

**DEVELOPMENT AND VALIDATION OF UV
SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS
ESTIMATION OF CILNIDIPINE AND BISOPROLOL FUMARATE IN
TABLET DOSAGE FORM**

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ABSTRACT

The UV spectrophotometric method for the simultaneous estimation of Cilnidipine and Bisoprolol Fumarate has been developed, using simultaneous equation method. The method is based on the measurement of absorbance of Cilnidipine and Bisoprolol Fumarate at their respective wavelength of 241 nm and 224 nm in methanol. It obeyed Beer's law in the concentration range 4-12 µg/ml and 2-6 µg/ml respectively with correlation coefficient 0.9993 for both Cilnidipine and Bisoprolol Fumarate. The results have been validated as per ICH guidelines.

KEYWORDS: Cilnidipine, Bisoprolol Fumarate, Simultaneous

estimation, Validation.

INTRODUCTION

Cilnidipine is chemically 1, 4 - Dihydro-2, 6-dimethyl-4-(3-nitriophenyl)-3, 5-pyridinedicarboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester. It is a dihydropyridine calcium channel blocker and used for treatment for Hypertension. 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, 5]

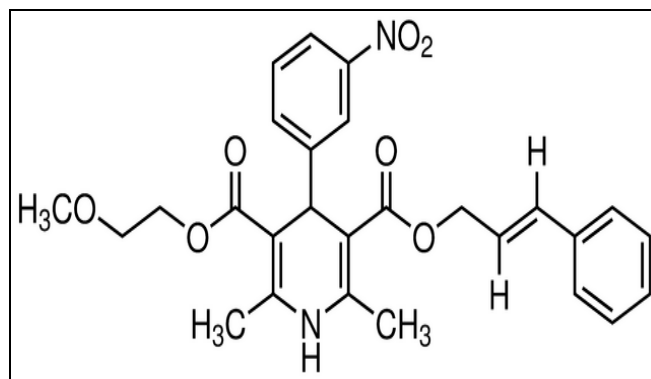


Figure 1: Structure of Cilnidipine.

Bisoprolol Fumarate is chemically (RS)-1-{4-[(2-isopropoxy ethoxy) methyl] phenoxy}-3-(isopropyl amino) propan-2-ol. It is a cardioselective β_1 – adrenergic blocker and used for treatment of Hypertension and Angina Pectoris. It is freely soluble in ethanol and methanol. Molecular formula of Bisoprolol Fumarate is $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ and molecular weight is 766.96 g/mol.^[2, 5]

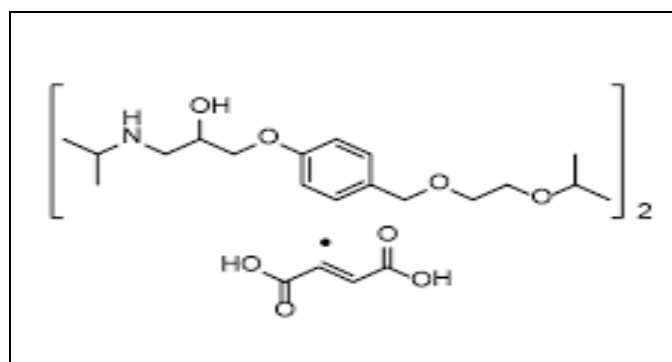


Figure 2: Structure of Bisoprolol Fumarate.

Literature review reveals that several methods such as HPLC, HPTLC, UV Spectrophotometry, UPLC etc. Methods have been reported for the individual drugs as well as in combination with others drugs in formulation.^[6, 19] But no method was reported for the simultaneous estimation of Cilnidipine and Bisoprolol Fumarate in tablet dosage form by UV-Spectrophotometric 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.^[20]

MATERIAL AND METHODS

Instrument and Apparatus

- Double beam UV-Visible Spectrophotometer (Lab India, model UV 3000⁺, Software – UV Win) having two matched quartz cells with 1 cm light path.
- Digital weighing balance, Contech
- Ultra sonicator
- Volumetric flasks – 10,50,100 ml
- Pipettes – 1,2 and 10 ml

Reagent and Chemicals

Cilnidipine was gifted by J.B. chemicals and Pharmaceuticals Pvt. Ltd., Daman and Bisoprolol Fumarate was gifted by Mangalam Drugs and Organic Limited, Vapi. The dosage form containing Cilnidipine 10mg and Bisoprolol Fumarate 5 mg (BESICOR C5) was procured from the market. The solvent used was Methanol. (AR Grade).

Method development

- ❖ Selection of solvent for Cilnidipine and Bisoprolol Fumarate

Methanol was selected as solvent for developing spectral characteristics of Cilnidipine and Bisoprolol Fumarate. The selection was made after evaluating the solubility of Cilnidipine and Bisoprolol Fumarate in different solvent.

❖ SELECTION OF WAVELENGTH

The standard solution of Cilnidipine (10 µg/ml) and Bisoprolol Fumarate (5 µg/ml) were scanned separately in the UV range of 200 – 400 nm. Spectra show 241 nm as the λ_{\max} of Cilnidipine and 224 nm as the λ_{\max} of Bisoprolol Fumarate.

➤ PREPARATION OF STOCK SOLUTION

Cilnidipine Standard Stock Solution ((200 µg/ml)

A 100 mg of Cilnidipine was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000µg/ml Cilnidipine. From this solution 20 ml was transferred to 100 ml volumetric flask and volume was made up to the mark to give a solution contain 200 µg/ml of Cilnidipine.

Bisoprolol Fumarate standard stock solution (100 µg/ml)

A 100 mg of Bisoprolol Fumarate was weighed and transferred to a 100ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml Bisoprolol Fumarate. From this solution 10 ml was transferred to 100 ml volumetric flask and volume was made up to the mark to give a solution contain 100 µg/ml of Bisoprolol Fumarate.

➤ PREPARATION OF CALIBRATION CURVE

Calibration Curve for the Cilnidipine (4-12 µg/ml)

Appropriate volume (0.4, 0.6, 0.8, 1.0 and 1.2 ml) of aliquot from standard Cilnidipine stock solution was transferred to different 10 ml volumetric flask. The volume was adjusted to the mark with methanol to obtain concentration of 4,6,8,10 and 12 µg/ml. Absorbance at 241 nm was measured and concentration vs. absorbance graph was plotted. The straight line equation was determined.

Calibration curve for the Bisoprolol Fumarate (2-6 µg/ml)

Appropriate volume (0.2, 0.3, 0.4, 0.5 and 0.6 ml) of aliquot from standard Bisoprolol Fumarate stock solution was transferred to different 10 ml volumetric flask. The volume was adjusted to the mark with the methanol to obtain concentration of 2,3,4,5 and 6 µg/ml. absorbance at 224 nm was measured and concentration vs. absorbance graph was plotted. The straight line equation was determined.

❖ Development of Simultaneous equation:

If a mixture of two components a and b are present in x% w/v and y% w/v respectively, by measuring the absorbance of mixture at two wavelengths λ_1 and λ_2 , the concentration or amount of components a and b can be estimated.^[4]

C_x and C_y the concentration of Cilnidipine and Bisoprolol Fumarate respectively in the diluted sample:

$$C_x = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

$$C_y = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

Where, a_{x1} and a_{x2} : Absorptivity of Cilnidipine at 241 nm

a_{y1} and a_{y2} : Absorptivity of Bisoprolol Fumarate at 224 nm

A_1 : Absorbance of Cilnidipine at 241 nm

A₂: Absorbance of Bisoprolol Fumarate at 224 nm

❖ Assay of tablet formulation

An quantity of powder equivalent to 10 mg Cilnidipine and 5 mg Bisoprolol Fumarate was transferred into 100 ml volumetric flask containing methanol, sonicated for 15 min and diluted to mark with the same solvent to obtain 200 µg/ml Cilnidipine and 100 µg/ml Bisoprolol Fumarate. The resulting solution was filtered using filter paper. From the above solution 0.2 ml Cilnidipine and 0.1 ml Bisoprolol Fumarate was transferred in to 10 ml volumetric flask and diluted with solvent and taken the absorbance.

➤ METHOD VALIDATION

• Linearity and Range

The linearity was determined at the range of 4-12 µg/ml for Cilnidipine and 2-6 µg/ml Bisoprolol Fumarate. Absorbance of above linearity solution preparations were taken at each concentration was calculated and graph of absorbance vs. concentration was plotted.

• Limit of Detection and Limit of Quantitation

The LOD and LOQ of the drug were calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

• Precision

1. Repeatability

Standard solution of 8 µg/ml Cilnidipine and 4 µg/ml Bisoprolol Fumarate were prepared and spectra were recorded. Absorbance was measured of the same concentration solution six times and %RSD was calculated.

2. Intermediate precision for the proposed method was determined by estimating standard solution of Cilnidipine and Bisoprolol Fumarate for 3 different concentrations for the three times on the same day (intra day) and on the three different days (inter day). The results are in terms of %RSD which is less than 2%.

- **Accuracy**

Recovery studies were carried out by spiking of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of spiked bulk drug samples. These solutions were subjected to reanalysis by the proposed method and results are calculated.

RESULT AND DISCUSSION

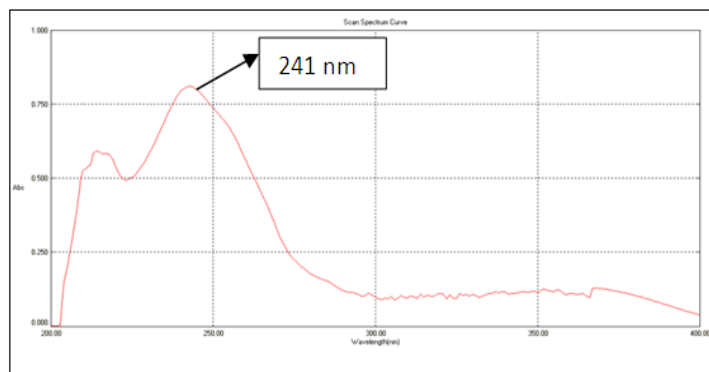


Figure 3: Selection of wavelength for Cilnidipine.

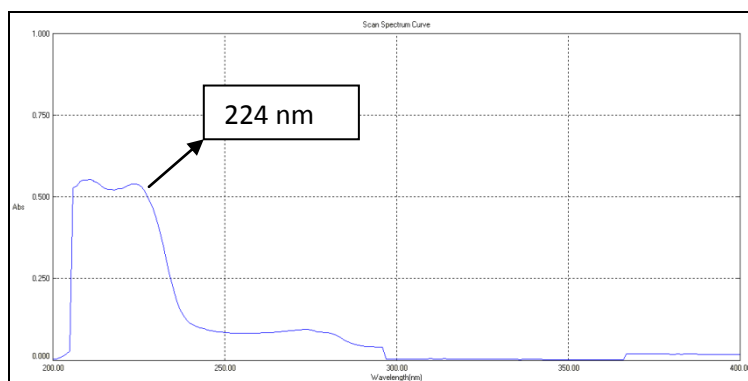


Figure 4: Selection of wavelength for Bisoprolol Fumarate

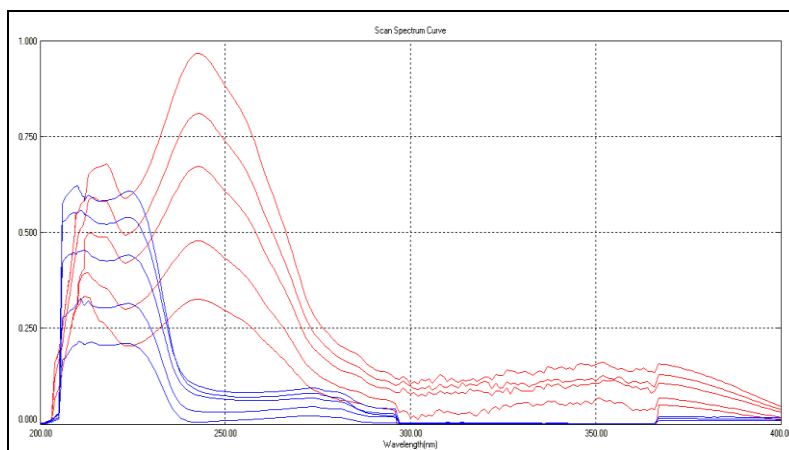


Figure 5: Overlay spectra of Cilnidipine and Bisoprolol Fumarate.

❖ Linearity

- Cilnidipine

Table 1: Calibration curve of Cilnidipine.

Conc. (µg/ml)	At 241 nm		At 224 nm	
	Mean ± SD (n=5)	%RSD	Mean ± SD (n=5)	%RSD
4	0.325 ± 0.004	1.235	0.202 ± 0.002	1.106
6	0.472 ± 0.007	1.559	0.303 ± 0.002	0.890
8	0.666 ± 0.003	0.526	0.423 ± 0.004	1.063
10	0.774 ± 0.003	0.452	0.496 ± 0.004	0.873
12	0.974 ± 0.005	0.577	0.599 ± 0.003	0.662

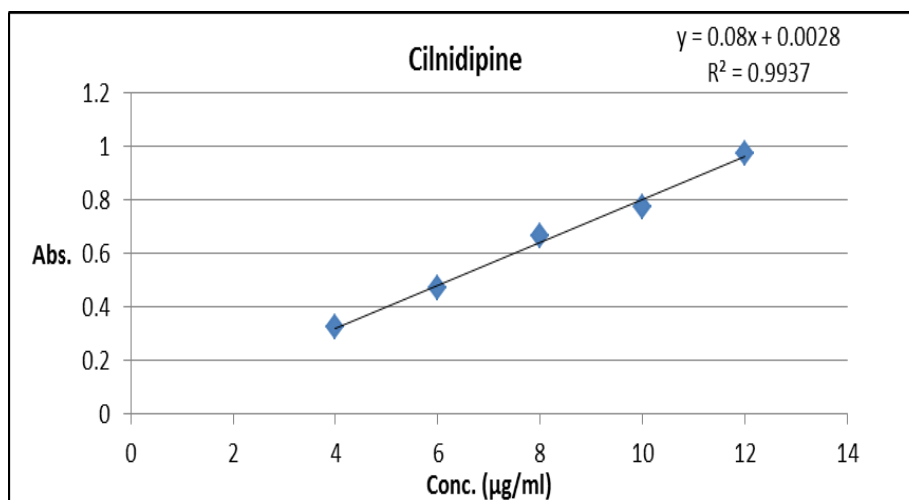


Figure 6: Calibration curve of Cilnidipine at 241 nm.

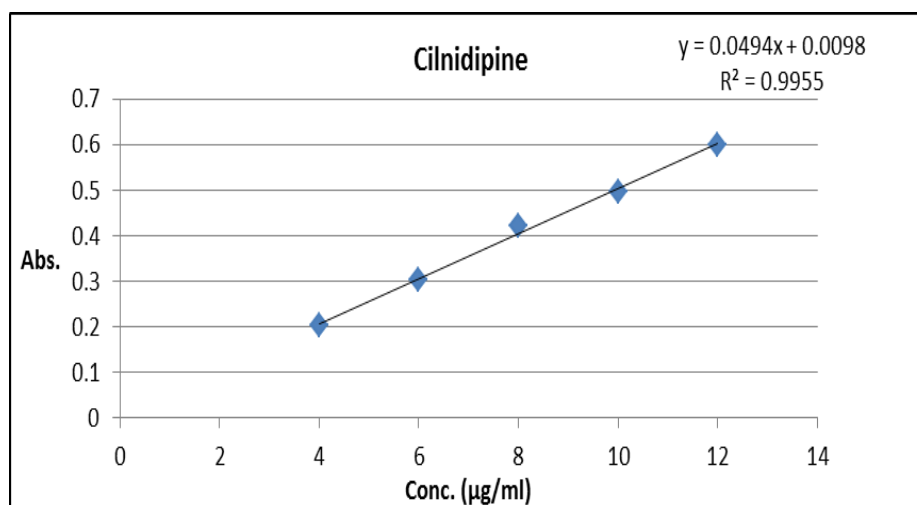


Figure 7: Calibration curve of Cilnidipine at 224 nm.

• Bisoprolol Fumarate

Table 2: Calibration curve of Bisoprolol Fumarate.

Conc. (µg/ml)	At 224 nm		At 241 nm	
	Mean ± SD (n=5)	%RSD	Mean ± SD (n=5)	%RSD
2	0.205 ± 0.003	1.872	0.023 ± 0.0004	1.106
3	0.308 ± 0.003	1.089	0.046 ± 0.0007	0.890
4	0.437 ± 0.004	1.073	0.071 ± 0.0011	1.063
5	0.530 ± 0.005	1.002	0.093 ± 0.0018	0.873
6	0.609 ± 0.003	0.652	0.106 ± 0.0019	0.662

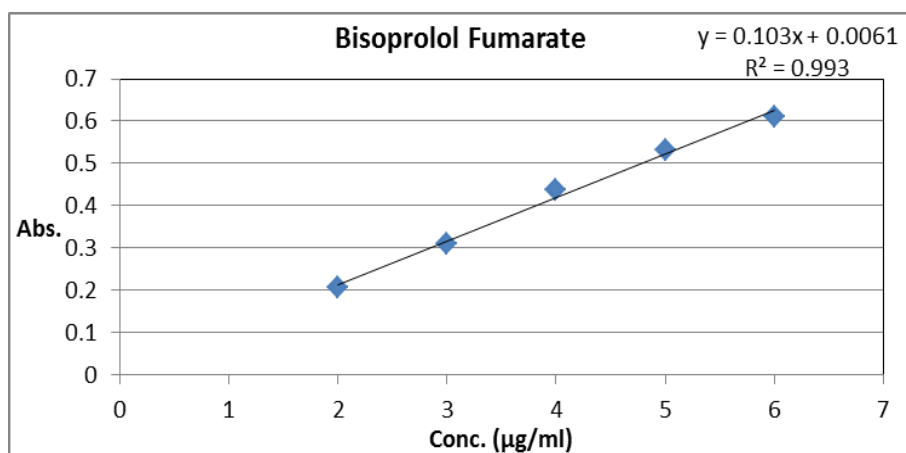


Figure 8: Calibration curve of Bisoprolol Fumarate at 224 nm.

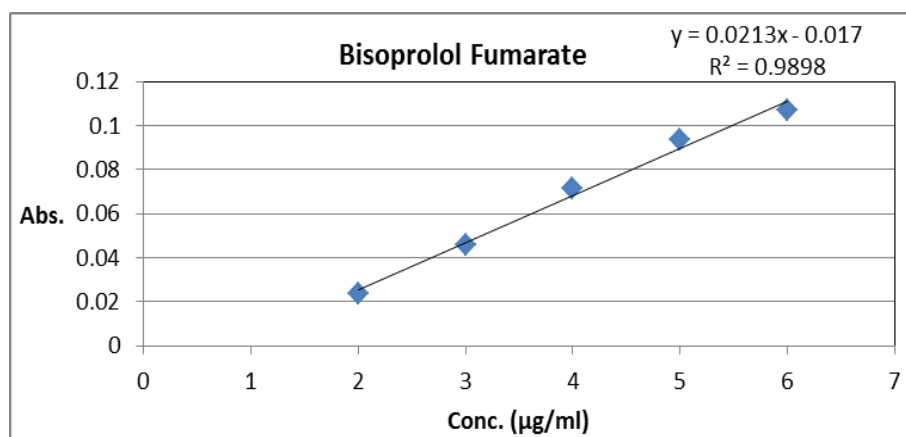


Figure 9: Calibration curve of Bisoprolol Fumarate at 241 nm.

❖ LOD and LOQ

Table 3: LOD and LOQ data for Cilnidipine and Bisoprolol Fumarate.

Characteristics	Cilnidipine	Bisoprolol Fumarate
Slope	0.08	0.103
LOD (µg/ml)	0.959	0.507
LOQ (µg/ml)	2.908	1.538

❖ PRECISION

Table 4: Intra-Day and Inter-Day Precision.

Drug	Conc. (µg/ml)	Intra- Day		Inter-Day	
		Mean Abs. ± SD (n=3)	%RSD	Mean Abs. ± SD (n=3)	%RSD
Cilnidipine	4 (50%)	0.322 ± 0.002	0.645	0.324 ± 0.004	1.246
	8 (100%)	0.667 ± 0.002	0.676	0.665 ± 0.003	0.527
	12(150%)	0.973 ± 0.007	0.815	0.981 ± 0.018	1.860
Bisoprolol Fumarate	2 (50%)	0.209 ± 0.002	0.992	0.213 ± 0.002	1.242
	4 (100%)	0.433 ± 0.002	0.580	0.437 ± 0.006	1.396
	6 (150%)	0.603 ± 0.002	0.438	0.616 ± 0.009	1.472

• Repeatability

Table 5: Repeatability data of Cilnidipine and Bisoprolol Fumarate.

Characteristics	Cilnidipine	Bisoprolol Fumarate
Mean Abs. (n=6)	0.667	0.436
SD	0.005	0.004
%RSD	0.823	1.061

❖ ACCURACY

Table 6: Accuracy data of Cilnidipine.

% Level	Amount of sample taken (µg/ml)	Amount of standard added (µg/ml)	Total amount (µg/ml)	Standard amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80	10	8	18	8.070	100.887	99.665 ± 1.255	1.259
	10	8	18	7.870	98.379		
	10	8	18	7.978	99.729		
100	10	10	20	9.830	98.302	98.868 ± 1.400	1.416
	10	10	20	9.783	97.839		
	10	10	20	10.046	100.462		
120	10	12	22	12.237	101.980	101.294 ± 0.875	0.864
	10	12	22	12.037	100.308		
	10	12	22	12.191	101.594		

Table 7: Accuracy data of Bisoprolol Fumarate.

% Level	Amount of sample taken (µg/ml)	Amount of standard added (µg/ml)	Total amount (µg/ml)	Standard amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)	% RSD
80	5	4	9	3.928	98.214	98.315 ± 1.045	1.063
	5	4	9	3.892	97.321		
	5	4	9	3.976	99.404		
100	5	5	10	4.892	97.857	98.809 ± 0.952	0.963
	5	5	10	4.940	98.809		
	5	5	10	4.988	99.761		
120	5	6	11	5.964	99.404	99.537 ± 1.195	1.201
	5	6	11	5.904	98.412		
	5	6	11	6.047	100.793		

❖ **Analysis of marketed formulation****Table 8: Assay of Marketed formulation.**

Drug	Std Abs.	Sample Abs.	Tablet amount (mg)	% Assay	Avg % Assay ± SD	%RSD
Cilnidipine	0.670	0.662	10	98.805	99.25 ± 0.258	0.533
		0.655	10	97.761		
		0.658	10	98.208		
Bisoprolol Fumarate	0.432	0.429	5	99.305	98.611 ± 0.694	0.704
		0.423	5	97.916		
		0.426	5	98.611		

CONCLUSION

It concludes that the developed methods are 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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