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Development and validation of UV spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk drugs and combined dosage form

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ABSTRACT

A simple, precise and economical UV spectrophotometric method has been developed for the simultaneous estimation of Cilnidipine and Metoprolol Succinate in their combined dosage form. Method is Absorbance ratio (Q-analysis method) which is based on measurement of absorption at wavelength of 230.60 nm and 223.40 nm i.e. iso-absorptive point of Cilnidipine and Metoprolol Succinate and λ_{max} of Metoprolol Succinate respectively. Linearity was observed in the concentration range of 2-10 µg/ml for Cilnidipine and 10-50 µg/ml for Metoprolol Succinate . The accuracy of method was assessed by recovery studies and was found to be within range of 99-101% for both Cilnidipine and Metoprolol Succinate . The developed method was validated with respect to linearity, accuracy (recovery) and precision. The results were validated statistically as per ICH guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of Cilnidipine and Metoprolol Succinate in the mixture.

Keywords: Cilnidipine, Metoprolol Succinate, Q-analysis, UV spectrophotometry.

INTRODUCTION

Cilnidipine (CIL) [1-3] is chemically 2-Methoxyethyl (2E)-3-phenyl-2-propen-1-yl 2, 6-dimethyl-4(3-nitrophenyl)-1, 4-dihydro-3,5-pyridinedicarboxylate (Fig.1) and it belongs to calcium channel blocker class of oral antihypertensive drugs . CIL acts as anti-hypertensive and lowers the blood pressure level by blocking calcium channel and increasing contraction of heart.

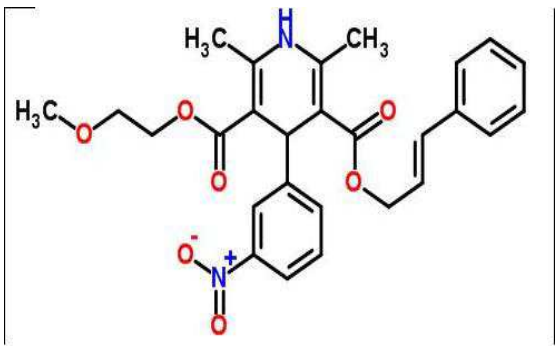

Fig. 1: Structure of Cilnidipine

Metoprolol Succinate (METO)[3-7]: Metoprolol Succinate (METO) is an anti-hypertensive drug and it belongs to sympatholytics beta adrenergic antagonists class of it. Metoprolol Succinate is chemically bis[(2RS)-1-[4- 2 methoxy ethyl) phenoxy]-3-[(1-methyl ethyl) amino] propan-2-ol] butanedioate (Fig.2). Metoprolol Succinate belongs to the class of selective β-1 receptor blocker , class of anti-hypertensive drugs which act by competitive antagonism of catecholamines at peripheral (especially cardiac) adrenergic neuron sites, leading to decreased cardiac output.

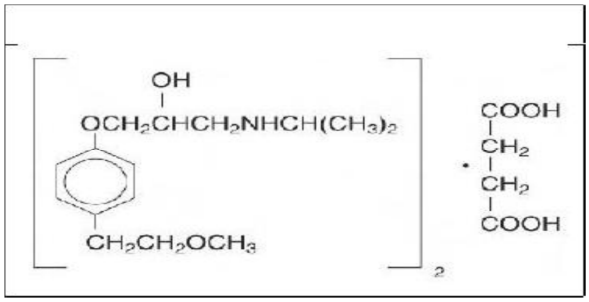

Fig. 2: Structure of Metoprolol Succinate

Metoprolol is official in BP 2009[4] and USP 2007[5] and potentiometric titration method and liquid chromatography method are given in BP 2009 and USP 2007 respectively.

Extensive literature survey revealed that methods were reported for the estimation of Cilnidipine[8-13] and Metoprolol succinate[14-41] alone and in combination with other drugs but there is no method reported for this combination .So, an attempt has been made to develop an accurate, precise and economically viable RP-HPLC method for the simultaneous estimation of combination of interest in the current research.

The objective of present study was to develop and validate the UV spectrophotometric method for simultaneous estimation of *Cilnidipine and Metoprolol Succinate* by asorbance ratio method. UV spectroscopy is simple, precise, accurate and economical technique for estimation of drugs. Using UV spectroscopy the two drugs can be estimated simultaneously without any prior separation.

MATERIALS AND METHODS

Instruments:
UV double beam spectrophotometer of Simadzu-1800 with spectral band width of 1nm and wavelength accuracy of ±0.3 nm was used for analytical work along with matched quartz cell of length 1cm. The analysis was carried by using UV Solutions 2.42 software. All the weighing was carried out on the Reptech electronic weighing balance, Sonication of samples was carried out by Metrex sonicator.

Materials and reagents:
Cilnidipine was gift sample by J.B. Chemicals and Pharmaceuticals Pvt. Ltd., Ankleshwar. Metoprolol Succinate was supplied as gift sample by Zydus Cadila, Ahemdabad. The analytical grade methanol was purchased from Rankem Pvt. Ltd. (India). Tablets (Cilacar-M) were purchased from local pharmacy. The distilled water was used for analytical work and rinsing of clean glass wares.

Preparation of stock solution and selection of wavelength for analysis:
Standard stock solutions of Cilnidipine and Metoprolol Succinate were prepared separately by adding 100mg of drug to methanol taken in 100ml volumetric flasks and then sonicated for five minutes and the volume was made up with methanol. The resulting solutions contain 1mg/ml of the drug. The stock solutions of CIL and METO were further diluted with methanol to obtain the concentration of 10µ g/ml and 50µ g/ml respectively. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200nm. From the resulting spectra λmax for CIL and METO were calculated separately (Fig. 3, 4). The overlay spectra of CIL and METO were also recorded. From the overlay spectra iso- absorptive point of CIL and METO was calculated (Fig. 5).

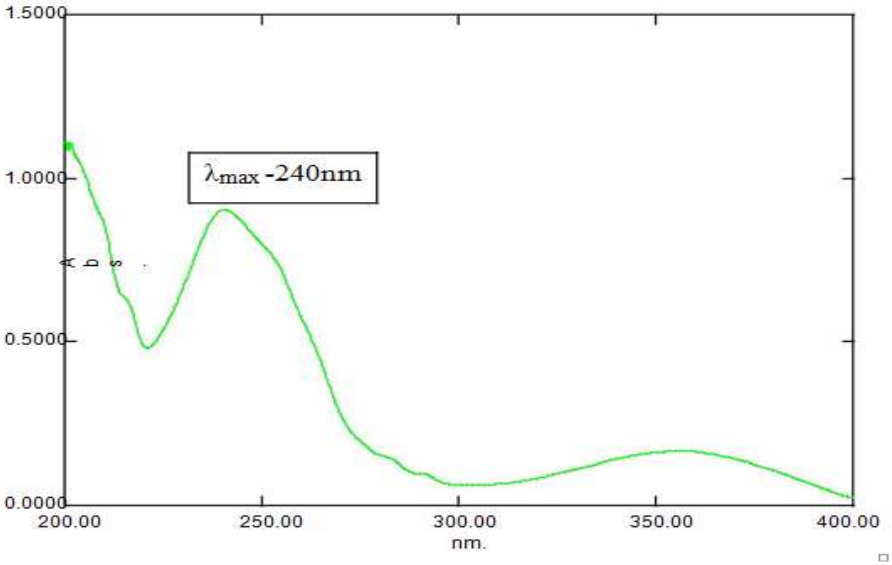

Fig. 3: UV spectra of Cilnidipine

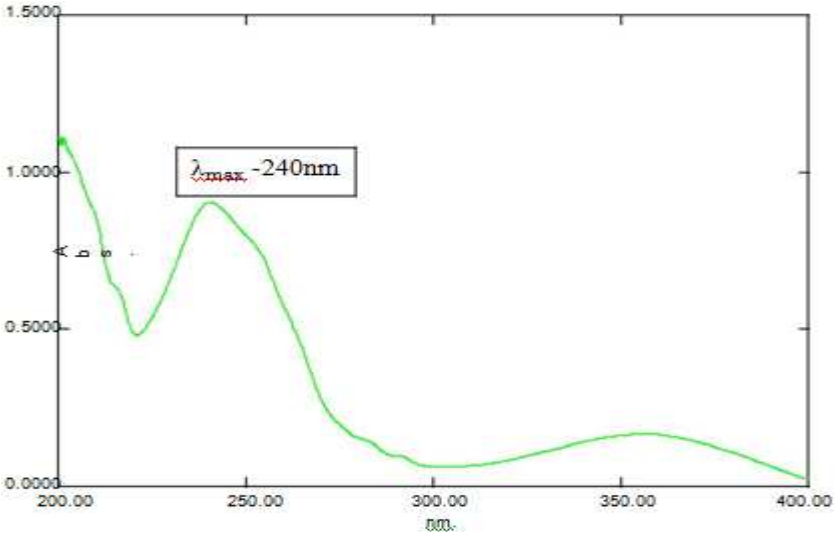

Fig. 4: UV spectra of Metoprolol succinate

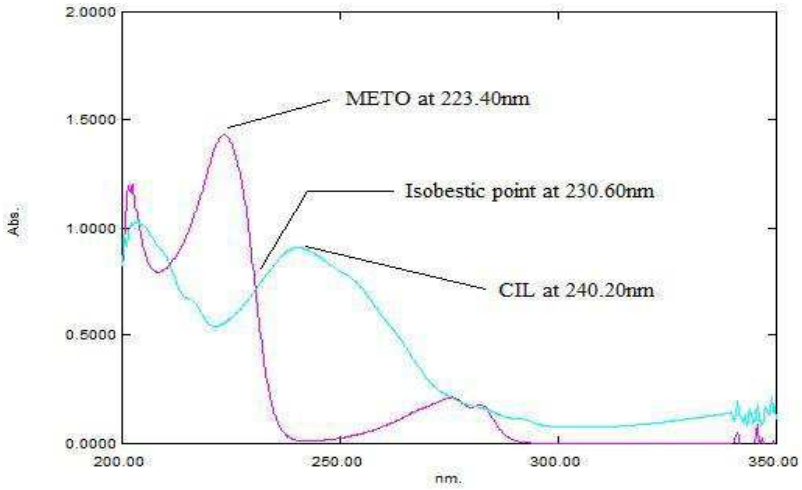

Fig.5: Overlay spectra of Cilnidipine and Metoprolol Succinate

Method : Absorbance ratio method(Q-analysis method):

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property

that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length e.g. two dilutions of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λmax of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an iso- absorptive point. A series of standard solutions of CIL and METO in the concentration range of 2-10µg/ml and 10-50µg/ml respectively were prepared in methanol and the absorbance of these solutions were measured at 230.60nm (iso- absorptive point) and 223.40nm (λmax of METO) (Fig. 5). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

The concentration of two drugs in mixture was calculated by using the following equations:

$$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$$

$$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$$

Where,
ax1 = A (1%, 1cm) of CIL at 230.40nm
ay1 = A (1%, 1cm) of METO at 230.40nm
ax2 = A (1%, 1cm) of CIL at 223.40nm
ay2 = A (1%, 1cm) of METO at 223.40nm

A1 and A2 are the absorbances of mixture at 230.40 nm and 223.40 nm. Cx and Cy are the concentrations of CIL and METO in gm/100 ml respectively in sample solution.

Qm= A2 / A1, Qx = ax2 / ax1 and Qy= ay2 /ay1

Assay of tablets by method :
Tablet Cilacar M 10/50 contains 10mg of Cilnidipine and 50mg of Metoprolol succinate equivalent to Metoprolol tartarate. It is marketed by J.B Chemicals and pharmaceutical Ltd. Fixed dose combination of Cilnidipine and Metoprolol Succinate is approved for marketing in India. Twenty tablets were weighed and triturated in a mortar pestle and the tablet powder equivalent to 100 mg of CIL and 500 mg of METO was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with methanol and 0.6 ml of this solution was further diluted to 10 ml with methanol. Absorbance of the resulting solution was measured at 223.40 nm and 230.60 nm against methanol. The concentration of CIL and METO can be obtained by using following equations,

$$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$$

$$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$$

Method validation:
The UV spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameters like linearity, precision and accuracy were evaluated.

Linearity:
Linearity was studied by diluting standard stock solution of CIL 2-10 µg/ml and METO 10-50 µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for CIL and METO are shown in Fig. 6,7,8 and 9 and data is presented in Table 1.

Precision:
Repeatability: 0.6 ml of working standard solution of CIL (100µg/ml) w as transferred to 10 ml volumetric flask. 3 ml of working standard solution of METO (100 µg/ml) was transferred to another 10 ml volumetric flask. The volume was adjusted up to mark with methanol in both the flask to get 6 µg/ml solution of CIL and 30µg/ml solution of METO . The absorbances of solutions were measured spectrophotometry six times and % RSD was calculated.The data is represented in Table 2.

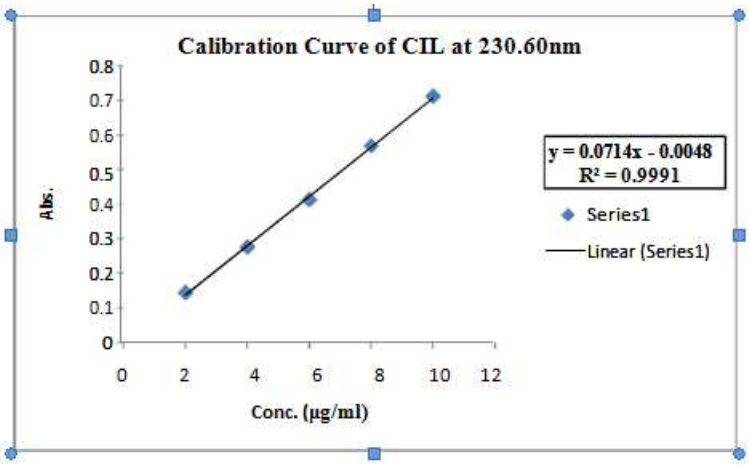

Fig.6. Calibration curve for CIL at 230.60 nm in methanol

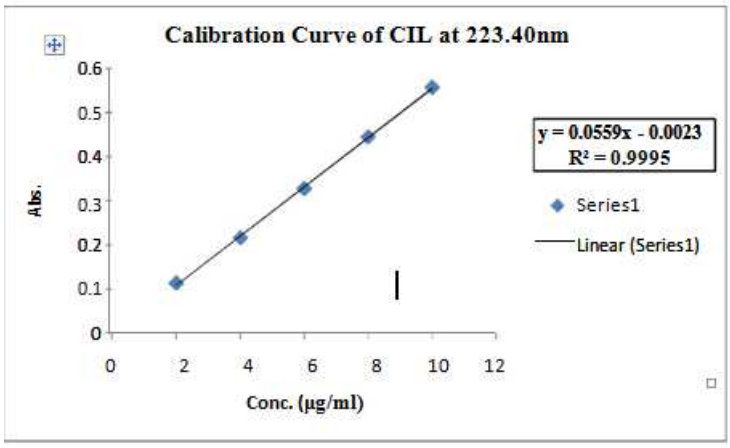

Fig.7.Calibration curve for CIL at 223.40 nm in methanol

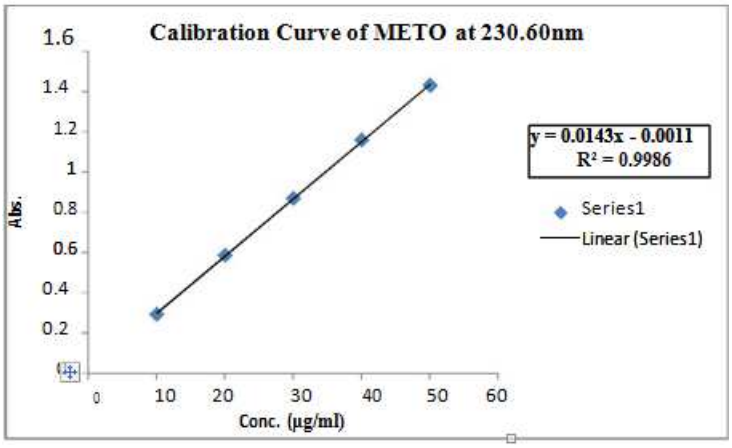

Fig.8. Calibration curve for METO at 230.60 nm in methanol

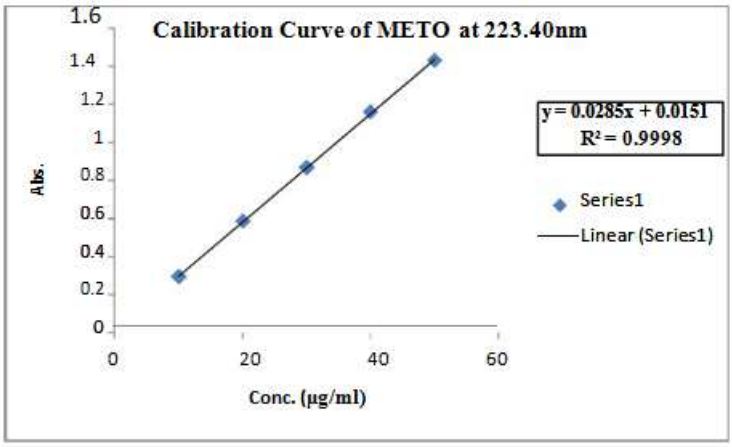

Fig.9. Calibration curve for METO at 223.40 nm in methanol

Intermediate precision: intermediate precision is studied in terms of intraday and inter-day precision. Three concentrations of CIL and METO were selected in a mixture and analyzed by method (n=3). For intraday, the analysis was carried out at different intervals on the same day and for inter day, the analysis was carried on different days. Table 3. give the results for intraday and inter-day studies respectively.

Accuracy*:*

To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level (80,100 and 120%). At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated. Table 4. shows the result for accuracy of the method.

Table 1 : Regression analysis of calibration curves and summary of validation parameters

Sr.No.	Parameter	CIL		METO	
		230.60	223.40	230.60	223.40
1.	Linearity range (µg/ml)	2 -10	2 -10	10 – 50	10 – 50
2.	Slope	0.0714	0.0559	0.0143	0.0285
3.	Intercept	0.0048	0.0023	0.0011	0.0151
4.	Limit of Detection (µg/ml)	0.0529	0.0909	0.1647	0.1281
5.	Limit of Quatificatiion (µg/ml)	0.16058	0.2757	0.4993	0.3884

Table 2 : Repeatability data of CIL and METO

Drugs	%R.S.D.	
	230.60 nm	223.40 nm
CIL	0.688	0.525
METO	0.673	0.330

Table 3: Precision data

Drug	Intraday Precision(%RSD)		Interday Precision (%RSD)	
	230.60 nm	223.40 nm	230.60 nm	223.40 nm
Cilnidipine	0.708-1.070	0.828-1.078	1.874-2.0576	1.881-2.016
Metoprolol Succinate	0.787-1.095	0.526-1.030	1.813-2.211	1.058-1.675

Table 4: Recovery study data for CIL and METO (n=3)

Drug	Pre-analyzed conc. (µg/ml)	Drug added (µg/ml)	230.60 nm		223.40 nm	
			Conc.recovered	% Recovery	Conc.recovered	% Recovery
CIL	4	**0**	4.02	100.50	4.03	100.76
		3.2	3.18	99.38	3.18	99.37
		4	4.04	101.11	4.09	101.53
		4.8	4.79	99.08	4.81	100.25
METO	20	**0**	19.93	99.68	20.03	100.15
		16	16.09	100.56	15.96	99.79
		20	20	100.00	19.96	99.83
		24	23.92	99.68	24.00	100.00

Table 5 : Analysis of marketed formulation (n=3)

Tablet batch no.	Actual Conc. (µg/ml)		Mean conc. obtained ± S.D. (µg/ml)		% Conc. of Label claim	
	CIL	METO	CIL	METO	CIL	METO
XCW3004	6	30	6.01 ± 0.1311	29.93 ± 0.4090	100.16	99.76
XCW3005	6	30	5.90 ± 0.0981	30.34 ± 0.2557	98.33	101.13

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient, precise and accurate way for simultaneous analysis of Cilnidipine and Metoprolol succinate in its bulk and pharmaceutical dosage form. Absorbance maxima of METO at 223.40 nm and isoabsorptive point 230.60 nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 2-10µg/ml for CIL and 10-50µg/ml for METO with respective correlation coefficients of 0.9986 and 0.9998 respectively. The % RSD for repeatability (n=6), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for CIL and METO were found within the range of 99% and 101%. Values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of this method. The assay for CIL and METO was found to be 100.77± 0.58 and 98.63± 0.37 respectively. The % RSD value for both CIL and METO were found to be less than2%. In this study simultaneous estimation of Cilnidipine and Metoprolol Succinate were carried out by absorbance ratio method satisfactorily.

CONCLUSION

Based on the results obtained, it is found that the developed UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, sensitive and economical. These can become effective analytical tool for routine quality control of Cilnidine and Metoprolol succinate bulk drug combination and its combined pharmaceutical dosage form without any prior separation of components.

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