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Development and Validation of Spectrophotometric Method for Clopidogrel bisulfate in pure and in film coated tablet dosage form

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ABSTRACT

Clopidogrel bisulfate belongs to the class of P2Y12 ADP platelet receptors inhibitor. The aim of this study was to develop simple, sensitive, cost effective, accurate, precise and rapid ultraviolet (UV) Spectrophotometric method for the estimation of Clopidogrel bisulfate in pure form and its formulations. For the estimation of Clopidogrel bisulfate, solvent system employed was triple distilled water pH 1 instead of Acetonitrile and wavelength of detection was 222 nm. The developed method was used to estimate the total drug content in commercially available tablet formulations of Clopidogrel bisulfate.

Key Words: Spectrophotometric determination, Acetonitrile Triple distilled water, Clopidogrel bisulfate, Validation.

INTRODUCTION

Plavix (Clopidogrel bisulfate) is a thienopyridine class inhibitor of P2Y12 ADP platelet receptors. Chemically it is methyl (+)-(S)-α-(2-chlorophenyl)-6, 7-dihydrothieno [3, 2-c] pyridine-5(4H) acetate sulfate (1:1). The empirical formula of Clopidogrel bisulfate is $C_{16}H_{16}ClNO_2S•H_2SO_4$ and its molecular weight is 419.9. , CAS Number: 113665-84-2, Brands: Plavix (75mg), structural formula (Fig. I) and Space-filling model of Clopidogrel. (Fig. II)[5] Clopidogrel bisulfate is a white to off-white powder. It is practically insoluble in water at neutral pH but freely soluble at pH 1. It also dissolves freely in methanol, dissolves sparingly in methylene chloride, and is practically insoluble in ethyl ether. It has a specific optical rotation of about +56°.Plavix for oral administration is provided as either pink, round, biconvex, debossed, film-coated tablets containing 97.875 mg of Clopidogrel bisulfate which is the molar equivalent of 75 mg of Clopidogrel base or pink, oblong, debossed film-coated tablets containing 391.5 mg of Clopidogrel bisulfate which is the molar equivalent of 300 mg of Clopidogrel base. Each

tablet contains hydrogenated castor oil, hydroxypropylcellulose, mannitol, microcrystalline cellulose and polyethylene glycol 6000 as inactive ingredients. The pink film coating contains ferric oxide, hypromellose 2910, lactose monohydrate, titanium dioxide and triacetin. The tablets are polished with Carnauba wax. Literature survey reveals that only HPLC methods are available for the estimation of Clopidogrel bisulfate alone, in combination with other drugs and in its dosage form, no UV Spectrophotometric method using triple distilled water in staid of Acetonitrile as solvent was found in literature [7]. The present investigation has been undertaken to develop simple UV Spectrophotometric method for the estimation of Clopidogrel bisulfate in pure form and its formulations [1].

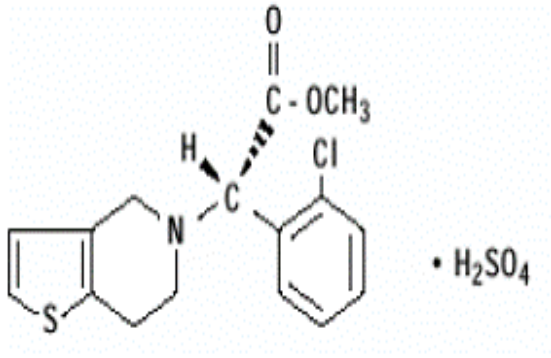

Figure I. Structural formula of Clopidogrel bisulfate

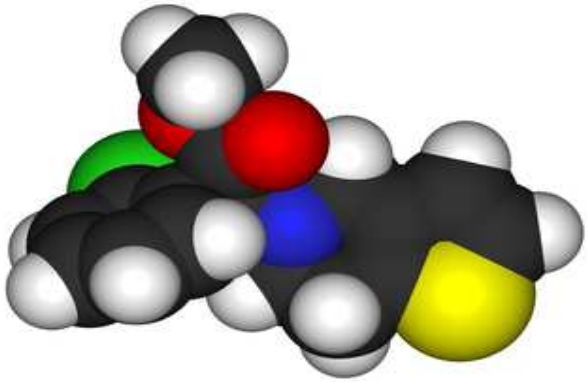

Figure II. Space-filling model of Clopidogrel bisulfate

MATERIALS AND METHODS

Clopidogrel bisulfate pure drug was obtained as a gift sample from Cadila pharma ltd (Ahmadabad, India). PLAVIX® (75mg) tablet were purchased from local market. All the reagents in this assay along with triple distilled water were of analytical grade.

Apparatus

Spectral analysis were made on a Jasco Spectrophotometer, Model- V-630 (Japan), was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with automatic wavelength correction with a pair of 10 mm quartz cells. Glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

Standard Stock Solution:

Accurately weighed Clopidogrel bisulfate (10mg) was transferred to a 100ml volumetric flask, dissolved in 10ml with distilled water pH 1 and made up the volume up to the mark with same. A stock solution contained 100µg/ml of Clopidogrel bisulfate [4]**.**

Determination of absorbance maximum:

Weighed an accurate amount of 10mg Clopidogrel bisulfate was dissolved in 10ml with distilled water Ph 1and diluted up to100ml by same to obtain a 100µg/ml conc. of Clopidogrel bisulfate in solution. This solution was subjected to scanning between 200-400 nm (fig. III). The effect of

dilution on absorption maxima was studied by diluting the above solution to 40- 70µg/ml and scanned from 200-400nm [8].

Preparation of calibration curve for Clopidogrel bisulfate:
Stock solutions of Clopidogrel bisulfate were pipette out in to a series of six volumetric of 10ml. The volume in each volumetric flask was made up to the mark with distilled water pH 1. It produced the concentration range of 40-70 µg/ml. The absorbance of the solution was measured at 222 nm against distilled water pH 1 as a blank in Table no. I. The calibration curve was given in figure III, Spectra of 50µg/ml solution of Clopidogrel bisulfate in distilled water pH 1solution (Fig.no.IV) and statistical parameters are summarized in Table no.IV.

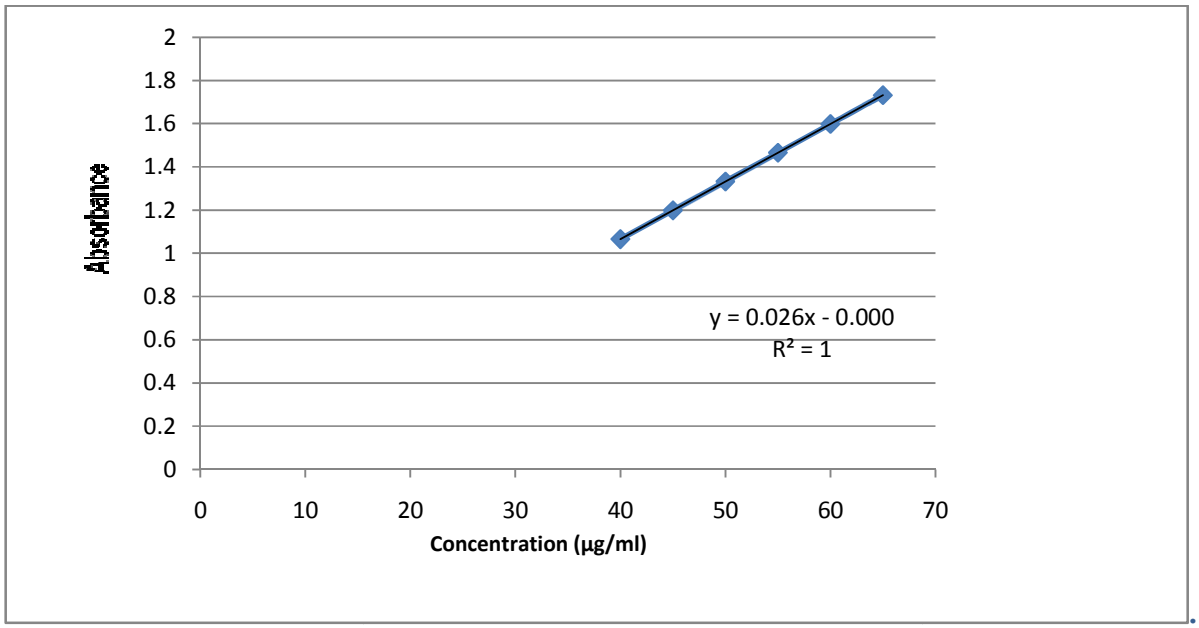

Figure III. Calibration Curve of Clopidogrel bisulfate at 220 nm

Table. I Results of least square regression analysis and absorbance of UV methods for the estimation

Sr. no.	Concentration, µg/ml	Absorbance Mean(n=6)
1	40	1.065
2	45	1.198
3	50	1.332
4	55	1.465
5	60	1.598
6	65	1.731

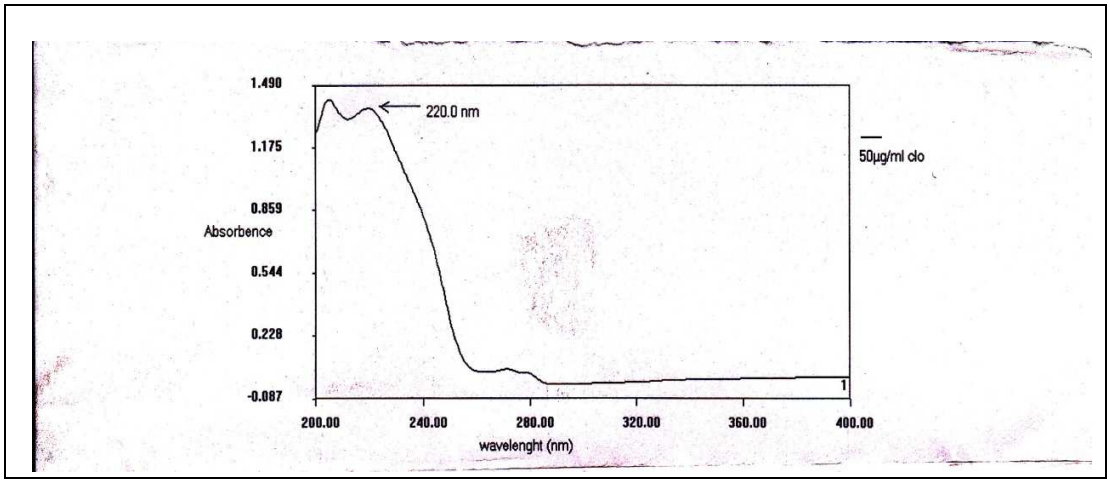

Figure. IV Scan Spectra of 50µg/ml solution of Clopidogrel bisulfate in distilled water pH 1solution.

Table. II Calibration curve points of the proposed method for the estimation of Clopidogrel bisulfate

Sr. no.	Parameters	Values
1	Absorption maxima, nm	220
2	Beer's law limit, µg/ml	40-70
3	Molar absorptivity, 1 mole-1/cm-1	3.202×10^2
4	Regression equation	Y=0.026x+0.00
5	Slope(b)	1
6	Y-Intercept(a)	0.0175
7	1/slope	78.43
8	Correlation coefficient(f)	1
9	LOD and LOQ	0.4ppm and 2ppm

Analysis of Marketed Tablet Formulation:

Accurately weighed the 20 tablets of Plavix® and fine powdered. The powder equivalent to 100mg of Clopidogrel bisulfate was transferred to 100ml volumetric flask and 20ml distilled water pH 1 solution is added and sonicated for 15 minutes to dissolve the Clopidogrel bisulfate in it and made the volume to mark with same. The solution was filtered through Whatmann filter paper No.40. 10 ml of this was diluted with distilled water pH 1 solution diluted with same as blank. The concentration of Clopidogrel bisulfate present in marketed tablet formulation were determined, Table no.III [2].

Table no.III Result of Analysis of Clopidogrel bisulfate in Tablet Plavix®75mg.

Tablet Sample	Label Claim, mg/tablet	Actual content found, mg±S.D	Percent Actual content found, ±S.D	% CV	% Recovery ± SD
Plavix®75mg Tab.(n=3)	75mg	74.71±0.167	99.62±0.224	0.225	99.64 ±0.236

Method Validation

Accuracy (Recovery studies): [3]

Recovery studies were performed to judge the accuracy of the method. 1ml of standard formulation (100mcg/ml) was taken in three 10 ml volumetric flask and to it 80%, 100% and 120% (i.e. 0.8ml, 1.0ml and 1.2ml) of working standard solution (100mcg/ml) added respectively and made the volume up to the mark. The respective absorbance at 220 nm was recorded against the blank. The amount of added concentration was determined from the obtained absorbance values and percent recovery was determined for formulation Table no.IV.

Table. IV Clopidogrel bisulfate estimation in dosage form in recovery studies by proposed method

Sr. no.	Concentration of added amount of drug in the final dilution,(µg/ml)	Recovery, (µg/ml)	Percent Recovery (%)	Mean recovery ±SD	CV
1	8	7.91	79.1		
2	10	9.89	98.9	98.87± 0.975	0.958
3	12	11.86	118.6		

Precision: [6]

The Precision of the proposed method was ascertained by actual determination of ten replicates of fixed concentration of the drugs within the Beer's range and finding out the absorbance by the proposed method. From this absorbance, mean, SD, %RSD was calculated. The readings were shown in Table no.IV.

Table. IV Precision Reading of Clopidogrel bisulfate

Sr. no.	Concentration (µg/ml)	Absorbance	Statistical analysis
1	50	1.364	Mean 1.360
2	50	1.351	S.D. 0.975
3	50	1.361	%RSD.91.98.
4	50	1.363	
5	50	1.360	
6	50	1.332	
7	50	1.365	
8	50	1.346	
9	50	1.266	
10	50	1.346	

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method, it was found that Clopidogrel bisulfate obeys linearity within the concentration ranges 1-100µg/ml. The developed estimation method proved to be accurate (accuracy varies between 10.2-5.5%) and precise (Intra day precisions were less than 4.5%).The method has been validated for the range 40-70 µg/ml using distilled water Ph 1solution. The method is linear over this concentration range as indicated by the *F*-test for lack of fit. Analyte recovery was better than 90% at all points on the standard curve, Intraday precision was better than 5% CV, while accuracy was between 98-100% of nominal over this range of the estimation.

CONCLUSION

The developed UV Spectrophotometric method for the estimation of Clopidogrel bisulfate was found to be simple and useful with high accuracy, precision, repeatability. Sample recoveries in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non Interference of formulation excipients in the estimation. In the selected solvent system (distilled water pH 1 solution), drugs were stable for more than 48 hours, thus suggesting that samples need not be estimated immediately after collection. The developed method was found to be stability specific and was validated as per ICH guidelines (1994, 1996 and 2005) and statistical method.

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