# UV-VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF ASSAY OF CLOPIDOGREL TABLET FORMULATION

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***ABSTRACT-Safe, rigid, accurate, precise and sensitive method of spectrophotometric estimation in UV-region has been developed for the assay of Clopidogrel. The method has been developed and validated using 0.1N ethanolic HCl, which does not showing any interference in spectrophotometric estimations. All the parameters of the ICH Q2R1 for the analytical procedure and validation were performed and validated statistically using RSD and %RSD.***

***Keywords- Ultraviolet, Parts per million.***

## INTRODUCTION

Spectroscopy is the study of the interaction between matter and electromagnetic radiation. Historically, spectroscopy originated through the study of visible light dispersed according to its wavelength, by a prism. Later the concept was expanded greatly to include any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopy and spectrography are terms used to refer to the measurement of radiation intensity as a function of wavelength.[1,2]

### Introduction to Clopidogrel

Clopidogrel is used alone or together with aspirin to lessen the chance of a heart attack or stroke. It is given to patients who have already had a heart attack, severe chest pain, or a stroke, or to patients with other circulation problems that could cause a stroke or heart attack.

A heart attack or stroke may occur when a blood vessel is blocked by a blood clot. Clopidogrel is a platelet inhibitor. It reduces the chance that a harmful blood clot will form by preventing platelets from clumping together in the blood. Clopidogrel may also increase the chance of serious bleeding in some people.[3]

Clopidogrel may also be used instead of low-dose aspirin if the patient is allergic to aspirin and are considered to be at risk of having a heart attack or stroke. For example, if patient:

- have high cholesterol
- have high blood pressure
- have diabetes
- smoke

### How it works

Antiplatelet medicines reduce the risk of clots forming in the blood. This cuts the risk of having a stroke or heart attack. Normally, when there is a cut or break in a small blood vessel, a blood clot forms to plug the hole until the blood vessel heals.

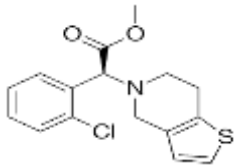
Small cells in the blood called platelets make the blood clot. When a platelet detects a damaged area of a blood vessel, it produces a chemical that attracts other platelets and makes them stick together to form a blood clot.

Clopidogrel reduces the ability of the platelets to stick together and reduces the risk of clots forming.

### Clopidogrel and low-dose aspirin

Sometimes, Pateint may be given both low-dose aspirin and clopidogrel to take together for a period of treatment. Taken together, they are very effective, but there is a higher risk of bleeding, usually in the gut. This risk increases with age. This combination treatment should usually be taken for no longer than 9-12 months. After this period, low-dose aspirin is taken.[4]

### Description




**Systematic (iupac) name: methyl (2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridine-5-yl)acetate**

It is a P2Y12 Platelet Inhibitor

**Mechanism of action:** The search for active antiplatelet drugs within the original chemical class of the thienopyridines, led to the discovery of clopidogrel, a novel ADP-selective agent whose antiaggregating properties are several times higher than those of ticlopidine. The antiaggregating properties of this compound are well known and, very recently, new results have clarified its mechanism of action. Clopidogrel is active only after intravenous or oral administration, and no circulating activity has been found in the plasma of treated animals or human volunteers. Experiments in rats have demonstrated that the antiaggregating activity was caused by a shortlasting metabolite generated in the liver by a cytochrome P450-dependent pathway. The antiaggregating property of clopidogrel is caused by an inhibition of the binding of ADP to its platelet

receptors, and more specifically to the low affinity receptors, the high affinity binding sites being unaffected by clopidogrel. Several events in the ADP activation process, including adenylyl cyclase down-regulation, protein tyrosine phosphorylation, activation of the GPIIb-IIIa complex, fibrinogen binding, aggregation and release, were inhibited by clopidogrel and indicate their close relationship with the activation of a low affinity receptor by ADP. In contrast, binding of ADP to its high affinity binding sites (clopidogrel-resistant receptors) induced shape change, cytosolic calcium increase and phosphorylations of several other proteins, some events which were clopidogrel-sensitive. Thus, clopidogrel not only constitutes a potent antithrombotic drug in humans but also a good tool to study the effect of ADP on platelets.[5]

**Metabolism:** After oral administration, clopidogrel is rapidly absorbed. Owing to its extensive metabolism, clopidogrel is not detected in human plasma. Clopidogrel is a prodrug that is absorbed in the intestine [6,7] and activated in the liver [8]

The conversion of clopidogrel to its active metabolite requires two sequential oxidative steps. The first step leads to formation of 2-oxo-clopidogrel, followed by the conversion of 2-oxoclopidogrel to the active metabolite. CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4/5 are implicated as cytochrome P450 enzymes involved in the metabolism of clopidogrel. However, the relative importance of the individual enzymes and which part of the reaction they are involved in are controversial, showed that clopidogrel was converted into 2-oxo-clopidogrel by cytochrome P450 monooxygenase-dependent metabolism **in vitro** and that hydrolysis of 2-oxo-clopidogrel generates the active metabolite. Several publications indicate a major role for CYP3A4 [9,10]

Other in-vitro studies showed that CYP1A2, CYP2B6, and CYP2C19 were capable of forming the 2-oxo-clopidogrel form from clopidogrel in liver microsomes. When 2-oxo-clopidogrel was used as a substrate, the enzymes CYP3A4, CYP2C9, CYP2C19, and CYP2B6 produced the active metabolite [11,12]CYP2C19 contributes substantially to both oxidative steps and that CYP3A4 contributes substantially to the second oxidative step. In a competing metabolic reaction, about 85% of the drug is hydrolyzed to an inactive carboxylic acid derivative by esterases [13,14]. The active metabolite of clopidogrel contains a thiol group which binds to a free cysteine on the P2RY12 receptor and irreversibly blocks ADP binding and receptor activation .Once this blockage has occurred, platelets are affected for their entire lifespan of approximately 7–10 days.

**Aim of Present Work**

This work deals with the validation of the developed method for the assay of Clopidogrel from its dosage form (tablets). Hence, the method can be used for routine quality control analysis and also stability.

**The aim and scope of the proposed work are as under:**

- To develop suitable spectrophotometric method for assay of Clopidogrel tablet.
- Perform the validation for the method.

**Experimental:**

**Materials**

Clopidogrel standard. Clopidogrel tablets containing 75 mg Clopidogrel and the inactive ingredient used in drug matrix were obtained from market. Analytical grade ethanol and HCl were obtained.

**Diluent preparation**

0.1N Ethanolic HCl is used as a diluent.

**Standard preparation**

10 mg drug was dissolved in 15 ml 0.1N ethanolic HCl and was shaken well. Then 85 ml diluent was added to it to adjust the volume up to 100 ml (100 ppm). From that 5 ml was taken and volume was adjusted up to 50 ml with diluent.

**Test preparation**

20 tablets were weighed and powdered. Powdered tablet equivalent to 100 mg of Clopidogrel was weighed and taken into 100 ml volumetric flask then 15 ml of 0.1N ethanol HCl was added and shaken well to dissolve it after that 85 ml of diluent was added to adjust the volume up to 100 ml. From that 1 ml of solution was withdrawn and taken in 100 ml volumetric flask. The volume was adjusted with diluent up to 100 ml.

**Instrumentation**: UV-Visible single beam spectrophotometer with quartz cell(1cm).

**RESULT AND DISCUSSION**

**Development and optimization of the spectrophotometric method**

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of clopidogrel, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, and other chromatographic conditions.

Our preliminary trials were by using different compositions of diluents consisting 0.1N NaOH, HCL, 0.1N ethanol NaOH, 0.1N ethanolic HCl. By using diluent consisted of 0.1N ethanolic HCl best result was obtained and degassed in an ultrasonic bath.

**Selection of wavelength**

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank (Figure 1).

Clopidogrel shows $\lambda_{max}$ at 230. The proposed analytical method is simple, accurate and reproducible.

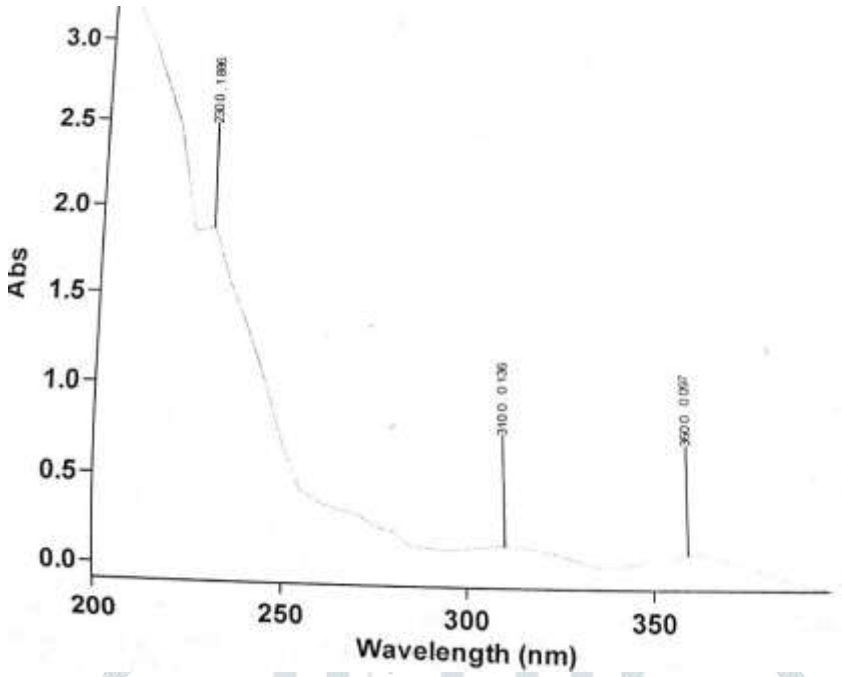



**Figure 1: Selection of wavelength**

**Method validation**

**1. Linearity:** Eight points calibration curve were obtained in a concentration range from 5-40 ppm for Clopidogrel. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.08763 x Conc + 0.06220 with correlation coefficient 0.99936.

**TABLE 1 : CONC. VS ABS. TABLE FOR LINEARITY STUDY**

| S.NO | Concentration(ppm) | Absorbance at 250 nm |
|---|---|---|
| 1 | 5 | 0.1267 |
| 2 | 10 | 0.2468 |
| 3 | 15 | 0.3527 |
| 5 | 25 | 0.6337 |
| 6 | 30 | 0.7662 |
| 7 | 35 | 0.8965 |
| 8 | 40 | 1.0302 |

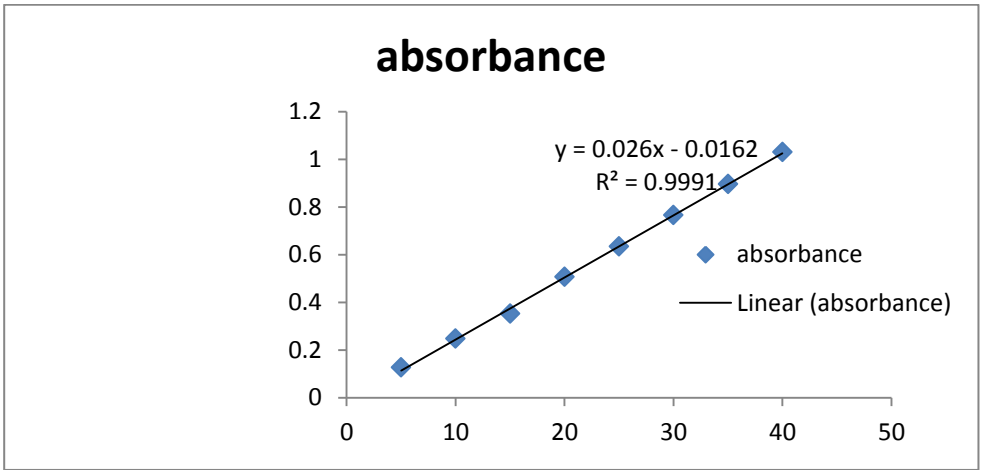



**Figure2 : Linearity Curve For Clopidogrel**

**2. Precision:**

**2.1 Repeatability:** Pipette out 1 ml working solution and transfer into 10 ml volumetric flasks. Dilute it to 10 ml with ethanol to get 10 µg/ml solution. Six separate 10 µg/ml solutions of the drug was prepared. Absorbance of the resultant solutions was measured at 230 nm using ethanol as blank. The result obtained is summarized in the table below.

**Table 2: Repeatability Study Of Clopidogrel**

| Nominal Conc.(µg/ml) | Absorbance | Observed Conc.(µg/ml) | Mean Conc.(µg/ml) | SD | %RSD |
|---|---|---|---|---|---|
| 10 | 0.2430<br>0.2356<br>0.2308<br>0.2308<br>0.2370<br>0.2299 | 9.9<br>9.6<br>9.4<br>9.4<br>9.6<br>9.4 | 9.55 | 0.000851 | 0.3598 |

**2.2 Intra-Day Precision:** Pipette out 1, 1.5 and 2.3 ml working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with ethanol to get solution of concentrations 10, 15 and 23µg/ml respectively. Absorbance of the resultant solutions was measured at 230 nm using ethanol as blank. Such three studies were performed within a day at 0,3 and 6 hrs interval. The result obtained is summarized in the table below.

**Table 3 : Intra Day Precision Study Of Clopidogrel**

| Nominal Conc. (µg/ml) | Absorbance | | | Observed Conc.(µg/ml) | | | Mean Conc. (µg/ml) | SD | %RSD |
|---|---|---|---|---|---|---|---|---|---|
| | 0 hr | 3 hrs | 6 hrs | 0 hr | 3 hrs | 6 hrs | | | |
| 10 | 0.2410 | 0.2429 | 0.2438 | 9.8 | 9.8 | 9.8 | 9.8 | 0.00143 | 0.58930 |
| 15 | 0.3793 | 0.3791 | 0.3825 | 15.1 | 15.2 | 15.2 | 15.16 | 0.00093 | 0.36865 |
| 23 | 0.6017 | 0.5816 | 0.5838 | 23.8 | 23 | 23.1 | 23.3 | 0.00051 | 0.08819 |
| **Mean** | | | | | | | | | 0.3487 |

**2.3 Inter-Day Precision:** Pipette out 1, 1.5 and 2.3 ml working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with ethanol to get solution of concentrations 10, 15 and 23 µg/ml respectively. Absorbance of the resultant solutions was measured at 230 nm using ethanol as blank. Such three studies were performed for three day at 0, 24 and 48 hrs interval. The result obtained is summarized in the table below.

**Table 4 : Inter Day Precision Study Of Clopidogrel**

| Nominal Conc. (µg/ml) | Absorbance | | | Observed Conc.(µg/ml) | | | Mean Conc. (µg/ml) | SD | %RSD |
|---|---|---|---|---|---|---|---|---|---|
| | 0 hr | 24 hrs | 48 hrs | 0 hr | 24 hrs | 48 hrs | | | |
| 10 | 0.2410 | 0.2429 | 0.2438 | 9.8 | 9.8 | 9.8 | 9.8 | 0.00143 | 0.5893 |
| 15 | 0.3791 | 0.3807 | 0.3795 | 15.1 | 15.2 | 15.2 | 15.16 | 0.00093 | 0.36865 |
| 23 | 0.6007 | 0.5825 | 0.5835 | 23.8 | 23 | 23.1 | 23.3 | 0.00051 | 0.08819 |
| **Mean** | | | | | | | | | 0.3487 |

**3. Accuracy:**

Pipette out 10 ml working solution and transfer into 10 ml volumetric flasks. Nine such transfer are made. Spike three of the solutions with 1 ml of working solution and dilute each to 10 ml with ethanol to get 10 µg/ml solution. Spike another three of the solutions with 1.5ml of working solution and dilute each to 10 ml with ethanol to get 15 µg/ml solution. Spike last three of the solutions with 2.3 ml of working solution and dilute each to 10 ml with ethanol to get 23 µg/ml solution. Absorbance of the resultant solutions were measured at 230 nm using ethanol as blank. The result obtained is summarized in the table below.

**Table 5 : Accuracy Study Of Clopidogrel**

| Recovery at | Nominal Conc.(µg/ml) | Absorbance | Observed Conc. ( µg/ml) | % Recovery |
|---|---|---|---|---|
| 80% | 10=8+2 | 0.2410 | 9.8 | 98 |
| 80% | 10=8+2 | 0.2429 | 9.8 | 98 |
| 80% | 10=8+2 | 0.2438 | 9.8 | 98 |
| 100% | 15=8+7 | 0.3791 | 15.1 | 100.6 |
| 100% | 15=8+7 | 0.3807 | 15.2 | 101.33 |
| 100% | 15=8+7 | 0.3795 | 15.1 | 100.6 |

| | | | | |
|---|---|---|---|---|
| 120% | 23 = 8+15 | 0.5825 | 23 | 100 |
| 120% | 23 = 8+15 | 0.5816 | 23 | 100 |
| 120% | 23 = 8+15 | 0.5835 | 23.1 | 100.6 |
| **Mean** | | | | 99.681 |

**4. e(1%,1cm), Absorptibity and Molecular Absorptibity:**

**Table6: The Absorbance, $e^{1\%}$1cm, Absorptivity and Molar Absorptivity values of Clopidogrel in different concentration at $\lambda_{max}$=230nm**

| S.No | Conc (µg/ml) | Absorbance | Absorbance/Conc. (A/C) | E(1%,1cm) (A/C x 10000) | Absorptivity (E(1%,1cm)/10) | Molecular Absorptivity |
|---|---|---|---|---|---|---|
| 1 | 5 | 0.1267 | 0.02534 | 253.4 | 25.34 | 8154.9188 |
| 2 | 10 | 0.2468 | 0.02468 | 246.8 | 24.68 | 7982.5176 |
| 3 | 15 | 0.3527 | 0.02351 | 235.1 | 23.51 | 7565.9882 |
| 4 | 20 | 0.5064 | 0.02532 | 253.2 | 25.32 | 8148.4824 |
| 5 | 25 | 0.6337 | 0.02534 | 253.4 | 25.34 | 8154.9188 |
| 6 | 30 | 0.7662 | 0.02554 | 255.4 | 25.54 | 8219.2828 |
| 7 | 35 | 0.8965 | 0.02561 | 256.1 | 25.61 | 8241.8102 |
| 8 | 40 | 1.0302 | 0.02575 | 257.5 | 25.75 | 8286.865 |
| **Mean** | | | | 251.36 | 25.136 | 8094.347 |

**CONCLUSION:** The present analytical method was validated as per ICH(Q2(R1) guideline and it meet to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate. The present analytical method can be used for its intended purpose.

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