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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND OLMESARTAN MEDOXOMIL IN BULK AND TABLET DOSAGE FORM BY HPTLC

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ABSTRACT

Cilnidipine and Olmesartan medoxomil in combined dosage form has been developed and validated. Sample and standard solutions of Cilnidipine and Olmesartan medoxomil were applied to precoated silica gel G 60 F254 HPTLC plates and the plates were developed with Methanol: Toluene: Ethyl acetate: Acetic acid in the ratio 2.5: 5.5: 2.0: 0.1 (v/v/v/v) as mobile phase. The Rf value for cilnidipine and Olmesartan medoxomil was found to be 0.66 and 0.43 respectively The detection was performed at 254 nm. The calibration curve was found to be linear between 100 to 200 ng/spot for cilnidipine and 200 to 400 ng/spot for Olmesartan medoxomil with correlation coefficients 0.995 and 0.996 for Cilnidipine and Olmesartan medoxomil respectively. The LOD and LOQ were found to be 23.057ng/spot and 69.86 ng/spot for Cilnidipine and 39.18ng/spot and 118.750 ng/spot for Olmesartan medoxomil respectively. The results have been validated statistically as per ICH guidelines.

Keywords: Validation, Olmesartan medoxomil, Cilnidipine, HPTLC

1. INTRODUCTION

Cilnidipine (CILNI), chemically, 1,4-Dihydro- 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2-methoxyethyl(2E)-3-phenyl-propenyl ester is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals.[1] Olmesartan medoxomil (OLME) chemically is 2,3-dihydroxy-2-butenyl-(1-hydroxy-1-methylethyl)-2-propyl-1-[P-(O-1H-tetrazole-5 ylphenyl)benzyl] imidazole-5-carboxylate, cyclic 2,3-carbonate. Olmesartan medoxomil is a prodrug, which after ingestion liberates the only active metabolite, Olmesartan. Olmesartan is a competitive and selective angiotensin 2 receptor antagonist. The hydrolysis of OLME occurs readily by the action of esterase which is present abundantly in the gastrointestinal tract, liver and plasma and is used alone or with other antihypertensive agents to treat hypertension [1].

Literature survey reveals spectrophotometric [2-4], reverse phase high-performance liquid chromatography (RP-HPLC) [5, 6] and high performance thin layer chromatography (HPTLC) [7, 8] methods for the determination of CILNI either as a single or in combination with other drugs in pharmaceutical preparations. Analytical methods reported for OLME includes spectrophotometric [9, 10], HPLC [11-13], and HPTLC [14, 15] either as a single drug or in combination with other drugs. No HPTLC method of analysis has yet been reported for simultaneous analysis of CILNI and OLME. This paper describes a rapid, accurate, economical and validated high-performance thin layer chromatographic (HPTLC) method for the simultaneous quantification of these compounds in bulk and tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All the chemicals and reagents used were of analytical grade. Cilnidipine were obtained as gift sample from J B Chemicals and Pharmaceuticals, Daman and Olmesartan medoxomil were obtained as gift sample from Macleods Pharmaceuticals limited, Mumbai. The commercial formulation for combined dosage form was purchased from local market.

2.2. Instrumentation

Pre-coated silica gel 60F254 aluminium plates (10 x 10 cm, 250 µm thickness; Merck, Germany), Automatic TLC sampler 4 (Camag, Switzerland), twin trough chamber (10 x 10 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner 4 (Camag, Switzerland), winCATS version 1.4.6 software (Camag, Switzerland) were used in the study. Ultrasonic bath (PowerSonic405, Hwashin technology, Korea) and Electronic balance Shimadzu AX200, (Shimadzu Corporation, Japan) were used in the study.

2.3. Preparation of standard solutions

Stock solutions for measurements were prepared by dissolving Cilnidipine and Olmesartan medoxomil separately in methanol to obtain concentration of 1000µg/ml for each

compound. For calibration, by diluting the stock standard solution with methanol in 10 ml standard volumetric flasks series of solutions were prepared containing 100, 120, 140, 160, 180, 200 ng/band for Cilnidipine and 200, 240, 280, 320, 360, 400 ng/band for Olmesartan medoxomil. Mixed standard solution containing final concentration 10 µg/ml of Cilnidipine and 20 µg/ml of Olmesartan medoxomil was prepared from the stock solutions.

2.4. Preparation of Sample solution

Twenty tablets of brand Nexovas-o (Macleods Pharmaceuticals Ltd.) containing 10 mg of Cilnidipine and 20 mg of Olmesartan medoxomil were weighed, average weight determined and finely powdered. Appropriate quantity of powder equivalent to 10 mg of Cilnidipine and 20 mg Olmesartan medoxomil was accurately weighed and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol and shaken vigorously for 5 minutes. The solution was then sonicated for 20 minutes and filtered through the Whatman filter paper no.41. Necessary dilutions of filtrate were made with methanol to get final concentration 10 µg/ml of Cilnidipine and 20 µg/ml of Olmesartan medoxomil.

2.5. Selection of mobile phase

A trial and error method was used to select the optimised mobile phase. The solvent system of Methanol:Toluene:Ethylacetate:Acetic acid in the ratio 2.5:5.5:2.0:0.1 (v/v/v/v) was the most appropriate mobile phase for the HPTLC analysis of Cilnidipine and Olmesartan medoxomil in methanol as solvent.

2.6. Application of standard solutions

Separate HPTLC pre-coated plates of silica gel G 60 F254 (10x10) were employed for the spotting of standard solutions. 10 µl to 20 µl of standard solutions of concentration 100, 120, 140, 160, 180 and 200 ng/band of Cilnidipine and 200, 240, 280, 320, 360 and 400ng/band for Olmesartan medoxomil standard solutions were applied in the six tracks respectively in two different plates.

2.7. Application of sample solution

10µl of the mixed standard solution of 10µg/ml for cilnidipine and 20µg/ml for Olmesartan medoxomil was applied. The same procedure was repeated with the sample solution prepared from tablet dosage form. After application the position of spots were visualized and confirmed under UV cabinet at 254nm.

2.8. Development of spot

Twin Trough chamber containing 10 ml of mobile phase system was used for developing the spotted plates and saturated for 15 minutes. The plates were dried after development and viewed under UV lamp to evaluate the spot obtained. The spots were uniform and there was no tailing.

2.9. Scanning by HPTLC scanner

After setting up the instrument parameters, the spot was scanned from 200-400nm and the spots showed maximum absorption at 254nm using the Camag TLC scanner 4. (Figure 1) The Rf values were found to be 0.66 for cilnidipine and 0.43 for Olmesartan medoxomil. Typical chromatograms obtained for Cilnidipine RS and Olmesartan medoxomil RS separately and of sample. (Figure 2, 3 and 4)

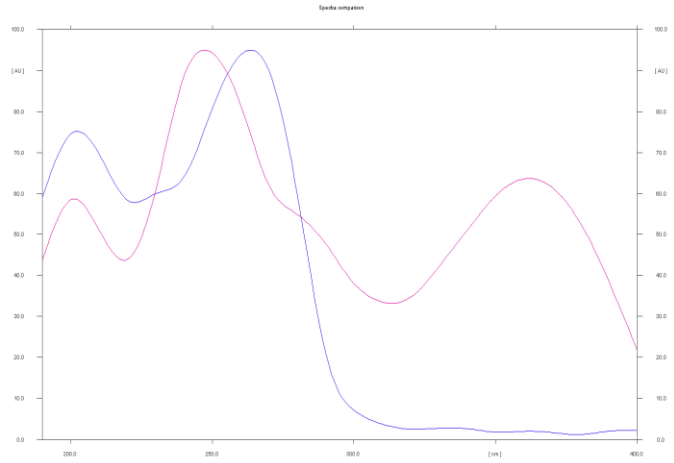

Figure 1. Overlain spectra of CILNI and olme from 200-400nm

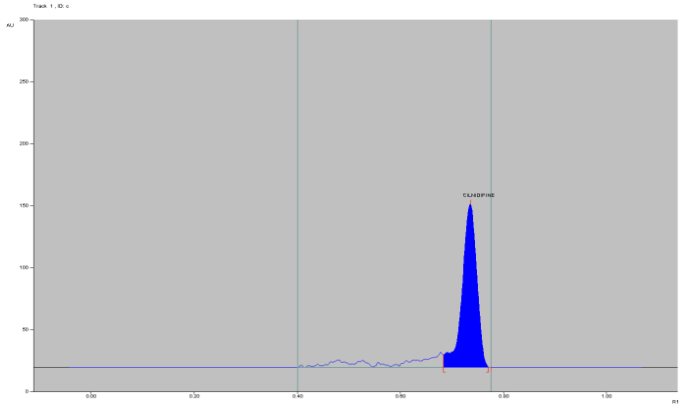

Figure 2 Typical chromatograms obtained for Cilnidipine

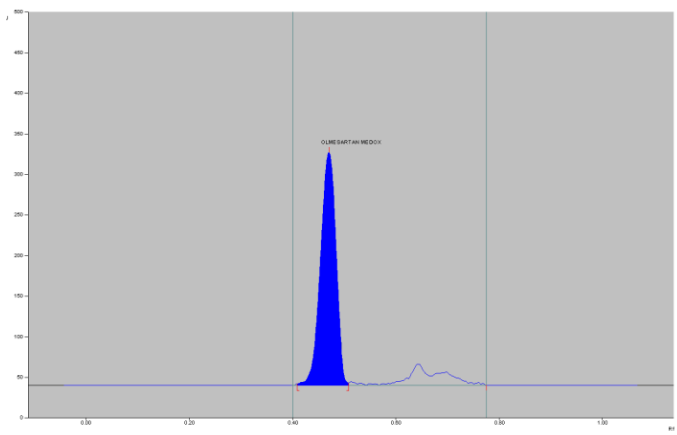

Figure 3 Typical chromatograms obtained for Olmesartan medoxomil

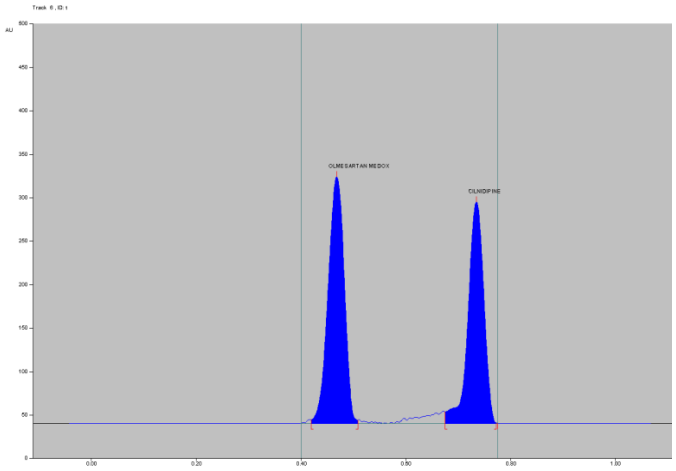

Figure 4 Typical chromatograms obtained for sample Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision and specificity, in accordance with ICH guidelines [16].

2.10. Calibration curve

Response to Cilnidipine and Olmesartan medoxomil was linear in the concentration ranges 100-200ng/spot for Cilnidipine and 200-400 ng/spot for Olmesartan medoxomil respectively. The regression equations for Cilnidipine and Olmesartan medoxomil were y = 13.41x + 726.0and y = 4.140x + 584.8 respectively, where y is response and x the concentration of drug. The correlation coefficients were 0.995 and 0.996 respectively. [Table 1 and Table 2]

Table 1: Concentration, Rf, and Area of Cilnidipine peak

Amount ng/spot	Rf	Area
100	0.66	2061.67
120	0.66	2332.48
140	0.66	2588.31
160	0.66	2890.90
180	0.66	3195.52
200	0.66	3361.50

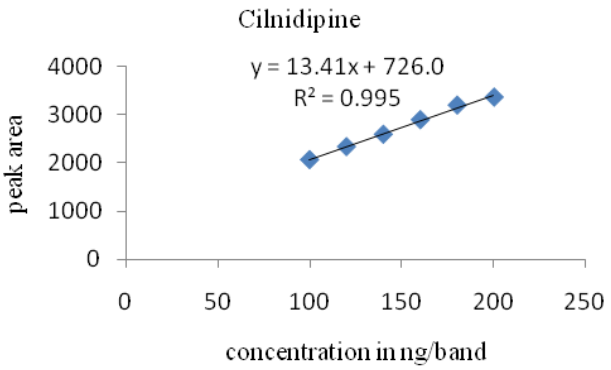

Figure 5 Calibration curve for CILNI

Table 2: Concentrations, Rf, and Area of Olmesartan medoxomil peak

Ammount (ng/spot)	Rf	Area
200	0.43	1425.31
240	0.43	1589.9
280	0.43	1709.54
320	0.44	1906.79
360	0.44	2077.51
400	0.43	2252.5

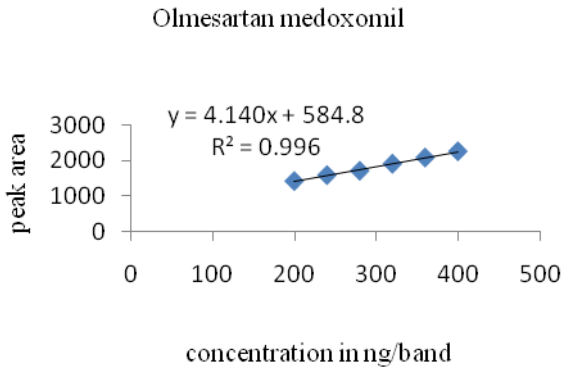

Figure 6 Calibration curve for OLME

2.11. Assay for marketed preparation

Twenty tablets of Nexvas-o containg 10mg of Cilnidipine and 20mg of Olmesartan Medoxomil were weighed and average weight was determined, tablets were triturated to fine powder. Tablet powder equivalent to 10 mg of Cilnidipine and 20mg of Olmesartan Medoxomil was transferred in 100 ml volumetric flask and were dissolved in methanol then the solution was ultrasonicated for 20 min. and filtered through Whatman filter paper No. 41.The filtrate was appropriately diluted with Methanol to obtain 10 µg/ml of Cilnidipine and 20 µg/ml of Olmesartan Medoxomil. The plate was developed under previously described chromatographic conditions.

Table 3: Assay for marketed formulation

Brand name	Drug	Label claim	Amount of drug found	% Amount of drug found
Nexovas-o Tablet	CILNI	10 mg	9.8 mg	98.10
	OLME	20 mg	19.98 mg	99.81

2.12. Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Cilnidipine and Olmesartan medoxomil by the standard addition method. Known amount of standard of cilnidipine and Olmesartan medoxomil (80%, 100%, and 120%) were added to sample solutions of tablet

dosage forms. The amounts of Cilnidipine and Olmesartan medoxomil were estimated by substituting values in the regression equations (y = 13.41x + 726.0 and y = 4.140x + 584.8). The % recovery found to be between 98.48-100.87 % indicates that the method is accurate.

Table 4: Recovery Studies

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	%Recovery*	%RSD
	80	64	145.37	100.05	1.13
CILNI	80	80	161.39	100.87	1.37
	80	96	173.57	98.61	0.19
	160	128	286.61	99.51	0.23
OLME	160	160	315.15	98.48	0.33
	160	192	346.83	98.53	0.85

Table5: Statistical evaluation of precision studies

Drug	Drug content %*	Std. Dev.	%RSD	SE
Inter-Day Precision				
CILNI	99.70	0.408	0.409	0.16
OLME	99.66	0.334	0.335	0.13
Intra-Day Precision				
CILNI	98.11	0.809	0.825	0.33
OLME	99.06	0.214	0.216	0.028

2.13. Precision studies

The precision of the method was checked by repeatedly scanning (n = 6) standard solutions of Cilnidipine and Olmesartan medoxomil 100 ng/band and 200ng/band respectively on the same day and on different days. The RSD values were found to be below 2% which indicate that the proposed methods are precise.

2.14. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and the LOQ of the drug were calculated using the equations 3.3 σ /S and 10 σ /S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

2.15. Specificity

Specificity study was performed by analyzing standard of drugs and samples. The spot for OLME and CILNI in sample was confirmed by comparing the Rf value and spectra of the spot with that of standards.

3. RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 100-200 ng/spot for CILNE and 200-400 ng/spot for OLME with r^2 = 0.995 and 0.996 respectively in mobile phase Methanol:Toluene:Ethyl acetate:Acetic acid in the ratio 2.5:5.5:2.0:0.1v/v/v/v. The detection was done at 254nm Rf value was found to be 0.66 and 0.43 for Cilnidipine and Olmesartan medoxomil respectively. The proposed method was also evaluated by the assay of commercially available tablet and % assay was found to be 98.10 % for CILNI and 99.81% for OLME. The accuracy of the proposed method was studied by recovery studies at three levels (80%, 100% and 120%). The % recovery was found to be in the range of 98.61 to 100.87 for CILNI and 98.48 to 99.51for OLME. The precision of the proposed method was studied by interday and intraday precision. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. The summary of validation parameters of proposed HPLC method is given in Table 6.

Table 6: Summary of validation parameters

Parameter	CILNI	OLME
Linearity range (ng/ spot)	100-200	200-400
Correlation co-efficient	0.995	0.996
Slope (m)	13.41	4.140
Intercept (c)	726.0	584.8
Precision (intraday) %RSD	0.825	0.216
Precision (interday) %RSD	0.409	0.335
Accuracy	98.61-100.87	98.48-99.51
LOD (ng /spot)	23.05	39.18
LOQ (ng /spot)	69.86	118.75

4. CONCLUSION

The developed and validated HPTLC method is found to be rapid, accurate, precise and economical, thus can be used for routine analysis of Olmesartan medoxomil and Cilnidipine in combined tablet dosage form.

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