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RP-HPLC Method Development and Validation for Simultaneous Estimation of Cilnidipine and Bisoprolol Fumarate in Tablet Dosage Form

Hetal Patel*, Dulendra P. Damahe, Sachin B. Narkhede

SMT B.N.B. Swaminarayan Pharmacy College, Salvav, Vapi, India

Abstract : The objective of the recent study was to develop a simple, accurate and precise RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Cilnidipine and Bisoprolol Fumarate using mobile phase (mixture of a Phosphate Buffer: Methanol 60:40) as the solvent. The proposed method involves the measurement of Retention time at analytical wavelength 225 nm was selected. The Retention time of Cilnidipine and Bisoprolol Fumarate was found to be 4.053 and 5.730 respectively. The linearity of the proposed method was investigated in the range of 10-30 µg/ml for Cilnidipine and 5-15 µg/ml for Bisoprolol Fumarate respectively. The method was validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less 2 % RSD.
Keywords : RP-HPLC method, Cilnidipine, Bisoprolol Fumarate, Validation.

Introduction

Cilnidipine (INN) 1,4 – Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester is a calcium channel blocker. Cilnidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking developed by Fuji Viscera pharmaceutical Company, Japan and Ajinomoto, Japan and approved to come into market for the first time and used for high blood pressure treatment in 1995. Cilnidipine is approved for use in Japan, China, India, Korea and some European countries. . It is freely soluble in methanol. Molecular formula of Cilnidipine is $C_{27}H_{28}N_2O_7$ and molecular weight is 492.52 g/mol.[1, 3, 5]

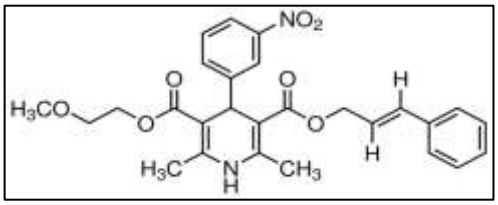

Figure 1: Structure of Cilnidipine

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BisoprololFumarate is chemically (RS) -1- {4-[(2 – isopropoxyethoxy) methyl] phenoxy } -3-(isopropyl amino) propan -2-ol. It is β_1 selective 2nd generation drug. B – blocker lacking intrinsic sympathomimetic activity; suitable for once daily administration in angina, hypertension and CHF. It is official in United State Pharmacopoeia. . It is freely soluble in ethanol and methanol. Molecular formula of Bisoprolol Fumarate is $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$ and molecular weight is 766.96 g/mol.[1,2,5]

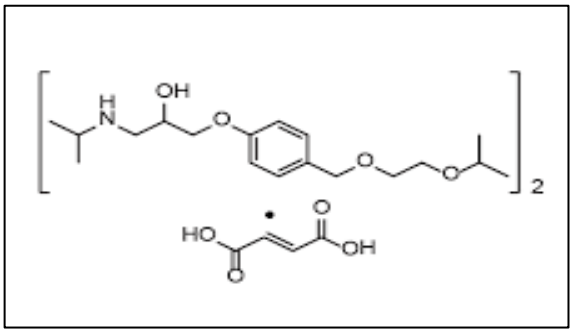

Figure 2: Structure of BisoprololFumarate

Literature review reveals that several methods such as HPLC, HPTLC, UV Spectrophotometry, UPLC etc [7-20].Methods have been reported for the individual drugs as well as in combination with others drugs in formulation. But no method was reported for the simultaneous estimation of Cilnidipine and Bisoprolol Fumarate in tablet dosage form by HPLC method. Therefore main objectives of study were to develop simple, accurate and precise method for estimation of Cilnidipine and Bisoprolol Fumarate. Validation of the developed method done in accordance with ICH guidelines.[6]

Materials and Method

Instrument and Apparatus

HPLC analysis is carried out using Shimadzu SCL 10 VP equipped with a UV-Visible detector, Shiseido – C_{18} (250*4.6mm, 5 µm) column.

Reagents and chemicals

Cilnidipine bulk powder was gifted by Prayosha health care, Ankleshwar and Bisoprolol Fumarate was gifted by Mangalam Organic Limited, Vapi. The dosage form containing Cilnidipine 10 mg and Bisoprolol Fumarate 5 mg (BESICOR C) was procured from the local market.

Chromatographic Method

Chromatographic condition

1. Stationary phase: Shiseido – C_{18} (250×4.6mm, 5 µm)
2. Mobile phase: Phosphate buffer (pH-3.5) : Methanol (60 : 40)
3. Detection wavelength: 225 nm
4. Flow rate: 1.0ml/min
5. Run time: 20 min

Preparation of Stock Solution

- **Preparation of Cilnidipine standard stock solution (1000µg/ml)**

Accurately weighed 100 mg Cilnidipine was transferred into 100 ml volumetric flask and dissolved in methanol and volume was made up to the mark.

- **Preparation of Bisoprolol Fumarate Standard stock solution (1000 µg/ml)**

Accurately weighed 100 mg Bisoprolol Fumarate was transferred into 100 ml volumetric flask and dissolved in methanol and volume was made up to the mark.

- **Working standard solution of Cilnidipine and BisoprololFumarate**

Pipette out 2 ml solution of Cilnidipine and 1 ml solution of Bisoprolol Fumarate from standard stock solution and transferred into 100 ml volumetric flask and dissolved in methanol and volume was made up to the mark to obtain 20 µg/ml Cilnidipine and 10 µg/ml Bisoprolol Fumarate.

Method Validation

1) Specificity: It is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix. Specificity of developed method was established by spiking of Cilnidipine and BisoprololFumarate in hypothetical placebo and expressing that analytes peak was not interfered from excipients.

2) Linearity and range: The linearity expressed in term of correlation co-efficient of linear regression analysis. Aliquots of standard solution of Cilnidipine in range of 10-30 µg/ml and Bisoprolol Fumarate in range of 5-15 µg/ml was prepared from standard solution and injected to system with stated chromatographic condition and analysed. The mean area and standard deviation were calculated.

3) LOD and LOQ: LOD and LOQ for Cilnidipine and Bisoprolol Fumarate were calculated as per ICH guidelines using equation LOD=3.3 σ/S and LOQ=10σ/S. Where σ is the SD of the response and S is the slope of the calibration curve.

4) Precision: The method was validated in terms of intraday and interday precision. The solution contains 20µg/ml Cilnidipine and 10 µg/ml Bisoprolol Fumarate was injected six times for repeatability study. Inter day and intraday study was performed by injecting 10, 20, 30 µg/ml solution of Cilnidipine and 5, 10, 15 µg/ml solution of Bisoprolol Fumarate. The %RSD for precision study was found less than 2%.

5) Accuracy: it was determined by calculating recovery of Cilnidipine and BisoprololFumarate by standard addition method. Known amount of standard solution were added to a quantified test solution. Each solution was injected in triplicate and the recovery was calculated by measuring peak areas.

6) Analysis of Tablet Formulation: The proposed method was tested by analyzing the commercially available marketed formulation.

Results and Discussion

- **Method development**

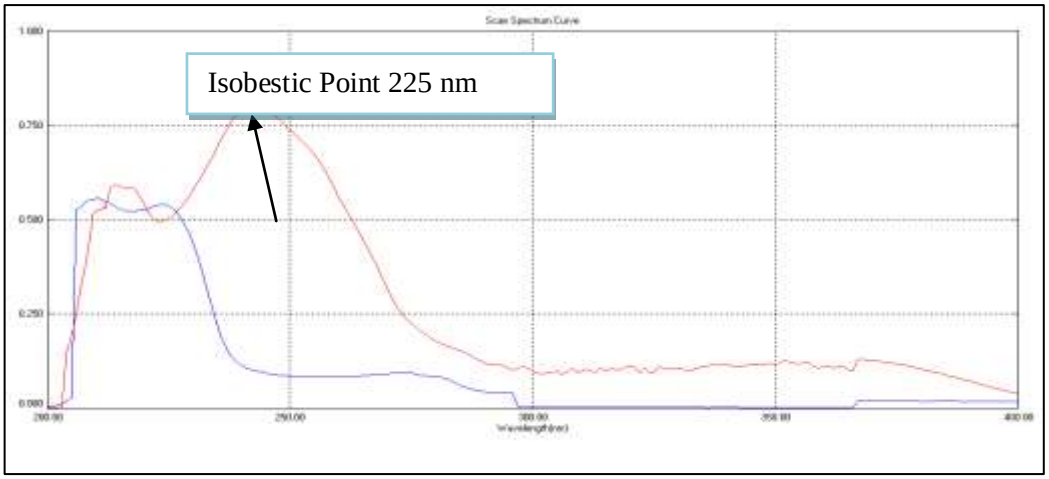

Figure 3: Determination of detection wavelength

- **Final mobile phase**

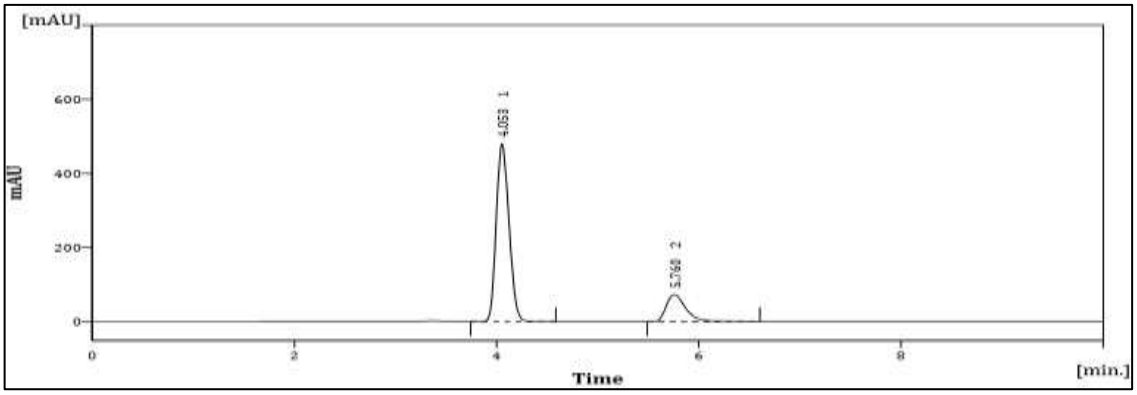

Figure 4: Final mobile phase of Cilnidipine & Bisoprolol Fumarate

- **Linearity**

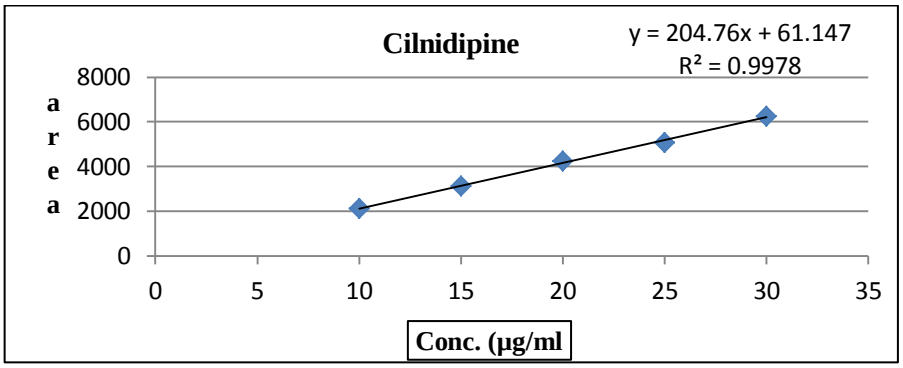

Figure 5: Calibration curve of Cilnidipine

Table 1: Linearity of Cilnidipine

Sr. No.	Conc. (µg/ml)	Peak area Mean±S.D (n=5)	%RSD
1.	10	2106.517 ± 14.778	0.701
2.	15	3118.136 ± 16.540	0.530
3.	15	4240.74 ± 28.289	0.667
4.	20	5063.793 ± 38.004	0.750
5.	25	6252.735 ± 42.737	0.683

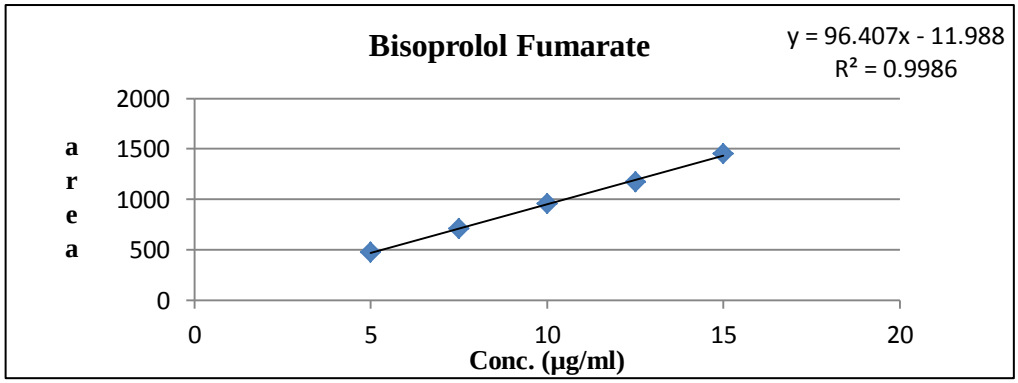

Figure 6: Calibration curve of Bisoprolol Fumarate

Table 2: Linearity of Bisoprolol Fumarate

Sr. No.	Conc. (µg/ml)	Peak area Mean±S.D (n=5)	%RSD
1.	5	477.003 ± 7.672	1.608
2.	7.5	706.571 ± 12.419	1.757
3.	10	956.065 ± 10.019	1.048
4.	12.5	1170.795 ± 16.502	1.409
5.	15	1449.98 ± 13.419	0.925

- **LOD & LOQ**

Table 3: Data of LOD & LOQ

Parameters	Cilnidipine	Bisoprolol Fumarate
Mean Slope	204.7	96.40
Intercept	61.14	11.98
LOD (µg/ml)	1.419	0.570
LOQ (µg/ml)	4.300	1.729

- **Precision**

- **Intra-Day**

Table 4: Intra-Day Precision of Cilnidipine and Bisoprolol Fumarate

Drug	Conc. (µg/ml)	Intraday Precision	
		Peak Area Mean ± S.D (n=3)	%RSD
Cilnidipine	10	2084.872 ± 17.352	0.832
	20	4214.644 ± 25.460	0.604
	30	6311.199 ± 44.181	0.700
BisoprololFumarate	5	470.089 ± 9.036	1.922
	10	958.572 ± 7.737	0.807
	15	1432.252 ± 21.309	1.487

- **Inter-Day**

Table 5: Inter-Day Precision of Cilnidipine and Bisoprolol Fumarate

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- **Repeatability**

Table 6: Repeatability of Cilnidipine&Bisoprolol Fumarate

Drug	Conc. (µg/ml)	Peak Area Mean ±S.D (n=6)	%RSD
Cilnidipine	20	4234.991 ± 12.740	0.300
BisoprololFumarate	10	959.131 ± 15.961	1.664

- **Accuracy**

Table 7: Accuracy data of Cilnidipine

% Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Standard amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80	10	8	18	7.899	98.741	98.775 ± 0.566	0.573
	10	8	18	7.858	98.226		
	10	8	18	7.948	99.358		
100	10	10	20	9.859	98.595	99.956 ± 1.288	1.288
	10	10	20	10.115	101.156		
	10	10	20	10.011	100.118		
120	10	12	22	11.974	99.787	99.831 ± 0.673	0.674
	10	12	22	11.901	99.181		
	10	12	22	12.063	100.526		

Table 8:Accuracy data of BisoprololFumarate

%Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Standard amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80	5	4	9	3.918	97.964	99.559 ± 1.358	1.358
	5	4	9	4.026	100.666		
	5	4	9	3.977	99.430		
100	5	5	10	5.008	100.177	101.309 ± 1.179	1.164
	5	5	10	5.126	102.533		
	5	5	10	5.072	101.453		
120	5	6	11	6.064	101.072	99.718 ± 1.435	1.439
	5	6	11	5.992	99.867		
	5	6	11	5.892	98.213		

- **Assay**

Table 9: Assay of Marketed Formulation

Drug	Std Peak Area	Sample peak area	Tablet amount (mg)	% Assay	Avg % Assay ± SD	%RSD
Cilnidipine	4306.721	4275.624	10	99.277	98.673 ± 0.633	0.642
		4221.186	10	98.013		
		4252.006	10	98.729		
BisoprololFumarate	972.827	953.826	5	98.046	98.598 ± 0.677	0.686
		966.543	5	99.354		
		957.203	5	98.393		

Conclusion

It concludes that the developed method is simple, accurate and precise and suitable for the routine analysis. The developed methods were validated as per ICH guidelines and were found to be within limit.

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References

1. Tripathi KD. Essential of Medical Pharmacology. 6th ed., Jaypee brothers, Medical Publishers (P) Ltd, New Delhi: 2008, pp. 1543-1545.
2. The United States Pharmacopoeia (USP 29), The National Formulary (NF 24). United State Pharmacopieal Convection Inc. Rockville, U.S.A: 2006, pp. 292-294.
3. The Japanese Pharmacopoeia. The Ministry of Health,Labour and Welfare. 17th ed., Japan: 2016, pp. 514, 704,705.
4. SankarSR.Text book of Pharmaceutical analysis. 4th ed., New Delhi: 2010, pp. 2-10 to 2-11.
5. The Merck Index. An Encyclopedia of Chemicals, drugs and Biologicals, USA, 2006, pp. 379,211.
6. ICH Harmonised Tripartite Guidelines. Validation of Analytical Procedures: Text and Methodology Q2 (R1). International conference on Harmonization, Geneva, Switzerland, 2005; 1-13.
7. Kadam A, Hamrapurkar P, Patil S, Manoharan M, Suryangandha A .Development and Validation on Stability Indicating RP-HPLC Method for the estimation of Cilnidipine in Bulk and Pharmaceutical Dosage Form. International Journal of Pharmaceutical Science Review and Research, 2015; 32: 177-181.
8. Ahmed M, Rashmi R, Kuppast j. RP-HPLC method development and validation for simultaneous estimation of Cilnidipine and Olmesartan Medoxomil in combined tablet dosage form. World Journalof Pharmacy and Pharmaceutical Sciences, 2014; 4: 785-795.
9. Hinge MA, Desai DK, Patel ES. Simultaneous estimation of Cilnidipine and Metoprolol Succinate by RP-HPLC. Scholars Research Library, 2015; 7: 333-340.
10. Kachave N, Kale M, Wagh D. Simultaneous estimation of Cilnidipine and Valsartan by RP-HPLC in Tablet Formulation. Eurasian Journal of Analytical Chemistry, 2016; 11: 245-253.
11. Pawar P, Gandhi SV, Shelar SU.Simultaneous RP-HPLC estimation of Cilnidipine and Telmisartan in combined Tablet Dosage Form. Pleagia Research Library, 2013; 4: 6-10.
12. Patel MP, Patel KP, Patel DB. Development and Validation of Analytical Method for Simultaneous Estimation of Cilnidipine, Chlorthalidone and Telmisartan in Tablet Dosage Form. World journal of Pharmacy and Pharmaceutical Sciences, 2016; 5: 1228-1241.
13. Rupareliya RH, Joshi HS, Khosla E. Stability Indicating Simultaneous Validation of Telmisartan and Cilnidipine with Forced Degradation Behaviour Study by RP_UPLC in Tablet Dosage Form. International Journal of Pharmaceutical Quality Assurance, 2016; 7: 39-45.
14. Minase As, Dole MN. Development and Validation of Analytical Method for Simultaneous Estimation of Cilnidipine and OlmesartanMedoxomil in Bulk and Tablet Dosage form by HPTLC. Journal of Advanced scientific Research, 2014; 5: 34-38.
15. Patel ND, Mehta RS, Captain AD, Chavda AA. Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Cilnidipine and Nebivolol Hydrochloride in Tablet Dosage Form. Journal of pharmaceutical Science and Bioscientific Research, 2017; 7: 140-147.
16. Patel DC, Tandel JN, Shah SK. Stability Indicating assay method development and validation for Nebivolol Hydrochloride and Cilnidipine in Pharmaceutical Dosage Form. International Journal of Institutional Pharmacy and Life Science, 2016; 6: 108-120.
17. Bhoya PN. Development and Validation of TLC-Densitometry method for Simultaneous estimation of Bisoprolol Fumarate and Hydrochlorothiazide in Bulk and Tablets. Journal of Chromatography Separation Technique, 2013; 4: 1-4.
18. KonamK,Soujanya J, Sasikala M, Kumar AK. Development and Validation of RP-HPLC Method for the Determination of Bisoprolol Fumarate Tablets. International Journal of Research in Pharmaceutical and Nano science, 2013; 2: 57-67.
19. Patil VS, Talele A., Narkhede SB. Development and Validation of Chromatographic and Spectrophotometric Method for Simultaneous Estimation of Amlodipine Besilate and Bisoprolol Fumarate in Tablet Dosage Form. European Journal of Biomedical and Pharmaceutical Science, 2017; 4: 502-514.

20. Vora DN, Kadav AA. Development and Validation of a Simultaneous HPLC method for simultaneous estimation of Bisoprolol Fumarate and Amlodipine Besylate from Tablets. Indian Journal of Pharmaceutical Sciences. 2008; 70: 542-546.

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