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Development and validation of second order derivative methods for quantitative estimation of clopidogrel in bulk and pharmaceutical dosage form

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ABSTRACT

Simple and precise UV spectrophotometric method by second order derivative was developed and validated for the estimation of clopidogrel bisulphate from bulk and its tablet formulation. The standard and sample solutions of clopidogrel bisulphate were prepared in 0.1 N hydrochloride acid. Clopidogrel bisulphate was estimated at 207.5 nm for the second order derivative UV-spectrophotometric method. Beer's law was obeyed in the concentration range of 10 to 35 µg / ml with coefficient of correlation value 0.9991. The method was tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation was of 1.2081% for the above method respectively. The proposed method was successfully applied for the determination of clopidogrel bisulphate in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed method is simple, easy to apply, low-cost and require relatively inexpensive instruments.

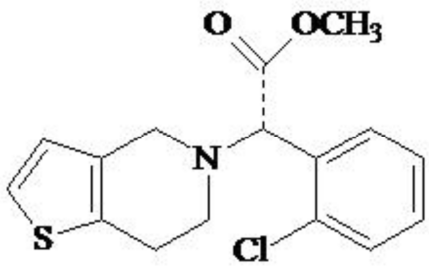
Keywords: Clopidogrel bisulphate, UV spectroscopy, second derivative spectroscopy.

INTRODUCTION

Clopidogrel bisulfate, chemically (+)-(S) -(2-chlorophenyl)- 6,7-dihydrothieno [3,2-c] pyridine- 5(4H)-acetic acid methyl ester sulphate is a potent oral antiplatelet 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. It 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 UV spectrophotometric 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.

Fig. 1: Chemical Structure of Clopidogrel



MATERIALS AND METHODS

Shimadzu UV-1800 was used with 10 mm matched quartz cell to measure absorbance of solution.

A Shimadzu analytical balance with 0.01 mg was used.

CHEMICAL AND REAGENTS

Reference standard of clopidogrel bisulphate was obtained from reputed firm with certificate analysis. All spectral absorbance measurements were made on Shimadzu UV-1800 with 10 mm matched cell.

PREPARATION OF STANDARD SOLUTION

About 10 mg of standard clopidogrel bisulphate was weighed accurately and transferred in 100 ml of volumetric flask. About 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes. The volume was adjusted up to the mark with 0.1 N hydrochloric acid to give concentration as 100 µg /ml.

Estimation from tablets

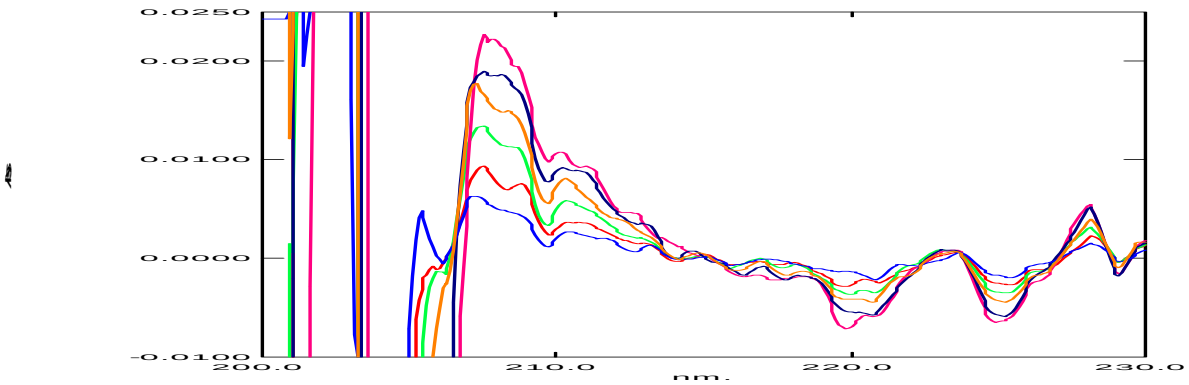
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of clopidogrel bisulphate was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

Experimental

Method

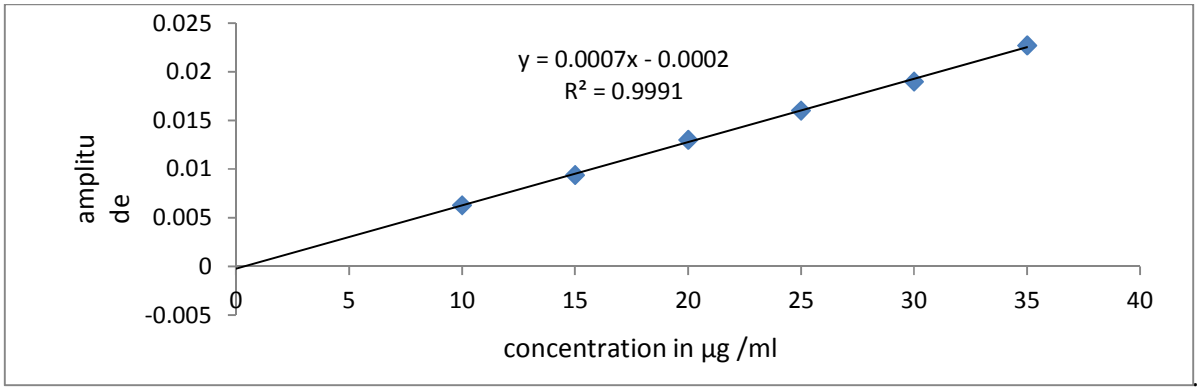
For the selection of analytical wavelength, 10 µg /ml solution of clopidogrel bisulphate was scanned in the spectrum mode from 350 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 207.5 nm. (Fig. 2).

Fig. 2.Overlay spectra of Second order derivative spectrum of clopidogrel bisulphate (10-35 µg/ml) showing absorbance at 207.5 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of clopidogrel bisulphate was pipette out and volume was adjusted with 0.1 N hydrochloric acid as solvent. Solutions were scanned between 350 nm to 200 nm in spectrum mode. The second order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 207.5 nm by using 0.1 N hydrochloric acid as blank. The calibration curve was prepared in the concentration range of 10 to 35 µg/ml. (Fig. 3)

Fig. 3. Calibration curve for clopidogrel bisulphate at 207.5 nm by second order derivative Spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	values
Detection Wavelength (nm)	207.5
Beer Law Limits (µg/ml)	10-35
Correlation coefficient(r^2)	0.9991
Regression equation (y=b+ac)	
Slope (a)	0.0007
Intercept (b)	-0.0002

Validation

Accuracy

Accuracy of the proposed method was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table 2.

Table 2: Results of recovery of clopidogrel bisulphate for second order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery (%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
10	0	9.9319	99.3197	0.1799	1.8121
10	5	15.0793	100.529	0.1296	0.8594
10	10	20.0907	100.453	0.2019	1.0051
10	15	25.0793	100.317	0.2898	1.155
				Mean =0.2003	Mean =1.2081

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 3.

Table 3: Precision- method precision

Experiment no.	Weight of clopidogrel bisulphate taken in mg	values
1	10	10.1583
2	10	9.8412
3	10	9.8412
4	10	9.6825
5	10	9.8412
6	10	10.8517
	Standard deviation	0.1944
	%RSD	1.9595

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of clopidogrel bisulphate was transferred to 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with0.1 N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

Solution was scanned between 350 nm to 200 nm in spectrum mode. The second order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 207.5 nm by using 0.1 N hydrochloric acid as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell 207.5 nm for second order derivative. Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of clopidogrel bisulphate was estimated by comparison with standard at 207.5 nm for second order derivative, table 4.

Table 4: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	values
(A)	Intra-day precision (n=3) Amount found ±% RSD	100.317 % 1.1555
(B)	Inter-day precision (n=3) Amount found ±% RSD	99.319 % 1.8121
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	1.9595

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 5.

Table 5: Values of results of LOD and LOQ

parameters	values
Limit of Detection (µg/ml)	0.5327
Limit of Quantification (µg/ml)	1.6142

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of clopidogrel bisulphate sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of clopidogrel bisulphate was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by

different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The second order derivative method is useful for routine analysis of clopidogrel bisulphate in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 10 to 35 µg/ml and given in table 1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2. The percentage recovery value indicates noninterference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of method is its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed method is fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of clopidogrel bisulphate in pharmaceutical formulation.

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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] Dr. 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.