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Pharmaceutical Sciences **Research Article……!!!**

‘‘DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTROPSCOPIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND METOPROLOL SUCCINATE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS”

Manzoor Ahmed, Shriya V A*, Vijayakrishna C Aradhya, Narayana Murthy G.

Department of pharmaceutical Analysis, National College of Pharmacy, Balraj Urs Road, Shimoga-577201, Karnataka, India

KEYWORDS:

Cilnidipine, Metoprolol Succinate , First Order Derivative Method and Second Order Derivative Method.

FOR CORRESPONDENCE:

Shriya V A*

ADDRESS:

Department of Pharmaceutical Analysis, National College of Pharmacy, Balraj Urs Road, Shimoga-57720, Karnataka, India.

ABSTRACT

In the present work two simple, accurate and economical spectroscopic methods have been developed for the simultaneous estimation of Cilnidipine and Metoprolol Succinate in bulk drug and pharmaceutical dosage form by using methanol as a solvent. Method A is First Order Derivative Method which is based on the measurement of absorbances at 231nm and 219nm for the estimation of Cilnidipine and Metoprolol Succinate. Linearity range was found to be 2-10µg/ml and 5-25µg/ml with (r^2 =0.9998, %RSD =1.5413-0.4999 and r^2= 0.9999, %RSD=0.5319-0.2778) for Cilnidipine and Metoprolol Succinate respectively. LOD of both the drugs were 0.132891µg/ml and 0.10576µg/ml and LOQ were found to be 0.40270µg/ml and 0.32049µ/ml for Cilnidipine and Metoprolol Succinate respectively. Second Order Derivative Spectroscopy method is based on the measurement of absorbance at two selected wavelengths 240nm and 223nm for the estimation of Cilnidipine and Metoprolol Succinate. Linearity range was found to be 2-10µg/ml and 5-25µg/ml with (r^2= 0.9993 , % RSD= 0.8167-0.3412 , r^2=0.9999 , %RSD=0.2526-0.7984) for Cilnidipine and Metoprolol Succinate respectively. LOD of both drugs was 0.07277µg/ml and 0.04488µg/ml respectively. LOQ of 0.2205µg/ml and 0.136µg/ml for Cilnidipine and Metoprolol Succinate respectively.

INTRODUCTION:

Cilnidipine is a dihydropyridine calcium antagonist. Chemically it is 3-(E)-3-Phenyl-2-Propenyl 5-2-methoxyethyl 2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate Compared to with other calcium antagonist , Cilnidipine can act on N type calcium channel that existing sympathetic nerve end and besides act on L type calcium channel that is similar to most of the calcium antagonist . Cilnidipine is unique Ca^{2+} channel blocker as it has inhibitory action on sympathetic N type Ca^{2+} channels along with its effect on L type Ca^{2+} channel. Cilnidipine decreases the blood pressure safely and effectively without excessive blood pressure reduction /Tachycardia. Cilnidipine acts on L type of calcium channel of blood vessels by blocking the incoming calcium and suppressing contraction of blood vessesls, therby reducing blood pressure[1-2].

Metoprolol belongs to class of drugs known as beta blockers. Chemically it is bis-(1-[4-(2-methoxyethyl)phenoxy]-3-[(propan-2-yl)amino]propan-2-ol), Butanoic acid. It works by blocking the action of certain natural chemicals in the body such as epinephrine, on heart and blood vessels. This effect lowers the heart rate, blood pressure and strain on the heart. Metoprolol is selective beta blocker commonly employed as succinate or tartarate derivative depending if formulation is designed extended release or immediate release. Metoprolol is indicated for the treatment of angina, heart failure, myocardial infarction, atrial fibrillation, hypertension[3].

The combination of Cilnidipine and Metoprolol Succinate is used in treatment of Hypertension and lowers blood pressure effectively[4].

On literature survey it was found that cilnidipine[5-7] has been estimated individually and in combination with other drugs. Metoprolol Succinate[8-10] also estimated individually with other drugs. It was found that only one method has been reported for the simultaneous estimation of Cilnidipine and Metoprolol Succinate in their combined dosage form. Hence in the view of the need for a suitable method for routine analysis in combined formulations, attempts were made to develop simple, precise and accurate spectroscopic estimation for the titled drugs and extend for their determination in pharmaceutical formulations. The present UV spectroscopic methods were validated according to ICH guidelines.

MATERIALS AND METHODS:

INSTRUMENT

For UV-Visible spectroscopy methods , Shimadzu model 1800 double beam UV-Visible spectrophotometer with a special band width of 1±0.2nm, wavelength accuracy of ±0.3nm and a pair of

cuvettes having 1cm path length was used. volumetric flasks used for the preparation of standard solutions and sample solutions were calibrated before use.

CHEMICALS AND REAGENTS

Methanol.

DRUG SAMPLE

Standard Cilnidipine was obtained as gift samples from J.B Chemicals and Pharmaceuticals .Ltd , Mumbai. Standard Metoprolol Succinate was procured from Brawn Labouratories Ltd, Faridabad , Haryana.

Preparation of Standard Solutions:

100mg of Standard Cilnidipine and 100mg of Standard Metoprolol Succinate were weighed separately and transferred into two different volumetric flasks. Both the drugs were dissolved in 50ml of methanol by sonication and then volume was made up to the mark with methanol to obtain the final concentration of 1000µg/ml (Stock A solution).

From the above stock A solution 10ml of aliquot was pipetted out into two different 100ml volumetric flasks and volume was made up-to the mark with methanol to obtain a concentration of 100µg/ml (Stock B solution).

From the above stock B solution further dilutions were made to get the concentration range from 2-10µg/ml of Cinidipine and 5-25µg/ml of Metoprolol Succinate .

Preparation of Sample Solutions:

Commercially available tablet formulation CILCAR-M was purchased, each tablet contains 10mg of Cilnidipine and 25mg of Metoprolol Succinate. 20 tablet were weighed and a powder equivalent to 100mg of Metoprolol Succinate and 40mg of Cilnidipine was weighed accurarately and transferred to a volumetric flask, dissolved in 50ml of methanol and the content was kept in sonicator for 15min. The solution was filtered through whatmann filter paperNo. 41, finally the volume was made upto the mark with methanol to obtain the concentration of 1000µg/ml of METO and 400µg/ml of CIL and this solution was used as stock "A" Solution of the sample.

From the above stock "A" solution, 10ml of aliquot was pipetted out and transferred to a 100ml volumetric flask. The volume was made upto 100ml with methanol to obtain the concentration of 1000µg/ml of METO and 400µg/ml of CIL and this solution was used as stock "A" Ssolution of the sample.

From the above stock "A" solution, 10ml of aliquot was pipetted out and transferred to a 100ml volumetric flask. The volume was made upto 100ml with methanol to obtain the concentration of 100µg/ml of METO and 40µg/ml of CIL(stock "B" solution of sample).

0.4ml of stock B solution was pippetted out and transferred to a 10ml volumetric flask and diluted upto the mark to get 10µg/ml of METO and 4µg/ml of CIL.

METHODOLOGY

Method A(First Order Derivative Method).

The standard solutions of both the drugs were scanned in the spectrum mode from 400-200nm using UV Spectrophotometer. Cilnidipine showed maximum absorbance 240nm and Metoprolol succinate at 223nm. These spectrum were converted into first order derivative spectroscopy method. Zero crossing point of both the drugs were obtained and wavelengths were selected in a manner such that at zero crossing of one drug, the other drug shows a substantial absorbance. From the first order derivative spectra of standard cilnidipine and Metoprolol Succinate, zero crossing was found at 223nm and wavelength selected for their estimation was 231nm for CIL and 219nm for METO respectively.

Method B(Second Order Derivative Method).

The most characteristic feature of second order derivative is a negative band with minimum at the same wavelength as the maximum on the zero order band . It also shows two additional positive bands either side of the main band. From the second order derivative spectra of standard CIL, METO wavelength selected for their estimation was 240nm and 223nm respectively.

VALIDATION PARAMETER:

Validity of the proposed methods were confirmed by performing linearity, limit of detection, limit of quantification, accuracy, precision and stability studies as per the ICH guidelines.

Linearity

CIL showed excellent linearity in range of 2-10µg/ml and METO showed excellent linearity in range of 5-25µg/ml. The coefficient of correlation are listed in Table 1.

Limit of detection and Limit of Quantification.

The detection and quantification limits were estimated using the linearity curve parameters. The LOD was calculated as 3.3 times the standard deviation of the intercept to the slope of the curve. The LOQ was 10 times the standard deviation of the intercept to the slope of the curve. The low LOD and LOQ values are tabulated in Table 1, indicating the good sensitivity of the proposed methods.

Precision

The precision of the established procedures was also assessed in terms of intra and inter-day by analyzing five concentrations of both analytes in the calibration curve range. For intra-day, solutions were analyzed six times in a day and these solutions were investigated for three succeeding days for inter day precision. The percentage relative standard deviation was calculated and presented in Table**.** The result showed low percentage RSD, which confirmed that the proposed methods were precise.

Accuracy

Accuracy of the developed methods were examined by assaying different concentration of both the analytes in the calibration concentration range. The accuracy of the methods was expressed in terms of the percent recovery. The Recovery studies were carried out are three different levels i.e .,80%, 100%, 120%

Figures:

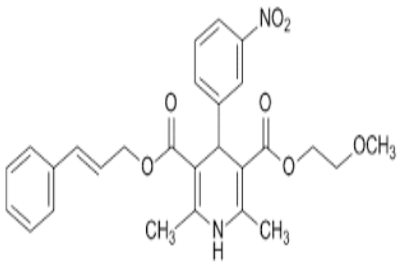

Fig 1. Structure of Cilnidipine

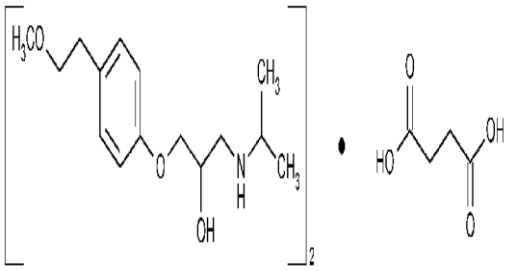

Fig 2. Structure of Metoprolol Succinate

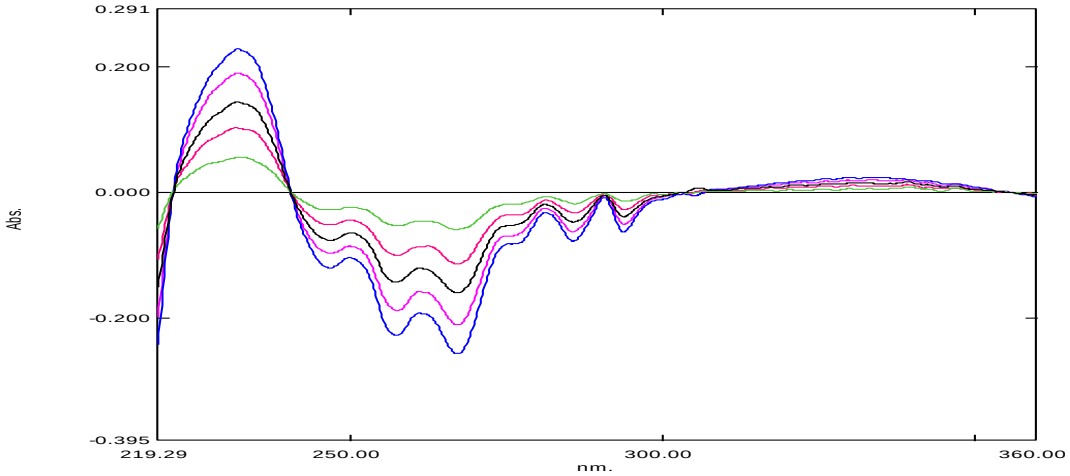

Fig 3. Overlay spectrum of CIL at 231nm by First Order derivative method

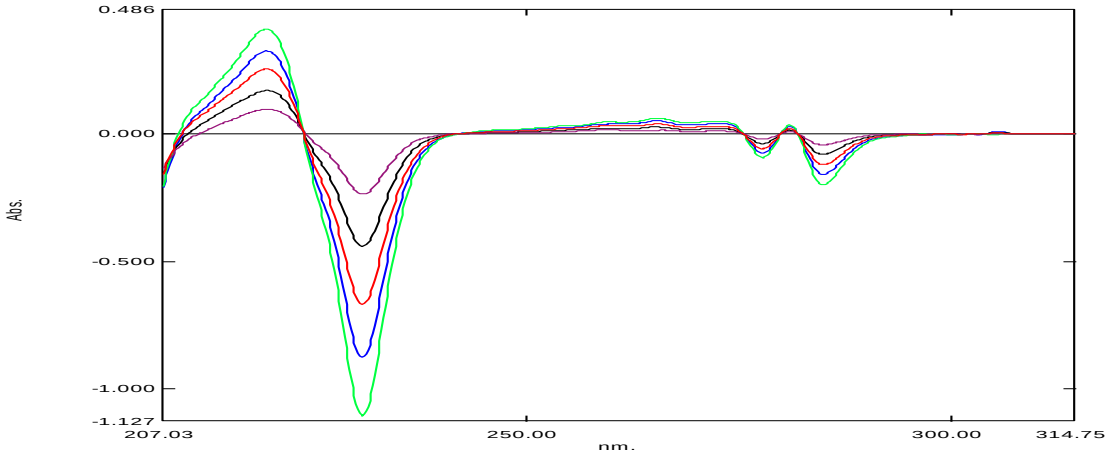

Fig 4. Overlay of First Order Derivative Spectrum of METO at 219nm.

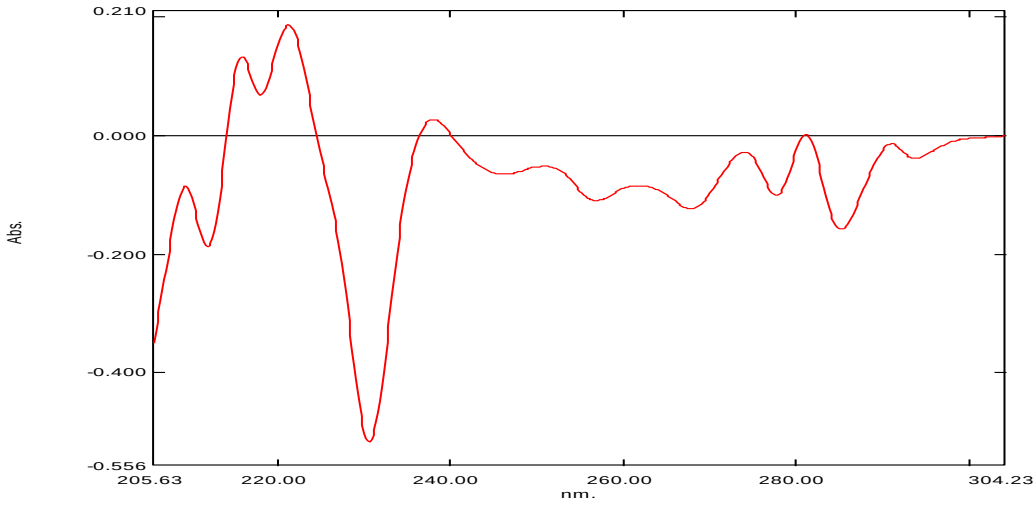

Fig 5. Spectra of CIL and METO at 231nm and 219nm in formulation.

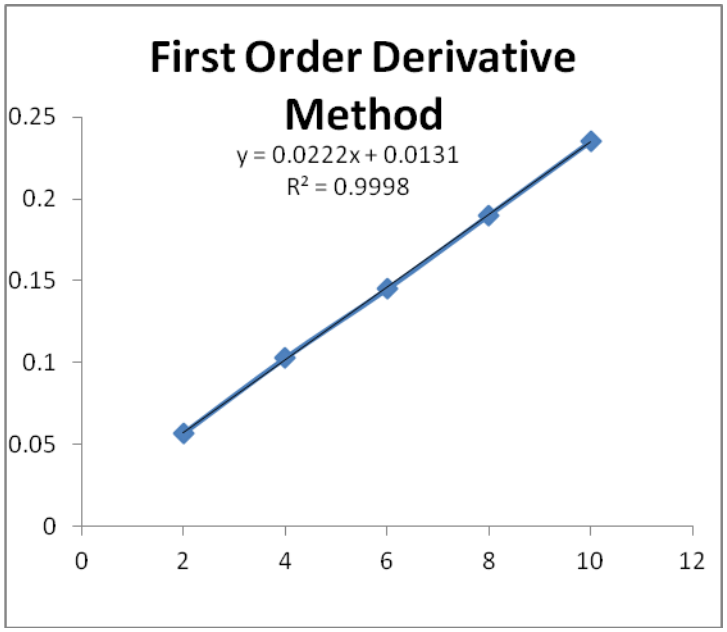

Fig 6. Calibration of CIL at 231nm by First Order Derivative Method.

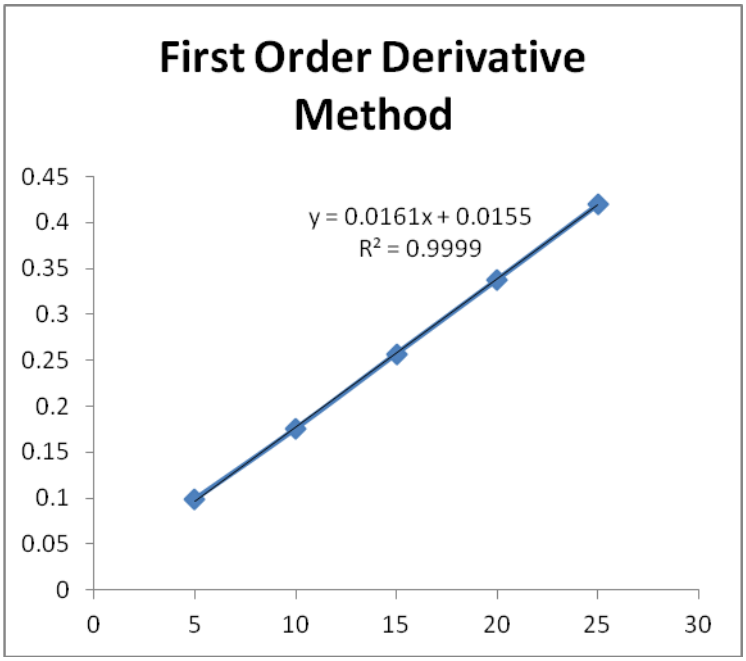

Fig 7. Calibration of METO at 219nm by First Order Derivative method.

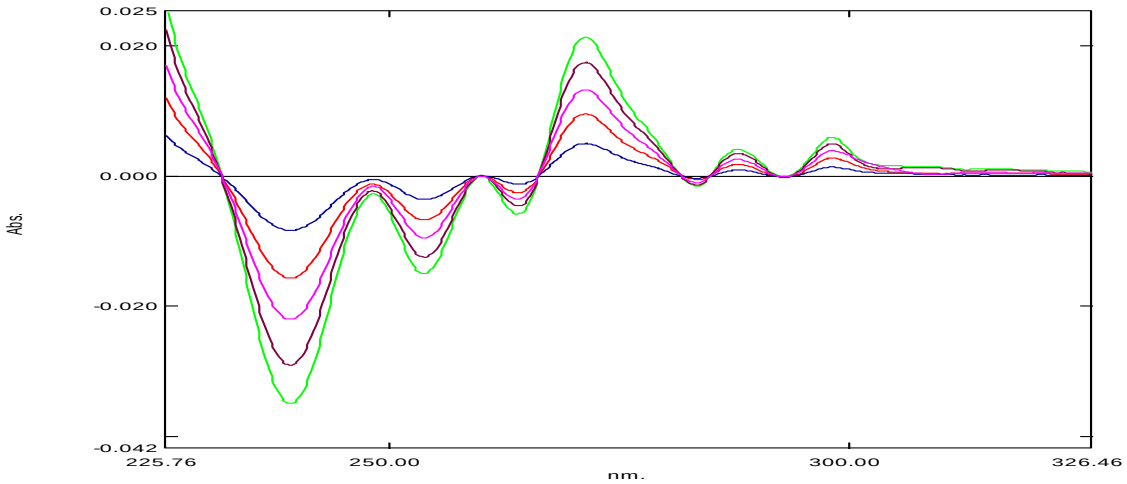

Fig 8. Overlay Spectrum of CIL at 240nm by Second Order Derivative method.

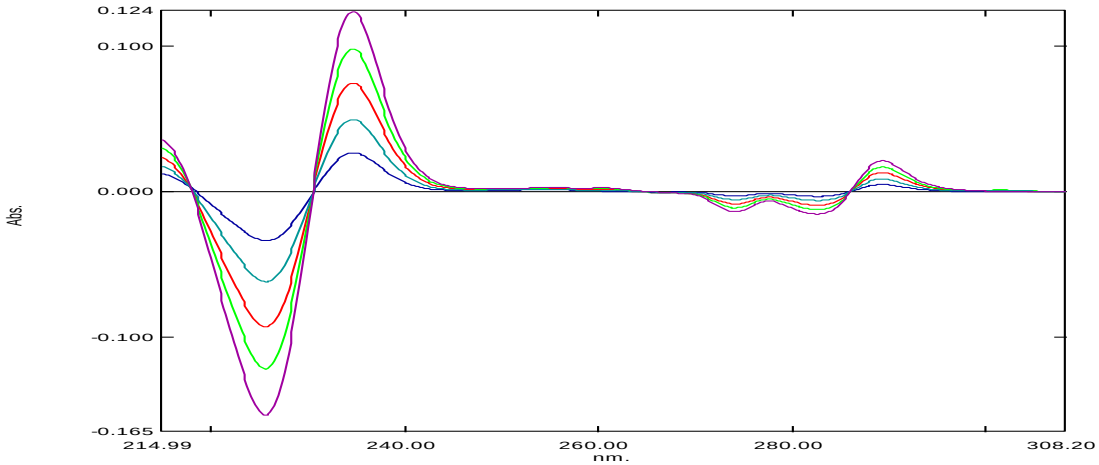

Fig 9. Overlay Spectrum of METO at 223nm by Second Order derivative method.

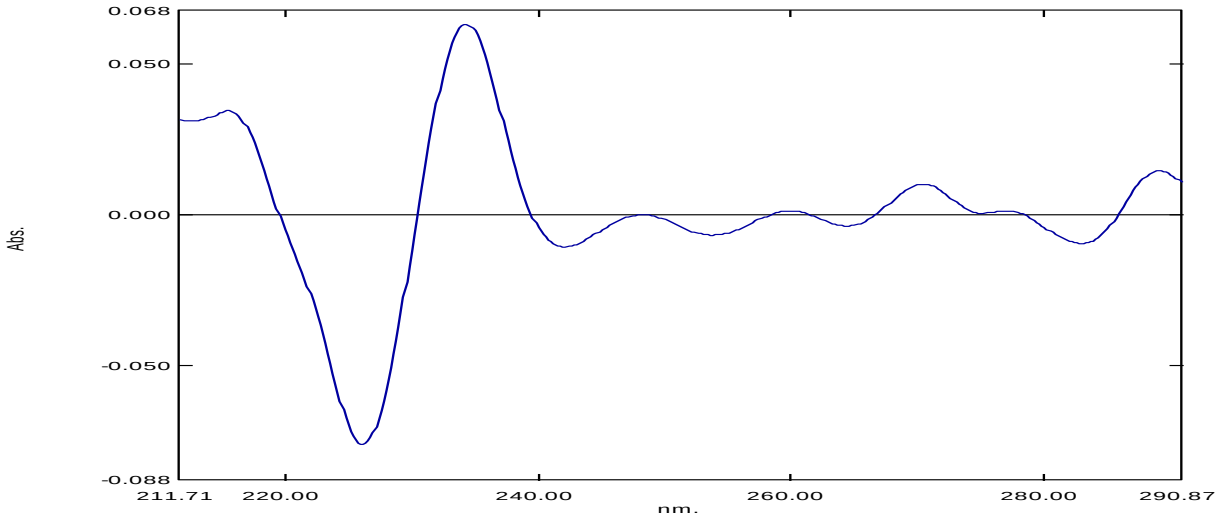

Fig 10. Spectra of CIL and METO at 240nm and 223nm in formulation

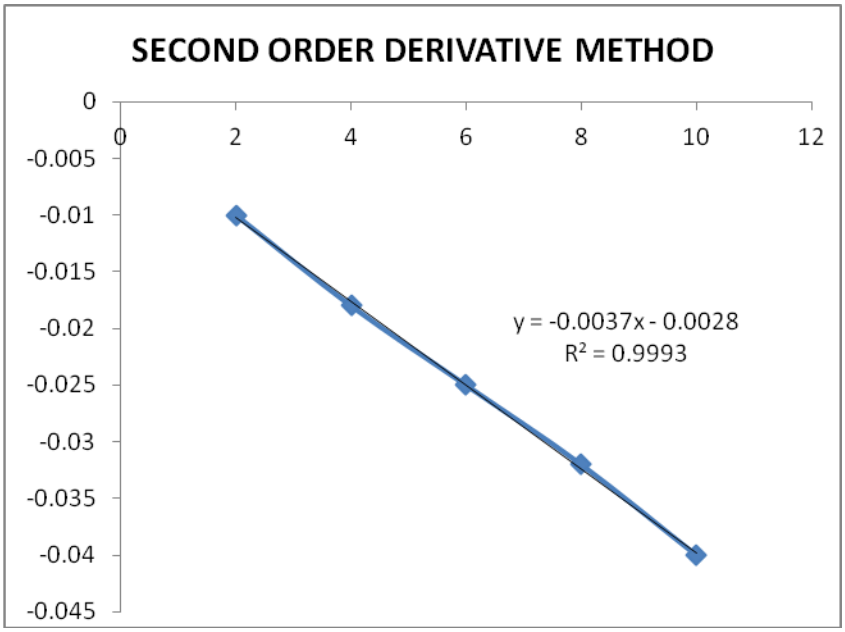

Fig 11. Calibration of CIL at 240nm by Second Order Derivative Method.

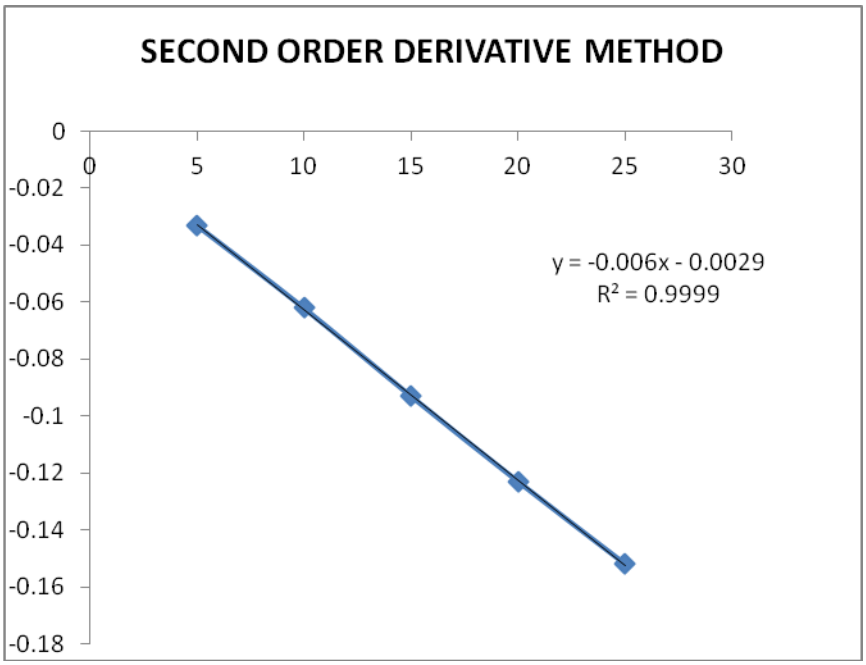

Fig 12. Calibration of METO at 223nm by Second Order Derivative Method

Tables

Table 1: Optical and validation parameters of UV Spectrophotometric methods.

UV Spectroscopy		First Order Derivative Method		Second Order Derivative Method	
Parameters		**CIL**	**METO**	**CIL**	**METO**
Linearity range (µg/ml)		2-10	5-25	2-10	5-25
λmax / wavelength range (nm)		231	219	240	223
Coefficient of correlation		0.9998	0.9999	0.9993	0.9999
Slope*(m)		0.0222	0.0161	0.0037	-0.006
Intercept*(c)		0.0116	0.0155	-0.0028	-0.0029
Accuracy (%RSD)	**80%**	0.56332	0.39190	0.7938	0.2268
	100%	0.99220	0.991313	0.3572	0.2755
	120%	0.40732	0.222680	0.7886	0.0463
Precision (%RSD)	**Intra-day**	0.28799	0.25657	0.84391	0.70963
	Inter-day	1.5764	0.04718	0.0089	0.0045
Limit of Detection (µg/ml)		0.1328	0.1057	0.07277	0.04488
Limit of Quantification (µg/ml)		0.4027	0.3204	0.2205.	0.136

TABLE 2 : Statistical validation data for Accuracy determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Method A		Method B	
				Total amount recovered (µg)	% Recovey	Total amount recovered (µg)	% Recovery
80%	CIL	4	3.2	7.21	100.13	7.16	99.44
	METO	10	8	17.95	99.72	17.89	99.38
100%	CIL	4	4	7.98	99.75	8.01	100.1
	METO	10	10	19.87	99.35	19.93	99.65
120%	CIL	4	4.8	8.73	99.20	8.79	99.88
	METO	10	12	21.87	99.54	21.95	99,77

TABLE 3 : Statistical validation data for Intra-day precision.

Components	Method A		Method B	
	CIL	METO	CIL	METO
Mean	99.644	100.47	100.125	99.994
Standard deviation	0.28795	0.6317	1.36702	0.38668
Relative standard deviation	0.28795	0.6287	1.36531	0.38669

n*=6

TABLE 4 : Statistical validation data for inter-day precision.

Components	Method A		Method B	
	CIL	METO	CIL	METO
Mean	99.889	99.861	99.1	99.94
Standard deviation	0.5443	0.6617	1.2337	1.4730
Relative standard deviation	0.5449	0.6627	1.2347	1.4730

n*=3

ABBREVATIONS:

UV: Ultra violet, % RSD: Percentage Relative Standard Deviation, CIL: Cilnidipine , METO: Metoprolol Succinate .

RESULTS AND DISCUSSION

In First order derivative spectroscopy method, the absorbance was measured at 231nm and 219nm for the estimation of CIL and METO respectively (fig 3and 4). Linearity was shown in the range of 2-10µg/ml and 5-25µg/ml (fig 6 and 7) for both CIL and MET respectively.

In Second Order derivative spectroscopy method, the absorbance was measured at 240nm and 223nm for the estimation of CIL and METO respectively (fig 8 and 9) amd Linearity was shown in the range of 2-10µg/ml and 5-25µg/ml (fig 11 and 12) for both CIL and METO respectively.

CONCULSION:

A simple, accurate and reproducible UV spectrophotometric methods were established for concurrent quantification of CIL and METO. Methanol has been used as a solvent. Analysis of laboratory prepared mixture confirmed the accuracy, specificity and also absence of interference from formulation excipients.

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