The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CLOPIDOGREL BISULPHATE

(Pembangunan dan Penentusahkan Kaedah RP-HPLC Terhadap Klopidogrel Bisulfat)

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Abstract

A novel, simple, precise, accurate and economical method has been developed and validated for the estimation of Clopidogrel bisulphate. Chromatographic analysis was performed on Hypersil BDS C_{18} (250 × 4.6 mm, 5 µm) column. Mobile phase used was potassium dihydrogen orthophosphate buffer: acetonitrile in a ratio of 32:68, pH was adjusted to 4.0 using orthophosphoric acid and 0.1 ml of triethyl amine was added for peak sharpness. Isocratic analysis was performed on SPD-20A double beam UV spectrophotometer at a detection range of 220 nm with a flow rate of 1.0 ml/min. The retention time for clopidogrel bisulphate was 3.847 min. The linear response was found to be in a concentration range of 50-150 µg/ml with a correlation coefficient of 0.999. The mean recovery was found to be 100.67 %. Intraday and Interday precision variations were found to be 1.88 and 0.863 respectively, which are within the limit of % RSD not more than 2. Hence the developed method is simple, fast, accurate and reproducible. It is suitable for routine quality control analysis of clopidogrel bisulphate.

Keywords: RP-HPLC, validation, clopidogrel bisulphate, linearity, accuracy, precision

Abstrak

Satu kaedah baru yang mudah, tepat, jitu dan ekonomikal telah dibangunkan dan disahkan terhadap analisa klopidogrel bisulfat. Analisis kromatografi telah dijalankan menggunakan turus Hypersil BDS C18 (250 × 4.6 mm, 5 µm). Fasa bergerak yang digunakan ialah larutan penampan kalium dihidrogen ortofosfat : asetonitril dalam nisbah 32 : 68, pH telah diubah kepada nilai 4.0 menggunakan asid ortofosforik dan 0.1 ml larutan trietil amine ditambah untuk ketajaman puncak. Analisis isokratik telah dijalankan ke atas SPD- 20A spektrofotometer UV pada julat pengesanan 220 nm dengan kadar aliran 1.0 ml / min. Retensi masa bagi klopidogrel bisulfat adalah 3. 847 min. Sambutan linear didapati berada dalam julat kepekatan 50-150 µg ml dengan pekali korelasi adalah 0.999. Min pemulihan semula adalah 100. 67 %. Variasi kejituan ujian Intra dan Inter-hari didapati masing – masing adalah 1.88 dan 0.863 yang berada dalam julat had % RSD tidak lebih daripada 2. Maka kaedah yang dibangunkan adalah mudah, cepat, tepat dan keboleh ulangan. Ia sesuai untuk analisis rutin kawalan mutu bagi klopidogrel bisulfat.

Kata kunci: RP- HPLC, Penentusahkan, klopidogrel bisulfat., Kelinearan , Ketepatan, Kejituan

Introduction

Clopidogrel bisulphate is an antiplatelet agent or platelet aggregation inhibitor. The IUPAC name of clopidogrel bisulphate is Methyl (+)-(S)-α-(2-chlorophenyl)-6,7dihydrothieno [3,2-c]pyridine-5(4H)acetate sulfate (Figure 1).The drug is practically insoluble in water at neutral pH, but freely soluble at pH 1[1]. It is used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease [2].

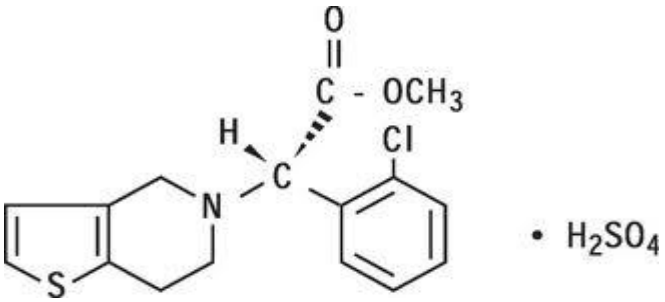

Figure 1. Structure of clopidogrel bisulfate [1]

The quantitative analysis was made on Shimadzu prominence system using reverse phase chromatographic method to obtain less retention time. Hence clopidogrel bisulphate is insoluble in water; RP-HPLC method was used. Several analytical methods which are commonly used to identify the drug concentration and for quantification of drugs for the determination of clopidogrel bisulphate are Spectrofluorometry [3], LC-MS/MS [4], HPLC [5, 6], HPTLC [7, 8] and potentiometry method [9].

The aim of this study was to develop a RP-HPLC method, which could be employed for the routine analysis of the drug clopidogrel bisulphate.

Materials and Methods

Chemicals and Reagents

Working standard of clopidogrel bisulphate was obtained from Sidmak India (pvt) Ltd Valsad, India having purity > 99.2%. HPLC grade Acetonitrile and Water was procured from Merck, Rankem Ltd., Mumbai, India. Phosphate buffer (pH 4.0) used as diluent and all chemical reagents used were HPLC grade.

Instrumentation

Spectral scan was made on a Shimadzu UV 1800 pc double beam spectrophotometer, with spectral bandwidth of 190-800 nm, wavelength accuracy of + 0.3 nm with automatic wavelength corrections using a pair of 10 mm quartz cells. All Spectral measurements were done using Spinchrom software.

Chromatographic Conditions

Chromatographic analysis was performed on Thermo Hypersil BDS C_{18} (250 × 4.6 mm, 5 µm) column with a mobile phase used as potassium dihydrogen orthophosphate buffer adjusted pH 4.0 with orthophosphoric acid: acetonitrile in a ratio of 32:68 and 0.1 ml of triethyl amine was added for peak sharpness. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system. Isocratic analysis was performed on SPD-20A double beam UV spectrophotometer at a detection range of 220 nm at a flow rate of 1.0 ml/min. The injection volume was 20 µl.

Preparation of Phosphate Buffer

Dissolve 10 g of Potassium dihydrogen orthophosphate and add 0.1ml of triethyl amine in 1000 ml distilled water. Adjust pH 4.0 with orthophosphoric Acid.

Preparation of Standard Solution

An accurately weighed quantity of 25mg clopidogrel bisulphate transferred in 25ml volumetric flask, dissolved with 10 ml of acetonitrile and volume was made up to the mark with acetonitrile sonicated for 10 min. From that solution pipette out 1 ml in 10 ml volumetric flask and volume was made up to the mark with mobile phase to make final Concentration 100 µg/ml.

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Procedure

Equilibrate the column with mobile phase at prescribed condition until a stable baseline is achieved. Separately inject diluent, standard solution and test preparation into liquid chromatography. Measure the response for all peaks.

Results and Discussion

The developed method was validated for parameters like linearity, accuracy, precision, robustness, ruggedness, sensitivity and system suitability [10, 11].

Method development and optimization

Optimized chromatographic conditions for estimation of clopidogrel bisulphate are finalized using Column Thermo Hypersil BDS C_{18} (250×4.6 mm, 5 µm) consisting of mobile phase acetonitrile: phosphate buffer pH 4.0 in a ratio of 68:32. HPLC pump mode was made Isocratic with a flow rate of a 1.0 ml/min over the detection wavelength range of 220 nm. Standard spectrum of clopidogrel bisulphate is as shown in Figure 2.

Injection volume was made with Rheodyne injector 7725i of 20 µl. The run time was 10 min. The retention time for clopidogrel bisulphate was found to be 3.8 min.

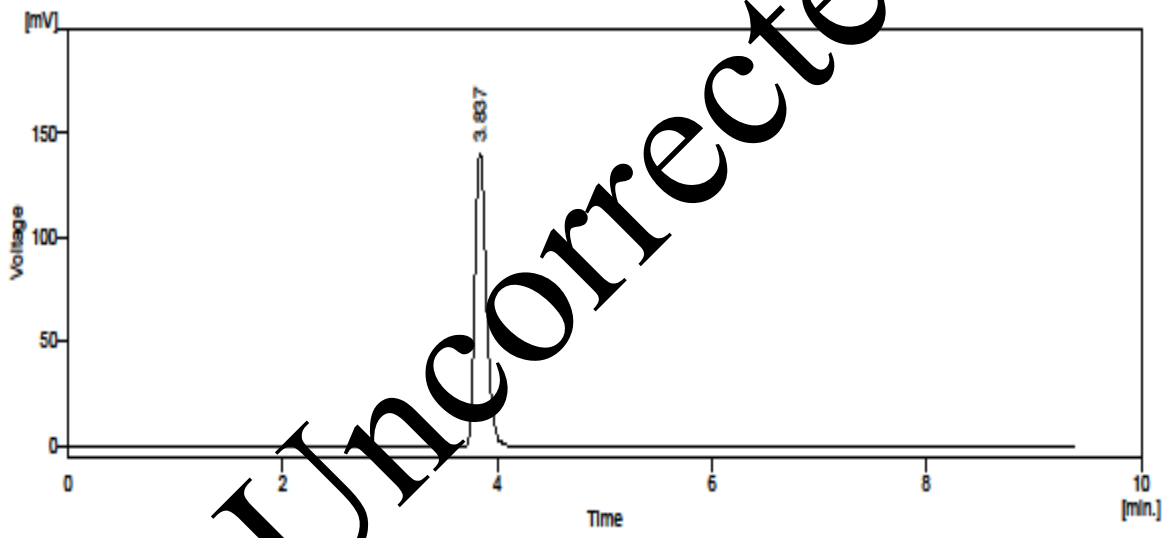

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Figure 2. Chromatogram of standard preparation of Clopidogrel bisulphate

System Suitability

In these studies, 5 replicate injections of the drug at a concentration of 100 µg/ml were injected and evaluated for the parameters such as Retention time, theoretical plates and tailing factor. The results obtained from the System Suitability studies were calculated and recorded in Table 1.

Table 1. System suitability parameters for Clopidogrel bisulphate

Name	Retention Time	Area	% Area	Theoretical Plate Count	Tailing Factor
Clopidogrel bisulphate	3.8	1037.18	100	6073	1.63

Linearity

The linear response was found in a concentration range of 50-150 µg/ml. The linearity plot is drawn in figure 3 and the correlation co-efficient (r^2), y-intercept and slope of regression line were found to be 0.999, 9.521, -2.834 respectively and recorded in Table 2.

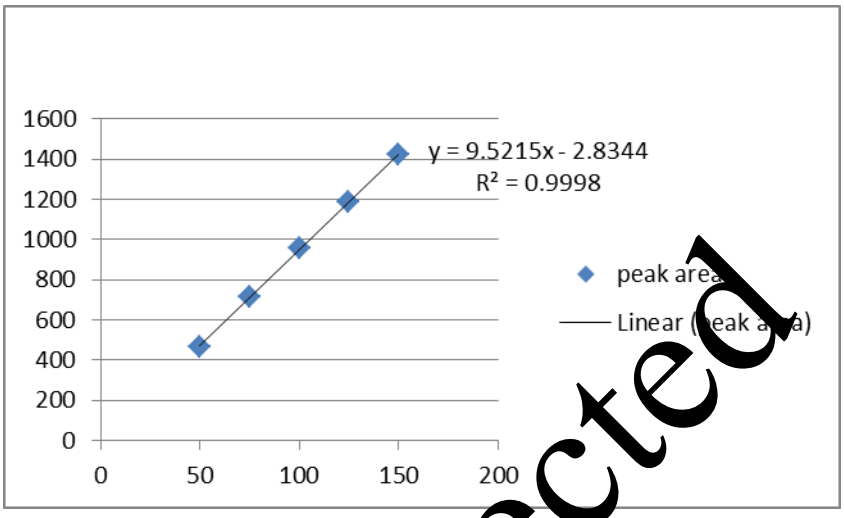

Figure 3. Calibration curve of clopidogrel bisulphate at 220 nm.

Table 2. Linearity study for clopidogrel bisulphate

Injection No.	Concentration (µg/ml)	Area
1	50	467.510
2	75	715.577
3	100	954.103
4	125	1187.576
5	150	1421.576

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Accuracy

The accuracy was determined at three levels: 80, 100 and 120 %. From the peak area of the drug, percentage recovery (%) was calculated for 80, 100 and 120 %, which are 99.78, 101.54 and 100.70 % respectively. Percentage recovery was found to be in the limit of 98 – 102 % and recorded in Table 3. Hence, the method termed as accurate.

Precision

The precision expressed as standard deviation or Relative standard deviation.

Intraday Precision

In this study, six injections of standard solution of 100 µg/ml were injected into the chromatographic system in different time intervals within a day. % RSD for retention time was found to be 0.768 and for area 1.88, which are within limit of % RSD not more than 2 and recorded in Table 4.

Table 3. Accuracy study for clopidogrel bisulphate

Accuracy level	Amount added	Area	Amount recovered	% Recovery (Mean)	SD	% RSD
80%		1708.768	80.66			
80%	80	1696.497	79.39	99.78 %	5.71	0.336
80%		1696.798	79.42			
100%		1913.944	101.90			
100%	100	1903.472	100.82	101.54 %	4.94	0.258
100%		1913.944	101.90			
120%		2098.460	121.00			
120%	120	2082.636	119.36	100.7 %	11.02	0.525
120%		2109.498	122.14			

*average of three determinations

Table 4. Intraday precision of clopidogrel bisulphate

Injection No.	Retention Time (min)	Area
1	3.810	952.166
2	3.780	950.675
3	3.780	949.230
4	3.847	975.080
5	3.853	990.675
6	3.830	950.788
Average	3.817	959.135
SD	0.029	18.05
% RSD	0.768	1.88

Uncorrected

Interday Precision

In this study, six injections of standard solution of 100 µg/ml were injected into the chromatographic system at different days. % RSD for retention time and for area was found to be 0.863 and 1.99 respectively, % RSD NMT 2 and recorded in Table 5.

Robustness

The robustness was obtained by altering the flow rate by 1.0 ml/min (altered flow rate 0.8 ml/min and 1.2 ml/min) pH 4.0 (altered pH 3.9 and 4.1). The results were obtained from robustness study were calculated. Percentage RSD for flow rate and for pH were found to be 1.49 and 1.03 respectively, % RSD NMT 2, and recorded in Table 6. Hence the method termed as robust.

Table 5. Interday Precision of Clopidogrel bisulphate

Injection No.	Retention Time	Area
1	3.810	952.166
2	3.780	936.951
3	3.780	949.230
4	3.853	995.554
5	3.863	1017.480
6	3.837	970.751
Average	3.821	969.355
SD	0.033	28.96
% RSD	0.863	1.99

Table 6. Robustness study of clopidogrel bisulphate

Condition	Modification	Mean Area ± SD*	% RSD	Mean % RSD
Flow Rate (ml/min)	0.8 ml/min	1041.542 ± 17.83	1.71	1.49
	1.2 ml/min	902.667 ± 11.43	1.27	
pH	3.9	952.471 ± 7.63	0.80	1.03
	4.1	968.01 ± 12.18	1.26	

*average of three determinations

Uncorrected

Ruggedness

The ruggedness of the method was determined by carrying out the experiment by different operators. Percentage RSD were calculated and recorded in Table 7. Result obtained by different analyst and different day were found within acceptable limit, which shows that test method is rugged.

Table 7. Ruggedness study of Clopidogrel bisulphate

Injection no.	Analyst I	Analyst II
	Area	Area
1	479.040	480.981
2	486.911	493.290
Average	482.976	487.136
SD	3.940	6.150
% RSD	0.810	1.260

Limit of Detection and Quantification (LOD and LOQ)

The Limit of Detection and Quantification**,** LOD and LOQ data was calculated and recorded in Table 8. LOD and LOQ were found to be 1.8728, 5.6752 ppm respectively.

Table 8. Limit of Detection and Quantification in this study

Parameters	Clopidogrel bisulphate
LOD (µg/ml)	1.8728
LOQ (µg/ml)	5.6752

Conclusion

The developed analytical method is validated as per ICH guidelines. A rapid and simple RP-HPLC method for determination of clopidogrel bisulphate has been developed and validated. The method involved a mobile phase consisting of acetonitrile: phosphate buffer (pH 4.0) 68:32 used at 220 nm. The retention time was 3.837 min at a flow-rate of 1.0 ml/min and the injection volume was 20 µl. The total run time for an assay was approximately 10 min. This proposed method was validated for parameters including linearity, accuracy, sensitivity, precision, ruggedness and robustness. The present study is concerned for the development and validation of simple, specific, sensitive, accurate and precise RP-HPLC method for the determination of clopidogrel bisulphate for routine analysis work.

Acknowledgement

The authors wish to thank Dr. Anjan Paul from Sidmak India (pvt) Vatsad, Gujarat for providing drug sample and Mr. Ketan Patel from Molecule Laboratory, Ahmadabad, Gujarat for providing the facilities to carry out this research work.

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