JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

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

Shubhada Pawar*, Ashpak Tamboli, Snehal Patil

Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India

Article History:

Received 09 Jun 2020
Accepted 17 July 2020
Available online 10 Aug 2020

Citation:
Pawar S., Tamboli A., Patil S. RP-HPLC Method Development and Validation for Simultaneous Estimation of Bisoprolol Fumarate and Cilnidipine in Pharmaceutical Dosage Form. **J Pharm Sci Bioscientific Res**. 2020. 10(2):149-155

For Correspondence:

Shubhada Pawar

Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India.

(www.jpsbr.org)

ABSTRACT:

A simple, precise, and accurate RP-HPLC method has been developed and then validated for simultaneous estimation of Bisoprolol Fumarate and Cilnidipine in pharmaceutical formulations. RP-HPLC method was performed on (C18) Inertsil ODS 3V column (150*4.6mm,5µm) and buffer: methanol in ratio (20:80 %v/v) as mobile phase at flow rate of 1.0ml/min and UV detection at wavelength 231nm. The retention time for Bisoprolol Fumarate was 2.84min and Cilnidipine was 1.518min. The obtained calibration curve was linear in the concentration range of Bisoprolol Fumarate is 5-25µg/ml and for Cilnidipine is 10-50µg/ml. The limit of detection for Bisoprolol Fumarate and Cilnidipine were found to be 2.0252µg/ml and 1.0121µg/ml respectively. The limit of quantification for Bisoprolol Fumarate and Cilnidipine were found to be 6.1370µg/ml and 3.0948µg/ml respectively. The proposed method was found to be fast, accurate and reproducible and can be used for simultaneous estimation of these drugs in tablet. The developed method was successfully validated as per ICH guideline.

KEY WORDS:

Bisoprolol Fumarate, Cilnidipine RP-HPLC method development and Validation.

INTRODUCTION

Bisoprolol Fumarate:

Bisoprolol Fumarate is 2- proponal, 1-[4-[[2-(1-Methylethoxy) ethoxy] methyl]-3-[(1-methyl ethyl) amino] -, (±)-, (E)-2-butenedioate. Bisoprolol fumarate is a synthetic beta1 –selective, cardioselective adrenoceptor blocking agent. It possesses an asymmetric carbon atom in its structure and is provided as a racemic mixture. The S (-) enantiomer is responsible for most of the β-blocking activities. It is a white crystalline powder, which is readily soluble in water, methanol, ethanol, and chloroform. It is official in USP.[1,5,6]

Molecular Formula: $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$.

Molecular Weight: 767.0 g/mol.

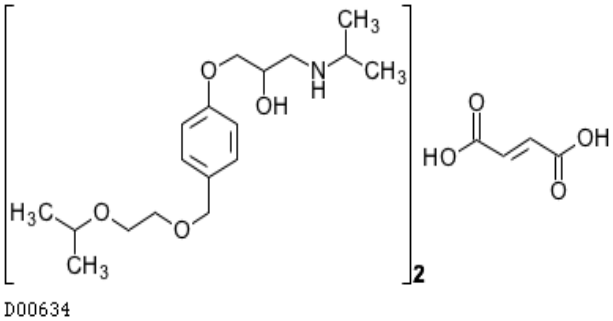

Figure 1 Structure of Bisoprolol Fumarate

Cilnidipine:

Cilnidipine is 1, 4- Dihydro- 2, 6- dimethyl-4-(3-nitrophenyl)-3, 5-pyridinedicaboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester. Cilnidipine is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. It has greater

selectivity for vascular smooth muscle. A class of drugs that act by selective inhibition of calcium influx through cell membranes or on the release and binding of calcium in intracellular pools. Since they are inducers of vascular and other smooth muscle relaxation, they are used in the drug therapy of hypertension and cerebrovascular spasms, as myocardial protective agents and in the relaxation of uterine spasms. [1,2,4]

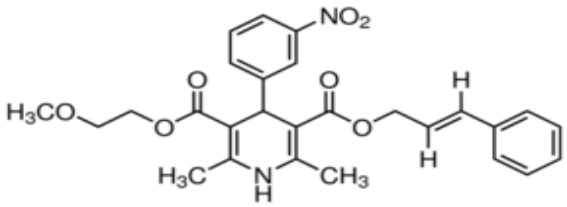

Figure 2 Structure of Cilnidipine

Molecular Formula: $C_{27}H_{28}N_2O_7$.

Molecular Weight: 492.5g/mol.

Literature review suggest few RP-HPLC, HPTLC, UV spectroscopic and stability indicating HPLC determination were performed. [2-12] The main objective of present study to develop a simple, accurate, sensitive, less retention time and economic RP-HPLC method development of Bisoprolol Fumarate and Cilnidipine in pharmaceutical dosage form and validation were performed according to ICH guideline.[13-14]

EXPERIMENTAL

Instrumentation:

Chromatography was performed with Systronic HPLC system provided with Hamilton Syringe, auto sampler and UV detector**.**

Reagent and Materials:

Bisoprolol Fumarate [bulk drug] used were of analytical reagent grade purchased from Unichem laboratories Ltd, Pharmaceutical Company in Goa Industrial Estate, Goa, India. Cilnidipine was gifted by J.B. chemical and pharmaceutical Pvt. Ltd., Mumbai. Methanol (AR grade) was purchased from Research lab finechem. Industries Mumbai and double distilled water was used throughout the analysis.

Chromatographic Method
Chromatographic condition

1) Buffer: 0.1%Formic acid in water
2) Mobile Phase: Buffer: Methanol (20:80%v/v)
3) Column: (C18) Inertsil ODS 3V column (150*4.6mm,5µm)
4) Flow Rate: 1.0ml/min
5) Temperature: Ambient
6) Volume: 10µl
7) Detector: 231nm

Preparation of mobile phase:

1000ml mobile phase was prepared by mixing 800ml methanol with 200ml buffer (0.1% formic acid) solution.

Degassing of mobile phase:

The above prepared mobile phase was degassed by using sonicator then sonicate mobile phase for 15min to avoid disturbance caused due to dissolved gases in mobile phase.

Filtration of mobile phase:

The degassed mobile phase was filtered through 0.45µ filters to avoid the column clogging due to smaller particles.

Preparation of standard stock solution:

10mg of Bisoprolol Fumarate and 10mg of Cilnidipine were weighed accurately and transferred to a separate 10ml volumetric flask, dissolved in sufficient quantity of mobile phase then sonicated for 15min and diluted to 10ml with the same solvent so as to get the concentration of 1000µg/ml. From the respective standard stock solution, working standard solution was prepared containing 5µg/ml of Bisoprolol Fumarate and 10µg/ml of Cilnidipine separately in mobile phase.

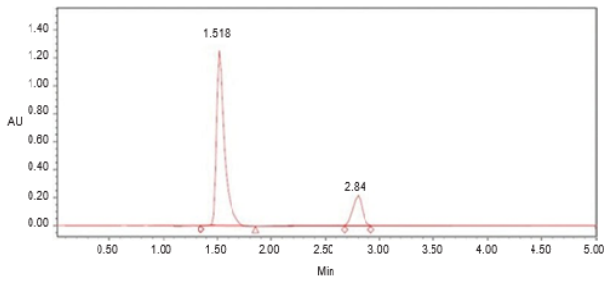

Figure 3 Chromatograph of standard mixture of Cilnidipine and Bisoprolol Fumarate at 231nm wavelength.

Preparation of sample solution (Tablet formulation analysis):

Ten tablets each containing 5mg of Bisoprolol Fumarate and 10mg of Cilnidipine was crushed and powered then accurately weighed tablet powder equivalent to 5mg of Bisoprolol Fumarate and 10mg of Cilnidipine then transferred in 10ml volumetric flask and was diluted with mobile phase then ultrasonication was done and volume

made to 10 ml(1000µg/ml of Bisopolol Fumarate and 1000µg/ml of Cilnidipine) with mobile phase then further dilution were made with mobile phase to get final concentration of 5µg/ml of Bisoprolol Fumarate and 10µg/ml of Cilnidipine. Then solution was filtered by 0.45µm nylon membrane filters by using vacuum filter. Then prepared above solution was injected and area was recorded for each drug. Then determine % assay for each drug.

Table 1: Assay of Cilnidipine and Bisoprolol Fumarate

Sr. No.	Bisoprolol Fumarate			Cilnidipine		
	Peak area	Amount recovered (µg/ml)	% Recovery	Peak area	Amount Recovered (µg/ml)	% Recovery
1	588.111	4.9980	99.961	2208.518	9.8121	98.121
2	585.101	4.9323	98.647	2220.512	9.9340	99.340
3	587.430	4.9831	99.663	2231.514	10.0458	100.458
4	586.450	4.9618	99.236	2208.416	9.8111	98.111
5	591.489	5.0717	101.435	2230.432	10.0348	100.348
6	590.231	5.0443	100.886	2213.421	9.8620	98.620
Mean	588.1353	4.998589	99.97177	2218.802	9.916689	99.16689
%RSD	0.403685	1.036616	1.03616	0.469397	1.154448	1.067327

METHOD VALIDATION [13-14]

Linearity:

The linearity of the proposed RP-HPLC method was evaluated by analyzing a series of different concentrations (n=5) for each of the two drugs. The calibration curve showed (Fig.4 and 5) good linearity in the range of 5-25µg/ml for Bisoprolol Fumarate with Correlation co-efficient of 0.995 and 10-50µg/ml for Cilnidipine with Correlation co-efficient of 0.999. Results shown in table 2 and 3.

Table 2 : Linearity of Bisoprolol Fumarate

Sr. No.	Conc.(µg/ml)	Peak Area
1	5	577.112
2	10	807.589
3	15	1069.801
4	20	1302.89
5	25	1474.97

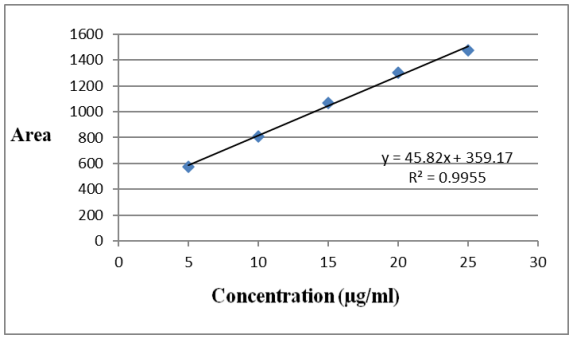

Figure 4 Linearity of Bisoprolol Fumarate

Table 3: Linearity of Cilnidipine

Sr. no.	Conc. (µg/ml)	Peak Area
1	10	2205.518
2	20	3217.144
3	30	4239.1
4	40	5163.697
5	50	6152.701

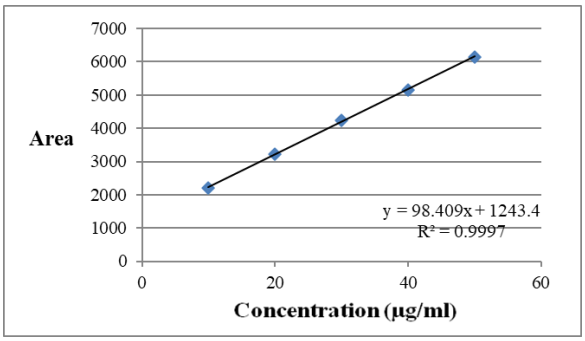

Figure 5. Linearity of Cilnidipine.

Precision:

Intra-day precision:

Standard solution containing (5,10,15µg/ml) of Bisoprolol Fumarate and(10,20,30µg/ml) of Cilnidipine were analyzed three times on the same day and % R.S.D was calculated. The result of intraday precision is given in table no.4 and 5.

Table 4: Intra-day precision study of Bisoprolol Fumarate

Conc. (µg/ml)	Area	% Recovery	Mean % Recovery ± SD	Mean% Recovery±%RSD
	583.112	97.779		
5	5850114	98.652	97.925	
	582.117	97.344	±0.666	
	807.587	97.880		99.825
10	818.581	100.279	99.698	±1.210
	821.581	100.934	±1.607	
	1061.801	102.240		
15	1048.800	100.349	101.853	
	1066.807	102.269	±1.352	

Table 5: Intra-day Precision Study of Cilnidipine

Conc. (µg/ml)	Area	% Recovery	Mean % Recovery ± SD	Mean% Recovery ±%RSD
	2205.51	97.816		
10	2212.514	98.527	98.426	
	2216.518	98.934	±0.566	
	3217.141	100.312		100.228
20	3222.14	100.566	100.684	±0.402
	3234.144	101.176	±0.444	
	4239.1	101.493		
30	4248.101	101.798	101.573	
	4237.102	101.426	±0.198	

Inter-day precision:

Standard concentration containing (5,10,15µg/ml) and (10,20,30µg/ml) of Cilnidipine were analyzed three times on the different day and % R.S.D was calculated. The result of inter-day precision are given in table no. 6and 7.

Table 6: Inter-day precision study of Bisoprolol Fumarate

Conc. (µg/ml)	Area	% Recovery	Mean % Recovery ± SD	Mean% Recovery ±%RSD
	582.110	97.341		
5	589.111	100.397	99.524	
	590.109	100.833	±1.902	
	807.585	97.879		100.132
10	814.591	99.408	99.699	±1.704
	825.588	101.808	±1.980	
	1045.80	99.913		
15	1054.81	101.223	101.174	
	1062.81	102.387	±1.237	

Table7: Inter-day precision study of Cilnidipine.

Conc. (µg/ml)	Area	% Recovery	Mean % Recovery ± SD	Mean % Recovery ±%RSD
	2206.51	97.918		
10	2228.51	100.153	99.781	
	2239.51	101.271	±1.707	
	3217.14	100.312		100.743
20	3231.14	101.023	100.955	±0.879
	3241.14	101.531	±0.612	
	4239.1	101.493		
30	4229.12	101.155	101.494	
	4249.1	101.832	±0.338	

Accuracy:

The % recovery of the proposed method was performed by the standard addition technique, by adding a known amount of standard drug at three different levels (50%, 100% and 150%) to the tablet sample. Accuracy was expressed as percentage recovery in Table 8and 9.

Table 8: Recovery study for Bisoprolol Fumarate

%Level	Conc.(µg/ml)	% Recovery	%RSD

	Sample	Std		Mean % Recovery ±SD (n=3)	
			99.1	99.2	
50%	5	2.5	98.9	±0.360	0.363
			99.6		
			100.2	99.76	
100%	5	5	99.7	±0.404	0.405
			99.4		
			100	100.03	
150%	5	7.5	100.2	±0.152	0.152
			99.2		

Table 9: Recovery study for Cilnidipine

% Level	Conc.(µg/ml) Sample	Std.	% Recovery	Mean % Recovery ±SD (n=3)	%RSD
			99.5		
50%	10	5	99.3	99.4 ±0.1	0.100
			99.4		
			100.1		
100%	10	10	99.5	99.733	0.322
			99.6	±0.321	
			99.3		
150%	10	15	100.1	99.8±0.435	0.436
			100		

Table10: System suitability data of Bisoprolol Fumarate and Cilnidipine

Parameter	Bisoprolol Fumarate	Cilnidipine	Acceptance Criteria
Retention Time (min)	2.84	1.518	±10
Theoretical plate	2100	3728	>2000
Tailing Factor	1.91	1.78	<2.00

LOD and LOQ:

LOD and LOQ are calculated by using slopes and intercepts of the calibration curves for the both the drug.

LOD values are calculated by using following formula:

LOD = 3.3 σ/S
σ = standard deviation of y-intercept of regression line.
S= slope of the calibration curve.

Bisoprolol Fumarate:- 2.025137µg/ml. Cilnidipine:- 1.021299µg/ml.

LOQ values are calculated by using following formula:

LOQ = 10 σ/S
S = Standard deviation of the response.
S= slope of the calibration curve.
. Bisoprolol Fumarate:- 6.137083µg/ml. Cilnidipine:- 3.094844µg/ml

SPECIFICITY:

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix. The peak purity of Bisoprolol Fumarate and Cilnidipine were determined by comparing the Retention time of standard Bisoprolol Fumarate and Cilnidipine good correlation was obtained between the Retention time of standard and sample of Bisoprolol Fumarate and Cilnidipine. The results are given in table no.11.

Table 11: specificity Parameter

Parameter	Bisoprolol Fumarate	Cilnidipine
Tailing factor	1.91	1.78
Retention time	2.84	1.518

Robustness:

This was done by minute change in the chromatographic conditions and found to be unchanged by minute change like ± 2% change in volume of organic solution of mobile phase.

RESULT AND DISCUSSION:

The simultaneous estimation of Bisoprolol Fumarate and Cilnidipine in pharmaceutical formulation was done by RP-HPLC. In that developed method mobile phase consist of 200ml of buffer with 800ml of methanol. Then finally mobile phase was filtered by using 0.45µ nlyon membrane filter paper and sonicate for 15min. The flow rate for that method development was found to be optimum at 1ml/min resulting in short retention time and good baseline stability. The proposed Chromatographic system was found suitable for effective separation and

quantization of Bisoprolol Fumarate and Cilnidipine was 2.84 and 1.518min respectively. The assay of Bisoprolol Fumarate and Cilnidipine in pharmaceutical formulation was found to be 99.97% and 99.16%. The linearity range for BISO and CIL were found to be 5-25µg/ml and 10-50µg/ml respectively and it show good linearity with regression coefficient 0.995 and 0.999 respectively. The LOD values were found to be2.025137 µg/ml and 1.021299µg/ml and LOQ values were found to be 6.137087µg/ml 3.094844µg/ml for both BISO and CIL respectively. The all parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. From the recovery studies data, it was found that the mean % recovery was within the limits, indicated high accuracy of the developed method. System suitability parameters were also found satisfactory; hence the developed analytical method would be considered as robust.

CONCLUSION:

The proposed RP-HPLC method employed here proved to be simple, fast, accurate, precise, reproducible, sensitive, economic, short analysis time and robust, thus can be used for routine analysis of BISO and CIL in pharmaceutical dosage form.

ACKNOWLEDGEMENT:

The authors are thankful to Sahyadri College of Pharmacy Methwade (Sangola), Maharashtra, for giving permission to carry out my work and special thanks for J. B. Chemical and pharmaceutical Pvt. Ltd., Mumbai for providing gift sample of cilnidipine & Unicheme Laboratories Ltd., Goa for Bisoprolol Fumarate.

REFERENCE:

1. Indian Pharmacopoeia. Volume II. “Government of India ministry of health and family welfare, published by Indian Pharmacopoeial commission”, Government of India Ghaziabad, 2018:1392, 1616.
2. Patel N.D., Mehta R.S., Captain A.D. Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Nebivolol Hydrochloride and Cilnidipine in Tablet Dosage Form. J Pharm Sci Bioscientific Res. 2017, 7(1);140-147.
3. Patel H., Damahe D.P., Narkhede S.B. RP-HPLC Method Development and Validation for Simultaneous Estimation of Cilnidipine and Bisoprolol Fumarate in Tablet Dosage Form. International Journal of Chem Tech Research, 2019;12: (1): 269-276
4. Prajapati Ketulkumar M. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Cilnidipine and Irbesartan in its Pharmaceutical Dosage Form. World Journal of Pharmacy and Pharmaceutical Sciences, 2017;6(5);1017-1026.
5. Baokar Shrikrishna B., Erande Ritesh S., Shaikh Surfraj G. Analytical Method Development and Validation for Simultaneous Determination of Bisoprolol Fumarate and Amlodipine Besylate. Indo American Journal of Pharmaceutical Research. 2011:2(1);100-110.
6. Ganipisettya V. N. R. Novel Reversed-phase Liquid Chromatographic Method for the Simultaneous Determination of Potential Impurities of Bisoprolol Fumarate and Hydrochlorothiazide in a Fixed Dosage Form. Separation Science and Technology 2016:51(8):1362-1369.
7. Chandani S., Patel C. J. Development and Validation of Stability Indicating Chromatographic Method for Simultaneous Estimation of Cilnidipine and Irbesartan in Pharmaceutical Dosage Form. World Journal of Pharmaceutical Research (2018) , 7(8): 1124-1135.
8. Satyavati D., G. Akshay Kumar. Validated RP-HPLC Method for Simultaneous Estimation of Chlorthalidone and Cilnidipine in API and Tablet Dosage Form. J Sci Res Pharma, 2018:7(11):124-129.
9. Sachdev Khushboo. Simultaneous estimation of Cilnidipine and Metaprolol Succinare Combination in its Tablet Dosage Form by HPLC Detection Method. Asian Journal of Biochemical and Pharmaceutical Research, 2015: 5(2); 250-260.
10. Minase A. S., Dole M. N. Development and Validation of Analytical Method for Simultaneous Estimation of Cilnidipine and Olmesartan Medoxomil in Bulk and Tablet Dosage Form by HPTLC. Journal of Advanced Scientific Research,2014;5(3):34-38.
11. Balakrishna T. Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Cilnidipine. Journal of Drug Delivery and Therapeutics, 2020; 10(1):97-100.
12. Patel H. Development and Validation of UV Spectrophotometric Method for the Simultaneous Estimation of Cilnidipine and Bisoprolol Fumarate

in Tablet Dosage Form. World Journal of Pharmaceutical Research,2018;7(11): 616-627.

13. ICH Q2A Text on validation of analytical procedure, International conference on harmonization. Tripartite guideline. 1994; 1-5.

14. ICH Q2B Validation of analytical procedure: methodology, International conference of harmonization. Tripartite guideline, 1994; 1-10.