A simple and sensitive Spectrophotometric method for determination of clopidogrel from tablets

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ABSTRACT

A simple, sensitive, rapid and accurate spectrophotometric method has been developed for the estimation of clopidogrel in pharmaceutical formulations. The proposed method was based on the formation of chloroform extractable complex of clopidogrel with phenol red. The absorbance of the extractable ion pair complex is measured at the wavelength of maximum absorbance 482 nm against the reagent blank. Results obtained are statistically validated and found to be reproducible.

Key words: Spectrophotometry, phenol red, clopidogrel,
Pharmaceutical and Formulation

MATERIALS AND METHOD

Instrument:

All measurement were done on Milton Roy 1001spectrophotometer by using 10 mm matched quartz cuvettes.

Hydrochloric acid (0.1N):

10 g of 36% HCl (Merck) is dissolved in 1000ml of distilled water.

Phenol Red (100µg/ml):

Phenol Red is prepared by dissolving 80 mg of Phenol Red (Fischer scientific) in 100 ml of methanol and 5 ml of this stock solution is dissolved in 40 ml methanol.

0.1 M Potassium Hydrogen Phthalate : It is preparedby dissolving 2.0422 grams of Potassium Hydrogen Phthalate (Fischer Scientific) in 100 ml of distilled water.

Buffer solution (pH 3.6):

Buffer solution (pH 3.6) is prepared by mixing 100 ml of 0.1 M potassium hydrogen phthalate (20.422 gm of Potassium Hydrogen Phthalate (Fischer scientific) in 1000 ml of distilled water) in 12.6 ml of 0.1M HCl(10 g of 36% HCl (Merck) is dissolved in 1000ml of distilled water) and pH of the solution is adjusted to pH 3.6.

INTRODUCTION

The chemical designation is methyl (2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetate;sulfuric acid. Clopidogrel bisulfate is a thienopyridine with antiplatelet activity. Clopidogrel bisulfate irreversibly alters the platelet receptor for adenosine diphosphate (ADP), thereby blocking the binding of ADP to its receptor, inhibiting ADP-mediated activation of the glycoprotein complex GPIIb/IIIa, and inhibiting fibrinogen binding to platelets and platelet adhesion and aggregation. Molecular formula of Clopidogrel bisulfate is $C_{15}H_{12}N_2O_2$. Molecular weight of Clopidogrel bisulfate is 321.82 g/mol. Clopidogrel bisulfate is a white to off-white powder. It is practically insoluble in water at neutral pH but freely soluble at pH 1Several analytical methods have been reported for assay of clopidogrel including spectrophotometric method[1-23], RP-HPLC method[24-38], HPLC method [39-42], LC method [43-45], Solid method [46-49], Nano particles method [50,51].

This paper describes a rapid, simple, sensitive and economical spectrophotometeric methods for the determination of clopidogrel in phamaceutical formulations forms. The main purpose of the present study was to establish relatively simple, sensitive and validated visible spectrophotometric methods for the determination of clopidogrel in pharmaceutical dosage forms.

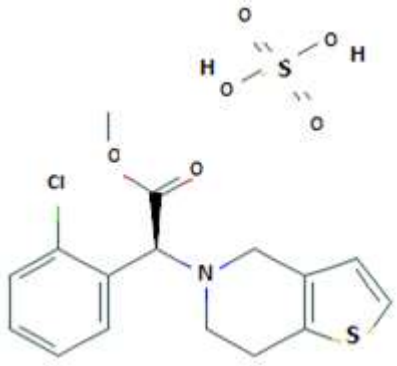

Fig.1. The chemical structure of clopidogrel

MATERIALS AND METHODS

Instrument:

All measurement were done on Milton Roy 1001spectrophotometer by using 10 mm matched quartz cuvettes.

Materials:

All chemicals used are of A.R. grade and were purchased from S.D. fine chemicals and LOBA-Chemi, Mumbai. Doubled distilled water were used for preparation of solutions

Buffer solution (p^H 3.6):

Buffer solution (pH 3.6) is prepared by mixing 100 ml of 0.1 M potassium hydrogen phthalate (20.422 gm of Potassium Hydrogen Phthalate (Fischer scientific) in 1000 ml of distilled water) in 12.6 ml of 0.1M HCl(10 g of 36% HCl (Merck) is dissolved in 1000ml of distilled water) and pH of the solution is adjusted to pH 3.6.

Preparation of standard stock solution: The standard stock solution (1mg/ml) of clopidogrel was prepared by dissolving 100 mg of clopidogrel in 100 ml distilled water. The working standard solutions of clopidogrel were obtained by appropriately diluting the standard stock solution with the same solvent.

Preparation of Calibration curve:

Various aliquots of the standard clopidogrel, solution ranging from 0.5-2.5 ml are transferred into a series of separating funnel. To each flask, 0.5 ml of phenol red solution, 1.0 ml of buffer solution and 5 ml of chloroform are added. Reaction mixture in each funnel is shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer is separated out and absorbance is measured at 482 nm, against the

reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of clopidogrel, solution. The calibration curve is found to be linear over a concentration range of 50 to 250 μg/mL of clopidogrel,. The amount of clopidogrel, present in the sample is estimated from the calibration graph. The results are presented in fig.2

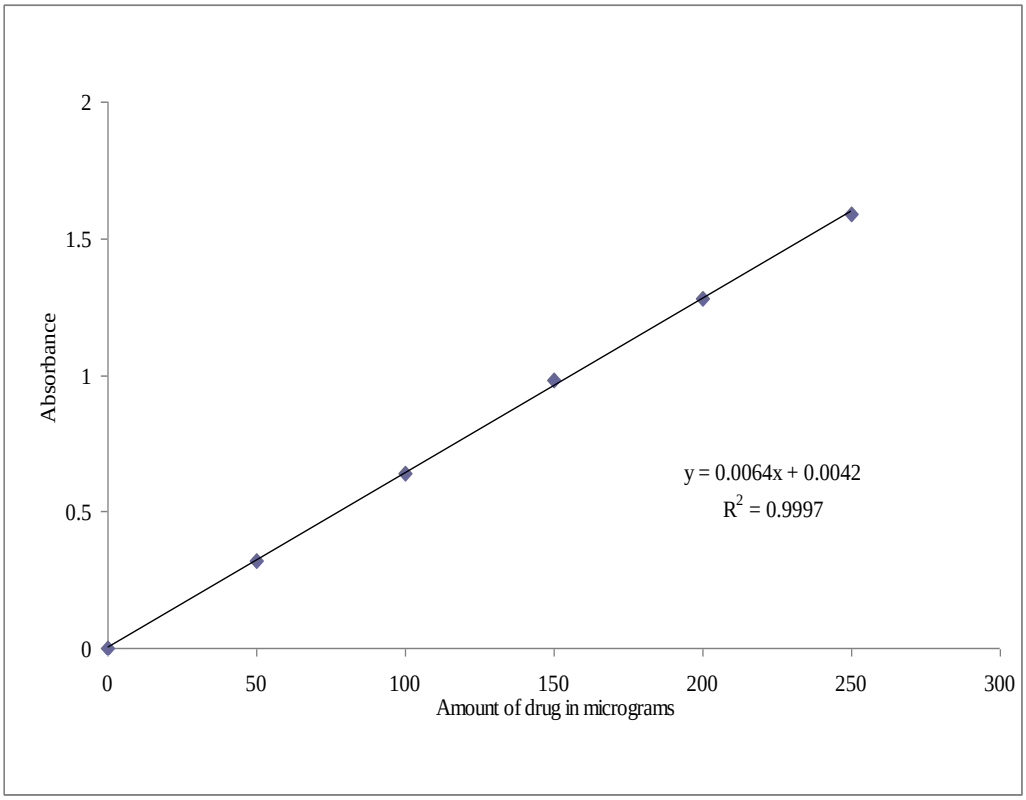

Fig.2:Calibration curve of clopidogrel

Assay of pharmaceutical Formulations:

Ten tablets of clopidogrel, are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of clopidogrel, was taken in a 50 ml volumetric flask containing 25 ml of methanol, sonicated for 20 minutes. The resultant solution is filtered through Whatman filter paper No. 41 into another 50 ml volumetric flask. The filter paper was washed several times with methanol. The washings were added to the filtrate and the final volume was made up to the mark with methanol and treated as per the procedure of the calibration curve. Amount of the drug present in sample was computed from respective calibration curve. The results are present in table.2

Table. 2:
Optical characteristics of the proposed methods

parameters	Proposed method
λmax (nm)	482
Beer's law limit (µg/ml)	50-250
Molar absorptivity (l mole-^{1} cm-^{1})	4.43x10^{3}
Sandell's sensitivity (µg cm-^{2} / 0.001 absorbance unit)	0.0253
Regression equation (Y = bC +a)	Y=0.0064x+0.0042
Slope (b)	0.0064
Intercept (a)	0.0042
Correlation coefficient (r)	0.9997

*Y = a+bx, where Y is the absorbance and X concentration in µg/ml

Table. 4.3.4:

Assay of clopidogrel in tablet formulations

Tablets	Labeled amount(mg)	*Amount found (mg)±S.D*	% label claim	%RSD*	*t value
Tablet 1	75	75.15±0.31	100.2	0.4137	1.079
Tablet 2	75	75.01±0.27	100,01	0.3662	0.0814
Tablet 3	75	74.95±0.23	99.93	0.3175	0.4699

***Average of five determinations**

Results and discussions:

Clopidogrel, treated with phenol red dye at 3.6 pH. The resultant solution is extracted with chloroform. The ion pair complex is form in extractable chloroform layer. The absorbance of the extractable ion pair complex is measured at 482 nm against the reagent blank (prepared in a similar manner devoid of drug solution). The calibration curve (concentration vs absorbance) is linear over the range of 50-250 μg/mL of clopidogrel,. The optical characteristics of the proposed method such as absorption maxima, Beer´s law limits, molar absorptivity and Sandell´s sensitivity are presented in Table 1. The molar absorptivity and Sandell´s sensitivity values shows sensitivity of the method. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized in the Table 1. The value of correlation coefficient was 0.999, which indicated the good linearity of calibration lines. The percent relative standard deviation calculated from the five measurements of clopidogrel, shown in Table 2. The % RSD is less than 2, which indicates that the method has good reproducibility. The values of standard deviation values are low, indicates high accuracy and reproducibility of the method. The 't' calculated values are compares well with the theoretical value of 2.78 there by indicating that there is no significant difference between proposed method and official method. There is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of clopidogrel, in bulk drugs samples and pharmaceutical formulations.

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Table.1: Optical characteristics of the proposed method

parameters	**Proposed method**
Wavelength (nm)	482
Beer's limits, mcg/ml	50-250
Sandell's , sensitivity, (μg cm^{-2})	0.1421
Molar absorptivity, (L mol^{-1} cm^{-1})	$1.42x10^2$
Regression equation, Y*	Y= 0.0064x+0.0042
Correlation coefficient, (r)	0.9997
Intercept (a)	0.0064
Slope (b)	0.0042

Table.2: Assay of Clopidogrel in pharmaceutical preparations

Formulation	**Labeled amount**	***Amount found (mg±S.D)**	**% RSD**	***t value**
Tablet 1	75	75.15±0.31	0.4137	1.0791
Tablet 2	75	75.01±0.27	0.3662	0.0814
Tablet 3	75	74.94±023	0.3175	0.4699

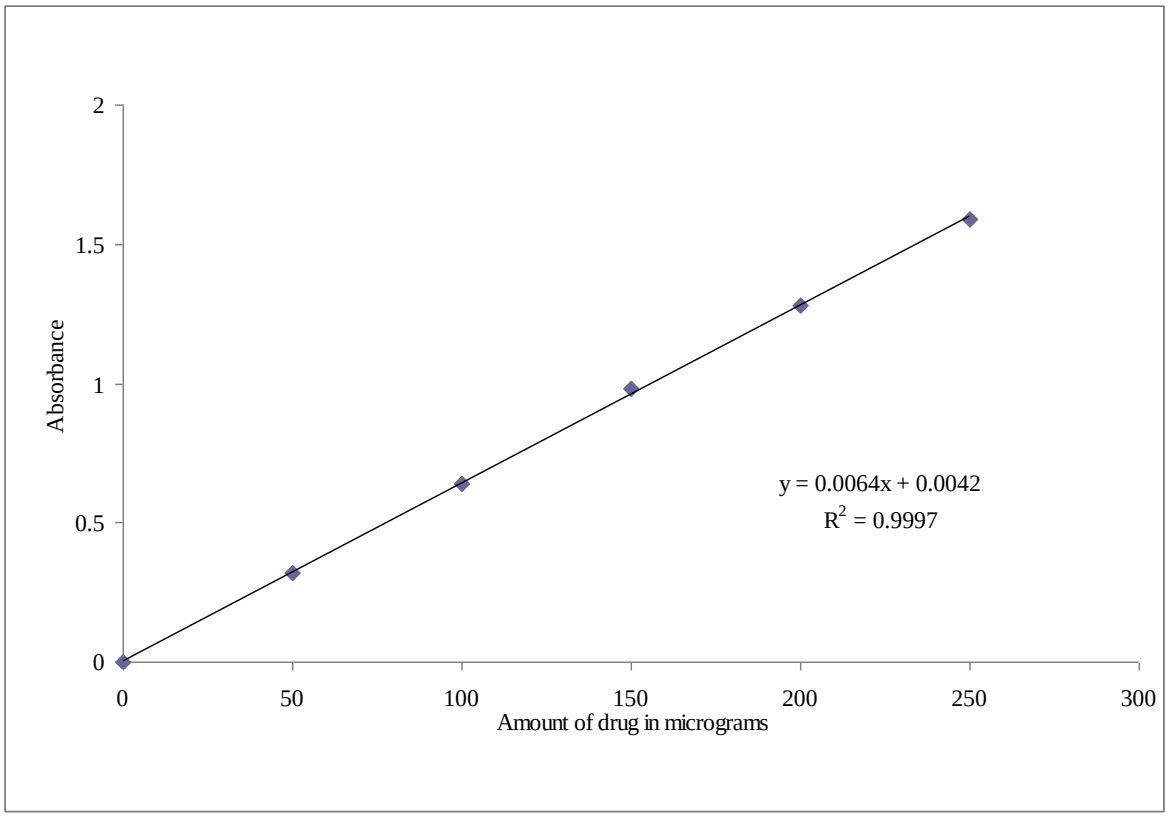

Fig.2: Calibration curve of clopidogrel