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Simultaneous RP-HPLC estimation of cilnidipine and telmisartan in combined tablet dosage form

Prajakta Pawar, Santosh V. Gandhi*, Padmanabh B. Deshpande, Suvarna Vanjari and Swapnil U. Shelar

Department of Pharmaceutical Analysis, A. I. S. S. M. S. College of Pharmacy, Pune, MH, India

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ABSTRACT

This paper describes a simple, accurate, and validated reverse-phase high-performance liquid chromatographic method for the simultaneous quantification of Telmisartan and Cilnidipine as the bulk drug and in tablet dosage forms. Separation was carried out on Jasco HPLC system equipped with HiQ sil C_{18} HS column (250 × 4.6 mm i.d.) and PDA detector using Methanol: 40 mM Potassium dihydrogen ortho phosphate buffer (pH 3) (90:10, v/v)) as the mobile phase, and detection was carried out at 245 nm. Results were linear in the range of 1-10 μg mL^{-1} for Cilnidipine and 5-30 μg mL^{-1} 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, RP-HPLC, Tablet dosage form

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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), 4′-[1(1,4′ - Dimethyl-2′-propyl [2,6′-bi-1H-benzimidazol]-1′-yl) methyl] - [1,1′-biphenyl] -2-carboxylic acid is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor [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 a 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 a single drug or in combination with other drugs.

To the best of our knowledge no HPLC method of analysis has yet been reported for simultaneous analysis of CILNI and TELMI. This paper describes a simple, accurate, and validated reverse-phase high-performance liquid chromatographic (RP-HPLC) 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 from J. B. Chemicals and Pharmaceuticals Ltd. (Mumbai) and FDC Ltd. (Goa), India respectively used as such without further purification. 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 (HPLC grade), Potassium dihydrogen phosphate (AR grade), Ortho phosphoric acid (AR grade) purchased from Merck specialties Pvt. Ltd. (Mumbai, India) and double distilled water were used in analysis.

Instrumentation and Chromatographic Conditions

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pumpand MD 2010 PDA detector and JASCO Borwin PDA 1.5 version software was used for analysis. Separation was carried out on HiQ sil C_{18} HS (250 x 4.6 mm i.d.) column using as mobile phase methanol: 40 mM potassium dihydrogen ortho phosphate buffer (pH 3) (90:10, v/v) at flow rate of 1 mL min^{-1} .Samples were injected using Rheodyne injector with 50 µL loop and detection was carried out at 245 nm.

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 µg mL^{-1} from which 1 ml of solution was further diluted to 10 mL with mobile phase to get a working standard solution having concentration 100 µg mL^{-1} for both the drugs.

Procedure for 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. One mililitre of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the mobile phase to get solution of concentration 10 µg mL^{-1} for TELMI and 2.5 µg mL^{-1} for CILNI. 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 injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

System Suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg mL^{-1} and 10 µg mL^{-1} of CILNI and TELMI respectively. The resolution, peak asymmetry, number of theoretical plates, and HETP were calculated as represented in Table1.

Table 1: System suitability parameters for RP-HPLC method

Sr. No.	Parameters	TELMI	CILNI
1	Theoretical Plates	7251.12	5886.45
2	HETP (cm)	0.0034477	0.004247
3	Asymmetry Factor	1.01	0.90
4	Resolution [a]	--	14.96

[a] *With respect to previous peak.*

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. Mean retention time and standard deviation was found to be 2.587 ± 0.0014 for TELMI and 5.720 ± 0.0032 for CILNI respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

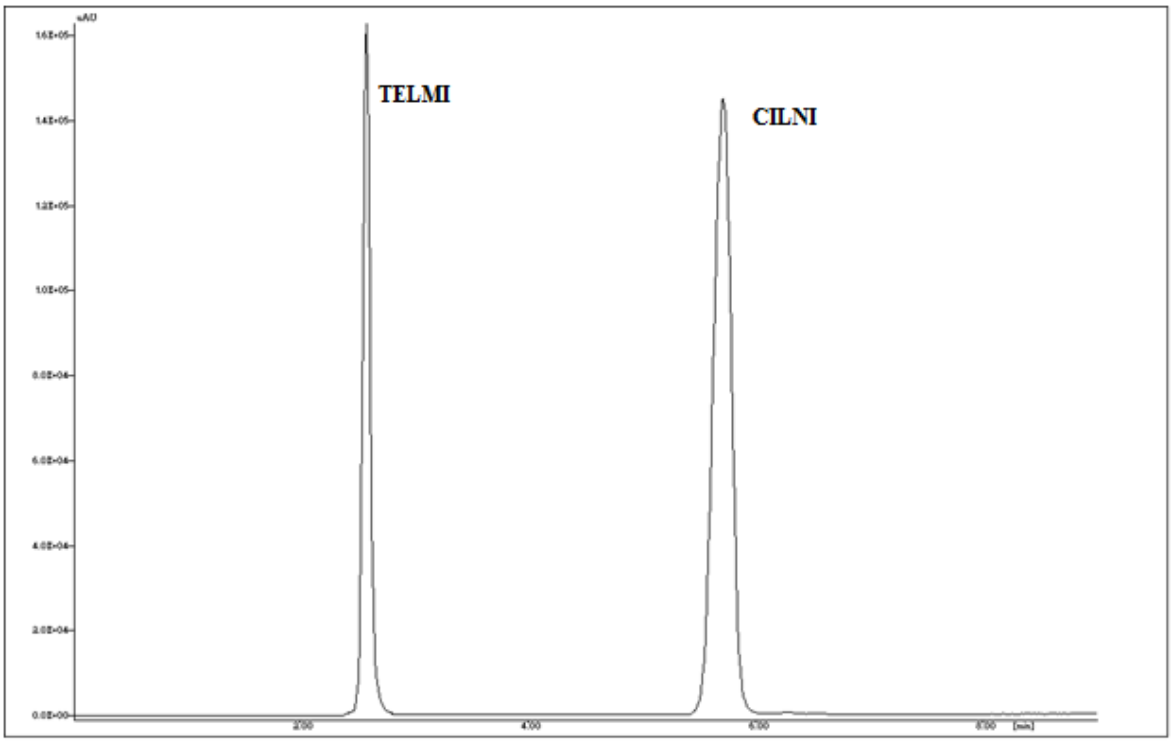

Figure 1: Representative chromatogram obtained for standard mixture of TELMI (10 µg mL^{-1}, 2.587 min), CILNI (10 µg mL^{-1}, 5.720 min)

Method Validation

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

Linearity

Aliquots 0.5, 1.0, 1.5, 2, 2.5 and 3 mL of working standard solution of TELMI (100 µg mL^{-1}) and 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of CILNI (100 µg mL^{-1}) were transferred in a series of 10 mL volumetric flasks and the volume was made up to the mark with mobile phase. Six replicates per concentration were injected and chromatograms were recorded. The peak areas were recorded and calibration curve was plotted of peak area against concentration of drug.

Precision

One set of three different concentrations of mixed standard solutions of TELMI and CILNI were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. For Inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses.

Accuracy

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 80, 100 and 120 %. The percentages of recoveries were calculated, the results of which are represented in Table 2.

Table 2: Recovery studies of TELMI and CILNI

Drug	Amount taken (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount found (µg mL^{-1})	% Recovery[a]	% R.S.D.[a]
TELMI	10	8	17.92	99.60	0.91
	10	10	19.95	99.78	0.45
	10	12	21.96	99.83	0.67
CILNI	2.5	2	04.47	99.40	0.27
	2.5	2.5	05.01	100.37	0.38
	2.5	3	05.52	100.39	0.42

[a] *Average of three determinations; RSD is the relative standard deviation*

Limit of detection and Limit of quantitation

Limit of detection and Limit of quantitation were calculated as 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.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase (1 ± 0.05 mL min^{-1}), a wavelength at which the drugs were recorded (245 ± 1 nm). One factor at the time was changed to estimate the effect. The solutions containing 20 µg mL^{-1} of TELMI and 6 µg mL^{-1} of CILNI were applied onto the column. A number of replicate analyses (n = 3) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 5-30 µg mL^{-1} for TELMI and 1-10 µg mL^{-1} for CILNI with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablets containing TELMI and CILNI. The % assay was found to be 100 ± 0.64 for TELMI and 99.769 ± 0.67 for CILNI (mean ± S.D., n = 6). The % recovery was found to be in the range of 99.60 to 99.83 for TELMI and 99.40 to 100.39 for CILNI. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD <2), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed HPLC method is given in Table 3.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Parameters	TELMI	CILNI
Linearity range (µg mL^{-1})	5-30	1-10
Correlation co-efficient	0.996	0.999
Slope (m)	61086	100462
Intercept (c)	41839	11995
LOD[a] (µg mL^{-1})	0.60	0.28
LOQ[b] (µg mL^{-1})	1.81	0.86
Accuracy (% Recovery)	99.60-99.83	99.40-100.39
Precision (% R.S.D.)[c]		
Intraday (n[d] = 3)	0.38-0.82	0.25-0.93
Inter day (n = 3)	0.12-0.89	0.24-0.89

[a] *LOD = Limit of detection* [c] *R.S.D. = Relative standard deviation*
[b] *LOQ =Limit of quantitation* [d] *n = Number of determination*

CONCLUSION

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

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