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Development and validation of reversed-phase high-performance liquid chromatography method for simultaneous estimation of Nebivolol and Cilnidipine in combined tablet dosage form

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ABSTRACT

Objective: A simple, precise and accurate reversed phase highperformance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Nebivolol HCl and Cilnidipine in tablet formulation.

Methods: The adequate separation was carried out using Cosmocil C18 column (250 mm x 4.6 mm, 5 µm particle size), mixture of Methanol and water (OPA PH 3) 65:35 % v/v as a mobile phase with a flow rate of 1.0 ml/min and the effluent was monitored at 260 nm using UV detector. The retention time of Nebivolol HCl and Cilnidipine were 3.741 min and 5.301min respectively.

Results: Linearity for Nebivolol HCl and Cilnidipine were found in the range of 5-25 µg/ml and 10-50 µg/ml (R2 = 0.999) respectively. The accuracy of the present method was evaluated at 50%, 100% and 150%. The % recoveries of both drugs were found to be in range of 99.67- 100.11% and 99.120-100.520% for Nebivolol HCl and Cilnidipine respectively. Precision studies were carried out and the RSD values were less than two. The method was found to be robust.

Conclusions: The proposed method was found to be specific, accurate, precise and robust can be used for simultaneous estimation of these drugs in tablet dosage form.

Keywords: Nebivolol HCl, Cilnidipine, Reversed phase HPLC, Validation

I. INTRODUCTION

Nebivolol HClis named chemically as 1-(-6-Fluorochroman-2-yl) - {[2- (6-fluorochroman-2-yl)-2-hydroxyethyl] amino}-ethanol (Fig 1), epitomizes the class of Benzopyrans, β1 receptor blocker with nitric oxide potentiating vasodilation effect used in treatment of hypertension[(1)]. Nebivolol lowers the heart rate and blood pressure and as well as prevent the release of renin, which is a hormone produced by the kidney may also bind beta 2 receptors [(2,3)]. Numerous analytical methods have been reported for the estimation of NEB as alone as well as in combination with other drugs. They include spectrophotometric methods [(5,6)] HPLC [(7,8),] TLC [(9)],

Cilnidipine1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine_carboxylic acid 2-methoxyethyl(2e)-3-phenyl-propenyl ester (fig. 2) is a novel and unique calcium channel blocker that possesses a slow-onset, long-lasting vasodilation. Cilnidipine is used in the treatment of hypertension. Cilnidipine shows first pass mechanism. Cilnidipine is used in combination with other drugs like telmisartan, Olmesartan. Cilnidipine and its formulations are not official in any pharmacopeias [(4)].

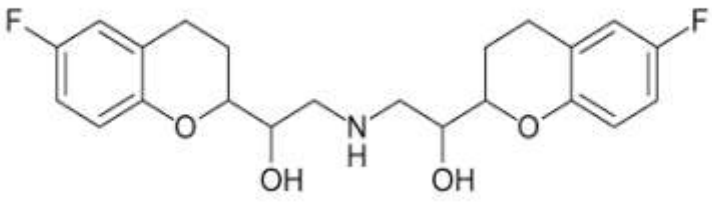

Fig 1 Chemical Structure on Nebivolol

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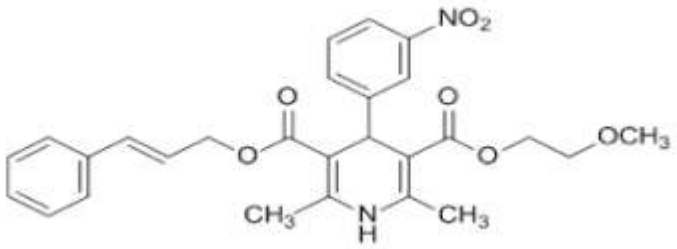

Fig 2 Chemical Structure on Cilnidipine

Literature survey suggests various RP-HPLC, HPTLC, spectroscopic, stability indicating HPLC estimations were performed [10-15]**.** There is still very increasing significance for development of more specific, accurate, precise and rapid method for determination of cilnidipine and nebivolol especially in human plasma as well as drug formulations [16]. The effort was to develop and validate simple, precise, and accurate reversed phase RP-HPLC method for simultaneous determination of both the drugs in their combined dosage form [16]. The % RSD of robustness was found to be less. The results obtained from the validation suggest that the method was found to be precise, accurate, linear and robust enough and the method was also found to be economical.

II. MATERIALS AND METHODS

Equipment's

Analytical technologies HPLC 3000 series system was used for method development and validation. Data acquisition was performed with HPLC workstation software. The elution was achieved on Cosmocil C18 (250mm x 4.6ID, Particle size: 5 micron) column. Digital balance (PGB 100, Wenser High Precision Balance), Ultrasonic cleaner (WUC- 4L, Wenser Ultra Sonicator), pH meter (Systonic) and Pipettes and volumetric flask (Borosil) used during study.

Reagents and materials

Nebivolol and Cilnidipine were obtained as gift samples from Micro labs and Intas Pharma respectively. Nebivolol and Cilnidipine combined dosage form tablets were purchased from local market. HPLC grade methanol, Water and Ortho phosphoric acid was used.

Chromatographic conditions

The column used was kept at room temperature and the eluent was monitored at 260 nm using UV detector. The mixture of Methanol and water with ortho phosphoric PH 3.0 in proportion of 65:35 % v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The manual injection volume was 20µl.

Preparation of stock solution

An accurately weighed quantity of standard Nebivolol (10 mg) and cilnidipine (10 mg) Were transferred to 100 ml volumetric flasks and volumes were made up to mark with mobile phase to get 1000 PPM of Nebivolol and 1000 PPM of Cilnidipine

Preparation of mobile phase

35 ml HPLC grade water, pH 3 was adjusted with Ortho phosphoric acid and the volume was made up to mark with HPLC grade 65 ml methanol. Above solution filtered with vacuum filter using filter membrane and solution was sonicated for degassing.

Nebivolol and Cilnidipine sample stock solution

20 tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg Nebivolol and 10 mg of Cilnidipine was weighed accurately and transferred to a 100 ml volumetric flask. Added 60 ml mobile phase and sonicated for 30 min and made-up volume with mobile phase to produce a test solution having 100 µg/ml NEB and 100 µg/ml CIL and filtered through a Whatman Filter Paper no. 42. The resulted test solution was then analysed for assay determination.

III. METHOD VALIDATION

Calibration curve (Linearity)

A series of standard solutions 5-25 µg/ml of Nebivolol and 10-50 µg/ml of Cilnidipine were prepared. The sample of 20 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 is plotted and from this the

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correlation coefficient and regression equation were produced. The calibration data of Nebivolol and Cilnidipine is given in Table 3, while Figures 4 and 5 represent linearity graphs of both drugs respectively. The respective plot should be linear passing through the origin and the R^2 value should not be less than 0.992

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, peak area, tailing factor and resolution. The repeatability of these parameters was checked by injecting three times the test solution of 10 µg/ml Nebivolol and 20 µg/ml Cilnidipine. The results shown in Table 1 were within acceptable limits.

Acuracy (% Recovery)

Accuracy was determined by calculating recovery of Nebivolol and Cilnidipine by the standard addition method. The known amounts of standard solutions of Nebivolol (5, 10 and 15 µg/ml) and Cilnidipine (10, 20 and 30 µg/ml) were added to a pre quantified test solutions of Nebivolol (10 µg/ml) and Cilnidipine (20 µg/ml). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results are shown in Table 4. The mean recovery should be in the range of 99-101% and the % RSD should not be less than 2%

Precision

The method was validated in terms of intra-day inter-day precision. The solution containing 10µg/ml of NEB and 20 µg/ml of CIL was injected six times for repeatability study. Inter-day and intra-day study were performed by injecting 5, 15 and 25 µg/ml of Nebivolol and 10, 30 and 50 µg/ml of Cilnidipine solutions three times for each aliquot. The %RSD for precision study should not be less than 2% as shown in Table 5

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.

Robustness

Robustness was carried by varying two parameters from the optimized chromatographic conditions which is change in flow rate (±0.2 ml/min) and change in wavelength (±2 nm) no significant change was observed

IV. RESULTS AND DISCUSSION

UV-Visible Spectroscopy

UV absorption of 10 µg/ml solution of nebivolol and cilnidipine was generated and the absorbance was taken in the range of 200-400 nm. λ_{max} of Nebivolol and cilnidipine in methanol was found to be 282 nm to 242 nm respectively. The Iso-absorptive point was found to be 260 nm as per the below graphs.

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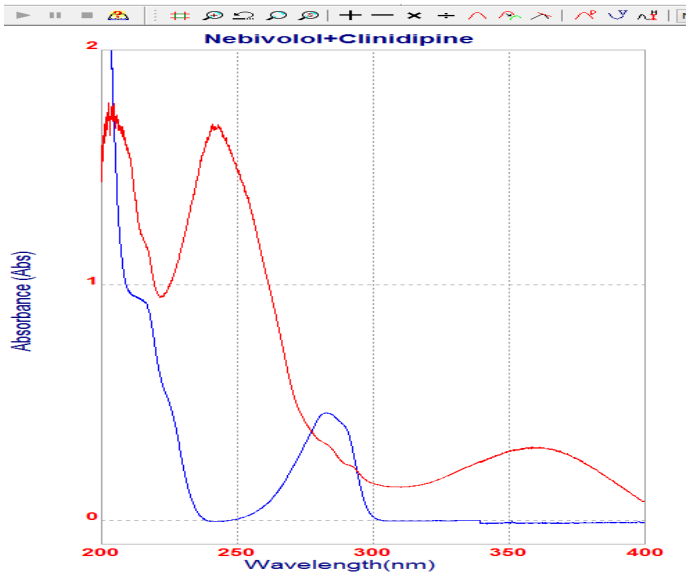

Fig 3 Iso-absorptivepoint

Table 1: Results for system suitability parameters

Parameters	**Nebivolol (mean*)**	**Cilnidipine (mean)***
Retention time (min)	3.741	5.301
Theoretical Plate	6746	7261
Tailing factor	1.18	1.29
Resolution	3.82	

*= average of three determinations,

To optimize the RP-HPLC parameters, various mobile phase trials were taken. A satisfactory separation and good peak symmetry were found in a mixture of Methanol: water (OPA PH3.0) 65:35 v/v and 1.0 ml/min flow rate gave resolution and peak shape. The effluent was monitored at 260nm using UV-detector. As it was shown in Figure 3 the retention time of Nebivolol and Cilnidipine were 3.741 min and 5.301 min respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification and Robustness. Linearity of Nebivolol and Cilnidipine were in the range of 5-25 µg/ml and 10-50µg/ml respectively. The proposed method enables rapid and simultaneous analysis of both drugs for various available formulations without any excipient's interference. The method can be used for routine analysis of marketed products of Nebivolol and Cilnidipine in combined tablet formulation. System suitability test parameters for Nebivolol and Cilnidipine for the RP-HPLC method are reported in Table1. The optical and regression characteristics and validation parameters are reported in Table 2.

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Table 2: Optical and regression characteristics and validation parameters of HPLC method for analysis of NEBI and CIL.

Parameter	Nebivolol	Cilnidipine
Calibration Range	5-25 µg/ml	10-50 µg/ml
Regression Equation	Y=44634x+ 44239	Y= 45593x+ 80679
Slope(m)	44634	45593
Intercept (c)	44239	80679
Correlation co-efficient (R^2)	0.999	0.9982
Detection Limit (µg/ml)	1.47 µg/ml	1.30 µg/ml
Quantitation Limit (µg/ml)	4.48 µg/ml	3.94 µg/ml

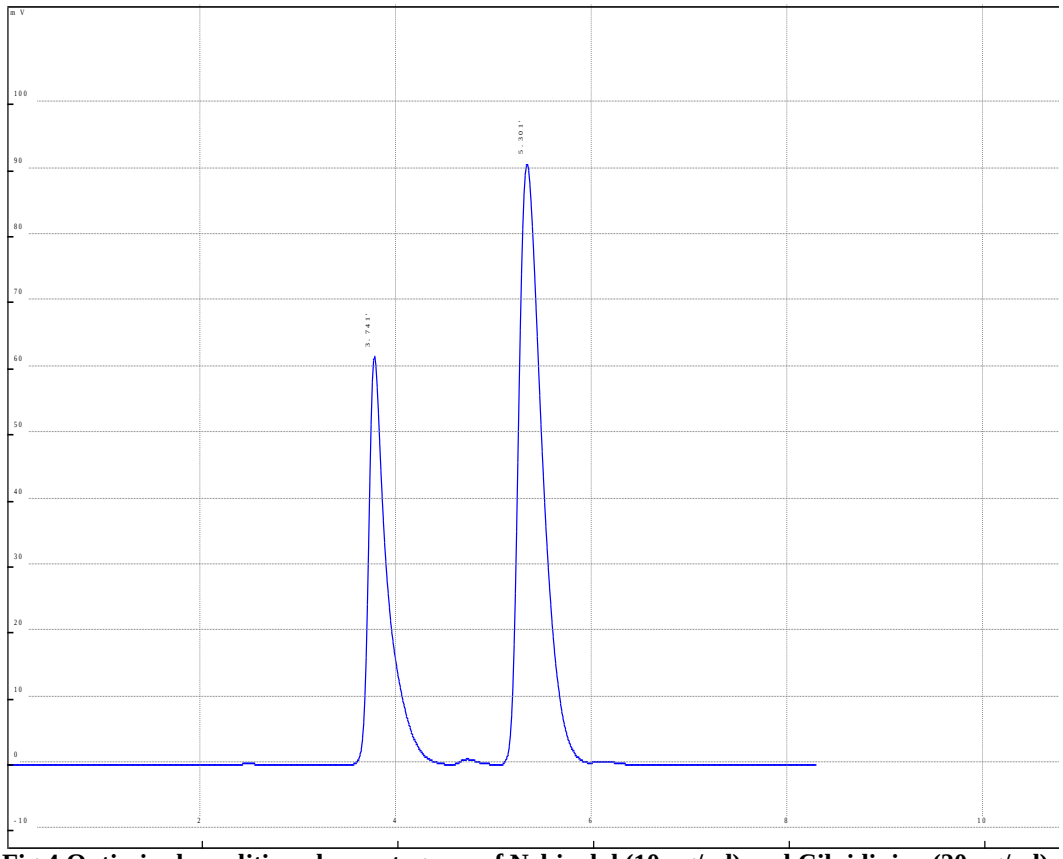

Fig 4 Optimised condition chromatogram of Nebivolol (10 µg/ml) and Cilnidipine (20 µg/ml)

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Table 3: Linearity study data for Nebivolol and Cilnidipine.

Concentration µg/ml	Area Nebivolol	Concentration µg/ml	Area Cilnidipine
5	167188	10	528481
10	413591	20	997007
15	636340	30	1433196
20	838847	40	1954073
25	1070420	50	2329603

Table 4: Recovery data for Nebivolol and Cilnidipine by HPLC method.

Drug	Level %	Amount taken (PPM)	Amount taken (PPM)	Area of Standard	Area of Sample	% Recovery
Nebivolol	50%	10	05	1433196	1434541	100.0938462
	100%	10	10	1954073	1953739	99.9829075
	150%	10	15	2329603	2326851	99.88186828
Cilnidipine	50%	20	10	636340	634587	99.72451834
	100%	20	20	838847	840085	100.1475835
	150%	20	30	1070420	1068794	99.84809701

Table 5: Precision study for Nebivolol and Cilnidipine

Drug	Concentration PPM	Interday Precision		Intraday Precision	
		Mean SD	% Found	Mean SD	% Found
Nebivolol	5	877.14	99.72	875.25	99.67
	15	1454.26	100.14	1453.12	100.09
	25	2668.52	99.84	2670.2	100.11
Cilnidipine	10	1171.75	100.09	1175.21	100.52
	30	2618.49	99.98	2612.54	99.12
	50	1651.38	99.88	1657.46	100.13

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Table 6: Robustness.

Parameter	Change level	Peak area	
		Nebivolol	**Cilnidipine**
Change in flow rate (±0.2ml/min)	0.8ml/min	411611	995700
	1.0ml/min#	413591	997007
	1.2ml/min	411113	999833
	Mean	**412105**	**997513**
	SD	**1310.781**	**2112.51**
	%RSD	**0.3180**	**0.211**
Change in wavelength (±2 nm)	260nm#	413591	997007
	258nm	414859	998371
	262nm	414251	994803
	Mean	**414234**	**996727**
	SD	**634.178**	**1800.4**
	%RSD	**1.15**	**0.18**

#= actual parameter as control standard

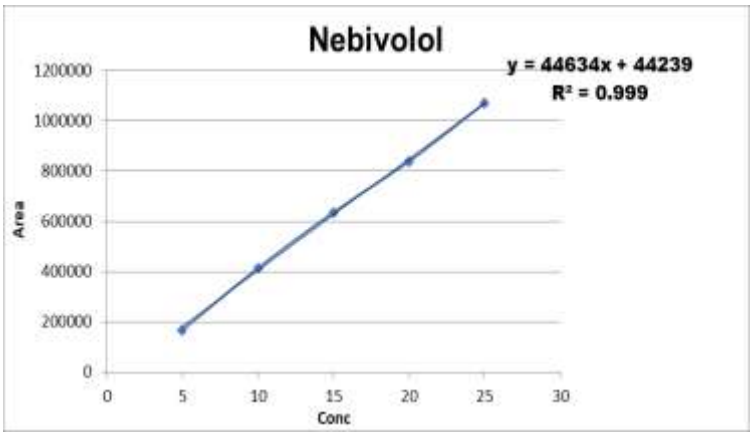

Fig 4: Calibration curve of nebivolol (5-25 µg/ml).

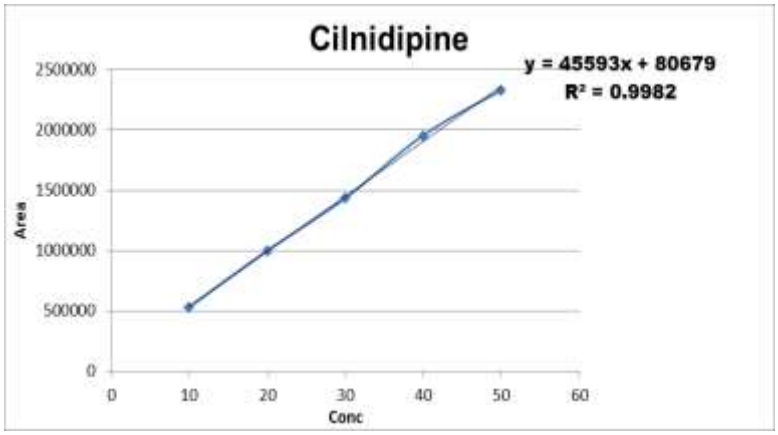

Fig 5: Calibration curve of Cilnidipine (10-50µg/ml).

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V. CONCLUSIONS

A RP –HPLC method was developed for the determination of Nebivolol and Cilnidipine. The method was validated as per the ICH guidelines and the method was found to be simple, precise, linear, accurate, rugged and robust enough

Results for validation parameters are in excellent agreement with label claim, which indicates that is no interference of additives in routinely used experiment. The proposed method is found to be accurate and precise, therefore proposed method can be used for routine analysis of Nebivolol HCl and Cilnidipine in tablet dosage form.

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