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Research Article

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF CILNIDIPINE AND TELMISARTAN IN COMBINED TABLET DOSAGE FORM

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ABSTRACT
A new simple high performance thin layer chromatography (HPTLC) method for determination of Cilnidipine and Telmisartan in combined dosage form has been developed and validated. The separation was carried out on Merck aluminium plates precoated with silica gel 60 F_{254} using Toluene: Methanol: Ethyl acetate (8: 2: 1, v/v/v) as the mobile phase, and detection was carried out at 260 nm. Results were linear in the range of 200-1200 ng/band for Cilnidipine and 800-4800 ng/band for Telmisartan. The method was successfully applied for the analysis of drugs in pharmaceutical formulation. Results of the analysis were validated statistically and by recovery studies.
Keywords: Cilnidipine, Telmisartan, High performance thin layer chromatography

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 that supply blood vessels [1].Telmisartan (TELMI) is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1(AT_1), with a binding affinity 3000 times greater for AT_1 than AT_2 [2].

Literature survey reveals reverse phase high-performance liquid chromatographic (RP-HPLC) [3], LC-MS [4, 5] and high performance thin layer chromatographic (HPTLC) [6] methods for the determination of CILNI either as single or in combination with other drugs in human plasma and in pharmaceutical preparations. Analytical methods reported for TELMI includes HPLC [7-12], Spectrophotometric [13-16], UPLC [17] and HPTLC [18] either as single drug or in combination with other drugs.

To the best of our knowledge no reports were found for simultaneous estimation of CILNI and TELMI in combined dosage form. This paper describes a simple, accurate and validated HPTLC method for the simultaneous quantification of these compounds as a bulk drug and in tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [19].

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade working standards CILNI and TELMI were obtained as gift samples from J. B. Chemicals and Pharmaceuticals Ltd. (Mumbai, India) and FDC Ltd. (Goa, India), respectively. The pharmaceutical dosage form used in this study was Cilacar T tablets (**J. B. Chemical and Pharmaceuticals Ltd., Mumbai, India)** labeled to contain 10 mg of CILNI and 40 mg of TELMI were procured from the local market. Methanol, Toluene, Ethyl acetate (all AR grade) were obtained from Sisco Research Laboratories (Mumbai, India).

Instrumentation and Chromatographic Conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 5mm, with 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) on precoated Silica gel 60 F_{254} plates (20 x 20 cm) with layer thickness 250µm (E. MERCK , Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm x 0.45 mm and scanning speed of 20 mm / sec was employed. The linear ascending development was carried out in 10 cm x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Methanol: Ethyl acetate (8: 2: 1, v/v/v)) as mobile phase. The optimized chamber saturation time was approximately 15 min. The development distance was 9 cm and the development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner 3 at 260 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400nm.

Preparation of Standard Stock Solutions

Standard stock solution of CILNI and TELMI was prepared separately by dissolving 10 mg of drug in 10 ml Methanol to get concentration of 1000 ng /µl.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 260 nm. So 260 nm was selected as the wavelength for detection Figure 1.

Preparation of Calibration Curves

The standard stock solutions of CILNI and TELMI (1000 ng/µl each) were applied by overspotting on TLC plate in range of 0.2, 0.4, 0.6, 0.8, 1, 1.2 µl and 0.8, 1.6 , 2.4, 3.2, 4, 4.8 µl respectively with the help of CAMAG 100 µl sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in six replicates was analyzed and peak areas were recorded. Calibration curves of CILNI and TELMI were plotted separately of peak area vs respective concentration of CILNI and TELMI.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of TELMI (2.5 mg of CILNI) was weighed and transferred to 10 ml volumetric flask containing about 6 ml of Methanol and ultrasonicated for 10 min and volume was made upto the mark with the Methanol. The solution was filtered through Whatman paper No. 41.and one µl volume of this solution was applied on TLC plate to obtain final concentration of 1000 ng/band for TELMI and 250 ng/band for CILNI. After chromatographic development peak areas of the bands were measured at 260 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Robustness Studies

In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs was examined. Factors varied were mobile phase composition (± 2 %), mobile phase saturation (± 10 %) , development distance (± 10 %), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to estimate the effect. Robustness of the method was checked at a concentration level of 600 ng/ band for CILNI and 2400 ng/ band for TELMI.

Recovery Studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Chromatogram was developed and the peak areas were noted. At each level of the amount, three determinations were carried out. The results of recovery studies were expressed as percent recovery and are shown in Table 1.

Precision

Set of three different concentrations in three replicates of mixed standard solutions of CILNI and TELMI were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. For Inter day variation study, three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves.

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of Toluene, Methanol and Ethyl acetate were examined (data not shown). Finally the mobile phase containing Toluene: Methanol: Ethyl acetate (8: 2: 1, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 260 nm. The retention factors for TELMI and CILNI were found to be 0.51 ± 0.008 and 0.71 ± 0.012 respectively. Representative densitogram of mixed standard solution of TELMI and CILNI is shown in Figure 2.

Straight-line calibration graphs were obtained in the concentration range 200-1200 ng/band for CILNI and 800-4800 ng/band for TELMI with high correlation coefficient > 0.998. The calibration plots obtained for CILNI and TELMI are shown in Figure 3 and Figure 4. The proposed method was also evaluated by the assay of commercially available tablets containing CILNI and TELMI. The % assay (Mean ± S.D.) was found to be 100.09 ± 0.43 for CILNI and 100.33 ± 0.75 for TELMI. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2), which demonstrated that the HPTLC method developed is robust.

For CILNI, the recovery study results ranged from 100.28 to 100.95 % with % RSD values ranging from 0.61 to 0.90. For TELMI, the recovery results ranged from 99.58 to 100.06 % with % RSD values ranging from 0.58 to 0.94. The method was found to be accurate and precise, as indicated by recovery studies as recoveries were close to 100 % and % RSD not more than 2. Intra-day variation, as RSD (%), was found to be in the range of 0.31–0.97 for CILNI and 0.36–0.88 for TELMI. Interday variation, as RSD (%) was found to be in the range of 0.49–0.88 for CILNI and 0.21–0.90 for TELMI. The summary of validation parameters of proposed method are given in Table 2.

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of CILNI and TELMI in combined tablet dosage form.

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Table 1: Recovery studies of CILNI and TELMI

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% RSD[a]
CILNI	400	200	601.72	100.28	0.72
	400	400	807.60	100.95	0.61
	400	600	1003.08	100.30	0.90
TELMI	1600	800	2401.64	100.06	0.94
	1600	1600	3186.56	99.58	0.83
	1600	2400	3998.33	99.95	0.58

*Average of three determinations.

Table 2: Summary of validation parameters of HPTLC method

Parameters	TELMI	CILNI
Linearity range (ng/ band)	800-4800	200-1200
Correlation co-efficient	0.999	0.999
LOD[a] (µg /ml)	188.28	36.48
LOQ[b] (µg/ ml)	570.54	110.56
Accuracy (% Recovery)	99.58-100.06	100.28- 100.95
Precision (% R.S.D.)[c]		
Intra day (n[d] = 3)	0.36– 0.88	0.31–0.97
Inter day (n = 3)	0.21–0.90	0.49–0.88

[a]LOD = Limit of detection.
[b]LOQ = Limit of quantitation.
[c]RSD = Relative standard deviation.
[d]n = Number of determinations

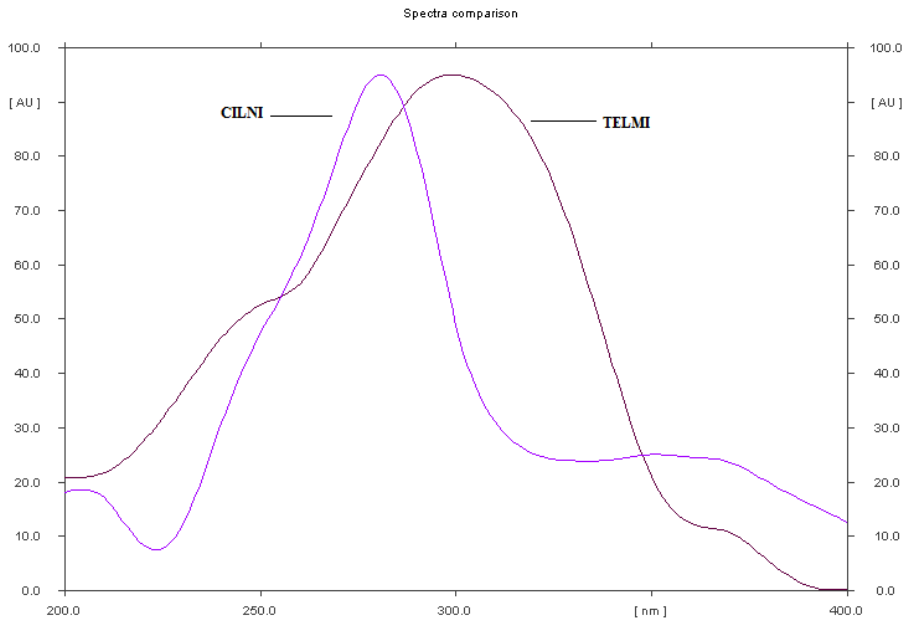

Figure 1: Overlain spectra of CILNI and TELMI from 200 to 400 nm

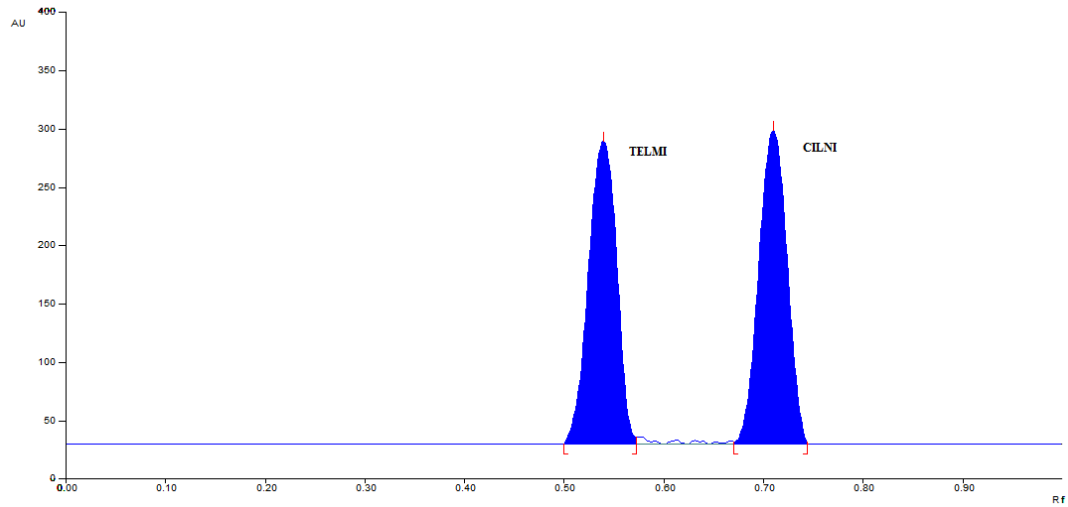

Figure 2: Representative chromatogram of mixed standard solution of TELMI (2400 ng/band, $R_f = 0.51 \pm 0.008$) and CILNI (600 ng/band, $R_f = 0.71 \pm 0.012$)

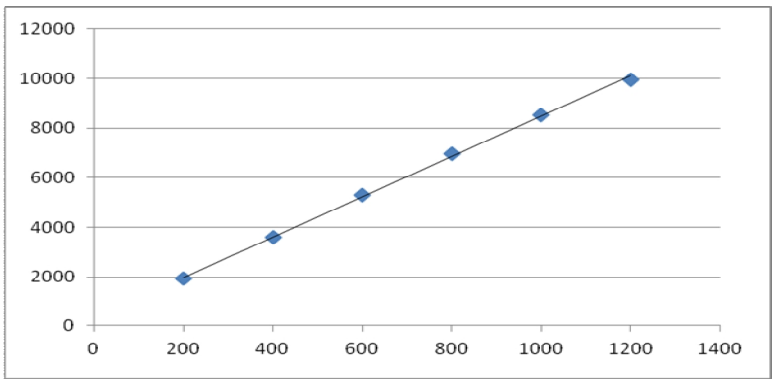

Figure 3: Calibration curve for CILNI

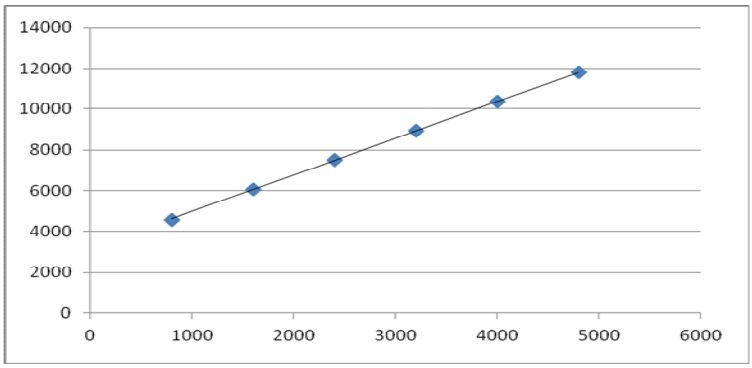

Figure 4: Calibration curve for TELMI

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