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Spectrophotometric methods for the estimation of nebivolol hydrochloride in pharmaceutical formulation

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Abstract
Two simple and sensitive spectrophotometric methods have been developed for the estimation of Nebivolol Hydrochloride HCl in bulk drug and pharmaceutical formulation. Method A is a UV Spectrophotometric method using Dimethylformamide as a solvent in which drug showed maximum absorbance at 282.5 nm. Beer's law was obeyed in the concentration range of 10-50 µg/mL with correlation coefficient 0.999. Method B is based on a charge transfer complexation with 0.1% w/v iodine in dichloromethane to form pink colored chromogen which showed maximum absorbance at 486.5 nm. Beer's law obeyed in the concentration range of 5-40 µg/mL with correlation coefficient 0.999. The results of the methods have been validated statistically and by recovery studies confirmed the accuracy and precision of the methods. The proposed methods are simple, sensitive and economical for quantitative determination of Nebivolol Hydrochloride HCl in bulk drug and pharmaceutical formulation.

Keywords: Nebivolol hydrochloride, spectrophotometric, validation, dimethylformamide, iodine

1. Introduction
Nebivolol Hydrochloride chemically 2, 2'-azanediylbis (1-(6-fluorochroman-2-yl) Dimethyl formamide) hydrochloride [1] (Fig.1) is a long acting cardio selective beta blocker currently used for the treatment of hypertension [2]. Nebivolol Hydrochloride 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 as a result it causes dilation of blood vessels. It is official in British pharmacopoeia. A survey of literature revealed that various analytical methods have been reported for determination of Nebivolol Hydrochloride including spectrophotometric [3-10], first order derivative spectrophotometry [11], spectro fluorimetric [12], high-performance liquid chromatography (HPLC) [13-17], HPTLC [18-19], liquid chromatography mass spectrophoometry (LCMS) [20-21]. Chromatographic methods for the determination of Nebivolol Hydrochloride concentrations require an automated system not available in many research laboratories. Therefore, it was considered worthwhile to develop rapid and sensitive methods suitable for the routine quality control analysis of the investigated drug. Spectrophotometry is still the most frequently used analytical technique for pharmaceutical analysis, providing practical and significant economic advantages compared to other methods In the proposed work, a successful attempt has been made to develop both UV and visible spectrophotometric methods with due consideration of accuracy, precision and sensitivity.

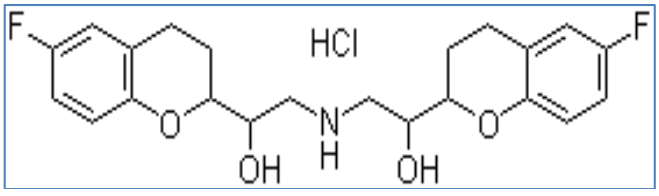

Fig 1: Structure of Nebivolol Hydrochloride

Materials and Methods

Instrumentation and Reagents

Spectral and absorbance measurements were made using ELICO-SL 210 UV-Visible double beam spectrophotometer with 10 mm matched quartz cells. Tablet formulation NEBICARD-5 (Nebivolol Hydrochloride Tablets) containing Nebivolol Hydrochloride 5 mg was used in the present study. Dimethylformamide, iodine, dichloromethane were of analytical grade acquired from S.D. Fine Chemicals, Mumbai.

Preparation of Reagents

Iodine (0.1%w/v) solution: It was prepared by dissolving 0.1 g of pure resublimed iodine in 20 mL of dichloromethane in 100 mL volumetric flask and the volume was made upto the mark with dichloromethane.

Preparation of standard stock solution (100 µg/mL) (Method A & B)

Weighed accurately about 10 mg of Nebivolol Hydrochloride and transferred it into a 100 mL volumetric flask. The content of the flask was dissolved with little quantity of Dimethylformamide and volume was made upto the mark with same.

Method A

Procedure for calibration curve

Aliquots of 1.0, 2.0, 3.0,4.0 and 5.0 mL of 100 µg/mL Nebivolol Hydrochloride standard solution were accurately transferred into a series of 10 mL volumetric flasks and volume was made up to the mark with (95%v/v) Dimethyl formamide. The absorbance of the resulting solutions was measured at 282.5 nm against blank. The absorbance spectra was shown in Fig.2. A calibration graph for Nebivolol Hydrochloride was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig 4.

Fig 2: UV Spectra of Nebivolol Hydrochloride (Method A)

Estimation of Nebivolol Hydrochloride in tablet formulation

Marketed tablet formulations containing 5 mg of Nebivolol Hydrochloride were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 10 mg of Nebivolol Hydrochloride was weighed and transferred to 100 mL volumetric flask. The contents of the flask were dissolved in about 40 mL Dimethylformamide with the aid of ultrasonication for 30 min. The solution was filtered through Whatmann filter paper no. 41.The filter paper was washed with Dimethylformamide. The washings were added to the filtrate and the final volume was made up to 100 mL with the Dimethylformamide to get a concentration of 100 µg/mL.From the above solution 3 mL was transferred to 10 mL calibrated volumetric flasks and made to the mark with the aid of Dimethylformamide to obtain concentration 30 µg/mL. The absorbance of the final sample corresponding to 30 µg/mL was recorded against the blank at 282.5 nm. The amount of drug in pharmaceutical formulation was calculated from calibration curve. The results of analysis of tablet formulations are recorded in Table 1.

Method B

Procedure for calibration curve

Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL of 100 µg/mL Nebivolol Hydrochloride standard solution were accurately transferred into a series of 10 mL volumetric flasks and to these 1.2 mL of 0.1% w/v iodine solutions were added to each flask, the content was mixed well and the flasks were allowed to stand for 10 min. The volume was brought to the mark with dichloromethane. The pink colour was developed and the solution of each was measured at 486.5 nm against the reagent blank. The absorbance spectrum was shown in Fig.3. A calibration graph for Nebivolol Hydrochloride was plotted by taking concentration of drug on x-axis and absorbance on y-axis and as presented in Fig 5.

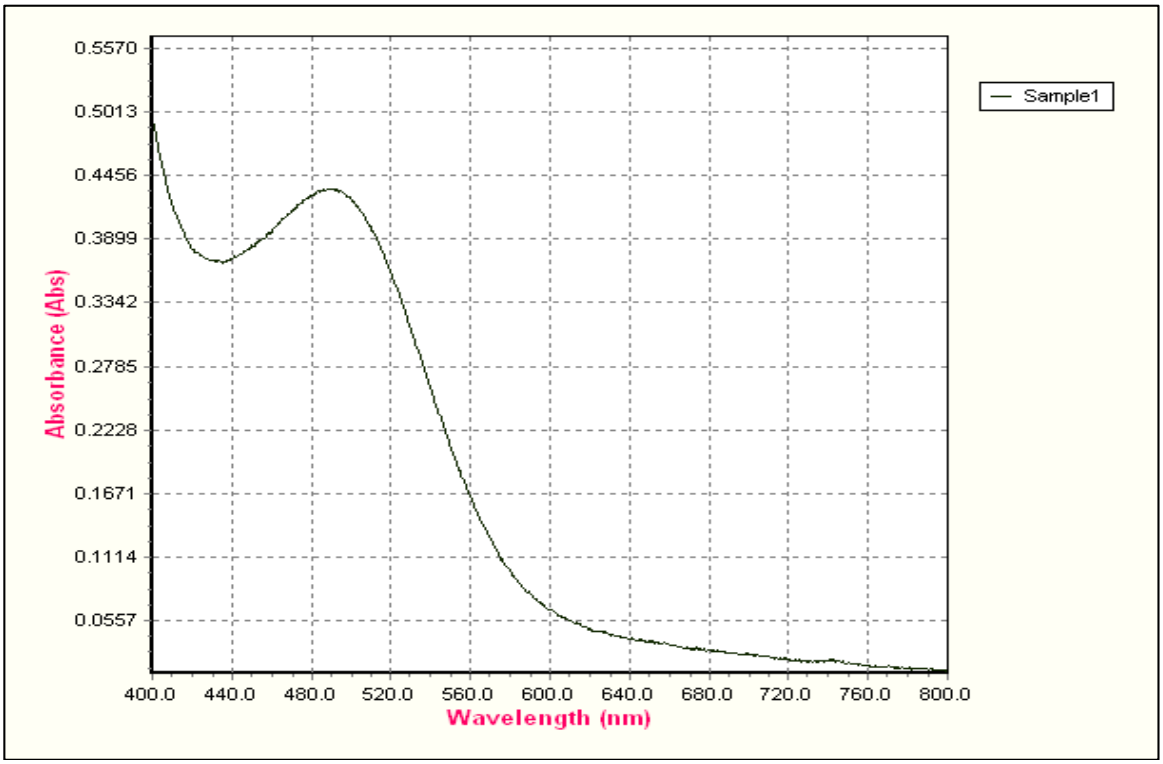

Fig 3: Visible Spectra of Nebivolol Hydrochloride (Method B)

Estimation of Nebivolol Hydrochloride in tablet formulation

Marketed tablet formulations containing 5 mg of Nebivolol Hydrochloride were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 10 mg of Nebivolol Hydrochloride was weighed and transferred to 100 mL volumetric flask. The contents of the flask were dissolved in about 40 mL Dimethylformamide with the aid of ultrasonication for 30 min. The solution was filtered through Whatmann filter paper no. 41. The filter paper was washed with Dimethylformamide. The washings were added to the filtrate and the final volume was made up to 100 mL with the Dimethylformamide to get a concentration of 100 µg/mL.From the above solution 3 mL was transferred to 10 mL calibrated volumetric flasks and to these 1.2 mL of 0.1% w/v iodine solutions was added, the content was mixed well and the flask was allowed to stand for 10 min. The volume was brought to the mark with dichloromethane. The pink colour was developed and the absorbance of the final sample corresponding to 30 µg/mL was measured at 486.5 nm against the reagent blank. The amount of drug in pharmaceutical formulation was calculated from calibration curve The results of analysis of tablet formulations are recorded in Table 1.

Table 1: Estimation of Nebivolol Hydrochloride in tablets

Methods	Assay Sample	Labeled found(mg)	Amount found(mg)	%Purity ± SD*
Method A	Nebicard-5	5 mg	4.88mg	97.80% ± 0.02
Method B	Nebicard-5	5 mg	4.95mg	97.80% ±0.049

*Average of six determinations

Results

Validation of analytical data

The method was validated in accordance with the current ICH guidelines.

Linearity and Range

The calibration graphs obtained by plotting the values of the absorbance versus the concentrations (µg/mL) were found to be linear over the concentration range of 10-50 µg/mL and 5-40 µg/mL for method A and B respectively. From the data obtained, co-relation coefficient, slope and y-intercept were calculated and the results were recorded in Table 2, 3 and 4**.** Ideally co-relation coefficient should be not less than 0.998 and the Beer's plots were shown in Fig.4 & 5.

Table 2: Calibration Data Table

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.1212
2	20	0.2514
3	30	0.3562
4	40	0.4831
5	50	0.5943

Table 3: Calibration Data Table

S. No.	Concentration (µg/ml)	Absorbance
	5	0.1051
1	10	0.2162
2	15	0.3249
3	20	0.4371
4	25	0.5428
5	30	0.6516
6	35	0.7693
7	40	0.8785

Table 4: Optical Characteristics of Nebivolol Hydrochloride

S. No.	Parameter	Method A	Method B
1.	Absorption Maxima(nm)	282.5	486.5
2.	Linearity Range(µg/ml)	10-50	5-40
3.	Regression Equation	y = 0.0119x +0.0037	y= 0.022x - 0.0035
4.	Slope(b)	0.0119	0.022
5.	Intercept(a)	0.0037	- 0.005
6.	Correlation Coefficient(r^2)	0.999	0.999
7.	%RSD	0.372	0.8
8.	Molar Absorptivity(litmol^{-1} cm^{-1})	5.314×10^3	8.826×10^3
9.	Sandell's Sensitivity(µg/cm^2/ 0.001 abs unit)	0.076	0.045
10.	% Range of errors (Confidence limits)		
	0.01%	0.3562	0.624
	0.05%	0.6023	0.991
11.	Limit of Detection (µg/ml)	0.2457	0.190
12.	Limit of Quantification (µg/ml)	0.7412	0.571

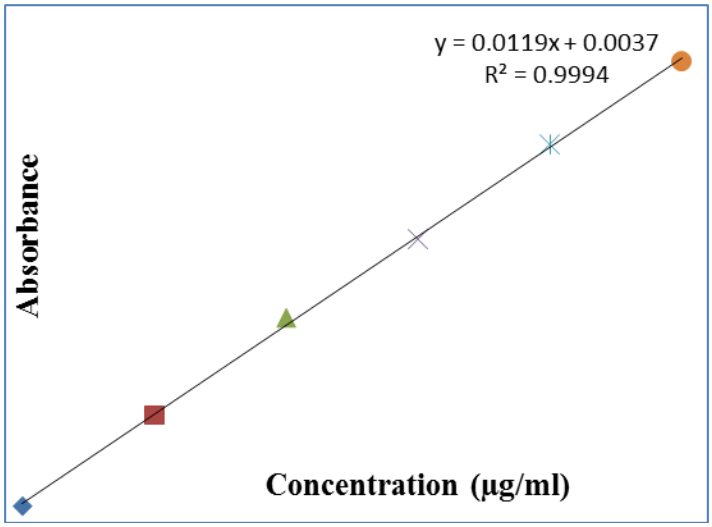

Fig 4: Beer's law plot of Nebivolol Hydrochloride (Method A)

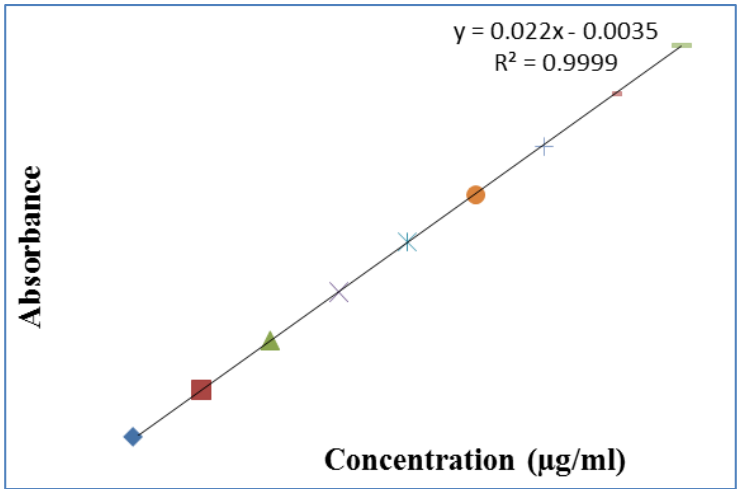

Fig 5: Beer's law plot of Nebivolol Hydrochloride (Method B)

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed condition. The system precision was analysed by six different solutions of same concentration and absorbances were noted. The result was indicated by% RSD. The results are shown in Table 4. Repeatability or Intra-day precision was investigated on six replicate sample solutions on the same day. Inter-day precision was assessed by analyzing newly prepared sample solutions in triplicate over three consecutive days. Both inter day and intraday precision was expressed as% RSD. The results were summarized in Table 5 & 6. The low value of% RSD for both methods indicates the high precision of the both methods.

Table 5: Intraday and Interday precision for method A

	Intraday		Interday	
Con. taken (µg/mL)	**Con. found* (µg/mL)**	**%RSD**	**Con. found* (µg/mL)**	**%RSD**
20	19.82	0.46	19.89	0.48
30	29.98	0.51	29.91	0.45
40	39.89	0.48	39.85	0.45

*average of six determinations

Table 6: Intraday and Interday precision for method B

	Intraday		Interday	
Con. taken (µg/mL)	**Con. found* (µg/mL)**	**%RSD**	**Con. found* (µg/mL)**	**%RSD**
20	19.98	0.34	19.95	0.38
25	24.93	0.36	24.96	0.31
30	29.95	0.36	30.14	0.35

*average of six determinations

Accuracy

Accuracy of the methods were determined by preparing solutions of different concentrations that is 80%, 100% and 120% of targeted drug concentration in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by% recovery. The results are shown in Table 7 & 8.

Table 7: Accuracy Results of the Method A

S. No	% Level of Recovery	Initial amount present (µg/ml)**	Amount of standard Added (µg/ml)**	Total Amount present (µg/ml)**	Standard Amount Recovered (µg/ml)**	% Recovery ± S.D**
1.	80	30	24	54	23.9	99.5 ± 0.281
2.	100	30	30	60	29.6	98.6 ± 0.312
3.	120	30	36	66	35.9	99.7 ± 0.318

** Average of three determinations

Table 8: Accuracy Results of the Method B

S. No	% Level of Recovery	Initial amount present (µg/ml)**	Amount of standard Added (µg/ml)**	Total Amount present (µg/ml)**	Total Amount Recovered (µg/ml)**	% Recovery ± S.D**
1.	80	30	24	54	23.8	99.4 ± 0.289
2.	100	30	30	60	29.7	99.8 ± 0.309
3.	120	30	36	66	35.8	99.7 ± 0.312

****** Average of three determinations.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 2 nm. For changes of conditions, the sample was assayed in triplicates (Table 9 and Table 10).

Table 9: Results of Robustness Study (method A)

Formulation	Amount of drug taken from tablet (mg)	At 280.5 nm	At 284.5 nm
		(n=3)%Assay±%RSD	(n=3)%Assay ±%RSD
NEBICARD-5	5	99.73±0.313	99.91±0.224

Table 10: Results of Robustness Study (method B)

Formulation	Amount of drug taken from tablet (mg)	At 484.5 nm	At 488.5 nm
		(n=3)%assay ±%RSD	(n=3)%assay ±%RSD
NEBICARD-5	5	99.61±0.423	99.84±0.516

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogeneous slot by two analysts using same operational and environmental conditions (Table 11 and Table 12).

Table 11: Ruggedness results of Method A

S. No	Labelled amount (mg)	Analyst 1		Analyst 2	
		Amount found (mg)	%Recovered ± S.D. * *	Amount found (mg)	%Recovered ± S.D. * *
1.	5 mg	4.96 mg	99.20 ± 0.022	4.98 mg	99.70±0.042

****** Average of six determinations

Table 12: Ruggedness results of Method B

S. No	Labelled amount(mg)	Analyst 1		Analyst 2	
		Amount found (mg)	%Recovered ± S.D. * *	Amount found (mg)	%Recovered ± S.D. * *
1.	5 mg	4.97 mg	99.20 ± 0.033	4.98 mg	99.80±0.041

****** Average of six determination

Limit of detection and Limit of quantitation

Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by using the formula based on standard deviation of the response and the slope. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the equations LOD = 3 σ /S and LOQ= 10 σ /S, where σ is standard deviation of intercept, S is slope of the line (Table 3).

Discussion

The present study was carried out to develop simple and sensitive spectrophotometric methods for determination of Nebivolol Hydrochloride in tablets. The method A is a UV spectrophotometric method in which the absorption spectrum of the drug Nebivolol Hydrochloride in Dimethylformamide exhibits an absorption peak at 282.5 nm. Linearity of the method was observed in the concentration range of 10-50 µg/mL with correlation coefficient(r^2=0.999).The proposed method was applied to pharmaceutical formulation and percent amount of drug estimated was found in good agreement with the label claim. The excipients used in the pharmaceutical preparation do not interfere in this analysis. The recovery experiment was carried out at three different levels i.e., 80%, 100% and 120%. The percentage recovery was found to be in the range 98.6-99.7%.The precision of the method was studied as an intra-day and inter-day and repeatability. A low value of% RSD indicated the precision of proposed method. The method was unaffected small variation in wavelength indicates the robustness of method.The method was unaffected by change in analyst indicate the ruggedness of method. The limits of Detection and Quantitation were 0.2457 and 0.7412 µg/mL respectively, indicates the sensitivity of the method.

Method B is charge transfer complexation reaction between Iodine in dichloromethane and the drug Nebivolol Hydrochloride resulting in the formation of yellow colored chromogen for which the absorbance was measured at 486.5 nm. This complex too has 1: 1 composition as determined by Job's method. Linearity of the method was observed in the concentration range of 5-40 µg/mL with correlation coefficient (r^2=0.999). The proposed method was applied to pharmaceutical formulation and percent amount of drug estimated was found in good agreement with the label claim. The excipients used in the pharmaceutical preparation do not interfere in this analysis. The recovery experiment was carried out at three different levels i.e., 80%, 100% and 120%. The percentage recovery was found to be in the range 99.4-99.8%. The precision of the method was studied as an intra-day and inter-day and repeatability A low value of% RSD indicated the precision of proposed method. The method was unaffected small variation in wavelength indicates the robustness of method.The method was unaffected by change in analyst indicate the ruggedness of method. The limits of Detection and Quantitation were 0.190 and 0.571 µg/mL respectively, indicates the sensitivity of the method.

Conclusion

The proposed UV and Visible Spectrophotometric methods for the determination of Nebivolol Hydrochloride in the bulk and its pharmaceutical dosage forms was found to be simple, precise, rapid, accurate and involved easy sample preparation. The linearity, reproducibility and recovery data confirms no major interference due to excipients in the tablet in the assay determination so this method can be used for routine quality control analysis of this drug in pharmaceutical dosage forms.

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