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Stability indicating RP-HPLC method development and validation for simultaneous estimation of cilnidipine and bisprolol fumarate in synthetic mixture

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ABSTRACT

A new simple, precise, accurate and selective stability indicating RP-HPLC method has been developed and validated for estimation of Cilnidipine and Bisoprolol fumarate in synthetic mixture. The method was carried out on Hypersil ODS C_{18} 5μ column (250 x 4.6 mm) with a mobile phase consisting of Acetonitrile: 0.025 M Dibasic phosphate buffer pH 3.0 with phosphoric acid (70: 30 v/v) and flow rate of 1 mL/min. Detection was carried out at 245 nm. The retention time for Cilnidipine and Bisoprolol fumarate was found to be 3.04 min and 15.114 min, respectively. The Cilnidipine and Bisoprolol fumarate followed linearity in the concentration range of 5 - 25 μg/mL (r^2= 0.998) and 2.5 - 12.5 μg/mL (r^2= 0.9993). The developed method was validated for linearity and range, accuracy, precision, and assay. Cilnidipine and Bisoprolol fumarate was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different Retention time. The proposed method can be used for routine analysis of Cilnidipine and Bisoprolol fumarate in synthetic mixture.

1. Introduction

Cilnidipine protects the end organs from the potentially damaging consequences that hypertension might have. The elderly, diabetics, and albuminurics are three groups of persons who can benefit from consuming it. Cilnidipine is being used by an increasing number of patients who are afflicted with chronic renal disease. High blood pressure is also referred to as hypertension in some circles. The force that is generated when the blood that is pumped by the heart presses against the walls of the blood vessels is referred to as blood pressure. A disorder that can cause irreversible damage to the blood vessels is high blood pressure, which is also known as hypertension. Bisoprolol is prescribed to patients with hypertension that is either mild or severe in severity. Treatment of illnesses such as heart failure, atrial fibrillation, and angina pectoris are examples of off-label usage of a drug.[1] The combination of these two drugs Cilnidipine and Bisoprolol is used in the treatment of Hypertension (high blood pressure). Cilnidipine and bisoprolol lower blood pressure effectively. Several analytical methods are available which can determine individually or in combination with another drug. From detailed review of literature, it was found that no analytical method is available for determination of this combination and its degradants from simulated mixture or formulation. So, for the same reason stability indicating RP — HPLC method was selected.[2–7]

2. Materials and Methods

CIL (99.98% pure) and BIS (99.96% pure) were obtained as gift sample for research purpose from, Cadila Healthcare Ltd., Sanand. Acetonitrile (HPLC grade), Orthophosphoric

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acid (LR grade) was purchase from S. D. fines.

2.1. Preparation of stock solution

1. BIS: Accurately weighed 5 milligram drug dissolved in 100 millilitre methyl alcohol (50 μg/millilitre). 1.0 millilitre from Stock Solution and make up to 10millilitre with mobile phase (5 μg/millilitre).
2. CIL: Accurately weighed 10 milligram drug dissolved in 100 millilitre methyl alcohol (100 μg/millilitre). 1.0 millilitre from Stock Solution and make up to 10millilitre with mobile phase (10 μg/millilitre).[8]

2.2. Selection of analytical wavelength

Working standards of BIS (10 μg/millilitre) and CIL (10 μg/millilitre) were scanned in UV 200 – 400 nanometer region and overlapped.[9–15]

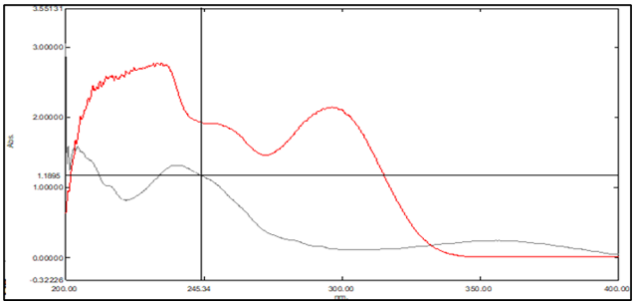

Fig. 1: Overlain UV Spectra of BIS (10 μg/mL) and CIL (50 μg/mL)

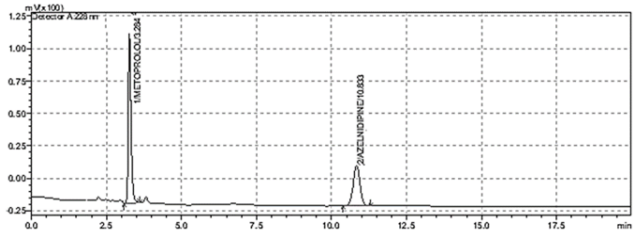

Fig. 2: Chromatogram of mixture of CIL and BIS using optimized chromatographic

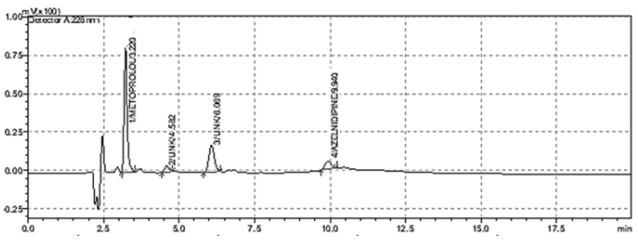

Fig. 3: Chromatogram of treated sample (Acid hydrolysis)

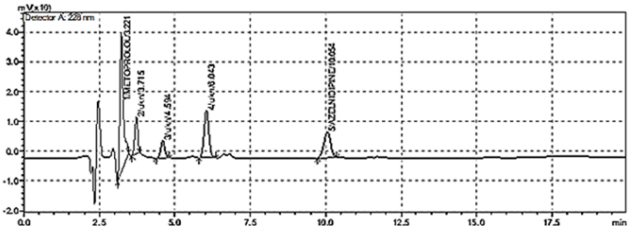

Fig. 4: Chromatogram of treated sample (Base hydrolysis)

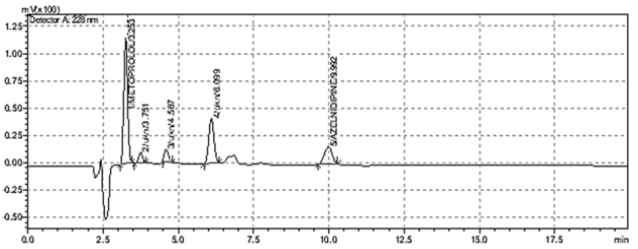

Fig. 5: Chromatogram of treated sample (Oxidative stress)

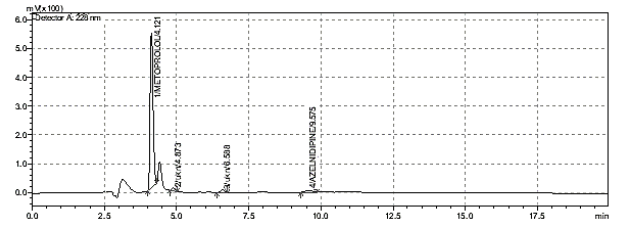

Fig. 6: Chromatogram of treated sample (Thermal stress)

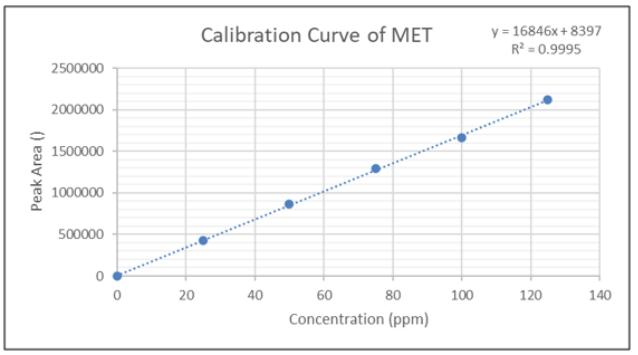

Fig. 7: Regression analysis of CIL (25 – 125 μg/mL)

3. Preparation of Solutions for Forced Degradation Studies

3.1. Acid induced hydrolysis

Accurately weighed amount corresponding to 10 mg of CIL and 5 mg of BIS were transferred to 10 ml volumetric flask and add 5 mL of 1 N HCl. Same solution was heated under reflux condition at 60°C for 1 hour on a hot

Table 1: Preparation of solution for accuracy studies

Concentration of stock solution	**25 μg/millilitre of CIL and 12.5 μg/millilitre of BIS**			
Volume taken from SS	-	2.0 millilitre	4.0 millilitre	8.0 millilitre
Amount of Placebo added	200 milligram	200 milligram	200 milligram	200 milligram
Volume made up with	10 millilitre	10 millilitre	10 millilitre	10 millilitre
Diluent	Mobile phase	Mobile phase	Mobile phase	Mobile phase
Final Concentration	-	5+2.5 μg/millilitre	10+5 μg/millilitre	20+10 μg/millilitre
Identification	Unspiked	50 % Spiked	100 % Spiked	150 % Spiked

Table 2: System suitability parameter of CIL and BIS

Parameter	**CIL (n=3)**			**BIS (n=3)**		
	Mean	± SD	RSD	Mean	± SD	RSD
Retention time (Rt)	3.04	0.01	1.52	15.12	0.01	0.31
Tailing Factor	1.31	0.02	1.36	1.19	0.02	0.67
Number of theoretical plates	12417	22.48	1.20	7583	32.42	1.69
Resolution (Rs)	Mean (9.84) SD (0.07) RSD (1.32)					

Table 3: Evaluation table of forced degradation studies

Stress Condition	**Area**	**CIL**	**BIS**	**% Degradation (CIL)**	**% Degradation (BIS)**
Acid Hydrolysis	Standard Area	488875	304876	13.77 %	10.41 %
	Observed Area	421543	273122		
Base Hydrolysis	Standard Area	488875	304876	14.00 %	12.87 %
	Observed Area	420431	265613		
Oxidative Stress	Standard Area	488875	304876	13.46 %	12.59 %
	Observed Area	423045	266478		
Thermal Degradation	Standard Area	488875	304876	12.59 %	10.97 %
	Observed Area	427324	271428		

Table 4: Linearity data of CIL

Sr. No.	**Conc. (μg/millilitre)**	**Mean**	**± SD (n=3)**	**RSD**
1	5	206751.4	7778.06	1.76
2	10	421172.6	6989.08	1.66
3	15	637635.2	3544.88	0.56
4	20	822667.2	7669.76	0.93
5	25	992189	4990.28	0.51

Table 5: Linearity data of BIS

Sr. No.	**Conc. (μg/millilitre)**	**Mean**	**± SD (n=3)**	**RSD**
1	2.5	196331.2	3558.13	1.81
2	5	307226.8	3120.45	1.02
3	7.5	438759.4	6720.61	1.53
4	10	561795.6	6669.63	1.19
5	12.5	660774.4	6020.37	0.91

Table 6: Repeatability data of CIL

Sr.No.	Concentration (μg/millilitre) (n=5)				
	5	**10**	**15**	**20**	**25**
1.	200248	428875	638666	822385	991345
2.	202345	417265	641256	834245	994212
3.	218688	422554	636386	820238	984177
4.	201921	425847	639725	823524	997512
5.	210555	411322	632143	812944	993699
Mean	206751.4	421172.6	637635.2	822667.2	992189
± SD	7778.06	6989.08	3544.88	7669.76	4990.28
RSD	1.76	1.66	0.56	0.93	0.50

Table 7: Repeatability data of BIS

Sr. No.	Concentration (μg/millilitre) (n=5)				
	2.5	**5**	**7.5**	**10**	**12.5**
1.	193633	304876	443844	561128	667288
2.	198289	304495	429834	567487	653798
3.	199899	306556	433731	569445	656845
4.	191541	312220	440899	553687	659176
5.	198294	307987	445489	557231	666765
Mean	196331.2	307226.8	438759.4	561795.6	660774.4
± SD	3558.13	3120.45	6720.61	6669.63	6020.37
RSD	1.81	1.02	1.53	1.19	0.91

Table 8: Intraday andinterday precision data of CIL

Concentration (μg/millilitre)	Intraday Mean	+ SD (n=3)	RSD	Inter-Day Mean	+ SD (n=3)	RSD
7.5	327058	3953.83	1.21	327186	4155.15	1.27
12.5	653869.66	6620.16	1.01	653918	6467.48	0.99
17.5	966840.33	10883.98	1.13	966737.66	10841.14	1.12

Table 9: Intraday andinterday precision data of BIS

Concentration (μg/millilitre)	Intraday Mean	+ SD (n=3)	RSD	Inter-Day Mean	+ SD (n=3)	RSD
2.5	196607.66	4369.61	1.22	197181.33	2999.54	1.52
7.5	434512.66	5527.43	1.27	434449.66	5498.80	1.27
12.5	661064.33	5393.07	0.82	660840	5089.32	0.77

Table 10: Accuracy data of CIL by HPLC method

Level of spiking	Amount of placebo (milligram)	Amount of drug added (μg/millilitre)	Amount of drug recovered (μg/millilitre)	% Recovery	% Mean Recovery ± SD (n=3)
		5	5.02	100.40	
50%	200	5	5.09	101.80	100.67 ± 1.03
		5	4.99	99.80	
		10	10.14	101.40	
100%	200	10	9.92	99.20	100.57 ± 1.19
		10	10.11	101.10	
		20	20.22	101.10	
150%	200	20	20.35	101.75	101.15 ± 0.58
		20	20.12	100.60	

Table 11: Accuracy data of BIS by HPLC method

Level of spiking	Amount of placebo (milligram)	Amount of drug added (μg/millilitre)	Amount of drug recovered (μg/millilitre)	% Recovery	% Mean Recovery ± SD (n=3)
		2.5	2.52	100.80	
50%	200	2.5	2.49	99.60	100.27 ± 0.61
		2.5	2.51	100.40	
		5	5.11	102.20	
100%	200	5	5.09	101.80	101.27 ± 1.29
		5	4.99	99.80	
		10	10.23	102.30	
150%	200	10	10.11	101.10	100.87 ± 1.56
		10	9.92	99.20	

Table 12: Assay of CIL and BIS by HPLC method

Drug	Amount taken (μg/millilitre)	Amount found (μg/millilitre) (Mean ± SD n=5)	% Assay (Mean ± SD n=5)
CIL	10	10.14 ± 0.05	101.4 ± 0.46
BIS	5	5.06 ± 0.04	101.2 ± 0.72

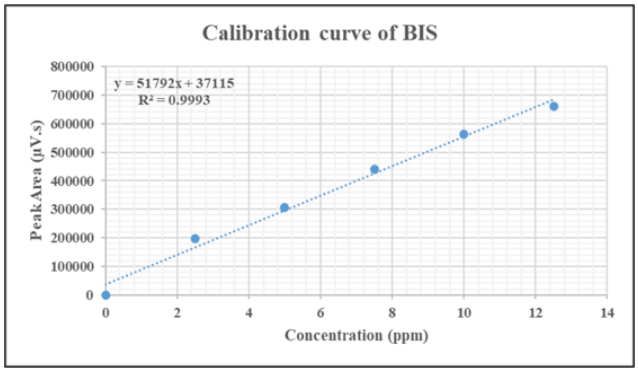

Fig. 8: Regression analysis of BIS (8 – 40 μg/mL)

plate. After the heating cool down the solution and were neutralized with 2 N NaOH and volume was raised to mark with diluent if necessary. 0.1 mL of previous solution was further diluted to 10 mL with diluent. The resulting solution have concentration of 10 μg/mL of CIL and 5 μg/mL of BIS (Treated sample). In similar way 0-hour sample (Only difference was heating condition was not provided) and blank (Only difference is there is no addition of API) were prepared. % Degradation of both components was calculated by comparing area of treated sample and control.[16]

3.2. *Base induced hydrolysis*

Same amount of API like in former case were transferred to 10 mL volumetric flask and volume of same was raised to the mark with 5 mL 1 N NaOH. Same solution was heated under reflux condition at 60°C for 1 hour on a hot plate. After the heating cool down the solution and were neutralized with 2 N HCl and volume was raised to mark with diluent if necessary. 0.1 ml of previous solution was further diluted to 10 mL with diluent. The resulting solution have concentration of 10 μg/mL of CIL and 5 μg/mL of BIS (Treated sample). In similar way 0-hour sample and blank sample were prepared. % Degradation of both components was calculated by comparing area of treated sample and control.[16]

3.3. *Hydrogen peroxide induced stress (Oxidative)*

Same amount of API like in former case were transferred to 10 mL volumetric flask and volume of same was raised to the mark with 5 mL 3% hydrogen peroxide. Same solution was heated under reflux condition at 60°C for 1 hour on a hot plate. After the heating cool down the solution and volume was raised to mark with diluent. 0.1 ml of previous solution was further diluted to 10 mL with diluent. The resulting solution have concentration of 10 μg/mL of CIL and 5 μg/mL of BIS (Treated sample). In similar way 0-hour sample and blank sample were prepared. % Degradation of both components was calculated by comparing area of treated sample and control.[16]

3.4. *Thermal stress*

Exact quantity of CIL and BIS like in previous cases were transferred to petri dish and exposed to 70 C° for 3 hours in hot air oven and residues were reconstituted with help of acetonitrile and transferred into 10 mL volumetric flask and volume of flask was raised with the mark with same solvent. 0.1 mL of resulting solution was further diluted to 10 ml with diluent. Above solution was chromatographed and % degradation was computed by comparing against standard concentration of CIL and BIS.[16]

4. Preparation of Solutions for Analytical Method Validation

4.1. Linearity and range

Preparation of CIL (25 to 400 μg/millilitre) and BIS (4 to 64 μg/millilitre). Master Stock Solution: 5 milligram CIL and 2.5 milligram BIS dissolved in 10 millilitre MeOH. Concentration of master stock solution (μg/millilitre) CIL+BIS (500+250) μg/millilitre. Volume of master stock solution (millilitre) 0.1 – 0.5. Final dilution in 10 millilitre volumetric flask. Volume make up was done with mobile phase. Concentration of final mixture (in μg/millilitre) 5+2.5 - 25+12.5. All above solutions were injected at volume of 20 μL into column by employing optimized chromatographic conditions.[17–20]

4.2. Intermediate precision (Repeatability)

Prepared standard mixtures having concentration of CIL (5 μg/millilitre to 25 μg/millilitre) and BIS (2.5 μg/millilitre to 12.5 μg/millilitre) were injected at volume of 20 μL into column by employing optimized chromatographic conditions. Each standard mixture was injected 5 time and peak area was monitored. Each concentration was monitored for repeatability by RSD.[17–20]

4.3. Accuracy

Accuracy of the analytical method has been performed by spiking of placebo with the standard. Placebo for the study was selected on the basis of reported formulation. And spiking of the placebo was performed at 50, 100 and 150 % of the target concentration. (Table 1)[17–20]

4.4. Assay

Sample Preparation: Composition of Synthetic Mixture: Composition of Placebo: HPMC (4 milligram), MCC (190 milligram), Magnesium stearate (4 milligram), Talc (2 milligram). Role of HPLC-Film forming agent, MCC-Directly compressible material, MS, gliding agent, Talk, lubricating agent CIL (10 milligram) and BIS (5 milligram) was taken into the volumetric flask (100 millilitre) and volume of the flask was raised to 100 milliliters with acetonitrile to give stock solution containing 100 μg/millilitre of CIL and 50 μg/millilitre of BIS. (Sonicate the solution for 10 minutes and filter the same from 0.45 Micro-meter Whatman filter paper.).

Test Solution: Withdraw 1.0 millilitre from above filtrate in 10 millilitre volumetric flask; make up the volume with mobile phase, which contain CIL+BIS = 10+5 μg/millilitre.[17–20]

5. Result and Discussion

5.1. Selection of analytical wavelength

Working standard of Metoprolol and Bisoprolol fumarate were scanned in UV range of 200 – 400 nm and overlapped Two iso-absorptive points were observed that is 240 nm and 254 nm (Figure 1). Therefore, 245 nm was selected as analytical wavelength for further trials. As well as both the compounds gives good intensity peak at 245 nm.

5.2. Optimized chromatographic condition

When method was operated using optimized chromatographic condition a well resolved peak of CIL and BIS was observed at 3.281 and 15.114 minutes respectively (Figure 2). All the system suitability parameters were within the guidelines.

5.3. Forced degradation studies

Optimized method was found to be stability indicating as it is able to separate all the degradation products in the presence of active ingredient. (Figures 3, 4, 5 and 6) No degradation product found to interfere with estimation of CIL and BIS in stressed samples. Even the stress given found to be optimum as % degradation observed was predictive in nature (below 15%). (Table 3)

6. Analytical Method Validation

6.1. Linearity and range

As per ICH guidelines, the value of r^2 should be greater than 0.995 and observed r^2 for given concentration range for CIL and BIS is 0.998 and 0.9993 respectively. Hence, we can say that developed method is linear over the range of 5 – 25 μg/mL and 2.5 – 12.5 μg/mL for CIL and BIS respectively show in Figures 7 and 8. Linearity data for both drugs is shown in Tables 4 and 5.

6.2. Repeatability

When all mixtures were analyzed at all concentration, calculated relative standard deviation at each level was found to be less than 2 so that method was found to be repeatable over the range of 5 – 25 μg/mL and 2.5 – 12.5 μg/mL for CIL and BIS respectively. Repeatability data are shown in Tables 6 and 7 for CIL and BIS respectively.

6.3. Method precision

For determining inter day and intraday precision, % RSD was monitored at selected concentration level which was found to be less than 2 so method was found to be precise for estimation of CIL and BIS. Data for intermediate precision are given in Table 8 and 9 for CIL and BIS respectively.

6.4. Accuracy study

Accuracy of the analytical method has been performed by spiking of placebo with the standard. Placebo for the study was selected on the basis of reported formulation. And spiking of the placebo was performed at 50, 100 and 150 % of the target concentration. (Tables 10 and 11).

6.5. Assay

When prepared synthetic mixture was analyzed by developed and validated method, % assay was found to be 101.4 for CIL and for 101.2 BIS (Table 12).

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8. Author Contribution

1. Pramod Kumar Goyal, Research Scholar. Maharishi Arvind University, Jaipur.
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4. Mohit Agarwal, Assistant Professor, NIMS Institute of Pharmacy, NIMS University, Jaipur.

9. Source of Funding

None.

10. Conflict of Interest

None.

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