



**IJPPR**

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article

May 2018 Vol.:12, Issue:2


© All rights are reserved by I. Lakshmi Prasanna et al.

## Spectrophotometric Determination of Clopidogrel in the Presence of Asprin (Clopin - A) and its Assay by Charge-Transfer Complex Method Using 2, 3-Dichloro-5, 6-Dicyano-1, 4-Benzoquinone (DDQ)



**IJPPR**  
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203



**I. Lakshmi Prasanna<sup>\*1</sup>, G. T. Naidu<sup>1</sup>, Nuzhath Fathima<sup>2</sup>, I. E. Chakravathy<sup>2</sup>, G. Abdul Huq<sup>3</sup>**

*1. Department of Physics, Rayalaseema University, Kurnool.*

*2. Department of Chemistry, Rayalaseema University, Kurnool.*

*3. Department of Chemistry, School of Sciences, Maulana Azad National Urdu University, Hyderabad.*

**Submission:** 22 April 2018

**Accepted:** 28 April 2018

**Published:** 31 May 2018

**Keywords:** Spectrophotometry, Clopidogrel, Asprin, Charge - Transfer Complex, DDQ, Pharmaceutical Formulations

### ABSTRACT

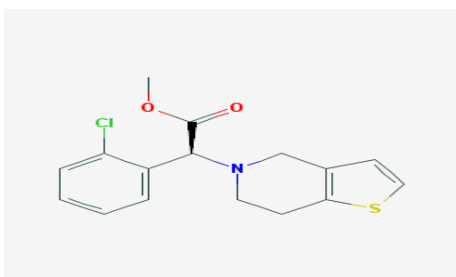
A simple, versatile and a new spectrophotometric method is proposed for the estimation of microgram quantities of the drug Clopidogrel in the presence of Asprin. It is also known as Clopin-A. The drug forms a Charge Transfer (CT) complex with 2,3 -Dichloro-5,6-Dicyano-1,4-Benzoquinone (DDQ), the stoichiometry of which is established as 1:1 by Job's continuous variation method. The wavelength of the maximum absorbance of the CT complex is found to be 440 nm. The absorbance values of the CT complex increased linearly with the increase in the amount of the drug Clopidogrel in the presence of Asprin. This suggests the suitability of the method for the determination of the drug in the range 10 µg/ml to 250 µg/ml. This also indicates the verification of the Beer-Lambert's Law in this range. The method is successfully applied to evaluate the assay of commercial tablets in pharmaceutical formulations for Clopidogrel and the results agreed very well. The molar absorptivity and Sandell Sensitivity of the method are  $4.827 \times 10^4$  lit/mol/cm and  $0.0086 \mu\text{g/ml/cm}^2$  respectively.



[www.ijppr.humanjournals.com](http://www.ijppr.humanjournals.com)

## INTRODUCTION

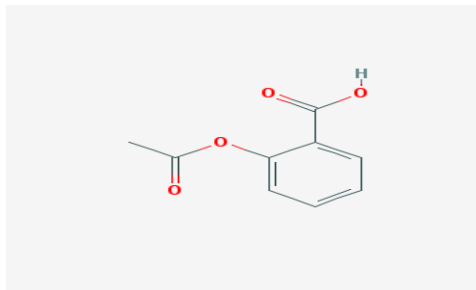
Clopidogrel was introduced in about 1982 and was approved for medical use in 1998. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. It is also used, along with acetylsalicylic acid (ASA, aspirin), for the prevention of thrombosis after placement of a coronary stent or as an alternative antiplatelet drug for people intolerant to aspirin<sup>1</sup>. Clopidogrel is an inhibitor of platelet activation and aggregation through the irreversible binding of platelets. Clopidogrel<sup>2-5</sup> is a prodrug and is metabolized to a pharmacologically active metabolite and inactive metabolites. It is used in the prevention of ischemic events, myocardial infarction, stroke syndrome, epilepsy, panic disorder. This medication is an anti-platelet agent, a drug that inhibits the ability of platelets to clump together as part of a blood clot. This medication is prescribed either alone or with other medications for prevention or treatment of stroke and heart attack (which are usually caused by blood clots) in persons who are at high risk.



**Fig. 1 Structure of Clopidogrel**

Aspirin, 2-acetoxy benzoic acid is cyclooxygenase inhibitor. It is used as an analgesic, antipyretic, anti-inflammatory and antithrombic agent. Clopidogrel bisulphate is methyl (s)-2-chlorophenyl (4,5,6,7-tetrahydrothieno-[3,2-C]pyridin-5-yl) acetate bisulphate, an ADP antagonist. It is used as an antithrombic agent. A capsule formulation containing 75 mg of aspirin and 75 mg of clopidogrel bisulfate is available in the market as Clopin- A75. A survey of biochemical literature revealed that spectrometric method was reported for the determination of aspirin in biological fluids. RP-HPLC methods were reported for the simultaneous estimation of aspirin, paracetamol, caffeine, and aspirin with atorvastatin. The spectrofluorimetric method was also developed for the estimation of aspirin and dipyridamole. Colorimetry, HPLC and gas chromatographic methods were described in the literature for the estimation of clopidogrel bisulfate. However less number of UV methods<sup>6-7</sup> for the simultaneous estimation of aspirin and clopidogrel bisulfate in combined dosage

forms have so far been reported. The present work describes the development of a simple, precise and accurate method for the estimation of clopidogrel bisulfate in the presence of Aspirin in capsules.



**Fig.2. Structure of Aspirin**

The tablet formulation is somewhat a new entrant in the Indian market. Aspirin is a well-studied drug, an official in all the pharmacopeias whereas clopidogrel is not official in any of these pharmacopeias. Several spectrophotometric and HPLC methods are reported for the estimation of aspirin in literature, whereas only a few HPLC methods are available for clopidogrel bisulfate. A spectrophotometric method was reported recently in literature for simultaneous analysis Aspirin and Clopidogrel where the analysis was done after hydrolyzing the drugs<sup>8-13</sup>. Rajput S.J et al have reported the chemometric simultaneous estimation of Clopidogrel in the presence of Aspirin<sup>14</sup>.

## **MATERIALS AND METHODS**

**Instrumentation:** A Single beam spectrophotometer Model SP-UV200 with 1 cm matched quartz cuvettes is employed throughout the study for all opticometric measurements.

### **Preparation of Reagents and Solutions:-**

#### **Clopidogrel solution:**

50 mg of pure Clopidogrel is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol. This is used as the stock solution of the drug. The working solution with concentration 100 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

**Aspirin solution:**

50 mg of pure Aspirin is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol. This is used as the stock solution of the drug. The working solution with concentration 100 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

**DDQ solution (0.1% w/v):**

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone which is abbreviated as DDQ is prepared by dissolving 100 mg of it in 100 ml of Acetonitrile.

All other chemical substances and reagents employed in the present investigation are of AR Grade only.

**RESULTS AND DISCUSSION**

Clopidogrel in the presence of Aspirin, when treated with 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone (DDQ), forms a Charge Transfer (CT) complex in which the drug Clopidogrel acts as a then-electron donor and the DDQ as the electron acceptor. This charge transfer (CT) complex formation reaction is spectrophotometrically monitored to develop the method of determination of the drug Clopidogrel in the presence of Aspirin. In the Process of carrying out detailed investigations, the various required optimum Parameters such as the wavelength of maximum absorbance ( $\lambda_{max}$ ), the effect of the concentration of DDQ on the absorbance of the Charge Transfer complex are established and the procedures adopted in each case are described as mentioned below:

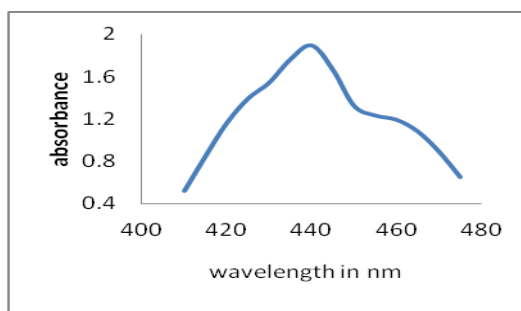
**Absorption Spectrum of CT Complex:**

The absorption spectrum of the CT complex formed between Clopidogrel and DDQ is obtained in order to fix the wavelength of maximum absorbance in the present study. The experimental

The procedure adopted is as follows:

1 ml of Clopidogrel solution (100 µg/ml), 1 ml of Aspirin solution (100 µg/ml), 2 ml of DDQ solution (0.1% w/v) and 2 ml methanol are taken in a 10 ml standard flask. The resulting solution is made up to the mark with distilled water. The contents of the flask are shaken well

and allowed to stand for a minute for equilibration. Then the absorbance values of the CT complex formed are measured in the wavelength range 410 nm to 475 nm against the reagent blank. The results obtained are used to draw a graph between the wavelength and the absorbance values. This graphical representation is called the Absorption spectrum, which is as shown in below Figure 3.



**Fig.3 Absorption Spectrum of Charge –Transfer complex of Clopidogrel with DDQ**

It is seen from the figure.1 of the absorption that the maximum absorbance is obtained at 440 nm. Hence, for all further studies, the wavelength of 440 nm is fixed.

**Effect of DDQ concentration:-** The effect of DDQ on the absorbance of the CT complex is studied by taking varying volumes (x ml ) of DDQ in a series of 10 ml standard flasks keeping the volume of Clopidogrel solution fixed at 2 ml. To each flask, 1 ml of methanol and 1 ml of aspirin is added followed by the addition of distilled water to make up each 10 ml flask to the mark. The absorbance of each solution is recorded at 440 nm against a suitable blank. The results obtained are tabulated in Table 1.

**Table 1: - Effect of DDQ on CT complex**

2 ml of Clopidogrel (100  $\mu\text{g}/\text{ml}$ ) +1 ml of Aspirin solution(100  $\mu\text{g}/\text{ml}$ ) + x ml of DDQ solution (0.1% w/v) + 1 ml of methanol + + (6-x) ml distilled water = Total volume kept at 10 ml each.  $\lambda_{\text{max}} = 440 \text{ nm}$

S. No	Vol. of Clopidogrel in ml	Vol. of Aspirin in ml	Vol. of DDQ solution x ml	Vol. of Methanol in ml	Vol. of distilled water in ml (6-x)	Total vol. in each flask in ml	Absorbance
1	2.0	1.0	0.5	1.0	5.5	10	1.21
2	2.0	1.0	1.0	1.0	5.0	10	1.28
3	2.0	1.0	1.5	1.0	4.5	10	1.32
4	2.0	1.0	2.0	1.0	4.0	10	1.38
5	2.0	1.0	2.5	1.0	3.5	10	1.48
6	2.0	1.0	3.0	1.0	3.0	10	1.47

From the data presented in Table.1 above, it is clear that 2.5 ml of DDQ solution are required for maximum absorbance. Hence, for all further studies, a volume of 2.5 ml of DDQ solution is fixed.

**Effect of concentration of drug Clopidogrel: Calibration Curve:** - This study pertains to the effect of the drug Clopidogrel concentration on the absorbance of the CT complex under the established optimal experimental conditions. The recommended procedure is as follows:-

**The recommended procedure for the determination of Clopidogrel: Applicability of Beer-Lambert's Law:-** Various aliquots ( x ml i.e., 0.5 ml to 2.5 ml) of Clopidogrel solution (100 µg/ml) are taken in a series of 10 ml standard flask. To each flask, 2.5 ml of DDQ solution (0.1% w/v), 1 ml of Aspirin solution (100 µg/ml), 1 ml of methanol followed by (5.5-x) ml of distilled water are added so as to make the total volume in each case at 10 ml. The contents of each flask are shaken well and allowed to stand for a minute for equilibration. The absorbance of each solution is measured at 440 nm against a suitable reagent blank, which is prepared in a similar manner but devoid of drug solution. The results obtained are mentioned in Table 2 and figure .4 as shown below.

**Table .2: Calibration Curve – Applicability of Beer – Lambert's Law**

x ml (0.5 ml to 2.5 ml) of Clopidogrel solution ( 100 µg/ml) + 1 ml of Aspirin solution (100 µg/ml) + 2.5 ml of DDQ solution (0.1% w/v) + +1 ml of Methanol + (5.5– x) ml distilled water = total volume kept at 10 ml in each case.  $\lambda_{\max} = 440 \text{ nm}$

S. N	Vol. in ml Clopidogrel (100 µg/ml) x ml	Amount of Clopidogrel in µg/ml	Vol.of Aspirin in ml	Vol.of DDQ in ml	Vol.of Methanol in ml	Vol.of distilled water in ml(5.5-x)	Total vol.in each flask in ml	Absorbance
1	0.5	50	1.0	2.5	1.0	5.0	10	0.34
2	1.0	100	1.0	2.5	1.0	4.5	10	0.67
3	1.5	150	1.0	2.5	1.0	4.0	10	0.98
4	2.0	200	1.0	2.5	1.0	3.5	10	1.34
5	2.5	250	1.0	2.5	1.0	3.0	10	1.50

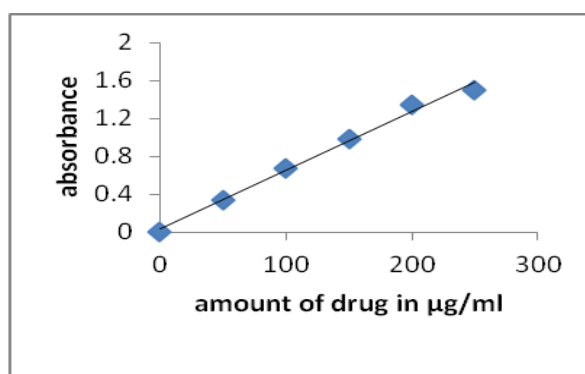


Fig.4: Calibration curve –Verification of Beer-Lambert’s Law

It is clear from the data presented in Table.2 that the absorbance values increased linearly with the increase for drug. This verifies the Beer-Lambert's law and suggests that the method can be suitably employed for the spectrophotometric quantitative determination of the drug Clopidogrel in the range 10 µg/ml to 250 µg/ml. The molar absorptivity and Sandell Sensitivity of the method are found to be  $4.827 \times 10^4$  lit/mole/cm and  $0.0086$  µg/ml/cm<sup>2</sup> respectively.

**Stoichiometric composition of Charge –Transfer Complex: Job’s continuous variation method:**

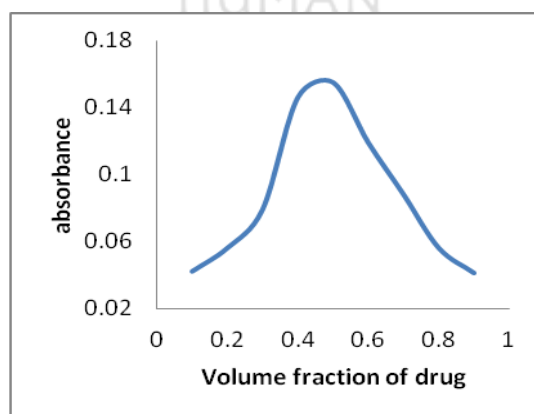
- The composition of the Charge – Transfer complex between the drug Clopidogrel in the presence of aspirin and the reagent DDQ is established by the Job's continuous variation method. In this, the equimolar concentrations ( $5 \times 10^{-4}$  M) of both the drug and DDQ are varied continuously keeping the total volume of mixed solution as constant at 10 ml. In each case, the absorbance is measured at 440 nm against a suitable blank. The data obtained are presented in table.3 and the figure. 5 & is as shown below:-

**Table .3: Job’s method of continuous variation**

0.5 ml to 4.5 ml of Clopidogrel solution ( $5 \times 10^{-4}$  M) + 2 ml of Aspirin solution ( $5 \times 10^{-4}$  M) + 4.5 ml to 0.5 ml of DDQ solution ( $5 \times 10^{-4}$  M) + + 3 ml Methanol = total volume kept at 10 ml in each case.  $\lambda_{\max}=440$  nm

S. No .	Vol.of Clopidogrel ( $5 \times 10^{-4}$ M) $V_1$ in ml	Vol.of Aspirin ( $5 \times 10^{-4}$ M) in ml	Vol.of DDQ ( $5 \times 10^{-4}$ M) $V_2$ in ml	Vol.of Methanol in ml	Total vol.in each flask in ml	Vol.fraction (x) of the drug ( $V_1/V_1+V_2$ )	Absorbance
1	0.5	2.0	4.5	3.0	10	0.1	0.042
2	1.0	2.0	4.0	3.0	10	0.2	0.056
3	1.5	2.0	3.5	3.0	10	0.3	0.079
4	2.0	2.0	3.0	3.0	10	0.4	0.146
5	2.5	2.0	2.5	3.0	10	0.5	0.155
6	3.0	2.0	2.0	3.0	10	0.6	0.119
7	3.5	2.0	1.5	3.0	10	0.7	0.088
8	4.0	2.0	1.0	3.0	10	0.8	0.056
9	4.5	2.0	0.5	3.0	10	0.9	0.041

The data in the above table are plotted in the form of a graph between volume fraction of the drug i.e., ( $V_1/V_1+V_2$ ) on X-axis and the absorbance values on Y-axis. The graph obtained is as shown below in figure .6.



**Fig.6: Job’s Continuous Variation Method**

From the graph shown above, it is found that one mole of the drug is reacting with 1 mole of DDQ, thereby establishing the stoichiometry of the Charge –Transfer complex as 1:1 (Drug: DDQ)



**Assay of Clopidogrel drug in pharmaceutical formulations:** - The recommended procedure for the quantitative micro determination of Clopidogrel drug is applied for the assay of the drug in the dosage form of the commercial tablets and also in pharmaceutical formulations. The assay is carried out as follows: 20 tablets of Clopidogrel are weighed and finely powdered. An accurately weighed portion of the powdered sample equivalent to 50 mg of Clopidogrel is taken in a 50 ml volumetric flask containing 25 ml of methanol and is sonicated for about 20 minutes. The resultant solution is filtered through Whatman filter paper No.41 into another 50 ml volumetric flask. The filter paper is washed several times with methanol and the washings are added to the filtrate. The final volume is made up to the mark with methanol. Now, 5 ml of the filtrate of the sample solution is diluted to 10 ml with methanol and treated as per the recommended procedure of calibration. From this, the amount of the drug present in the sample is computed from the calibration curve. The results obtained are as shown in table .4 below.

**Table .4: Assay of Clopidogrel in Tablets**

Sample	The labelled amount in mg	Amount found by present method $\pm$ SD*	Percentage of Label claim	%RSD	*t <sub>cal</sub>
Tablet I	20	20.002 $\pm$ 0.11	100.002	0.55	0.0406
Tablet II	20	20.002 $\pm$ 0.10	100.002	0.52	0.0425

## CONCLUSION

The calibration curve is linear up to 250  $\mu$ g/ml indicating the suitability of the proposed method for the spectrophotometric determination of Clopidogrel in the presence of Aspirin in the range of 10  $\mu$ g/ml to 250  $\mu$ g/ml. The standard deviation values are found to be low showing high accuracy and reproducibility of the method. The calculated 't' values are less than the 't' theoretical values with 4 degrees of freedom at 95% level of significance. This indicates that there is no observable difference between the proposed method and the standard method. Further, there is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentration of those present in general pharmaceutical preparations. Thus, the proposed method can be conveniently adopted for the routine analysis and estimation of Clopidogrel in the presence of Aspirin in pharmaceutical formulations.

## ACKNOWLEDGMENTS

The authors (ILP & NF) express their grateful thanks to the authorities of the Departments of Physics and Chemistry respectively of Rayalaseema University, Kurnool and the Central Instrumentation Cell of Prof. T Jayashankar Telangana State Agricultural University, Hyderabad. And the Management of Tirumala Engineering College, Hyderabad for providing necessary facilities. One of the authors (ILP) is also grateful to Tirumala Engineering College, Hyderabad, for their kind permission and constant encouragement to carry out the proposed Research Work.

## REFERENCES

1. Ahmed M, Biswas HU, Sadik G. Development of a spectrometric method for the determination of aspirin in blood samples. *J Med Sci.* 2001;1 suppl.2:61-62.
2. Mishra P, Dolly A. Development of UV spectrophotometric method for the determination of clopidogrel in tablet dosage form. *Indian J Pharm Sci* 2005;67:491-493.
3. Vocilkova L, Opatrilova R, Sramek V. Determination of Clopidogrel by Chromatography. *Curr Pharm Anal* 2009; 5: 424-431.
4. Mitakos A, Panderi I. A Validated LC method for the determination of clopidogrel in pharmaceutical preparations. *J Pharm Biomed Anal* 2002;28 suppl. 3-4, 431-438.
5. Kampe NS, Venkatachalam A. Estimation of clopidogrel from tablet dosage form by the gas chromatographic method. *Indian J Pharm Sci* 2005;67:128-129.
6. Sankar ASK, Vetrichelvan T, Venkappaya D. Simultaneous estimation of ramipril, acetylsalicylic acid and atorvastatin calcium by chemometrics assisted UV spectrophotometric method in capsules. *Acta Pharm* 2011;61:283-96.
7. Zenon Kokot, Kinga Burda, Simultaneous determination of Salicylic acid and Acetylsalicylic acid in Aspirin delayed release tablet formulation by second derivative UV spectrophotometry, *Journal of Pharmaceutical and Biomedical Analysis*, Volume 18, 1998, Pg no.871-875.
8. Anandakumar K, Ayyappan T, Raghu RV, Vetrichelvan T, Sankar ASK, Nagavalli D. RP-HPLC analysis of aspirin and clopidogrel bisulfate in combination. *Indian J Pharm Sci* 2007; 69: 597-599.
9. Patel RB, Shankar MB, Patel MR, Bhatt KK. Simultaneous Estimation of Acetylsalicylic Acid and Clopidogrel Bisulfate in Pure Powder and Tablet Formulations by HPLC and HPTLC. *JAOAC International* 2008; 91: 750-755.
10. Pankaj K, Kachhadia AS, Doshi HS. Validated Column HPLC Method for Determination of Aspirin and Clopidogrel in Combined Tablets in the Presence of Degradation Products Formed Under ICH Recommended Stress Conditions. *JAOAC Int* 2009; 92: 152-157.
11. Himani A, Neeraj K, Paradkar AR, Mahadik KR. Stability indicating HPTLC determination of clopidogrel bisulfate as a bulk drug and in the pharmaceutical dosage form. *J Pharm & Biomed Anal* 2003; 61: 581- 589.
12. Shinha PK, Mr inalini CD, Bothra KG. A validated stability indicating HPTLC method for determination of Aspirin and Clopidogrel Bisulfate in the combined dosage form. *Eur J Chem* 2009; 4: 152-160.
13. Anti D, Filipi S, Agbaba D. A simple and sensitive TLC method for determination of clopidogrel and its impurity SR 26334 in a pharmaceutical product. *Acta chromatogr* 2007; 18: 199-206.
14. Rajput SJ, George RK, Ruikar DB. Chemometric Simultaneous Estimation of Clopidogrel Bisulfate and Aspirin from Combined Dosage Form. *Indian J Pharm Sci* 2008; 70: 450- 454.