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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND CILNIDIPINE IN COMBINED TABLET DOSAGE FORM

Pavan Kumar.V*, G. Lokeswara, Dr. V.Haribaskar, Dr. M.Gobinath

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh

***Corresponding author e-mail:** pavanvarikuti87@gmail.com

ABSTRACT

A simple, accurate and precise method was developed for the simultaneous estimation of Metoprolol and Cilnidipine in Tablet dosage form. Chromatogram was run through Altima (150 x 4.6 mm, 5µ). Mobile phase containing Buffer (0.1%OPA) and Methanol in the ratio of 45:55 v/v was pumped through column at rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Metoprolol and Cilnidipine was 225nm. Retention time of Metoprolol and Cilnidipine was found to be 2.249min and 3.062 min. %Assay was obtained as 101.22% and 100.45% for Metoprolol and Cilnidipine respectively. The accuracy and reliability of the method was assessed by evaluation of linearity, precision (intra-day and inter-day % RSD >2), accuracy and specificity for Metoprolol and Cilnidipine in accordance with ICH guidelines.

Key Words: Metoprolol Succinate, Cilnidipine, RP-HPLC

INTRODUCTION

Metoprolol: Metoprolol (MTS) chemically (RS)-1-(Isopropylamino)-3-[4-(2-methoxy ethylephenoxy] propane-2-ol marketed under the trade name Lopresor among others, is a selective β_1 receptor blocker medication. It is used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate. It is also used to prevent further heart problems after myocardial infarction and to prevent headaches in those with migraines[1].

Description: Metoprolol is a cardioselective β1-adrenergic blocking agent used for acute myocardial infarction (MI), heart failure, angina pectoris and mild to moderate hypertension. It may also be used for supraventricular and tachyarrhythmias and prophylaxis for migraine headaches. Metoprolol is structurally similar to bisoprolol, acebutolol and atenolol in that it has two substituents in the para position of the benzene ring. The β1-selectivity of these agents is thought to be due in part to the large substituents in the para position[1].

Mechanism of Action: Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart. Beta(1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Cilnidipine: Cilnidipine (CLN) chemically 3-(2-methoxyethyl)-5-[(E)-3-phenylprop-2-enyl]-2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3,5-dicarboxylate. Cilnidipine (INN) is a calcium channel blocker. Cilnidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function. Compared with other calcium antagonists, Cilnidipine can act on the N-type calcium-channel that existing sympathetic nerve end besides acting on L-type calcium-channel that similar to most of the calcium antagonists[2].

Description: Cilnidipine is a dual L-/N-type calcium channel protein inhibitor and blocker. Cilnidipine has displayed renal and vascular protective effects and improved baroreflex sensitivity in patients with hypertension. It has also demonstrated neuroprotective effects in a rat focal brain ischemia model by removing free radicals and activating the phosphatidylinositol 3-kinase pathway.

Mechanism of action: Cilnidipine is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium[2].

MATERIALS AND METHODS

Materials: Metoprolol and Cilnidipine, Combination of Metoprolol and Cilnidipine tablets(Cilamet XL 25), distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instruments: HPLC instrument used was of Waters HPLC 2965 System with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer of PG Instruments T60 with spectral bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Metoprolol and Cilnidipine solutions.

Methods:

Preparation of buffer:

Buffer: 1ml of ortho phosphoric acid was transferred to 1000ml volumetric flask and make up the volume with water to produce 0.1% OPA solution.

Standard Preparation: Accurately Weighed and transferred 25mg of Metoprolol and 10mg Cilnidipine of working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation: 10 tablets were weighed and powdered and take 444 mg (equivalent to 25 mg of Metoprolol and 10 mg of Cilnidipine) was transferred into a 50mL volumetric flask, 15mL of diluents was added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent.

HPLC Optimized Method:

The HPLC method was optimized and developed with a simultaneous method for Metoprolol and Cilnidipine. The mixed standard solution (25 mg of Metoprolol and 10 mg of Cilnidipine) injected in HPLC by the followed chromatographic conditions. The chromatographic separation was achieved on a Altima (150 x 4.6 mm, 5µ)**.** The mobile phase consisting of Ortho Phosphoric Acid (0.1%) and Methanol in the ratio of (45:55 v/v). The flow rate of the mobile phase was 1.0 ml/min. Detection was monitored at wavelength of 225nm. The column temperature was ambient and injection volume was 10µl.

Column Used : Altima, 150 x 4.6 mm, 5µ.
Buffer used : 0.1% OPA
Mobile phase : Buffer and Methanol(45:55v/v)
Flow rate : 1ml/min
Diluent : Water and Methanol (50:50)
Wavelength : 225
Temperature : 30^{o}C
Injection Volume : 10µl

Method validation:

The method validation was done according to the ICH guidelines. The following validation Characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Metoprolol and Cilnidipine are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm of Metoprolol and 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, 30ppm of Cilnidipine

Accuracy:

Standard Preparation: Accurately Weighed and transferred 25mg of metoprolol and 10mg of Cilnidipine working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Preparation of 50% Spiked Solution: 222mg of drug was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labelled as Accuracy 50% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution: 444 mg of drug was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labelled as Accuracy 100% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 150% Spiked Solution: 666mg of drug was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labelled as Accuracy 150% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD Obtained for Metoprolol and Cilnidipine were 0.82 and 0.74 and was found to be within the limits

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Metoprolol and Cilnidipine were 0.71 and 0.54 and was found to be within the limits

LOD

Limit of detection was performed as sample very low concentration of analytes under ICH guidelines calculated by std deviation method for Metoprolol and Cilnidipine

LOQ

Limit of Quantification was calculated by std deviation method in sample was performed by very low concentration under ICH guidelines for Metoprolol and Cilnidipine

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 101.22% and 100.25% for Metoprolol and Cilnidipine respectively.

RESULTS AND DISCUSSION

The simultaneous estimation of Metoprolol and Cilnidipine was done by RP-HPLC and in the optimized method Chromatogram was run through Altima (150 x 4.6 mm, 5μ). The mobile phase consists of 450 volumes of Ortho phosphoric acid buffer and 550 volumes of Methanol and the pH was adjusted to be 3.0. The detection is carried out using PDA detector at 225nm. The solutions are flowing at the constant flow rate of 1.0 ml/min. The retention time for Metoprolol and Cilnidipine was 2.249 and 3.062 minutes respectively. Linearity ranges for Metoprolol and Cilnidipine were 12.5-75μg/ml and 5-30μg/ml respectively and the results were found for in the acceptable range as R^2 = 0.999 and 0.999 for Metoprolol and Cilnidipine respectively. LOD were 0.08 and 0.03ppm and LOQ were 0.25 and 0.08 ppm for Metoprolol and Cilnidipine respectively. Intraday Precision was performed and % RSD Obtained for Metoprolol and Cilnidipine were 0.82 and 0.74.Inter day precision was performed and the %RSD Obtained for Metoprolol and Cilnidipine were 0.71 and 0.54. %Assay was obtained as 101.22% and 100.45% for Metoprolol and Cilnidipine respectively.

CONCLUSION

A simple, accurate and precise method was developed for the simultaneous estimation of the Metoprolol and Cilnidipine in Tablet dosage form. The developed method provides good resolution between Metoprolol Succinate and Cilnidipine. All the parameters for these drugs met the criteria of ICH guidelines for method validation. The developed method is recommended for routine and QC analysis of the investigated drugs to provide simple and accurate quantitative analysis for the determination of Metoprolol and Cilnidipine.

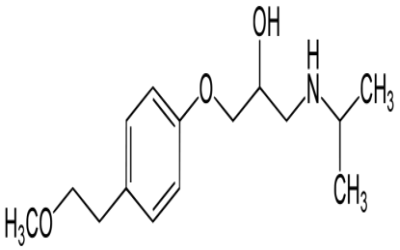

Fig.1: Chemical structure of Metoprolol

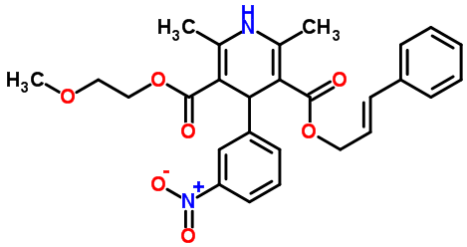

Fig.2: Chemical structure of Cilnidipine

Table 1 : System suitability studies of Metoprolol and Cilnidipine

Property	Metoprolol	Cilnidipine
Retention time (Rt)	2.249 min	3.062min
Theoretical plates (N)	2970± 63.48	6913 ± 63.48
Tailing factor (T)	1.74 ± 0.117	1.14± 0.117

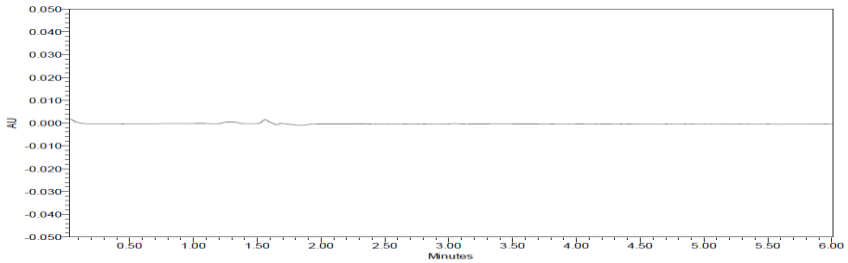

Fig.3: Chromatogram of blank

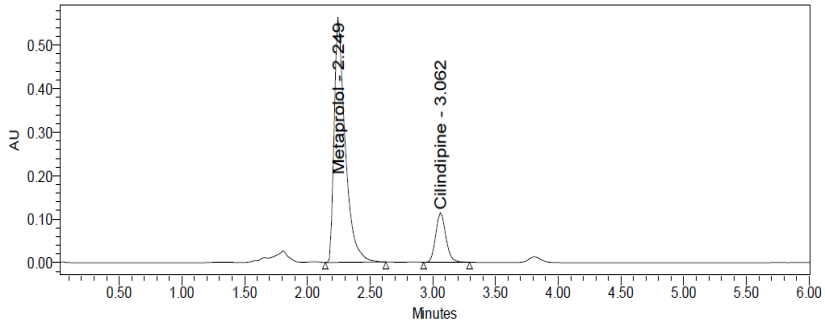

Fig.4: Optimized chromatogram of Metoprolol and Cilnidipine

Table 2 : Calibration data of Metoprolol and Cilnidipine method

S.no	Concentration Metoprolol (µg/ml)	Response	Concentration Cilnidipine (µg/ml)	Response
1	0	0	0	0
2	12.5	750975	5	155306
3	25	1660812	10	325225
4	37.5	2446103	15	468122
5	50	3260575	20	634394
6	62.5	4060241	25	806300
7	75	4810389	30	936233

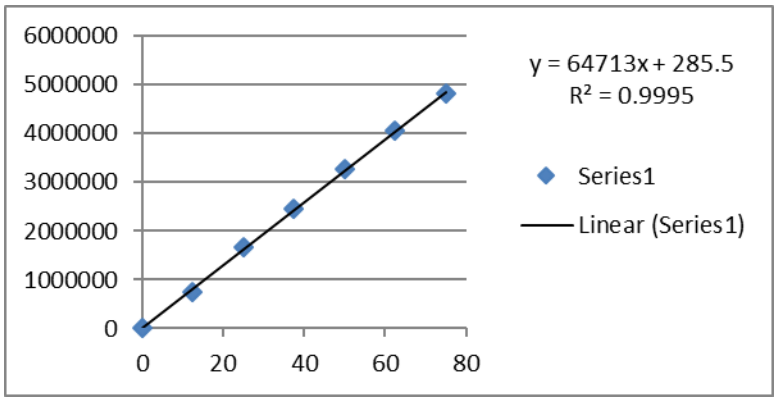

Fig.5 : Calibration curve of Metoprolol

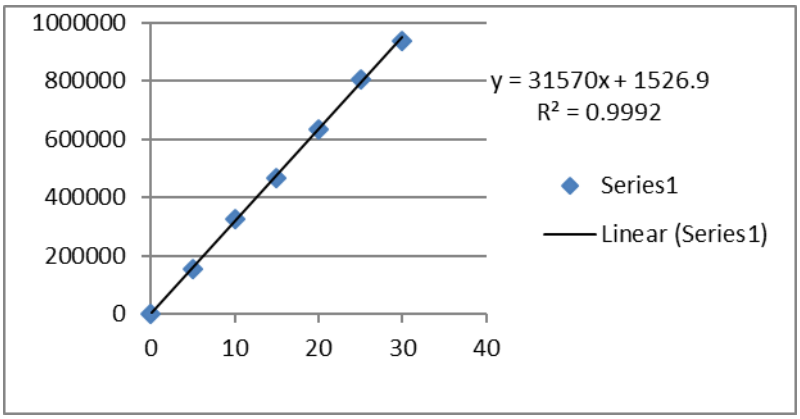

Fig.6: Calibration curve of Cilnidipine

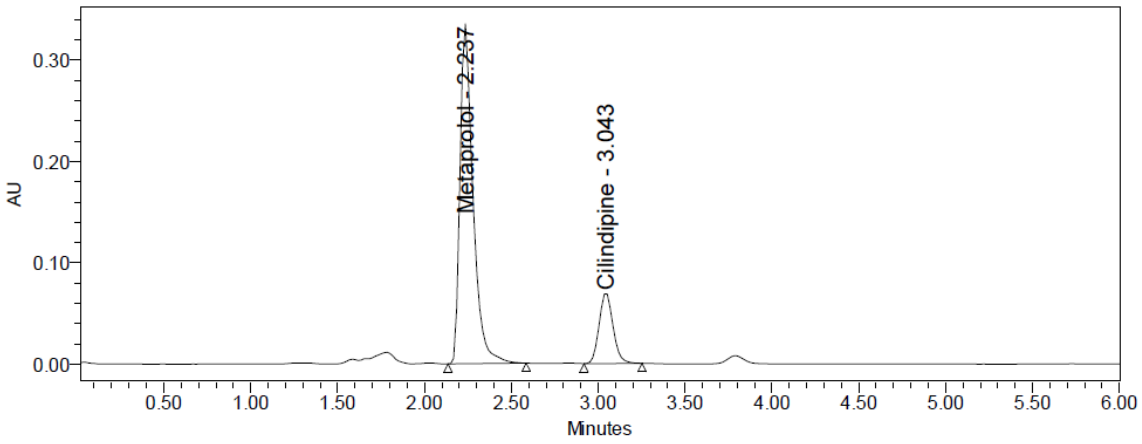

Fig.7: Accuracy 50% Chromatogram of Metoprolol and Cilnidipine

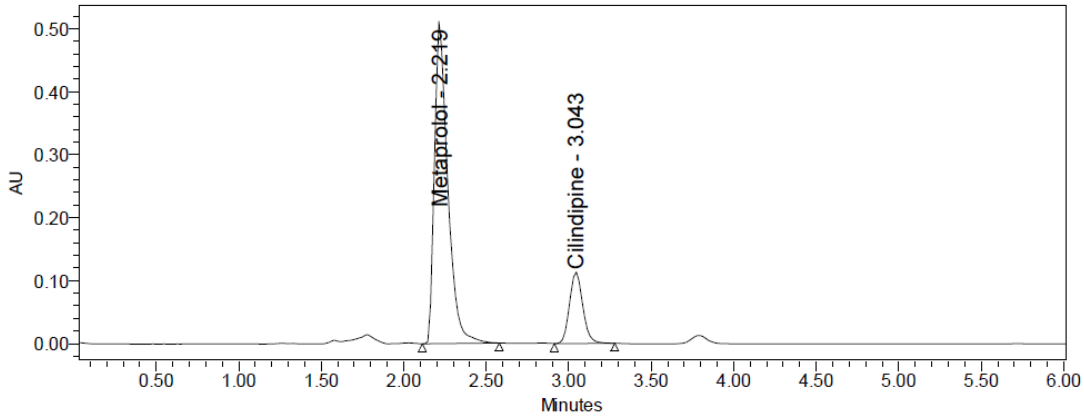

Fig.8: Accuracy 100% Chromatogram of Metoprolol and Cilnidipine

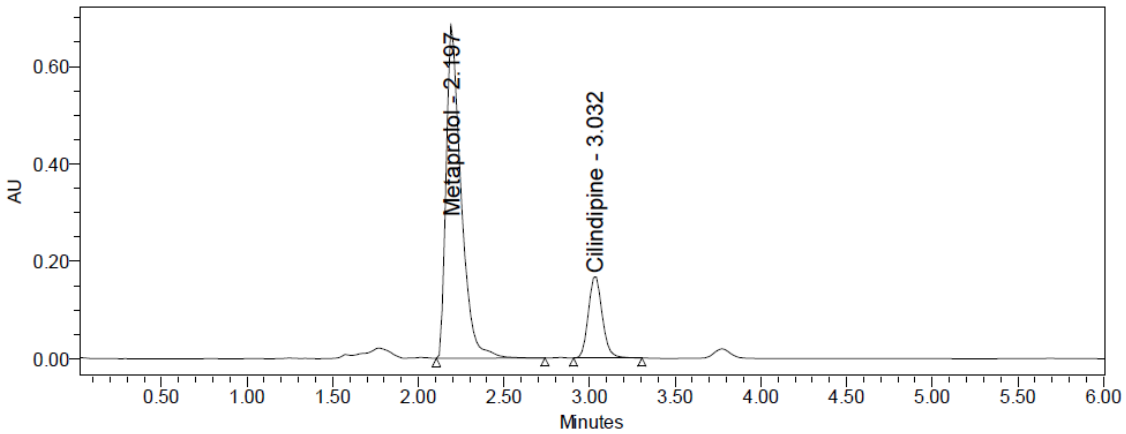

Fig.9: Accuracy 150% Chromatogram of Metoprolol and Cilnidipine

Table 3: Accuracy Table

Sample	Concentration (%) (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Metoprolol	25	100.24	100.50	1.22
	50	100.65	100.72	1.09
	75	98.76	100.96	0.16
Cilnidipine	10	101.37	100.50	1.22
	20	101.07	100.72	1.09
	30	101.96	100.96	0.16

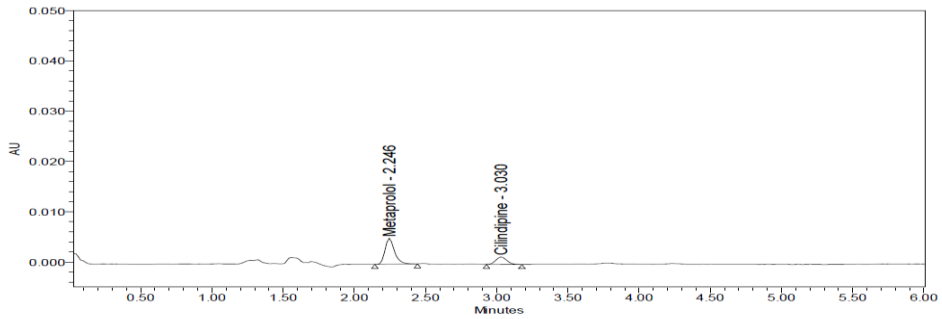

Fig 11: LOD Chromatogram of Metoprolol and Cilnidipine

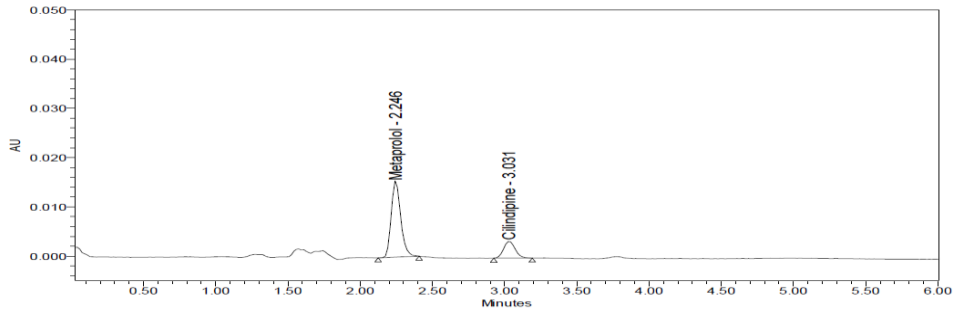

Fig 11: LOQ Chromatogram of Metoprolol and Cilnidipine

Table 4: Assay of Tablet

S. No.	Metoprolol %Assay	Cilnidipine %Assay
1	101.1538	101
2	100.3598	100.071
3	102.2635	100.906
4	100.8724	99.887
5	101.8773	99.8968
6	100.7952	100.914
AVG	101.22	100.45
STDEV	0.72	0.55
%RSD	0.71	0.54

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