OXIDATIVE SPECTROPHOTOMETRIC DETERMINATION OF CLOPIDOGREL IN THE PRESENCE OF ASPIRIN USING CERIC AMMONIUM NITRATE (CAN)

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***Abstract*: A simple, precise, accurate and versatile new spectrophotometric method is proposed for the estimation of micro determination of the drug Clopidogrel in the presence of Asprin using CAN. It is also known as Clopin-A. During the course of study, it is observed that acidic solution of the drug, when treated with CAN forms the oxidation product which is exploited for the development of spectrophotometric method for the determination and analysis of the drug. The oxidation product showed λ_{max} at 300 nm. Applicability of Beer- Lamberts law showed the linearity range for Clopidogrel in the presence of Aspirin is found to be 20 µg/ml to 200 µg/ml. Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. The molar absorptivity and the sandell sensitivity of the method are evaluated and the values are found to be to be 2.8162x 10^4 lit/ mole/cm and 0.0149 µg/ ml/ cm^2 respectively.**

***Keywords*: Spectrophotometry, Clopidogrel, Asprin, Oxidation method, CAN, Pharmaceutical Formulations.**

INTRODUCTION: Clopidogrel is on the World Health Organizatons 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. Clopidogrel [1-6] is an inhibitor of platelet activation and aggregation through the irreversible binding of platelets. It is used in the prevention of ischemic events, myocardial infraction, stroke syndrome, epilepsy, panic disorder. This medication is an anti-platelet agent, that is, 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. Structure of Clopidogrel is as shown in fig.1 below.

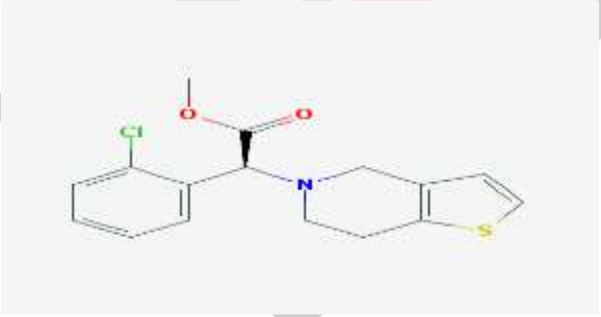

Fig. 1 Structure of Clopidogrel

Aspirin [7, 8] is a 2-acetoxy benzoic acid is cyclo oxygenase 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 bisulphate is availablein market as Clopin- A75[9-12]. A survey of literature revealed that spectrophotometric 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. Spectrofluorimetric method was also reported for the estimation of aspirin and dipyridamole. However less number of UV methods for the simultaneous estimation of aspirin and clopidogrel bisulphate in combined dosage forms has so far been reported. The present work describes the development of a simple, precise and accurate UV method for the estimation of Clopidogrel bisulphate in the presence of Aspirin capsules.Structure of Aspirin is as shown in fig.2 below.

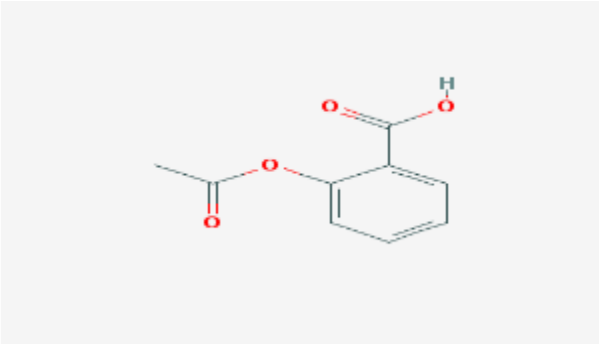

Fig.2. Structure of Aspirin

The tablet formulation is a somewhat new entrant in the Indian market. Several spectrophotometric and HPLC methods are reported for the estimation of aspirin in literature, whereas only a few HPLC methods are available for Clopidogrel bisulphate. A spectrophotometric method was reported recently in literature for simultaneously[13,14] analyzing ASP and CLP where the analysis was done after hydrolyzing the drugs.

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 50 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 50 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

Ceric Ammonium Nitrate (CAN) Solution: Dissolve 275 mg of Ceric Ammonium Nitrate in 500 ml of 2N Nitric acid.

2N Nitric Acid Preparation: 16 ml of Concentrated Nitric Acid is dissolved in 484 ml of water, and then its normality will become about 2 N.

Methyl Orange solution : 50 mg Methyl Orange is dissolved in 50 ml water and is diluted to get 50 µg/ml.from it take 5 ml and add 100 ml water then its concentration is equal to50 µg/ml.

RESULTS AND DISCUSSION:

Cerium (IV) is a good oxidizing agent like $KMnO_4$, $K_2Cr_2O_7$ etc. It has been used for quantitative determination of drugs based on the oxidation of drugs. The spectrophotometric methods involved addition of excess Ce (IV) and direct measurement of unreacted drugs at its λ_{max} or unreacted cerium is estimated by suitable dyes like Indigo Carmine, Methyl Orange, and oxidation of iodide, Fe^{2+} , Cr^{3+} which are readily oxidizable by cerium (IV). Methyl orange dye was used to estimate the unreacted Ce (IV).

Clopidogrel in the presence of Aspirin when treated with CAN with Methyl Orange as an indicator forms an oxidation reaction. This oxidation formation reaction is spectrophotometrically monitored to develop a method for the determination of the drug. In the process of carrying out detailed investigations, first of all, optimization of various parameters such as the wavelength of maximum absorbance (λ_{max}), the effect of the concentration of oxidizing agent CAN and an indicator Methyl Orange on the absorbance of the oxidation are established and the procedures adopted in each case are described as follows:

Absorption Spectrum of Oxidation reaction: The absorption spectrum of the oxidation reaction formed between Clopidogrel in the presence of Aspirin and CAN is obtained in order to fix the wavelength of maximum absorbance in the present study. The experimental procedure adopted is as follows:

1 ml of Clopidogrel solution (50 µg/ml), 1 ml of Aspirin solution (50 µg/ml), 1 ml of CAN, 1 ml of Methyl Orange 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 oxidation reaction formed are measured in the wavelength range 250 nm to 350 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 shown in figure 3 below.

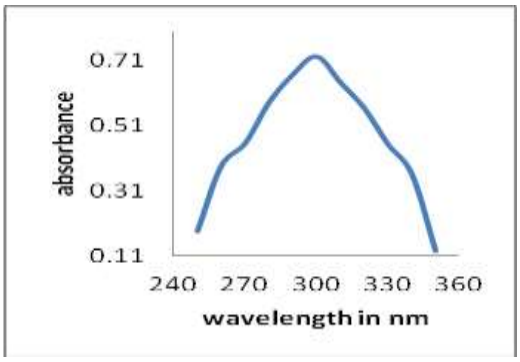

Fig: 3: Absorption Spectrum of Oxidation with CAN

It is seen from the above Fig.3 of the absorption spectrum, that the maximum absorbance is obtained at 300 nm. Hence for all further studies, the wavelength 300 nm is fixed.

Effect of CAN: The effect of CAN on the absorbance of the oxidation reaction is studied by taking varying volumes (x ml) of CAN in a series of 10 ml standard flasks. After taking x ml (0.5 ml to 3.0 ml) of CAN in each flask, 1 ml of drug solution of Clopidogrel, 1 ml of Aspirin, 1 ml of Methyl Orange are added and the resulting solution is made up to 10 ml using distilled water. The absorbance of each solution is recorded at 300 nm against a suitable blank and is as shown in Table.1 below.

Table 1: Effect of CAN on Oxidation

1 ml Clopidogrel solution(50 µg/ml) +1 ml of Aspirin solution (50 µg/ml) + x ml (0.5 ml to 3.0 ml) of CAN solution (10 µg/ml) + 1 ml of Methyl Orange + (7-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 300 nm**

S. No	Vol.of Clopido-grel(50 µg/ml) in ml	Vol.of Aspirin (50 µg/ml) in ml	Vol. of CAN Solution x ml	Vol.of Methyl Orange (50 µg/ml) in ml	Vol.of distilled water in ml (7-x)	Total Vol. in ml	Absorbance
1	1.0	1.0	0.5	1.0	6.5	10	0.424
2	1.0	1.0	1.0	1.0	6.0	10	0.670
3	1.0	1.0	1.5	1.0	5.5	10	0.894
4	1.0	1.0	2.0	1.0	5.0	10	1.217
5	1.0	1.0	2.5	1.0	4.5	10	1.428
6	1.0	1.0	3.0	1.0	4.0	10	1.427

It is observed that 2.5 ml of CAN solution is required for maximum absorbance. Hence for all further studies a volume of 2.5 ml of CAN solution is fixed.

Effect of volume of Methyl Orange: The effect of Methyl Orange on the absorbance of oxidation reaction is studied by taking varying volumes of (x ml) of Methyl orange solution in a series of 10 ml standard flasks keeping the volume of Clopidogrel solution fixed at 1 ml. To each flask, 1 ml of Aspirin solution, 2.5 ml of CAN are added followed by the addition of (5.5-x) ml of distilled water to make up each 10 ml flask to the mark. The absorbance of each solution is recorded at 300 nm against the suitable blank and is as shown in Table 2 below.

Table 2: Effect of Methyl Orange on Oxidation

1 ml of Clopidogrel solution (50 µg/ml) + 1 ml of Aspirin solution (50 µg/ml) +x ml (0.5 ml to 2.5 ml) of Methyl Orange solution (50 µg/ml) + 2.5 ml of CAN (10 µg/ml) + (5.5-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max} = 300 nm**

S. No	Vol.of Clopi-dogrel (50 µg/ml) in ml	Vol.of Aspirin (50 µg/ml) in ml	Vol.of CAN in ml	Vol. of Methyl Orange solution(50 µg/ml) x ml	Vol.of distilled water in ml (5.5-x)	Total vol.in ml	Absor-bance
1	1.0	1.0	2.5	0.5	5.0	10	0.709
2	1.0	1.0	2.5	1.0	4.5	10	0.861
3	1.0	1.0	2.5	1.5	4.0	10	0.981
4	1.0	1.0	2.5	2.0	3.5	10	0.680
5	1.0	1.0	2.5	2.5	3.0	10	0.806

It is observed that 1.5 ml of Methyl Orange solution is necessary to achieve maximum absorbance. Hence for all further studies a volume of 1.5 ml of Methyl Orange solution is required.

Effect of concentration of Drug Clopidogrel: This study pertains to the effect of the drug Clopidogrel concentration on the absorbance of the Oxidation reaction under the established optimal experimental conditions. The recommended procedure for the calibration curve and for the obedience of Beer-Lambert's Law for the quantitative spectrophotometric determination of the drug Clopidogrel is as follows.

Calibration Curve: Obedience of Beer - Lambert's Law: Various aliquots (x ml i.e., 0.5 ml to 2.5 ml) of Clopidogrel solution (50 µg/ml) are taken in a series of 10 ml standard flask. To each flask, 1 ml of Aspirin solution (50 µg/ml), 1.5 ml of Methyl Orange solution, 2.5 ml of CAN solution, are added followed by (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 300 nm against a suitable reagent blank which is prepared in a similar manner but devoid of drug solution and is as shown in Table 3 below.

Table 3: calibration curve: - obedience of Beer-Lambert's Law

x ml of Clopidogrel (50 µg/ml) + 1 ml of Aspirin (50 µg/ml) + 1.5 ml of Methyl Orange solution (50 µg/ml)+ 2.5 ml of CAN (10 µg/ml) + (5-x) ml distilled water = Total volume kept at 10 ml each. **λ_{max}= 300 nm**

S. No	Vol. in ml Clopidogr-el (50 µg/ml) x ml	Amount of Clopi -dogrel in micrograms	Vol.of Aspiri-n(50 µg/ml) in ml	Vol.of Methyl Orange(50 µg/ml) in ml	Vol.of CAN in ml	Vol.of distilled water in ml (5-x)	Total vol.in ml	Absorbance
1	0.5	25	1.0	1.5	2.5	4.5	10	0.166
2	1.0	50	1.0	1.5	2.5	4.0	10	0.332
3	1.5	75	1.0	1.5	2.5	3.5	10	0.493
4	2.0	100	1.0	1.5	2.5	3.0	10	0.667
5	2.5	125	1.0	1.5	2.5	2.5	10	0.835
6	3.0	150	1.0	1.5	2.5	2.0	10	1.024
7	3.5	175	1.0	1.5	2.5	1.5	10	1.178
8	4.0	200	1.0	1.5	2.5	1.0	10	1.344

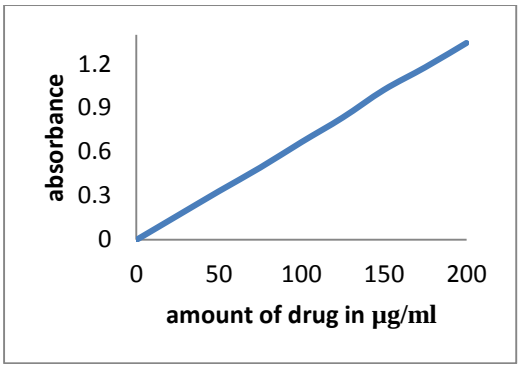

Fig. 4: Calibration curve –Verification of Beer-Lambert's Law

It is obviously clear from this calibration straight line as shown in above Fig.4 that the absorbance values increased linearly with the increase in the amount of the drug. This verifies the Beer-Lambert's Law and suggests that the method can be successfully employed for the spectrophotometric quantitative determination of the drug Clopidogrel in the range 10 µg/ml to 200 µg/ml. The molar absorptivitiy and the Sandell Sensitivity of the method are found to be 2.8162x 10^4 lit/ mole/cm and 0.0149 µg/ ml/ cm^2 respectively.

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 filtrate. The final volume is made upto the mark with methanol. Now, 5 ml of 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	Labelled amount in mg	Amount found by present method ±SD*	Percentage of Label claim	% RSD	*t_{cal}
Tablet I	20	20.016±0.15	100.016	0.75	0.2385
Tablet II	20	20.026±0.08	100.026	0.40	0.7177

* Average of 5 determinations based on label claim.

CONCLUSION:

The calibration curve is linear up to 200 µg/ml indicating the suitability of the proposed method for the spectrophotometric determination of Clopidogrel in the presence of Asprin in the range of 10 µg/ml to 200 µg/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 Asprin in pharmaceutical formulations.

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