HPTLC METHOD FOR THE ESTIMATION OF CILOSTAZOL IN BULK AND FORMULATION

JAYESH V. PATEL*, C. N. PATEL, P. U. PATELA
PANKAJ H. PRAJAPATI, I. S. ANAND and M. B. PATEL

Shri Sarvajanik Pharmacy College, MEHSANA (Guj.) INDIA
aShree S. K. Patel College of Pharma. Education and Research, Ganpat Vidyanagar, Kherva, MEHSANA – 382711 (Guj.) INDIA

ABSTRACT

A high performance thin layer chromatography (HPTLC) method was developed and validated for quantitative determination of cilostazol. The analysis was performed using a mobile phase composed of acetate: toluene: methanol: ether (2:2:1:0.5 v/v). The calibration graphs were linear (correlation coefficient \( r = 0.997 \)) in the studied concentration range of 100 to 800 ng/mL for cilostazole in HPTLC. Relative standard deviation of all the parameters for intraday and interday precision is 0.75 to 2 and the accuracy was greater than 98%. The limit of quantification of the drug was found to be 20.5 ng/spot and the limit of detection of the drug was found to be 6.765 ng/spot. So the proposed method is precise and accurate and can be applied directly and easily to the cilostazol.

Key words: Cilostazol, HPTLC

INTRODUCTION

Cilostazol is chemically 6-[4-1(cyclohexyl-1H-tetrazol-5-yl-butoxyl] 3-4-dihydro-2 (1H)-quinolinone¹, which is a phosphodiesterase inhibitor -². It is used in the treatment of peripheral artery disease. It is not official in any pharmacopoeia. It is listed in the Merck index complete drug reference. A survey of literature revealed HPLC³⁴ and LC/MS/MS⁵ methods for the determination of cilostazol and it’s metabolite in biological fluids and tablets. In the present investigation, an attempt has been made to develop a simple and economical HPTLC method with greater precision, accuracy, and sensitivity for the analysis of cilostazol in bulk and dosage forms.

There is no HPTLC method reported for the estimation of this drug as such and in formulations. Since HPTLC is easier and cheaper technique as compared to HPLC for

* Author for correspondence; E-mail:jayesh_pharma@yahoo.com
quality control and standardization of the dosage forms, an attempt has been made to develop a HPTLC method for the determination and quantification of the cilostazol in tablets. Thus, the aim of present work was to develop a simple, accurate and cost effective HPTLC method for determination of the said drug in tablet dosage form.

EXPERIMENTAL

Chemicals and materials

Analytical grade methanol was obtained from Finar Laboratories. Ethyl acetate, toluene and solvent ether were obtained from Merck India Limited, Mumbai. TLC aluminum plate percoated with silica gel 60 F$_{254}$ (10 cm × 10 cm) was obtained from E. Merck Limited, Mumbai.

Instrument

A Camag HPTLC system equipped with Linomat IV sample applicator was used. Camag TLC scanner 3 was used for scanning and integration software, CATS 4.02 (Switzerland) was used for interpretation of data.

Standard preparation

Stock solution of 1000 µg/mL cilostazol was prepared in 100 mL of methanol.

Chromatographic condition

The experiments were performed on a precoated silica gel G60 F$_{254}$ HPTLC plates (10 cm × 10 cm) using mobile phase comprising of ethyl acetate, toluene, methanol and ether (2 : 2 : 1 : 0.5). The plates were prewashed by methanol and activated at 110°C for 20 min prior to chromatography. Samples were applied as bands 6 mm long, at 6 mm intervals, under a stream of nitrogen. Ascending development to a distance of 8 cm was performed in saturated 20 cm × 10 cm twin trough TLC development chamber (Camag) at room temperature. The plate was scanned and quantified at 257 nm using slit dimension of 4.0 × 0.45 mm.

Linearity of detector response

Varying concentrations of the drug (100 ng/mL to 900 ng/mL) were prepared from the stock solution. The above concentrations were applied on the chromatographic plate. The plate was developed with mobile phase comprising of ethyl acetate, toluene, methanol and ether (2: 2: 1: 0.5) in twin trough chamber, to a distance of 8 cm after removal from
chamber, the plate was scanned and quantified at 257 nm. From the result obtained, the peak area was calculated. A linear relationship was observed between the concentration of peak area in the range of 100 ng/mL to 900 ng/mL.

**Sample preparation**

Twenty tablets were weighed and average weight of each tablet was calculated. An accurately weighed amount of sample equivalent to 100 mg of cilostazol was transferred into a 100 mL volumetric flask and extract was filtered through Whatman filter paper and residue was washed with 10 mL of methanol. The extract and washing were pooled and transferred to 100 mL volumetric flask and volume was made up to 100 mL with methanol.

**Assay**

From the above sample solution, 100 µg/mL was prepared and 6 µL was spotted in triplicate along with same concentration of standard solution on precoated silica gel G60 F254 HPTLC plate. The plate was developed and scanned as mentioned above. The peak area was recorded and the amount of cilostazol was estimated using calibration curve data.

**Recovery studies**

To study the accuracy and precision of the method, recovery experiment was performed by the method of standard addition to determine, if there are positive or negative interferences from excipients present in the formulation. The recovery of the added standard was studied at three different levels, each being analyzed in a manner similar to as described for assay. Each set of additions was repeated five times and the recovery of added standard was calculated.

**RESULTS AND DISCUSSION**

Several mobile phases were tried to accomplish good detection of cilostazol. After optimizing all parameters, chromatographic condition was obtained, which gave satisfactory chromatogram with best single and sharp peak of cilostazol. Linear correlation was obtained between peak areas and concentrations of cilostazol in concentration range of 100-800 ng/spot. Characteristic parameters for regression equation and correlation are given in Table 1. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression. The recovery experiments were carried out. The percent recoveries obtained were 98.79 to 100.2. The results of recovery study are given in Table 2. Intermediate precision was also determined. The low % CV values of intra-day (0.73-1.43) and inter-day (0.26-1.69) precision reveal that the proposed method is precise.
The limit of detection of the drug was calculated. LOD for cilostazol was found to be 6.765 ng/spot. The limit of quantification of the drug was calculated it was found to be 20.5 ng/spot for cilostazol.

Comparison of chromatogram of cilostazol in test with standard cilostazol showed no interference from the excipients

**Table 1. Optical and regression characteristics for analysis of cilostazol by HPTLC method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (ng/spot)</td>
<td>100-800</td>
</tr>
<tr>
<td>Limit of detection (LOD) (ng/spot)</td>
<td>6.765</td>
</tr>
<tr>
<td>Limit of quantification (LOQ) (ng/spot)</td>
<td>20.5</td>
</tr>
<tr>
<td>Regression equation ($y^* = a + bc$)</td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>7.558</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>743.48</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.997</td>
</tr>
</tbody>
</table>

**Table 2. Data of recovery study for cilostazol by HPTLC method**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (ng/spot)</th>
<th>Amount added (ng/spot)</th>
<th>Amount found (ng/spot)</th>
<th>% Recovery ± SD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cilostazol</td>
<td>200</td>
<td>100</td>
<td>296.37</td>
<td>98.79 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200</td>
<td>399.12</td>
<td>99.78 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>300</td>
<td>501.00</td>
<td>100.2 ± 1.09</td>
</tr>
</tbody>
</table>

**Table 3. Result of assay experiments**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labeled amount (mg)</th>
<th>Amount found (mg)</th>
<th>% Amount found ± SD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand-A</td>
<td>100</td>
<td>100.4</td>
<td>100.4 ± 0.39</td>
</tr>
<tr>
<td>Brand-B</td>
<td>100</td>
<td>100.9</td>
<td>100.9 ± 0.27</td>
</tr>
</tbody>
</table>
REFERENCES


6. ICH Harmonized Tripartite Guideline, Recommended for Adoption at Step 4 of the ICH Process on 6 November, 1966 by the ICH Steering Committee.

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