WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 1057-1065 Research Article ISSN 2278 – 4357

A VALIDATED RP-HPLC METHOD FOR PRASUGREL HYDROCHLORIDE

D. Pavan Kumar*[1,2], T. Naga Jhansi[1], G. Srinivasa Rao[2] and Kirti Kumar Jain[1]

[1]Dr. Reddy's Laboratories Ltd., Hyderabad 500 072, A.P., India.

[2]GITAM Institute of Science, Gitam University, Beach Rd, Rushi Konda, Visakhapatnam, 530045, A.P., India.

Article Received on
15 Dec. 2018,

Revised on 06 Jan. 2018,
Accepted on 18 Jan. 2019

DOI: 10.20959/wjpps20192-13128

***Corresponding Author**
Dr. D. Pavan Kumar
Dr. Reddy's Laboratories Ltd., Hyderabad 500 072, A.P., India.

ABSTRACT

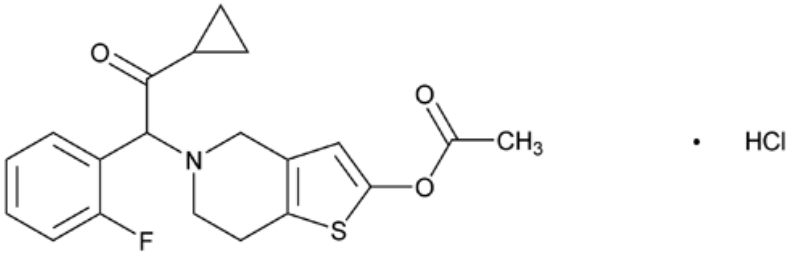
Prasugrel hydrochloride, (2S)-4-Oxo-[3-(trifluromethyl)-5,6-dihydro[1,2,4]triazolo [4,3-α] pyrazine-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butane-2-amine is a platelet inhibitor and an irreversible antagonist of P2Y12 ADP receptors and is used for the treatment of heart patients to prevents from forming harmful blood clots. Several bio-analytical and analytical methods have been reported for the analysis of Prasugrel hydrochloride. Recently stability indicated related substance by HPLC method was published in European Pharmacopoeia and United States Pharmacopeia, with six potential impurities.[1-2] Three of the in-house process impurities were not separated by pharmacopeia method. So, comprehensive study was taken to develop a single method for all these impurities and it should be stability indicating method. In this newly developed HPLC method included three in-house impurities other than the listed pharmacopeia impurities. This method is accurate, linear, specific, reproducible and stability indicating HPLC method with good Limit of Quantification and Limit of Detection of Prasugrel hydrochloride and its related impurities.

The chromatographic separation was achieved on YMC Pack Pro C18, 150 mm x 4.6 mm, 3µ particle size column. Using 25mM Potassium di hydrogen phosphate with 0.025% ortho phosphoric acid in water as mobile phase A and Mixture of Acetonitrile and water in the ratio of 80: 20 v/v as mobile phase B. Flow rate is 1.2mL/min in gradient mode. The column temperature was maintained at 45°C. Detection wavelength was set at 210 nm and the injection volume was 10 µL. Acetonitrile and water in the ratio 70:30(v/v) was used as a

diluent. The developed RP-HPLC method was validated according to ICH guidelines.[3] In this method the LOD and LOQ values for Prasugrel hydrochloride and all its related impurities were ranged from 0.2µg/mL to 0.8µg/mL. The percentage recovery for all impurities was ranged from 96% to 107 % w/w. The test solution and mobile phase were observed to be stable up to 48h after preparation. The validated method produced good results of precision, linearity, accuracy, robustness and ruggedness. The proposed method was found to be suitable precise, sensitive and accurate for the quantitative determination of related impurities in the bulk samples of Prasugrel hydrochloride API.

KEYWORDS: Prasugrel hydrochloride, Impurities, HPLC, UV Detector and Validation.

INTRODUCTION



Chemical Name: (2S)-4-Oxo-[3-(trifluromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α] pyrazine-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butane-2-amine.

Molecular formula: C20H21ClFNO3S

Prasugrel is a drug used to prevent formation of blood clots. It was developed by Daiichi Sankyo Co. and produced by Ube. Prasugrel was approved for use in Europe in February 2009 and in the US in July 2009.It is a platelet inhibitor and an irreversible antagonist of P2Y12 ADP receptors. In studies, prasugrel was more effective than the related clopidogrel but also caused more bleeding. Prasugrel is used with aspirin by patients with heart disease (recent heart attack, unstable angina) who undergo a certain heart procedure (angioplasty). This medication helps to prevent other serious heart/blood vessel problems (such as heart attacks, strokes, blood clots in stents). It works by blocking platelets from sticking together and prevents them from forming harmful blood clots. This "anti-platelet" effect helps to keep blood flowing smoothly in your body.[4,5]

MATERIALS AND METHODS

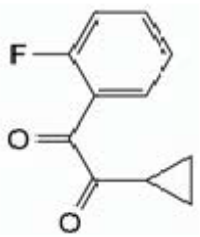
Samples and reagents

The development samples of Prasugrel hydrochloride and all impurities (Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E, Impurity-F, Impurity-G, Impurity-H and Impurity-I) were obtained from synthetic R&D laboratory of Dr. Reddy's Laboratories Ltd., CTO-VI, and Srikakulam, India. Reagents used for analysis, i.e., Potassium di hydrogen phosphate, Ortho phosphoric Acid (AR grade) and acetonitrile (HPLC grade) were obtained from Merck (India) Limited. Milli-Q grade water was used. The purity of all impurities were>95%.[6] Details of purity are as follows.

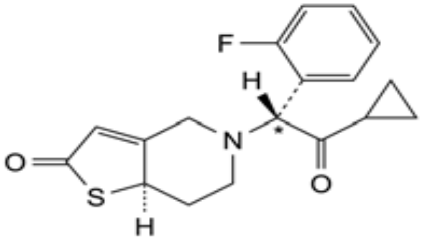
Table-1: Impurity details.

Name of impurity	Other name	Chemical name of the impurity	Purity (%)
Impurity-A	Prasugrel diketone	1-Cyclopropyl-2-(2-fluorophenyl) ethane-1,2-dione.	97.5
Impurity-B	Des fluoro prasugrel	5-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one	93.8
Impurity-C	Methyl Prasugrel	5-[(1RS)-2-Methyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7- tetrahydrothieno[3,2-c]pyridin-2-yl acetate	94.2
Impurity-D	4-Fluoro prasugrel	5-[2-Cyclopropyl-1-(4-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate	96.4
Impurity-E	Des acetyl hydroxy prasugrel	5-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-7a-hydroxy-5,6,7,7a-tetra hydrothieno[3,2-c]pyridin-2(4H)-one.	95.9
Prasugrel	Prasugrel	(2S)-4-Oxo-[3-(trifluromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α] pyrazine-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butane-2-amine	99.9
Impurity-F	3-Fluoro prasugrel	5-[2-Cyclopropyl-1-(3-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate.	96.6
Impurity-G	Desacetyl prasugrel	5-(2-Cyclopropyl-2-oxo-1-phenylethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate.	95.7
Impurity-H	Propionyl prasugrel	5-[(1RS)-2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7- tetrahydrothieno[3,2-c]pyridin-2-yl propionate	97.5
Impurity-I	Prasugrel chloro butyryl analogue	5-[5-Chloro-1-(2-fluorophenyl)-2-oxopentyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate.	95.2

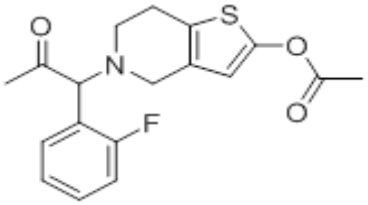
Impurity-A

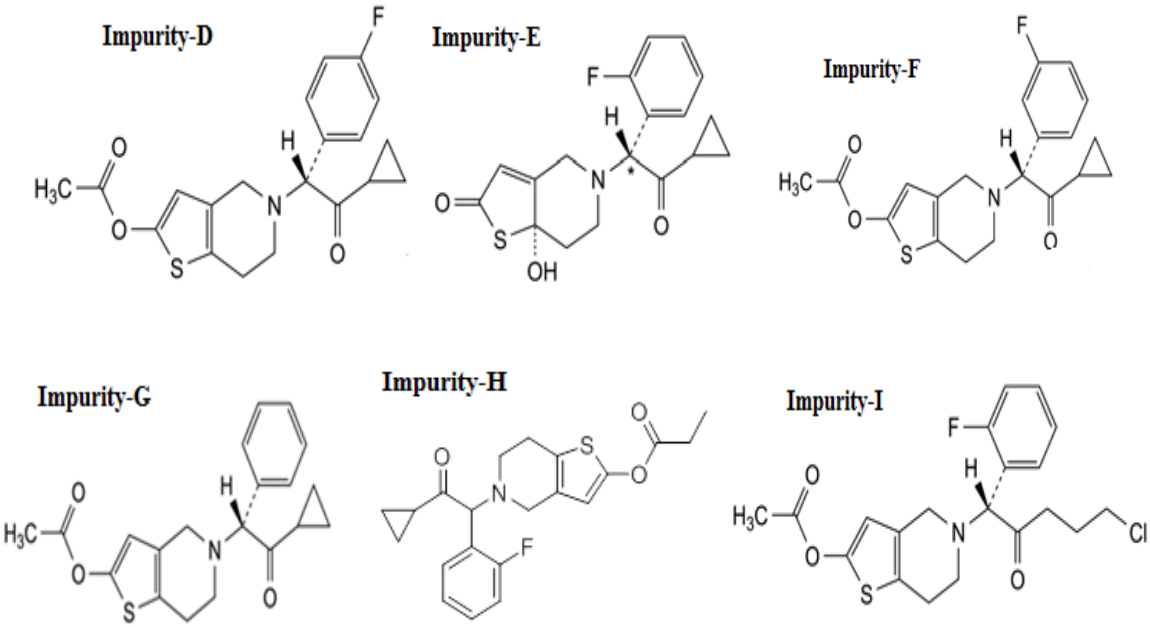


Impurity-B



Impurity-C





Instruments: A Waters Model Alliance 2695-separation module (Waters corporation, Milford, MA, USA) equipped with a waters 2998-photo diode array UV detector was used. Data was processed through Waters empower software.

Chromatographic Conditions: The analysis was carried out on YMC Pack Pro C18, 150 mm x 4.6 mm, 3µ particle size (Advanced Chromatography Technologies., Scotland) with a 25mM Potassium di hydrogen phosphate with 0.025% ortho phosphoric acid in water as mobile phase A and Mixture of Acetonitrile and water in the ratio of 80: 20 v/v as mobile phase B. The column temperature was maintained at 45°C. Flow rate was kept at 1.2 mL min^{-1} and the column eluent was monitored at 210 nm for 65 minutes Acetonitrile and water in the ratio of 70:30 (v/v) is used as diluent.[7-8]

Standard and Sample Preparation: Related substance was performed with 2.0 mg/mL test concentration. In related substance, all impurities are spiked 0.10% with respect to 2.0 mg/mL test concentration.

RESULTS AND DISCUSSION

Analytical method validation: Analytical method validation for the estimation of related substance by HPLC of Prasugrel hydrochloride API was performed according to ICH guidelines on Validation of Procedures.

System Suitability Evaluation: 0.1% of impurity spiked solution in the prasugrel standard is considered for SST criteria verification. Resolution between Prasugrel hydrochloride and impurity-F should not be less than 2.0.

Precision: The Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision for Related Substance: The repeatability of the related substance method was checked by six-fold analysis by spiking all the nine impurities at 0.10% to 2.0 mg/mL of Prasugrel hydrochloride test sample as well as the same study was performed on different day with different analyst for the evaluation of inter day and Intra-day variation and analyst. The % RSD of percent area of all the impurities in each six preparations were found well within the set acceptance limit, which conforms that the method is precise. The results are tabulated in Table-4.

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of detection (LOQ) of Prasugrel hydrochloride and all its impurities were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, LOQ values were established by injecting a series of diluted solution of known concentration. The LOQ values were confirmed by performing precision and accuracy verification.

The LOD and LOQ values of impurities are as tabulated below. The % RSD of percent area of all the impurities in six preparations at LOQ concentration were found less than 08, which conforms that the method is precise at LOQ Concentration. The results are tabulated in Table-4. The percentage recovery of each impurity ranged from 90 to 105, those values are listed in the Table-4. Recovery all the impurities are well within the acceptance limit.

Linearity: Linearity of the response for all the impurities was carried out from limit of quantification (LOQ) to 150% concentration of the specification limit, where specification limit is 0.1% of each impurity with respect to test concentration. Peak responses for all impurities were recorded and plotted the calibration curve for each impurity concentration verses response, the correlation coefficient obtained for each impurity was greater than 0.999 and results are listed in Table-4.

Accuracy: Known amount of each impurity addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of all impurities in the Prasugrel hydrochloride sample. The study was carried out in triplicate at LOQ, 0.05%, 0.1% and 0.15% of the analyte concentration (2.0mg/mL) and calculated the percentage recovery of all the nine impurities. The percentage recovery of each impurity ranged from 88 to 105, those values are listed in the Table-1. Recovery all the impurities are well within the acceptance limit.

Robustness: To determine the robustness of the method, chromatographic parameters are deliberate varied and the evaluated the change of resolution between Prasugrel hydrochloride and impurity-F. Experiments are conducted by varying the flow by ± 10% and column temperature by ±5ºC. Resolution between Prasugrel hydrochloride and impurity-F is illustrating and established the robustness of the method. Data is evaluated in the Table-1.

Solution Stability: The solution Stability of Prasugrel hydrochloride and its impurities in related substance method was carried out by spiking impurities at 0.1% level. All the prepared impurity solutions in volumetric flask were tightly capped and kept at room temperature for 48 hours. Content of all the impurities were determined initially, after 24hours and after 48hours. Results were indicated the sample solution is stable up to 48hours.

Mobile Phase solution stability was also established for Prasugrel hydrochloride related impurities with fresh sample solutions and holding the mobile phased for 48 hours. Freshly prepared sample solutions were analyzed initially, after 24 hours and after 48 hours with initially prepared mobile phase. Results were indicated the sample solution is stable up to 48hours.

Table. 2: System Suitability Results.

Parameter	Resolution Between Prasugrel and impurity-F	%RSD for six replicates of 0.1% standard
System Suitability (As is condition)	3.1	2.3
Robustness		
Flow Variation (1.0 mL/min)	3.5	2.7
Flow Variation (1.4 mL/min)	2.7	2.9
Column Temperature Variation (40º C)	2.9	3.1
Column Temperature Variation (50º C)	3.4	1.8

Table. 3: LOD and LOQ results.

Name of the impurity	LOQ (ug/ml)	LOD(ug/ml)
Impurity-A	0.813	0.268
Impurity-B	0.531	0.175
Impurity-C	0.790	0.261
Impurity-D	0.749	0.247
Impurity-E	0.773	0.255
Prasugrel	0.799	0.264
Impurity-F	0.771	0.255
Impurity-G	0.774	0.255
Impurity-H	0.526	0.174
Impurity-I	0.753	0.249

Table-4: Validation Data

Parameter	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E	Impurity-F	Impurity-G	Impurity-H	Impurity-I
RT about	4.535	14.876	17.306	24.807	26.599	35.801	44.417	47.301	48.752
LOQ (n=6)	2.2	7.9	4.9	6.7	2.8	8.8	6.3	2.4	8.0
100% (n=6)	1.6	0.8	1.2	1.3	0.9	1.2	1.4	0.7	3.4
100% (n=12)	1.7	2.4	1.9	2.4	1.5	1.7	1.4	2.5	4.4
r	0.9995	0.9990	0.9992	0.9990	0.9996	0.9994	0.9992	0.9995	0.9993
%Y-Intercept	-0.51	-0.32	1.1	0.88	-2.5	0.63	-1.6	3.1	-2.1
LOQ (n=3)	102.2	105.7	93.8	100.1	100.1	106.9	98.0	104.3	106.7
50% (n=3)	96.3	100.0	101.7	97.0	99.5	103.0	103.2	90.2	102.2
100% (n=6)	100.4	99.2	100.8	99.6	99.5	99.7	101.7	97.9	104.3
150% (n=3)	102.7	98.0	104.6	99.0	98.5	95.8	96.7	94.8	107.8

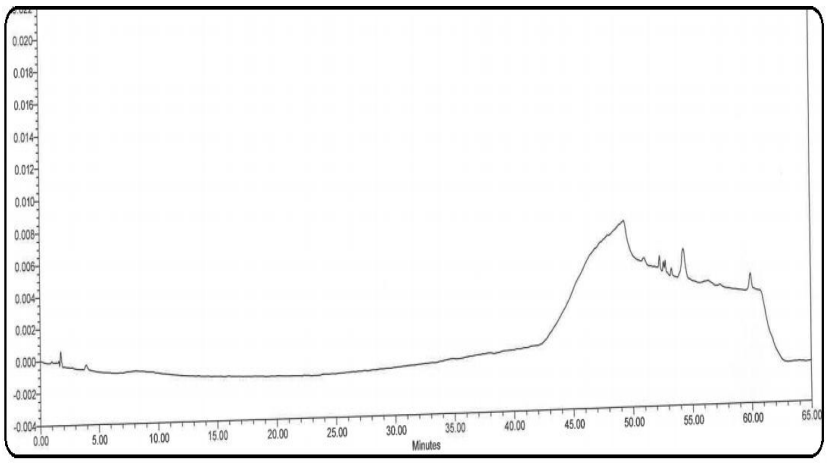


Fig. 1: Typical HPLC chromatogram for Blank of Prasugrel hydrochloride.

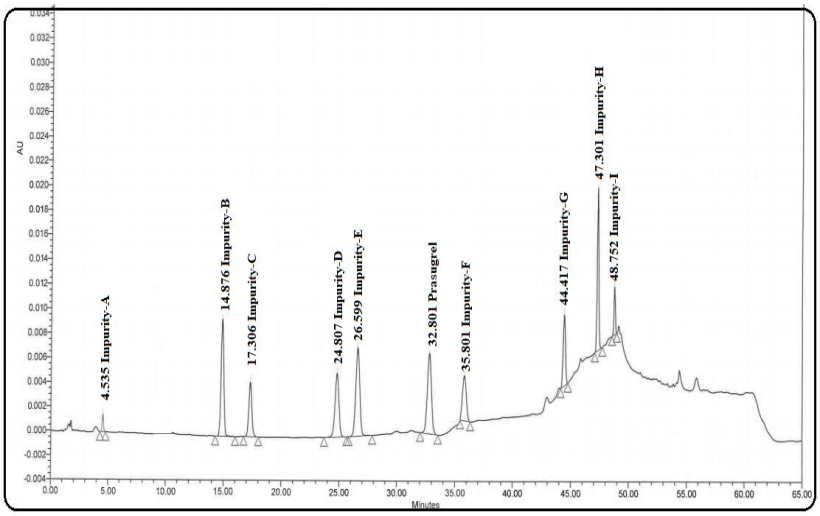


Fig. 2: Typical HPLC chromatogram for specificity of Prasugrel hydrochloride.

CONCLUSION

The LC method developed for quantitative and related substance determination of Prasugrel hydrochloride is precise, accurate, linear and specific. The method was fully validated, and the data found to be satisfactory for all the method validated parameters tested. The developed method can be conveniently used to determine the related substance of Prasugrel hydro chloride commercial samples and also stability samples.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd., for permitting this work to be published. Cooperation extended by all the colleagues of Analytical R&D, CTO-VI QC, and Process R&D division, is gratefully acknowledged.

REFERENCES

1. European Public Assessment Report for Efient EMA. 2009.
2. Bellemain-Appaix, A; Kerneis, M; O'Connor, SA; Silvain, J; Cucherat, M; Beygui, F; Barthelemy, O; Collet, J-P; Jacq, L; Bernasconi, F; Montalescot, G (2014) http://en.wikipedia.org/wiki/Prasugrel.
3. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology, 2005.
4. Farid, N. A.; Smith, R. L.; Gillespie, T. A.; Rash, T. J.; Blair, P. E. The Disposition of Prasugrel, A Novel Thienopyridine in Humans. Drug Metab. Dispos, 2007; 35: 1096–1104.

5. Lukram, O. Electrospray Ionization LC-MS-MS Validated Method for the Determination of the Active Metabolite (R-138727) of Prasugrel in Human Plasma and its Application to a Bioequivalence Study. Drug Test Anal, 2011.
6. Farid NA, McIntosh M, Garofolo F, Wong E, Shwajch A, Kennedy M, et al. Quantitative determination of clopidogrel active metabolite in human plasma by LC–MS/MS. Rapid Communications in Mass Spectrometry, 2007; 21(2): 169-179.
7. WIPO Patent Application WO=2010=070677.
8. Farid, N. A. Determination of the Active and Inactive Metabolites of Prasugrel in Human Plasma by Liquid Chromatography Tandem Mass Spectrometry. Rapid Common. Mass Spectrum, 2007; 21: 169–179.