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Der Pharmacia Lettre, 2015, 7 (5):333-340
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ISSN 0975-5071
USA CODEN: DPLEB4

Analytical method development and validation for simultaneous estimation of cilnidipine and metoprolol succinate by RP-HPLC

Madhuri A. Hinge*, Dhwani K. Desai, Rima N. Chavda, Dhaval R. Patel, Rashmi D. Shing and Ekta S. Patel

Department of Quality Assurance, Rofel Shri G. M. Bilakhia College of Pharmacy, Vapi

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ABSTRACT

The present work involves development and validation of RP-HPLC methods for simultaneous estimation of Cilnidipine and Metoprolol Succinate in their combined tablet dosage form. In HPLC method, Enable C18G (250 x 4.6 mm, 5 µm) column was used as stationary phase and Methanol: Water in the ratio 80: 20, (v/v) (pH adjusted to 3.5 with orthophosphoric acid) as mobile phase was used. The flow rate was 1 ml/min and both drugs were quantified at 231.0 nm. The retention time for Cilnidipine and Metoprolol Succinate was found to be 4.092 ± 0.12 min and 2.913 ± 0.18 respectively. The linearity range obtained for RP-HPLC method was 2 -10 µg/ml and 10 -50 µg/ml for Cilnidipine and Metoprolol Succinate respectively. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations.

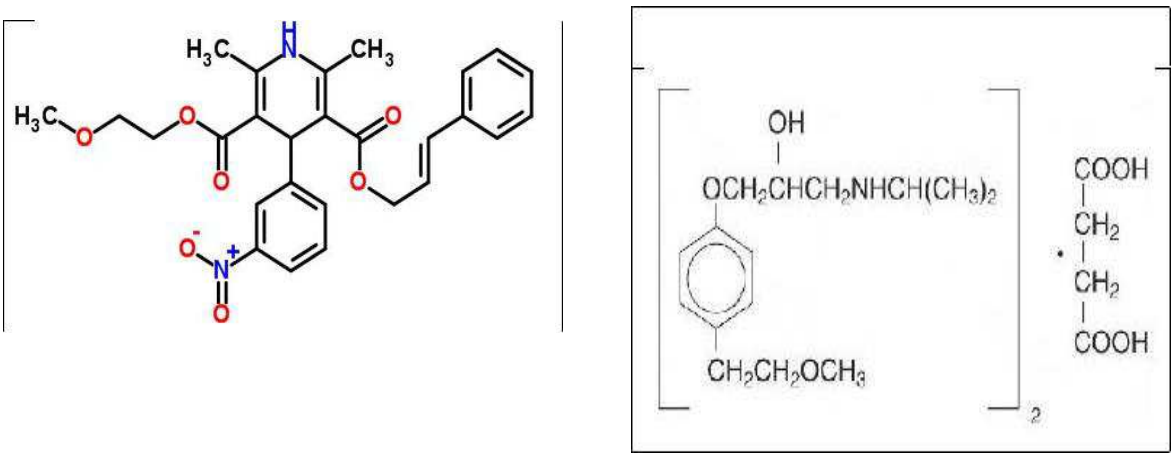
Keywords: Cilnidipine, Metoprolol Succinate, RP-HPLC method.

INTRODUCTION

Cilnidipine[1-3] is a dual blocker of L-type voltage gated calcium channels in sympathetic nerve terminals that supply blood vessels. It also dilates efferent and afferent arterioles. Used in Treatment of hypertension. Chemically it is described as 2-Methoxyethyl (2E)-3-Phenyl-2 propen-1-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridine dicarboxylate.

Metoprolol Succinate[3-7] is competitive antagonism of catecholamines at peripheral adrenergic neuron sites, Leading to decreased Cardiac output. It is Management of acute myocardial infection, angina pectoris, heart failure and hypertension. Chemically it is described as Bis[(2RS)-1-[4-(2-Methoxy ethyl)phenoxy]-3-[(1-methyl)amino]propan-2-ol]butanedioate. 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.

The chemical structure of Cilnidipine and Metoprolol succinate were shown in the figure 1.



Cilnidipine **Metoprolol Succinate**

Fig 1: chemical structures of a) Cilnidipine and b) Metoprolol Succinate

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.

MATERIALS AND METHODS

Equipments used:
HPLC Model was LC-2010C HT, Column was Enable C18 (250 x 4.6 mm, 5 µm), Injector: An auto injector, Detector: UV Detector used for analytical work. The analysis was carried by using LC Solution software. All the weighing was carried out on the Reptech electronic weighing balance, Sonication of samples was carried out by sonicator.

Chemicals 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.

Chromatographic Condition
C18 column, 250mm × 4.6 mm, 5µm was used for the chromatographic separation at a detection wave length of 231 nm. Mobile phase of composition Methanol: Water (80:20 v/v, pH- 3.5 adjusted with orthophosphoric acid) was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1.0 ml/min and the injection volume was 10.0 µl.

Preparation of mobile phase
A degassed mixture of Methanol and water in the ratio 80:20 (v/v) was prepared and pH was adjusted to 3.5 with orthophosphoric acid. The mixture was then filtered through 0.45 µ membrane filter and was degassed.

Preparation of standard solution of binary mixtures of Cilnidipine and Metoprolol Succinate
Accurately weighed 10 mg of Cilnidipine and 50 mg of Metoprolol succinate were transferred to 10 ml volumetric flask and diluted up to mark with mobile phase to give concentration of 1000 µg/ml of Cilnidipine and 5000 µg/ml of Metoprolol Succinate.

Preparation of working solution of binary mixtures of Cilnidipine and Metoprolol Succiante.
5.0 ml of standard stock solution was taken in 50ml volumetric flask and diluted up to the mark with mobile phase to get concentration of 100 µg/ml of Cilnidipine and 500µg/ml of Metoprolol Succinate.

Preparation of Sample Solution

Twenty tablets of Cilacar- M were weighed and crushed. Tablet powder equivalent to 10 mg of Cilnidipine and 50 mg of Metoprolol Succinate was weighed accurately and transferred to a 100 ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. the volume was made up with the mobile phase and filtered with Whatmann filter paper no.41. From this solution 0.6 ml was pipetted out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase. The areas of resulting peak were measured at 231 nm.

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Cilnidipine and Metoprolol Sccinate. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Methanol: Water (80:20 v/v, pH- 3.5 adjusted with orthophosphoric acid) using column Enable C18G, 250mm × 4.5 mm,5 µm.

Validation of RP-HPLC Method

System Suitability Study

Diluent was used as blank. Standard and sample containing 6 µg/ml Cilnidipine and 30 µg/ml Metoprolol Succinate were prepared and was injected in system as per stated condition. The mean values of system suitability parameters are shown in following Table 1.

Table 1: Mean values of system suitability study (n=5)

Parameters	Cilnidipine ± % RSD		Metoprolol Succinate ± % RSD
Retention time	4.090	± 0.124	2.913 ± 0.1794
Tailing factor	1.342	± 0.592	1.5148 ± 0.987
Theoretical plates	7681.60 ± 1.461		3283.53 ± 1.988
Resolution		7.197 ± 1.621	

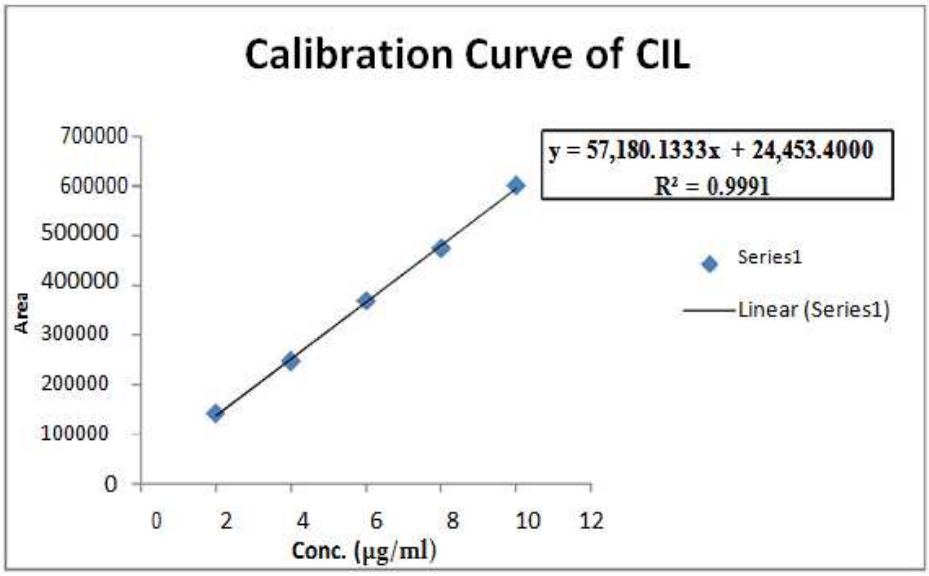


Figure 2: Calibration curve of Cilnidipine

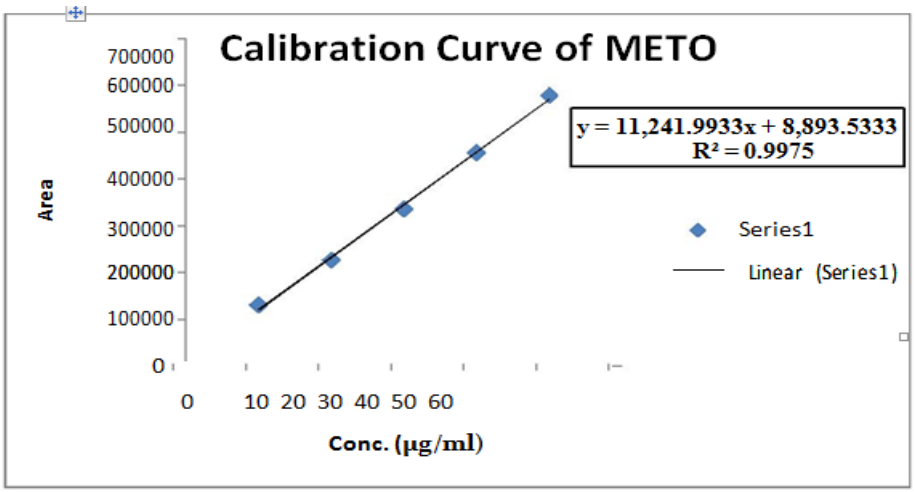


Figure 3: Calibration curve of Metoprolol Succinate

Linearity and Range

For the determination of linearity, appropriate aliquots were pipetted out from working stock solution to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 2-10 µg/ml of Cilnidipine and 10-50 µg/ml of Metoprolol Succiante. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Cilnidipine and Metoprolol succinate were shown in figure 2 and figure 3 and their corresponding linearity parameters were given in table 2.

Figure 4: Optimized Chromatogram of Cilnidipine and Metoprolol Succiante in Methanol : water (80:20,v//v, pH 3.5 adjusted with orthophosphoric acid)

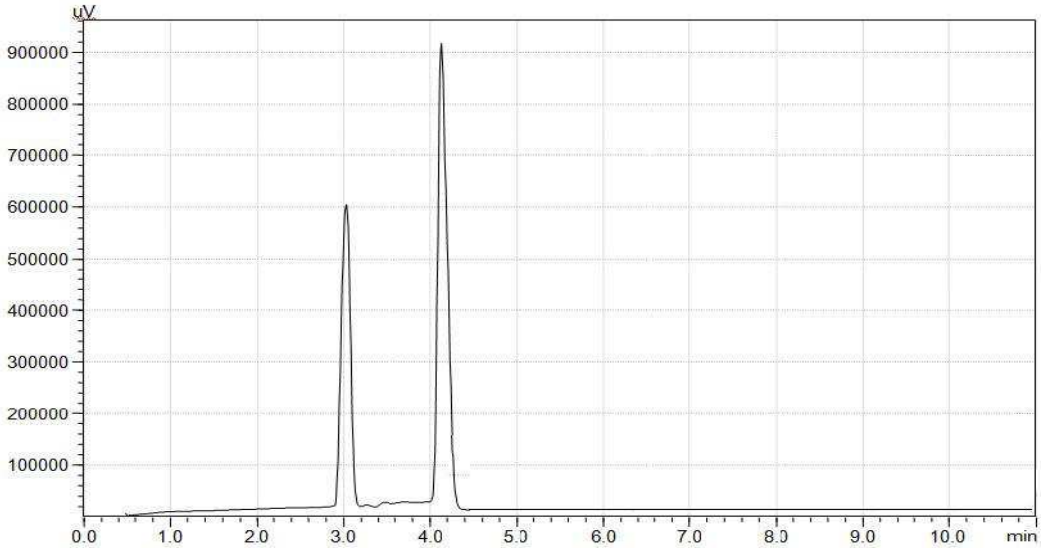


Table 2: Results for Linearity (n=3)

Parameters	Cilnidipine	Metoprolol Succinate
Slope	57180.1333	11241.9933
Y intercept	24453.4000	8893.5333
Correlation coefficient (r^2)	0.9991	0.9975
Regression equation	Y=57180.1333x+2445.4000	Y=11241.9933+8893.5333
Linearity range	2-10 µg/ml	10-50 µg/ml
LOD	0.05332 µg/ml	0.226354 µg/ml
LOQ	0.161577 µg/ml	0.685921 µg/ml

n= No. of determinants

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of (6 µg/ml of Cilnidipine and 30 µg/ml of Metoprolol Succinate) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in table 3.

Table 3: Results of precision (n=6)

Drug	Intraday Precision(%RSD)	Interday Precision (%RSD)
Cilnidipine	0.597 - 0.977	1.829 - 1.959
Metoprolol Succinate	0.506 - 0.737	1.758 - 1.993

n=No. of determinants

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in table 4.

Table 4: Results for Accuracy (n=3)

Recovery level	Cilnidipine				Metoprolol succinate			
	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery
	std	test			std	Test		
0%	0	4	4.03	100.76	0	20	20.09	100.58
80%	3.2	4	3.17	99.28	16	20	16.03	100.24
100%	4	4	3.99	99.98	20	20	19.93	99.68
120%	4.8	4	4.70	98.11	24	20	23.91	98.65
Mean recovery	98.11 – 100.76 %w/w				99.65 -100.24 %w/w			

n= No. of determinants

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients.

Limit of Detection (LOD) and Limit of Quantitation (LOQ).

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in table 2.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported.

Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ±2nm in the detection wave length and ±0.2ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in the table 5.

Table 5: Results for Robustness

Parameters (n=3)	%RSD	
	Cilnidipine Maleate	Metoprolol Succinate
Mobile Phase +2	0.617	0.824
Mobile Phase -2	1.34	0.342
Flow rate +2	0.472	1.533
Flow rate -2	0.608	0.395
pH +2	1.202	0.950
pH -2	0.867	1.043

n= No. of determinants

Assay of Marketed Formulations

10.0 µl of sample solution of concentration of 6 µg/ml Cilnidipine and 30 µg/ml of Metoprolol Succinate was injected into chromatographic system and the peak responses were measured and shown in the figure 5. The solution

was injected three times in to the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples.

Figure 5: A typical chromatogram for assay of marketed formulation containing 6 µg/ml of Cilnidipine and 30 µg/ml Metoprolol Succinate

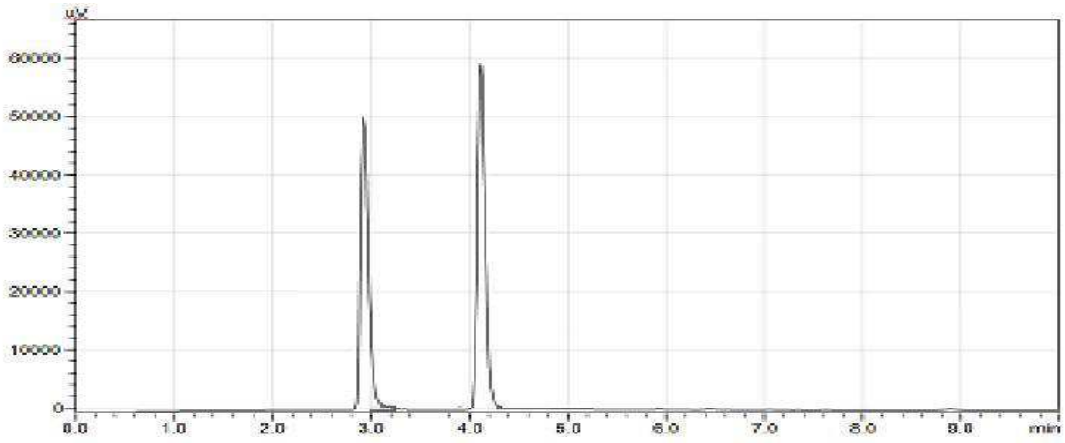


Table 6 : Analysis of marketed formulation (n=3)

Tablet batch no.	Actual Conc. (µg/ml)		Mean conc. obtained ± S.D. (µg/ml)		% Conc. of Label claim	
	Cilnidipine	Metoprolol Succiante	Cilnidipine	Metoprolol Succiante	Cilnidipine	Metoprolol Succiante
XCW3004	6	30	6.03 ± 0.095	29.92 ± 0.376	100.50	99.73
XCW3005	6	30	5.99 ± 0.119	29.89 ± 0 .365	99.83	99.63

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Methanol and water in the ratio 80:20 v/v pH adjusted to 3.5 with orthophosphoric acid was selected as mobile phase because of better resolution and symmetric peaks. Cilnidipne and Metoprolol Succinate were found to show appreciable absorbance at 231.0 nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Cilnidipine and Metoprolol succinate at different RTs was shown in figure 4.

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Cilnidipine and Metoprolol succinate at 4.09 min and 2.9 min respectively without any interferences. The parameters were given in table 1.

Concentration range of 2-10 µg/ml for Cilnidipine and 10-50 µg/ml for Metoprolol Succinate were found to be linear with correlation coefficients 0.9991 and 0.9975 for Cilnidipine and Metoprolol Succinate respectively. The results were given in table 2.

The proposed method was found to be precise and reproducible with % RSD of 1.829-1.959 and 1.758-1.993 for Cilnidipine and Metoprolol Succinate respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 98.11 to 100.76% w/w and 99.65 to 100.58% w/w for Cilndipine and Metoprolol Succinate respectively. This indicates that the method was accurate. Values obtained were given in table 4.

The limits of detection for Cilnidipine and Metoprolol Succinate were found to be 0.053 µg/ml and 0.22 µg/ml

respectively and the limits of quantitation were 0.16 µg/ml and 0.68 µg/ml respectively. Values were represented in table 2.
The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination. Values obtained were given in table 6.

The method was found to be robust after changing the conditions like, flow rate (±0.2 ml), mobile phase composition and pH (± 2). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

CONCLUSION

A new RP-HPLC method has been developed for simultaneous estimation of Cindipine and Metoprolol Succinate in marketed formulation. The method gave good resolution for both the drugs with a short analysis run time within 6 min. The developed method was validated. It was found to be novel, simple accurate precise, sensitive and cost effective. Hence the proposed RP-HPLC method is suitable for routine assay of Cilnidipine and Metoprolol Succinate in pharmaceutical dosage form in quality control laboratories.

Acknowledgment
The authors are thankful to the J.B. Chemicals and Pharmaceuticals Pvt. Ltd., Ankleshwar for providing the gift sample of Cilnidipine and Zydus Cadila, Ahemdabad for providing gift sample of Metoprolol Succinate. The authors are also thankful to the Principle, Rofel Shri G. M. Bilakhia College of Pharmacy, VAPI, India, for providing the required facilities to carry out this research work.

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