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STABILITY INDICATING HPLC DETERMINATION OF CILOSTAZOL IN PHARMACEUTICAL DOSAGE FORMS.

JOSE KURIEN*1 AND P. JAYASEKHAR2

1College of Pharmaceutical Sciences, Govt. Medical College, Kottayam, Kerala, India 686 008.
2Oman Medical College, Post Box.620, Postal Code:130 Azaiba, Muscat, Sultanate of Oman

ABSTRACT

A simple, selective, precise and stability indicating high performance liquid chromatographic (HPLC) method of analysis of Cilostazol in pharmaceutical dosage form was developed and validated. The chromatographic conditions consisted of a reversed phase C18 column (250 × 4.6 mm, 5µm) using a mobile phase containing a mixture of acetonitrile and acetic acid (0.1% aqueous solution) in the ratio of 50:50. Flow rate was 1ml/min. Detection was carried out at 260nm. The retention time of Cilostazol was 8.2 min. Cilostazol was subjected to acid and alkali hydrolysis, oxidation, photochemical and thermal degradation. The linear regression analysis data for the calibration plot showed a good linear relationship in the concentration range of 10 – 200 µg/ml. The value of correlation coefficient, slope and intercept were 0.99961, 44.25 and 130.3 respectively. The limit of detection and limit of quantification were 3 µg/ml and 10 µg/ml respectively. The method was validated for precision, accuracy, ruggedness and robustness. The drug was stable under acidic, basic, ultraviolet and thermal degradation conditions, but undergoes oxidative degradation in the presence of peroxides. All the peaks of degraded products were resolved from the active pharmaceutical ingredient with significant different retention times. This method is proposed to differentiate the drug from its degradation products very precisely; hence it can be employed as a stability indicating one.

KEY WORDS: RP-HPLC; Cilostazol; stability indicating; dosage forms; validation.

*Corresponding author

JOSE KURIEN
College of Pharmaceutical Sciences, Govt. Medical College, Kottayam, Kerala, India 686 008.
INTRODUCTION

Cilostazol is chemically 6-[4-1(-cyclohexyl-1H-tetrazol-5-yl-butoxy)] 3-4-dihydro-2(1H)-quinolinone. Cilostazol and its metabolites are cyclic adenosine monophosphate (cAMP) phosphodiesterase III inhibitors, inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation. Therefore, Cilostazol is used for the treatment of intermittent claudication resulting from peripheral arterial disease. Cilostazol is commercially available as single and combined formulations. Cilostazol is official in United States Pharmacopoeia 2009. USP describes HPLC method for the assay of Cilostazol and its tablets, using a column packed with octadecylsilanized silica gel with a mobile phase of water, acetonitrile and methanol (10:7:30) equipped with a 254 nm detector and a flow rate of 1ml/min. There are limited reports regarding determination of Cilostazol in pharmaceutical dosage forms and biological fluids. These works include HPLC, UV spectrophotometric and potentiometric methods to determine Cilostazol in pharmaceutical dosage forms. The assay of Cilostazol in the human plasma and mouse serum are also reported by HPLC methods. The aim of the present study was to develop a simple, validated and rapid HPLC method for routine analysis of Cilostazol in tablets. The HPLC method was studied following official guidelines, evaluating the main parameters and the procedures that can be applied to consider a stability indicating assay. The International Conference on Harmonization (ICH) requires stability indicating methods to be used for assay of a drug in test samples. Stability indicating methods should be suitable for determination of the drug during hydrolysis (at various pH values), oxidation, photolysis and thermal degradation. According to FDA guidance document, a stability indicating method accurately measures the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities.

MATERIALS AND METHODS

1) Chemicals and Reagents
Cilostazol Reference Standard was supplied by Glenmark Pharmaceuticals Ltd, Mumbai, India. Acetonitrile HPLC grade and Acetic acid GR procured from Merck, India. High purity water was prepared with Elix-milli-Q system. Sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from Qualigens, India.

2) Instrumentation
The HPLC instrument used was AGILENT 1260 RRLC system equipped with a photodiode array detector PDA 1290 infinity.

3) Chromatographic Conditions
Chromatographic separation was achieved on a reverse phase column Phenomenax C₁₈ (250 × 4.6 mm, 5μm) at ambient temperature using a mobile phase consisting of a mixture of acetic acid (0.1% aqueous solution) and acetonitrile in the ratio of (50:50) at a flow rate of 1ml/min.
Detection was carried out at 260 nm. The mobile phase system after preparation was filtered through a membrane filter (0.22 µm) and sonicated for 10 minutes. The pH of the mobile phase was set at 3.6. Injection volume used for assay and degradation studies was 20 µl. The retention time of Cilostazol was 8.2 min.

4) Standard Preparation
Stock solution of Cilostazol (1mg/ml) was prepared by dissolving appropriate amounts of the compounds in methanol. A series of working standard solutions of Cilostazol were prepared by the appropriate dilution of the above mentioned stock solution in the mobile phase to get concentration range of 10.0-200.0 µg/ml. Cilostazol standard solutions were found to be stable during the analysis time (Fig 3).

5) Sample Preparation
Ten tablets of Cilostazol (Stiloz-50) were accurately weighed and powdered. Average weight of a tablet was determined. A quantity of Cilostazol tablet powder equivalent to 25mg of Cilostazol was dissolved in methanol and made up to volume in a 50 ml volumetric flask. The solution was sonicated for 15 minutes. A 1ml aliquot was transferred to 10 ml volumetric flask and diluted to volume with the mobile phase. 20 µl of the solution was injected into the HPLC system. Peak area was measured at retention time of 8.2 min (Fig 4).

6) Preparation of Calibration Graph
Linearity of response for Cilostazol assay method was determined by preparing and injecting solutions having concentrations from 10 to 200 µg/ml of Cilostazol (Fig 2).

7) System Suitability
The system suitability test is performed to ensure that the HPLC method was suitable to the analysis intended. A standard solution containing 40 µg/ml of Cilostazol was injected in triplicate. Chromatographic factors like peak area, retention time, theoretical plates, and tailing factors were determined and the relative standard deviation (RSD) for each factor was calculated.

8) Method Validation
The HPLC method was validated by evaluation of the analytical parameters including specificity, linearity, precision, accuracy and robustness. The stability indicating capability was determined by forced degradation conditions, including testing heat, light, oxidation and acid and basic degradation.

i) Specificity
Specificity was examined for the non-degraded and degraded samples. The accelerated degradation studies conducted were the following:

a) Acidic degradation: 25 mg of Cilostazol tablet powder was dissolved in 40ml mobile phase in a 50 ml volumetric flask and then added 1ml of 2 N HCl, heated at 80°C for 24 hours. Cooled, neutralized the solution and made up to 50ml with the mobile phase. The solution was filtered through a 0.45µm filter. 20 µl solution was injected into the column.

b) Alkaline degradation: 25 mg of Cilostazol tablet powder was dissolved in 40 ml mobile phase in a 50 ml volumetric flask and then added 1ml of 2 N NaOH, heated at 80°C for 24 hours. Cooled, neutralized the solution and made up to 50ml with the mobile phase. The solution was filtered through a 0.45 µm filter. 20 µl solution was injected into the column.

c) Oxidative degradation: 25 mg of Cilostazol tablet powder was dissolved in 40 ml mobile phase in a 50 ml volumetric flask and then added 1 ml of 15% H2O2 solution, after 30 minutes neutralized the solution and made up to 50ml with the mobile phase. The solution was filtered through a 0.45 µm filter. 20 µl solution was injected into the column.

d) Thermal degradation: 25 mg of Cilostazol tablet powder was dissolved in 40ml mobile phase in a transparent glass bottle and kept at 80°C for 24 h. Transferred the contents into a 50ml volumetric flask and made up with the mobile phase. The solution was filtered through a 0.45 µm filter. 20 µl solution was injected into the column.

e) UV degradation: 25 mg of Cilostazol tablet powder was exposed to sunlight for 24 h. Then it was dissolved in 40 ml mobile phase
and made up to 50 ml with the mobile phase. The solution was filtered through a 0.45 µm filter. 20 µl solution was injected into the column.

**ii) Linearity**
Cilostazol reference solutions were prepared at concentrations of 10, 20, 40, 80, 120, 160, 180 and 200 µg/ml. Calibration curves were constructed and linearity was determined statistically by linear regression analysis.

**iii) Precision**
The precision of the assay method was calculated by repeatability (intra-day) and intermediate precision (inter-day). The repeatability of the methods was determined on six sample solutions during the same day for three concentrations of Cilostazol. Intermediate precision was studied by comparing the assays performed on two different days.

**iv) Accuracy**
The accuracy was determined by the recovery of known amounts of Cilostazol reference standard added to the samples. The results were expressed as the percentage of Cilostazol reference standard recovered from the sample. All solutions were prepared in triplicate and assayed.

**v) Limit of Detection (LOD) and Quantification (LOQ)**
The LOD and LOQ parameters were determined from the regression equation of Cilostazol: LOD = 3.3 Sy/a, LOQ = 10 Sy/a; where Sy is the standard error and a is the slope of the corresponding calibration curve.

**vi) Robustness**
The robustness of the procedure was evaluated after changing the following parameters: the composition of the mobile phase (content of acetonitrile in the range %); the mobile phase flow rate (0.8-1.2 ml/min) and temperature (25 ± 2°C). For each parameter change its influence on the retention time, resolution and peak characteristics (height, area and width) were evaluated.

![Figure 2: Calibration Curve of Cilostazol](image-url)
Figure 3
Chromatogram of Cilostazol Standard

Figure 4
Chromatogram of Cilostazol Tablet assay
Figure 5
Chromatogram of acid degraded sample

Figure 6
Chromatogram of alkali degraded sample
Figure 7
Chromatogram of peroxide degraded sample

Figure 8
Chromatogram of photo degraded sample
RESULTS AND DISCUSSION

1) Method development
The chromatographic conditions were optimized with a view to develop a stability indicating assay method. The experimental studies revealed that the column, Phenomenax C$_{18}$ (250 × 4.6 mm, 5µm) was most suitable, since it produced best chromatographic performance and acceptable peak characteristics including high resolution and very good sensitivity. The chromatographic conditions finally comprised of a mobile phase consisting of a mixture of acetic acid (0.1% aqueous solution) and acetonitrile in the ratio of (50:50) at a flow rate of 1ml/min using Phenomenax C$_{18}$ column (250 × 4.6 mm, 5µm) and detection was carried out at 260nm.

2) Calibration curve
Under the above mentioned experimental conditions, a linear relationship was established by plotting Cilostazol concentrations against the corresponding peak areas. The response was linear over the range of 10 to 200µg/ml of Cilostazol with a correlation coefficient, slope and intercept of 0.99961, 44.25 and 130.3 respectively (Fig 2). Under the applied chromatographic conditions, the LOD and LOQ of Cilostazol were 3µg/ml and 10µg/ml respectively.

3) Validation of the method
a) Precision
The intraday and interday precision values were calculated for three concentrations of Cilostazol. The RSD values were less than 1%, demonstrating that the method was precise (Table1).

b) Accuracy
The accuracy of the method was established by recovery studies (Table 2). Results indicate that the individual recovery of Cilostazol ranges from 99.70% to 100.02% with mean recovery of 99.89% and %RSD of 0.45%. The recovery of the Cilostazol by proposed method is satisfactory as %RSD is not more than ±2.0% and mean recovery between 98.0 – 102.0%.

c) Ruggedness and Robustness
The method robustness and ruggedness was determined by changing the following parameters: composition of the mobile phase, mobile phase flow rate in the range of 0.8-1.2 ml/min, temperature in the range 23-27°C and pH in the range 3.0-4.0. With the deliberate aforementioned change in parameter, the
effects on the retention time and peak resolution, peak shape and peak area (height and width) were evaluated. No significant changes in the resolution and shapes of the peak and retention time were observed when temperature and flow rate were modified. Modifications of the composition of the mobile phase by changing the organic-to-inorganic ratio resulted in the essential changes of retention time and resolution in the determination of Cilostazol.

4) Analysis of marketed formulation
The drug content was found to be 99.95% with %RSD of 0.16 (Table 3). It was noted that no degradation had occurred in the marketed formulation that were analyzed by this method. The low RSD value indicated the suitability of this method for routine analysis of Cilostazol in pharmaceutical dosage forms.

5) Stability indicating property
The chromatogram of no stress treatment sample (as control) showed no additional peak (Fig 4). The chromatogram of acid and alkali degraded samples showed no degradation in the peak intensity at retention time of 8.2 min (Fig 5 & 6). The chromatogram of H$_2$O$_2$ degraded sample showed a 20% decrease in intensity of peak at 8.2 min and an additional small peak at retention time of 2.3 min corresponding to degraded component (Fig 7). There was no decrease in intensity of peak at 8.2 min in photo degraded sample (Fig 8) and thermal degraded sample (Fig 9). Thus Cilostazol was found to be stable to acid, alkali, heat and sunlight. The lower retention times of the degraded components indicated that they were polar than the analyte itself (Table 4).

6) Detection of the related impurities
The sample solution showed no additional peaks other than the principal peak. Hence related impurities are not present in the market sample.

<table>
<thead>
<tr>
<th>Spiked concentration (µg/ml)</th>
<th>Measured concentration ± SD (µg/ml)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>19.96 ± 0.06</td>
<td>0.30</td>
</tr>
<tr>
<td>40</td>
<td>39.92 ± 0.10</td>
<td>0.26</td>
</tr>
<tr>
<td>80</td>
<td>80.06 ± 0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>39.84 ± 0.19</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount of Standard added to pre-analyzed sample (µg/ml)</th>
<th>Amount recovered (mg)</th>
<th>%Recovery ± SD</th>
<th>%RSD</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>19.88</td>
<td>99.42 ±0.53</td>
<td>0.53</td>
<td>0.33</td>
</tr>
<tr>
<td>40</td>
<td>40.65</td>
<td>101.62 ±0.39</td>
<td>0.39</td>
<td>5.80</td>
</tr>
<tr>
<td>60</td>
<td>60.03</td>
<td>100.05 ±0.21</td>
<td>0.21</td>
<td>5.77</td>
</tr>
<tr>
<td>*n=3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Label Claim</th>
<th>Amount found ± SD</th>
<th>% of Label claim ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mg</td>
<td>49.97mg ± 0.08</td>
<td>99.95 ± 0.16</td>
</tr>
<tr>
<td>*n=3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4

Degradation studies of Cilostazol

<table>
<thead>
<tr>
<th>Condition</th>
<th>Degradation</th>
<th>Additional peaks</th>
<th>Peak purity$^a$</th>
<th>Tail peak$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid, 2N HCl, heated at 80°C for 24 Hr</td>
<td>Nil</td>
<td>-</td>
<td>99.8</td>
<td>1.11</td>
</tr>
<tr>
<td>Base, 2N NaOH at 80°C for 24 Hr</td>
<td>Nil</td>
<td>-</td>
<td>98.8</td>
<td>1.13</td>
</tr>
<tr>
<td>Oxidative, 15% H$_2$O$_2$ heated for 30 min</td>
<td>20%</td>
<td>2.3 min</td>
<td>98.9</td>
<td>1.12</td>
</tr>
<tr>
<td>Thermal, Heating at 80°C for 24 Hr</td>
<td>Nil</td>
<td>-</td>
<td>99.8</td>
<td>1.10</td>
</tr>
<tr>
<td>Light, Exposed to sunlight for 24 Hr</td>
<td>Nil</td>
<td>-</td>
<td>99.5</td>
<td>1.17</td>
</tr>
</tbody>
</table>

$^a$Peak purity values in the range of 98-100 indicate a homogeneous peak
$^b$Tail peak <1.50 indicates symmetry of peak.

Table 5

Results from system suitability determination

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean$^*$</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area</td>
<td>1100.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Retention time</td>
<td>0.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>70053</td>
<td>1.76</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.972</td>
<td>0.69</td>
</tr>
</tbody>
</table>

$^*$Mean of three replicates.

CONCLUSION

The developed HPLC technique is simple, precise, accurate and stability indicating. As the Cilostazol is sensitive to degradation, selectivity is an important validation parameter. Statistical analysis proves that the method is reproducible and selective for the analysis of Cilostazol in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, under all stress conditions using HCl, NaOH, H$_2$O$_2$, heat and UV light, it can be employed as a stability indicating one.

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Conflict of Interest: Declared none

REFERENCES


