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RESEARCH ARTICLE

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ASSAY OF CLOPIDOGREL BY VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

Three simple and sensitive spectrophotometric methods have been developed for the estimation of Clopidogrel (CLP) in pure and pharmaceutical dosage forms. This method is based on the formation of ion-pair complexes of the drug with acidic dye Solochrome black T (SBT : λ_{max} 510 nm) , This method is based on Oxidation of the drug with Phenanthroline (Fe(III)/PTL: λ_{max} 515 nm) and This method is based on oxidation followed by complex formation when the drug reacts with potassium permanganate ($KMnO_4$ λ_{max} 410nm). These methods have been statistically evaluated and found to be precise and accurate.

KEY WORDS

Clopidogrel , Solochrome black T, Phenanthroline , potassium permanganate

INTRODUCTION

Clopidogrel (CLP) which is chemically methyl 2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetate is an inhibitor of ADP-induced platelet aggregation acting by direct inhibition of adenosine diphosphate (ADP) binding to its receptor and of the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa. A number of methods such as UPLC, LCMS were reported for the estimation of CLP. Literature survey reveals that spectrophotometric methods have not been reported for its quantitative determination in its pure form and pharmaceutical formulations. In the present investigation three simple and sensitive spectrophotometric methods have been developed for the determination of CLP. The developed methods involve the formation of colored chloroform extractable complexes with SBT, Fe(III)/PTL and $KMnO_4$. The complexes showed absorption maximum at 510nm ,515nm and 410nm respectively. Beers law is obeyed in the concentration ranges of 15-35µg/ml ,10-50µg/ml and 2.5-12.5 µg/ml respectively. The results of analysis for the three methods have been validated statistically and by recovery studies.

EXPERIMENTAL

Preparation of reagents:

1. Solochrome Black T Solution: 0.5 g of SBT dye was dissolved in 100 ml of distilled water.
2. PTL solution : prepared by dissolving 198 mg of 1,10 phenthroline in 100 ml 0.1 N HCL.
3. $FeCl_3$ solution : About 162 mg of anhydrous ferric chloride was accurately weighed & dissolved in 100 ml of distilled water. 33.3 ml of above stock solution was further diluted to 100 ml with water.
4. Potassium permanganate (0.05%w/v) : 25 mg in 50ml distilled water
5. Standard drug solution: About 100mg of Clopidogrel was accurately weighed and dissolved in 100 ml of water to obtain a stock solution of 1 mg/ml. This solution was further diluted with distilled water to get working standard solution of 100 µg/ml.

ASSAY PROCEDURES:

Method A: Aliquots of working standard solution of CLP ranging from 1.5-3.5 ml were transferred into a series of 125 ml separating funnels. To these 1 ml of of SBT dye were added. The total volume of aqueous phase was adjusted to 10 ml with distilled water and 10 ml of chloroform was added. The contents were shaken for 2 minutes. The two phases were allowed to separate and the absorbance of the Pink colored chromogen was measured at 510 nm against reagent blank and the amount of CLP present in the sample solution was computed from its calibration curve.

Method B: Aliquots of working standard solution of CLP ranging from 1-5 ml were transferred into a series of 10ml volumetric flasks. To this 1 ml of ferric chloride solution was added. Then 2 ml of PTL solution was added & volume in all volumetric flasks were equalized with water. The total contents were boiled for 30 min. The contents were cooled to room temperature and add 2ml of ortho phosphoric acid .The absorbance of the colored chromogen was measured at 515nm against reagent blank and the amount of CLP present in the sample solution was computed from its calibration curve.

Method C: Aliquots of working standard solution of CLP ranging from 0.5-2.5 ml were transferred into a series of 10ml volumetric

flasks. To this 1 ml of Potassium Permanganate solution was added and allowed to stand for 20 minutes. The total volume was made up to 10ml with water. The absorbance of the cherry red colored chromogen was measured at 410nm against reagent blank the amount of CLP present in the sample solution was computed from its calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as beers law limits, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation, percent range of error(0.05 and 0.01 confidence limits) were calculated for both the methods and results are summarized in Table 1. The values obtained for the determination of CLP in Pharmaceutical formulations (Tablets) by the proposed methods are presented in Table 2. Studies reveal that the common excipients and other additives usually present in the Tablets did not interference in the proposed methods.

Table-1

Optical characteristics, precision and accuracy of the proposed method

Parameters	Method A	Method B	Method C
λ_{max} (nm)	510	515	410
Beer's law limit(µg/mL)	15-25	10-50	2.5-12.5
Sandell'sensitivity(µg/cm^2/0.001abs.unit)	0.05 4	0.1227	0.0197
Molar absorptivity(litre.mole^{-1}.cm^{-1})	5.0781× 10^4	3.873× 10^3	21.274×10^3
Regression equation(Y*)			
Slope(b)	0. 1872	0.007	0.002
Intercept(a)	-0.006	0.009	0.011
Correlation coefficient(r)	0.9996	0.998	0.998
%Relative standard deviation**	0.69	0.66	1.56
%Range of error			
0.05 significance level	0.52	0.551	1.304
0.01 significance level	0.76	0.816	1.929

**Y = a + bx, where 'Y' is the absorbance and x is the concentration of Clopidogrel in µg/mL*

***For six replicates*

Table-2

Estimation of Clopidogrel in Pharmaceutical Formulations

Formulations (Tablets)	Labelled amount(mg)	Amount found* by proposed method			% recovery** by proposed method		
		Method A	Method B	Method C	Method A	Method B	Method C
Tablet 1	75	74.82	74.73	74.86	99.75	99.26	99.58
Tablet 2	75	74.05	74.89	74.61	99.84	99.78	99.84
Tablet 3	75	74.65	74.96	74.09	99.48	99.45	99.45

** Average of six determinations*

***Recovery of amount added to the pharmaceutical formulation (Average of three determinations)*

CONCLUSION

The proposed methods are applicable for the assay of drug CLP and have an advantage of wider range under Beers law limits. The proposed methods are simple, selective and reproducible and can be used in the routine determination of CLP in pure form and formulations with reasonable precision and accuracy.

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