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Stability indicating HPTLC method for simultaneous estimation of Cilnidipine and Telmisartan in their combined dosage form


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Abstract

A sensitive, selective and precise high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of Cilnidipine and Telmisartan both as a bulk drug and in formulation. The method employed HPTLC aluminum plates pre-coated with silica gel 60F-254 as the stationary phase while the solvent system was chloroform: methanol: toluene (6:0.7:2 v/v/v). The Rf values of Cilnidipine and Telmisartan were observed to be 0.59 and 0.30 respectively. The densitometric analysis was carried out in absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed a good linear relationship as the stationary phase while 60F-254. The method employed HPTLC, Validation, Stability indicating and quantification for Cilnidipine and Telmisartan were found to be 14.95 and 10.37 ng/spot, 45.32 and 31.43 ng/spot respectively. Statistical analysis showed that the method is repeatable, selective and precise. Cilnidipine and Telmisartan were subjected to acid, base, peroxide and UV-induced degradation. In stability tests the drugs were susceptible to acid and basic hydrolysis, oxidation and photodegradation. Statistical analysis proved the method is repeatable, selective, and accurate for estimation of Cilnidipine and Telmisartan. Because the method could effectively separate the drugs from their degradation products, it can be used as a stability-indicating method.

Keywords: Cilnidipine, Telmisartan, HPTLC, Validation, Stability indicating and validation.

INTRODUCTION

Cilnidipine (CIL) is a light yellowish powder. Chemically it is 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl(2E)-3phenyl-2-propenyl ester (Löhn et al., 2002) (Figure 1a).

It is an antihypertensive agent and calcium channel blocker. Cilnidipine is a dual L-/N-type calcium channel protein inhibitor and blocker. Cilnidipine has displayed renal and vascular protective effects and improved baroreflex sensitivity in patients with hypertension. Telmisartan (TEL) is white crystalline powder. Chemically, it is 4′-((4-Methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl)methyl)biphenyl-2-carboxylic acid (Löhn et al., 2002; Indian Pharmacopoeia, 2007) (Figure 1b).

It is very soluble in methanol and practically insoluble in water. It is Angiotensin-converting Enzyme Inhibitors and Angiotensin II Type 1 Receptor Blockers agents. The mechanism by which Telmisartan is an angiotensin II receptor blocker (ARB) that shows high affinity for the angiotensin II receptor type 1 (AT1), with a binding affinity 3000 times greater for AT1 than AT2. It has the longest half-life of any ARB (24 hours) and the largest volume of distribution (Löhn et al., 2002). The combination of CIL and TEL is indicated as antihypertensive agents. (Lee et al., 2010)

Literature survey revealed that Cilnidipine can be estimated by spectrophotometry (Safi, 2013; Chaudhri

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and Bhalerao, 2012; Safhi and Yagaina, 2013) and by liquid chromatographic methods (George, 2005; Lee et al., 2012; Zhang et al., 2007) individually or in combination with other drugs, and Telmisartan can be estimated by spectrophotometry (Sharma et al., 2012; Tatane, 2011; Pandey et al., 2011; Rathod et al., 2012; Palled et al., 2006) and by liquid chromatographic methods individually or in combination with other drugs (Surekha et al., 2012; Rao et al., 2013; Kumar et al., 2011). Two methods UV spectroscopy (Haripriya et al., 2013) , RP-HPLC (Pawar et al., 2013) have been reported for the estimation of Cilnidipine and Telmisartan in their combined dosage form. The reported methods are highly sophisticated, costly, time-consuming and require special sample preparation. HPTLC method is considered to be a good alternative, and it should be widely explored as an important tool in routine drug analysis.

Present study involves development of a high performance thin liquid chromatographic method for the determination of CIL and TEL in combination dosage form. A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces the time and cost of analysis, minimizes exposure risks, and significantly reduces disposal problems of toxic organic solvents, thereby reducing the possibilities of environment pollution. The aim of the present work was to develop an accurate, repeatable, and specific HPTLC method for the determination of CIL and TEL both as a bulk drug and in formulation in the presence of their degradation products, as stipulated by the ICH guidelines. The proposed method was validated according to ICH guidelines (ICH, 1996 and 2005) and its updated international convention.

Experimental

HPTLC Instrument

The samples were applied with a sample syringe (Hamilton, Switzerland) using Camag Linomat 5 (Switzerland) sample applicator on pre-coated silica gel aluminum plate 60 F254, (10 cm x 10 cm with 0.2 mm thickness, E. Merck, Germany). Camag TLC scanner was used for the densitometric scanning of the developed chromatogram. All the drugs and chemicals were weighed on Mettler Toledo electronic balance.

Chemicals and Reagents

Analytically pure CIL and TEL were obtained as gratis samples from J.B Chemicals and Pharmaceuticals, Daman India and Akums Drugs and Pharmaceuticals limited, Hardwar India respectively. AR grade methanol, chloroform and toluene were procured from Sisw Research Lab, Mumbai, India, Astron chemicals, Ahmedabad, India and Chiti-Chem Corporation, Baroda, India respectively. Tablet formulation (CILACAR T, J.B chemicals and pharmaceuticals Ltd., Mumbai, India) containing labeled amount of 10 mg of cilnidipine and 40 mg of telmisartan were purchased from local market.

Chromatographic System

Sample Application

Standards and formulation samples of CIL and TEL were applied on the HPTLC plates in the form of narrow bands of 8 mm length and with 9 mm distance between two bands. Samples were applied under a continuous drying stream of nitrogen gas.

Mobile Phase and Development

Plates were developed using a mobile phase consisting of chloroform: methanol: toluene (6:0.7:2 v/v/v). Linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapors for 15 min at ambient temperature. Ten milliliters of the mobile phase (5 mL in the trough containing the plate and 5 mL in the other trough) was used for each development and was allowed to migrate a distance of 80 mm. After development, the HPTLC plates were dried.
Densitometric Analysis

Densitometric scanning was performed in the absorbance mode under control by winCATS planar chromatography software (ver.4.2). The source of radiation was the deuterium lamp, and bands were scanned at 254 nm. The slit dimensions were 6 mm length and 0.45 mm width, with a scanning rate of 20 mm/s. Concentrations of the compound chromatographic were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using a linear regression equation.

Preparation of Standard Stock Solution

Stock solutions were prepared by accurately weighing 25 mg of CIL and 25 mg of TEL transferring to 25 mL volumetric flask containing a few mL of methanol. The flask was swirled and sonicated for few minutes to dissolve the solids. Volume was made up to the mark with methanol to yield a solution containing 1000 μg/mL of CIL and TEL, respectively. Aliquot from the stock solution of CIL and TEL were appropriately diluted with methanol to obtain working standard solution of 100 μg/mL of CIL and TEL respectively.

Validation

Validation of developed HPTLC method was done with respect to following parameters.

Linearity of Calibration Curves

Linearity of the method was determined by constructing calibration curves at six concentration levels over a range of 100-600 ng/band for CIL and 400-2400 ng/band for TEL. The calibration curves were developed by plotting peak area versus concentration (n = 6) with the help of the winCATS software.

Accuracy

The accuracy of the method was determined by calculating recoveries of CIL and TEL by method of standard additions. Known amount of CIL (0, 80, 100, 120 ng/spot) and TEL (0, 80, 100, 120 ng/spot) were added to a pre quantified sample and the amount of CIL and TEL were estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

Precision

Precision was evaluated in terms of intraday and interday precisions. Intraday precision were determined by analyzing sample solutions of CIL and TEL at three levels covering low, medium, and high concentrations of the calibration curve three times on the same day (n = 3). Interday precision was determined by analyzing sample solutions of CIL and TEL at three levels covering low, medium, and high concentrations over a period of 3 days (n = 3). The peak areas obtained were used to calculate mean and RSD values.

Robustness

Small changes in the chamber saturation time, and change in mobile phase, and the effects on the results were examined. Robustness of the method were determined in triplicate at a concentration level of 400 ng/band for CIL and 1600 ng/band for TEL, and the mean and RSD of peak areas were calculated.

Analysis of Marketed Formulations

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10mg of CIL and 40 mg of TEL were accurately weighed and transferred to a 10 mL volumetric flask. A few mL (5 mL) of methanol was added to the above flask and flask was sonicated for 5 min. The solution was filtered using Whatman filter paper No.1 in another 10 mL volumetric flask and volume was diluted to the mark with the methanol. 1 mL aliquot from the above solution was taken in 10 mL volumetric flask and diluted to mark with methanol to obtain final concentration of 100 μg/mL for CIL and TEL was obtained. The possibility of interference from other components of the tablet formulation in the analysis was studied. From the developed chromatogram spot area and Rf values were determined.
Forced Degradation of CIL and TEL

Acid Catalyzed Induced Degradation

To 1 ml of working standard solutions of Cilnidipine and Telmisartan separately, each of conc. 1,000 mcg/ml, 2 ml of 0.5 N HCl was added and kept at room temperature for 1 hour. The solutions were diluted to 10 ml with methanol. Appropriate volume of resultant solution was applied on TLC plate (300 ng/spot and 1200 ng/spot of Cilnidipine and Telmisartan respectively, and densitograms were analyzed.

Degradation under Alkali Catalyzed Hydrolytic Condition

To 1 ml of working standard solutions of Cilnidipine and Telmisartan separately, each of conc. 1,000 mcg/ml, 2 ml of 0.5 N NaOH was added and kept at room temperature for 1 hour. The solutions were diluted to 10 ml with methanol. Appropriate volume of resultant solution was applied on TLC plate (300 ng/spot and 1200 ng/spot of Cilnidipine and Telmisartan respectively, and densitograms were analyzed.

Degradation under Oxidative Conditions

To 1 ml of working standard solutions of Cilnidipine and Telmisartan separately, each of conc. 1000 mcg/ml, 2 ml of 12% H₂O₂ was added and kept at room temperature for 1 hour. The solutions were diluted to 10 ml with methanol. Appropriate volume of resultant solution (300 ng/spot and 1200 ng/spot of Cilnidipine and Telmisartan respectively) was applied on TLC plate and densitograms were analyzed.

Photo-Degradation Studies

Photolytic degradation studies were carried out by exposure of drugs to UV light up to illumination of 200 watt hours/square meter and subsequently cool fluorescent light to achieve an illumination 1.2 million Lux. Hr. (ICH, 1996).

RESULTS AND DISCUSSION

Optimization of the Mobile Phase

To develop the HPTLC method of analysis of CIL and TEL for routine analysis, selection of the mobile phase was carried out on the basis of polarity. A mobile phase that would give a dense and compact band with an appropriate Rₜ value for CIL and TEL were desired. Various mobile phases such as methanol–ethylacetate, chloroform-methanol, methanol – ethyl acetate-toluene, were evaluated in different proportions. A mobile consisting of chloroform: methanol: toluene(6:0.7: 2, v/v/v) gave good separation of CIL and TEL from its matrix. It was also observed that chamber saturation time and solvent migration distance were crucial in the chromatographic separation as chamber saturation time of less than 15 min and solvent migration distances greater than 80 mm resulted in diffusion of the analyte band. Therefore, chloroform: methanol: toluene (6:0.7: 2, v/v/v) mobile phase with a chamber saturation time of 15 min at 25°C and solvent migration distance of 80 mm was used. These chromatographic conditions produced a well-defined, compact band of CIL and TEL with optimum migration at Rₜ0.59 and 0.30 respectively (Figure 2).

Validation

Linearity and calibration curves

Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte. The method were found to be linear in a concentration range of 100-600 ng/band \((n = 6)\) for CIL and 400-2400 ng/band \((n = 5)\) for TEL with respect to peak area. Figure 3 displays a three-dimensional overlay of HPTLC densitograms of the calibration bands of CIL and TEL at 254 nm. The regression data shown in Table 1 reveal a good linear relationship over the concentration range studied, demonstrating the suitability of the method for analysis.

Accuracy

Accuracy of an analytical method is the closeness of test results to the true value. It was determined by the application of analytical procedure to recovery studies, where a known amount of standard is spiked into pre-analyzed samples solutions. Results of the accuracy studies from excipients matrix are shown in Table 2; recovery values demonstrated the accuracy of the method in the desired range.

Precision

The precision of an analytical method expresses the degree of scatter among a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of an analytical procedure within a laboratory over a short period of time.
Figure 2. Densitogram of CIL and TEL using mobile phase chloroform: methanol: toluene(6:0.7:2 v/v/v)

Figure 3. Three dimensional overlay of HPTLC densitograms of calibration bands of CIL

Table 1. Regression Analysis of Calibration Curve

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CIL</th>
<th>TEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng/spot)</td>
<td>100-600</td>
<td>800-2400</td>
</tr>
<tr>
<td>Slope</td>
<td>7.66</td>
<td>1.943</td>
</tr>
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<td>Standard deviation of slope</td>
<td>0.116432</td>
<td>0.005263</td>
</tr>
<tr>
<td>Intercept</td>
<td>395.6</td>
<td>1026.6</td>
</tr>
<tr>
<td>Standard deviation of intercept</td>
<td>34.71868</td>
<td>6.107373</td>
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<tr>
<td>Correlation coefficient</td>
<td>0.997</td>
<td>0.996</td>
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Table 2. Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CIL</th>
<th>TEL</th>
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<tr>
<td>Rf</td>
<td>0.59</td>
<td>0.30</td>
</tr>
<tr>
<td>Detection limit (ng/spot)</td>
<td>14.95</td>
<td>10.37</td>
</tr>
<tr>
<td>Quantitation limit (ng/spot)</td>
<td>45.32</td>
<td>31.43</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.4-100.09%</td>
<td>100.7-101.1%</td>
</tr>
<tr>
<td>Precision (RSD, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Intra-day precision (n=3)</td>
<td>0.66–1.37%</td>
<td>0.18-1.01%</td>
</tr>
<tr>
<td>• Inter-day precision (n=3)</td>
<td>0.72-1.15%</td>
<td>0.19-0.36%</td>
</tr>
<tr>
<td>Repeatability study (%RSD) (n=6)</td>
<td>0.96%</td>
<td>0.22%</td>
</tr>
</tbody>
</table>

Table 3. Robustness Studies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal condition</th>
<th>Change in condition</th>
<th>Change in % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber Saturation time</td>
<td>20 min</td>
<td>18 min</td>
<td>0.47</td>
</tr>
<tr>
<td>Mobile phase ratio</td>
<td>6:0.7:2</td>
<td>6:0.5:2.5</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>7:0:7:3</td>
<td></td>
<td>1.15</td>
</tr>
</tbody>
</table>

by the same operator with the same equipment, whereas interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days. The results obtained are shown in Table 2. In all instances, RSD values were less than 5%, confirming the precision of the method. Repeatability of the scanning device was studied by applying and analyzing CIL sample (400 ng/spot) and TEL sample (1600 ng/spot) seven times. RSD was less than 5% (Table 2), which was well below the instrumental specifications.

Limit of Detection and Limit of Quantification

Under the experimental conditions used, the lowest amount of CIL and TEL that could be detected (LOD) were found to be 14.95 ng/spot and 10.37 ng/spot, and the lowest amount of CIL and TEL that could be quantified (LOQ) were 45.32 ng/spot and 31.43 ng/spot.

Robustness

The low values of RSD (Table 3) obtained after introducing small, deliberate changes in parameters of the developed HPTLC method confirmed its robustness.

Analysis of marketed formulation

Marketed formulation was analyzed using proposed method which gave percentage recoveries of 99.4-100.09% for CIL and 100.7-101.1% for TEL. A well resolved band at Rf 0.59 and 0.30 were observed in the chromatogram of CIL and TEL, and no interference from the excipients present in the marketed tablet formulation was observed.

Degradation Behavior

HPTLC studies on CIL and TEL under different stress conditions suggested following degradation behavior.

Hydrolytic Studies

Acidic Condition

Drugs, CIL and TEL showed 25.4% and 38.9% degradation under acidic hydrolysis at room temperature respectively. Additional peaks were observed for CIL at Rf 0.44 and for TEL at 0.06, 0.1 and 0.29 respectively. (Figure 4).

Alkaline condition

CIL degraded under alkaline condition (2 ml of 0.5N NaOH) in short period of time. Three new peaks were observed for product of CIL formed under these conditions at Rf 0.45, 0.49 and 0.57. CIL and TEL degraded to about 13.1% and 63.11% under alkaline condition (2 ml of 0.5N NaOH) after keeping for 1 hour. One peak was observed for degradation product of CIL at Rf 0.44 and for TEL three peaks were observed at Rf 0.07, 0.1 and 0.32. (Figure 5).
Oxidative Studies

CIL and TEL showed 15.7% and 39.54% degradation upon treatment with 12% H$_2$O$_2$ at room temperature. Reduction in area of peak of CIL was observed and three other peaks of degraded product were found at Rf 0.45, 0.49 and 0.57 and for TEL one peak of degraded product was observed at Rf 0.16 upon treatment with 12% H$_2$O$_2$ at room temperature. (Figure 6).

Photolytic Studies

Under photolytic studies, additional peaks were observed of CIL at Rf 0.49 and two peaks were observed of TEL at Rf 0.12 and 0.2. This indicates stability of drugs upon exposure to white fluorescent light and UV light for specified period. The forced degradation study results are summarized in Table 4. (Figure 7).

CONCLUSION

Introducing HPTLC method in pharmaceutical analysis represents a major step in quality assurance. A specific, accurate and precise HPTLC analytical method has been developed for the simultaneous estimation of Cilnidipine and Telmisartan as bulk and in pharmaceutical formulation. From the above study, we can conclude that the CIL and TEL undergo degradation to different extent under different, above mentioned, stress conditions. In this study, the products formed after
Figure 6. Densitogram of mixture in H2O2 degradation

Figure 7. Densitogram of mixture in photo degradation

Table 4. Degradation Studies

<table>
<thead>
<tr>
<th>Sample exposure conditions</th>
<th>No. of degradation products ($R_i$)</th>
<th>Drug remained (ng)</th>
<th>% degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIL</td>
<td>TEL</td>
<td>CIL</td>
</tr>
<tr>
<td>0.5N HCl, 1 hr, RT</td>
<td>1</td>
<td>3</td>
<td>223.8</td>
</tr>
<tr>
<td></td>
<td>(0.44)</td>
<td>(0.06,0.1,0.29)</td>
<td></td>
</tr>
<tr>
<td>0.5N NaOH, 1 hr, RT</td>
<td>1</td>
<td>3</td>
<td>260.7</td>
</tr>
<tr>
<td></td>
<td>(0.44)</td>
<td>(0.07,0.1,0.32)</td>
<td></td>
</tr>
<tr>
<td>12% H2O2, 1 hr, RT</td>
<td>3</td>
<td>1</td>
<td>252.9</td>
</tr>
<tr>
<td></td>
<td>(0.45,0.49,0.57)</td>
<td>(0.16)</td>
<td></td>
</tr>
<tr>
<td>Photo degradation, 12 hr</td>
<td>1</td>
<td>2</td>
<td>196.8</td>
</tr>
<tr>
<td></td>
<td>(0.49)</td>
<td>(0.12,0.2)</td>
<td></td>
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</tbody>
</table>
forced decomposition studies were resolved from the bulk drug response. From the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the response of drugs. It confirms that degradation product of drug can be separated from the drug by this method. The developed method is simple, accurate, precise, and specific. It is proposed for routine analysis of these drugs in the presence of degradation products in stability study. Statistical analysis proves that the method is suitable for the analysis of Cilnidipine and Telmisartan as a bulk drug and in pharmaceutical formulation without any interference from the excipients. The method was successfully validated in accordance with ICH guidelines. The method can be used to determine the purity of drug available from various sources.

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