Development and Validation of Derivative UV-Spectropotometric Methods for Quantitative Estimation of Clopidogrel in Bulk and Pharmaceutical Dosage Form

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Abstract: A simple, precise and economical first-order (Method A), Area Under Curve [AUC] (Method B) UV-Spectrophotometric methods have been developed and validated for the estimation of Clopidogrel in bulk and its tablet formulation. The solutions of standard and sample were prepared in 0.1 N HCl. Clopidogrel was estimated at 219 nm for the first order UV-Spectrophotometric method, while area under the zero order spectrum of Clopidogrel was measured in between 221 nm to 231 nm for AUC method. Beer’s law was obeyed in the concentration range of 10 - 30 μg / ml with $r^2$ value 0.999 for first order method. Similarly in AUC method, Beer’s law was obeyed in the concentration range of 10 - 30 μg / ml with $r^2$ value 0.999. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation, which was within the range of 0.169 % to 0.519 % for the above two methods. The proposed methods were successfully applied for the determination of Clopidogrel in tablet formulations. In addition, the proposed methods are simple, easy to apply, low-cost, and requires relatively inexpensive instruments.

Keywords: Clopidogrel, Method Validation, UV-Spectrophotometric methods.

1. INTRODUCTION:
Clopidogrel bisulfate, chemically (+)-(S)- (2-chlorophenyl)- 6,7-dihydrothieno [3,2-c] pyridine- 5(4H)-acetic acid methyl ester sulphate is a potent oral antiplatelet agent often used in the treatment of coronary artery disease, peripheral vascular disease and cerebro vascular disease. The structure of Iloperidone is shown in Figure 1. The mechanism of action of clopidogrel is irreversible blockade of the adenosine diphosphate (ADP) receptor P2Y12 and is important in platelet aggregation, the cross-linking of platelets by fibrin. The blockade of this receptor inhibits platelet aggregation by blocking activation of the glycoprotein IIb/IIIa pathway. It is not in any pharmacopoeia. Literature survey reveals the estimation of Clopidogrel bisulfate in pharmaceutical formulations by various chemometric², HPLC³-⁶, HPTLC⁷-⁸, TLC⁹, and an LC- ESI-MS-MS¹⁰ method was developed. There is no spectrophotometric method for Clopidogrel bisulfate, hence to develop simple, sensitive and accurate method.

Fig. 1: Chemical Structure of Clopidogrel
2. MATERIALS AND METHODS:

2.1 Instrument and reagents
Spectral scans were made on a Shimadzu UV spectrophotometer, model 1800 (Shimadzu, Japan) with spectral bandwidth of 0.5 nm, wavelength accuracy of ± 0.3 nm with automatic wavelength corrections using a pair of 10 mm quartz cells. All spectral measurements were done using UV-Probe 2.33 software. Clopidogrel sample was supplied by Cadila Pharmaceutical Ltd. Ahmedabad, India as a gift sample and used as such 0.1 N HCl used and purchased from S. D. Fine Chemicals Ltd., India.

2.2 Preparation of standard drug solution
100 mg standard Clopidogrel was weighed accurately and transferred to a 100 ml volumetric flask and dissolved in 0.1 N HCl. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 μg/ml. From this solution, 10 ml of solution was pipetted out and placed into 100 ml volumetric flask. The volume was made up to mark with methanol to give a solution containing 100 μg/ml. Further dilutions with 0.1 N HCl were made from this stock solution to get required concentration.

2.3 Preparation of sample solution
To determine the content of Clopidogrel in conventional tablet (label claim: 75 mg Clopidogrel per tablet), twenty tablets were weighed, their mean weight was determined and finely powdered. Tablet powder equivalent to 100 mg of Clopidogrel was weighed and transfer into 100 ml volumetric flask then dissolved with methanol and further diluted up to the mark. It was kept for ultra-sonication for 30 min; this was filtered through Whatman filter paper No. 41 and then the final dilution was made with 0.1 N HCl to get the final stock solution of 1000 μg/ml. From this stock solution, various dilutions of the sample solution were prepared and analyzed.

2.4 Calibration curve

Method A: Absorption Maxima Method
For the selection of analytical wavelength, 20 μg/mL solution of Clopidogrel was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the spectrum (Figure 2,3), λ max of Clopidogrel, 219 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-30 μg/mL at 219 nm.

Method B: Area under Curve Spectroscopic method
In this method, 20 μg/mL solution of Clopidogrel was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The Absorption spectra thus obtained were AUC from zero order. The AUC spectra (Figure 4,5) measured between 221 nm to 231 nm. The calibration curve for Clopidogrel was plotted in the concentration range of 10-30 μg/mL.

Fig. 2. Overlay first order derivative spectra of Clopidogrel (20 μg/ml) showing absorbance at 219 nm
Fig. 3. Calibration curve for Clopidogrel at 262nm by First order derivative spectroscopy

Fig. 4. Overlay AUC spectra of Clopidogrel (20 µg/ml) showing area from 221 nm to 231nm.

Fig. 5. Calibration curve for Clopidogrel by AUC spectroscopy
Table-1: Validation Parameters.

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>First order derivative method</th>
<th>AUC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Wavelength (nm)</td>
<td>219</td>
<td>221-231</td>
</tr>
<tr>
<td>Linearity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (µg/ml)</td>
<td>10-30</td>
<td>10-30</td>
</tr>
<tr>
<td>Slope</td>
<td>0.009</td>
<td>0.017</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.0113</td>
<td>0.058</td>
</tr>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>0.345</td>
<td>0.175</td>
</tr>
<tr>
<td>% RSD of Intraday Precision (20 µg/ml)</td>
<td>0.40</td>
<td>0.21</td>
</tr>
<tr>
<td>% RSD of Interday Precision (20 µg/ml)</td>
<td>0.48</td>
<td>0.25</td>
</tr>
<tr>
<td>Accuracy* (% Recovery ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% (10 µg/ml)</td>
<td>98.33±0.162</td>
<td>99.23±1.68</td>
</tr>
<tr>
<td>100% (20 µg/ml)</td>
<td>101±0.165</td>
<td>98.46±0.881</td>
</tr>
<tr>
<td>150% (30 µg/ml)</td>
<td>99.44±0.05</td>
<td>99.76±0.54</td>
</tr>
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</table>

*Mean ±SD are obtained from 6 determinations.

Table 2: Analysis of tablet formulation

<table>
<thead>
<tr>
<th>METHOD</th>
<th>Label claimed (mg)</th>
<th>Amount found (mg)</th>
<th>%Recovery(± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First order derivative method 75</td>
<td>74.99</td>
<td>99.96 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>AUC method             75</td>
<td>75.07</td>
<td>100.16 ± 0.11</td>
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</table>

**Average of six determinations

3. RESULT AND DISCUSSION
The first order, AUC UV-spectroscopic methods is for routine analysis of Clopidogrel in bulk and formulations. The spectroscopy methods applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures[11-13]. Clopidogrel has the absorbance maxima at 219 nm (Method A), and in the AUC spectra measured between 221 nm to 231 nm (Method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 2-10 µg/ml and given in Table 1. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analyzed tablet powder sample and shown in Table 1. The percentage recovery value indicates non interference from excipients used in formulation. The result of analysis of marketed formulations is shown in Table 2. The reproducibility and accuracy of the method was found to be good, which was evidenced by low standard deviation.

4. CONCLUSION
The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. It can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of Clopidogrel in pharmaceutical formulations.

5. ACKNOWLEDGEMENT
We would like thank to Cadila Pharmaceutical Ltd. Ahmedabad, India for providing reference sample of Clopidogrel to facilitate this work, and also we are grateful to the Principal, Bharathi College of Pharmacy, Bharathi Nagara, for providing necessary facilities to carry out this work.
6. REFERENCES


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