Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):907-913

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Development and validation of stability indicating HPTLC method for determination of Prasugrel

T.C. Borole, R. Mehendre, M. C. Damle*, K.G. Bothara

Department of Pharmaceutical Chemistry, AISSMS College of Pharmacy, Pune, Maharashtra, India

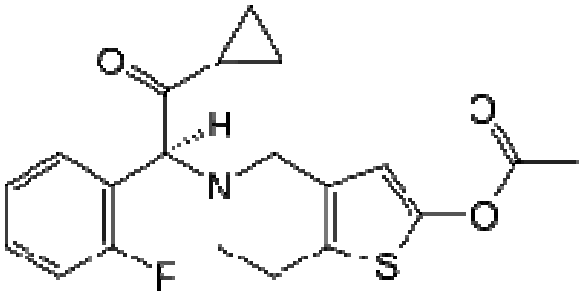
ABSTRACT

A sensitive, selective, precise and stability indicating (in accordance with ICH guidelines) High-Performance Thin Layer Chromatographic method of analysis for Prasugrel was developed, to resolve drug response from that of their degradation products. The method employed TLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of Dichloromethane: Methanol (9.9:0.1v/v). This system was found to give compact spot for Prasugrel (R_f value 0.58±0.03). Prasugrel was subjected to stress test conditions like acid, alkali, neutral hydrolysis, oxidation, dry heat and photo degradation. The spot for product of degradation was well resolved from the drug. Densitometric analysis of drug was carried out in the absorbance mode at 254 nm. The linear regression data for the calibration plots showed good linear relationship with r^2 was 0.995 in the concentration range of 300-1500ng/band. The result indicates that the drug was susceptible to degradation, to different extent in different conditions.

Keywords: Prasugrel, HPTLC, Stability indicating

INTRODUCTION

Prasugrel chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetra hydrothieno[3,2-*c*]pyridin-2-yl acetate. It is a member of the thienopyridine class of ADP receptor inhibitors, like ticlopidine and clopidogrel.These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to $P2Y_{12}$ receptors. Prasugrel inhibits adenosine diphosphate–induced platelet aggregation more rapidly, more consistently, and to a greater extent than do standard and higher doses of clopidogrel in healthy volunteers and in patients with coronary artery disease.



Literature survey revealed that some analytical methods like LC-MS[1,2] have been reported for estimation of Prasugrel butno HPTLC method was reported for its analysis. Hence the objective was to develop a simple, sensitive, accurate and precise method for determination of Prasaugrel.

To the best of our knowledge there is no report found for the stability indicating assay method as per ICH guidelines, for Prasugrel by HPTLC method. This paper describes a simple, accurate, sensitive, economic, simple and validated stability indicating HPTLC method for Prasugrel as the bulk drug.

The drug stability test guidelines[3] Q1A (R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assay should be stability indicating. The aim of the present study accordingly was to establish inherent stability of the Prasugrel through stress studies under a variety of ICH recommended test conditions.

EXPERIMENTAL SECTION

Materials

Prasugrel was provided as a gift sample by Alkem Laboratories, Mumbai. Drug was used without any further purification. All other reagent required for experimentationwas analytical reagent (AR) grade. Chemicals used for this experiment were Dichloromethane (AR grade), Methanol (AR grade), NaOH (AR grade), HCl (AR grade), and H_2O_2 (AR grade). These chemicals were purchased from SD-FINE Chemicals.

Instruments and chromatographic conditions

Chromatographic separation of drug was performed on TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) purchased from E. Merck, Germany. The sample was applied onto the plate as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 in the range of 200-400 nm and operated by win CATS software (V 1.4.3, Camag).

Selection of Detection Wavelength

After chromatographic development, bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that drug showed considerable absorbance at 254 nm. So, the wavelength of detection was 254nm selected.

Method development

Method development, for resolution of Prasugrel from its degradation product, was started with development of densitogram with neat solvents and combinations of Chloroform, Toluene,

Methanol, Acetone, and Carbon tetrachloride, Dimethyl Sulfoxide, Dichloromethane and Benzene. Finally Dichloromethane: Methanol (9.9:0.1 *v/v*) was selected as mobile phase with a good resolution at R_f 0.58.

Preparation of standard solution
Standard stock solution of Prasugrel was prepared by dissolving 25 mg of drug in 25 ml of methanolto get concentration of 1000µg/ml. The solution was then further diluted with methanol to get working standard solutions of 100µg/ml.From thissolution a quantity of 3,6,9,12 and 15 µL was applied on pre-coated silica gel G plate as aband of length 4mm at a distance of 10mm from both x-axisand y-axis. This plate was developed in developmentchamber using selected mobile phase.

Stress Degradation studies:
Degradation under acid catalyzed hydrolytic condition
1 ml of working standard solution of Prasugrel of conc. 1000µg/ml was mixed with 1 ml of 0.1 N HCl. The solution was diluted to 10 ml with methanol and kept for 4 hours. Appropriate volume of resultant solution (12µL) was applied on TLC plate and densitogram was developed.
Degradation under alkali catalysed hydrolytic condition
1 ml of working standard solution of Prasugrel of each conc. 1000 mcg/ml was mixed with 1ml of 0.01N NaOH. The solutions were diluted to 10 ml with methanol and keptit for 5min. Appropriate volume of resultant solution (12µL) was applied on TLC plate and densitogram was developed.

Degradation under neutral hydrolytic condition
5 ml of working standard solutions of Prasugrel of each conc. 1000 mcg/ml were mixed with 5 ml of water. The solutions were diluted to 50 ml with methanol and refluxed for 1 hour. Appropriate volume of resultant solution (12µL) was applied on TLC plate and densitogram was developed.

Degradation under oxidative condition
5 ml of working standard solution of Prasugrel of conc. 1000 mcg/ml was mixed with 5 ml of 30% H_2O_2. The solutions were diluted to 50 ml with methanol and refluxed for 1 hour. Appropriate volume of resultant solution (12µL) was applied on TLC plate and densitogram was developed.

Degradation under dry heat
Dry heat studies were performed by keeping drug samples in oven (100^0 C) for a period of 6 hours. Samples were withdrawn at appropriate time, dissolved in methanol and diluted to get 100mcg/ml as final concentration. Appropriate volume of resultant solution (12µL) was applied on TLC plate and densitogram was developed.

Photo-degradation studies
Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr in photostability chamber (Make : Newtronics). Sample was removed form chamber, weighed, dissolved and diluted get 100mcg/ml as final conc. appropriate volume of resultant solution (12µL) was applied on TLC plate and densitogram was developed.

Method Validation
The developed method was validated as per ICH guidelines[4]

Linearity and Range
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample, was studied by analyzing five concentrations of the drug, and process was repeated for five times each. It was done over the range of 300-1500ng/band.

Precision
Precision of the system was evaluated by analyzing six independent sample preparations obtained from homogenous sample and % RSD value obtained was calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day variation.

Accuracy
To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Mean percentage recovery was determined.

Limit of detection and limit of quantitation
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the Standard Deviation of the Y-intercept and the Slope, detection limit (DL) may be expressed as:

$$DL = \frac{3.3\,\sigma}{S}$$

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the Standard Deviation of the Response and the Slope, The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where,
σ = the standard deviation of the response for the lowest conc. in the range
S = the slope of the calibration curve.

Specificity
The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on TLC scanner 3 in the range of 200-400 nm using WinCats software version 1.4.3.

RESULTS AND DISCUSSION

Development of the optimum mobile phase
TLC procedure was optimized with a view to develop a stability-indicating assay method. The drug reference standard was spotted on the TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve Prasugrel from its degradation product. Best suited mobile phase were found to be Dichloromethane: Methanol in the ratio of

9.9:0.1(v/v). Developed mobile phase resulted in resolution for Prasugrel with R_f0.58 ± 0.03. Well-defined spots were obtained when the chamber was saturated with the mobile phase at room temperature. The representative densitogram is shown in Fig. 1.

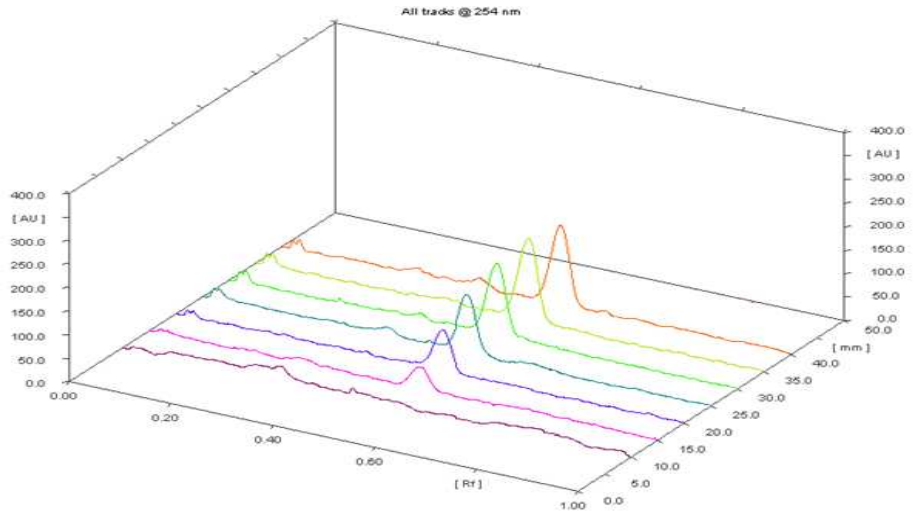


Fig.1 Densitogram of Prasugrel linearity

Validation of the developed stability-indicating method:

Linearity

The data obtained in the linearity experiments was subjected to linear-regression analysis. A linear relationship between peak areas and concentrations was obtained in the range of 300-1500ng /band with r^2 0.995.

Precision

The developed method was found to be precise as the % RSD value for repeatability studies was less than 1%, where as the %RSD for inter-day precision was higher than that of repeatability study.

Accuracy

Excellent recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80 %, 100 %, 120 % level) indicated the mean recovery between 98% to 102% for Prasugrel.

Limit of Detection

The limit of detection as calculated by standard formula as given in ICH guidelines was found to be 286.75ng/band for Prasugrel.

Limit of Quantitation

The limit of Quantitation as calculated by standard formula as given in ICH guidelines was found to be 869ng/band for Prasugrel.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found within limit, indicating the non interference of any other peak of degradation product, impurity or matrix.

Degradation pattern observed:

Under alkaline condition 45.84% degradation of Prasugrel was observed and 1 peak for degradation product was observed at R_f = 0.34(Fig. 2). Spectra of degradation product, differs from that of Prasugrel spectra(Fig. 3).

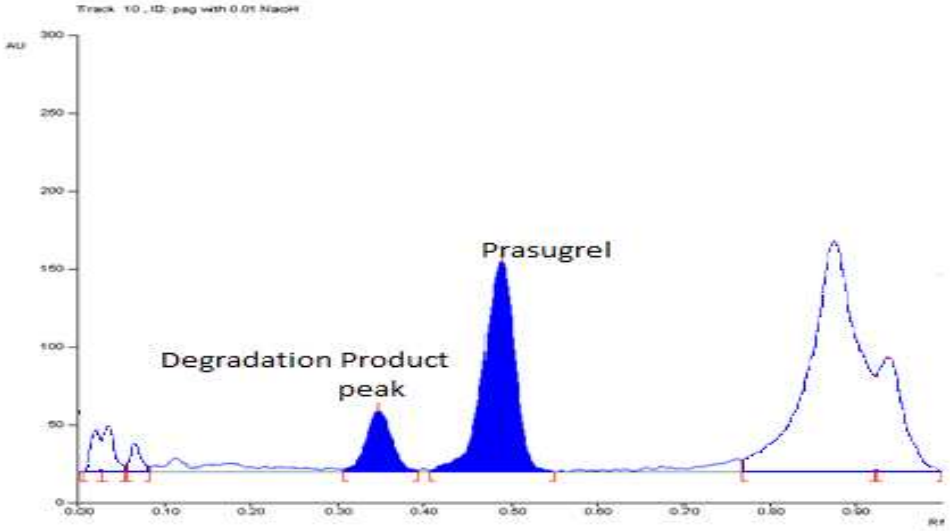


Fig.2: Densitogram of Prasugrel after alkaline hydrolysis

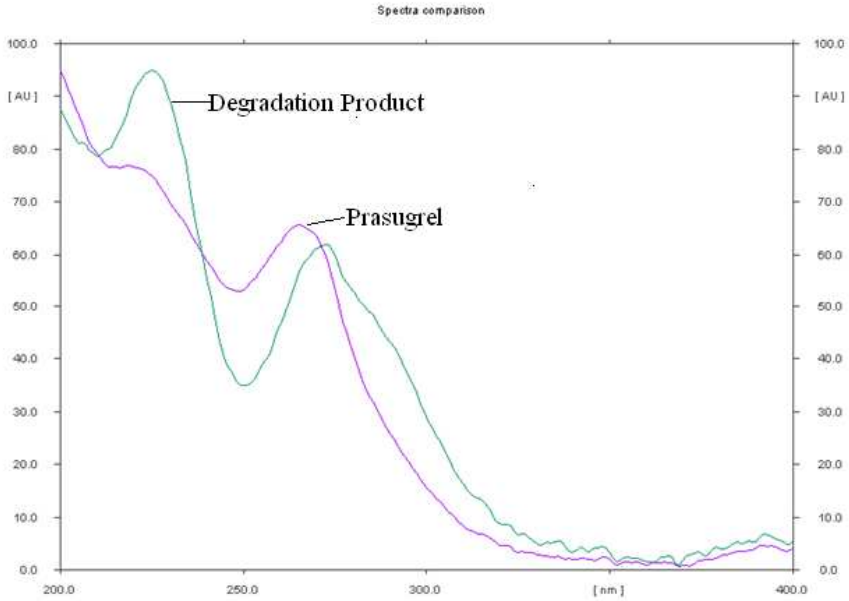


Fig. 3: Overlain UV spectra of Prasugrel and hydrolytic degradation product

Under the acidic condition, 62.43% degradation was found. There is no peak for degradation product. However, the area for Prasugrel peak was reduced.

In Neutral hydrolysis Prasugrel was not degraded. This indicates stability of Prasugrel to Neutral hydrolysis.

In oxidative study, 71.56% degradation was observed. There is no peak for degradation product

After the dry heat treatment 50% degradation of Prasugrel occurred. No peaks of degradation were obtained. However, the area for Prasugrel peak was reduced.

Degradation of Prasugrel was 22.60% upon exposure to UV light and 23.48% upon exposure to Fluorescent light (after UV light exposure)in photo stability chamber.

CONCLUSION

Thus Prasugrel appears to be prone to hydrolytic, thermal and oxidative degradation and should be protected accordingly. This developed HPTLC method can be used for stability monitoring of Prasugrel.

Acknowledgement
The authors are thankful to AlkemLaboratories,Mumbaifor providing a working standard of Prasugrel. The authors are also thankful to the Principal, AISSMS College of pharmacy for providing necessary facilities and constant encouragement.

REFERENCES

[1] ER Tian; KJ Ruterbories; EM Verburg; GJ Weerakkody; FN Kurihara.*American Society for Pharmacology and Experimental Therapeutics*,**2007**,35(6), 917-921.
[2] NA Farid; M McIntosh; F Garofolo; E Wong; A Shwajch; M Kennedy; M Young; P Sarkar; K Kawabata; M Takahashi; H Pang. *Rapid Communications in Mass Spectrometry*,**2007**, 21(2), 169-179.
[3] ICH, Q1A (R2), Stability Testing of New Drug Substances and Products, Geneva, Feb.**2003**.
[4] ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology International conference on Harmonization, Geneva, **2005**, 1-13.