A VALIDATED HPTLC METHOD FOR THE DETERMINATION OF CLOPIDOGREL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple, selective and precise high performance thin layer chromatographic method was developed and validated for the determination of Clopidogrel in bulk drug and in formulation. The method uses aluminium plates pre-coated with silica gel 60F254 as the stationary phase and hexane: methanol: chloroform: ammonia (16:2:1.5:0.5, v/v/v/v) as solvent system. This system gave compact spot for Clopidogrel (Rf: 0.65 ± 0.02). Densitometric analysis of Clopidogrel was performed in the absorbance mode at 254nm. The linear regression analysis data for the calibration plot showed good linear relationship over a concentration range of 1 – 10 µg spot⁻¹. The values of correlation coefficient, slope and intercept were 0.998, 661.6 and -4.333 respectively. The method was validated for precision, robustness and recovery. The limit of detection and limit of quantification were 0.0786 and 0.785 µg spot⁻¹, respectively.

KEYWORDS: HPTLC; Clopidogrel; dosage forms; validation.

INTRODUCTION
Clopidogrel bisulfate [1] is official in USP 2007. It is chemically (+)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid methyl ester sulphate, an inhibitor of adenosine diphosphate (ADP) induced platelet aggregation. It [2] acts by direct inhibition of ADP binding to its receptor and of subsequent ADP mediated activation of glycoprotein
GPIIb/IIIa complex. It is used as an effective drug for reducing the incidence of ischemic strokes, heart attacks or claudicating due to vascular diseases such as atherosclerosis.

The United States Pharmacopoeia 2007 recommended a reverse phase HPLC method with UV detection at 220 nm for determination of Clopidogrel bisulphate in tablets.

Literature survey reveals that there are different types of assay methods for the determination of Clopidogrel in pharmaceutical dosage forms. These include chemometric, spectrophotometric, TLC, HPTLC, HPLC, LC-MS and voltametric methods. The analysis of carboxylic acid metabolite of Clopidogrel in the plasma and serum are reported by HPLC, LC-MS/MS, GC-MS and capillary zone electrophoresis methods.

However, most of these methods either require very expensive instruments and reagents or complicated procedures. Hence, there is a need for simple, rapid and reproducible method for the routine analysis of Clopidogrel in pharmaceutical dosage forms.

Today TLC is rapidly becoming a routine analytical technique due to its low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase and thus reducing the analysis time and cost per sample as compared to HPLC. The aim of the present study was to develop a simple, validated and rapid HPTLC method for routine analysis of Clopidogrel in tablets. The HPTLC method was studied following official guidelines, evaluating the main parameters and the procedures and validated according to ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents
Clopidogrel bisulfate was supplied by Sun Pharmaceutical Industries Limited, Gujarat. Clopidogrel Tablets were purchased from local market. All the chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

HPTLC Instrumentation
The samples were spotted in the form of 6mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 F254 (10 × 10 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were pre-washed with methanol and
activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 4.00 × 0.30 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of hexane:methanol:chloroform:ammonia (16:2:1.5:0.5, v/v/v/v) and 10 ml of mobile phase was used. Linear ascending development was carried out in a 10×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2). The length of the chromatogram run was approximately 8 cm, subsequent to development; the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WINCats software.

**Preparation of standard solution and linearity study:** An accurately weighed quantity of 10 mg of Clopidogrel was transferred to 10 ml volumetric flask, dissolved in methanol and made up to mark with the same solvent to obtain concentration 1 µg/µl. Standard solutions of 1, 2, 5, 7, 10 and 15 µl of Clopidogrel was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtain the concentration of 1, 2, 5, 7, 10 and 15 µg spot⁻¹. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated six times.

**Method validation**

**Precision:** Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (5µg spot⁻¹ of Clopidogrel). The intra and inter-day variation for the determination of Clopidogrel was carried out at three different concentration levels of 2, 5 and 7 µg per spot in triplicate.

**Limit of detection (LOD) and limit of quantification (LOQ):** In order to determine detection and quantification limit, Clopidogrel concentrations in the lower part of the linear range of the calibration curve were used. Clopidogrel solutions of 1, 2, 5, 7, 10 and 15 were prepared and applied in triplicate. The LOQ and LOD were calculated using equation LOD=3.3 × N/B and LOQ=10×N/B, where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

**Specificity:** The specificity of the method was ascertained by analyzing standard drug and sample. The spot of Clopidogrel in sample was confirmed by comparing the Rₜ values and spectra of the spot with that of standard. The peak purity of Clopidogrel was assessed by
comparing the spectra at three levels, i.e., peak start(S), peak apex(M) and peak end(E) positions of the spot.

**Ruggedness:** Ruggedness of the method was performed by spotting 5µg spot\(^{-1}\) of Clopidogrel by two different analyst keeping same experimental and environmental conditions.

**Accuracy:** The analyzed samples were spiked with extra 80, 100 and 120% of the standard Clopidogrel and the mixtures were analyzed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

**Robustness:** By introducing small changes in the mobile phase composition, the effects of the results were examined. Mobile phases having different compositions of hexane:methanol:chloroform:ammonia was tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60±5ºC for 2, 5 and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20 and 40 min.

**Application of proposed method to Tablet formulation:** 20 tablets (CLOPILET 75 mg) were weighed and finely powdered. A quantity of Clopidogrel powder equivalent to 10mg of Clopidogrel was dissolved in methanol, sonicated for 20 minutes and made up to volume in a 10ml volumetric flask. After filtration through 0.41 µm filter (millifilter, Milford, MA), 5µl and 7µl of the solution were spotted followed by development and scanning as described in standard preparation. The analysis was repeated in triplicate.

**RESULTS AND DISCUSSION**

**Development of optimum mobile phase:** TLC procedure was optimized with a view to develop a sensitive and reproducible assay method for Clopidogrel. Initially, different solvent systems were tried based on trial and error method. The solvent system, hexane: methanol: chloroform, (v/v/v) gave good resolution for Clopidogrel, but typical peak nature was missing. Finally, the mobile phase consisting of hexane: methanol: chloroform: ammonia (16:2:1.5:0.5, v/v/v/v) gave a sharp and well defined peak at R\(_f\) value of 0.65. Well defined
spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

**Calibration curve:** The linear regression data for the calibration curves showed good linear relationship over the concentration range 1-10µg spot⁻¹. Linear regression equation was found to be \( Y=661.6x-4.333 \) \( (r^2=0.998) \).

**Validation of method**

**Precision**

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (\%RSD). The results depicted revealed high precision of the method presented in Table 1.

**LOD and LOQ:** Detection limit and quantification limit was calculated by the method described above. The LOQ and LOD were found to be 0.0786 and 0.785 µg spot⁻¹ respectively. This indicates the adequate sensitivity of the method.

**Recovery studies:** The proposed method when used for extraction and subsequent estimation of Clopidogrel from the pharmaceutical dosage form after spotting with 80, 100 and 120% of additional drug; afforded good recovery of Clopidogrel. The amount of drug added and the % recovery are listed in Table 2.

**Specificity:** The peak purity of Clopidogrel was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., \( r^2 \) (S,M)=0.999 and \( r^2 \) (M,E)=0.9998. Good correlation \( (r^2=0.999) \) was also obtained between standard and sample spectra of Clopidogrel.

**Robustness of the method:** The standard deviation of peak areas was calculated for each parameter and \%RSD was found to be less than 2%. The low values of \%RSD values as shown in Table 3 indicated robustness of the method.

**Analysis of the marketed formulation:** A single spot at \( R_f \) 0.65 was observed in the chromatogram of the drug samples applied from the tablets. There was no interference from excipients. The % drug content and \%RSD were calculated. The low \%RSD value indicated the suitability of this method for the routine analysis of Clopidogrel in pharmaceutical dosage forms.
Fig 1. Structure of Clopidogrel

![Structure of Clopidogrel](image)

Fig 2. Calibration Curve of Clopidogrel

![Calibration Curve of Clopidogrel](image)

Fig 3. Chromatogram of Clopidogrel

![Chromatogram of Clopidogrel](image)
Table 1. Intraday and inter-day precision studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. µg/spot</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Amount found*±SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>2</td>
<td>100.08±1.2</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>99.98±0.35</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>99.70±0.26</td>
<td>0.26</td>
</tr>
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</table>

*n=3

Table 2. Recovery Studies of Clopidogrel

<table>
<thead>
<tr>
<th>Label claim (mg/tablet)</th>
<th>Amount of Standard drug added (%)</th>
<th>Drug recovered* (%)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0</td>
<td>99.86</td>
<td>0.15</td>
</tr>
<tr>
<td>75</td>
<td>80</td>
<td>99.98</td>
<td>0.21</td>
</tr>
<tr>
<td>75</td>
<td>100</td>
<td>99.92</td>
<td>0.38</td>
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<tr>
<td>75</td>
<td>120</td>
<td>99.80</td>
<td>0.64</td>
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*n=6

Table 3. Robustness of the method*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S.D. of peak area</th>
<th>% RSD</th>
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<tr>
<td>Mobile phase composition</td>
<td>25.04</td>
<td>0.76</td>
</tr>
<tr>
<td>Mobile phase volume</td>
<td>29.73</td>
<td>0.90</td>
</tr>
<tr>
<td>Development distance</td>
<td>24.21</td>
<td>0.73</td>
</tr>
<tr>
<td>Activation of TLC plate</td>
<td>19.21</td>
<td>0.59</td>
</tr>
<tr>
<td>Duration of saturation</td>
<td>20.41</td>
<td>0.62</td>
</tr>
<tr>
<td>Time from spotting to chromatography</td>
<td>24.61</td>
<td>0.74</td>
</tr>
<tr>
<td>Time from chromatography to scanning</td>
<td>15.57</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*n=6

Table 4. Results of analysis of Clopidogrel Tablet by proposed method*

<table>
<thead>
<tr>
<th>Label Claim</th>
<th>Amount found ± SD</th>
<th>% of Label claim ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>75mg</td>
<td>74.97mg ± 0.19</td>
<td>99.96 ± 0.25</td>
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</table>

*n=3

CONCLUSION
The developed HPTLC technique was simple, precise, accurate and economical. As the Clopidogrel is sensitive to degradation, selectivity is an important validation parameter. Statistical analysis proves that the method is reproducible and selective for the analysis of Clopidogrel in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various sources.
ACKNOWLEDGEMENT
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REFERENCES