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Research Article

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Development and Validation of RP-HPLC Method for Prasugrel

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ABSTRACT

A reverse-phase liquid chromatographic (RP-LC) method was developed for the assay of Prasugrel in bulk. The Chromatography was performed on Kromasil C18 column. The eluted compounds were monitored by UV detection at 257nm using mobile phase methanol-potassium dihydrogen orthophosphate (pH 2.2; 10mM) (70:30, v/v). The method was statistically validated for linearity, accuracy, precision and repeatability. The linearity of prasugrel was demonstrated in concentration range 15-75µg/mL. The limit of detection and quantitation were 10 and 50ng/mL. The method developed was precise, accurate and specific for estimation of prasugrel in bulk.

INTRODUCTION

The development and validation of a method for drug substance is a process of finding out the condition that can separate the main peak response from the response obtained due to other components, and assuring that the results obtained from the developed method meets the predetermined specification.

Prasugrel is a platelet inhibitor of the thienopyridine class of ADP receptor inhibitor. It is chemically described as *(RS)* -5 - [2 - cyclopropyl - 1 - (2 - fluorophenyl) - 2 - oxoethyl] - 4, 5, 6, 7-tetrahydrothieno [3, 2 - *c*]pyridin – 2 - yl acetate. Prasugrel is a prodrug which is rapidly metabolized to active and inactive metabolites [5].

Literature survey revealed that few methods are reported for determination of prasugrel in human plasma using LC-MS/MS [3]. HPTLC [2] and UV-spectrophotometric [1] methods are also been reported for determination of prasugrel, but no HPLC method is been reported for determination of prasugrel.

EXPERIMENTAL SECTION

Chemical and Reagents

HPLC grade Acetonitrile and Methanol was obtained for RFCL Limited (New Delhi, India), Potassium dihydrogen orthophosphate and Ortho-phosphoric acid was procured for Qualigens fine chemicals (Mumbai, India). HPLC grade water obtained from Millipore Water Purification System (Molsheim, France) was used throughout the study.

Instrumentation

To determine the wavelength at which the compound shows maximum absorbance, it was scanned in the UV range of 200-400nm on a UV-visible spectrophotometer of Perkin Elmer (Shelton, CT, USA) **(Fig. 2. UV scan of Prasugrel)**

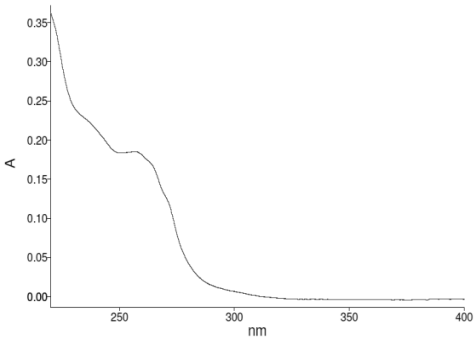

Fig. 2. UV scan of Prasugrel

A High-Performance Liquid Chromatography (HPLC) system from Perkin Elmer (Shelton, CT, USA) was used for analysis, which consisted of online degasser, sample injector (Rheodyne sample loop 20µL), UV – visible detector (series 200), pump (reciprocating, series 200), computer system loaded with Total Chrome Navigator (version 6.3.1). Other equipments used were pH meter, Weighing Balance and Sonicator.

Chromatographic Conditions

All the analyses were performed on isocratic mode. Chromatography was carried out using Kromasil C18 column (250 X 4.6 mm i.d, 5µ). The mobile phase consisted of Methanol and Buffer (pH 2.2) in a ratio of 70:30 (v/v), degassed by use of an ultrasonicator and filtered through 0.45µ nylon filter. Chromatography was performed at room temperature at a flow rate of 1.0mL/min. The UV detection was made at 257nm.

Preparation of Solutions

A stock solutions of Prasugrel (1000µg/mL) was prepared in methanol and diluted further with diluent (MeOH: Buffer, 1:9) to obtain a standard solution of 50µg/mL.

Method Validation

The method was validated according to the ICH guideline for the validation of analytical procedures. The parameters validated were linearity, accuracy, precision, detection limits, quantitation limits and repeatability.

Table No. 1: Optimization of Chromatographic Conditions

Mobile phase composition				Sample Preparation	RT	Remark
Acetonitrile	Buffer pH 2.67	70	30	Methanol	3.04	Peak merged with blank
Acetonitrile	Buffer pH 2.67	50	50	Methanol	3.53	Peak merged
Acetonitrile	Buffer pH 2.67	50	50	Buffer pH 2.67	3.30	Peak merged
Acetonitrile	Buffer pH 2.67	30	70	Buffer pH 2.67	9.88	Tailing observed
Acetonitrile	Buffer pH 2.35	40	60	Methanol	4.36	Tailing observed
Methanol	Buffer pH 2.35	40	60	Methanol	11.4	Tailing observed
Methanol	Buffer pH 2.35	50	50	Methanol: Buffer 1:9	5.45	Slight tailing
Methanol	Buffer pH 2.2	70	30	Methanol: Buffer 1:9	5.35	Peak shape good

RESULTS AND DISCUSSION

Optimization of Chromatographic procedure

The chromatographic conditions were optimized so as to obtain a good peak shape. Detection was performed at 257 nm, the λ_{max} of Prasugrel.

There has been not single LC method reported for Prasugrel. However an LC-tandem MS method has been reported by Farid et al. [3] for the determination of active and inactive metabolites of Prasugrel in human plasma. An UV spectrophotometric method is also been reported by Ashok Kumar et al. [1] employed 0.1N HCl as a diluent and result were observed at 249 nm. A HPTLC stability indicating method was developed by Damle et al. [2], it was observed that drug showed degradation to different extent in different conditions.

As an intial guide for the selection mobile phase, a standard solution of Prasugrel was injected in the system and Retention time (Rt) was observed using mobile phase containing various ratios MeOH, ACN and KH_2PO_4 buffer (pH 2.67, 2.35 and 2.2). The results are shown in **Table no. 1** [7]. The chromatographic conditions were finalized taking into consideration the peak shape and retention time of the Prasugrel. A representative chromatogram of Prasugrel is shown in **Fig No. 3.**

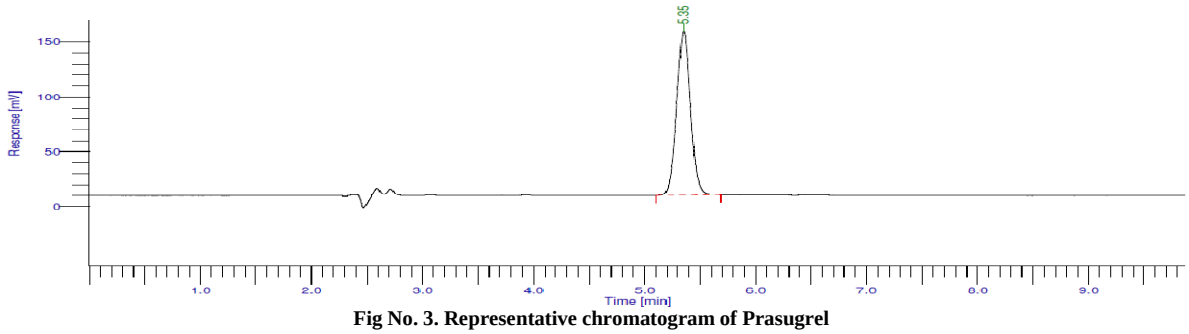

Fig No. 3. Representative chromatogram of Prasugrel

Validation of Assay Method[4]

Linearity

The calibration curve of Prasugrel was linear over the concentration range of 15.0 to 75.0µg/mL. Thus Method is more sensitive to that of already developed method by K. Vanitha Prakash *et al* [6]. The equation of the standard curve based on the ratio of the peak area of prasugrel to the prasugrel concentration was $y = 23964x + 7418$, $r^2 = 0.996$.

Repeatability

The study was carried out by injecting 6 injection of 50µg/mL standard solution and results were calculated in terms of RSD. The % RSD for repeatability should be not more than 2.

Table 2: Repeatability of the Method

Sr. No.	Area
1	1234026.35
2	1238750.47
3	1227268.84
4	1234964.30
5	1201398.57
6	1211666.76
Mean	1224679.22
SD	14894.10
%RSD	1.22

Precision

The intraday precision was evaluated by analysis of three different standard concentrations of 25.0, 50.0 and 75.0 µg/mL on the same day. The interday precision was calculated by carrying out the analysis on different day. The results are shown in Table 3. The precision in indicated in terms of RSD.

Table 3. Precision of the Method

Concentration (µg/mL)	Relative Standard Deviation (%)	
	Intraday [a)]	Inter day [b)]
25	0.20	1.04
50	1.13	0.93
75	0.53	1.05

a) Analyzed of same day (n =3)

b) Analyzed of different day (n=3)

Accuracy

The Accuracy for prasugrel was measured by testing recovery on three different preparation of prasugrel in the concentration level 80, 100 and 120% of the prasugrel. The accuracy was determined by comparing the found concentration with added concentration. The results are shown in Table 4. The obtained results show that the proposed method is accurate.

Table 4. Accuracy of the Method

Level (%)	Amount Added (ppm)	Amount Found (ppm) [a)]	% Recovery [b)]
80	40	40.04	100.10
100	50	50.42	100.85
120	60	59.83	99.72

a) Mean (n=3)

b) (Found Concentration/Added Concentration) x 100

Detection Limit and Quantitation Limits

Detection limit and Quantitation limit were also determined. Detection Limit was 10 ng/mL and Quantitation Limit was 50ng/mL.

CONCLUSION

The RP-LC assay method developed for Prasugrel is precise, accurate and specific. The method can be used for determination of prasugrel in bulk and in pharmaceutical formulation.

REFERENCES

[1] AA Kumar, A Kumar, D Gowri Sankar, *Pharmanest- Int. J. Adv. Pharm. Sci*. **2011**, 2(1), 37-39.
[2] TC Borole, R Mehendre, MC Damle, KG Bothara, *J. Chem. Pharm. Res*. ,**2010**, 2(4), 907-913.
[3] NA Farid, M McIntosh M, F Garofolo, E Wong, A Shwajch, M Kennedy, M Young, P Sarkar, K Kawabata, M Takahashi, H Pang, *Rapid Communication in Mass Spectrophotometry*, **2007**, 21(2), 169-179.
[4] International Conference on Harmonization, ICH Q2B, Validation of Analytical Procedures, Methodology, **1996**.
[5] Wishart, D.,Knox, C. ,Law , V., *et al.*, 'DrugBank 3.0: a comprehensive resource for 'omics' research on drugs' [Online], Available : http://www.drugbank.ca/drugs/DB06209 [Accessed: 28 April **2011**].
[6] BM Ishaq, KV Prakash, GK Mohan, *J. Chem. Pharm. Res*., **2011**, 3(4):404-409.
[7] CK K and B Srivastava, *J. Chem. Pharm. Res.*, **2010**, 2(2): 519-545.