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DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR CILNIDIPINE AND METOPROLOL SUCCINATE IN THEIR COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Current research work describes rapid high performance thin layer chromatographic determination of Cilnidipine and Metoprolol Succinate form its combined pharmaceutical Dosage Form. The mention drugs were spotted on silica gel F_{254} TLC plates under pure nitrogen stream by Linomat TLC spotter. Separation was carried out by using Chloroform, Ethyl acetate, Methanol and Triethylamine as mobile phase in ratio of 9:2:0.5:0.5 v/v/v/v. Developed TLC plates were scanned by CAMAG TLC scanner and detection was carried out at 280 nm. R_f value of separated drugs was found to be 0.58 and 0.37 for Cilnidipine and Metoprolol Succinate respectively. The developed method was validated as per ICH guidelines by studying various validation parameters like Accuracy, Precision, Robustness, LOD, LOQ and solvent stability. The developed and validated method was successfully applied for determination of Cilnidipine and Metoprolol Succinate from its combined Pharmaceutical Dosage Form.

KEYWORDS

Cilnidipine, Metoprolol Succinate, High performance Thin Layer chromatography, densitometry analysis and Analytical method validation.

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INTRODUCTION

Cilnidipine is chemically O3-(2-methoxyethyl) O5-[(E) - 3 - phenylprop - 2- enyl] 2, 6-dimethyl-4- (3-nitrophenyl)-1,4-dihydropyridine- 3,5-dicarboxylate (Figure No.1). CIL is not official in Pharmacopoeia. Cilnidipine is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. It has greater selectivity for vascular smooth muscle. It has little or no action at

the SA or AV nodes and -ve inotropic activity is rarely seen at therapeutic doses.[1-5]Literature survey revealed that various, UV spectroscopy[6-7], Chromatographic[8-9] methods and LC/MS/MS10 method have been reported for quantitative estimation of CIL in pharmaceutical Dosage Form and biological fluids individually or in combination with other drugs.

Metoprolol Succinate is chemically, 1-[4-(2-methoxyethyl)phenoxy]- 3-(propan-2-ylamino) propan-2-ol (Figure No.2). METO is class of "Beta-blocker". Metoprolol completes with adrenergic neurotransmitters such as catecholamines for binding at β1 adrenergic receptors in the heart β1 receptor blockage result in a decrease in heart rate, cardiac output & blood pressure. Metoprolol is use in hypertension, angina, acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, congestive heart failure[11-15]. METO is official in IP[16], USP[17]. The review of literature revealed that various analytical methods involving Spectrophotometry[18-23], HPTLC[24-26] and HPLC[27-32] have been reported for METO in pharmaceutical formulation and biological fluids individually or in combination with other drugs.

The aim of study is to develop and validate high performance thin layer chromatographic method[33] for quantitation of Cilnidipine and Metoprolol Succinate in tablet dosage form as per ICH guidelines[34]. Finally the developed method was successfully applied for determination of tablet containing 10 mg of Cilnidipine and 50 mg of Metoprolol Succinate

MATERIAL AND METHODS

Material

Analytically pure CIL and METO were kindly Gifted by GIDC Industrial Estate, Ankleshwar Gujarat, India and Novartis Pharmaceutical corp as gratis samples. AR HPLC Methanol was used as solvent. Tablet of CIL and METO in combined dosage form, Cilacar was procured from local market. Silica Gel F_{254} plates was purchased from E Merck India Pvt. Ltd. Mumbai. Chloroform, Ethyl acetate, Methanol and Triethaylamine used was of purchased from S.D. fine Chemicals.

Instrument and Experimental Conditions

HPTLC analysis was carried out on silica gel $60F_{254}$ HPTLC plates (10 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 µL syringe and operated with settings of band length, 6 mm; distance between bands, 5 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin trough chamber previously saturated for 30 min with the mobile phase for a distance of 7 cm. For densitometry analysis, the spots on the air dried plate were scanned with the Scanner III at 254 nm using the deuterium source. Photograph of developed plates were taken by REPROSTAR camera at 280 nm.

Preparation of working solutions:

Based upon trial and error at laboratory scale finally it was decided to prepare stock solution of 200 µg/ml 1000 µg/ml of CIL and METO respectively.

Final mobile phase consisting Chloroform, Ethyl acetate, Methanol and Triethaylamine of 9:2:0.5:0.5 v/v/v/v in the ratio was placed in CAMAG TLC chamber and saturation was performed for 20 min. Final Separation was achieved using above mobile phase.

Analytical Method validation

Preparation of calibration curves/ Linearity Range

For preparation of linearity range, Synthetic mixture containing 200 mcg/ml of CIL and 1000 mcg/ml of METO was prepared and 100 µl Hamilton syringe was filed and aliquots of 1 µl, 2µl, 3µl, 4µl, 5µl, 6µl was applied under pure nitrogen stream to give rise to spots containing CIL in range of 200-1200 ng/spot and METO 1000-6000 ng/spot. Spotted plates were developed under stated condition and dried plates were scanned at 280 nm. Procedure was repeated for further 6 times (total n=6). Finally mean area was plotted against concentration (ng/spot) with help of WINCATS software.

Accuracy studies (Recovery)

Accuracy studies were performed by spiking test solution with standard solution. Accuracy studies

were performed at spiking level of 50, 100 and 150 % of target concentration. Here stock solution containing 2000 µg/ml of CIL and 10,000 µg/ml of METO was prepared from tablet formulation. Resulting solution was filtered and 0.2 ml of solution was transferred to each four 10 ml volumetric flask. Now from standard stock solution of 1000 µg/ml of CIL and 10,000 µg/ml of METO various aliquots were transferred to each 10 ml volumetric flask. Volume was made up to mark with methanol and 6 µl of solution was applied from each volumetric flask on to plate. Procedure was repeated for further 2 times and mean recovery for each level was calculated (n=3).

Method Precision (Repeatability)

For repeatability studies the linearity studies was repeated for 6 times without changing the syringe and position of plates. Data are collected from each set and Mean area, standard deviation and Coefficient of variance was calculated.

Intermediate Precision (Reproducibility)

Precisions of the proposed HPTLC methods were determined by analyzing mixed standard solution of CIL and METO at 3 different concentrations (400,800,1200 µg/spot for CIL and 2000, 4000, 6000 µg/spot for METO) 3 times on the same day (intraday precision) and on 3 different days (Interday Precision). The results are reported in terms of relative standard deviation (RSD).

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

Limit of detection and Quantitation was performed based upon signal to noise ration of instrument (Instrumental LOD and LOQ) and also performed as per ICH guidelines by using mean of slope and standard deviation intercept from calibration curve.

$LOD = 3.3 \times \sigma / S$, $LOQ = 10 \times \sigma / S$

Where, σ =the standard deviation (SD) of the response and

S = The SD of the *y* - intercept of the regression line.

Specificity Studies

The excipients such as Starch, Lactose and magnesium stearate were spiked into a reweighed quantity of drugs to assess the specificity of the methods. The peak area was measured to determine the quantity of the drugs.

Robustness

Robustness was performed by changing various method parameters like Composition of mobile phase, Size of TLC Chamber, Saturation time and plate pretreatment. Finally effect of these changes was observed for change in R_F value and change in peak area. Spot stability was observed by performing 2 - dimensional HPTLC development using the same mobile phase.

Analysis of Marketed formulation

Powder 10 tablets (Average weight of tablet 560 mg), and take powder equivalent to 10mg Cilnidipine and 50 mg of Metoprolol succinate. Dissolve power in 50 ml volumetric flask with 50 ml of Methanol. Sonicate for 10 minutes and make up volume up to mark with methanol. Filter above solution for whatmann filter paper (0.45 micron). Take 2 µl of above solution and dilute up to 10 ml with methanol. (Stock solution: 400 ng/ml of Cilnidipine and 2000 ng/ml of Metoprolo Succinate). From above solution apply 6 µl of volume on to pretreated Silica Gel F^{254} plates.

RESULTS AND DISCUSSION

Method optimization

Several mobile phases were tried to accomplish good separation of CIL and METO final separation was achieved using Chloroform, Ethyl acetate, Methanol and Triethylamine as mobile phase in ratio of 9:2:0.5:0.5 v/v/v/v .The developed plate was analyzed by densitometry and densitogram was recorded to check resolution (Figure No.4). R_f value of CIL and METO was found to be 0.58 ± 0.016 and 0.37 ± 0.007 respectively. For quantitation spots were scanned at 280 nm (Figure No.3). Finally all chromatographic conditions were optimized (Table No.1).

Analytical Method Validation

Linearity and range

The method was found to be linear with concentration of 200 -1200 ng/spot of CIL and 1000-6000 ng/spot of METO (Table No.2 and 3) as r^2 value was found to be 0.9978 for CIL and 0.9985

(Figure No.7 and 8). Overlain spectra in D view also showed a good linearity (Figure No. 5 and 6).

Method Precision (Repeatability studies)

Method was found to be repeatable as value of coefficient of variance was found to be less than 2 for CIL and METO at all concentration.

Intermediate Precision (Reproducibility)

Method was found to be reproducible as value of coefficient of variance was found to be less than 2 for CIL and METO at all given concentration for both interday and intraday.

Accuracy Study

Accuracy was performed by by spiking method at 50, 100, 150 % of target concentration. Recovery was found in the range of 98.50-99.75 % for CIL and 99.43-100.19s % for METO (Table No.4).

Determination of LOD and LOQ

Instrumental LOD was found to be 400 ng/spot for CIL and 2000 ng/spot for METO when found mathematically the value found was 1.19697 and 0.82099 for CIL and METO respectively. Instrumental LOQ was found to be 3.62719 and 2.487849 ng/spot of CIL and METO respectively (Table No.5 and 6).

Specificity Studies

The method was found to be specific as there was no interference from the commonly used excipients. Analysis was performed with the peak purity was found to be 0.996 and 0.998 for CIL and METO respectively.

Robustness

Minor modification were made in method parameters and changes were observed in peak are and R_f value. And it was found that the method was found to be robust as there was no significant change in peak area and R_f value except in chamber saturation time where less saturation time leads to significant change in peak area and R_f value (Table No.7).

Assay of Marketed Formulation

The developed method applied for quantitation of CIL and METO from its combined dosage form value was found to be 99.86±0.1477 and 99.94±0.0945 respectively (Table No.8).

Table No.1: Optimized Chromatographic conditions for CIL and METO

S. No	Parameter	Condition
1	Mobile phase	Chloroform:Methanol:Ethyl acetate: Triethylamine(9.5:2:0.5:0.5,v/v/v/v)
2	Diluent	mobile phase
3	Stationary phase	Silica gel G F_{254}
4	Distance run	70 mm
5	Chamber dimensions	10 x 10 cm
6	Saturation time	20 minutes
7	Temperature	Ambient
8	Detection wavelength	280 nm
9	R_f value	Cilnidipine: 0.37±0.01169 Metoprolol Succinate: 0.58±0.00894

Table No.2: Result of calibration readings for CIL by HPTLC method (n = 6)

S. No	Concentrations ng/spot	Area Mean ± S.D. (n=6)	Coefficient of Variation
1	200	3812.53 ± 71.1210	1.865
2	400	3989.63 ± 77.9825	1.954
3	600	4124.25 ± 80.7383	1.957
4	800	4301.9 ± 71.1396	1.653
5	1000	4418.91 ± 73.1245	1.654
6	1200	4588.61 ± 70.2736	1.531

Table No.3: Result of calibration readings for METO by HPTLC method (n = 6)

S. No	Concentration (ng/spot)	Area Mean ± S.D. (n=6)	Coefficient of Variation
1	1000	1209.45± 16.1017	1.331
2	2000	2007.70 ± 32.0126	1.594
3	3000	2628.75 ± 38.0669	1.448
4	4000	3258.86 ± 41.7269	1.280
5	5000	4044.46 ± 49.6623	1.227
6	6000	4759.21 ± 54.7948	1.151

Table No.4: Accuracy data for CIL and METO at 50, 100 and 150 % of target concentration n= 3 determination

% Level of Recovery	Amount of drug in sample (ng/spot)	Amount of standard Added (ng/spot)	Amount of drug recovered ± SD (ng/spot)	% Recovery ± SD
	CIL (ng/spot)	CIL (ng/spot)	CIL (ng/spot)	% CIL
Unspike	400	-	-	-
50 %	400	200	200.07 ± 1.3804	100.03±0.6902
100 %	400	400	397.75 ± 4.4901	99.43 ±1.1201
150 %	400	600	601.15± 2.5508	100.19±0.4251
	METO (ng/spot)	METO (ng/spot)	METO (ng/spot)	% METO
Unspike	2000	-	-	-
50 %	2000	1000	997.61±2.5518	99.75±0.2516
100 %	2000	2000	1976.64± 19.35	98.82±0.9696
150 %	2000	3000	2955.20±5.7923	98.50±0.1908

Table No.5: Determination of LOD and LOQ for Cilnidipine (n=3)

Determination of LOD (Limit of Detection)	
From mathematical Equation	Standard deviation of intercept:45.37205
	Mean of slop:125.0885
	Equation: 3.3x σ /s
	Result :1.196975
Determination of LOQ (Limit of Quantitation)	
From mathematical Equation	Standard deviation of intercept: 45.37205
	Mean of slop: 125.0885
	Equation: 10 x σ /s
	Result :3.627196

Table No.6: Determination of LOD and LOQ for Metoprolol Succinate (n=3)

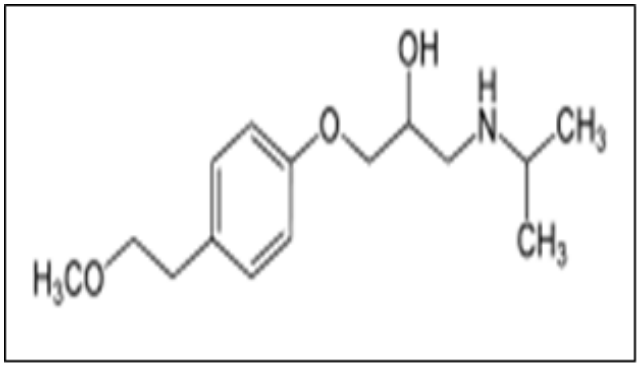
Determination of LOD (Limit of Detection)	
From mathematical Equation	Standard deviation of intercept:145.484
	Mean of slop: 584.7784
	Equation: 3.3x σ /s
	Result :0.82099
Determination of LOQ (Limit of Quantitation)	
From mathematical Equation	Standard deviation of intercept: 145.484
	Mean of slop: 584.7784
	Equation: 10 x σ /s
	Result :2.487849

Table No.7: Robustness studies for Cilnidipine and Metoprolol succinate

Change in parameters		CIL 400 ng/spot		METO 2000 ng/spot	
		Area ± S.D	**C.V**	**Area ± S.D**	**C.V**
Concentration of mobile phase	9.5:2:0.5:0.5	3923.86±49.112	1.25	1199.7±20.306	1.6926
	8:2:0.5:0.5	3912.76±69.317	1.77	1195.4±21.000	1.7567
Size of TLC chamber	20*20 cm	3857.93±40.502	1.04	1217.86±23.655	1.9423
	10*10 cm	3919.08±65.80	1.67	1206.73±21.522	1.7835
Plate pretreatment	Without treatment	3937.53±58.980	1.49	1991.63±23.525	1.1812

Table No.8: Assay result of Marketed Formulation (n=3)

Formulation	Drug	Amount taken (ng/spot)	Amount Found (ng/spot) (n = 3)	Labeled claim (mg)	Amount found per Tablet (mg)	% Label Claim± S.D
CILACAR	CIL	400	399.45± 0.5908	10	9.986	99.86±0.1477
	METO	2000	1998.87± 1.7644	50	49.97	99.94±0.0945



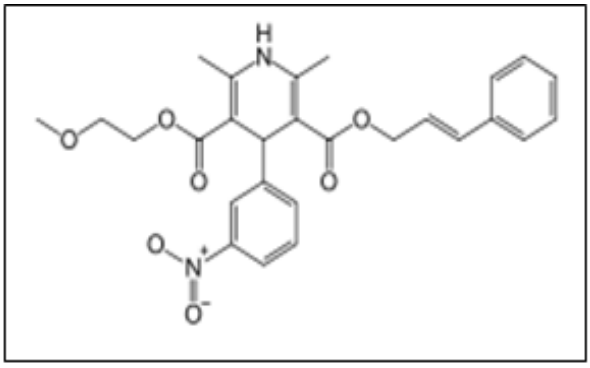


Figure No.1 and 2: Chemical structure of CIL and METO

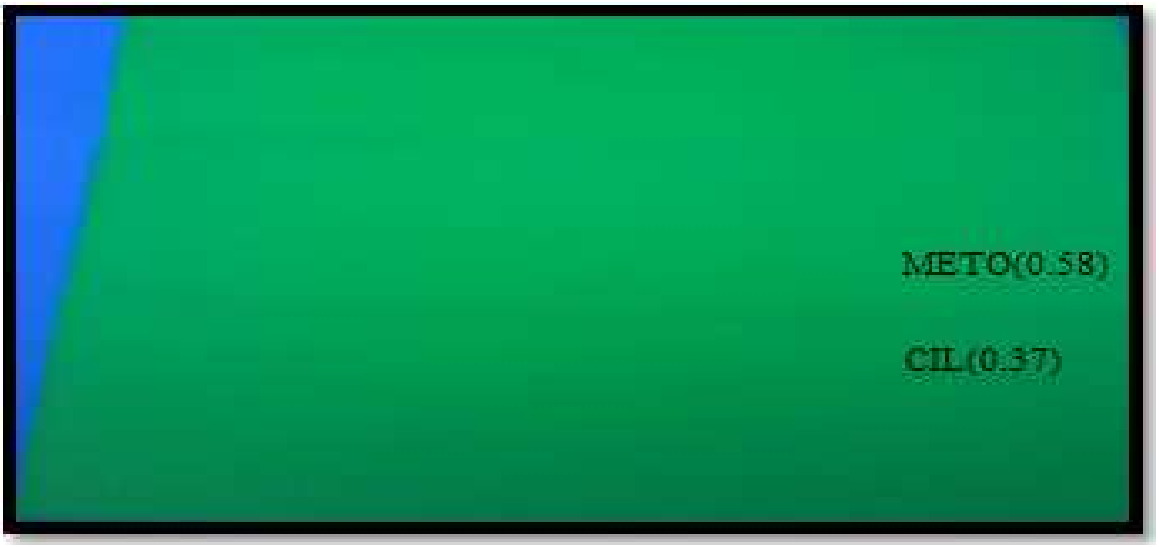


Figure No.3: Photograph of developed Plate in final Mobile phase

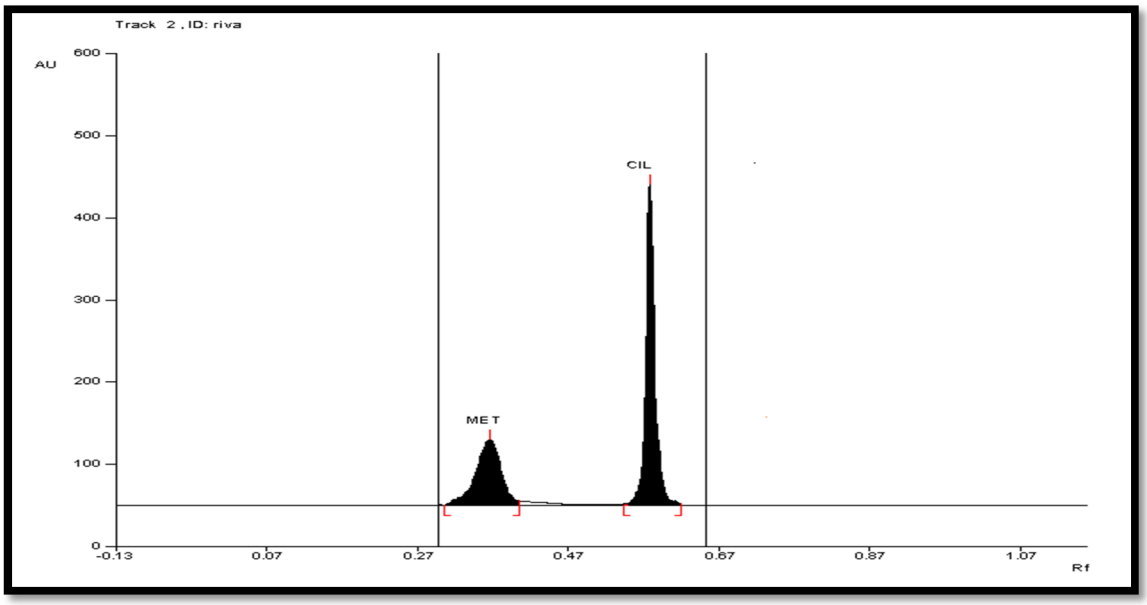


Figure No.4: Densitogram of standard solution of market formulation containing CIL and METO 400 ng/spot and 2000 ng/spot respectively using mobile phase Chloroform: Ethyl Acetate :Methanol: Triethylamine (9.5:2:0.5:0.5, v/v/v/v)

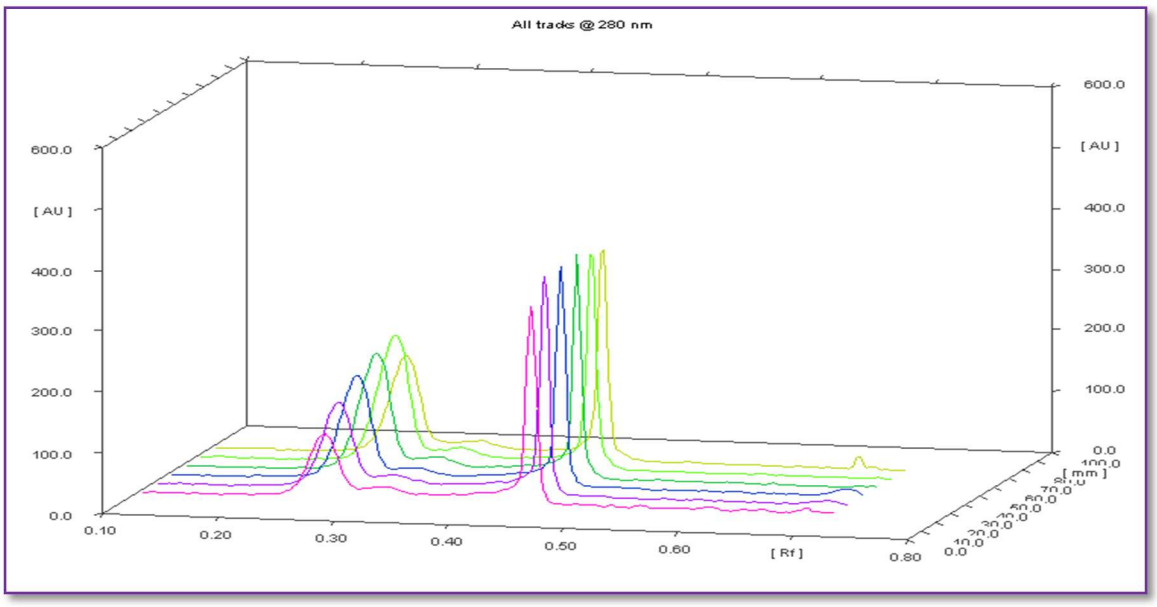


Figure No.5: Overlain view of all tracks of CIL and METO at 280nm

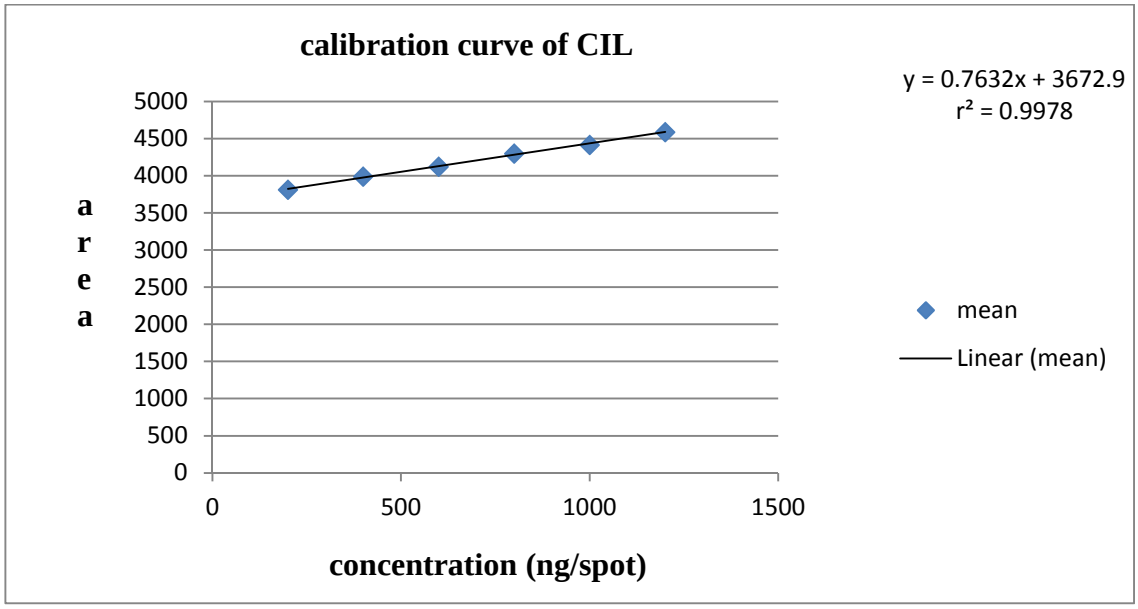


Figure No.6: Calibration Curve of CIL by HPTLC method

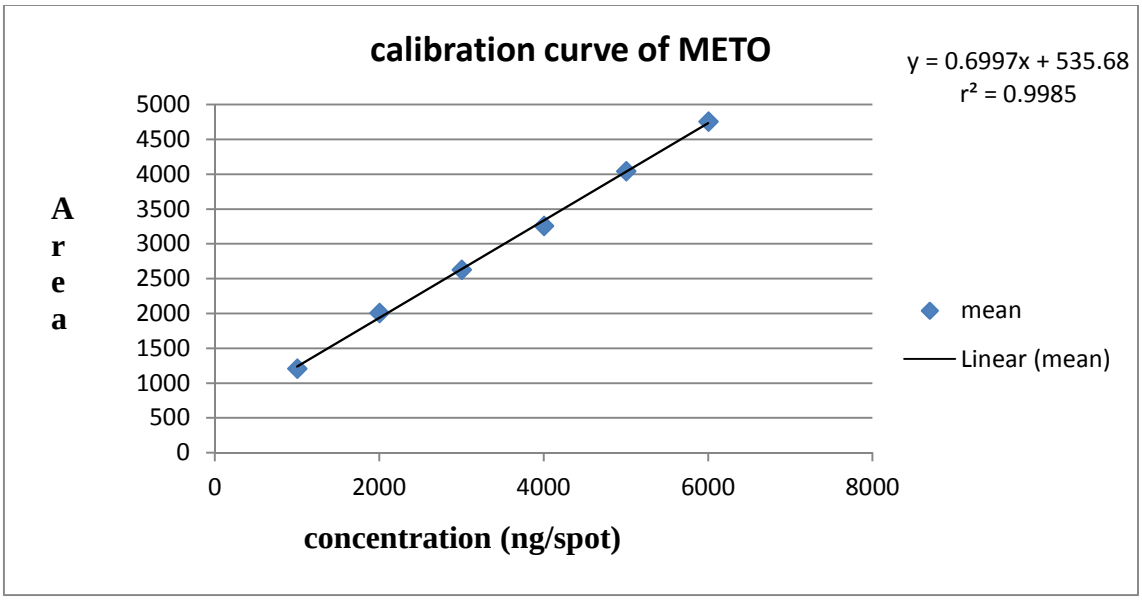


Figure No.7: Calibration Curve of METO by HPTLC method

CONCLUSION

The HPTLC method was successfully developed and validated as per ICH guidelines and was successfully applied for rapid determination of Cilnidipine and Metoprolol Succinate from its combined pharmaceutical Dosage Form. The developed and validated HPTLC method for CIL and METO was found to be simple, specific and cost effective for analysis of CIL and METO in their combined dosage form. The additives usually present in the pharmaceutical formulations of the assayed analytes did not interfere with determination of CIL and METO. The method can be used for the routine simultaneous analysis of CIL and METO in pharmaceutical preparations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. CIS Pharma Conferences, http://www.drugs.com/international/cilnidipine.html.
2. http://en.wikipedia.org/wiki/Cilnidipine.
3. J.B. Chemicals and Pharmaceuticals Ltd, Drug Information http://www.medindia.net/drug-price/cilnidipine/cilacar-10-mg.htm.
4. http://www.reachinformation.com/define/Cilnidipine.aspx.
5. Takahara A. Cilnidipine: a new generation Ca channel blocker with inhibitory action on sympathetic neurotransmitter release, *Cardiovasc Ther*, 27(2), 2009, 124-139.
6. Pankaj P, Chaudhari, Bhalerao A. Method validation for spectrophotometric estimation of cilnidipine, *International journal of pharmacy and pharmaceutical sciences*, 4(5), 2012, 96-98.
7. Faimida Jahan, Avnish Jain, Sumeet Prachand, Arun Kumar, Gupta *et al*. Simultaneous Estimation of Telmisartan and Cilnidipine in

Solid Dosage Form, *International Journal of Pharmaceutical and Research Science*, 1(1), 2012, 32-42.

8. Prajakta Pawar, Santosh V. Gandhi, Padmanabh B. Deshpande, Suvarna Vanjari and Swapnil U. Shelar. Simultaneous RP-HPLC estimation of cilnidipine and telmisartan in combined tablet dosage form, *Pelagia Research Library Der Chemica Sinica*, 4(2), 2013, 6-10.
9. Pawar Prajakta, Deshpande Padmanabh, Gandhi Santosh, Bhavnani Vandana. High Performance Thin Layer Chromatographic Determination of cilnidipine and Telmisartan in Combined Tablet Dosage Form, *International Research Journal of Pharmacy*, 3(6), 2012, 219-222.
10. Kyeong-Ryoon Lee A, Yoon-Jee Chae A , Jong-Hwa Lee A , Dae-Duk Kim A , Saeho Chong A, Chang-Koo Shim A *et al*. Quantification of cilnidipine in Human Plasma by liquid chromatography-mass spectrometry, *Journal of Liquid Chromatography and Related Technologies*, 35, 2012, 308-320.
11. http://en.wikipedia.org/wiki/Metoprolol.
12. http://www.everydayhealth.com/drugs/metoprolol-succinate-er.
13. http://www.rxlist.com/toprol-xl-drug.htm.
14. http://www.rxlist.com/toprol-xl-drug/side-effects-interactions.htm.
15. http://www.rxlist.com/toprol-xl-drug/clinical-pharmacology.htm.
16. Indian pharmacopoeia, *Govt. of India "Ministry of health and family welfare" the controller and publication, Delhi,* 16(1), 2007, 761-763.
17. United States Pharmacopoeia and National Formulary, (24th) Asian Edition, *The United States Pharmacopoeia Convention Inc, U.S.A*, 1593-1596.
18. Moreshwar N Kulkarni, Rajeshwar V Kshirsagar and Dinesh M Sakarkar. Development of Spectrophotometric method for determination of Metoprolol Succinate, *International Journal of ChemTech Research*, 1(4), 2009, 1273-1277.
19. Jadhav A S, Tarkase K N and Deshpande A P. Quantitative Determination of Metoprolol Succinate in bulk and tablet dosage form through comparative study of UV and derivative Spectroscopy, *Scholars Research Library Der Pharmacia Lettre*, 4(3), 2012, 763-767.
20. Disha D Patel and Mehul M Patel. Simultaneous Estimation of Metoprolol Succinate and Olmesartan Medoxomil in Pharmaceutical Dosage Form by UV Spectroscopy", *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 3(2), 202, 935-939.
21. Patel Prashant B, Marolia Bhavin P, Shah Sailesh A, Shah Dinesh R. Second order derivative spectrophotometric method for simultaneous estimation of Telmisartan and metoprolol in tablet dosage form, *International research journal of pharmacy*, 3(5), 2012, 259-62.
22. Disha D Patel and Mehul M Patel. Simultaneous Estimation of Metoprolol Succinate and Olmesartan Medoxomil in Pharmaceutical Dosage Form by UV Spectroscopy, *International Journal of Research in Pharmaceutical and Biomedical Sciences,* 3(2), 2012, 935-39.
23. Prajakta S Nawale, Atul A Shirkhedkar, Sanjay J Surana, and Amod S Patil. Normal and Reversed-Phase HPTLC Methods for Simultaneous Estimation of Telmisartan and Metoprolol Succinate in Pharmaceutical Formulation, *Analytical Chemistry*, 4(1), 2012, 1-6.
24. Yogeshvary S Bhangale, Santosh V Gandhi, Padmanabh B Deshpande, Sachin E Potawale. A Validated HPTLC method for simultaneous estimation of Metoprolol succinate and Isosorbide mononitrate in combined capsule dosage form, *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(4), 2012, 217-220.
25. Sagar B Wankhede, Nitin R Dixit and Sohan S. Chitlange. Stability Indicating HPTLC method for Quantitative Determination of Atorvastatin calcium and Metoprolol succinate in Capsules, *Scholars Research Library, Der Pharmacia Lettre*, 3(1), 2011, 1-7.

26. Mitesh D Phale and Purnima D Hamrapurkar. A Validated and Simplified RP- HPLC of Metoprolol Succinate from Bulk Drugs, *Asian J. Research Chem*, 2(2), 2009, 119-122.
27. Bhargavi Durga K, Naga Mounika I, Shajhan SK, Srinivasa rao N, Sarath Chandra. RP- HPLC method for estimation of Metaprolol in bulk drug, *International journal of pharmaceutical research and development*, 1(2), 2012, 151-157.
28. Aqil M, Ali A, Ahad M, Sultana Y, Najmi A K. Validated HPLC method for estimation of Metoprolol in Human plasma, *ACTA Chromatographica*, 19, 2007, 130-140.
29. Prasada Rao C H M M, Rahaman S A, Ragjendra Prasad Y, Gangi Reddy P. RP-HPLC method of simultaneous estimation of Amlodipine Besylate and metoprolol in combined dosage form, *International journal of pharmaceutical research and development*, 2(9), 2010, 69 - 76.
30. Mital Gosai, Rupal Tanna, Kashyap Thumar, Jasmin Chikhalia. RP-HPLC method for simultaneous estimation of Metoprolol succinate and Clopidogrel bisulphate from tablet dosage, *Inventi Rapid: Pharm Analysis and Quality Assurance*, 3, 2012, 1-7.
31. Chandra Bose RJ, Sivanseya G, Krishna kumar D. Validated Rp-Hplc Method for the Simultaneous Estimation of Ramipril and Metoprolol Tartrate in Bulk and Tablet Dosage Form, *Asian Journal of Biochemical and Pharmaceutical Research*, 1(2), 2011, 171-177.
32. Silkan Patel, Mehul Patel, Jagdish Kakadiya *et al*. Development and Validation of High Performance Thin Layer Chromatographic Method for Levosulpiride and Pantoprazole Sodium in Combined Dosage Form, *Inventi Rapid: Pharm Analysis and Quality Assurance*, 1(2), 2013, 1-8.
33. ICH Harmonized Tripartile Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), *International Conference on Harmonization, Geneva, Switzerland*, 2005.

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