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Original Research Article

QUANTITATIVE ANALYSIS OF CILNIDIPINE AND TELMISARTAN IN TABLETS BY HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY WITH ULTRAVIOLET ABSORPTION DENSITOMETRIC DETECTION

[a]M.HARIPRIYA*, [b]Dr. P. JAYASEKHAR

[a]College of Pharmaceutical Sciences, Government Medical College, Trivandrum, Kerala-695011

[b]Oman Medical College, Azaiba, Post box 620, Postal code.130 Muscat , Sultanate of Oman

Author for Correspondence: riyasiva@yahoo.co.in

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ABSTRACT

A novel HPTLC method for the simultaneous estimation of Cilnidipine and Telmisartan in two component dosage forms has been developed and validated. Standard and sample solutions of cilnidipine and telmisartan were applied to precoated silica gel G 60 F_{254} HPTLC plates and the plates were developed with Toluene: Ethyl acetate: DMF in the ratio 6.5: 3.0: 0.5 (v/v), as mobile phase. UV detection was performed densitometrically at 260 nm. The R_f value for cilnidipine and telmisartan was found to be 0.47and 0.17 respectively. The correlation coefficients 0.9917 and 0.9852 for telmisartan respectively. The calibration curve was found to be linear between 100 to 1200 ng/spot for both cilnidipine and telmisartan. The limits of detection and quantitation were found to be 12.6 and 38.28 ng/spot, respectively for cilnidipine and 61.05 and 185.0 ng/spot, respectively for telmisartan. The results have been validated statistically as per ICH guidelines. The developed method can be successfully employed for the simultaneous determination of cilnidipine and telmisartan in marketed tablet formulation.

Key words: Cilnidipine; Telmisartan; HPTLC; Validation.

INTRODUCTION

Cilnidipine (CIL) O3-(2-methoxyethyl) *O*5-[(*E*)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine- 3,5-dicarboxylate is a novel and unique dihydropyridine calcium antagonist that possesses a slow-onset, long-lasting vasodilating effect[1].

Telmisartan (TEL), 4-((2-n-propyl-4-methyl-6-(1- methylbenzimidazol – 2 – yl) – benzimidazol – 1 - yl) methyl) biphenyl-2- carboxylic acid, 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].

Cilnidipine and Telmisartan similarly improves vascular damage in hypertensive patients[3]. Literature review revealed spectrophotometric and RP-HPLC method for the estimation of these drugs individually. Only very few methods are established for the simultaneous estimation of Cilnidipine and Telmisartan in two component dosage forms. The purpose of this research was to establish a simple, rapid and cost effective HPTLC method and, after validation in accordance with International Conference on Harmonization (ICH) guidelines to use the method for analysis of the drug content of tablets.

Materials

All the chemicals and reagents used were of analytical grade. Cilnidipine and Telmisartan hydrochloride were obtained as gift samples from J B Chemicals and Pharmaceuticals, Daman and Akums Drugs and Pharmaceuticals limited, Hardwar. The combined dosage form was purchased from local market. Methanol HPLC was procured from SD fine-chem. limited; Mumbai.Ethyl acetate HPLC grade and DMF HPLC grade were obtained from LobaChemie, Mumbai and CDH (P) Ltd., New Delhi.

Equipment

Microsyringe (Linomat syringe ,Camag, Switzerland), pre-coated silica gel 60F254 aluminium plates (10 x 10 cm, 250 µm thickness; Merck, Germany), Linomat 5 applicator (Camag, Switzerland), twin trough chamber (20 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. Class A volumetric glass wares were used.

EXPERIMENTAL METHODS

Preparation of Standard solutions

Stock solutions for measurements were

prepared by dissolving cilnidipine and telmisartan separately in methanol to obtain concentration of 1000ng/µl for each compound. For calibration, series of solutions were prepared containing 50,100,200,400,600 ng/µl cilnidipine and telmisartan each, by diluting the stock standard solution with methanol in standard volumetric flasks (10ml).

Mixed standard solution containing final concentration 100 µg/ml of cilnidipine and 400 µg/ml of telmisartan was prepared from the stock solutions.

Preparation of Sample solution

Twenty tablets of brand Cilacar T (J B Chemicals and Pharmaceuticals) containing 10 mg of cilnidipine and 40 mg of telmisartan were weighed, average weight determined and finely powdered. Appropriate quantity of powder equivalent to 10 mg of cilnidipine and 40 mg telmisartan was accurately weighed, transferred to a 10 ml volumetric flask and volume was made up to 10 ml with methanol and shaken vigorously for 15 minutes. The solution was then sonicated for 5 minutes and filtered through the Whatman filter paper no.41. Necessary dilutions of filtrate were made with methanol to get final concentration 100 µg/ml of cilnidipine and 400 µg/ml of telmisartan.

Selection of mobile phase

A trial and error process was done to select the appropriate mobile phase. The solvent system of Toluene: Ethyl acetate: DMF in the ratio 6.5: 3.0: 0.5 was the most appropriate solvent system for the HPTLC analysis of cilnidipine and telmisartan in methanol.

Application of standard solutions

Separate HPTLC pre-coated plates of silica gel G 60 F_{254} (10x10) were employed for the spotting of standard solutions. 2µl of standard solutions of concentration 50,100, 200, 400, and 600µg/ml of Cilnidipine and Telmisartan standard solutions were applied in the five tracks respectively in two different plates.

Application of sample solution

2µl of the mixed standard solution of 100µg/ml for cilnidipine and 400µg/ml for telmisartan was applied. The same procedure was repeated with the sample solution prepared from tablet dosage form.

After application the plate was taken out and the position of spots were visualized and confirmed under UV cabinet at 254nm.

Development of spot

Twin Trough chamber containing 10ml of mobile phase system ,saturated for 30 minutes, was used for the developing the spotted plates. 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.

Scanning by HPTLC scanner

After setting up the instrument parameters, the spot was scanned from 200 – 400nm and the spots showed maximum absorption at 260nm using the CAMAG TLC scanner IV. The R_f values were found to be 0.47 for cilnidipine and 0.17 for telmisartan. Typical chromatograms obtained for Cilnidipine RS and Telmisartan RS separately and of sample are shown in fig.(1) and (2) and (3) respectively. The peak areas and peak heights were noted.

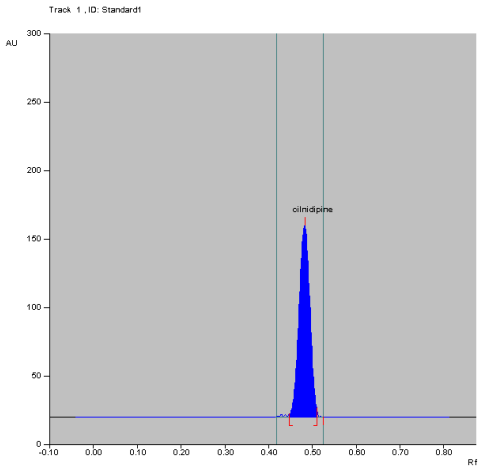

Fig.1. Typical chromatograms obtained for Cilnidipine RS

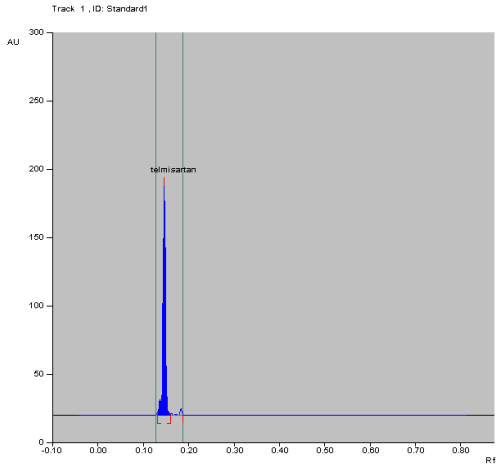

Fig.2. Typical chromatograms obtained for Telmisartan RS

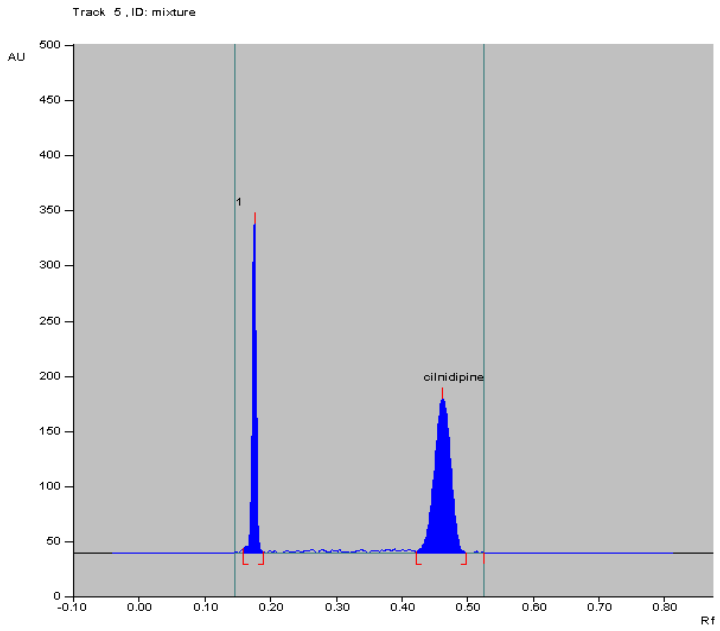

Fig.3. Typical chromatograms obtained for sample

METHOD VALIDATION[4]

Calibration curve (linearity of the method-area wise)

Response to cilnidipine and telmisartan was linear in the concentration ranges 100–1200ng spot^{-1}, respectively.

The regression equations for cilnidipine and telmisartan (n = 6) were

y = 17.93x + 1650.5 and

y = 2.9413x + 565.13 respectively,

where y is response and x the amount chromatographed. The correlation coefficients r, were 0.9917 and 0.9852 respectively, over these concentration ranges. The data is given in Table 1 and Table 2.

Table.1. Rf factor, height and area of Cilnidipine peak

Vial	Rf	Amount- ng/spot	Height	Area
S1	0.48	100	100.2	2011.2
S2	0.47	200	140.84	3343.3
S3	0.47	400	234.96	5867.6
S4	0.47	800	353.25	9287.4
S5	0.47	1200	431.36	11951.9

Table.2. Rf factor, height and area of Telmisartan peak

Vial	Rf	Amount- ng/spot	Height	Area
S1	0.16	100	108.9	662.3
S2	0.17	200	162.84	782.20
S3	0.17	400	247.42	1539.27
S4	0.17	600	325.66	1899.69
S5	0.17	1200	378.40	2212.94

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of cilnidipine and telmisartan by the standard addition method. Known amount of standard of cilnidipine and telmisartan (80%, 100%, and 120%) were added to sample solutions of tablet dosage forms. The amounts of cilnidipine and telmisartan were estimated by substituting values in the regression equations as given above. (Table 3)

The values prove that the method is accurate.

Method Precision (Repeatability)

The precision of the instruments was checked by repeatedly scanning (n = 6) standard solutions of cilnidipine and telmisartan (100 µg/ml). The RSD values were found to be below 2% which indicate that the proposed methods are repeatable. (Table 3).

Intermediate Precision (Reproducibility)

The intermediate precision for the proposed method was determined by estimating standard solution of cilnidipine and telmisartan for three different concentrations for three times on the same day(intraday) and on three different days(interday). The results are reported in terms of relative standard deviation (RSD). The RSD values were found to be below 2% which indicate that the proposed methods are reproducible (Table 3).

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

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 X Std.deviation/Slope,and LOQ = 10 X Std.deviation /Slope

The data are provided in Table 3.

Table.3.Summary of validation parameters for the proposed methods

Parameter	Cilnidipine	Telmisartan
Accuracy %	98.85	97.56
Precision (RSD, %)		
Repeatability(n=6)	0.98	0.72
Intraday(n=3)	0.88-1.25	0.66-0.84
Interday(n=3)	0.96-1.32	1.22-1.46
LOD	12.6 ng/spot	61.05 ng/spot
LOQ	38.28 ng/spot	185.0 ng/spot

Table.4.Compilation of results of commercial formulation

Brand name	Company	Formulation	Label Claim	Amount (mg)
Cilacar T	J B Chemicals & Pharmaceuticals	Tablet	cilnidipine 10mg telmisartan 40mg	09.64±0.22 38.97±0.43

CONCLUSION

A simple, sensitive HPTLC method has been developed for the estimation of Cilnidipine and Telmisartan in pure and in combined pharmaceutical dosage form. The stationary phase was pre-coated plates of silica gel G 60 F_{254} and the mobile phase used was Toluene: Ethyl acetate: DMF in the ratio 6.5:3.0:0.5v/v. The R_f value was found to be 0.47and 0.17 for cilnidipine and telmisartan respectively. The plate was scanned and quantified at 260nm. The calibration curve of Cilnidipine and Telmisartan were found to be linear. The Cilnidipine and Telmisartan content in dosage forms estimated by the proposed method were found to be in agreement with the label claim.

The validation of the developed method was performed in accordance with ICH guidelines. The accuracy of the proposed method was studied by recovery studies at three levels. The precision of the proposed method was studied by

repeatability and intermediate precision. The Limit of detection and Limit of quantitation was calculated.

The proposed method was found to be accurate and precise, so this method can be used for routine analysis of Cilnidipine and Telmisartan in pure and combined dosage form.

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