Spectrophotometric Determination of Clopidogrel in Pure Powder and in Tablets

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ABSTRACT

The charge transfer complex formed by the interaction between the electron acceptor, chloranilic acid and an electron donor clopidogrel was adopted for the assay of the drug in pure powder and in tablets. Chloranilic acid was found to form 1:1 molecular complex with clopidogrel with maximum absorption band at 540 nm. The absorbance was linear (r = 0.9990) over the concentration range of 5 – 40 µg/ml with molar absorptivity of 1.97 x 10³ Lmol⁻¹cm⁻¹. The effects of variables such as reagent concentration, time of reaction, colour stability and interference from excipients have been investigated to optimize the procedure. The results have been validated analytically and statistically. The proposed method has been successfully applied for the determination of clopidogrel in pharmaceutical formulations. Results indicated that the method is accurate, precise and reproducible (relative standard deviation < 2%).

Keywords: Clopidogrel, Spectrophotometric method

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INTRODUCTION

The term charge transfer complex denotes a certain type of complex which results from interaction of an electron acceptor and an electron donor with the formation of weak bonds [1]. Charge transfer complex results from a donor-acceptor mechanism of Lewis acid-base reaction between two or more different chemical constituents [2]. The formation of electron donor-acceptor complexes can be rapidly assessed for its validity as a simple quantitative determination of methldopa [3], hexamethyletetramine [4], flunarizine dihydrochloride [5], amino acid [6] and pyrimethamine [7], moxifloxacin [8], risperidone [9], paroxetine [10]. Chloranilic acid; 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone, has been used for the determination of some organic compounds containing lone pair of electrons [11-14]. These complex reactions were the basis of the development of sensitive spectrophotometric method for the determination of clopidogrel. Clopidogrel is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease and cerebrovascular disease. The drug works by irreversibly inhibiting a receptor called P2Y₁₂, an adenosine diphosphate (ADP) chemo receptor on platelet cell membranes.

EXPERIMENTALS

Apparatus

UV-visible spectrophotometer (Jenway 6305) with 1 cm quartz cells was used for all measurements.

Materials and reagent solutions

Clopidogrel (Ranbaxy Nigeria Limited), chloranilic acid was obtained from Sigma – Aldrich Co. Ltd., USA. The chloranilic acid was prepared in dioxin at concentration of 0.10 % (w/v). All solvents and other chemicals used throughout this study were of analytical grade.

Pharmaceutical dosage forms

Clopidogrel tablets were labeled to contain 75 mg clopidogrel per tablet.

Preparation of standard solution

Accurately weighed amount (75 mg) of clopidogrel was transferred into a 100 ml volumetric flask and dissolved in 60 ml of methanol and diluted to volume with methanol to provide a stock solution. The stock solution was diluted with methanol to obtain the suitable working concentrations.

Preparation of sample for analysis
Ten tablets were weighed, finely powdered. An accurately weighed quantity (0.208 g) of the powdered ingredient was transferred into a 100 ml volumetric flask, dissolved in about 60 ml of methanol. The contents of the flask were swirled, sonicated for 5 minutes and then diluted to volume with methanol. The mixture was mixed well, filtered through Whatman paper (No 14) and the first portion of the filtrate was rejected, a measured volume (20 ml) of the filtrate was transferred to a 10 ml volumetric flask and diluted quantitatively with methanol to give a working solution containing 20 µg/ml.

**General assay procedure**

Aliquots of standard or sample solution were transferred into a 10 ml volumetric flask. Then 1 ml of chloranilic acid (0.10% w/v) was added. The reaction solution were mixed well and allowed to stand at room temperature for 20 minutes. The solutions were completed to volume with dioxin. The absorbance readings were measured at λ max 540 nm against reagent blank treated similarly.

**Stoichiometry of the complex species**

The Job’s method of continuous variation [15] was employed. Equimolar solutions (9.75 x 10⁻⁵ M) of clopidogrel and chloranilic acid were prepared. The drug was prepared in methanol and in dioxan for chloranilic acid. Series of 10 ml portions master solutions of the drug and the reagent were made up comprising different complimentary ratios (0:10, 1:9 ... 9:1, 10:0 inclusive) in 10 ml volumetric flasks. The reactions were allowed to proceed under the optimum conditions reported under the general assay procedure. The absorbance readings of the resulting solutions were measured at λ max 540 nm against reagent blanks treated similarly.

**RESULTS AND DISCUSSION**

**Absorption spectra**

Chloranilic acid in a solution of dioxane displayed an absorption peak at 430 nm while mixing the chloranilic acid with a solution of clopidogrel in methanol resulted in a change of the yellow-pink colour of the chloranilic acid to purple. As a consequence, the absorption band of chloranilic acid showed a bathochromic shift. This was indicative of charge transfer complex formation. The charge transfer complex between clopidogrel and chloranilic acid exhibited an absorption band at 540 nm (Fig. 1). In other words, the interaction between clopidorel and chloranilic acid is a charge transfer complexation between the n-donor clopidogrel and the π-acceptor followed by the formation of radical anion according to the following scheme:

\[
D + A \rightarrow [D^\times A^-] \rightarrow D^+ + A^- 
\]

**Concentration of chloranilic acid and Reaction time**

The effect of chloranilic acid solution was investigated by carrying out the reaction using 1 ml of chloranilic acid solution of different concentrations varying from 0.01 – 1.0% (w/v). It was found that the absorbance increased by increasing the concentration of chloranilic acid up to 0.10% w/v. Higher concentrations of chloranilic acid solution had no effect on the values of absorbance readings in all cases.
The study of effect of time on the complex revealed that the optimum time for compete reaction was 20 minutes and longer time had neither enhancement nor negative effect on the reaction time.

**Job’s plot for clopidogrel-chloranilic acid complex**

The determination of the mole ratio of reactions in the complexes is based on the method of continuous variation as proposed by Job [13]. The plot (Fig. 5) indicated a 1:1 clopidogrel-chloranilic acid interaction which may be represented as

$$A + D = [D:A]$$

Where A is the acceptor (chloranilic acid), D is the donor (clopidogrel) and [D:A] represents the complex formed. This indicates the presence of only one possible binding site for chloranilic acid in clopidogrel. The absorbance of the complex obtained experimentally was used to calculate the molar absorptivity.
Beer's plot for clopidogrel-chloranilic acid complex

Beer's law was obeyed. At 540 nm, linear relationship was obtained \((r = 0.990)\) between the absorbance and the concentration over the entire range studied. The regression equation of the line as derived by the method of least square is

\[ A_{540 \text{ nm}} = 0.043[D] + 0.020 \]

Where \([D]\) is the concentration of clopidogrel in \(\mu\text{g/ml}\).

**Interference studies**

To explore the effect of some common excipients for tablets on the analytical performance of the proposed method, samples were prepared by mixing known amount of clopidogrel with various amounts of the excipients (Table 1). The analysis of these samples showed no interference was found from the tested excipients with the proposed method. The recovery values were \(99.88 \pm 0.27 \text{ – } 102.20 \pm 0.85\). The obtained good recoveries ensured the suitability of the method for the analysis of dosage forms without interference from the common excipients.

### Table 1: Characteristics and statistical data for the regression equation of the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Lambda_{\text{max}}) (nm)</td>
<td>540</td>
</tr>
<tr>
<td>Beer's law limit ((\mu\text{g/ml}))</td>
<td>5 - 40</td>
</tr>
<tr>
<td>Molar absorptivity (L/mole/cm)</td>
<td>1.97 \times 10^3</td>
</tr>
<tr>
<td>Color stability (h)</td>
<td>2</td>
</tr>
<tr>
<td>Regression equation (Y*)</td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.043</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>+ 0.020</td>
</tr>
<tr>
<td>Correction coefficient (r)</td>
<td>0.9990</td>
</tr>
<tr>
<td>Limit of detection ((\mu\text{g/ml}))</td>
<td>1.20</td>
</tr>
</tbody>
</table>

\*\(Y = a + bc\), where \(c\) is the concentration of analyte (\(\mu\text{g/ml}\)) and \(Y\) is the absorbance unit.

The validity of the regression equation stated for the calibration of clopidogrel-chloranilic acid complex was assessed in its determination on pure sample and in tablet dosage form (Table 2).

### Table 2: Validation of the method in pure and tablets.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pure sample</th>
<th>Tablets(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Mean recovery (%)(^b)</td>
<td>100.75</td>
<td>100.58</td>
</tr>
<tr>
<td>SD</td>
<td>1.69</td>
<td>0.51</td>
</tr>
<tr>
<td>(t)-calculated(^c)</td>
<td>1.21</td>
<td>1.05</td>
</tr>
</tbody>
</table>

\(^a\) Caplor tablets manufactured by Ind-Swift limited (A), Clopicure tablets manufactured by Hinglaj labs of India (B), Clopid tablets manufactured by Genix Pharma ltd (C).

\(^b\) Mean for ten determination; percentage recovery from the label claim amount

\(^c\) The value for \(t\)-theoretical at \(\alpha = 0.025\) is 1.96

Recovery experiments carried out on clopidogrel in pure sample and in tablet dosage forms showed high quantitative recoveries with low standard deviations. The performance of the proposed method was judged through calculation of the Student's \(t\)-test value. A 95% level of probability, the calculated values of \(t\) do not exceed the theoretical values. This is an indication that the proposed method gives results not significantly different from the true values, according to label claim, and tends further to confirm the high accuracy of the method. The presence of auxiliary substances used in the tablet manufacture did not interfere with the accuracy of the method since the clopidogrel was extracted before analysis. However, as there was no official method for the analysis of the dosage forms, the tablets were subjected to the analysis of their contents of the studied drug only by the proposed method (Table 3).

### Table 3: Recovery of clopidogrel in tablet – pure sample mixtures by the proposed method.

<table>
<thead>
<tr>
<th>Amount of clopidogrel in tablet (mg)</th>
<th>Amount of pure clopidogrel added (mg)</th>
<th>Amount of Clopidogrel found (mg)</th>
<th>Recovery (%)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0</td>
<td>75.263</td>
<td>100.35±0.93</td>
</tr>
<tr>
<td>75</td>
<td>4</td>
<td>80.477</td>
<td>101.87±0.56</td>
</tr>
<tr>
<td>75</td>
<td>6</td>
<td>80.936</td>
<td>99.92±0.82</td>
</tr>
<tr>
<td>75</td>
<td>8</td>
<td>82.893</td>
<td>99.87±0.43</td>
</tr>
<tr>
<td>75</td>
<td>10</td>
<td>84.987</td>
<td>99.89±0.51</td>
</tr>
</tbody>
</table>

Values are mean of five determination ± SD
CONCLUSION

Clopidogrel was found to form a charge transfer complex with chloranilic acid in 1:1 stoichiometry, with maximum absorption at 540 nm. The complex was found to be stable for over 24 hours at room temperature. Application of this method in the assay of clopidogrel showed that it is accurate and precise for the rapid assay of clopidogrel in pure sample and in dosage forms as presented in the recovery results.

REFERENCES


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