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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR NEBIVOLOL HYDROCHLORIDE IN BULK DRUG USING MIXED HYDROTROPIC SOLUBILISATION

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ABSTRACT

An analytical method for the validation of NEBIVOLOL HYDROCHLORIDE by UV spectrophotometry using hydrotropic solubilization is described. An attempt was made to preclude the use of corrosive organic solvents by the use of 6M Urea, 25% citric acid & 1% Sodium lauryl sulphate (SLS). The developed method has the measurement of absorptivity at 282 nm (absorption maximum of Nebivolol HCL). The method is simple, fast and accurate and has been applied successfully for the validation of Nebivolol HCL in pharmaceutical dosage forms. Accuracy was determined by recovery studies from marketed formulation and Ranges From 99.22 – 100.07 %. Precision of method was find out as repeatability, Day To Day and Analyst To Analyst variation and shows the values within acceptable limit.

KEY WORDS: Nebivolol, Mixed hydrotropy, UV spectrophotometric method**,** Validation.

INTRODUCTION

Nebivolol is a third-generation β (beta) 1 selective works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure. Nebivolol hydrochloride (NEB-H) chemically α, α'- [Iminobis (methylene)] bis [6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride is a white odourless powder used for the treatment of hypertension and heart failure. Its mode of action is lowering blood pressure

by reducing peripheral vascular resistance, and significantly increases stroke volume with preservation of cardiac output. The net hemodynamic effect of nebivolol is the result of a balance between the depressant effects of beta-blockade and an action that maintains the cardiac output. Nebivolol is the racemate (dl-nebivolol and d-nebivolol. It is a competitive and highly selective beta-1 receptor antagonist with mild vasodilating properties, possibly due to an interaction with the L-arginine/nitric oxide pathway. In animal models nebivolol has been shown to induce endothelium–dependent arterial relaxation in a dose dependent manner, by stimulation of the release of endothelial nitric oxide. Nitric oxide is produced in artery walls and acts to relax vascular smooth muscle cells. It also inhibits platelet aggregation and adhesion and may protect against vascular damage as it inhibits leukocyte activation and vascular smooth muscle cell proliferation .Fig. 1: Structure of Nebivolol hydrochloride

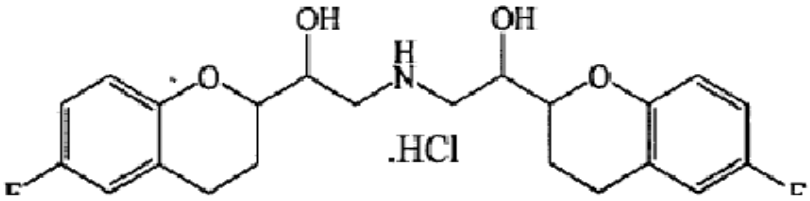

Fig. 1: Structure of Nebivolol hydrochloride

Mixed hydrotropic solubilization technique is the phenomenon to increase the solubility of poorly water-soluble drugs in the blends of hydrotropic agents, which may give miraculous synergistic enhancement effect on solubility of poorly water soluble drugs, utilization of it in the formulation of dosage forms of water insoluble drugs and to reduce concentration of individual hydrotropic agent to minimize the side effects (in place of using a large concentration of one hydrotrope a blend of, say, 5 hydrotropes can be employed in 1/5th concentrations reducing their individual toxicities. Sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents Extensive literature survey revealed that UV spectrophotometric methods, RP-HPLC methods and HPTLC methods has been reported. The primary objective of the present investigation was to employ the mixed hydrotropic solution to extract the drug from the dosage form and precludes the use of corrosive organic solvents. The aqueous solubility of nebivolol was enhanced to a great extent in the blends of 6M Urea and 25% citric acid,& 1% SLS.

EXPERIMENTAL

Instrumentation

UV experimentation was performed on **Shimadzu 1800** UV-visible spectrophotometer equipped with Photo Diode Array (PDA) detector, with 1 cm quartz cell. Citizen Digital Ultrasonic Cleaner was used for solubility purpose.

MATERIALS

All reagents used are AR grade and NEBIVOLOL HYDROHLORIDE was gift sample obtained from Dr. Reddy's Laboratories, Hyderabad and were used as reference standard. The tablet formulation was purchased from local market.

Preliminary Solubility Study

Solubility of drug was determined at 27 ±1°C. Nebivolol HCL (10mg) was added in 100 ml volumetric flask and 20 ml 6M urea plus 20 ml (25%) citric acid &1ml (1%) sodium lauryl sulphate was added in it. Then the final volume was made up with distilled water. The clear solution of Nebivolol HCL was obtained.

Preparation of Standard Stock Solutions

Nebivolol Hydrochloride Standard Stock Solution: (100µg/ml).

An accurately weighed Nebivolol HCL (10mg) was added in 100 ml volumetric flask and dissolved in 20 ml 6M urea plus 20 ml (25%) citric acid & 1ml (1%) SLS and volume was made up to the mark using distilled water to get final concentration (100µg/ml).

Study of Spectra and Selection of Wavelength

The aliquot portions of standard stock solutions of Nebivolol HCL were diluted appropriately with distilled water to obtain concentration 10 µg/mL of drug. The solutions of drug was scanned in the range of 400 – 200 nm. The UV absorbance spectrum of NEBIVOLOL HYDROCHLORIDE is shown in **Fig.2** from the spectrum wavelength selected for estimation of drug was 282nm as λ max of NEBIVOLOL HCL.

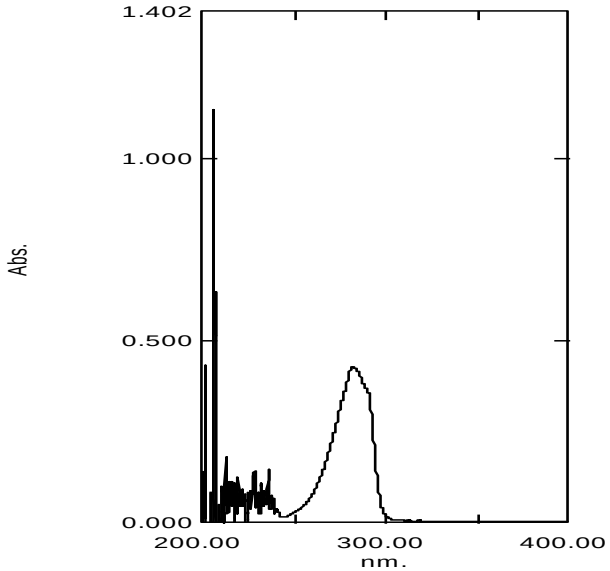

Fig.2 Uv-Absorbance Spectrum of Nebivolol Hcl at 282nm

Study of Linearity Curves

The aliquot portion of standard stock solutions of NEBIVOLOL HCL was diluted appropriately with distilled water to get a series of concentration from 25-100µg/mL for drug. The absorbance of this drug was measured at 282 nm respectively and calibration curve was plotted as concentration versus absorbance.

Linearity and Range

The suitable aliquots were taken to obtain 25, 50, 75, 100, µg/ml. from NEBIVOLOL HYDROCHLORIDE stock solution. The results are shown in table no. 2, Figure no. 3.NEBIVOLOL obeyed linearity in the concentration range of 25-100µg/ml respectively at λ max 282 nm with correlation coefficient (r2 > 0.99) in this case. Marketed brand of tablet was analyzed

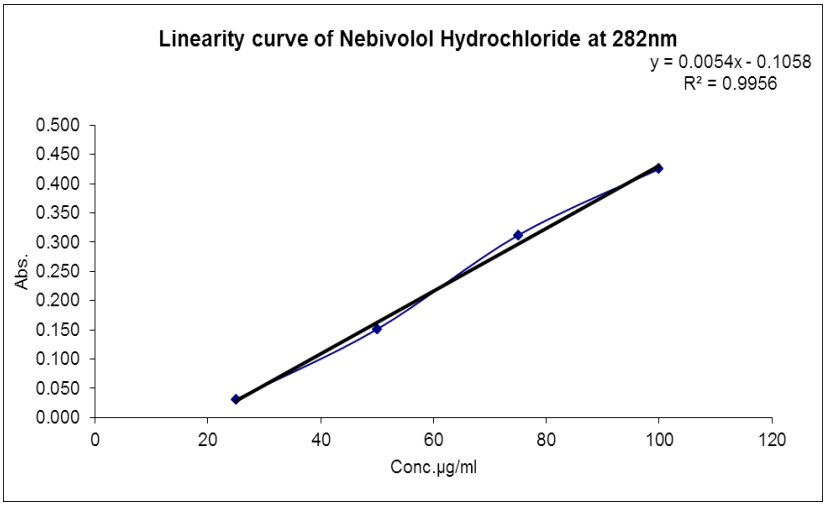

Fig.3 Linearity curve of Nebivolol Hydrochloride at 282nm.

Analysis of Marketed Formulation by Proposed Method

20 Tablets was accurately weighed, and reduced to fine powder. An accurately weighed powder sample equivalent to 10 mg of NEBIVOLOL HCL was transferred to 100 ml volumetric flask and 20 ml 6M urea plus, 20ml (25%) citric acid & 1ML(1%) SLS was added in it. Sonicate it for 20 min. The solution was filtered through Whatmann filter paper no. 41. The filtrate was further diluted with distilled water to get final concentration (100µg/ml). From this solution 10µg/ml was prepared. The absorbance of sample solution was measured at 282 nm and the results are shown in **Table No. 1.**

Table No. 1: Results of Application of Proposed Method for Analysis of Marketed Formulation.

Sample	Label Claimed	% Label Claim* ± SD	% RSD
NEBISTAR 2.5mg	NEBIVOLOL 2.5mg	111 ± 0.325	0.292

*Mean of Each 3 Reading

Validation of Method

Accuracy

Accuracy of each of the proposed method was ascertained on the basis of recovery studies performed by standard addition method as shown in the table no.2

Table No.2: Accuracy

S. No	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
1	80	75	60	0.568 ± 0.005	59.53 ± 0.31	99.22 ± 0.51
2	100	75	75	0.644 ± 0.0008	74.80 ± 0.20	99.73 ± 0.27
3	120	75	90	0.720 ± 0.001	90.07± 0.42	100.07 ± 0.46

*Mean of Each 3 Reading

Precision

Precision of the analytical method is expressed as the series of the measurement. It was ascertained by replicate estimation of the drug by the proposed method as shown in table no.3

Table No.3: Precision.

Conc.(µg/ml)	Inter day			Intra Day		
	Mean± S.D.	Amt. Found	%Amt. Found	Mean± S.D.	Amt. Found	%Amt. Found
50	0.151±0.002	51.13	102.27	0.146±0.002	50.13	100.27
75	0.311±0.002	83.27	111.02	0.311±0.003	64.30	85.73
100	0.427±0.002	106.40	106.40	0.474±0.003	115.80	115.80

*Mean of Each 3 Reading

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for no. of times. The results are shown in table no. 04

Table No.4: Repeatability.

S. No	Conc. (µg/ml)	Absorbance	Amt. Found	% Amt. Found
1	75	0.308	40.60	54.13
2	75	0.305	40.00	53.33
3	75	0.302	39.40	52.53
4	75	0.307	40.40	53.87
5	75	0.301	39.20	52.27
6	75	0.306	40.20	53.60
7	75	0.305	40.00	53.29
8	75	0.306	40.20	53.15
9	75	0.302	39.40	53.12
10	75	0.306	40.20	53.21
	Mean	0.3048	39.96	53.25
	S.D.	0.002	0.44	0.52
	%RSD	0.730	1.11	0.99

Label Claim

Table no.5

Brand Name: NEBISTAR 2.5mg. **Company: HETERO LAB LTD.**				
Amt taken (mg)	**Conc. (µg/ml)**	**Absorbance**	**Amt found**	**% Label Claim**
53.2	75	0.311	83.2	110
53.2	75	0.312	83.4	111
53.2	75	0.311	83.6	110
53.2	75	0.313	83.2	111
53.2	75	0.315	84	112
53.2	75	0.311	83.2	111
53.2	75	0.312	83.4	110
53.2	75	0.311	83.2	111
53.2	75	0.312	83.4	111
53.2	75	0.313	83.6	111
		MEAN	83.42	111
		SD	0.244	0.325
		%RSD	0.292	0.292

**mean of each 3 reading*

RESULT AND DISCUSSION

The method was optimized with view to develop a rapid, simple, accurate method for validation of Nebivolol HCL in bulk & pharmaceutical formulation. UV scanning at 400-

200nm of Nebivolol HCL shows that 282nm is the suitable wavelength for detection of drug.(fig.3).Nebivolol HCL shows linearity in the concentration range of 25-100µg/ml (r^2-0.995) respectively .Recovery study of drug were carried out for the accuracy parameter this study where carried out at three levels(80%, 100%, 120%) by standard addition method. The mean recovery was found to be 99.67% for Nebivolol HCL respectively. The developed method was found to be simple, precise, specific, & accurate Therefore this method can be applied for routine analysis of drugs in formulation & in bulk drug.

CONCLUSION

The developed method is suitable for validation of NEBIVOLOL HYDROCHLORIDE in pharmaceutical dosage form is accurate, precise, robust and rapid. Therefore this method can be applied for routine analysis of drugs in formulation & bulk drug.

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