STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF BISOPROLOL AND CLINIDIPINE BY RP-UPLC

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ABSTRACT:

The present study was aimed to develop a novel, simple, rapid accurate and precise, stability-indicating reversed-phase UPLC method for the simultaneous estimation of Bisoprolol and Clinidipine in tablet dosage forms.The chromatographic elution was achieved in isocratic mode using the combination of Bisoprolol and Clinidipine in the ratio of Acetonitrile and Buffer (60:40%v/v) using a X-Bridge phenyl C18 column which has specification (150mm× 4.6 mm, 3.5µm) and the flow rate of 1.0 ml/min and wavelength detection at 231 nm. The retention time obtained for Bisoprolol and Clinidipine was 1.918 min, 2.663 min respectively. Bisoprolol and Clinidipine their combination drug product were exposed to acidic, alkali, thermal, photolytic, and oxidative stress conditions. The current method was validated according to the ICH guidelines for accuracy, precision, linearity, specificity, and sensitivity.

KEYWORDS: Bisoprolol, Clinidipine, RP-UPLC, Stability Indicating, Forced Degradation Method Validation.

INTRODUCTION:

Bisoprolol

(1) Bisoprolol is a beta blocker medication and it is used in the treatment of heart diseases. It chemically named as (RS)-1-(4-[(2-Isopropoxyethoxy)methyl]phenoxy}-3-(isopropyiamino)propan-2-ol. It is a white crystalline powder with a molecular formula of $C_{18}H_{31}NO_4$ and molecular weight of 325.449 g/mole and soluble in ethanol and water (2) and its chemical structure is given in figure 1.

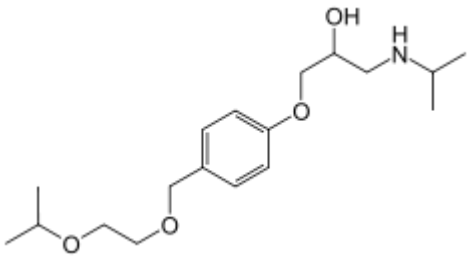

Figure 1: Chemical Structure of Bisoprolol

Clinidipine

(3) Clinidipine is a calcium channel blocker which is used in the treatment of hypertension. Its chemically named as 3-(2-methoxyethyl) 5-(2E)-3-phenylprop-2-en-1-yl2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. It is a white solid with a molecular formula of $C_{27}H_{28}N_2O_7$ and a molecular weight of 492.52 g/mole and insoluble in water and soluble in DMSO, ethanol (4). Its chemical structure is given in figure 2.

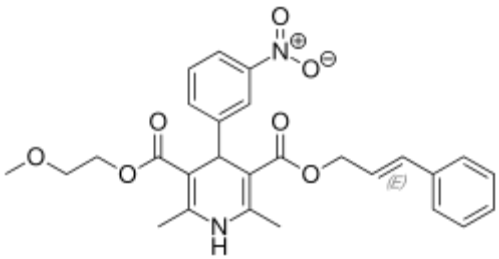

Figure 2: Chemical Structure of Clinidipine

(5, 6) From the literature survey it was concluded that only a few spectroscopic and liquid chromatographic procedures have been reported for the simultaneous estimation of Bisoprolol and Clinidipine. But there is no stability indicating method is available for the estimation of Bisoprolol and Clinidipine in pharmaceutical dosage forms (7, 8).

MATERIALS AND METHODS:

Chemicals and Solvents:

Solvents of UPLC grade like Acetonitrile, Water and Methanol was purchased from Ramkem Haryana, India. Sodium Hydroxide, Ortho- Phosphoric Acid was purchased from Fischer Scientific, Mumbai and India.

Instrumentation and Chromatographic Conditions:

The Present assay was carried out on Waters UPLC system equipped with photo diode array detector; auto sample injector integrated with Empower 2 Software was used in the current investigation. The isocratic mobile consisted of Acetonitrile and Buffer (70:30%v/v), flowing through the X-Bridge phenyl (150×4.6mm, 3.5µm) column. The mobile phase was pumped through the column at a flow rate of 1.0mL/min with a sample injection volume of 10µL.Detection of the analytes were carried out at a wavelength of 231nm

Preparation of Standard solution:

Working standards 20mg of Bisoprolol and 40mg of Clinidipine were accurately weighed and transferred into 100mL volumetric flask and 5 mL of diluent was added and sonicated for 30minutes.The final volume was made with diluent to get the concentration 2000 µg/mL of Bisoprolol, 4000 µg/mL of Clinidipine. From above stock solution 5mL was pipetted out into a 50mL volumetric flask and then made up to the final volume with diluent to get the concentration 200 µg/mL , 400 µg/mL of Bisoprolol and Clinidipine respectively.

Preparation of Sample solution:

10 tablets were weighed and crushed with a mortar and pestle and average weight was calculated. Accurately weighed and transferred equivalent amount of one tablet (160mg) was transferred to 25mL volumetric flask. To it 70 mL of diluent was added and sonicated for 30minutes; the further volume was made up with diluent. From the above stock solution 5mL was pipetted out and taken into a 50mL volumetric flask and made up to the volume with diluent and the finally to get the concentrations 200 µg/mL , 400 µg/mL of Bisoprolol and Clinidipine respectively.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT:

The present method reported is a new stability indicating RP-UPLC method development and validation for simultaneous estimation of Bisoprolol and Clinidipine in tablet dosage form. The method developed was proceeding within wavelength selection as 231nm. In order to get the optimized RP-UPLC method various mobile phases and columns were used to get better resolution. Finally the analysis was performed by using Acetonitrile and Buffer in the ratio of 60:40 at a flow rate 1.0mL/min at an injection volume of 10µL and separation was carried out by using X-Bridge Phenyl, C18 (150mm×4.6mm, 3.5µm). The resulting chromatograms were recorded and chromatographic parameters such as tailing factor, USP plate count and resolution were calculated

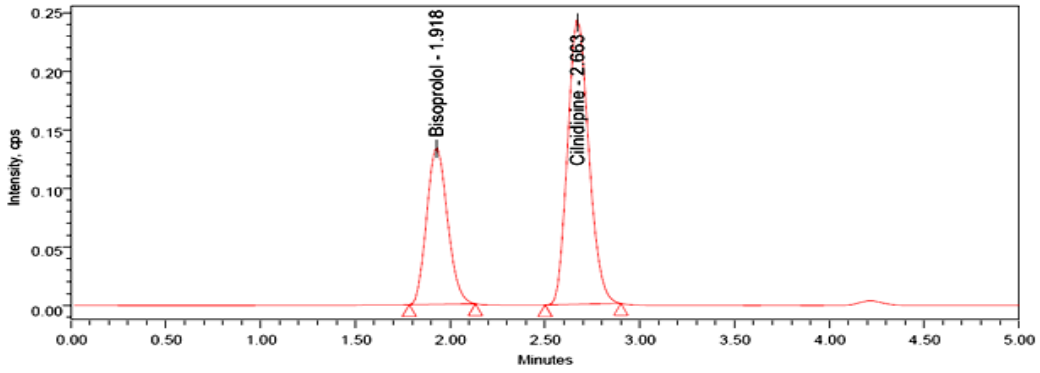

Figure 3: Optimized Chromatogram Method of Bisoprolol and Clinidipine

ANALYTICAL METHOD VALIDATION

[9] The proposed method was validated as per ICH guidelines. The parameters studied for validation were Specificity, Linearity, Accuracy, Precision, Robustness, System suitability, Limit of Detection, Limit of Quantification [10]

System suitability:

Six replicates of the working standard solution were prepared and injected 0.20µL solution to carry out system suitability parameters like Retention time, Peak area, USP Plate count, USP Resolution and USP Tailing. The theoretical plates were found to be not more than 5000 for all three drugs. The tailing factor was found to be less than 2.0 and resolution was less than 1.5. The system suitability parameters values are given in table 1.

Table 1: System suitability test parameter

S.No	Peak Name	Retention Time	Peak Area	USP Plate Count	USP Resolution	USP Tailing
1	Bisoprolol	1.918	1475621	2467	1.15	
2	Clinidipine	2.663	2558038	3687	1.11	3.57

Specificity:

A study to establish the interferences of blank and placebo was conducted. Analysis was performed on solution placebo and formulation as per test method. Chromatograms of blank, placebo and formulation solution showed no peaks at the retention time of Bisoprolol and Clinidipine peaks.

Linearity:

Volume of 10µL of each sample was injected in five times for each concentration level in triplicate into the chromatographic system and the chromatograms were recorded. Calibration curve was constructed by plotting the peak area versus drug concentration and these chromatograms are present in figures 4 to 5 and linearity data values are given in table 2.

Table 2: Linearity data of Bisoprolol and Clinidipine

Bisoprolol		Clinidipine	
Concentration(µg/mL)	**Peak Area**	**Concentration(µg/mL)**	**Peak Area**
2.00	149507	4.00	359101
5.00	401715	10.00	604301
10.00	763987	20.00	1289653
15.00	1157973	30.00	1895299
20.00	1454341	40.00	2578191
25.00	1887487	50.00	3188539
30.00	2192107	60.00	3770639

Regression equation y = 73429x+ 18769 R^2 = 0.9994	Regression equation y = 62754x+32037 R^2 = 0.9995

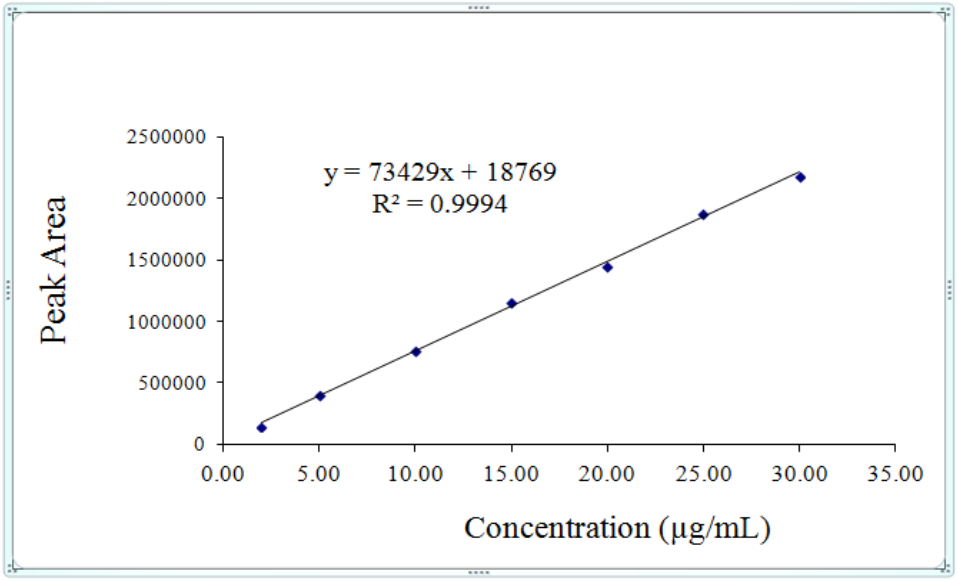

Figure 4: Standard calibration graph of Bisoprolol

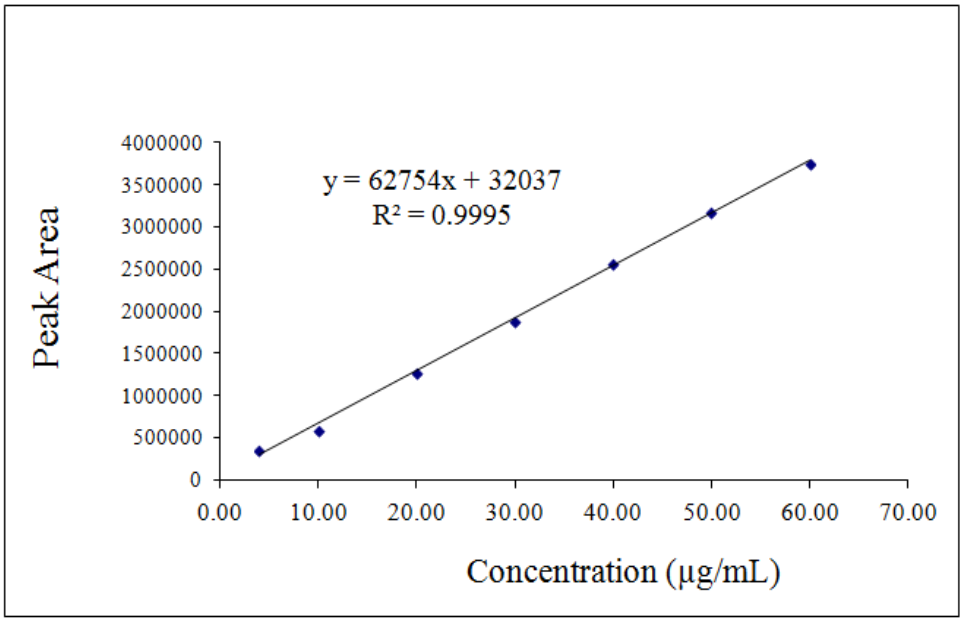

Figure 5 : Standard calibration graph of Clinidipine

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Series of very dilute LOD and LOQ solutions were prepared as per the test method and injected triplicate into the UPLC system. The LOD and LOQ were established based on signal to noise ratio.LOD and LOQ was established by identifying the concentration which given s/n ratio 3 and 10. The LOD and LOQ data values are given in table 3.

Table 3: Limit of Detection and Limit of Quantification data

S.No	Parameter	Measured values (µg/mL)	
		Bisoprolol	Clinidipine
1	LOD	0.03	0.05
2	LOQ	0.10	0.16

Method Precision:

10µL of working sample solution was injected six test preparations into the UPLC system and chromatograms were obtained. % RSD value of the peak area was calculated and method precision data are present in table 4.

Table 4: Method Precision Study for Bisoprolol and Clinidipine

Sample No.	Peak area response of drugs	
	Bisoprolol	Clinidipine
1	1469274	2578679
2	1499553	2568432
3	1484189	2543387
4	1485510	2579317
5	1464115	2538066
6	1483067	2518679
Mean	100.5	99.9
Std.Dev	0.861	0.983
%RSD	0.86	0.98

Intermediate Precision:

10µL of working standard preparations was injected six times into the chromatographic system on different days and chromatograms were recorded. % RSD value of Peak area was calculated. Intermediate precision data are given in table 5.

Table 5: Intermediate Precision data of Bisoprolol and Clinidipine

Sample No.	Peak area response of drugs	
	Bisoprolol	Clinidipine
1	1491234	2548786
2	1471328	2538367

3	1451384	2528542
4	1443784	2518143
5	1473128	2508357
6	1463354	2538687
Mean	99.7	98.8
Std.Dev	1.159	0.559
%RSD	1.16	0.57

Accuracy:

A known amount of Bisoprolol and Clinidipine at each three concentration levels 50%, 100% and 150% were added to a pre analyzed sample solution and injected in triplicate into the chromatographic system and the chromatograms were recorded. The mean percent recovery and % RSD of Bisoprolol and Clinidipine at each level was calculated and present in table 6 to 7.

Table 6: Data of Accuracy Studies of Bisoprolol

Spiked level	Amount spiked (µg/mL)	Amount Recovery (µg/mL)	% Recovery	Mean Recovery ± % RSD
50%	10	9.82	98.2	100.1±0.87
	10	9.95	99.5	
	10	9.96	99.6	
100%	20	20.15	100.8	
	20	20.13	100.7	
	20	19.88	99.4	
150%	30	30.48	101.6	
	30	29.94	99.8	
	30	30.3	101.0	

Table 7: Data of Accuracy Studies of Clinidipine

Spiked level	Amount spiked (µg/mL)	Amount Recovery (µg/mL)	% Recovery	Mean Recovery ± % RSD
50%	20.00	20.17	100.9	99.6±0.93
	20.00	19.81	99.1	
	20.00	19.98	99.9	
100%	40.00	40.5	101.3	
	40.00	39.67	99.2	

	40.00	40.34	100.9	
150%	60.00	59.14	98.6	
	60.00	59.31	98.9	
	60.00	59.02	98.4	

Robustness

The standard solution prepared as per test method was injected into the chromatograph at varied conditions of flow rate at ±0.1mL/min, mobile organic phase composition by ±10%. The results of robustness study are shown in table 8 to 9.

Table 8: Robustness study Results for Bisoprolol

Parameter	Optimized Conditions	Used Condition	Peak Area	Retention Time	Plate Count	Tailing Factor
Flow rate ±0.1mL/min	1.0mL/min	0.9mL/min	1256987	1.633	2165	1.19
		1.1mL/min	1752641	2.344	2583	1.05
Organic phase composition(5%v/v)		55:35	1194138	1.852	2341	1.15
	60:40	65:45	1694138	1.942	2574	1.12

Table 9: Robustness study Results for Clinidipine

Parameter	Optimized Conditions	Used Condition	Peak Area	Retention Time	Plate Count	Tailing Factor
Flow rate ±0.1mL/min	1.0mL/min	0.9mL/min	2356298	1.633	2165	1.19
		1.1mL/min	2945623	2583	2583	1.05
Organic phase composition(5%v/v)	60:40	55:35	2258502	1.852	2341	1.15
		65:45	2757542	1.942	3249	1.36

Forced Degradation Studies

A stress study was conducted to demonstrate the effective separation of degradation from the main analyte peaks of the sample when exposed to the following stress conditions. All the stressed samples were suitability diluted to required concentration with diluents and injected twice into the UPLC system by using optimized chromatographic conditions and the chromatograms were recorded and evaluated for the peak purity. The % degradation of Bisoprolol and Clinidipine were calculated. The chromatograms of the stressed samples were evaluated for peak purity as shown in the figures 6 to 7. These stress degradation results of Bisoprolol and Clinidipine are reported in tables 10 to 11

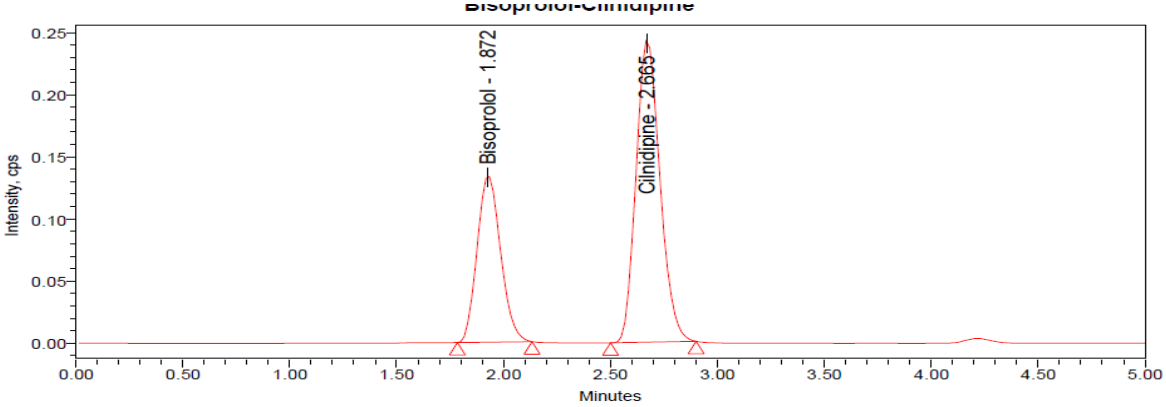

Figure 6: Chromatogram of Acid Degradation

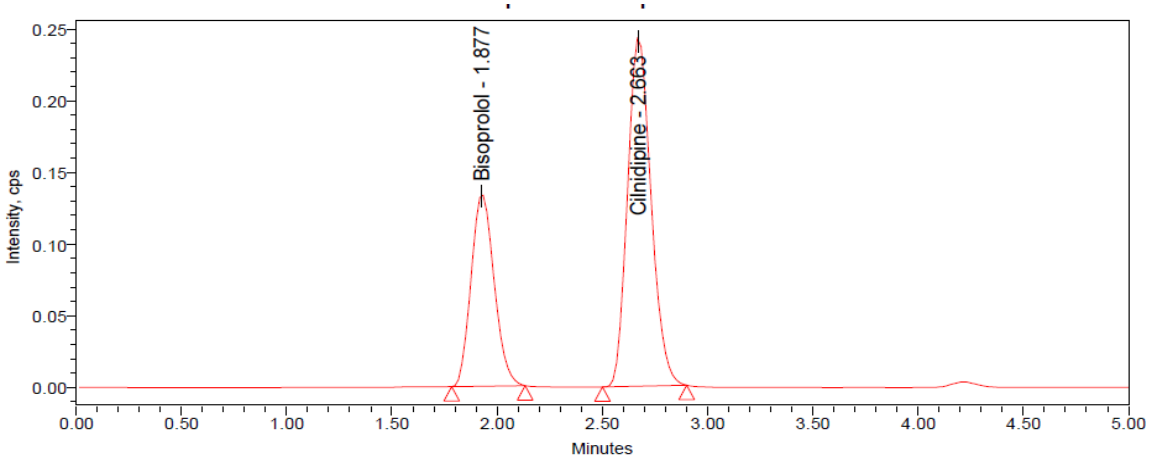

Figure 7: Chromatogram of Base Degradation

Table 10: Degradation studies of Bisoprolol

S.No	Degradation Conditions	% Peak Area	Purity Angle	Purity Threshold	% Degradation	Peak Purity
1	Acid	1412365	0.347	5.518	3.5	Passes
2	Base	1422343	0.329	5.134	2.8	Passes
3	Neutral	1403482	0.315	5.127	4.1	Passes
4	Peroxide	1430587	0.344	5.162	2.2	Passes
5	Thermal	1427540	1.347	5.119	2.4	Passes
6	UV light	1412748	0.332	5.123	3.5	Passes
7	Reduction	1408527	1.309	5.237	3.7	Passes

Table 11: Degradation studies of Clinidipine Drug

S.No	Degradation Conditions	% Peak Area	Purity Angle	Purity Threshold	% Degradation	Peak Purity
1	Acid	2472562	0.134	4.361	3.4	Passes
2	Base	2486957	0.237	6.826	2.9	Passes
3	Neutral	2479637	1.071	90	3.2	Passes
4	Peroxide	2469568	0.182	6.573	3.6	Passes
5	Thermal	2486352	1.063	9.165	2.9	Passes
6	UV light	2499637	1.064	9.341	2.4	Passes
7	Reduction	2441478	0.143	9.159	4.6	Passes

Analysis of Marketed Formulation:

The marketed tablet Besicor-C5 was analyzed separately by injecting 10µL of standard and sample solutions into the UPLC system and chromatograms were then recorded. The % assay values of the Bisoprolol and Clinidipine was found to be 98.2 and 98.8.

Conclusion

A new stability indicating RP-UPLC method was developed and validated for the simultaneous estimation of Bisoprolol and Clinidipine in pharmaceutical dosage form and it was validated as per ICH guidelines. This method represents simple, rugged, economic, selective, accurate, precision and stability indicating analytical procedure for simultaneous estimation of Bisoprolol and Clinidipine. Therefore, this method can be successfully applied to routine analytical purpose in bulk and tablet dosage forms.

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CONFLICTS OF INTEREST

No Conflicts of Interest

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