DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF CILNIDIPINE AND METOPROLOL SUCCINATE

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ABSTRACT

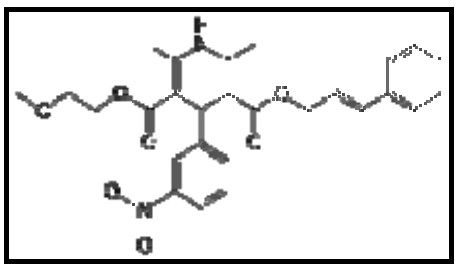
A simple, specific, accurate and precise reversed phase high performance liquid chromatographic method was developed and validated for the simultaneous estimation of Cilnidipine and Metoprolol Succinate, using a Cosmosil C18 (250 x 4.6 mm i.d.) column and a mobile phase composed of 0.05M potassium dihydrogen phosphate buffer: Methanol (70:30) pH 3.5 adjusted with ophosphoric acid and at flow rate of 1.0 ml/min. The retention times of Cilnidipine and Metoprolol Succinate were found to be 3.493 min and 5.960 min, respectively. Linearity was established for Cilnidipine and Metoprolol Succinate in the range of 12.5-37.5 µg/ml and 2.5-7.5 µg/ml, respectively. The percentage recoveries of Cilnidipine and Metoprolol Succinate were found to be in the range of 100.13-100.40 % and 99.24-100.29 %, respectively. The correlation coefficients for both components were found to be 0.999. The developed methods were validated according to ICH guidelines and values of linearity, accuracy, precision and other analysis were found to be in good accordance with the prescribed values. This method can be successfully employed for simultaneous quantitative analysis of Cilnidipine and Metoprolol Succinate in its dosage form.

The developed HPLC method was subjected to stability indicating studies for marketed formulation. Interfering peak from degraded products or solvent did not interfere with estimation of drugs and the developed method was found to be specific for estimation of Cilnidipine and Metoprolol Succinate.

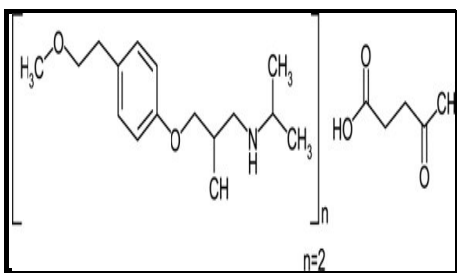
Key Words: Cilnidipine, Metoprolol Succinate, RP-HPLC, validation, stability

INTRODUCTION

Cilnidipine is a unique dihydropyridine derivative Ca2+ channel blocker with an inhibitory action on the sympathetic N-type Ca2+ channels. It has been clarified that cilnidipine exerts antisympathetic actions in various examinations from cell to human levels. Furthermore, its renoprotective, neuroprotective and cardioprotective effects have been demonstrated in clinical practice.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. At low doses, metoprolol selectively blocks cardiac β1-adrenergic receptors with little activity against β2-adrenergic receptors of the lungs and vascular smooth muscle. Receptor selectivity decreases with higher doses. Unlike propranolol and pindolol, metoprolol does not exhibit membrane-stabilizing or intrinsic sympathomimetic activity. Membrane-stabilizing effects are only observed at doses much higher than those needed for β-adrenergic blocking activity.



CILNIDIPINE



METOPROLO SUCCINATE

This combination though having different mechanism of action is effective in lowering high blood pressure with a single drug therapy. Cilnidipine is not official in IP, BP and USP.

Metoprolol succinate is official in IP and BP, EP and USP.

- Methods are available for estimation of individual drug & combination with other drugs.
- Literature survey reveals that only few analytical methods are reported for both the drugs
- Hence it was thought worthwhile to develop RP-HPLC methods for simultaneous estimation of Cilnidipine and Metoprolol succinate in its combined dosage form which is economical in terms of mobile phase composition, column, and run time.

MATERIALS AND METHODS

Water, Methanol , Acetonitrile, Orthophosphoric acid, KH_2PO_4 HPLC grade Procured from Finar Chemical pvt .Ltd.

TABLE 1: CHROMATOGRAPHIC CONDITION

OPTIMIZED HPLC CONDITION	OBSERVATION
Column	Cosmosil C18 (250 mm x 4.6 mm)
Mobile Phase	Phosphate buffer of (pH 3.5): Methanol 70:30 (v/v)
Detection Wavelength	230 nm
Flow Rate	1.0 ml/min
Injection Volume	20.0 µl
Run Time	10 min

Selection of wavelength

Both Metoprolol and Cilnidipine show reasonably good response at 230 nm. Standard solution of Metoprolol Succinate (25 µg/ml) and Standard solution of Cilnidipine (5 µg/ml) were scanned between 200-400 nm using UV-visible spectrophotometer. Wavelength was selected from the overlay spectra of above solutions

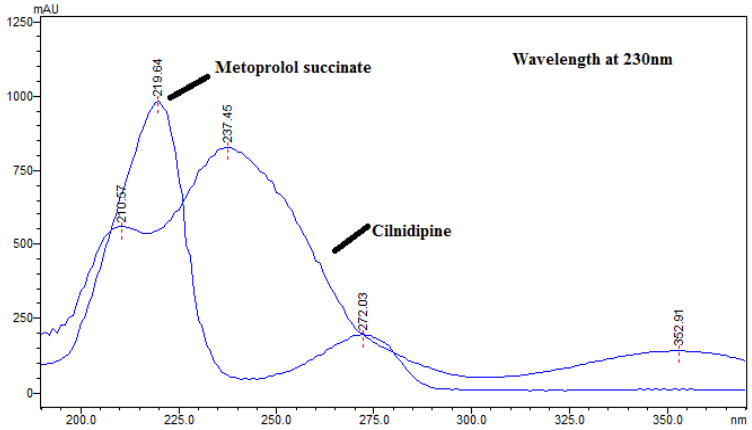


Fig. 1 Overlay UV Spectrum of Metoprolol Succinate and Cilnidipine showing selection of wavelength detection

Preparation of standard solutions:

(A) Preparation of Mobile Phase

Mix Phosphate buffer and methanol in proportion of 70:30 and set the pH 3.5 using 10% solution of o-phosphoric acid then sonicate it properly and filter the mobile phase through Whatman filter paper.

(B) Metoprolol Succinate standard stock solution: (250 µg/mL)

A 25 mg of Metoprolol Succinate was weighed and transferred to a 100 mL volumetric flask and dissolved in 25 ml mobile phase. The flask was sonicated for 10 minutes and volume was made up to the mark with mobile phase to give concentration of 250 µg/mL.

(C) Cilnidipine standard stock solution: (50 µg/mL)

A 10 mg of Cilnidipine was weighed and transferred to a 200 mL volumetric flask and dissolved in 25 ml mobile phase. The flask was sonicated for 10 minutes and volume was made up to the mark with mobile phase to give concentration of 50 µg/mL.

(D) Preparation of standard solution of binary mixtures of Metoprolol Succinate and Cilnidipine.

Accurately weighed 50 mg of Metoprolol Succinate and 10 mg of Cilnidipine were transferred to 200 ml volumetric flask and dissolved in 25 ml mobile phase. The flask was sonicated for 10 minutes and volume was

made up to the mark with mobile phase to give concentration of 250 µg/ml of Metoprolol Succinate and 50 µg/ml of Cilnidipine.

Linearity

The linearity for Metoprolol Succinate and Cilnidipine were assessed by analysisof combined standard solution in range of 12.5-37.5 µg/ml and 2.5-7.5 µg/ml respectively, in term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Precision

A. Repeatability

6 replicate of Standard solution containing Metoprolol Succinate (25µg/ml) and Cilnidipine (5µg/ml) were prepared and injected and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (12.5, 25, 37.5 µg/ml) of Metoprolol Succinate and (2.5, 5, 7.5 µg/ml) Cilnidipine were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (12.5, 25, 37.5 µg/ml) of Metoprolol Succinate and (2.5, 5, 7.5 µg/ml) Cilnidipine were analyzed three times on the same day and % R.S.D was calculated.

Accuracy

Recovery studies were carried out by addition of standard drugs to the sample at 3 different concentration levels (50, 100 and 150 %) taking into consideration percentage purity of added drug samples. It was determined by calculating the recovery of Metoprolol Succinate and Cilnidipine by standard addition method. Accuracy is the closeness of the test results obtained by the method to the true value. Was measured. The amount of Metoprolol Succinate was calculated at each level and % recoveries were computed.

Analysis of Tablet formulation:

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 25mg of Metoprolol succinate and 5 mg of Cilnidipine was transferred to 100 ml volumetric flask containing 50 ml of mobile phase, gentle shaking was carried out for 5 min and ultrasonicated for 5 min. The volume was made up to the mark with mobile phase to get the concentration of 250 µg/ml (METO) & 50µg/ml (CILN). The tablet sample solution was filtered through Whatman filter paper, 1 ml of the aliquot was transferred to a 10 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain a solution containing 25 µg/ml of Metoprolol Succinate and 5µg/ml of Cilnidipine. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The amount of each drug present in tablet was estimated from their respective calibration curve.

STABILITY STUDY:

ACID HYDROLYSIS:

Take 1.0 ml of aliquot from stock solution in 10 ml of volumetric flask. To this add 2.0 ml of 0.1N HCL and then solution is allowed to stand for 1 hrs and neutralized using 0.1N NaOH. Finally dilute upto mark using mobile phase to get final concentration 25 µg/ml of METO and 5 µg/ml of CILN.

ALKALI HYDROLYSIS:

Take 1.0 ml of aliquot from stock solution in 10 ml of volumetric flask. To this add 2.0 ml of 0.1N NaOH and then solution is allowed to stand for 4 hrs and neutralized using 0.1N HCL. Finally dilute upto mark using mobile phase to get final concentration of 25 µg/ml of METO and 5 µg/ml of CILN.

OXIDATIVE HYDROLYSIS:

Take 1.0 ml of aliquot from stock solution in 10 ml of volumetric flask. To this add 2.0 ml of 3% Hydrogen peroxide and then solution is allowed to stand for 4 hrs. Finally dilute upto mark using mobile phase to get final concentration of 25 µg/ml of METO and 5 µg/ml of CILN.

THERMAL HYDROLYSIS:

Take 1.0 ml of aliquot from stock solution in 10 ml of volumetric flask and then solution is allowed to stand for 1 hr at 60 ºC. Finally dilute up to mark using mobile phase to get final concentration of 25 µg/ml of METO and 5 µg/ml of CILN.

PHOTOLYTIC HYDROLYSIS:

Take 1.0 ml of aliquot from stock solution in 10 ml of volumetric flask and then solution is allowed to stand for 4 hrs in Sun light. Finally dilute up to mark using mobile phase to get final concentration of 25 µg/ml of METO and 5 µg/ml of CILN.

RESULTS AND DISCUSSION

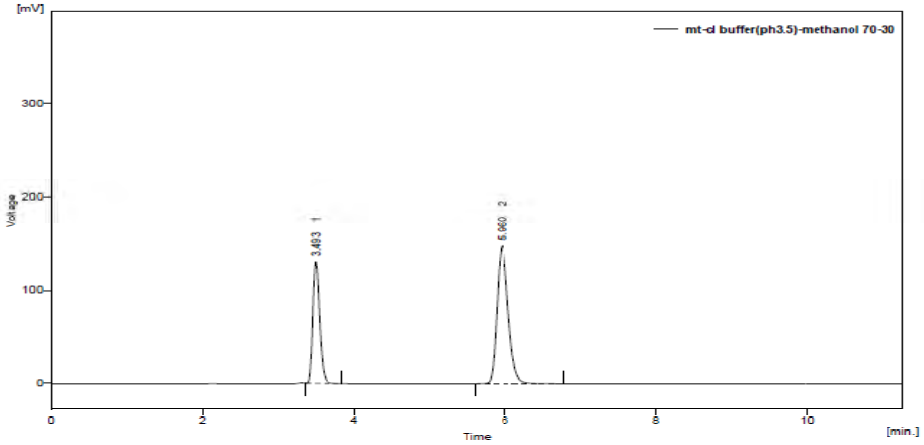


Fig-2 Chromatogram of Metoprolol succinate and Cilnidipine in Phosphate Buffer (pH3.5): Methanol (70:30 v/v) (Flow rate-1.0 ml/min)

Table 2: Linearity data for METO.

Sr.No.	Concentration (µg/ml)	Average peak Area (n=5)	%RSD (n=5)
1	12.5	418.803	1.694
2	18.75	615.287	1.286
3	25	826.623	1.153
4	31.25	1038.570	1.923
5	37.5	1243.970	0.586

Table 3: Linearity data for CILN

Sr.No.	Concentration (µg/ml)	Average peak Area (n=5)	%RSD (n=5)
1	2.5	779.227	1.846
2	3.75	1213.856	1.192
3	5	1607.224	0.976
4	6.25	1976.852	0.759
5	7.5	2380.826	1.107

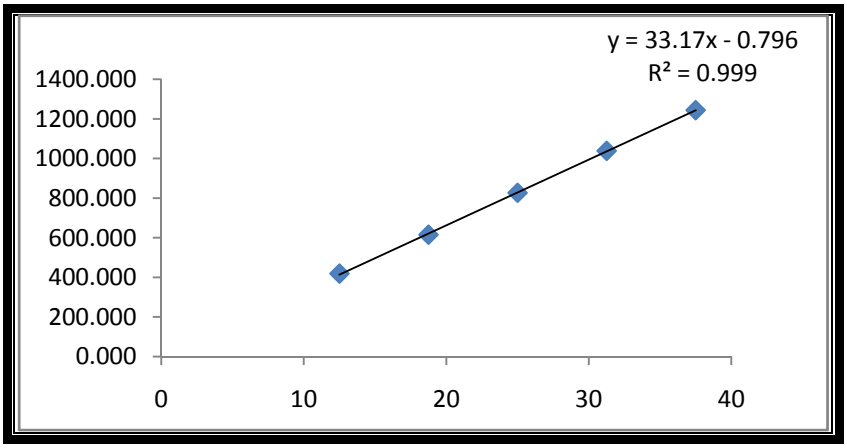


Fig-3: Calibration Curve of METO (12.5-37.5 µg/ml).

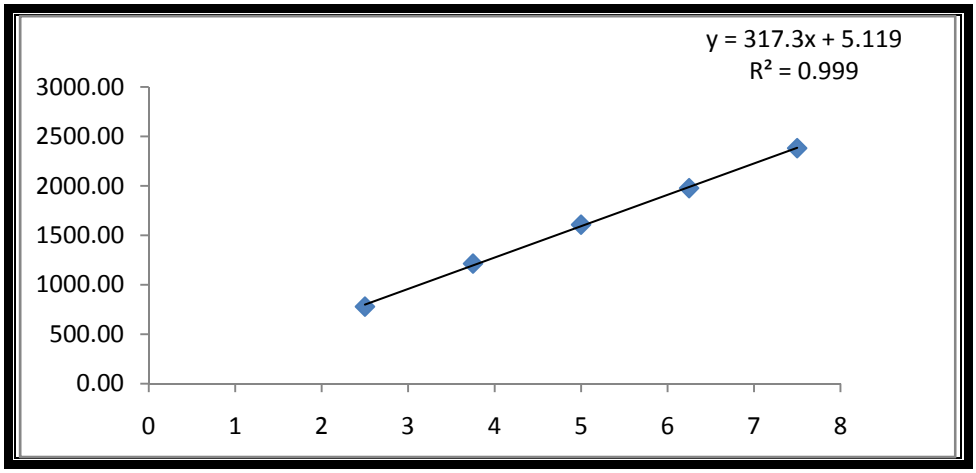


Fig.-4: Calibration Curve of CILN (2.5-7.5 µg/ml).

Table 4: Repeatability data for METO.

METOPROLOL SUCCINATE				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	25	815.333 826.673 821.677 829.063 824.911 825.723	823.897 ± 4.838	0.587

Table 5: Repeatability data for CILN.

CILNIDIPINE				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	5	1548.563 1570.188 1539.397 1564.387 1566.828 1568.401	1559.627 ± 12.607	0.808

Table 6: Intraday precision data for estimation of METO and CILN

	METO			**CILN**		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	12.5	407.381±4.295	1.054	2.5	775.002±4.52	0.584
2	25	821.725±6.001	0.730	5	1555.622±18.50	1.190
3	37.5	1221.650±3.072	0.251	7.5	2317.530±14.89	0.643

Table 7: Interday precision data for estimation of METO and CILN.

	METO			CILN		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	12.5	403.913±5.697	1.411	2.5	765.669±10.277	1.342
2	25	804.243±8.489	1.056	5	1523.974±23.458	1.539
3	37.5	1234.342±4.089	0.331	7.5	2342.321±21.223	0.906

Table 8: Recovery data for METO

Accuracy Level	**Conc. of Sample (µg/ml)**	**Amount of Standard Added (µg/ml)**	**Total Conc. (µg/ml)**	**Conc. Recovered (µg/ml)**	**% Recovery**	**Mean % Recovery ±SD**	**% RSD**
80%	12.5	10	22.5	10.036	100.36	100.40 ± 0.503	0.501
				9.993	99.93		
				10.093	100.93		
100%	12.5	12.5	25	12.415	99.32	100.13 ± 1.416	1.414
				12.413	99.31		
				12.721	101.77		
120%	12.5	15	27.5	14.963	99.75	100.26 ± 1.074	1.072
				15.224	101.50		
				14.930	99.54		

Table 9: Recovery data for CILN

Accuracy Level	**Conc. of Sample (µg/ml)**	**Amount of Standard Added (µg/ml)**	**Total Conc. (µg/ml)**	**Conc. Recovered (µg/ml)**	**% Recovery**	**Mean % Recovery ±SD**	**% RSD**
80%	2.5	2	4.5	1.995	99.74	100.29 ± 0.825	0.822
				1.998	99.89		
				2.025	101.24		
100%	2.5	2.5	5	2.471	98.85	99.76± 1.211	1.214
				2.482	99.29		
				2.528	101.13		
120%	2.5	3	7.5	2.993	99.75	99.94± 0.510	0.510
				3.015	100.51		
				2.986	99.54		

Table 10: Robustness

Sr.no	Parameter	variation	Average peak area (n=3)		% RSD	
			METO	CILN	METO	CILN
01	Flow rate	0.8	799.407	1513.247	0.549	1.016
		1.2	843.422	1598.838	0.490	0.692
02	pH	3.3	801.559	1524.457	0.432	0.280
		3.7	827.753	1567.420	0.646	1.174
03	Mobile phase ratio	68:32	772.490	1459.473	0.619	0.856
		72:28	834.397	1583.377	0.712	0.583

Table 11: Degradation study of METO and CILN.

Sr No	Parameter	Standard Area		Sample Area		Standard Degradation		Sample Degradation	
		METO	CILN	METO	CILN	METO	CILN	METO	CILN
1	**Acid**	617.025	1391.360	604.738	1378.179	24.32	10.15	25.83	11.00
2	**Base**	610.306	1199.110	572.101	1232.605	25.15	22.57	29.83	20.40
3	**Oxidation**	688.87	1250.069	689.332	1235.535	15.51	19.28	15.45	20.21
4	**Thermal**	696.716	1133.575	709.869	1090.441	14.55	26.80	12.94	29.58
5	**Photolytic**	627.612	1352.495	613.871	1375.586	23.02	12.66	24.71	11.17

CONCLUSION

The linearity of developed method for simultaneous estimation of Cilnidipine and Metoprolol Succinate by RP-HPLC was achieved in the range of 2.5-7.5 mg/ml for Cilnidipine and 12.5- 37 mg/ml (r2=0.999) for Metoprolol Succinate. The results of precision, recovery and all other validation parameters are within acceptance criteria. From the validation result, we can conclude that developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, and hence we can be used for the routine analysis of Cilnidipine and Metoprolol Succinate in quality control department.

Acknowledgments

The authors are thankful to Niksan pharmaceutical for providing gift samples.

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