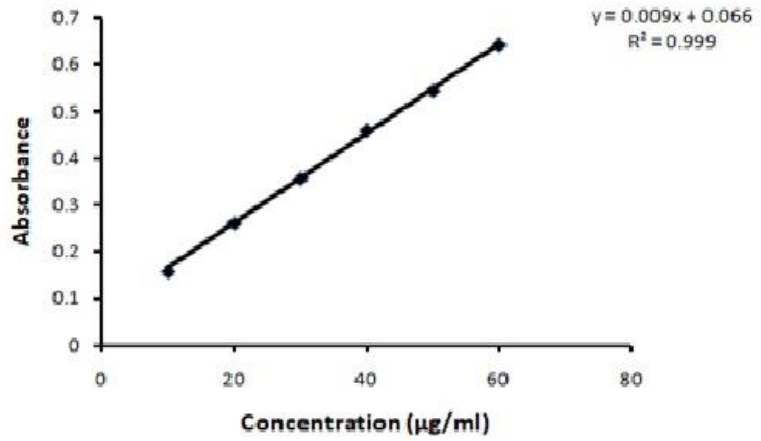

Figure 3: Calibration curve of Nebivolol hydrochloride with 0.01N HCl

Preparation of sample solution

Estimation of Nebivolol hydrochloride in pharmaceutical formulations, 20 tablets are weighted individually and find out their averages weight. For these 20 tablets finally crush with mortar pestle to get a fine powder. An equivalent weight of 10 mg Nebivolol hydrochloride tablet powder transferred to the 100 ml volumetric flask and upto 100ml with suitable diluents. The sample solution was sonicated at 15 minutes and filtered with the whattman filter paper (No. 41) to getting 100µg/ml stock solution. The stock solution further diluted with 0.01 N HCl preparing for suitable concentrations. The absorbance of these concentrations was

measured in UV spectrophotometer against 0.01N HCl as blank. And find out the drug content was calculated with the calibration curve of Nebivolol hydrochloride.

METHOD VALIDATION

According to the ICH guidelines, the propose method was validated. And the parameters are linearity, accuracy, precision, LOD & LOQ [14-15]. The critical parameters for Nebivolol hydrochloride is reported in table 1.

Parameters	Data
λ- Max	281nm
Beers law limit	10-60µg/ml
Regression equation	y = 0.009x±0.066
Correlation coefficient	r^2=0.999
Slope	0.009
Intercept	0.066
LOD	1.955
LOQ	5.925

Table 1: Critical parameters for Nebivolol hydrochloride

RESULTS AND DISCUSSIONS

Linearity

The linear in the concentration of the developed and validated method is 10-60µg/ml. The absorbance v/s concentration is plotted and correlation coefficient was find out (r^2=0.999). The linear equation was y= 0.009+0.066 shown in (figure 3).

Accuracy

The added of known amount of standard Nebivolol hydrochloride was spiked at different concentration and calculated percentage recovery from calibration curve. At the three levels 50%, 100%, 150% of standard Nebivolol hydrochloride was used in this method. The percentage recoveries result shown in table 2.

Table 2: Recovery data of Nebivolol hydrochloride in 0.01N HCl

Sr. No.	Label claim, tablet	Added amount	Recovered amount	% recovery	%RSD
1	5mg	2.5	7.45	99.33	1.16
2	5mg	5	9.96	99.60	0.71
3	5mg	7.5	12.52	100.16	0.42

*n=3 (Average of 3 determination)

Precision

The absorbance of known concentration was measured in different days. Intraday precision was performed in three times on same day and interday precision was performed three times on different days. The (%RSD) of the sample solution was calculated and the result of the percentage relative standard deviation shows to less than 2 that assemble to the measure for the developed and validated propose method. The precision data is reported in table 3.

ISSN: 0975-8585

Table 3: Precision data of Nebivolol hydrochloride

Drug	Conc. µg/ml	Intraday Amount of drug(µg/ml)	% relative standard deviation	Interday Amount of drug(µg/ml)	% relative standard deviation
Nebivolol hydrochloride	10	9.9±0.003126	1.18	9.7±0.001931	1.2
	20	20.5±0.002244	1.05	20.4±0.002126	1.05
	30	29.9±0.001577	1.44	29.7±0.002215	0.58

n*=3 (Averages of 3 determinations)

LOD and LOQ

According to the ICH guidelines, the limit of detection & the limit of quantification was calculated by (LOD =3.3ó/S and LOQ = 10 ó/S.) the standard deviation of the response and slop of the calibration curve.

Here, ó = the standard deviation of the response; S= slop of the calibration curve

The data is shown in table 4.

Table 4: LOD & LOQ data

LOD (µg/ml)	LOQ (µg/ml)
1.995	5.925

Experimental dissolution method

The dissolution method of Nebivolol hydrochloride (Nebicard 5 mg) was performed in USP type II apparatus with six tablets. The dissolution medium volume was 900ml and stirring speeds of 75 rpm. The medium temperature was set at 37±5ºC. The quantity of the withdrawn sample was 5ml and sampling time at 5, 10, 15, 20, 25, 30, 40 and 60 min. The sink condition of the dissolution procedure was maintained. The withdrawn sample was filtered with Whatman No. 41 filter paper & examined by UV spectrophotometer at 281 nm. The drug release was less than 85% within 45 min; according to the USFDA that was not satisfactory result [16]. To the experimental dissolution the drug release was greater than 85% within 45 minutes. The dissolution rate data as represent as (Table 5) and the dissolution study results are represented in (figures 4).

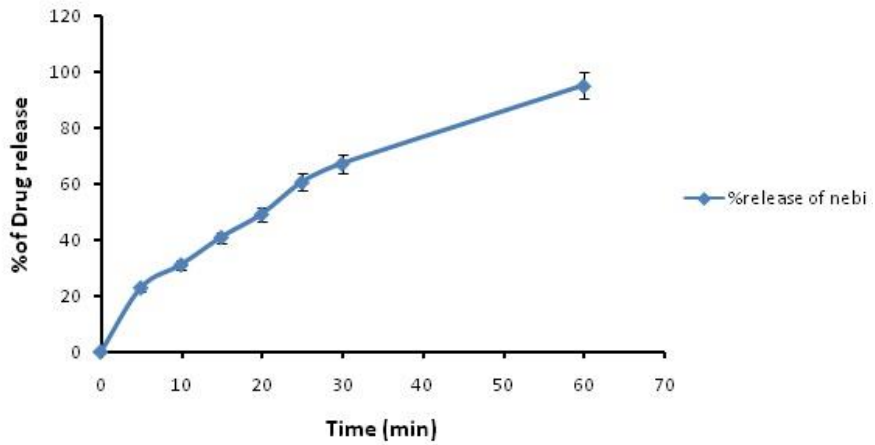

Figure 4: Dissolution profile of Nebicard tablet in 0.01 N HCl

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Table 5: Dissolution profile of Nebivolol hydrochloride tablet in 0.01N HCl

Sr. No.	Time	Avg. % Release
1	5	22.9
2	10	31.09
3	15	40.90
4	20	49.09
5	25	60.54
6	30	67.09
7	60	94.90

Assay of API in tablets

The assay of Nebivolol hydrochloride tablets was performed by this proposed method used in six tablets. The percentage purity of Brand I (Nebicard), Brand II (Nebinex) was found to be 99.80 %, 99.2 % respectively.

The result of assay is reported in table 6.

Table 6: Result of assay at Nebivolol hydrochloride formulation

Brands	Label claim	Amount found	% Amount found
Nebicard	5 mg	4.99	99.80
Nebinex	5 mg	4.96	99.20

CONCLUSION

The estimation of Nebivolol hydrochloride using UV spectrometric method was developed and validated according to the ICH guidelines. The proposed method is more effective and accurate. It can be easily used to routine quality control check of Nebivolol hydrochloride as API and pharmaceutical formulation in numerous pharmaceutical industries.

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