Int. J. Chem. Sci.: 10(1), 2012, 449-456
ISSN 0972-768X
www.sadgurupublications.com

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR CLOPIDOGREL BISULFATE IN BULK AND FORMULATIONS

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ABSTRACT

Clopidogrel bisulfate belongs to the class of inhibitor 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 (300 mg), structural formula (Fig. 1) and space-filling model of clopidogrel. (Fig. 2)[1]. 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

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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 copidogrel 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[2]. The present investigation has been undertaken to develop simple UV spectrophotometric method for the estimation of clopidogrel bisulfate in pure form and its formulations[3].

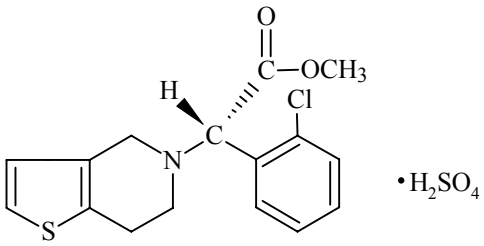

Fig. 1: Structural formula of clopidogrel bisulfate

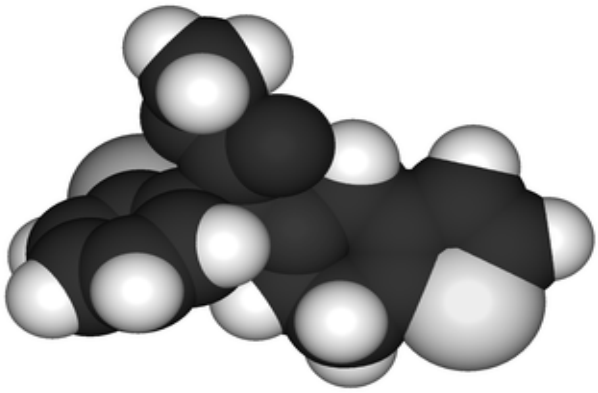

Fig. 2: Space-filling model of clopidogrel bisulfate

EXPERIMENTAL

Material

Clopidogrel bisulfate pure drug was obtained as a gift sample from Cadila Pharma Ltd. (Ahmedabad, India). PLAVIX® (75 mg), CAPLOR® (75 MG) and CLASS® tablets 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 (10 mg) was transferred to a 100 mL volumetric flask, dissolved in 10 mL with distilled water (pH 1) and made up to the volume with same. A stock solution contained 100 µg/mL of clopidogrel bisulfate was prepared[4].

Determination of absorbance maximum

Accurate amount of 10 mg clopidogrel bisulfate was weighed anddissolved in 10 mL with distilled water (pH 1) and diluted up to 100 mL by same to obtain a 100 µg/mL conc. of clopidogrel bisulfate in solution. This solution was subjected to scanning between 200-400 nm (Fig. 3). The effect of dilution on absorption maxima was studied by diluting the above solution to 40- 70 µg/mL and scanned from 200-400 nm[5].

Preparation of calibration curve for clopidogrel bisulfate

Stock solutions of clopidogrel bisulfate were pipette out in to a series of six volumetric flasks of 10 mL. 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 1. The calibration curve is given in Fig. 3, Spectra of 50 µg/mL solution of clopidogrel bisulfate in distilled water pH 1 (Fig. 4) and statistical parameters are summarized in Table 2.

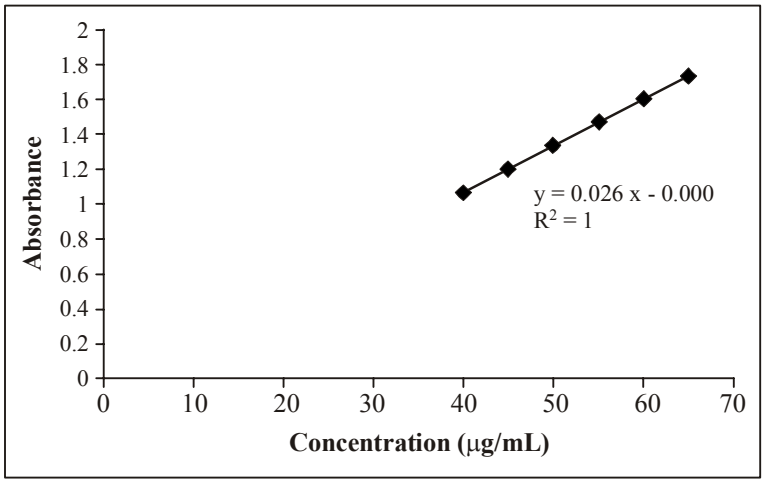

Fig. 3: Calibration curve of clopidogrel bisulfate at 220 nm

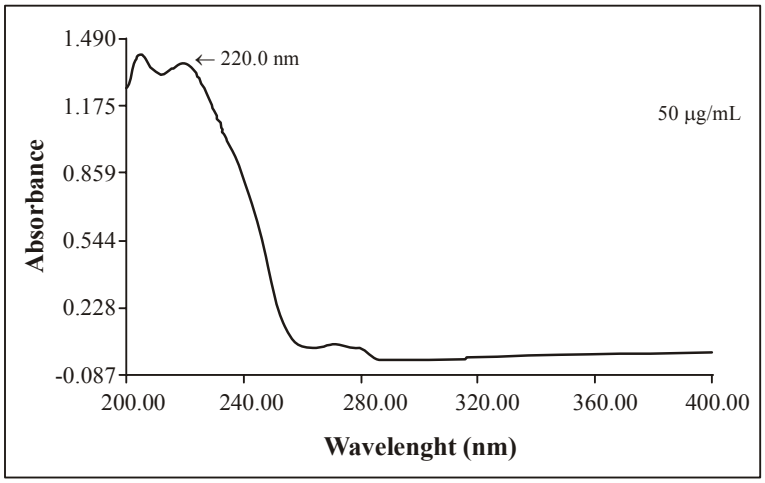

Fig. 4: Spectra of 50 µg/mL solution of clopidogrel bisulfate in distilled water (pH 1 solution)

Table 1: Results of least square regression analysis and absorbance of UV methods for the estimation

S. No.	Concentration (µg/mL)	Absorbance mean (n = 6)
1	40	1.065
2	45	1.198

Cont…

S. No.	Concentration (µg/mL)	Absorbance mean (n = 6)
3	50	1.332
4	55	1.465
5	60	1.598
6	65	1.731

Table 2: Calibration curve points of the proposed method for the estimation of Clopidogrel bisulfate

S. 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 = 0.026 x + 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	0.4 ppm and 2 ppm

*Indicates Y = a + b X when Y is absorbance and X is the concentration of clopidogrel bisulfate (µg/mL)

**denotes for six replicates

Analysis of marketed tablet formulation

Accurately weighed 20 tablets (of PLAVIX®, CAPLOR® and CLASS®) were finely powdered. The powder equivalent to 100 mg of clopidogrel bisulfate was transferred to 100 mL volumetric flask and 20 mL distilled water (pH 1 solution) was added and sonicated for 15 minutes to dissolve the clopidogrel bisulfate in it and volume was made 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) with same as blank. The concentration of clopidogrel bisulfate present in marketed tablet formulation were determined (Table 3)[6].

Table 3: Result of analysis of clopidogrel bisulfate in tablets

Tablet sample (n = 3)	Label claim, mg/tablet	Actual content found, mg ± S.D	Percent actual content found ± S.D	% CV	% Recovery ± SD
PLAVIX®	75 mg	74.71 ± 0.167	99.62 ± 0.224	0.225	99.64 ± 0.236
CAPLOR®	75 mg	74.65 ± 0.174	99.72 ± 0.492	0.461	99.58 ± 0.212
CLASS®	75 mg	74.82 ± 0.840	99.67 ± 1.121	1.023	99.54 ± 0.237

Method validation

Accuracy (Recovery studies)[7]

Recovery studies were performed to judge the accuracy of the method. 1 mL of standard formulation (100 µg/mL) was taken in three 10 mL volumetric flasks and 80%, 100% and 120% (i.e. 0.8 mL, 1.0 mL and 1.2 mL) of working standard solution (100 µg/mL) were added, respectively and the volume was made 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 4).

Table 4: Clopidogrel bisulfate estimation in dosage form in recovery studies by proposed method

S. 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[8]

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 the absorbance by the proposed method was find out. From this absorbance, mean, SD and % RSD was calculated. The readings are shown in Table 5.

Table 5: Precision reading of clopidogrel bisulfate

S. 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 1-100 µg/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 1) solution. This 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 and 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 were validated as per ICH guidelines (1994, 1996 and 2005)[9,10] and statistical method.

ACKNOWLEDGEMENT

Authors are sincerely thankful to P. D. V. V. P. F'C College of Pharmacy Ahmednagar for providing necessary facilities for work.

REFERENCES

1. R. B. Patel et al., Simultaneous Estimation of Acetylsalicylic Acid and Clopidogrel Bisulfate in Pure and Tablet Formulation by HPLC and HPTLC, J AOAVC International, **91**, 750-755 (2008).
2. A. H. Becket and J. B. Stenlake, Practical Pharmaceutical Chemistry, 4th Edition, CBS Publisher's Distributors, New Delhi ( ) pp. 275-337.
3. K. A. Anandakumar et al., RP-HPLC Analysis of Aspirin and Clopidogrel Bisulfate in Combination, Indian J. Pharma. Sci., **69**, 597-599 (2007).
4. P. B. Chaudhari et al., Stability Indicating Spectrophotometric Method for Determination and Validation of Clopidogrel Bisulfate in Tablet Dosage form, IJRAP, **1(2)**, 418-423 (2010).
5. G. D. Christian, Analytical Chemistry, 4th Edition, John Wiley and Sons, New York (1987) pp. 87-88.
6. RxList.com The Internet Drug Index for Prescription Drugs Medication and Pill Identifier Available form http://www.rxlist.com/plavix-drug.com.
7. S. J. Rajput et al., Chemometric Simultaneous Estimation of Clopidogrel Bisulfate and Aspirin from Combined Dosage Form, Indian J. Pharma. Sci., **70**, 450-454 (2008).
8. L. Vocilkova et al., Determination of Clopidogrel by Chromatography, Current Pharma Annul, **5**, 424-431 (2009).
9. ICH/CPMP Guidelines Q2A, Text on Validation of Analytical Procedures (1994).
10. ICH/CPMP Guidelines Q2B, Validation of Analytical Procedures-Methodology (1996).

Accepted : 30.10.2011