International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.5, pp 1571-1579, Sept-Oct 2014

Extractive Spectrophotometric Determination of Nebivolol Hydrochloride in Pharmaceutical Formulation and Biological Fluids

Tripti Sharma*[1], Dannana Gowrisankar[2], Sudam Chandra. Si[1]

[1]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar-751003, India
[2]Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, India
Address of correspondence: Tripti Sharma Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar-751003, India

*Corres.author: tripti_neema@yahoo.co.in, Tel.: +91 9937508852

Abstract: Three highly sensitive and simple spectrophotometric methods were developed to quantitate the drug Nebivolol Hydrochloride (NBH) in raw materials and in biological sample. The methods are based on extraction of NBH into chloroform as ion-pair complexes with bromophenol blue (BPB), cresol red (CR) and bromocresol purple (BCP) in acidic medium. The colored species exhibited absorption maxima at 410, 416 and 420 nm for BPB, CR and BCP, with molar absorptivity values of 2.90×10^5, 1.72×10^5 and 2.64×10^5 respectively. The reaction conditions were optimized to obtain the maximum color intensity. Beer's law was obeyed with a good correlation co-efficient 0.9980, 0.9986 and 0.9990 in the concentration ranges 1-20, 0.5- 15 and 2-20µgmL^{-1} for BPB, CR and BCP methods respectively. Sendell's sensitivities and detection limits were calculated and analyzed. The stoichiometry of the reaction was found to be 1:1 in all cases. The proposed method was successfully applied for the determination of NBH in tablets, spiked human plasma with good accuracy and precision. Statistical comparison of the results was performed using student's t-test and the F-test at 95% confidence level. The accuracy and precision of the proposed methods were not significantly different.
Keywords: Nebivolol Hydrochloride, Bromophenol blue, cresol red, bromocresol purple, ion-pair complex, spectrophotometric

Introduction

Nebivolol hydrochloride (NBH) 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 selectiveβ1-blocker with nitric oxide-mediated vasodilatory actions and beneficial effects on vascular endothelial function. It has been clinically used for the treatment of hypertension and chronic 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 NBH in pharmaceuticals and include spectrophotometry, TLC, LC, HPTLC, LC-MS [4-10]. Chromatographic methods for the determination of NBH 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 this paper, simple and sensitive extractive spectrophotometric methods for the analysis of NBH were described. The methods are based on the formation of chloroform soluble ion-association complexes of NBH with bromophenol blue (BPB), cresol red (CR) and bromocresol purple (BCP). The proposed method has the advantage of being rapid, simple, accurate, economic, sensitive and less-time consuming. The proposed method was applied for determining NBH in bulk, tablets, and spiked human plasma samples with satisfactory results.

Experimental

Apparatus

The spectrophotometric measurements were carried out using JASCO V360 Double beam UV- VIS Spectrophotometer with 1cm U.V. matched quartz cells. A REMI centrifuge with speed 50,000 rpm was used to carry out for the spiked plasma samples.

Reagent and chemicals

All chemicals and reagents were of analytical grade and water was always bidistilled water. Pharmaceutical grade NBH 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. Bromophenol blue (BPB), cresol red (CR) and bromocresol purple (BCP) were obtained from Merck, Darmstadt, Germany. Stock solutions (1.0×10^{-3} M) were prepared by dissolving the appropriate weight of BPB, CR and BCP in 10 mL chloroform and diluting to 100mL with chloroform. 4.425 ml of concentrated hydrochloric acid was diluted to 100 ml with distilled water.

Stock solutions

Stock standard solutions of NBH (1mg mL^{-1}) were prepared by dissolving an exact weight (100mg) of NBH in 10 mL of chloroform and further diluted to 100mL with chloroform. Working test solutions (100µg mL^{-1}) of NBH was prepared by dilution with chloroform. The solution was found to be stable for at least three days when kept in refrigerator.

General procedure for quantitation of NBH

Aliquots of standard drug solution (100 µgmL^{-1}) in the concentration range stated in Table 1 were transferred into a series of 50mL separating funnels. The volume in each separating funnel was adjusted to 2 mL with distilled water. 1 mL of 0.1N HCl was added to each separating funnels, followed by the addition of 1.0, 1.2 and 1.0 mL (1×10^{-3}M) of BPB, CR and BCP respectively to each series of separating funnel. The funnels were shaken vigorously with 10 mL of chloroform for 2min, and the allowed to stand at room temperature (25 ± 1^{0}C) for clear separation of the two phases. The separated organic phase was transferred to a 10 mL volumetric flask. Then the extract was made up to the mark with chloroform and mixed well. The absorbance of the yellow colored complex was measured at 410, 416, 420 nm for BPB, CR and BCP, respectively against a reagent blank prepared similarly omitting the drug (figure1). The calibration graphs are linear over the range the concentration ranges and are given in Table 1.

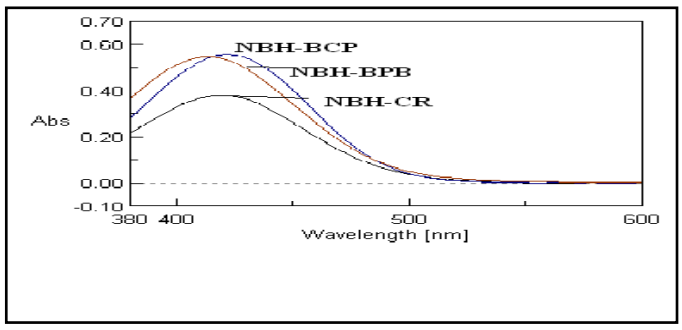


Figure 1 Absorption Spectrum of ion-association complexes of NBH(10µg mL^{-1}) with (1×10^{-3} M) BPB,CR and BCP against reagent blank

Table 1 Optical Characteristics and statistical analysis for the regression equation of the proposed Methods

Parameter	Extraction methods		
	BPB	CR	BCP
λmax, nm	410	416	420
Extracting solvents	chloroform	chloroform	chloroform
Complex stability (h)	3	6	5
Molar ratio(NBH:BPB or CR or BCP)	1:1	1:1	1:1
Beer's law limit. µg/mL	1-20	0.5-16	2-20
Molar absorptivity, L mol^{-1} cm^{-1}	2.90×10^5	1.72×10^5	2.64×10^5
Sandell sensitivity (µg mL^{-1})	0.0151	0.0256	0.0167
Limit of detection (LOD)(µg mL^{-1})	0.034	0.154	0.282
Limit of quantification (LOQ)(µg mL^{-1})	0.103	0.468	0.857
Slope (a) (mL µg mL^{-1})	0.055	0.032	0.043
Intercept (b)	0.102	0.070	0.164
Correlation coefficient (r)	0.9980	0.9986	0.9990
R.S.D. %	0.10	0.047	0.057

Y = a +bX where X is the concentration in µg mL^{-1}

Procedure for Tablets

Twenty tablets were weighed and pulverized. An amount of the powder equivalent to 10mg of NBH was weighed and transferred into a clean 100mL volumetric flask containing 10 mL of chloroform .After sonicating the sample for 20 min, the volume was brought up to the mark with distilled water and filtered through Whatmann No.42 filter paper. The drug content of an aliquot of this solution was obtained by applying the general procedure as described above.

Procedure for spiked human serum

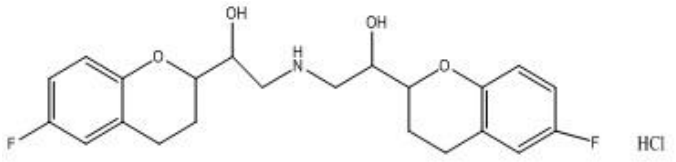
The appropriate amount of standard solutions of NBH was added to 1 mL of plasma sample. One mL of 10% (w/v) trichloro-acetic acid was added for each mL of the plasma for deproteination. The sample was centrifuged at 3500rpm for10 min. Two mL of protein-free supernatant was transferred into 10 mL volumetric flask and the above procedure was then followed.

Stoichiometric relationship

Job's method of continuous variation was employed to determine the stoichiometric ratios [11-12]. Equimolar (1×10^{-3}M) solution of NBH and BPB, CR and BCP were prepared. A series of solutions were prepared in which the total volume of drug and reagent was kept at 5mL. The reagents were mixed in various proportions and completed to 10 mL bidistilled water, following the above mentioned procedure.

Result and Discussion

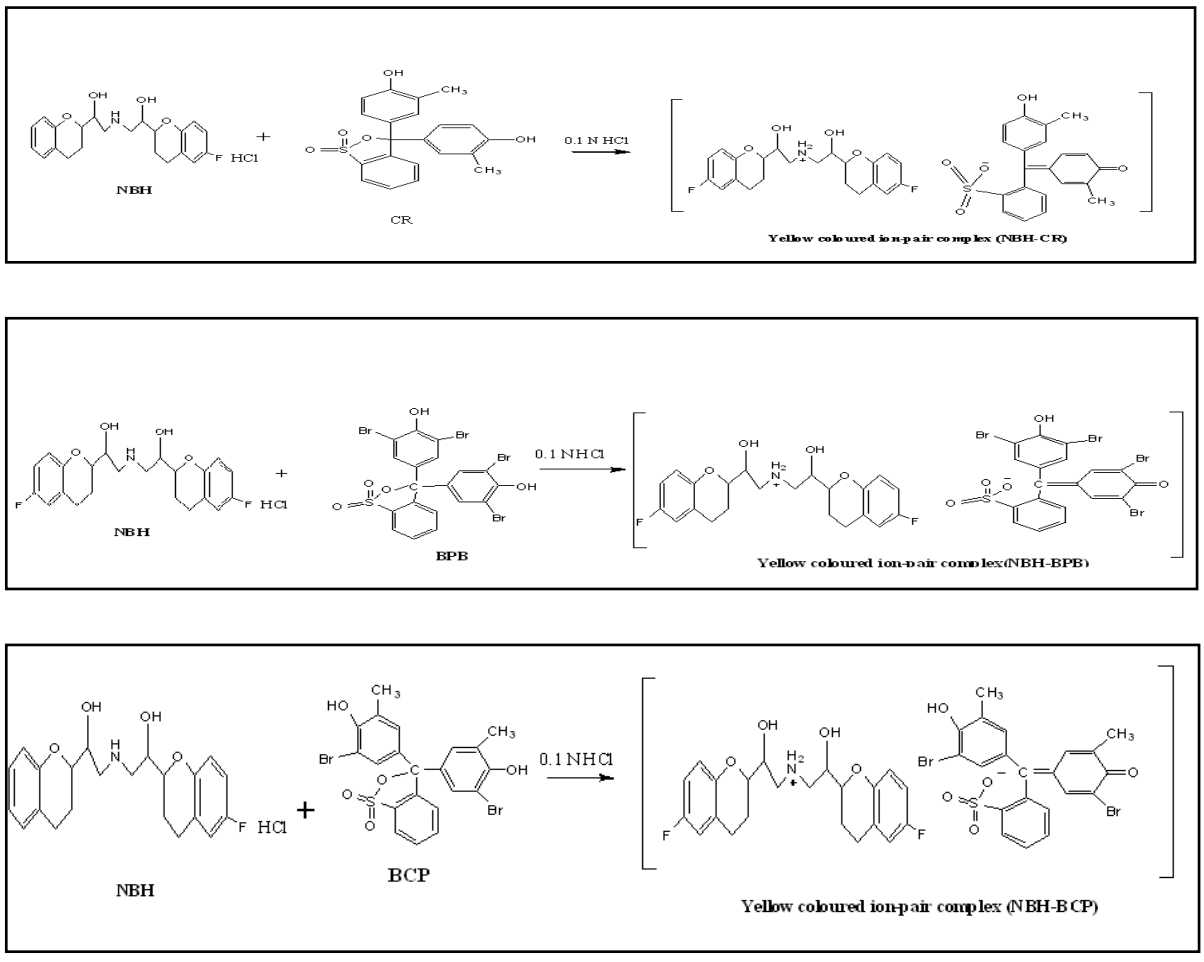
NBH was found to react with the dyes in chloroform medium to produce an intense and stable ion-pair complex. The results obtained in Methods were based on extractive spectrophotometry. The NBH exhibits basic character essentially because of the presence of secondary amino group. In acidic media, the secondary amino group of NBH is protonated, while sulphonic group present in BPB, CR and BCP undergoes dissociation. NBH involves an ion-pair association with BPB, CR and BCP under acidic conditions, which is extractable with chloroform from the aqueous phase, resulting in the formation of a yellow colored complex exhibiting maximum absorption at 410, 416 and 420nm against the corresponding reagent blank, respectively. The proposed reaction mechanisms of NBH with BPB, CR and BCP have been given in Scheme1.



Scheme 1 Structure of Nebivolol Hydrochloride (NBH)

Optimization of variables and method development

The experimental conditions were established by varying one variable and observing its effect on the absorbance of the colored species.



Scheme 2 Proposed reaction mechanism for ion-association complex of NBH with BPB, CR and BCP

Effect of solvent

In order to select a suitable solvent for preparation of the reagent solutions used in the study, a number of organic solvents such as chloroform, dichloromethane, acetonitrile, acetone, and dioxane were examined. Chloroform was best suited for the preparation of BPB, CR and BCP solutions. Similarly, the effect of the diluting solvent was studied for all methods and the results showed that chloroform formed sensitive and stable colored species in all the three methods. Therefore, chloroform was used for dilution throughout the investigation.

Effect of dye concentration

The influence of the concentration of BPB, CR and BCP on the intensity of the color developed at selected wavelength was studied (Fig-2). 1.0, 1.2 and 1.0 mL (1×10^{-3}M) of BPB, CR and BCP respectively was sufficient to produce maximum and reproducible color.

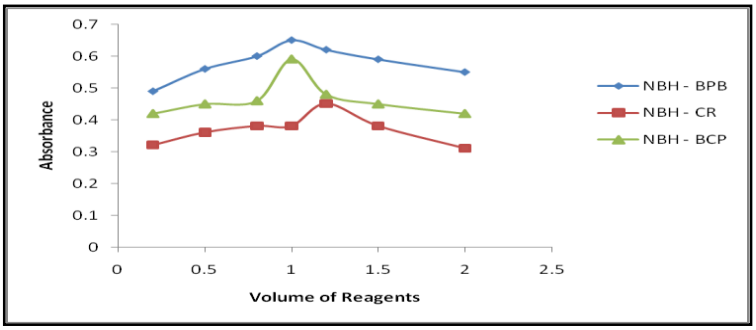


Figure 2 Effect of reagent concentration on the formation NBH–BPB complex, NBH–CR complex and NBH-BCP complex (NBH- 10µgmL^{-1})

Effect of reaction time

The optimum reaction time for the development of color at ambient temperature (25±2^{0}C) was studied and it was found that complete color development was achieved after 10min in all the three methods. The formed color was stable for more than 3 hours.

Stoichiometric ratio of the ion-association complex

Job's continuous variations graph for the reaction between NBH -BPB, CR and BCP shows that the interaction occurs on an equimolar basis via the formation of ion-pair complexes (Figure 3). The plot reached a maximum value at a mole fraction of 0.5 which indicated that a 1: 1 (NBH: BPB) (NBH:CR) and (NBH:BCP) ion-pair complexes are formed through the electrostatic attraction between positive protonated NBH and the anion of BPB, CR and BCP. The suggested mechanism of (NBH: BPB) (NBH:CR) and (NBH:BCP) ion pair complex formation is described in scheme 2

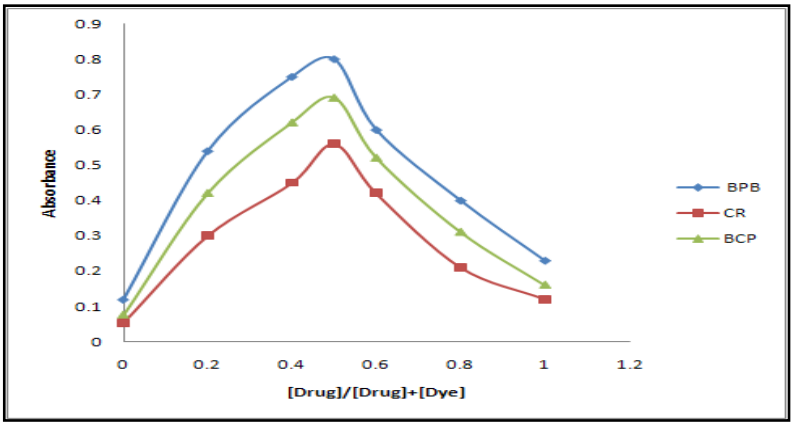


Figure 3 Job's method of continuous variation graph for reaction of NBH with BPB, CR and BCP

Method Validation

Analytical parameters

A linear relation was found to exist between absorbance and the concentration of NBH in the ranges given in Table 1.The calibration graph in each case is described by the equation:

Y = a +bX

where Y= absorbance, a =intercept, b= slope and X=concentration in µg mL^{-1} obtained by the method of least squares. 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 calculated as per the current ICH guidelines [13] are compiled in Table 1 and are indicative of the sensitivity of the methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

LOD = 3.3σ/s and LOQ = 10σ/s

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

Accuracy and precision

The accuracy and precision of the methods were evaluated by performing five replicate analyses on pure drug solution at three different concentration levels (within the working range). The relative error (RE %), an indicator of accuracy Table 2 was within 1.36 and within day precision, also called the repeatability, expressed as relative standard deviation (RSD %) was less than 2.15 indicating high accuracy and repeatability of the methods. The reproducibility of the methods also known as the day-to-day precision was evaluated by performing replicate analyses on pure drug solution at three levels over a period of five days, preparing all solutions afresh. The day-to-day RSD values Table 2 were less than 2% reflecting the usefulness of the methods in routine analysis.

Table 2 Evaluation of intra-day and inter-day precision and accuracy of the proposed procedure

Method	NBH taken (µg mL^{-1})	Intra day			Interday		
		NBH found (μgmL^{-1})[a]	%RSD[b]	%RE[c]	NBH found (μgmL^{-1})[a]	%RSD[b]	%RE[c]
BPB	5	4.96	1.83	0.64	5.01	0.61	0.28
	10	9.98	1.09	0.12	9.95	1.26	0.46
	15	15.04	1.02	0.28	15.07	1.29	0.48
CR	5	4.97	1.79	0.52	4.93	2.15	1.36
	10	9.94	0.81	0.62	9.95	1.27	0.54
	15	14.97	0.43	0.17	14.98	0.61	0.13
BCP	5	5.04	1.26	0.80	4.96	1.49	0.76
	10	10.03	1.05	0.26	10.04	0.65	0.42
	15	15.04	0.60	0.24	14.98	1.14	0.09

a Mean value of five determinations.
b Relative standard deviation (%).
c Relative error (%).

Robustness and ruggedness

Method robustness was tested by making small incremental changes in the volume of dyes and reaction time. To check the ruggedness, an analysis was performed by four different analysts; also by a single analyst performing analysis using four different cuvettes. The robustness and the ruggedness were checked at three different drug levels. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in Table 3.

Table 3 Robustness and ruggedness expressed as intermediate precision (% RSD).

Method	NBH taken (µg mL^{-1})	Method robustness		Method ruggedness	
		Parameters altered			
		Reagent volume (mL)[a] RSD (%) (n= 3)	Reaction time[b] RSD (%) (n= 3)	Inter-analysts[a] RSD (%) (n=4)	Inter-cuvettes[a] RSD (%) (n= 4)
BPB	5	1.81	2.62	0.93	1.50
	10	2.10	1.60	0.62	1.24
	15	1.64	0.83	1.09	1.36
CR	5	0.89	1.69	1.38	1.60
	10	1.28	1.45	0.86	1.06
	15	0.34	0.51	0.71	0.93
BCP	5	1.02	1.91	1.07	1.16
	10	0.63	1.08	0.85	1.22
	15	0.78	1.04	1.06	1.03

[a] In BPB and BCP methods, the volume of reagent was 0.8, 1.0 and 1.2 mL. In CR method, the volume of reagent was 1.0,1.2 and 1.4 mL.
[b] The reaction time was 9, 10 and 11 min for methods A, B and C.

Applications to Tablets

The proposed method has been successfully applied to the determination of NBH in commercial tablets purchased locally. The results are shown in Table 4. The results obtained were statistically compared with those obtained by the reference method [14], by applying the Student's t-test for accuracy and F-test for precision at 95% confidence level. Statistical analysis of the results did not detect any significant difference in the performance of the proposed method to the reference method with respect to accuracy and precision as revealed by the Student's *t*-value and variance ratio *F*-value.

Table 4. Results of analysis of tablets by the proposed method and statistical comparison of the results with the reference method

Tablet brand name	Label Claim (mg/tablet)	Found (Percent of label claim ± SD)[a]			
		Reference method	Proposed method		
			BPB Method	CR Method	BCP Method
Nebicard	5	100.29 ±1.17	100.24 ±1.32	99.14 ± 1.29	98.45 ±1.19
			t =0.47	t= 0.17	t=0.52
			F =0.82	F=0.85	F=0.97
Nebinex	5	100.11 ±0.42	100.45 ±0.88	99.70 ±0.82	99.54 ±1.72
			t =0.44	t =0.35	t =0.49
			F =0.17	F=0.21	F=0.18

[a] Mean value of five determinations.
(Tabulated *t*-value at the 95 % confidence level and for four degrees of freedom is 2.77). (Tabulated *F*-value at the 95 % confidence level and for four degrees of freedom is 6.39).

Application to spiked plasma sample

The proposed method was applied to the determination of NBH in spiked plasma by following the procedures described above. The recovery of the drug from spiked plasma analysis was calculated by triplicate analysis of plasma sample containing 5, 10 and 15 µg mL^{-1} NBH separately. The percentage recovery values of 96.42– 98.37 with standard deviation 1.24–1.97 % showed the non-interference of other materials present in plasma to the assay of NBH with considerable accuracy. The analytical results obtained for NBH in spiked plasma sample are presented in Table 5.

Table 5. Application of the proposed method to NBH concentration measurements in spiked plasma sample

Method	NBH in spiked plasma (µgmL^{-1})	NBH recovered [a] (percent ± SD)
BPB Method	5	97.56 ±1.88
	10	98.37±1.97
	15	97.96±1.78
CR Method	5	96.42±1.24
	10	95.65±1.53
	15	97.63±1.36
BCP Method	5	98.15±1.74
	10	97.7±1.53
	15	97.29±1.69

[a] Mean value of five determinations.

Conclusions

The proposed method for the estimation of NBH using BPB, CR and BCP is advantageous over many of the reported methods due to its sensitivity, rapidity and good agreement with the reference methods. The high recovery percentage and low relative standard deviation reflect the high accuracy and precision of the proposed method. Moreover, the method is easy, applicable to wide ranges of concentration, besides less time consuming and depend on simple reagents which are available. This offers economic and acceptable method for the routine determination of the cited drug. So, it is recommended for the routine determination in pure samples, pharmaceutical formulations and in plasma samples.

Acknowledgements

The authors express their sincere thanks to Cipla Pharmaceuticals Ltd., India for providing gift sample of Nebivolol Hydrochloride. Thanks are also due to the Professor and Chairman Siksha 'O' Anusandhan University, Bhubaneswar for providing laboratory facility to carry out the present research work.

References

1. Budavari S., The Merck Index, 13th ed. Merck & Co, Inc, Whitehouse Station, NJ, 2001, 1152.
2. Moen M, Wagstaff A., Nebivolol: A review of its use in management of hypertension and chronic heart failure, Drugs., 2006, 66, 1389–1409.
3. Sweetman S.C., Martindale-The Complete Drug Reference, 34th Ed. Pharmaceutical Press, London, UK, 2005, 650.
4. Shah D.A, Bhatt K.K, Mehta R.S, Baldania S.L., Determination of nebivolol hydrochloride and hydrochlorothiazide in tablets by first-order derivative spectrophotometry and liquid chromatography, J AOAC., 2008, 91,1075–1082.
5. Kachhadia P.K. Doshi A.S. Joshi H.S., Development and validation of a stability-indicating column high-performance liquid chromatographic assay method for determination of nebivolol in tablet formulation, J AOAC., 2008, 91,557–561.
6. Kamila M.M. Mondal N. Ghosh L.K. Gupta B.K., A validated UV spectrophotometric method for estimation of nebivolol hydrochloride in bulk and pharmaceutical formulation, Pharmazie, 2007, 62, 486-487.
7. Rajeswari K.R. Sankar G.G. Rao A.L. Raju D.B. Seshagiri Rao J.V.L.N., RP-HPLC method for the estimation of nebivolol in bulk and pharmaceutical dosage form, Asian. JChem, 2005, 17, 1259-1263.
8. Sahoo M .K. Giri R.K. Barik C.S. Kanungo S.K. Ravi Kumar B.V.V., RP-HPLC method for the estimation of nebivolol in tablet dosage form, E-Journal of Chem, 2009, 6, 915-919.
9. Reddy T.S. Devi P.S., Validation of a high- performance thin – layer chromatographic method, with densitometric detection, for quantitative analysis of Nebivolol hydrochloride in tablet formulations, J of Planar Chrom, 2007, 20,149-152.
10. Ramakrishna N.V. Vishwottam K.N. Koteshwara M. Manoj S. Santosh M. Varma D.P. Rapid quantification of nebivolol in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry, J Pharm. Biomed. Anal, 2005, 39, 1006-1013.
11. Job P., Formation and stability of inorganic complexes in solution, Ann. Chim, 1928, 9, 113–203.
12. Douglas A.S. Donald M.W., Principles of Instrumental Analysis. Holt, Rinehart, Winston, New York, 1971.
13. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.
14. Indian Pharmacopoeia, Government of India, New Delhi: Controller of Publications. II, 2010, 1290.

*****