_____

Determination of clopidogrel bisulphate in pharmaceutical dosage form by high performance liquid chromatography method

Rajan V. Rele

Central Research Laboratory, D. G. Ruparel College, Matunga, Mumbai
_____

ABSTRACT

Rapid and accurate reverse phase high performance liquid chromatography method is described for determination of clopidogrel bisulphate from the pharmaceutical dosage form. It was observed that Chromatopak peerless basic C18 (50 x 4.6 mm i.d.) with 3 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of ammonium acetate and methanol. The detection was carried out at wavelength 220 nm. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution with the linear range 50-150 µg/ ml. The method has been successfully used to assay of pharmaceutical dosage form i.e. tablets with good recoveries.

Key words: Clopidogrel bisulphate, Ammonium acetate, Methanol, HPLC.
_____

INTRODUCTION

In this communication a new RP-HPLC method is developed for assay of clopidogrel bisulphate in pharmaceutical dosage form. Clopidogrel bisulfate, chemically (+)-(S) -(2-chlorophenyl)- 6,7-dihydrothieno [3,2-c] pyridine-5(4H)-acetic acid methyl ester sulphate is a potent oral anti-platelet agent often used in the treatment of coronary artery disease, peripheral vascular disease and cerebro vascular disease.

The mechanism of action of clopidogrel is irreversible blockade of the adenosine di-phosphate (ADP) receptor P2Y12 and is important in platelet aggregation, the cross-linking of platelets by fibrin. The blockade of this receptor inhibits platelet aggregation by blocking activation of the glycoprotein IIb/ IIIa pathway. Literature survey reveals the estimation of Clopidogrel bisulfate in pharmaceutical formulations by various HPLC [1-5], spectrophotometric [6-10], TLC [11] methods for assay of clopidogrel bisulphate. In the proposed work simple, rapid and reliable reverse phase method is developed for the determination of clopidogrel bisulphate. The method can be used for the routine analysis. In the proposed method optimization and validation of this method are reported.

.MATERIALS AND METHODS

Materials:
Reference standard of clopidogrel bisulphate was obtained from reputed firm with certificate of analysis. HPLC grade methanol of Qualigens fine chemicals was used for chromatographic separation. Ammonium acetate was used of analytical reagent grade from S. D. fine chemicals, HPLC grade water was obtained using Millipore. Standard and sample solutions were prepared in diluent [water: acetonitrile (50:50 % v/v)].

INSTRUMENTATION
The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software.

Preparation Standard Stock Solution
Standard Solution was prepared by transferring appropriate amount of clopidogrel bisulphate in 100 ml volumetric flask and making volume with diluent [Water: acetonitrile (50:50 % v/v)] to get concentration of 1000 μg/ ml clopidogrel bisulphate.

Sample Solution
Twenty tablets were weighed accurately and average weight of each tablet was determined. A powdered tablet equivalent to 100 mg was weighed accurately. It was transferred into a 100 ml volumetric flask. It was dissolved in small quantity of diluent [water: acetonitrile (50:50 % v/v)] and diluted to 100 ml. volume using same diluent. It was further diluted to get 100 μg/ ml of clopidogrel bisulphate solution. It was sonicated for 15 minutes and filtered through Whatman filter paper no. 41. First few ml of the filtrate was discarded. The resulting solution was injected into the HPLC system.

Chromatographic conditions
Chromatographic separation was performed at ambient temperature on a reverse phase Chromatopak peerless basic C18 (50 x 4.6 mm i.d.) with 3 μ particle size column. Mobile phase was consisted of 0.01 M ammonium acetate and methanol (30:70 % v/v) with isocratic system. The solutions were filtered and degassed before use. The flow rate of the mobile phase was adjusted to 1.2 ml /min. The detector wavelength was set at 220 nm. The injection volume of the standard and sample solutions was 10 μl.

Method Development
Different columns containing octyl and octadecyl silane stationary phase were tried for the separation and resolution. It was found that Chromatopak peerless basic C18 (50 x 4.6 mm i.d.) with 3 μ particle size column more advantages over the columns. The drug solution was injected into the column and elution pattern of all the drug and resolution parameters were studied. In addition to this, UV spectrum of drug was recorded at the wavelength range from 200 to 400 nm and the response for optimization was compared. The choice of wavelength 220 nm was considered satisfactory, permitting the detection of drug with adequate sensitivity. (The spectrum of the drug is given in fig 1).

Fig 1: spectrum of clopidogrel bisulphate (100 μg /ml)

_____
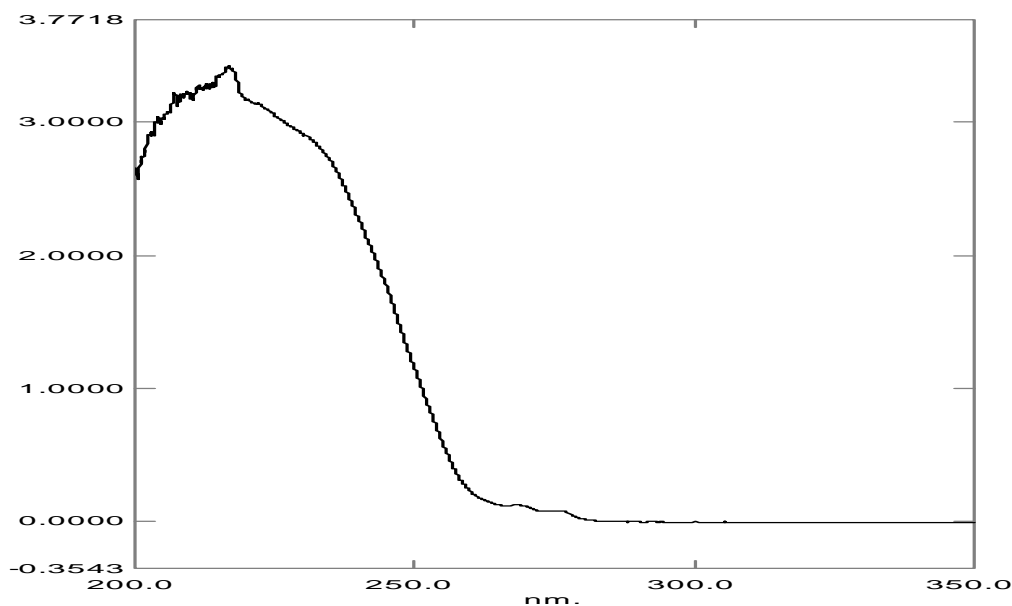
A typical chromatogram of the standard   and sample drug are given in fig.2,3.
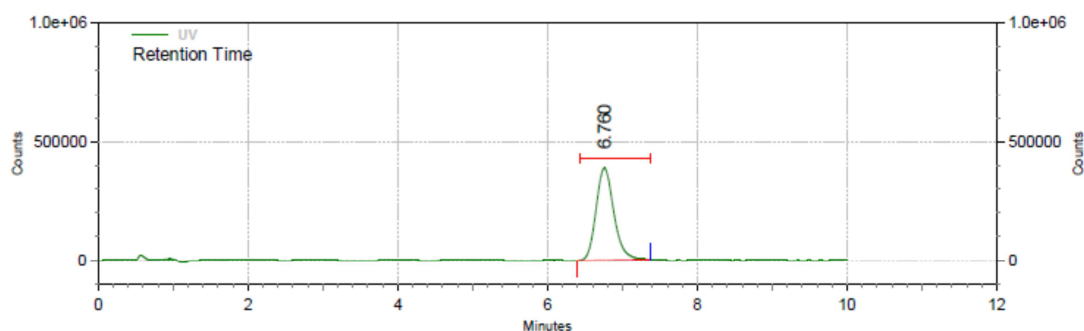

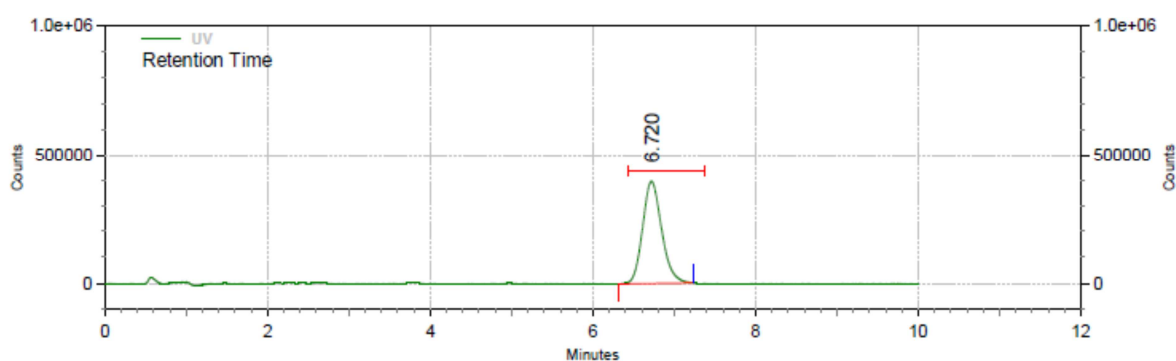**Fig -2: A Typical chromatogram of clopidogrel bisulphate (standard)**

Fig -3: A Typical chromatogram of clopidogrel bisulphate (sample)

The relative chromatographic figures of merit are reported in table -1.

Table – 1: System Performance Parameters for clopidogrel bisulphate (n = 5).

Retention time	Symmetry Factor	No. of plates
6.720 min.	1.18	3920

* Calculated at 5% peak height, + Calculated as $N = 16 \, (t_R/w)^2$

RESULTS AND DISCUSSION

METHOD VALIDATION
System suitability
System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates and symmetry factor were determined. The results are shown in table 1, indicating good performance of the system.

Linearity
Under the experimental conditions described above, linear calibration curve for drug was obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the drug i.e. (y) v/s concentration (x). The regression analysis data obtained is tabulated in Table -2. The linear range was 50 – 150 μg/ ml of clopidogrel bisulphate .

Table – 2: Linearity – Regression analysis data

Parameters	Values
Correlation Coefficient (r)	0.999
Intercept (y)*	-83201
Slope (m)*	64695

*For equation y = mx + c

_____

Accuracy

Accuracy of the proposed method was determined by applying the described method to synthetic mixture containing known amount of each drug corresponding to 50 %, 100% and 150% of the nominal concentration. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table 3.

Table - 3: Accuracy - %Recovery of each analyte

level	test	weight in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
50%	1	10.31	3307281	51.15	51.05	99.81	**100.03**
	2	10.34	3315208	51.15	51.03	99.76	
	3	10.26	3314912	51.15	51.42	100.53	
100%	1	10.05	6364544	102.3	100.79	98.52	**98.38**
	2	10.01	6332018	102.3	100.67	98.41	
	3	10.07	6356043	102.3	100.45	98.20	
150%	1	10.30	9903494	153.45	153.02	99.72	**100.03**
	2	10.25	9889015	153.45	153.55	100.06	
	3	10.23	9895020	153.45	153.94	100.32	
					Mean of % recovery		**99.48**

Precision

The method Precision was established by carrying out the analysis of powdered tablet containing the drug. The assay was carried out by using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limit indicating the repeatability of the method. The results obtained are tabulated in table - 4.

Table – 4: Precision – Method Precision

Test	Area	% assay
Test solution 1	6525467	100.54
Test solution 2	6441587	100.41
Test solution 3	6536467	100.80
Test solution 4	6444743	100.46
Test solution 5	6416932	100.03
Test solution 6	6388333	99.58
	Mean Assay	**100.30**

Robustness

The robustness of the method is determined as a measure of the analytical methods capability to be unaffected by small variation in method parameters.

The different variations are as given bellow:
Variation in mobile phase composition by ± 0.2 units
Variation in wavelength by ± 0.2 nm
Variation in flow rate by ± 0.2 ml

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Stability of Solution

Stock solution stability was checked for 24 hrs at room temperature. The drug solutions were found to be stable for the specified period. Stock Solution of sample and standard contain 1000 μg/ ml.

Method Application

The validated high performance liquid chromatographic method was applied to determination of clopidogrel bisulphate. Twenty tablets powder containing clopidogrel (75 mg) was used. A portion equivalent to 100 mg of clopidogrel bisulphate was weighed accurately and was dissolved in 50 ml of diluent. It was sonicated 10 minutes and further diluted to 100 ml to get a solution of concentration of 1000 μg/ ml of clopidogrel bisulphate. 1 ml of such solution is diluted to 10 ml to give concentration as 100 μg /ml. A 10 μl of this solution was injected into the

_____

chromatograph under the specified conditions. The analyte peaks were identified by comparison with observed retention times with those of respective standards. The peaks areas obtained were used to calculate the amount of drugs present. The assay results, expressed as mg/tablet, are shown in table 4, which indicates that the amount of drug in the product meets the requirements.

CONCLUSION

The proposed HPLC method provides a fast, accurate and rugged assay with stability indicating potential for clopidogrel bisulphate in tablet. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation in comparison to previous methods. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drugs to the pre analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery obtained indicates non- interference from the excipients used in the formulation.

The proposed method involves use of acetonitrile and ammonium acetate. Hence overall cost of analysis is less for proposed method. The retention time is 6.720 hence it is less time consuming and requires fewer chemicals due to less time for analysis. Hence it is more economical than previous methods. The proposed method has additional advantages over the existing methods and is more beneficial for analysis of such formulation than the previous methods.

Thus the proposed RP-HPLC method for the estimation of clopidogrel bisulphate in pharmaceutical dosage forms is precise, accurate, linear, robust, simple and rapid. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies.

REFERENCES

[1] Mohamad Ammar, Al-Khayat, Samer Haidar, Huda Mando, *Int. J. Pharm. Sci.Rev. Res.*, **2012**,14(2), , 1-5.
[2] Nawal A. Alarfaj, *Journal of Saudi Chemical Society*, **2012**,16,23-30.
[3] U.C.Mashelkar , Sanjay D.Renapurkar , *International Journal of ChemTech Research*, **2010**, 2(2), 822-829.
[4] Ajay Kumar Gupta,P.K.Patel **,** *international journal of scientific research*, **2014**,3(2), 59-60.
[5] A. Mounika, N. Sriram, *International journal of pharmacy and analytical research*,**2012**,1(1), 1-7,.
[6] B.anupama ,V.jagathi, A.aparna, M.madhubabu, V.Lakshmi Annapurna, *International Journal of Pharma and Bio Sciences*, **2011**, 2(2),105-108,.
[7] Chaudhari Pritam B., Pawar Pravin D. Narkhede Kiran P., *International journal of research in ayurveda and pharmacy*, **2010**,1(2), 418-423,.
[8] Saadet Dermiş, and Ekrem Aydoğan, *Commun.Fac.Sci.Univ.Ank.Series B*, **2009**, 55(1),1-16.
[9] Pravin B. Cholke , Raihan Ahmed, S. Z. Chemate and K. R. Jadhav, *Archives of Applied Science Research,* **2012**, 4 (1),59-64
[10] Suhas Gurav, R. Venkatamahesh, *International journal of ChemTech Research*, **2012**,4(2), 497-501.
[11] D. Antić, S. Filipić, and D. Agbaba, *Acta chromatographica*, **2007**,18, ,199-206.

.