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A HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF CLOPIDOGREL BISULPHATE IN TABLETS

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ABSTRACT
The present work describes validated Stability indicating High Performance Thin Layer Chromatographic (HPTLC) method for estimation of clopidogrel bisulphate in tablet. The method employed, TLC aluminum plates precoated with silica gel 60F-254 as a stationary phase and carbon tetrachloride:ethyl acetate:ammonia (5:0.3:0.2, v/v/v) as mobile phase. The wavelength selected for analysis was 230nm. Linerity was observed in concentration of 300-1500 ng. The percentage of labeled claim of clopidogrel was found 99.17 %. The recovery was 99.20±0.04 as per peak area. The proposed method was evaluated statistically in terms of accuracy, precision, specificity and reproducibility and can be used for routine analysis of Clopidogrel bisulphate in tablets.

Keywords: *Clopidogrel bisulphate; HPTLC.*

INTRODUCTION

Clopidogrel, methyl(+)-(S)-á-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-acetate is a new thienopyridine derivative chemically related to ticlopidine. It has been shown to prevent ischemic stroke, myocardial infarction and demonstrated clinically efficiency superior to that of aspirin. Clopidogrel inhibit ADP-induced platelet aggregation and is used therapeutically as antithrombotic agent.
In literature HPLC[1-3], gas chromatography[4]and spectroscopic[5,6] method are reported for its quantification in biological and pharmaceutical sample. In present work HPTLC method, for estimation of clopidogrel bisulphate in pharmaceutical dosage form is described.

EXPERIMENTAL

Chemical and Reagents

Clopidogrel (pure drug) was obtained as a gift sample form Zydus Cadila Pharmaceutical Ltd, Goa. Clopidogrel bisulphate tablet containing 75mg was purchased from local market. All chemicals, solvents and reagents used were of analytical grade.

Instruments

CAMAG-HPTLC system comprises CAMAG Linomat IV automatic sample applicator, CAMAG TLC scanner 3 with win CAT (1.3.0) software for interpretation of data and CAMAG Twin trough glass chamber for chromatograph development.

Methodology

Standard Solution - Standard stock solution was prepared by dissolving 75mg of CLPBS in 100ml of methanol and further diluted with methanol to get a final concentration of 75 mcg /ml of CLPBS.

Quantitation

Calibration curve was obtained by plotting peak area of CLPBS against the concentration over range of 300-1500ng/ml.

Chromatography

CAMAG-HPTLC system were used for quantitative evaluation. Chromatography was performed on aluminium plate precoated with silica gel 60F-254 TLC plate (20cmx10 cm,layer thickness 0.2mm) as a stationary phase and carbon tetrachloride : ethyl acetate : ammonia (5:0.3:0.2 v/v/v) as mobile phase. Samples were applied as 6mm long at 30mm interval under the stream of deuterium lamp. The plate were placed for saturation for 15 min. Chromatogram was evaluated by scanning in reflectance/absorbance mode at 230nm using slit dimension 6x0.45mm and quntitation was done using peak area.

Assay

Sample solution - Twenty tablets were weighed, crushed and ground to fine powder. An accurately weighed quantity of tablet powder equivalent to 75mg was transferred to 100ml volumetric flask containing 75ml of methanol, sonicated for 10 min and volume was adjusted to 100ml with methanol. Resulting solution was filtered using Whatmann filter paper 41.
A fixed volume of standard solution and sample solution were spotted as sharp bands on the plate. The plate was then developed in chamber saturated with mobile phase. After development the band were scanned at 230nm. On applying suitable dilution factor and

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comparing the peak area of standard and sample the amount of CLPBS per tablet was calculated. The representative densitogram of CLPBS obtained from tablet is shown in Fig. 1. The total amount of CLPBS determined and amount recovered in tablets dosage form by proposed method was found to be 99.42 %±0.42.

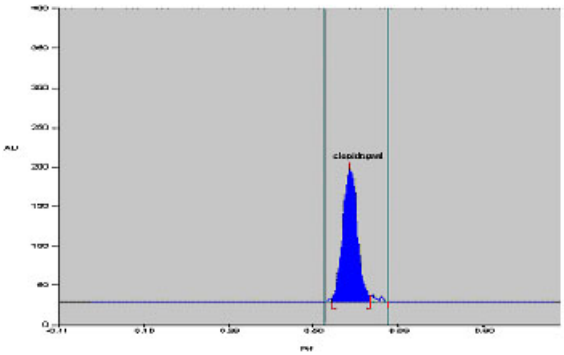

Fig.1: *Densitogram of standard CLPBS*

RESULT

Analytical method validation was performed for accuracy, precision, repeatability, specificity and robustness.

Accuracy

Accuracy of method was ascertained by performing recovery study using standard addition method to fix amount of preanalysed tablet powder (600ng). Pure drug was added at 50, 100 and 150% level respectively. The total amount of drug was determined by the above method and amount of pure drug recovered was calculated. The average % recovery was found to be 99.20%.

Precision

Precision of analytical method is expressed as S.D or %R.S.D of a series of measurements by replicate estimation of drug by proposed method (Table 1)

Table 1: *Results of Specificity study*

Sample	% of labeled claim CLPBS	Rf value of degraded product	Stability
Acid	97.46	0.48	Degraded
Base	76.49	0.02	Degraded
Oxide	105.14	0.65	Degraded
Dry Heat(100°c)	104.23	0.74	Degraded
Day light(25°C)	99.52	--	Stable

Ruggedness

It was studied by applying 900ng (n=5) for CLPBS by two different analysts keeping same experimental and environment condition. The %R.S.D was found to be 0.18 and 0.5 by two different analysts.

Robustness

Robustness of method was performed by applying 900ng of CLPBS by changing migration distance to 7, 7.5 and 8 cm respectively. R.S.D was found to be 0.6, 1, 1.8 at distance of 7, 7.5 and 8 cm respectively.

Stability indicating study

The Stability indicating ability of proposed method was investigated by deliberately degrading the sample preparation. The Stress condition applied were acidic (1N HCL), Alkaline medium (1N NaOH), Oxidizing condition (H_2O_2), Photochemical and dry heat degradation. The Rf value and % of degraded product under different stress condition is shown in Table 1.

CONCLUSION

From the above result it can be concluded that the proposed method is rapid, accurate, specific and sensitive. The low values of relative standard deviation indicate high precision of the method. The % recovery value obtained by standard addition method lies within standard limit, indicating the accuracy of the method. The proposed HPTLC method can be routinely employed for quality control analysis of CLPBS tablet.

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