International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.4, No.2, pp 497-501, April-June 2012

Development and Validation of Derivative UV-Spectropotometric Methods for Quantitative Estimation of Clopidogrel in Bulk and Pharmaceutical Dosage Form

Suhas Gurav[1]*, R. Venkatamahesh[2]

[1]Department of Pharmaceutical chemistry, Bharathi College of Pharmacy.
[2]Department of Pharmaceutical Analysis, Bharathi College of Pharmacy,
Bharathi Nagara, Mandya (District), Karnataka, India – 571 422.

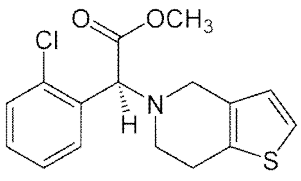
*Corres.author: suhasgurav07@gmail.com
Mobile No. 08088436467.

Abstract: A simple, precise and economical first-order (Method A), Area Under Curve [AUC] (Method B) UV-Spectropotometric methods have been developed and validated for the estimation of Clopidogrel in bulk and its tablet formulation. The solutions of standard and sample were prepared in 0.1 N HCl. Clopidogrel was estimated at 219 nm for the first order UV-Spectrophotometric method, while area under the zero order spectrum of Clopidogrel was measured in between 221 nm to 231 nm for AUC method. Beer's law was obeyed in the concentration range of 10 - 30 µg / ml with r^2 value 0.999 for first order method. Similarly in AUC method, Beer's law was obeyed in the concentration range of 10 - 30 µg / ml with r^2 value 0.999. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation, which was within the range of 0.169 % to 0.519 % for the above two methods. The proposed methods were successfully applied for the determination of Clopidogrel in tablet formulations. In addition, the proposed methods are simple, easy to apply, low-cost, and requires relatively inexpensive instruments.
Keywords: Clopidogrel, Method Validation, UV-Spectropotometric methods.

1. 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 structure of Iloperidone is shown in **Figure 1**. The mechanism of action of clopidogrel is irreversible blockade of the adenosine diphosphate (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. [1] It is not in any pharmacopoeia. Literature survey reveals the estimation of Clopidogrel bisulfate in pharmaceutical formulations by various chemometric[2], HPLC[3-6], HPTLC[7-8], TLC[9], and an LC- ESI-MS-MS[10] method was developed. There is no spectrophotometric method for Clopidogrel bisulfate, hence to develop simple, sensitive and accurate method.

Fig. 1: Chemical Structure of Clopidogrel



2. MATERIALS AND METHODS:

2.1 Instrument and reagents

Spectral scan were made on a Shimadzu UV spectrophotometer, model 1800 (Schimadzu, Japan) with spectral bandwidth of 0.5 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 UV-Probe 2.33 software. Clopidogrel sample was supplied by Cadila Pharmaceutical Ltd. Ahmedabad, India as a gift sample and used as such 0.1 N HCl used and purchased from S. D. Fine Chemicals ltd., India

2.2 Preparation of standard drug solution

100 mg standard Clopidogrel was weighed accurately and transferred to a 100 ml volumetric flask and dissolved in 0.1 N HCl. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. From this solution, 10 ml of solution was pipetted out and placed into 100 ml volumetric flask. The volume was made up to mark with methanol to give a solution containing 100 µg/ml. Further dilutions with 0.1 N HCl were made from this stock solution to get required concentration.

2.3 Preparation of sample solution

To determine the content of Clopidogrel in conventional tablet (label claim: 75 mg Clopidogrel per tablet), twenty tablets were weighed, their mean weight was determined and finely powdered.. Tablet powder equivalent to 100 mg of Clopidogrel was weighed and transfer into 100 ml volumetric flask then dissolved with methanol and further diluted upto the mark. It was kept for ultra-sonication for 30 min; this was filtered through Whatman filter paper No. 41 and then the final dilution was made with 0.1 N HCl to get the final stock solution of 1000 µg/ml. From this stock solution, various dilutions of the sample solution were prepared and analyzed.

2.4 Calibration curve

Method A: Absorption Maxima Method

For the selection of analytical wavelength, 20 µg/mL solution of Clopidogrel was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the spectrum (**Figure 2,3**), λ max of Clopidogrel, 219 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-30 µg/mL at 219 nm.

Method B: Area under Curve Spectroscopic method

In this method, 20 µg/mL solution of Clopidogrel was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The Absorption spectra thus obtained were AUC from zero order. The AUC spectra (**Figure 4,5**) measured between 221 nm to 231 nm The calibration curve for Clopidogrel was plotted in the concentration range of 10-30 µg/mL.

Fig. 2. Overlay first order derivative spectra of Clopidogrel (20 µg/ml)showing absorbance at 219 nm

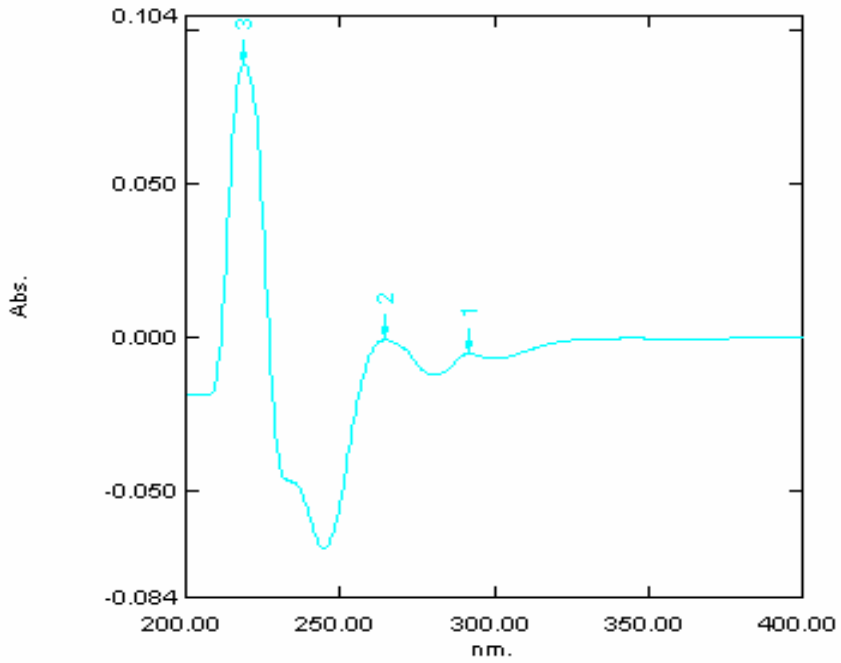


Fig. 3. Calibration curve for Clopidogrel at 262nm by First order derivative spectroscopy

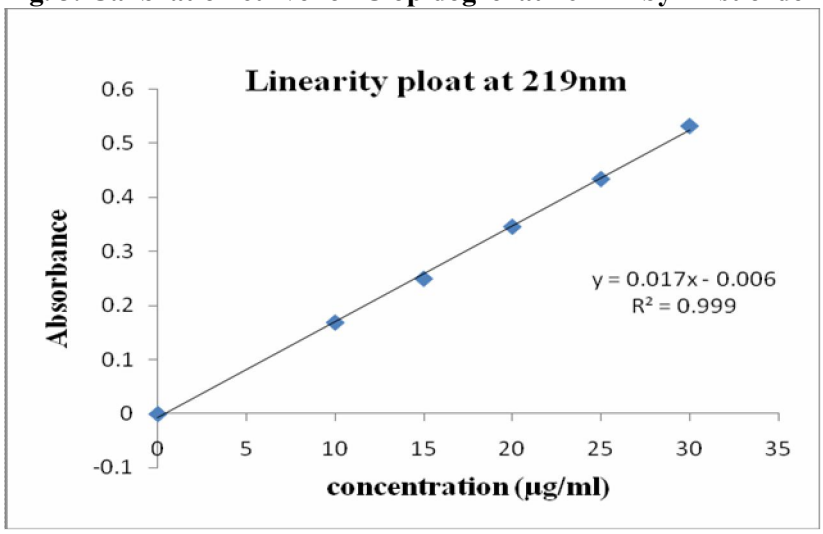


Fig. 4. Overlay AUC spectra of Clopidogrel (20 µg/ml) showing area from 221 nm to 231nm.

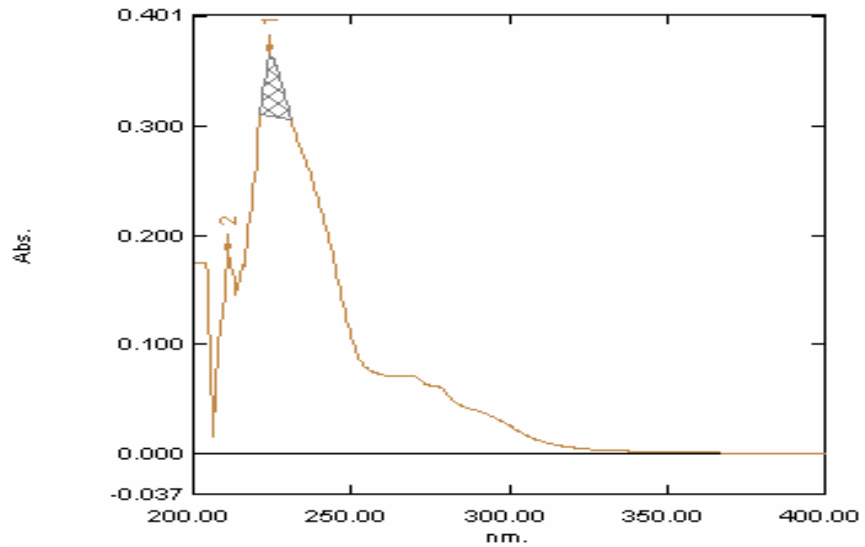


Fig. 5. Calibration curve for Clopidogrel by AUC spectroscopy

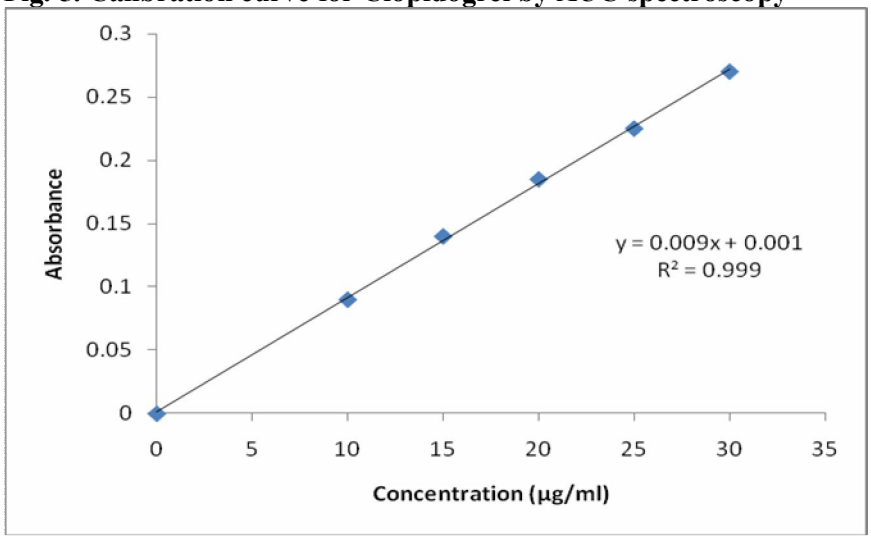


Table-1: Validation Parameters.

Validation Parameters		**First order derivative method**	**AUC method**
Detection Wavelength (nm)		219	221-231
Linearity	Range (µg/ml)	10-30	10-30
	Slope	0.009	0.017
	Intercept	0.001	0.006
	Correlation coefficient	0.999	0.999
Limit of Detection (µg/ml)		0.0113	0.058
Limit of Quantification (µg/ml)		0.345	0.175
% RSD of Intraday Precision	20 µg/ml	0.40	0.21
% RSD of Interday Precision	20 µg/ml	0.48	0.25
Accuracy* (% Recovery ± SD)	50%(10 µg/ml)	98.33±0.162	99.23±1.68
	100% (20 µg/ml)	101±0.165	98.46±0.881
	150% (30 µg/ml)	99.44±0.05	99.76±0.54

*Mean ±SD are obtained from 6 determinations.

Table :2. Analysis of tablet formulation

METHOD	**Label claimed (mg)**	**Amount found (mg)**	**%Recovery(± SD)**
First order derivative method	75	74.99	99.96 ± 0.09
AUC method	75	75.07	100.16 ± 0.11

**Average of six determinations

3. RESULT AND DISCUSSION

The first order, AUC UV-spectroscopic methods is for routine analysis of Clopidogrel in bulk and formulations. The spectroscopy methods 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 [11-13].Clopidogrel has the absorbance maxima at 219 nm (Method A), and in the AUC spectra measured between 221 nm to 231 nm (Method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 2-10 µg/ml and given in Table 1. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analyzed tablet powder sample and shown in Table 1. The percentage recovery value indicates non interference from excipients used in formulation.The result of analysis of marketed formulations is shown in Table 2.The reproducibility and accuracy of the method was found to be good, which was evidenced by low standard deviation.

4. CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. It can be concluded that the proposed methods are 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 in pharmaceutical formulations

5. ACKNOWLEDGEMENT

We would like thank to Cadila Pharmaceutical Ltd. Ahmedabad, India for providing reference sample of Clopidogrel to facilitate this work, and also we are grateful to the Principal, Bharathi College of Pharmacy, Bharathi Nagara, for providing necessary facilities to carry out this work.

6. REFERENCES

1. RxList.com The Internet Drug Index for prescription drugs, medications and pill identifier, Available from: http://www.rxlist.com/plavix-drug.htm.
2. Rajput SJ, George RK, Ruikar DB. Chemometric Simultaneous Estimation of C lopidogrel Bisulfate and Aspirin from Combined Dosage Form. Indian J Pharm Sci 2008; 70: 450- 454.
3. Anandakumar K, Ayyappan T, Raghu RV, Vetrichelvan T, Sankar ASK, Nagavalli D. RP-HPLC analysis of aspirin and clopidogrel bisulphate in combination. Indian J Pharm Sci 2007; 69: 597-599.
4. Patel RB, Shankar MB, Patel MR, Bhatt KK. Simultaneous Estimation of Acetylsalicylic Acid and Clopidogrel Bisulfate in Pure Powder and Tablet Formulations by HPLC and HPTLC. JAOAC International 2008; 91: 750-755.
5. Vocilkova L, Opatrilova R, Sramek V. Determination of Clopidogrel by Chromatography. Cur Pharm Anal 2009; 5: 424-431.
6. Pankaj K, Kachhadia AS, Doshi HS. Validated Column HPLC Method for Determination of Aspirin and Clopidogrel in Combined Tablets in the Presence of Degradation Products Formed Under ICH Recommended Stress Conditions. JAOAC Int 2009; 92: 152-157.
7. Himani A, Neeraj K, Paradkar AR, Mahadik KR. Stability indicating HPTLC determination of clopidogrel bisulphate as bulk drug and in pharmaceutical dosage form. J Pharm & Biomed Anal 2003; 61: 581- 589.
8. Shinha PK, Mr inalini CD, Bothra KG. A validated stability indicating HPTLC method for determination of Asprin and Clopidogrel Bisulfate in combined dosage form. Eur J Chem 2009; 4: 152-160.
9. Anti D, Filipi S, Agbaba D. A simple and sensitive TLC method for determination of clopidogrel and its impurity SR 26334 in pharmaceutical product. Acta chromatogr 2007; 18: 199 206.
10. Patel NK, Subbaiah G, Shah H, Kundlik, Shrivastav M, Pranav S. Rapid LC-ESI-MS-MS Method for the Simultaneous Determination of Clopidogrel and its Carboxylic Acid Metabolite in Human Plasma. J Chromatogr Sci 2008; 46: 867-875.
11. ICH, Q2A Text on Validation of Analytical Procedures; 1994.
12. ICH, Q2B Validation of Analytical Methodology; 1996.
13. ICH, Q2 (R1) Validation of Analytical Procedures: text and methodology; 2005.

*****