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Using an Innovative Quality-By Design Approach for Development and Validation of RP-HPLC Method For Simultaneous Estimation of Nebivolol HCl and Cilnidipine In the API and Tablet Dosage Form.

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ABSTRACT

The present study describes a simple, accurate, precise and cost effective reverse phase High Performance Liquid Chromatographic method for determination of Nebivolol HCl & Cilnidipine in bulk and marketed tablet formulation. Optimization was done by response surface methodology, applying a three level Box-Behnken design. Three factors selected were methanol concentration in mobile phase, flow rate and pH. The separation was carried on Chemsil C_{18} (250 mm x 4.6ID mm, Particle size: 5µ. Detection was done using UV detector at isobastic point 268 nm. The developed method employed mobile phase methanol: water (85:15v/v), (TEA-0.5% v/v in water, pH 3.6 Adjusted with 10% OPA) and flow rate 1.25 ml/min, which was optimized with the help of design expert-11 software. High linearity of the developed method was confirmed over concentration range of 100 – 180 µg/ml for Nebivolol HCl and 200-360 µg/ml for Cilnidipine with correlation coefficient of 0.999 and 0.999 respectively. The percentage RSD for precision of the method was found to be less than 2%. The percentage recoveries for Nebivolol HCl and Cilnidipine were found to be in range of 90.14-102.06 w/v and 94.07-106.62 w/v. The LOD and LOQ for Nebivolol HCl and Cilnidipine were found to be 0.98 ug/ml, 2.97 ug/ml and 7.42 ug/ml, 22.50 ug/ml respectively. Peaks were obtained at retention time of 3.21 and 7.06 min for NEBI and CIL respectively.The proposed method was found to be specific, precise, accurate, robust and can be successfully used to determine the drug contents of marketed tablet formulation in pharmaceutical industry.

KEYWORDS: RP-HPLC, QbD, Nebivolol HCl, Cilnidipine

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INTRODUCTION

The quality of HPLC methods has become increasingly important in a QbD environment. The purpose is to verify robustness and ruggedness in the early method development stage to ensure method performance over the lifetime of the product. Otherwise, if a non-robust or non-rugged method is adapted, significant time and resource may be required to redevelop, revalidate and retransfer analytical methods. According to literature survey, there are quite a few publications on HPLC method development strategy but the method development approaches for RP-HPLC specifically focused on pharmaceutical development in a QbD environment have not been widely discussed. Therefore, there is an unmet need to develop a systematic HPLC method development approach for pharmaceutical development using QbD principles to ensure the quality of the method throughout the product lifecycle.[1-3]

Nebivolol HCl (1S)-1-[(2S)-6-fluoro-3,4-dihydro-2H-chromen-2-yl]-2-[[(2S)-2[(2R)6fluoro-3,4-dihydro-2H-chromen-2-yl]-2-hydroxyethyl]amino]ethanol hydrochloride is White powder, Practically insoluble in water, soluble in DMSO, methanol, DMF and ethanol. Nebivolol HCl lowers blood pressure (BP) by reducing peripheral vascular, and significantly increases stroke volume with preservation of cardiac output

Cilnidipine 3-0-(2-Methoxy ethyl) 5-0-[(E)-3-Phenylpro-2-enyl]2,6 dimethyl-4(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate is yellowish crystalline powder, practically insoluble in water, soluble in DMSO, methanol, ethyl acetate and ethanol. Cilnidipine act on the n-type calcium channel that existing sympathetic nerve end, besides acting on l-type calcium channel that similar to most of the calcium antagonists. Structures of Nebivolol HCl and Cilnidipine are shown in figure I and II.[4]

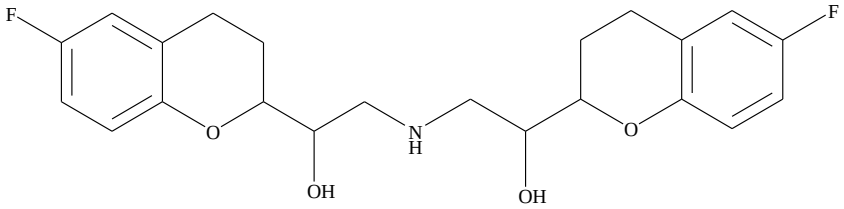

Figure No 1: Chemical structure of Nebivolol HCl [17-18]

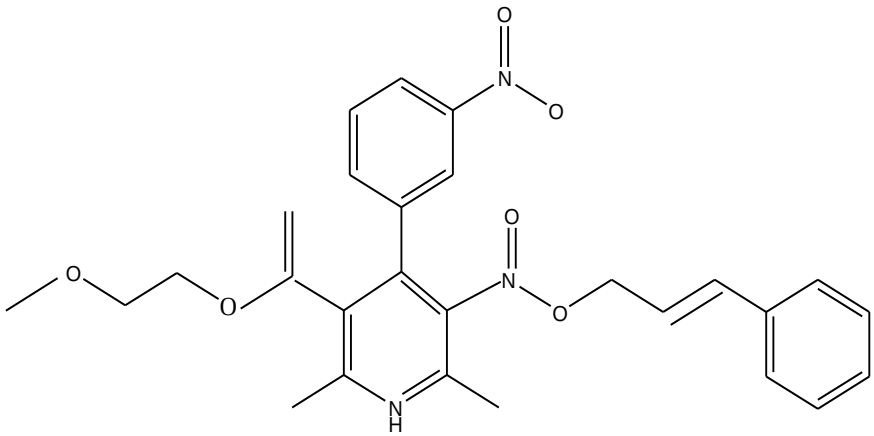

Figure No 2: Chemical structure of Cilnidipine [17-18]

MATERIALS AND METHODS

API: Nebivolol HCl and Cilnidipine were kindly procured as gift sample from Pure chem Pvt. Ltd, Ankaleshwar, Gujarat. Methanol (HPLC grade), ortho phosphoric acid (AR grade), triethyl amine (analytical Grade) and water (HPLC grade) was purchased from Modern chemical laboratory, Nashik, Maharashtra, India.

INSTRUMENTS

For analytical purpose HPLC was performed on waters 1525 separation module containing Waters 2489 (UV-Visible Detector) equipped with manual injector and Breeze 2 software. A reverse phase analytical column Chemsil C_{18} (250 x 4.6 mm ID, particle size 5 µm) was used.

SOFTWARE FOR QBD: Design expert-11

EXPERIMENTAL WORK

METHOD DEVELOPMENT BY QBD APPROACH AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

To develop a suitable RP-HPLC method for the determination of Nebivolol HCl and Cilnidipine, different mobile phases like methanol: water(95:05% v/v), methanol: water (90:10% v/v), methanol: water (85:15% v/v), methanol: water (75:25 v/v) at pH 3.5 were tried at different flow rates of 1 and 1.2 ml/min. The mobile phase methanol: water (85:15% v/v), (TEA-0.5% v/v in water, pH 3.5 Adjusted with 10% v/v OPA) at a flow rate 1.20 ml/min gave sharp peak and it was selected as middle level (0) for designing of DOE. After DOE, optimized and robust method was obtained from design space- mobile phase methanol: water (85:15% v/v), (TEA-0.5% v/v in water, pH 3.6 Adjusted with 10% v/v OPA) at a flow rate 1.25 ml/min gave sharp peak with good symmetry The retention time were found to be 3.21 and 7.06 min respectively. The detection

response was measured at isobastic wavelength 268 nm and column was maintained at ambient temperature throughout study.

DESIGN OF EXPERIMENT:

3^3 randomized response surface designs with a box-behnken design were used with 17 trial runs to study the impact of three factors on the two key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all 3 possible combinations. The mobile phase compositions (X1), Flow rate(X2) & pH (X3) were selected as independent variables and retention time (RT), theoretical plate number (TPN) & asymmetry factor were selected as dependent variables. The resulting data were fitted into Design Expert 11 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of Mobile phase composition, flow rate, and pH on dependent variables.[5]

PREPARATION OF STANDARD STOCK SOLUTION.[6-7]

In HPLC, accurately weighed about 25 mg Nebivolol HCl and 50 mg of Cilnidipine in 25 ml of volumetric flask. Dilute it to the mark with mobile phase to get concentration 1000 ug/ml of Nebivolol HCl and 2000 ug/ml of Cilnidipine. Take 1 ml of sample solution in 10 ml of volumetric diluted with mobile phase to get concentration 100 ug/ml Nebivolol HCl and 200 ug/ml of Cilnidipine.

PREPARATION OF SAMPLE STOCK SOLUTION:[6-7]

20 tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 25 mg Nebivolol HCl and 50 mg Cilnidipine was weighed accurately and transferred to a 25 ml volumetric flask. Add 15 ml mobile phase and sonicate for 30 min and made up volume with mobile phase to produce test solution having 1000 ug/ml of Nebivolol HCl and 2000 ug/ml Cilnidipine and filtered through a whatman filter paper no. 42. Take 1ml filtred in 10 ml volumetric flask and dilute with mobile phase to get concentration 100 ug/ml Nebivolol HCl and 200 ug/ml Cilnidipine. The resulted test solution was then analyzed for assay determination.

PREPARATION OF MOBILE PHASE:

(Methanol: Water (85:15 % v/v), TEA-0.5% v/v in water, pH 3.6 Adjusted with 10% v/v OPA)

An accurately measured 0.5 ml of triethyl amine in 100 ml volumetric flask, followed by the addition of 95 ml HPLC grade water, pH 3.6 was adjusted with 10% OPA, volume was made up to mark with HPLC grade water. The 15 ml of above solution was mixed with 85 ml of methanol and final solution was sonicated for degassing.

METHOD VALIDATION.[8]

SYSTEM SUITABILITY PARAMETERS

System suitability tests were performed to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters monitored for system suitability includes retention time, theoretical plate number, tailing factor, Peak area and resolution. The repeatability of these parameters was checked by injecting six times the test solution of 100 µg/ml Nebivolol HCl and 200 µg/ml Cilnidipine. The results shown in Table 1 were within acceptable limits.

Table no-1 : SYSTEM SUITABILITY

parameters	NEBI RESULTS	CIL Results	Acceptance Criteria
Retention time	3.23	7.19	-
Theoretical plates	4010	7502	>2000
Resolution		14.18	>2
Tailing Factor	1.03	1.5	<2

SPECIFICITY: (ASSAY)

Specificity was performed by the assay. Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting standard and sample preparations. Chromatograms were recorded and retention times from standard and sample preparations were compared for identification of analytes. The results shown in table no-II were within acceptable limits. Chromatogram shown in figure no-3: a,b.

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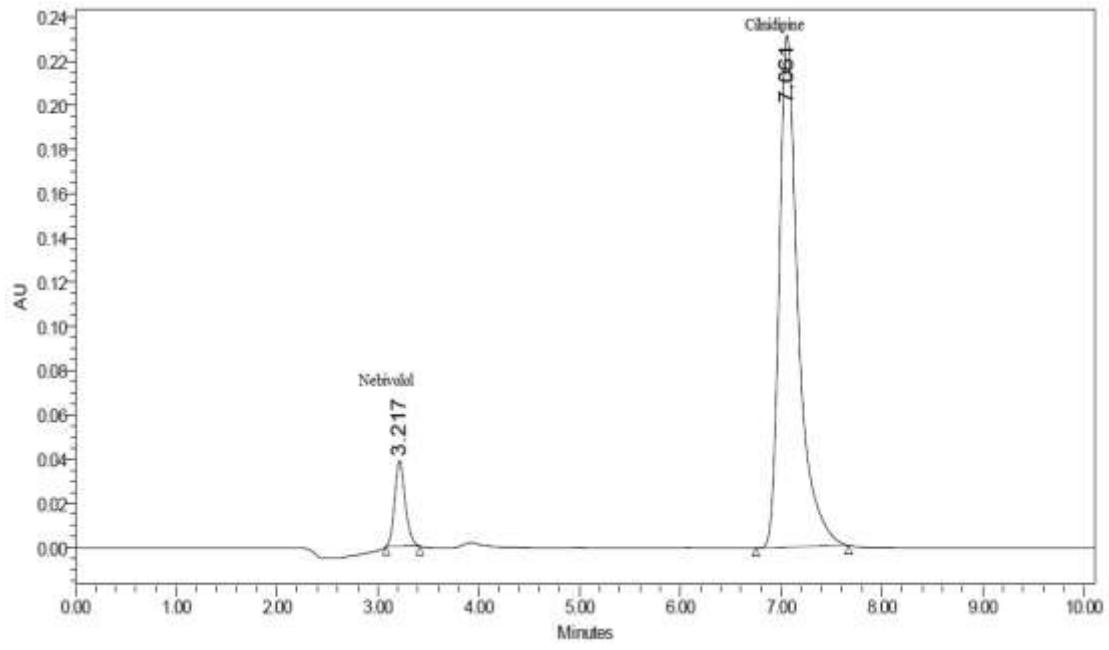
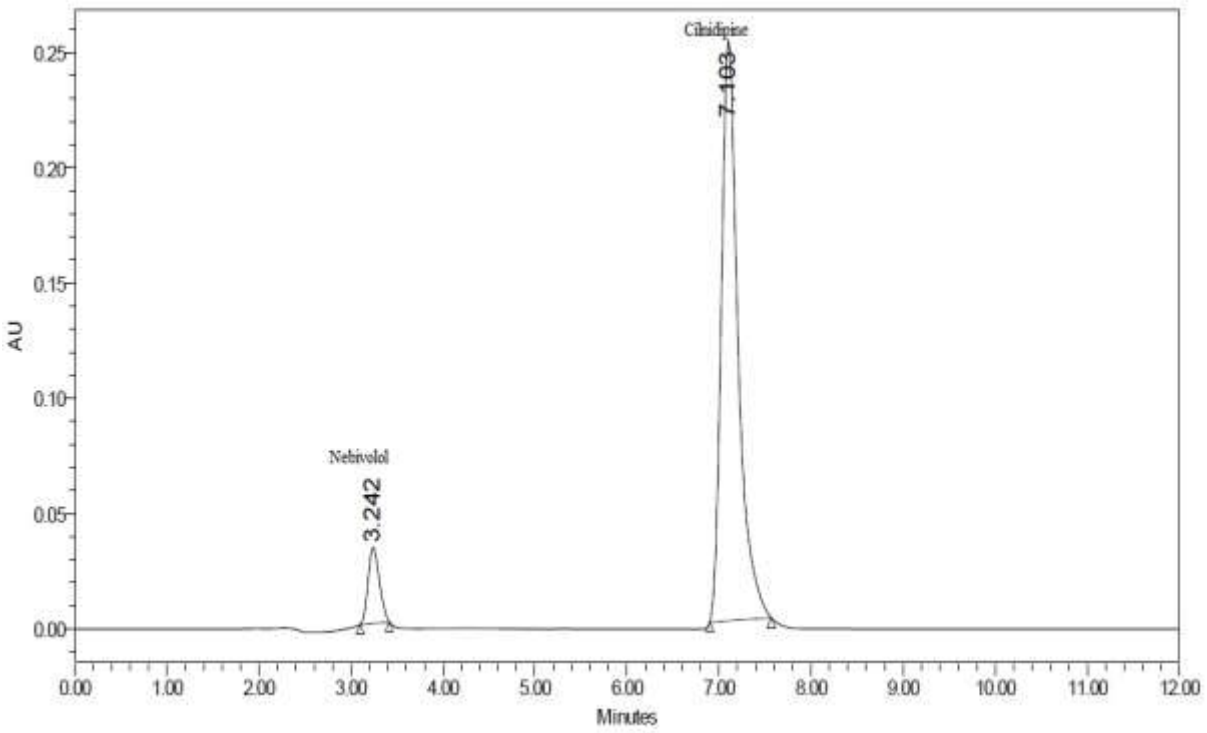


Figure No 3: Specificity :(Assay) a) Standard run



b) Test Run

Table No-2: Analysis of Marketed Formulation (Specificity/Assay)

Name	Area of std	Area of Test	CS (ug/ml)	CT (ug/ml)	ASSAY (%)
NEB	282467	280218	100	99.2038008	99.2038008
CIL	3015320	3188029	200	211.4554342	105.7277171

LINEARITY:

A series of standard solutions 100-180 µg/ml of Nebivolol HCl and 200-360 µg/ml of Cilnidipine were prepared. An aliquot of 10 µL of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration (µg/ml) is plotted and from this the correlation coefficient and regression equation were generated. Figure IX and V represent linearity graphs of both Nebivolol HCl and Cilnidipine . The results shown in table no-3 were within acceptable limits

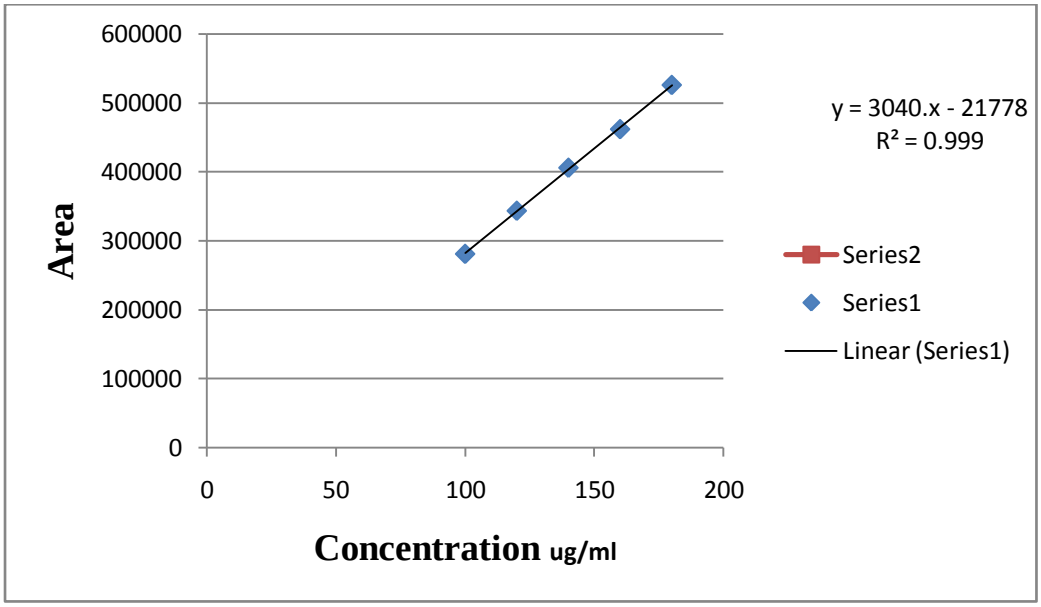


Figure no 4: Linearity graph of Nebivolol HCl

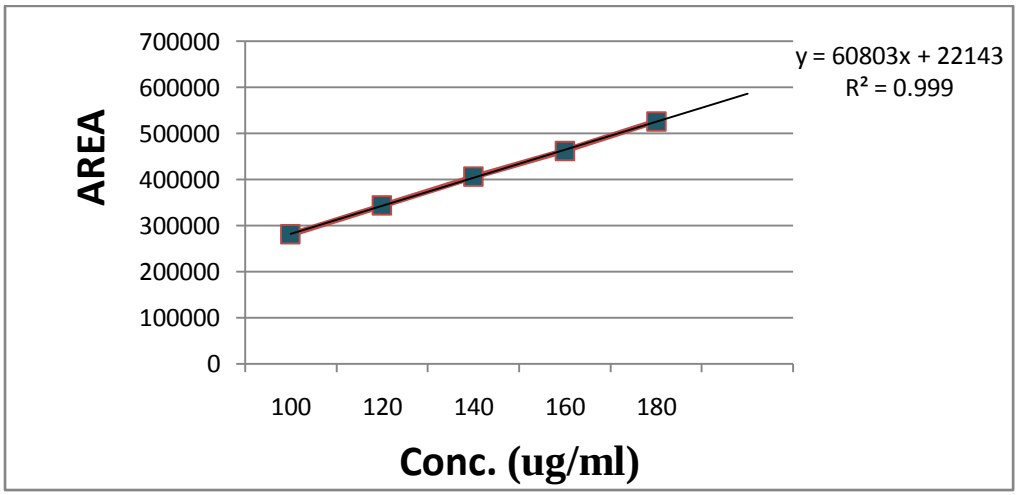


Figure no 5: Linearity graph of Cilnidipine

Tabel no-3: Linearity study for NEBIVOLOL HCl and CILNIDIPINE

Nebivolol HCL				Cilnidipine		
Sr no.	**Con. (ug/ml)**	**Avg Area***	**%RSD**	**Con (ug/ml)**	**Avg Area*2**	**%RSD2**
1	100	281243	0.35	200	3188160	0.91
2	120	343726	0.7	240	3806886	0.56
3	140	406125	0.59	280	4357256	1.18
4	160	462039	1.6	320	4851425	1.76
5	180	526105	0.12	360	5505743	0.99

CALIBRATION CURVE STUDY:

A series of standard solutions 100-180 µg/ml of Nebivolol HCl and 200-360 µg/ml of Cilnidipine were prepared. An aliquot of 10 µL of each solution was injected once for each standard solutions and peak area was observed. Plot of peak area versus the concentration is plotted and from

this the correlation coefficient and regression equation were generated. The calibration data of Nebivolol and Cilnidipine is given in Table no- 4.

Table no-4: Calibration study of NEBIVOLOL HCl and CILNIDIPINE for LOD and LOQ

Nebivolol HCL					Cilnidipine			
Sr no.	Con. (ug/ml)	Area*	Regression	Slope	Con.(ug/ml)	Area*2	Reggresion2	Slope2
1	100	280787			200	3406789		
2	120	343230			240	4024975		
3	140	405962	0.999	3084	280	4618774	0.999	31385
4	160	468057			320	5198827		
5	180	526862			360	5835431		

ACCURACY:

Accuracy study was determined at three different level 80 %, 100 % and 120 % of the target concentration 80 µg/ml of Nebivolol HCl and 160 µg/ml of Cilnidipine in triplicate. The result obtained for Nebivolol hydrochloride and Cilnidipine are shown in **table no- 5 and 6.**

Table no-5: Accuracy data for NEBIVOLOL HCl

Accuracy level %	Amount of standard added	Amount of Tab Added	Amount Found(ug/ml)	% Recovery (mean±SD)	%RSD
80	64	80	131		
80	64	80	129	90.14 ±0.639	0.7
80	64	80	129		
100	80	80	161		
100	80	80	59	100 ±0.744	0.74
100	80	80	159		
100	96	80	179		
120	96	80	179	102.06 ±0.173	0.17
120	96	80	179		

Table no-6 : Accuracy data for CILNIDIPINE

Accuracy Level %	Amount of Std. added	Amount of Tab Added	Amount Found (ug/ml)	% Recovery (mean± SD)	%RSD
80	128	160	270		
80	128	160	270	94.04 ±0.003	0.004
80	128	160	270		
100	160	160	323		
100	160	160	320	100 ±1.33	1.33
100	160	160	315		
120	192	160	375		
120	192	160	373	106.62 ±0.33	0.31
120	192	160	376		

PRECISION:

Precision studies included the following studies:

1. REPEATABILITY (INTRA-DAY PRECISION)

The precision of the analytical method was studied by analysis of multiple samplings of homogeneous sample. Precision was estimated by repeatability by analyzing six trials of a homogeneous sample of 100 µg/ml of Nebivolol HCl and 200 µg/ml of Cilnidipine and % RSD was calculated. **(Table 7)**

Table no-7 : INTRADAY PRECISION OR REPEATABILITY

DRUG	Target con.(ug/ml)	Peak area	Mean±SD	%RSD
Nebivolol	100	280422		
	100	280071	280830	
	100	280510	±916.02	0.32
	100	280795		
	100	282467		
	100	279952		
Cilnidipine	200	2986385		
	200	3038962	3036997	
	200	3033694	±31385	1.03
	200	3080450		
	200	3015320		
	200	3016559		

Table no-8: INTERDAY PRECISION

DRUG	Target conc. (ug/ml)	Peak area	Mean±SD	%RSD
Nebivolol	100	279455		
	100	280575	280122.4	
	100	279906	±466.960134	0.16
	100	280724		
	100	280071		
	100	279952		
Cilnidipine	200	3098940		
	200	3000432	3024218	
	200	3016347	±43536	1.4
	200	2988812		
	200	3038962		
	200	3016559		

Standard solutions containing 100 µg/ml, of Nebivolol HCl and 200 µg/ml, of Cilnidipine were analyzed on second day of repeatability as per the guidelines ICHQ2 (R1) and % RSD was calculated. **(Table 8)**

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response and S = Slope of calibration curve.

Data for calibration curve shown in table no-9

Table no-9 : LOD and LOQ

DRUGS	LOD (ug/ml)	LOQ(ug/ml)
Nebivolol	0.98	2.97
Cilnidipine	7.42	22.50

ROBUSTNESS:

Robustness was carried by varying three parameters from the optimized chromatographic conditions. No significant change was observed. Data for Robustness shown in table no-10.

Table no 10: Robustness

Parameters	Change level	Nebivolol Area	Cilnidipine Area
Flow Rate(±0.2)	1.15	281479	3051597
	1.25#	280422	2986285
	1.35	281107	2983289
	Mean	281002	3007057
	SD	536	38601
	%RSD	0.19	1.55
Wavelength(±2)	266	280320	3086384
	268#	280422	2986285
	270	280840	3060067
	Mean	280527	3044245
	SD	275	51891
	%RSD	0.98	1.7
pH (±0.2)	3.4	281014	3065484
	3.6#	280422	2986285
	3.8	281864	3035309
	Mean	281100	3029026
	SD	724	39971
	%RSD	0.25	1.31

RESULTS AND DISCUSSION:

1. METHOD OPTIMIZATION FOR LEVEL SELECTION

In order to provide base for method optimization by AQbD, as well as for selection of CMA's (CPP's) and CQA's, preliminary study had to be conducted. Firstly, four different mobile phases and two stationary phases (Orochem C_{18} and Chemsil C_{18}, 5 um particle size, 25 cm column length) along with different flow rate and pH were investigated in order to find the best performing method for this analysis. According to it, stationary phase, mobile phase, flow rate and pH that most suited were stationary phase (Chemsil C_{18}), mobile phase methanol: water (85:15% v/v), (TEA-0.5% v/v in water, pH 3.5 adjusted with 10% v/v OPA) and flow rate 1.20 ml/min and it was selected as middle level in DOE. Method optimization by QbD approach are given in detail in **2.5.2) design space and control space.**

2) METHOD Development STRATEGY BY QBD.[11-12]

In this research paper, development strategy by QbD is divided in to the 6 steps (1) Analytical target profile (2) critical quality attributes, (3) Risk assessment (4) Critical process parameters, (5) Design of experiment with screening and optimization steps,design space that includes model building, working point selection and verification, then method validation (6) control strategy.

2.1) *Analytical Target Profile Or Critical Material Attributes*

In this research work, ATP like stationary phase, mobile phase composition, flow rate, pH, temperature, injection volume, wavelengths were selected.

2.2) Critical Quality Attributes (Cqa's)

The impact of ATP on critical quality attributes like retention time of both drugs, plate count, tailing factor and resolution were studied and observed.

2.3) Risk Assessment:

In an early risk assessment, the critical parameters should be identified. That could be CMA's or Independent variables (method factors) which may affect the dependant variables (method responses). During this study, the risks were identified were column and injection volume.20 uL injection volume show bell shape peak while orochem column shows the less plate count of Nebivolol HCl. Therefore, this risk were analyses, evaluated and control by changing the column with Chemsil C_{18} and injection volume was reduced to 10 uL.

2.4) Critical Process Parameters:

As the result of the risk assessment, the 3 parameters mobile phase composition, flow rate, pH were optimized in preliminary study- after choosing the best stationary phase and injection volume due to their strong effect on dependant variables.

2.5) Design Of Experiment And Design Space:

Screening experiment for selection of mobile phase composition, flow rate and pH. The screening experiment were performed response surface methodology, applying a 3^3 Box-Behnken design using Design Expert 11 software. in box-behnken design, 3 levels were selected for 3 factors. Based on 3 level and 3 factors, 17 trial Runs were performed, from total 17 runs, 12 runs are different while 5 runs are same. Due to this, there are only 13 runs shown in Design space. Translation of coded levels in actual value and layout of actual design of DOE shown in table no-11 and 12.

After performing the 17 runs, the ANOVA was studied for 3 factors which show that the model of Mobile phase composition, Flow rate and pH are significant. From this study it was concluded that, retention time of Nebivolol hydrochloride and Cilnidipine drug, Resolution, plate count and tailing factor were more critically affected by above 3 factors. The dependant variables or responses selected for this factors was retention time of Nebivolol HCl ,Retention time of Cilnidipine, and Resolution.

Table no- 11: Translation of coded levels in actual values

Concentration of Factors			
Level of Variable	Flow Rate(ml/min)	pH	Mobile PhaseComposition
Low Level (-1)	1	3	75:25:00
Medium Level (0)	1.2	3.5	85:15:00
High Level (1)	1.4	4	95:05:00

Table no- 12: Layout of Actual Design of DOE

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A:Mobile Phase	B:Flow rate	C:pH	Retention 1	Retention 2	Resolution
5	1	85	1.2	3.5	3.07	7.2	15.59
10	2	75	1.2	3	4.47	21.44	35.44
16	3	85	1	3	3.86	9.31	16.15
2	4	95	1	3.5	2.74	5.1	9.84
17	5	85	1.4	3	2.69	7.67	17.76
8	6	75	1	3.5	4.77	17.68	31.03
3	7	85	1	4	3.48	9.85	18.67
11	8	85	1.2	3.5	3.13	7.48	16.59
14	9	85	1.2	3.5	3.15	8.15	18.1
12	10	85	1.2	3.5	3.25	8.35	17.86
15	11	85	1.4	4	2.86	6.31	12.26
4	12	95	1.2	3	2.18	4.55	10.87
9	13	95	1.4	3.5	1.92	3.43	7.95
13	14	85	1.2	3.5	3.17	7.74	16.75
7	15	75	1.4	3.5	4.14	18.14	27.36
1	16	95	1.2	4	2.97	4.45	5.76
6	17	75	1.2	4	5.4	19.86	23.43

2.5.1 Model Assessment For The Retention Time Response As Dependent Variable:

After entering the data in Design Expert software, fit summary applied to data after which "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. Polynomial equation in coded terms

Final Equation in Terms of Coded Factors:

R1 = 3.15-1.12 x A-0.4050 x B+0.1888 x C-0.0475 x AB-0.0350 x AC+0.1375 x BC+0.3855 x A^2-0.1470 x B^2-0.2155 x C^2

R2= +7.79-7.45 x A-0.7987 x B-0.3125 x C-0.5325 x AB+0.3700 x AC-0.4750 x BC-1379 x A^2-0.4973 x B^2+0.9902 x C^2

Rs = +17.71-10.32 x A-1.29 x B-2.56 x C2

where,

R1 and R2 - Retention time or responses of Nebivolol HCl and Cilnidipine.

Rs- Resolution or third response of experimental design.

A- Mobile Phase

B- Flow Rate

C- pH

AB-Mobile Phase+Flow Rate Interaction

AC-Mobile Phase+pH Interaction

BC- Flow Rate+pH Interaction

A^2- Mobile Phase^2

B^2- Flow Rate^2

C^2- pH^2

A, B, C is linear equation which is shown by counter plot, AB,AC,BC is 2 factorial equation shown by curvature plot, while whole equation is Quadratic equation shown by elliptical plot. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. . **After ANOVA counter plots for all 3 responses was obtained, are shown in figure no-6,7,8 : a, b, c.**

a) **Figure No 6: Counter plot for retention time of Nebivolol HCl (Flow rate vs Mobile Phase strength)**

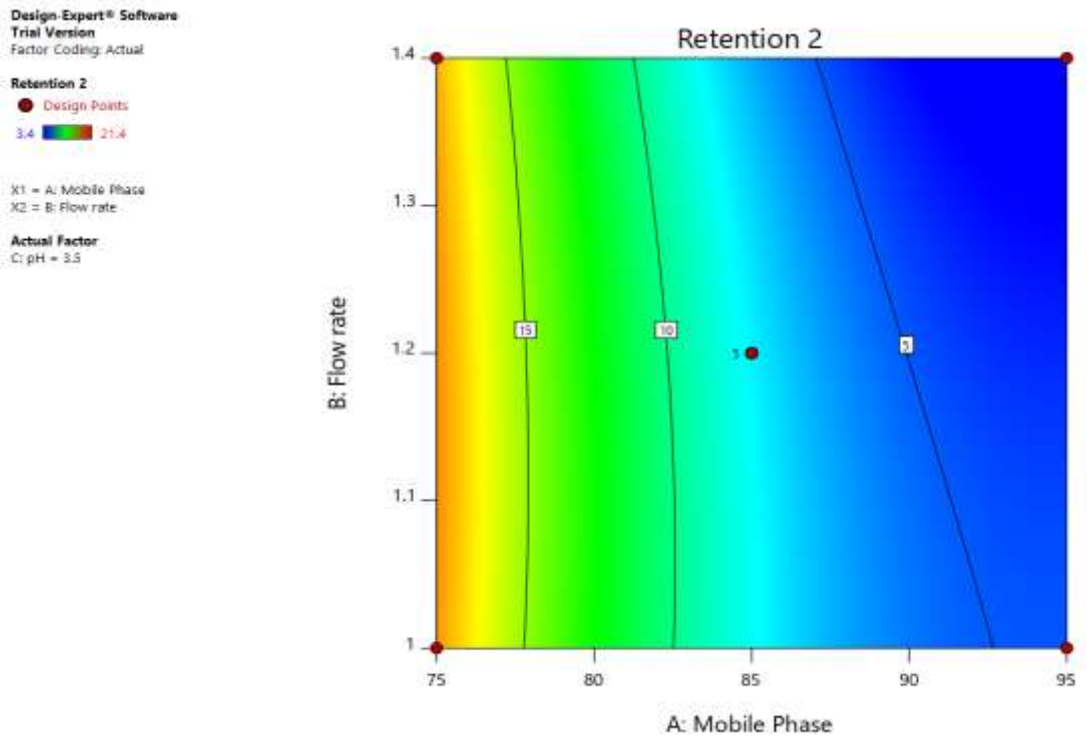


Figure no 7: Counter plot for retention of Cilnidipine (Flow rate vs Mobile Phase strength)

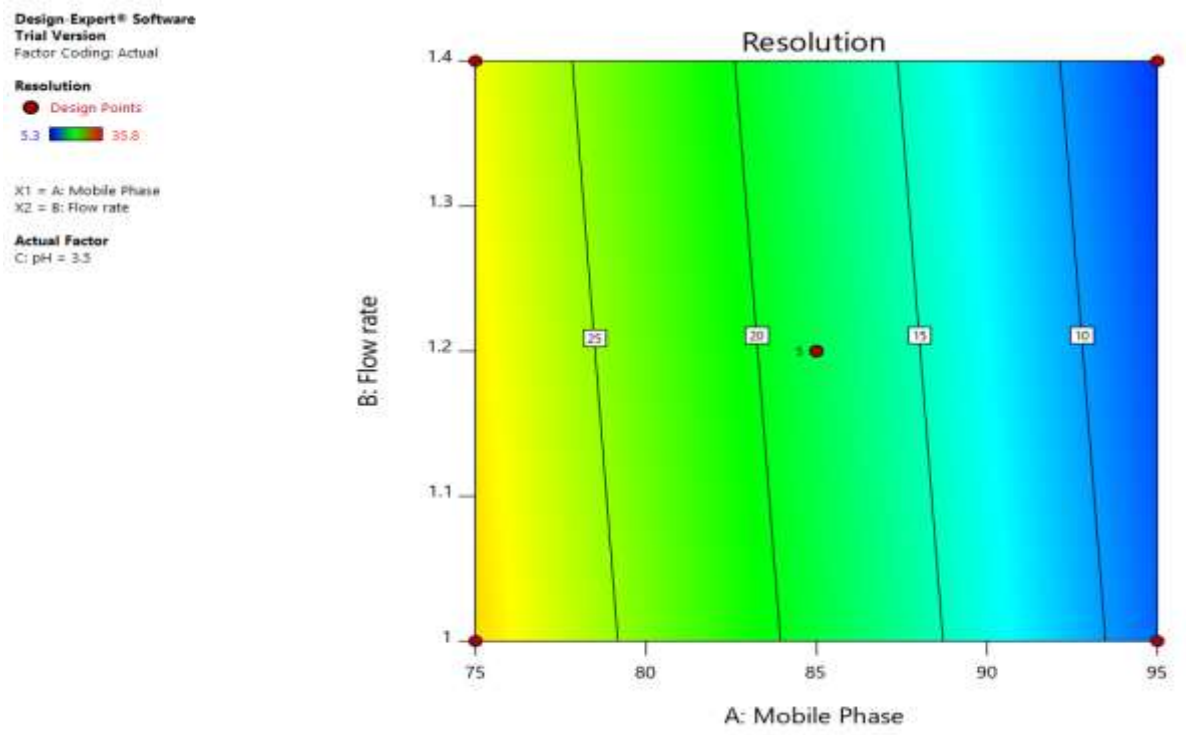


Figure No 8: Counter plot for Resolution (Flow rate vs Mobile Phase strength

2.5.2 Design Space And Control Space

Established Design space was explored and working point was selected on the basis of practical considerations with sufficient surrounding design space.

After processing and checking the accuracy of data, the method operable design region was obtained for independent variables (mp ratio, flow rate and pH) and dependant variables (Retention time 1, Retention time 2 and Resolution). In this work, Design space for mobile phase found from- 75 % to 95 %, Flow rate- 1 to 1.4 ml/min and pH is 3.6 which is actual factor. While control space was obtained at Mobile phase composition- 85:15 % v/v, flow rate-1.25ml/min, pH-3.6. The chromatographic method in design space is considered as robust region. Quite large yellow area gives surface where the changes of CMA's do not give variation in CQA's. Gray region in design space diagram is working space. Typical design space are shown in figure no-9 and optimized chromatographic condition by QbD approach are shown in table no:13.

Table no-13: Optimized Chromatography Condition by Qbd

Parameters Condition2	Description
Column name	Chemsil C18 (250mm x 4.6 mm ID, Particle size: 5 micron)
Detector	UV-3000-M
Injection Volume	10 µl
Wavelength	268 nm
Mobile Phase	Methanol : Water(85:15v/v %),TEA-0.5% v/v, pH-3.6 Adjust by 10% v/v OPA
Programme	Isocratic
Flow Rate	1.25 ml/min

1. In accordance with the requirement of ICH Q8 guidelines, regarding " design space" in product development, method operable design region can also be established in the method development phase, which could serve as a source for robust and cost effective method. MODR is the operating range for the critical process parameters (similar to Critical Quality Attributes) that produces result which consistently meet the goals set out in the ATP. MODR permits the flexibility in the various input method parameters to provide the expected method performance criteria and method response without resubmission to FDA.

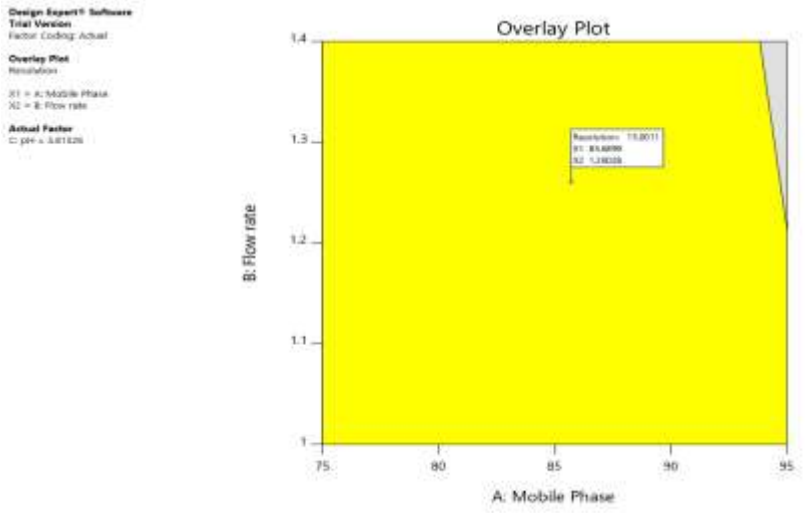


Figure no 9: Design Space or MODR

2.5.3 Working Point Selection and Verification

From the previously constructed design space (MODR) or control space, the working point was selected by visual examination looking for the highest critical resolution (Rs) and best robustness of method. At this point small changes of critical process parameter-pH, mobile phase composition, flow rate have no negative influence on the separation of two drugs. This working point was found in control space at mobile phase composition-85:15% v/v, flow rate-1.25 ml/min pH-3.6 and a predicted chromatogram is shown in figure no 10.

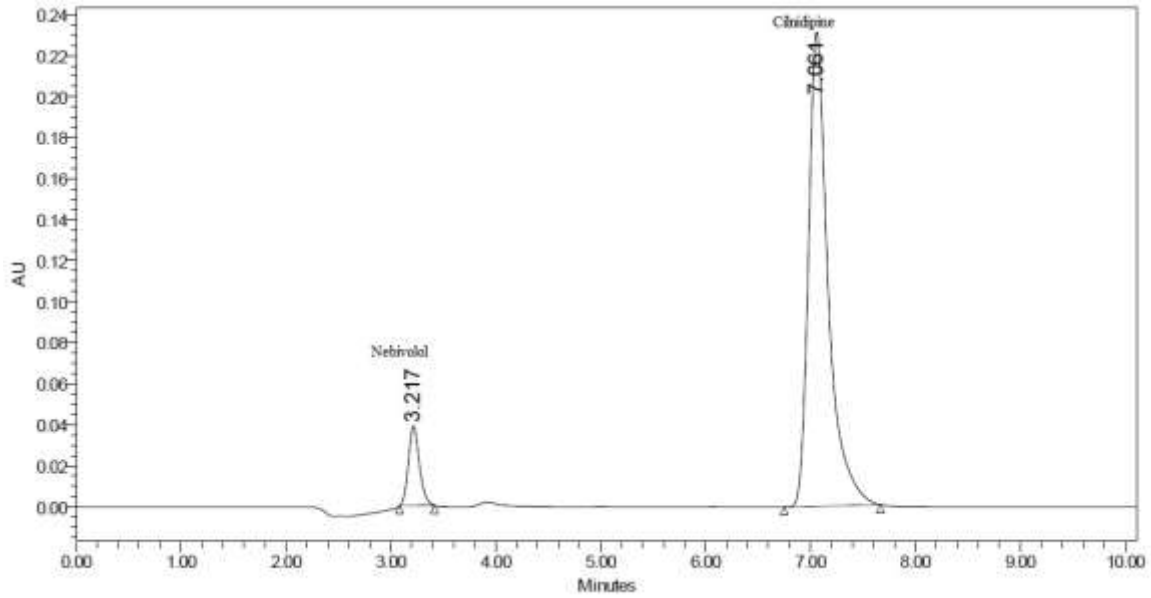


Figure No 10 : Standard Run

2.5.3 Method Validation

A validation study in compliance to the ICH guideline Q2 (R1) was performed. An important part of validation is robustness of developed method. The ICH Q2 (R1) define the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate changes in method parameter.

The robustness of the developed method was studied by doing small changes in flow rate (1.25±0.10), pH(3.6 ±0.2) and Wavelength (268±2).

2.6 Control Strategy

Control strategy is derived from various data collected during method development phase and method verification process. This data correlation will predict the ability of method to meet ATP criteria and control strategy including the overall monitoring of method parameters that significantly influence method. Therefore, the only one control element which is needed in our control method strategy is system suitability parameters.

SUMMARY AND CONCLUSION

In this project, as per our objective RP-HPLC method was developed by implementing QbD methodology with mobile phase methanol: water (85:15 v/v). The flow rate used was 1.25 ml /min, pH-3.6 and UV detection was carried out at isobastic wavelength 268 nm. The retention time for Nebivolol HCl and Cilnidipine was found to be 3.21 and 7.06 min.

Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, risk assessment, design of experiment and validation. System suitability test ensures that the analytical system is working properly and can

give accurate and precise results. A system suitability test includes tailing factor, number of theoretical plates, area, resolution etc. The results of all system suitability parameters were acceptable in their limits defined by official guidelines. Moreover, the lower solvent consumption along with the short analytical run time of 10 min leads to a cost effective and environmentally friendly chromatographic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for Nebivolol HCL and Cilnidipine

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