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Original Article

DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND METOPROLOL SUCCINATE IN BULK AND COMBINED DOSAGE FORM

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ABSTRACT

Introduction: Analysis of pharmaceutical product is very important as it concerned with quality of life. Cilnidipine is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. It has greater selectivity for vascular smooth muscle.When Metoprolol competes with adrenergic neurotransmitters such as catecholamine 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. This Combine dosage form is used in treatment of hypertension, angina pectoris, and respiratory tract infection.

Objective: The objective is to develop a Simple, Precise, Accurate and Rapid UV Spectrophotometric method for simultaneous estimation of Cilnidipine and Metoprolol succinate. to develop Simple, Precise, Rapid, and accurate RP-HPLCmethod for simultaneous estimation of Cilnidipine and Metoprolol succinate. To perform complete validationof newly developed analytical methods as per ICH Guideline.

Methods: In UV-Spectrophotometric method, estimation of Cilnidipine and Metoprolol succinate was carried out at 240 nm and 224 nm by Q-Absorbance ratio method. Absorbance uses the ratio of absorbance at two selected wavelengths, one which is an Iso-absorptive point and other being the λmax of one of the two components. From the overlay spectra of two drugs, it is evident that CIL and METO show an Iso-absorptive point at 231 nm. The second wavelength used is 224 nm, which is λmax of METO. In RP-HPLC method for Cilnidipine and Metoprolol succinate, chromatographic separation was carried out on Shimandzu Phenomenex-luna C18 (250 x 4.6mm, 5 µ) (Spincotech Pvt. Ltd.)in size using mobile phase Acetonitrile: Water (90:10 v/v)and detected at 231 nm.

Results: For UV Spectophometric method Linearity of Cilnidipine and Metoprolol succinate were found to be 2 – 10 µg/ml and 10 - 50 µg/ml and for RP-HPLC method Linearity were found to be 1 - 11 and 5 - 55 µg/ml respectively for both the drugs. For this two developed and validated methods the %RSD for precision was found to be less than 2% and the % recovery was found to between 98-102 %.

Conclusion: All developed and validated methods were found to be simple, accurate, economical, robust and reproducible. There was no interference of any degradants and excipient in the determination of drugs from formulation so all the methods can be successfully applied for routine QC analysis.

Keywords: Cilnidipine, Metoprolol Succinate,Q-Absorbance Ratio, Simultaneous Estimation method Validation.

INTRODUCTION

Cilnidipine 3-O-(2-methoxyethyl) 5-O-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3 nitro- phenyl)-1,4-dihydropyridine-3, 5-dicarboxylate (fig.1) Cilnidipine is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. It has greater selectivity for vascular smooth muscle [1]. Metoprolol succinate (RS)-1-(Isopropyl amino)-3-[4-(2 methoxyethyl) phenoxy] propan-2-ol (fig.2). Metoprolol competes with adrenergic neurotransmitters such as catecholamine 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 [1].

Cilnidipine is not official in IP, BP and USP. Metoprolol succinate is official in USP [2]. From Literature survey, various methods (UV [3-7], HPLC[8-12],HPTLC [13-14]) were reported for the analysis of individual drug and in combination with other drug but no method were reported for simultaneous estimation of Cilnidipine and Metoprolol succinate. Hence, the purpose of the present work was to develop and validate Q-absorbance ratio spectrophotometric method for simultaneous estimation of Cilnidipine and Metoprolol succinate in combined dosage form.

MATERIAL AND METHODS

Instruments

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents

The bulk drug Cilnidipine was obtain from N Cube pharmaceutical Pvt.Ltd. Bavla, Ahmedabad and Metoprolol succinate was obtain from Intas pharmaceutical Ltd. Ahmedabad Fixed dose of Combined dosage form of Cilnidipine 10 mg and Metoprolol succinate 50 mg, Cilacar M Tablet Procured From Market (Mfg. By Akuma Drugs &Pharmaceutical Ltd.,Ranipur,Haridwar).Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

Selection of a Solvent

Methanol was selected as solvent for studying spectral characteristic of drugs.

Preparation of Standard Solution

(A) Preparation of Standard Solution of Cilnidipine

Preparation of Standard Stock Solution of Cilnidipine (100µg/ml)

Accurately weighed quantity of CIL 10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol and diluted up to mark with Methanol to give a stock solution having strength of 100µg/ml.

Preparation of Working Standard Solution of Cilnidipine

From the above stock solution pipette out 0.2 mL, 0.4mL, 0.6mL, 0.8mL, and 1mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with methanol to Produce concentration 2,4, 6, 8 and10 µg/mL respectively.

(B) Preparation of Standard Solution of Metoprolol succinate

Preparation of Standard Stock Solution of Metoprolol succinate (100µg/ml)

Accurately weighed quantity of METO10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol and diluted up to mark with Methanol to give a stock solution having strength of 100µg/ml.

Preparation of Working Standard Solution of Metoprolol succinate

From the above stock solution pipette out 1 mL, 2 mL, 3 mL, 4 mL, and 5mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with methanol to Produce concentration 10,20, 30, 40 and 50 µg/mL respectively

Selection of Analytical Wavelength

To determine wavelength for measurement, standard spectra of CIL and METO were scanned between 200-400 nm against Methanol.

Absorbance maxima were obtained at 240 nm and at 224 nm for CIL and METO respectively and Iso-absorptive point were obtained at 231 nm.

Preparation of Calibration Curve

(A)Calibration Curve for Cilnidipine

Calibration curve for CIL consists of different concentrations of standard CIL solution ranging from 2 - 10 µg/ml. The solutions were prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard solution of CIL (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol.

The absorbance of the solutions was measured at 224 nm and 231 nm against Methanol as a blank.

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

(B) Calibration Curve for Metoprolol succinate

Calibration curve for METO consists of different concentrations of standard METO solution ranging from 10 - 50 µg/ml. The solutions were prepared by pipetting out 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the working standard solution of METO (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol.

The absorbance of the solutions was measured at 224 nm and 231 nm against Methanol as a blank.

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

Preparation of Sample solution

Twenty tablets were weighed and crushed to powder. The quantity of the powder equivalent to 50 mg of Metoprolol succinate and 10 mg of Cilnidipine was transferred to a 100 ml volumetric flask. The content was mixed with Methanol (70 ml) and sonicated for 20 min to dissolve the drug as completely as possible.

The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to mark with Methanol Metoprolol succinate (500 µg/ml) & Cilnidipine (100 µg/ml)].

An aliquot of this solution (1 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol to make final concentration of Metoprolol succinate (50 µg/ml) and Cilnidipine (10 µg/ml)

Validation

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-10 µg/ml and 10-50 µg/ml for CIL and METO respectively (n = 5).

The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for CIL and METO were calculated.

Precision

(A)Repeatability

Aliquots of 0.6ml of working standard solution of CIL (100 µg/ml) were transferred to a 10 ml volumetric flask. Aliquots of 3.0ml of working standard solution of METO (100 µg/ml) were respectively transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 6µg/ml solution of CIL and 30µg/ml solution of METO.

The absorbance of solution was measured spectrophotometry six times and % RSD was calculated.

(B) Intraday precision

Aliquots of 0.4, 0.6, and 0.8 ml of working standard solution of CIL (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 2.0, 3.0 and 4.0 ml of working standard solution of METO (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 4,6 and 8µg/ml solution of CIL and 20, 30 and 40µg/ml solution of METO.

Solution was analyzed 3 times on the same day spectrophotometry and % RSD was calculated.

(C) Interday Precision

Aliquots of 0.4, 0.6, and 0.8 ml of working standard solution of CIL (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 2.0, 3.0 and 4.0 ml of working standard solution of METO (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 4,6 and 8µg/ml solution of CIL and 20, 30 and 40µg/ml solution of METO.

Solution was analyzed 3 times on the 3 different days spectrophotometry and % RSD was calculated.

Limit of Detection (LOD)

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

$LOD = 3.3 * SD/Slope$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

Limit of Quantification (LOQ)

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

$LOD = 10 * SD/Slope$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

Accuracy

The accuracy of the method was determined by calculating recovery of CIL and METO by the standard addition method.

Aliquots of 0.32, 0.4, and 0.48 ml of working standard solution of CIL (100 µg/ml) were added at 80, 100 and 120 % level to pre-analyzed 0.4 ml sample solutions of CIL and METO (100 µg/ mL of CIL and METO) transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 7.2, 8 and 8.8µg/ml solution of CIL.

Aliquots of 1.6, 2.0, and 2.4 ml of working standard solution of METO (100 µg/ml) were added at 80, 100 and 120 % level to pre-analyzed 2 ml sample solutions of CIL and METO (100 µg/ mL of CIL and METO) transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 36, 40 and 44µg/ml solution of METO.

Absorbance of solution was measured at selected wavelengths for CIL and METO.

The amount of CIL and METO was calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation.

Accuracy was assessed using three concentrations and three replicates of each.

Q-Absorbance Ratio Method

• Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an Iso-absorptive point and other being the λmax of one of the two components.

• From the overlay spectra of two drugs, it is evident that CIL and METO show an Iso-absorptive point at 231 nm. The second wavelength used is 224 nm, which is λmax of METO (fig. 6.2.1)

• Five working standard solutions having concentration 2, 4, 6, 8 and 10 µg/mL for CIL and 10, 20, 30, 40 and 50 µg/mL for METO were prepared in methanol and the absorbance at 231 nm (Iso-absorptive point) and 224 nm (λmax of METO) were measured and absorptivity coefficients were calculated.

• The absorbance of the sample solution (10 µg/ml of CIL and METO) i.e. A_1 and A_2 were recorded at 231 nm (Iso-absorptive point) and 224 nm (λmax of METO) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1

• Relative concentration of two drugs in the sample was calculated using following equations.

$$C_X = [(Q_M - Q_Y)/ (Q_X - Q_Y)] \times A_1/a_{X1} \ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

$$C_Y = [(Q_M - Q_X)/ (Q_Y - Q_X)] \times A_1/a_{Y1} \ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

The Q-values and absorptivitie for both drugs were calculated as follows,

Q_M = Absorbance of Sample solution at 224 nm (A_2)/ Absorbance of Sample solution at 231 nm (A_1)

Q_X = Absorptivity of CIL at 224 nm (ax_2)/ Absorptivity of CIL at 231nm (ax_1)

Q_Y = Absorptivity of METO at 224 nm (ay_2)/ Absorptivity of METO at 231 nm (ay_1)

Where, A_1 and A_2 are absorbance of mixture at 231 nm and 224 nm;

QX And QY are Q value of CIL and METO respectively;

a_{X1} and a_{Y1} are absorptivitie of CIL and METO at 231 nm;

a_{X2} and a_{Y2} are absorptivitie of CIL and METO at 224 nm.

• The analysis procedure was repeated 3 times with sample solution.

RESULTS AND DISCUSSION

A reliable Q absorption ratio method was developed for simultaneous estimation Cilnidipine and Metoprolol succinate in combined pharmaceutical formulation by UV Spectrophotometry. Beers law was obeyed in concentration range of 2-10 µg/ml Cilnidipine and 10-50µg/ml for Metoprolol succinate at 240 nm and 224 nm wavelengths, respectively. The correlation coefficient Cilnidipine and Metoprolol succinate was found to be R^2= 0.999 and 0.998. The mean % recoveries were found to be in the range of 101.13-101.52% and 98.35-101.27% for Cilnidipine and Metoprolol succinate respectively. The LOD and LOQ were 0.040µg/ml and 0.121µg/ml of Cilnidipine 0.23µg/ml and 0.72µg/ml of Metoprolol succinate, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analyte, which can be applied for the analysis ofCilnidipine and Metoprolol succinate in combined pharmaceutical formulation.

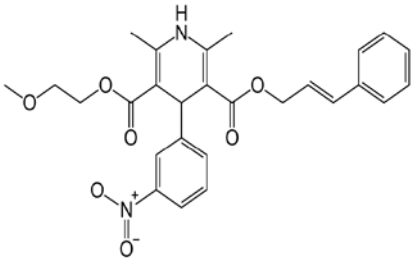

Fig. 1: structure of cilnidipine

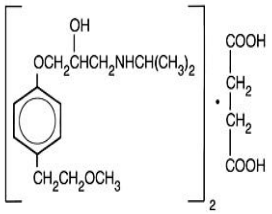

Fig. 2: structure of metoprolol succinate

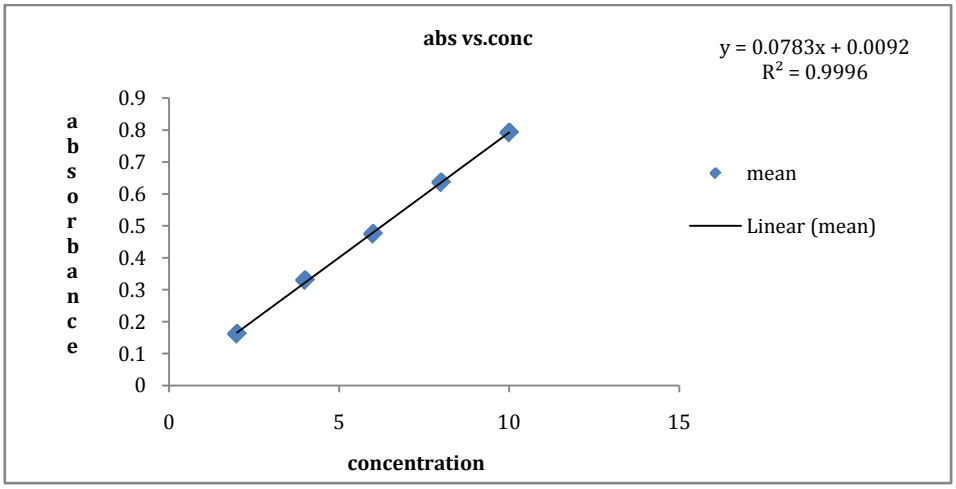

Fig. 3: Calibration curve for CIL at 231 nm (Iso-absorptive Point)

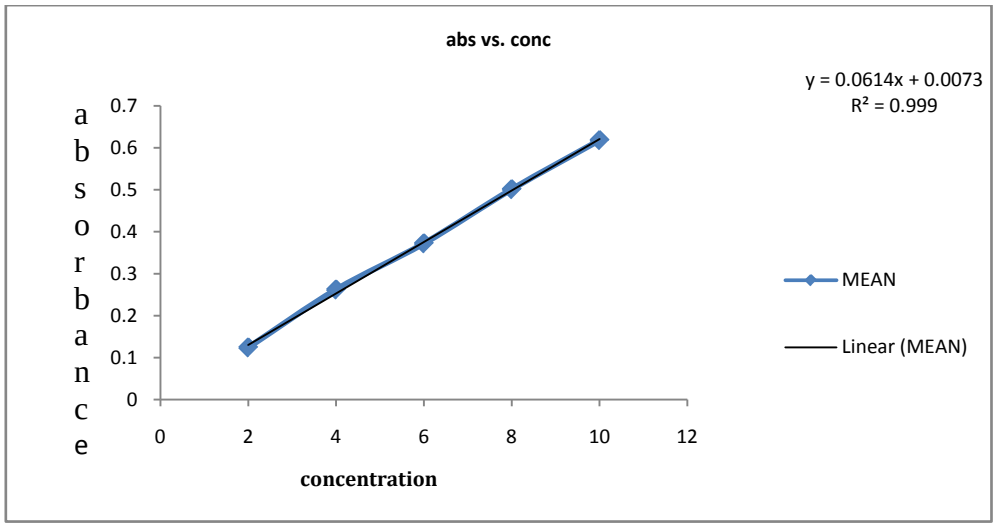

Fig. 4: Calibration curve for CIL at 224 nm (λmax of METO)

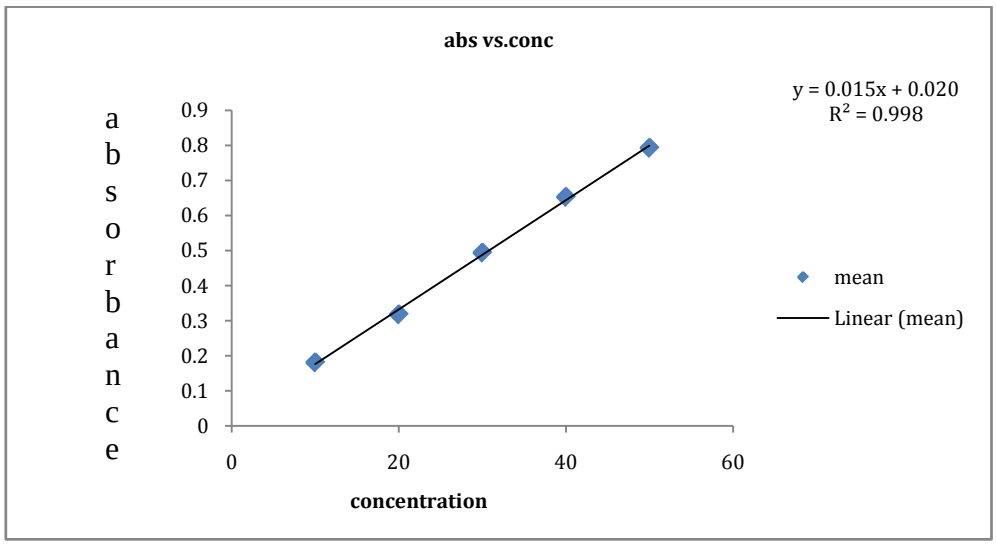

Fig.5: Calibration curve for METO at 231 nm (Iso-absorptive Point)

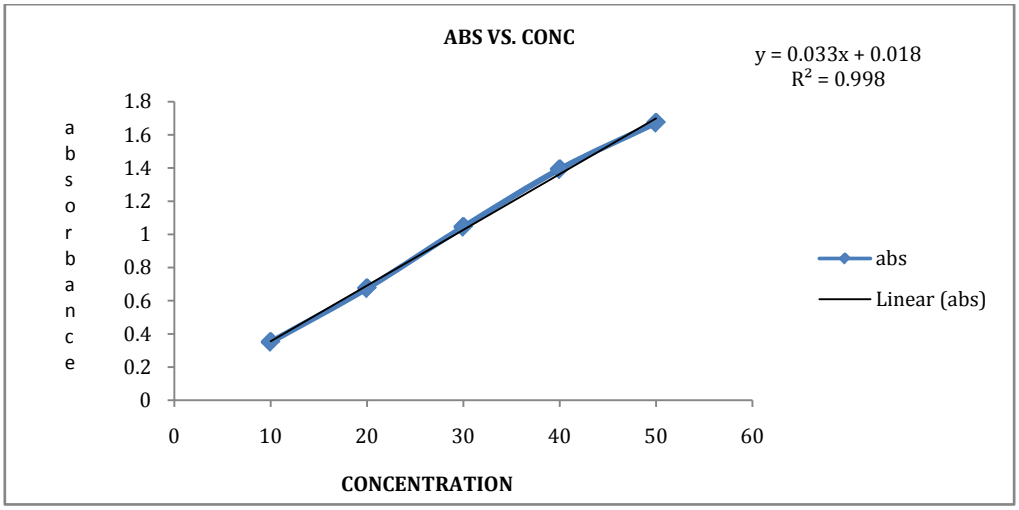

Fig.6: Calibration curve for METO at 224 nm (λmax of METO)

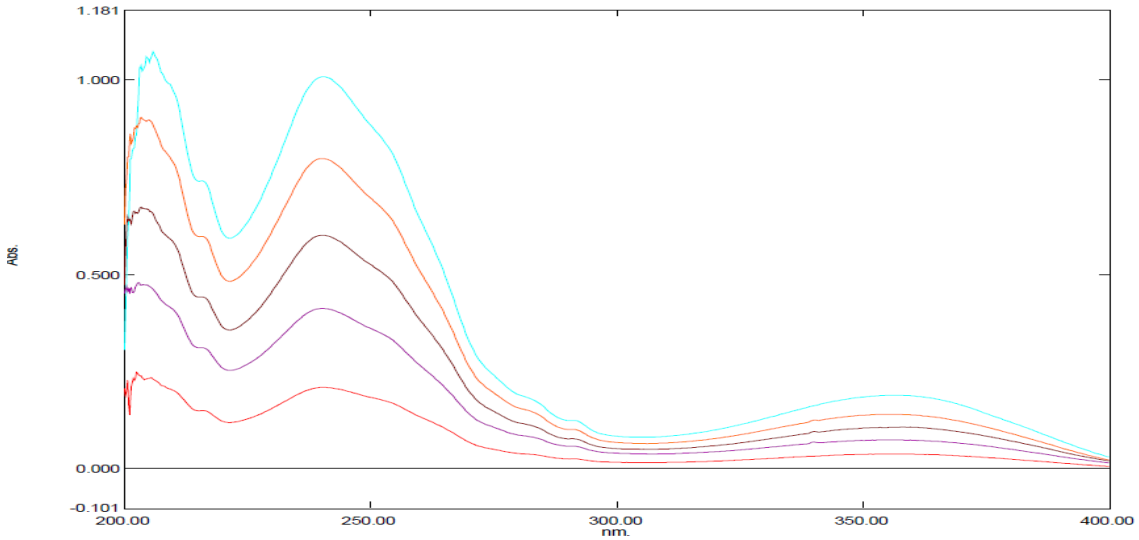

Fig. 7: Overlay spectra of CIL (2-10 µg/ mL)

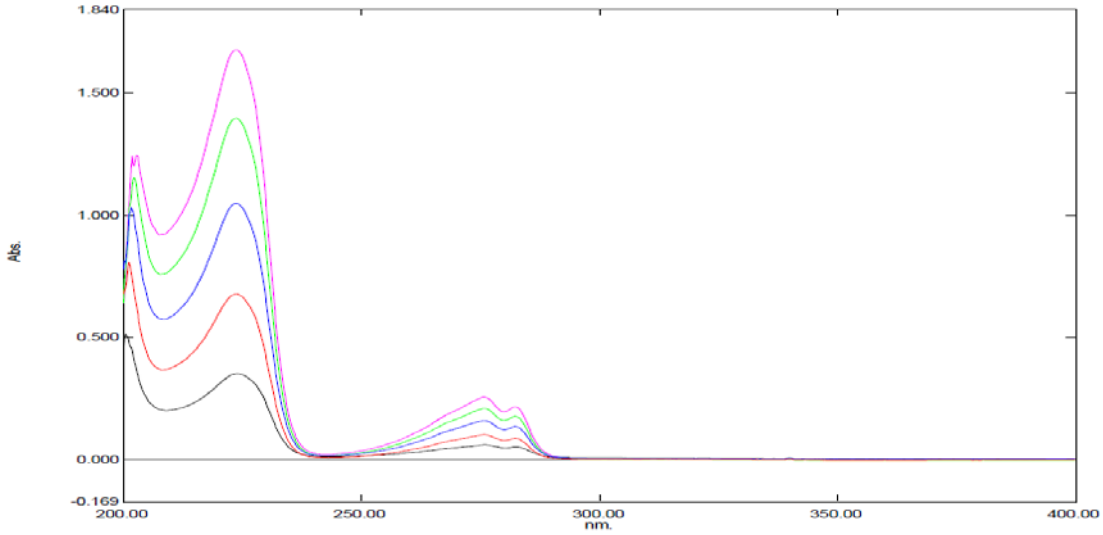

Fig. 8: Overlay spectra of METO (10-50 µg/ mL)

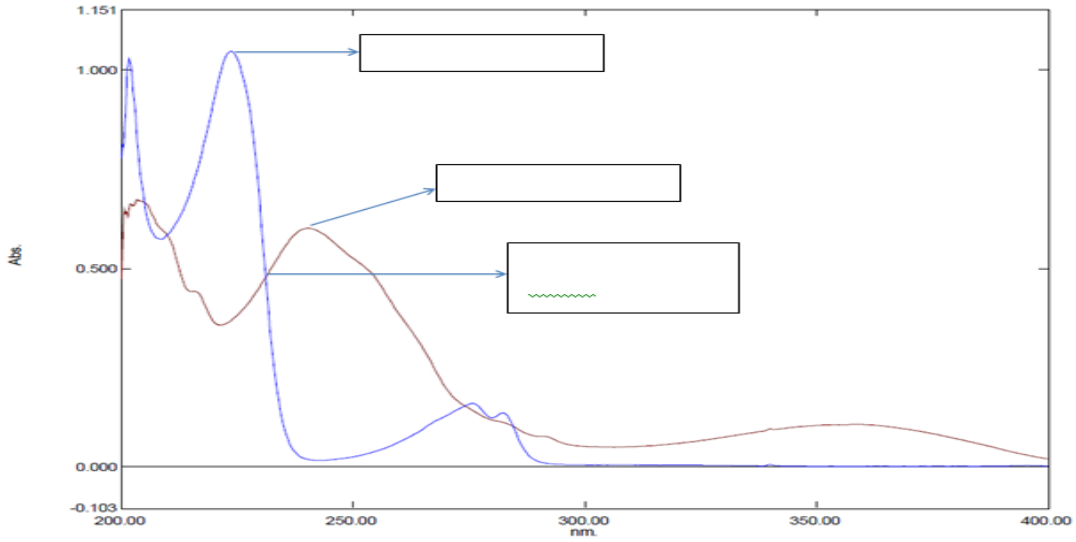

Fig. 9: Overlain Absorption Spectra of CIL and METOShowingIso-absorptive Point (231 nm) in Methanol.

Table 1: Recovery study (Accuracy) for Cilnidipine in tablet formulation

Drug	Level	Amount of sample taken (µg/ml)	Amount of Std. spiked (µg/ml)	Total Amt. of Drug found	Amt. of Std. Recover Mean (n=3)	% Recovery	SD* (n=3)	%RSD
	80		3.2	7.2	7.31	101.52	0.0033	0.42
CILN	100	4	4	8	8.1	101.25	0.0102	1.22
	120		4.8	8.8	8.9	101.13	0.0089	0.97

Table 2: Recovery study (Accuracy) for Metoprolol succinate in tablet formulation

Drug	Level	Amount of sample taken (µg/ml)	Amount of Std. spiked (µg/ml)	Total Amt. of Drug found	Amt. of Std. Recover Mean (n=3)	% Recovery	SD* (n=3)	%RSD
	80		16	36	35.40	98.35	0.0002	0.45
MET	100	20	20	40	40.20	100.51	0.0012	0.17
	120		24	44	44.56	101.27	0.0017	0.22

Table 3: Analysis of Pharmaceutical Dosage form

Tablet Formulation	Label claim (mg)		Amount found (mg)		% Assay ± S.D (n=3)	
Cilacar M	**CIL**	**METO**	**CIL**	**METO**	**CIL**	**METO**
	10	50	9.85	51.22	98.5% ± 0.8165	102.45% ± 0.0249

Table 4: Optical regression characteristics and Validation parameters

Parameters	Cilnidipine		Metoprolol succinate	
Wavelength range (nm)	231	224	231	224
Beer's Law Limit (µg/ml)	2-10	2-10	10-50	10-50
Regression equation (y = mx+c)	Y= 0.0783X + 0.0092	Y= 0.0614X + 0.0073	Y= 0.0156X + 0.0201	Y= 0.0336X + 0.0188
Standard deviation of the Y- intercepts of the 5 calibration curves	0.00095		0.000915	
Mean slope of the 5 calibration curves	0.0783		0.01272	
Correlation coefficient (r2)	0.999	0.999	0.998	0.998
Method Precision (Repeatability) (%RSD, n=6)	0.25	0.32	0.24	0.09
Intraday Precision (%RSD, n=3)	0.12-0.78	0.33-0.65	0.24-0.41	0.15-0.24
Interday Precision (%RSD, n=3)	0.74-1.59	0.64-1.59	0.65-1.42	0.35-1.59
LOD (µg/ml)	0.040		0.23	
LOQ (µg/ml)	0.121		0.72	
%ASSAY	98.5		102.45	

CONCLUSION

The proposed Spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of CIL and METO in combined dosage form. The method utilizes easily available and cheap solvent for analysis of CIL and METO hence, the method is economic for estimation of CIL and METO in combined dosage form. The common excipients and additives are usually present in the combined dosage form do not interfere in the analysis of CIL and METO in method, Hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture or combined pharmaceutical formulation.

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