Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

DEVELOPMENT OF RP-HPLC METHOD AND ITS VALIDATION FOR ESTIMATION OF PRASUGREL HYDROCHLORIDE

M. Alagar Raja*[1], Lingaswamy Vuyyala[1], David Banji[1], K.N.V. Rao[1], D. Selva Kumar[2]

[1*]Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Cherlapalli, Nalgonda, Andhra Pradesh, India-508001.

[2]School of Pharmacy, Taylors University, Subanjaya, Malaysia.

ABSTRACT

A simple, rapid and reproducible RP-HPLC method was developed and validated for the estimation of Prasugrel Hydrochloride in tablet dosage form. A Zorbax, SB-Phenyl 250x4.6mm, 5µ in isocratic mode, with mobile phase containing Buffer (pH 6.5 with Triethylamine and Potassium dihydrogen ortho phosphate): Acetonitrile: Water (90:10) [40:60 (v/v)] was used. The flow rate was 1.2 ml/min and the analyte was monitored at 235 nm. The retention time for Prasugrel HCl was 11.5 mins. Linearity was obtained in the concentration range of 50ppm to 150ppm with correlation coefficient of 0.999. The percentage recovery of Prasugrel was found to be in the range of 98%-102%.

KEYWORDS

Prasugrel, RP-HPLC, Isocratic, Actonitrile and Zorbax.

Author for Correspondence:

Alagar Raja. M,
Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Cherlapalli, Nalgonda, Andhra Pradesh, India.

Email: madurairaja@hotmail.com

INTRODUCTION

Prasugrel Hydrochloride chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]- 4,5,6,7-tetrahydrothieno [3,2- c] pyridin-2-ylacetate hydrochloride[1]. (Figure No.1). It is a member of the thienopyridine class of ADP receptor inhibitors. These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to P2Y12 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[2,3]. Literature review

revealed that few analytical methods have been reported like UV and HPTLC, HPLC for its analysis of pure drug[5-10]. The purpose of this study was to develop simple, rapid, precise, specific and accurate RP - HPLC method for the estimation of the drug in pure and in pharmaceutical dosage forms. The method was validated by evaluation of the linearity, presion, accuracy and as per ICH guidelines[4].

MATERIAL AND METHODS

Apparatus

A Zorbax, SB-Pheny l250x4.6mm, 5µ in isocratic mode, with mobile phase containing Buffer (P^H 6.5 with Triethylamine and Potassium dihydrogen ortho phosphate) Acetonitrile: Water (90:10) [40:60 (v/v)] was used.

Reagent and Materials

A Zorbax, SB-Phenyl 250x4.6mm, 5µ in isocratic mode, with mobile phase containing Buffer (P^H 6.5 with Triethylamine and Potassium dihydrogen ortho phosphate) Acetonitrile: Water (90:10) [40:60 (v/v)] was used.

Preparation Standard Stock Solution

Accurately Weighed and transferred Prasugrel HCl equivalent to 10 mg of Prasugrel Working Standard into a 100 ml clean dry volumetric flask, and 60 ml of diluent was added, sonicated for 10 minutes, and diluted to volume with diluent.

Chromatographic conditions

A Zorbax, SB- Phenyl (250 x 4.6mm; 5µm) was the column used for separation. Mobile phase consisting of a mixture of Triethylamine: buffer (1.36g potassium dihydrogen phosphate in 1000 ml water, pH-6.5 adjusted with Ophosphoric acid) in the ratio of 40:60 v/v delivered in isocratic mode at a flow rate of 1.2 ml/min quantified at 235 nm. The mobile phase was filtered through a 0.45 nylon filter and sonicated for 25 min.

Method development

Buffer (1.36g Potassium Dihydrogen Phosphate in 10000 ml water, pH-6.5 adjusted with O-phosphoric acid) and triethylamine in different proportions were tried and finally buffer (1.36g Potassium Dihydrogen Phosphate in 1000 ml water, pH-6.5 adjusted with O-phosphoric acid) and methanol (40:60v/v) was selected as an appropriate mobile phase which gave good resolution.

Procedures

Sample preparation

Weighed and powdered 20 tablets. Transferred the powder equivalent to 10 mg of Prasugrel into 100 ml of clean, dry, volumetric flask and, to this added 60 ml of diluent and sonicated for about 10 minutes, further made up the volume with diluent and then filtered through 0.45 micron filter and use the filtrate after discarding the first 5 ml.

Standard solution for Prasugrel

Accurately Weighed and transferred Prasugrel HCl equivalent to 10 mg of Prasugrel Working Standard into a 100 ml clean dry volumetric flask, and 60 ml of diluent was added, sonicated for 10 minutes, and diluted to volume with diluents.

Calibration curve

Accurately measured volume of working standard solution of Prasugrel Hydrochloride was transferred into a series of 100ml volumetric flasks and diluted appropriately with mobile phase. 20µl of each solution was injected under operating chromatographic conditions. Calibration curves were obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range of 10µg/ml to 50µg/ml.

Method Validation

Precision

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (% RSD) for response factor was calculated. The intraday % RSD of Prasugrel hydrochloride was found to be 0.12. In the intraday variation studies, injections of standard and sample solutions were made on three consecutive days and % RSD was calculated. The interday % RSD for Prasugrel hydrochloride was found to be 99.3. From the data obtained the developed RP-HPLC method was found to be precise.

Repeatability

Repeatability was obtained by injecting 6 injections of 100µg/ml and % RSD was found to be 0.41.

Accuracy (Recovery studies)

The accuracy of the developed analytical method was determined by recovery experiments. The recovery studies were checked at three different concentration levels (98 and 102%). The analyzed samples yielded high recovery values from the proposeded method. The % recovery results of the method are given in below Table No.1.

Linearity

The method was linear in the range of 10µg/ml to 100µg/ml for Prasugrel Hydrochloride. Linear Regression data were calculated.

RESULTS AND DISCUSSION

A simple, rapid and reproducible RP-HPLC method was developed and validated for the estimation of Prasugrel Hydrochloride in tablet dosage form. A Zorbax, SB-Phenyl 250x4.6mm, 5µ in isocratic mode, with mobile phase containing Buffer (pH 6.5 with Triethylamine and Potassium dihydrogen ortho phosphate) Acetonitrile: Water (90:10) [40:60 (v/v)] was used. The flow rate was 1.2 ml/min and the analyte was monitored at 235 nm. The retention time for Prasugrel HCl was 11.5 mins. Linearity was obtained in the concentration range of 50ppm to 150ppm with correlation coefficient of 0.999. The percentage recovery of Prasugrel was found to be in the range of 98%-102%.

Table No.1: Result data of Recovery Studies

S.No	Sample No	Spike level	µg/mL Added	µg/mL found	% Recovery
1	1	50%	50.0	50.8	101.3
2	2	50%	50.0	50.6	---
3	3	50%	50.0	50.5	---
4	1	75%	78.0	79.1	101.4
5	2	75%	78.0	79.0	---
6	3	75%	78.0	79.1	
7	1	100%	99.5	99.5	99.9
8	2	100%	99.5	99.5	
9	3	100%	99.5	99.4	
10	1	125%	125.0	123.9	100.2
11	2	125%	125.0	126.1	
12	3	125%	125.0	125.9	
13	1	150%	158.2	159.8	101.4
14	2	150%	158.2	160.9	
15	3	150%	158.2	160.5	
Acceptance Criteria		The accuracy (recovery) for the average of triplicate at each concentration level should be within 98.0% to 102.0%.			

Table No.2: Result data of Validation Parameters

S.No	Parameter	Acceptance criteria	Observation
1	System suitability	a) % RSD of Peak Area should be NMT 2 b) Theoretical Plates should be NLT 2500 c) USP Tailing should be NMT 2	0.07 4061 1.52
2	Linearity	NLT 0.999	0.999
3	Precision	a) RSD of % Assay results should be NMT 2.0 b) The Assay of Prasugrel Hydrochloride tablets should be within 98%-102%	0.12 99.3
4	Accuracy	Mean Recovery of Prasugrel at each spike level should be within 98%-102%	99.03 % -101.30% w/w

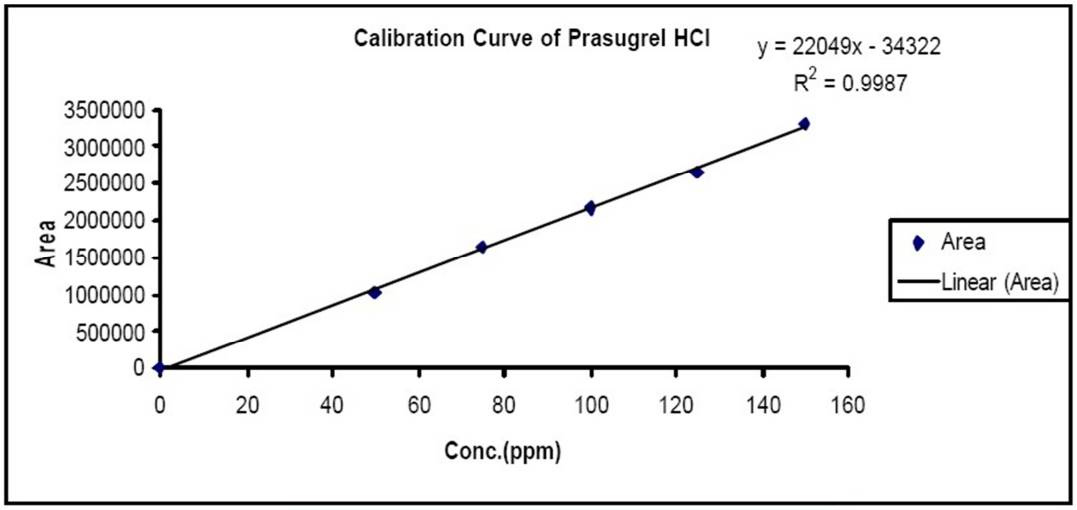

Figure No.1: Calibration Curve of Pasurgel HCL

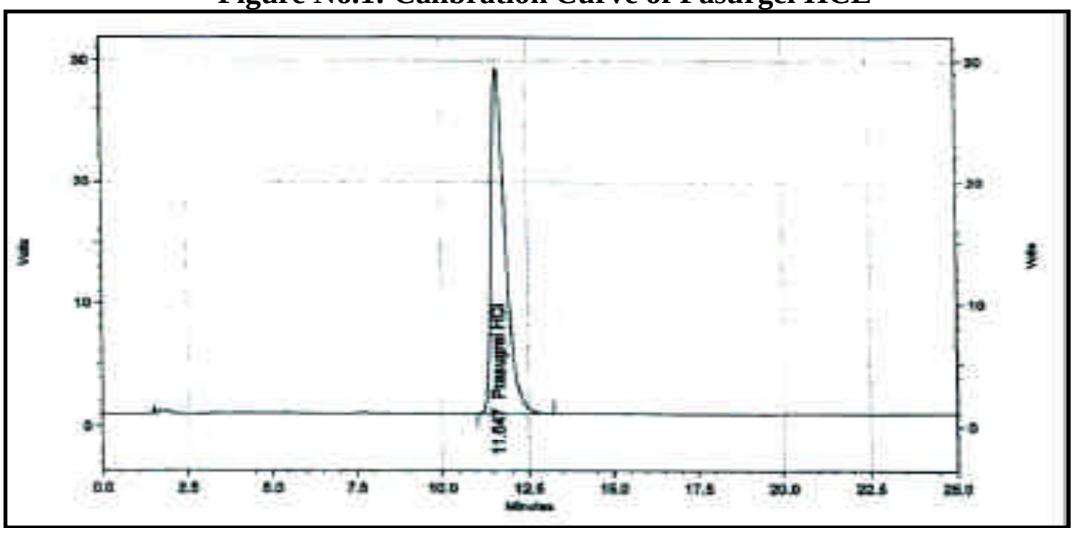

Figure No.2: Chromatogram of Standard

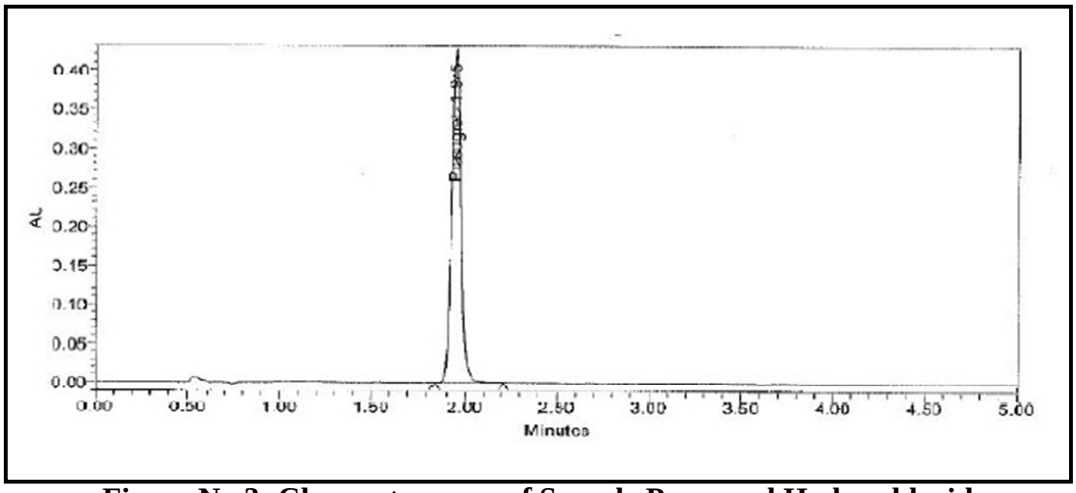

Figure No.3: Chromatograms of Sample Prasugrel Hydro chloride

CONCLUSION

Literature review revealed that no RP-HPLC method is yet reported for the estimation of Prasugrel Hydrochloride in tablet dosage form. Therefore, it was thought worthwhile to develop simple, precise, accurate RP-HPLC method for estimation of Prasugrel Hydrochloride in tablet dosage form.

ACKNOWLEDGEMENT

This work was supported by Nalanda College of Pharmacy, Charllapalli, and Nalgonda, for their continuous support and encouragement and for providing the necessary facilities.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Beckett H, Stenlake J B. Practical Pharmaceutical Chemistry, *C.B.S.Publications,* Part I, 4th Edn, 448-465.
2. Williard H H, Merit L L, Dean F A and Settle F A. Instrumental methods of analysis, *C.B.S. Publishers, New Delhi*, 7th Edn, 2002, 1036-1058.
3. Srikanth I, Sharmila P, Vijaya bharathi K, Raju M, Lakshma M, Nagarjuna K A. Validated Reverse Phase HPLC Method for the Estimation of Prasugrel Hydrochloride in Pharmaceutical Dosage Forms, *J Inno trends Pharma Sci*, 2(5), 2011, 140-148.
4. Usha Rani G, Chandrasekhar B, Devanna N. Analytical Method Development and Validation of Prasugrel in Bulk and its Pharmaceutical Formulation using HPLC, *J pp Pharma Sci*, 01(07), 2011, 172-175.
5. Borole T C, Mehendre R, Damle M C, Bothara K G. Development and Validation of Stability indicating HPTLC Method for Determination of Prasugrel, *J Che, Pharma Res*, 2(4), 2010, 907-913.
6. Kishore R, Venkateswara R A, Lavanya P, PaniKumar A D, Rama Krishna, Subba Reddy P V. Development of Validated RP-HPLC Method for the Estimation of Prasugrel HCl in Pure and Pharmaceutical Formulations, *Pharmacy Res*, 4(9), 2011, 3105-3110.
7. Pulla R P, Sastry B S, Prasad Y R, Raju N A. Estimation of Prasugrel in Tablet Dosage Form by RP-HPLC, *Int J Chem Res*, 2(3), 2011, 34-36.

Please cite this article in press as: Alager raja M *et al.* Development of RP-HPLC method and its validation for estimation of Prasugrel Hydrochloride, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 3(3), 2015, 90 - 94.