Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):2356-2363

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Liquid chromatographic method development and validation for assay and dissolution of nebivolol hydrochloride in tablet dosage form

Deepak Sharma*[1], Rajesh Yadav[2], Rajesh Asija[3]

[1]*Sunrise University, Alwar, Rajasthan, India*
[2]*Rajasthan University of Health Science, Jaipur, Rajasthan, India*
[3]*Maharishi Arvind Institute of Pharmacy, Mansarovar, Jaipur, Rajasthan, India*

ABSTRACT

A simple, rapid, precise and accurate RP-HPLC method was developed and validated for dissolution profiling of Nebivolol Hydrochloride in tablet dosage forms. As per official records, the dissolution media for NebivololHCl Tablets is 900ml, 0.01 N HCl and for 45 Minutes time duration, on USP II (Paddle) Dissolution apparatus, at 50 RPM and at 37.0°C ± 0.5°C. The chromatographic development for Dissolution samples was achieved in a SHISEIDO CAPCELLPAK, CAP C-18, 4.6mm X 250mm, 5µ, (Column No AKAB06451) as a stationary phase and Methanol: Water (pH 3.0) in a ratio of 60:40 as eluent, at a flow rate of 1.0 ml/min. UV detection was performed at 280 nm. The retention time of Nebivolol Hydrochloride was found to be 4.9 min. The results of analysis were validated statistically and by recovery studies. Linearity, accuracy and precision were acceptable in the ranges of 5-50µg/ml. The method was found suitable for Dissolution profiling and the results of Percentage Drug Release were within the USP Dissolution Limits.

Key words: Dissolution, Liquid chromatography, RP-HPLC, Validation, Nebivolol Hydrochloride.

INTRODUCTION

NebivololHCl is, α, α' [Iminobis (methylene) bis [6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol]; α, α'-(imino-dimethylene) bis [6-fluoro-2-chromanmethanol], is a β1-Blockers (Anti-Hypertensive). Nebivolol is the racemate (dl-nebivolol) of the enantiomers l-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 [1]. It works by reducing the peripheral vascular resistance and significantly increasing the stroke volume with preservation of cardiac output [2]. It is an official drug and also listed in the Merck Index with Merck no. 6519 [3]. It is official in Indian Pharmacopoeia [4] and British Pharmacopoeia [5] Literature survey reveals spectrophotometric methodand RP-HPLC method [6, 7, 8, 9] for its assay and spectrophotometric method for dissolution study of NebivololHCl [10] but there is no RP-HPLC estimation for dissolution profiling of nebivolol in different dissolution media. This paper presents simple, accurate and reproducible an RP-HPLC method for determination of NebivololHCl in tablet dosage form in different dissolution media. The reported method is helpful in determination of Nebivolol during dissolution study.

EXPERIMENTAL SECTION

Materials

NebivololHCl was received as gift sample from Glenmark Pharmaceuticals Ltd., Goa, India. All other chemicals used were of HPLC Grade or pharmaceutical or analytical grade purchased from local supplier (Merck). The marketed preparation of Nebivolol Hydrochloride Tablets was purchased from local market.

Instrument

The Scan Graph for absorbance maxima measurements were made on double beam UV visible spectrophotometer (Shimadzu, Kyoto, Japan, model UV - 1800) with matched quartz cuvettes. Chromatographic data was acquired on Gradient controlled CYBER LAB, USA RP-HPLC, LC-P100 PLUS (Pump Serial No. 08090870) with UV Detector LC-UV100 PLUS (UV Detector Serial No.08090665). Stationary phase column used was SHISEIDO CAPCELLPAK, CAP C-18, 4.6mm X 250mm, 5µ, (Column No AKAB06451). Electrolab Tablet Dissolution Tester TDT-06P was used for dissolution studies.

Methods

Preparation of standard drug solution

The stock solution (100 µg/ml) of NebivololHCl RS was prepared by dissolving accurately about 10mg of pure drug in 25 ml of methanol and shake for 15 min and the volume was made up to 100 ml with 0.01N HCl(http://www.accessdata.fda.gov/scripts/CDER/dissolution/dsp_SearchResults_Dissolutions.cfm) and then further suitable aliquots were made in dissolution media, filtered through millipore filter (0.45micron) and sonicated before use.

Mobile Phase

Finally optimized mobile phase was prepared by mixing Methanol: Water (pH 3.0) in a ratio of 60:40. pH of water was adjusted with Orthophosphoric Acid, filtered through 0.45µ milipore filter, sonicated and used.

Dissolution Media

As per official records [11], dissolution study of immediate release tablets of NebivololHCl is performed in dissolution media of 0.01N HCl for 45 minutes. So, the dissolution profile for NebivololHCl Tablets was conducted in 0.01N HCl for 45 minutes. Dissolution parameters are given in Table 1.

Table 1: Dissolution Parameters

Parameters	Description
Dissolution Apparatus Type	USP II (Paddle)
Dissolution Media	0.01N HCl, 900ml
RPM	50
Time Points	10, 20, 30, 45 minutes
Temperature	37.0°C ±0.5°C
Sample Volume	10 ml
Filter	0.45µ Teflon Syringe filter

Study of Absorbance Maxima

5ml of the stock solution was further diluted to 100ml with 0.01N HCl to obtain sample solutions of concentrations within Beer-Lambert's range and this solution was scanned in the wavelength range of 200-400nm. UV-spectra is presented in Figure 1.

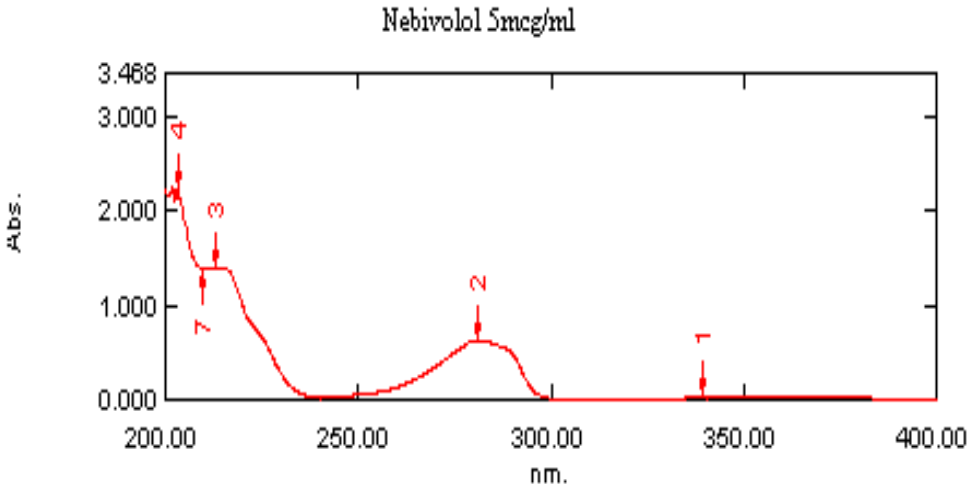

Figure 1: UV-Spectra of Nebivolol Hydrochloride in 0.01N HCl

Selection of Chromatographic Conditions

Various combinations of various solvents in different proportion were studied for best suitable chromatographic conditions. Finally optimized chromatographic conditions are given in Table 2.

Table 2: Chromatographic Conditions

S.No	Parameters	Optimized Conditions
1.	Column	ODS, 5 µ, 4.6X250 mm
2.	Mobile Phase	MeOH : Water (pH 3.0) :: 60:40
3.	Flow Rate	1.0 ml/min.
4.	Wavelength of Detection	2 nm
5.	Injection Volume	40 µl

System Suitability Parameter: System suitability parameters are shown in Table 3.

Table 3: System Suitability Parameters

Parameters	Observation	Limit
Retention Time	5.148	-
Theoretical Plates	2964	>2000
Tailing Factor	1.29	<2.0

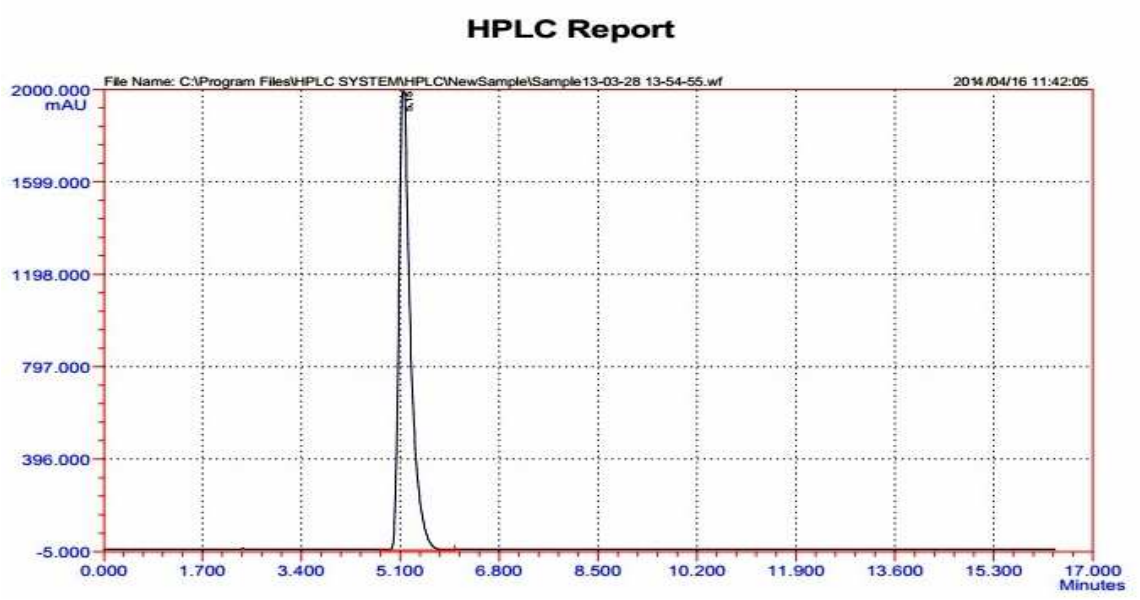

Figure 2: A typical RP-HPLC Chromatogram for Nebivolol Hydrochloride (2796543)

Table 4: Calibration curve

Conc. Mcg/ml	Area Under Curve						Average AUC	Slope	Intercept	r^2
	1	2	3	4	5	6				
5	279654	288726	257893	273666	289729	285677	279224	56359	16545	0.9994
10	559380	546734	558374	568776	537242	568979	556581			
15	838962	831543	842234	817964	818939	848243	832981			
20	1118616	1154904	1031572	1094664	1158916	1142708	1116897			
25	1298270	1443630	1319465	1386330	1248645	1328385	1337454			
30	1677924	1732356	1547358	1641996	1738374	1714062	1675345			
35	1957578	2021082	1805251	1915662	2028103	1999739	1954569			
40	2237232	2309808	2063144	2189328	2317832	2285416	2233793			
45	2416868	2619853	2421023	2499462	2675061	2571631	2533983			
50	2696554	2926087	2930857	2766263	2697529	2857767	2812510			

r^2 - Regression coefficient

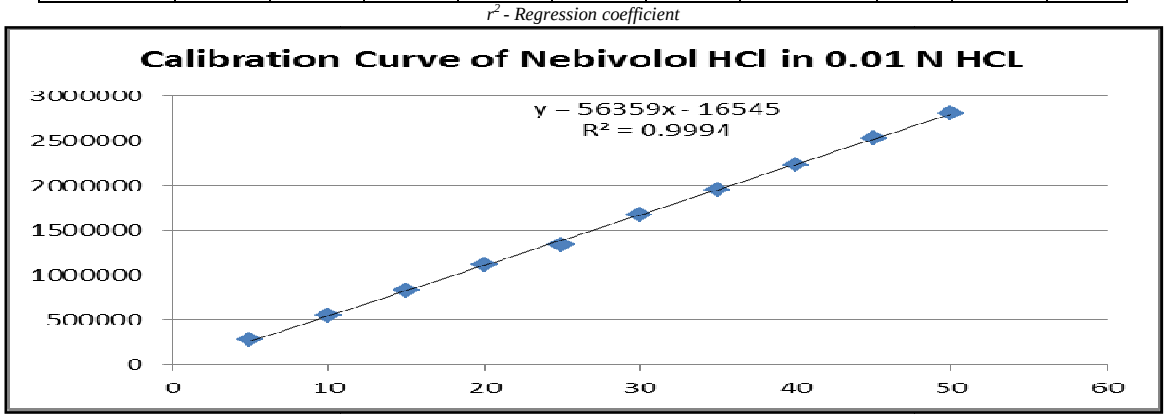

Figure 3: Calibration curve of Nebivolol Hydrochloride

Calibration Curve

Calibration curve was prepared in the concentration range of 5-50 ppm, for which the coefficient of regression was found to be near 1. The results are shown in Table 4 and Figure 3.

Assay of Tablet Formulation

Twenty tablets were individually weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 10 mg NebivololHCl was taken in 100 ml volumetric flask and dissolved in 25 ml of methanol and shake for 15 min; it was further diluted up to the mark with 0.01 N HCl. The solution was mixed and filtered and 5ml of the filtrate was further diluted to 50ml with 0.01 N HCl to obtain sample solutions of desired concentrations. The HPLC chromatogram of resulting solution was measured at 280nm wavelengths for the estimation of nebivolol. The results of the assay are shown in Table 5.

Table 5: Results for assay of Nebivolol Hydrochloride in pharmaceutical preparation

Labeled amount (mg)	Observed amount ± SD	%RSD
5	99.98±0.67	0.7154

Table 6: In-Vitro Dissolution Data for Nebivolol Hydrochloride

Tab.No.		**0 min**	**10 min.**	**20 min.**	**30 min.**	**45 min.**
1		0	33558	110049	200832	229342
2		0	30783	91753	190983	218983
3		0	28164	93472	177796	216074
4		0	34738	92566	159945	209334
5		0	36156	97632	199977	210047
6		0	38737	125167	212901	239705
Calculations:		**% Drug Release**				
Tab.No.		**0 min**	**10 min.**	**20 min.**	**30 min.**	**45 min.**
	1	0	13.8	45.3	82.6	94.3
	2	0	12.7	37.7	78.5	90.0
	3	0	11.6	38.4	73.1	88.8
	4	0	14.3	38.1	65.8	86.1
	5	0	14.9	40.1	82.2	86.4
	6	0	15.9	51.5	87.5	98.6
Calculations:		**% Cumulative Drug Release**				
Tab.No.		**0 min**	**10 min.**	**20 min.**	**30 min.**	**45 min.**
	1	0	13.8	45.3	82.9	95.1
	2	0	12.7	37.8	78.8	90.8
	3	0	11.6	38.5	73.4	89.5
	4	0	14.3	38.1	66.1	86.7
	5	0	14.9	40.2	82.5	87.1
	6	0	15.9	51.6	87.9	99.4
Average		**0.0**	**12.7**	**40.5**	**78.4**	**91.8**
Minimun		0.0	11.6	37.8	73.4	89.5
Maximum		0.0	13.8	45.3	82.9	95.1
SD		0.00	1.11	4.16	4.78	2.92
% RSD		**#DIV/0!**	**8.7**	**10.3**	**6.1**	**3.2**
Limits			**NLT85 in the specified time limit of 45 min.**			

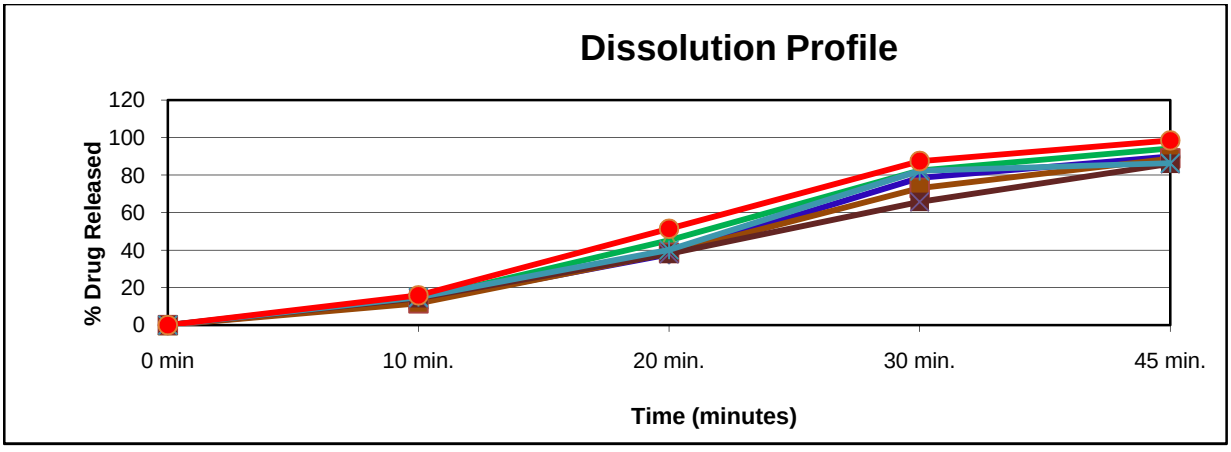

Figure 4: Dissolution Profile

In vitro dissolution studies

The *in vitro* drug release rate method of tablet is official in USP. It was carried out using USP dissolution testing apparatus II (paddle type) at 50 rpm. The dissolution test was performed using 900 ml of 0.01N HCl as described in

the USP monograph. [4] Dissolution test was carried out 0.01 N HCl for a period of 45 min.. The temperature of the dissolution medium is maintained at 37 ± 0.5°C. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at regular intervals and replaced with the same volume of pre-warmed fresh dissolution medium. The samples were filtered through a 0.45 µm membrane filter. The amount of drug release was determined from the comparison with standard response of pure drug. The results of *in vitro* dissolution are shown in Table 6 and Figure 4.

Validation of Analytical Method

The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity and Range: The calibration curve for RP-HPLC method was obtained with concentrations of the standard solutions 5-50 µg/mL of NebivololHCl. The solutions were injected in six replicates. Linearity was evaluated by regression analysis, which was calculated by the least square regression method and plot of residual method. The result of linearity is given in table 7, 8 and figure 5, 6, and results for range are shown in table 9.

Table 7: Linearity

Linearity range	5-50 µg/Ml
R2	0.9994

Table 8: Linearity by plot of residual method

Conc. mcg/ml	Experimental	Theoritical	Residual
	E	T	T-E
5	279224	265250	-13974
10	556581	547045	-9536
15	832981	828840	-4141
20	1116897	1110635	-6262
25	1337454	1392430	54976
30	1675345	1674225	-1120
35	1954569	1956020	1451
40	2233793	2237815	4022
45	2533983	2519610	-14373
50	2812510	2801405	-11105

Table 9: Table for Range for NebivololHCl

Range	NebivololHCl	Remarks
Linearity Range	5-50mcg/ml	As per lineartiy Plot
Target Range	4.0, 5.0, 6.0 mcg/ml	80-120% of target conc. (5ppm)
Working Range	0.151 - 50 mcg/ml	LOQ-higest point on linearity plot

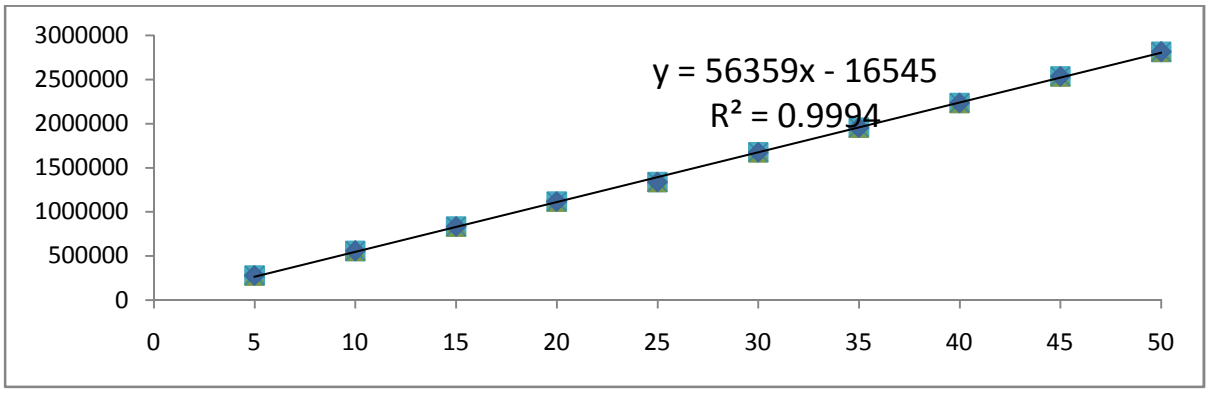

Figure 5: Linearity plot for NebivololHCl

Precision: Precision of the proposed method was checked by analyzing the samples at three different time intervals of the same day (intraday precision) as well as on different days (interday precision). Also, the same was analyzed by repeatability method. Results are shown in table 10, 11.

Accuracy: To check the degree of accuracy of the proposed RP-HPLC method, recovery studies were performed in triplicate by standard addition method. Results are shown in table 12.

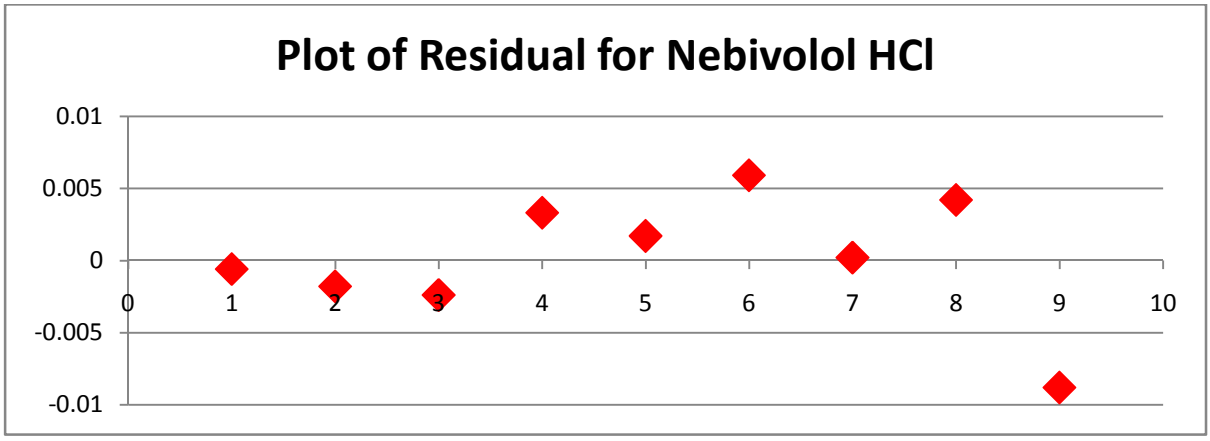

Figure 6: Linearity Plot (By residual method) for Nebivolol HCl

Table 10: Precision by repeatability

S.No.	AUC	Conc. (mcg/ml)
1.	350237	5.920828971
2.	248876	4.12234071
3.	264279	4.39564222
4.	323237	5.44175731
5.	297355	4.98252276
6.	300179	5.032630103
Avg.	**297360.5**	**4.9826203**
SD	**37198.37**	**0.660025**
RSD	**0.13**	0.13

Table 11: Intra-day and Inter-day Precision

Intraday /InterdayPrecision		4 mcg/ml (80% Target Conc)			5 mcg/ml (Target Conc.)			6 mcg/ml (120% target Conc)		
		AUC (mean of 3)	Conc (mg)	%Purity	AUC (mean of 3)	Conc (mg)	%Purity	AUC (mean of 3)	Conc (mg)	%Purity
Day 1	Time 1	212830	4.070	101.75%	270897	5.100	102.00%	327425.249	6.103	101.72%
	Time 2	208819	3.999	99.97%	265989	5.013	100.26%	323430.523	6.032	100.54%
	Time 3	210213	4.023	100.59%	259608	4.900	98.00%	321509.245	5.998	99.97%
Day 2	Time 1	208908	4.000	100.01%	269251	5.071	101.42%	321655.214	6.001	100.01%
	Time 2	204377	3.920	98.00%	265864	5.011	100.22%	327316.476	6.101	101.69%
	Time 3	213338	4.079	101.97%	265115	4.998	99.95%	327876.121	6.111	101.85%
Day 3	Time 1	208245	3.989	99.71%	259631	4.900	98.01%	315330.607	5.889	98.14%
	Time 2	205459	3.939	98.48%	265256	5.000	100.00%	315922.377	5.899	98.32%
	Time 3	208911	4.000	100.01%	266828	5.028	100.56%	321800.621	6.003	100.06%
Avg.		**209010.982**	**4.00213**	**100.05%**	**265382.193**	**5.002345556**	**100.05%**	**322474**	**6.0153489**	**100.26%**
SD		**2947.32325**	**0.05229552**	**0.01307388**	**3784.4595**	**0.06714916**	**0.0134298**	**4664.61**	**0.082766**	**0.0137943**
RSD		**0.01410**	**0.01307**	**0.01307**	**0.01426**	**0.01342**	**0.01342**	**0.01447**	**0.01376**	**0.01376**

Table 12: Data for Recovery Studies

Conc. (µg/ml)	AUC before Spiking	Conc. before Spiking (µg/ml) C1	Conc. of Std. added C2	AUC found after Spiking (µg/ml) C3	Conc. found after Spiking (µg/ml) C3	% Recovery (C3-C1) *100/C2	Mean ± SD	RSD	SE
5	297996	4.9939	1	354547	5.9973	99.96%	99.92 ± 0.011	0.01078	0.00359
				355002	6.0054	100.09%			
				349176	5.9020	98.37%			
	300043	5.0302	2	410023	6.9816	99.74%			
				416975	7.1050	101.50%			
				411387	7.0058	100.08%			
	296547	4.9682	3	471175	8.0667	100.83%			
				469862	8.0434	100.54%			
				449173	7.8537	98.17%			

Limit of Detection and Limit of Quantitation:
LOD and LOQ were calculated as per the formula given below:

L.O.D. = 3.3× (SD/S)

Where, SD = Standard deviation of the response
S = Slope of the calibration curve

L.O.Q. = 10× (SD/S)

Where, S.D = the standard deviation of response
S = the slope of the calibration curve

Results are shown in the Table 13.

Table 13: LOD and LOQ

LOD (µg/mL)*	0.0500
LOQ (µg/mL)*	0.15143

RESULTS AND DISCUSSION

Different proportions of methanol, water, acetonitrile and 0.05M phosphate buffer was tried for selection of mobile phase. Ultimately, Methanol and water of pH 3.0 in a proportion of 60:40 v/v respectively was finalized as the mobile phase. Figure 2 shows typical chromatogram obtained from the analysis of standard solution of Nebivolol Hydrochloride using the proposed method. The Rt for Nebivolol Hydrochloride was found to be 5.148, at a flow rate of 1.0 mL/min. The chromatogram was recorded at 280 nm.

The proposed RP-HPLC method, allows a rapid and accurate quantitation of Nebivolol Hydrochloride in tablet preparation. The absorption spectrum of Nebivolol Hydrochloride in 0.01 N HCl is shown in Figure 1. Wavelengths selected for analysis are 280 nm (λmax of Nebivolol Hydrochloride). Calibration curves were constructed in the concentration range of 5-50 µg/mL. Beer's law was obeyed over this concentration range, and the coefficient of regression of the drug was found to be nearer to 1 (Table 4 and Figure 3). Linearity of the method was determined by residual curve method and regression analysis. Precision was calculated as interday and intraday variations given drug. Percent relative standard deviations for estimation of Nebivolol Hydrochloride under intraday and interday variations were found to be less than 2 (Table 8). The accuracy of proposed method was determined by recovery studies (Table 9), indicating an agreement between the true value and found value.

CONCLUSION

The proposed RP-HPLC method were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. The proposed method was found to be simple, accurate and reproducible for routine estimation of Nebivolol Hydrochloride in different dissolution media. The standard deviation, percentage recovery indicates precision and accuracy of the method.

Acknowledgement
Authors are thankful to Glenmark Pharmaceuticals Ltd., Goa, INDIA for providing gift samples of nebivolol hydrochloride. The authors also express their gratitude to Maharishi Arvind Institute of Pharmacy, Jaipur for extending laboratory facilities to carry out the work.

REFERENCES

[1] O.Uhlir, *Drug Invest*, **1991**, 3 (1), 107-110.
[2] CIPLADOC- Cipla Therapeutic Index for Nebivolol Hydrochloride and Amlodipine Besilate, Available online at link: http//:www.cipladoc.com Accessed on 12th September **2013**.
[3] S. Budavari. The Merck Index, 12TH Edition, Merck Research Laboratories, Division of Merck & Co. Inc., White House Station, New Jersey,**1996;** 1103.
[4] Indian Pharmacopoeia, Vol. II, The Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, Ghaziabad, **2010;** 1681.
[5] British Pharmacopoeia, 5th Edition, The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA), The Department of Health: Great Britain, **2005;** 1327-28.
[6] A. A. Shirkhedkar; P. M. Bugdane; S. J. Surana, *J. Chromatographic Science*, **2010**, 48, 109-113.
[7] B. Yilmaz. *Int. J. Pharm. Sciences Review and Research*, **2010**, 1(2), 14-17.
[8] A. L. Rao; K. R. Rajeswari; G.G. Sankar. *J. Chem. Pharm. Res.*, **2010**, 2(1), 280-282.
[9] D. A. Shah; K. K. Bhatt; R. S. Mehta; S. L. Baldania; T. R. Gandhi. *Indian J. Pharm. Sci.* **2008**, 70(5), 591–595 (**2008**).
[10] L. J. Patel; B. N. Suhagia; P. B. Shah. *Indian J. Pharm. Sci*. 69(4): 594–596 (**2007**).

[11] USFDA, method for dissolution of Nebivolol Hydrochloride Tablets as per USP. Available online at link: http://www.accessdata.fda.gov/scripts/CDER/dissolution/dsp_SearchResults_Dissolutions.cfm, accessed on 22nd April, **2014**.