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Research Article

DEVELOPMENT AND VALIDATION OF A REVERSE-PHASE LIQUID CHROMATOGRAPHIC METHOD FOR RELATED SUBSTANCES OF PRASUGREL FOR 5 AND 10MG TABLETS

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ABSTRACT

Objective: The main objective of current study was to develop and validate RP-HPLC, simple, precise, accurate and specific chromatographic method for the determination of related substances of Prasugrel in pharmaceutical formulations.

Methods: A high performance liquid chromatograph instrument and Inert Sustain C18, 75 x 4.6 mm, 3 µm were used for determination of Prasugrel and its related substances. Buffer was prepared by using 1.36 g of potassium phosphate monobasic in 1000 mL of water, adjust the pH of this solution to 3.30 with dilute orthophosphoric acid solution and mix well. Filter through 0.45 µm nylon filter and degas. The buffer used as a mobile phase-A The mobile phase-B was prepared by mixing Acetonitrile and Water in the ratio of 90:10(v/v).The flow rate of 1.0 mL/min was set with gradient program, the temperature of column compartment maintained at 30°C and Ultra violet detection done at 235nm wavelength.

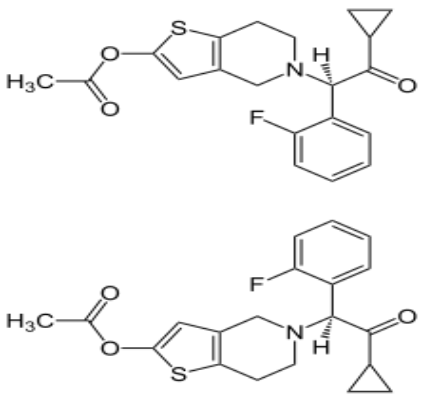
Results: The correlation coefficient (≥ 0.998) shows the linearity of response against concentration over the range of LOQ to 200%. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Conclusion: The developed and validated high performance liquid chromatographic method was suitable for determination of Prasugrel and its related substances in pharmaceutical formulations which was more useful with respect to regular Laboratory analysis.

Keywords: Prasugrel, Related substances, validation, RP-HPLC, ICH guidelines.

INTRODUCTION

Prasugrel is a member of the thienopyridine class of ADP receptor inhibitors and reduces the aggregation ("clumping") of platelets by irreversibly binding to $P2Y_{12}$ receptors [1,2].The chemical name of Prasugrel (RS)-5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate. The empirical formula of Prasugrel is $C_{20}H_{20}FNO_3$ with molecular weight 373. Prasugrel produces inhibition of platelet aggregation to 20 µM or 5 µM ADP, as measured by light transmission aggregometry [3].A simple HPLC method was developed for determination of Prasugrel in formulations by using 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) [4]. Another RP-HPLC method available for estimation of Prasugrel in tablet dosage form by using Xterra RPC18 250X4.6mm, 5µ column mobile phase 0.03M K2HPO4 in water pH 3.2.and Acetonitrile (25:75) [5].There was some other methods also available for determination of Prasugrel [6-8]. All the above methods are used for determination of Prasugrel in tablet form. But there was no method for determination of related substances of Prasugrel in Tablet dosage form. The novelty of present research work is a new RP-HPLC method developed for determination of related substance of Prasugrel with accurate, precise and selectively. The developed method was validated as for ICH guidelines [9-12]. The chemical structure of Prasugrel shown below fig-1



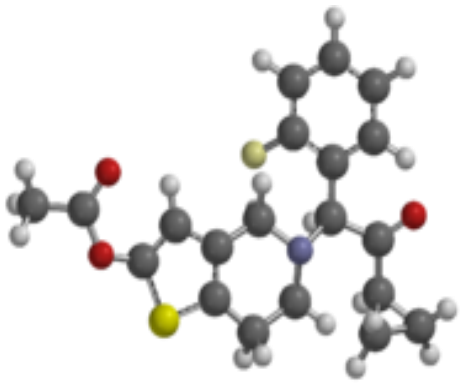


Fig. 1: Structure of Prasugrel

MATERIALS AND METHODS

Chemicals

Qualified standards for drug substance and impurities were obtained from Bio Leo Analytical laboratory and were used without any further purification. The chemicals like potassium phosphate monobasic and acetonitrile (ACN) were purchased from Merck, Mumbai. Millipore water generated from Millipore Water System. The analytical column used was Inert Sustain C18, 75 x 4.6 mm, 3 µm.

Instruments

A Waters Alliance HPLC system equipped with a quaternary pump was used for method development and validation studies. Second HPLC system Agilent 1200 series with high pressure liquid chromatographic instrument provided with Auto sampler and VWD UV detector, thermostatted column compartment connected with EZ Chrome software.

Standard Stock preparation

Weighed and transferred 25 mg Prasugrel into 100 mL amber colored volumetric flask, add 50 mL diluent, sonicated to dissolve

and diluted to volume with diluent. Dilute 5 mL of this solution to 50 mL with diluent and mix well Further dilute 2 mL to 50mL with diluent, Acetonitrile: Water (90:10).

Placebo preparation

Transferred equal amount of the placebo powder present in 100 mg tablet into 100 mL volumetric flask, add 70 mL of diluent, sonicated to dissolve for 20 min and dilute to volume with diluent. Further filtered the solution through 0.45 µm nylon filter.

Preparation of sample for 5 mg

Transferred 20 tablets into 100 mL amber colored volumetric flask, add 70 mL of diluent, sonicated to dissolve for 20 min and dilute to volume with diluent. Further filter the solution through 0.45 µm nylon filter.

Preparation of sample 10 mg

Transferred 10 tablets into 200 mL amber colored volumetric flask add 120 mL of diluent, sonicate to dissolve for 20 min and dilute to volume with diluent. Further filter the solution through 0.45µm Nylon filter.

Chromatographic conditions

The chromatographic column used was Inert Sustain C18, 75 x 4.6 mm, 3 µm particle size. The gradient method was employed with the mobile phase-A was buffer. The mobile phase-B was prepared by mixing Acetonitrile and Water in the ratio of 90:10(v/v).The column temperature was maintained at 30.0°C and detection was monitored at a wavelength of 235 nm. Injection volume was 20 µl and the mobile phase flow was set at 1.0 mL/min. The mobile phase-B was used as diluents for preparation of solutions. The gradient program was given in Table -1.

Table 1: Gradient Program

Time in (min)	%Mobile phase-A	%Mobile phase-B	Flow rate (mL/min)
0	75	25	1.0
20	70	30	1.0
30	65	35	1.0
45	50	50	1.0
65	40	60	1.0
70	75	25	1.0
75	75	25	1.0
85	75	25	1.0

Method validation

The developed method for determination of Prasugrel and its related substances was validated for system suitability along with method selectivity, specificity, linearity, range, precision (Repeatability and Intermediate precision),accuracy, limits of detection and Limit of quantification according to the ICH guidelines. The system suitability was conducted using diluted standard preparation and evaluated by injecting six replicate injections. Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of component that may be expected to be present, such as impurities, degradation products and matrix components. Performed the specificity parameter of the method by injecting Diluent, standard preparation, and sample preparation spiked with impurities into the chromatographic system and evaluated by making three replicate injections. Performed the linearity with Prasugrel standard and impurities in the range of LOQ to 200% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system. The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements. The system precision was conducted using all the impurities spiked to Prasugrel and evaluated by making six replicate injections. The Accuracy of the method by recoveries of all the impurities was determined by analyzing Prasugrel sample solutions spiked with each impurity at three different concentration levels ranging from LOQ to 200%. The LOD and LOQ were determined for Prasugrel and each of the impurities based on signal to noise ration method.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Method development includes selection of appropriate chromatographic conditions/factors like detection wavelength, selection, optimization of stationary and mobile phases. The longer wavelength of 235 nm was selected since it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Prasugrel and its related substances. Moreover, all related impurities are also detected satisfactorily at the same wavelength and hence it is selected as detection wavelength. Preliminary development trials were performed with various C_{18} columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Inert Sustain C18, 75 x 4.6 mm, 3 µm there was a substantial increase in the theoretical plates (15810) with a significant improvement in the peak shape. It also produced adequate resolution between Prasugrel and its related substances.

System suitability

The RSD from six replicate injections of diluted standard preparation was 0.36 %. Theoretical plates for Prasugrel peak 15810.The system suitability of Prasugrel given in the table-2.

Table 2: System Suitability results of Prasugrel

Parameters	Observed Results	Acceptance criteria
Theoretical plates	15810	NLT 3000
Asymmetry	0.94	NLT 2.0
% RSD of standard preparation	0.36	NMT 5

Selectivity

Performed the specificity parameter of the method by injecting standard preparation, sample preparation, placebo preparation, Impurities and sample spiked with impurities into the chromatographic system. This was further demonstrated by determining the peak purity of analyte and known impurity peaks. The results of the peak purity values of analyte and known impurities were given in the below table-3. The chromatograms of standard and sample with its impurities shown in the figure-2, 3

Table 3: Selectivity results of Prasugrel calcium

Compound Name	Peak Purity
Prasugrel	1.000
Desfluoro impurity	1.000
Acetyl impurity	1.000
Desoxo impurity	1.000
4-Fluoro impurity	1.000
Desacetyl impurity	1.000

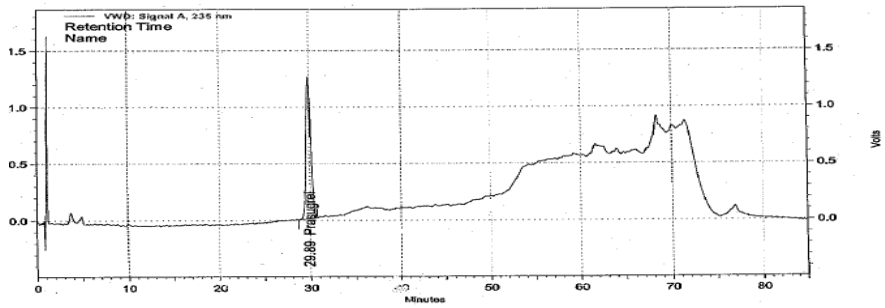
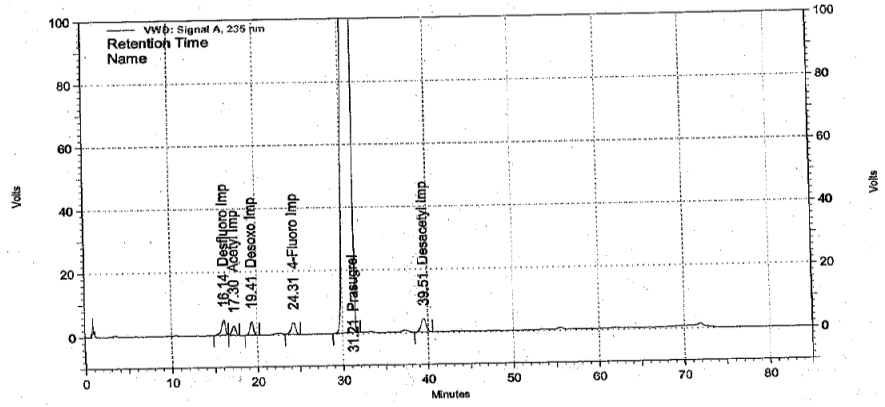


Fig. 2: Chromatogram of standard of Prasugrel



VWD: Signal A, 235 nm Results

Name	Retention Time	Area	Area Percent	Relative RT
Desfluoro Imp	16.14	2452085	0.63	0.52
Acetyl Imp	17.30	1424158	0.36	0.55
Desoxo Imp	19.41	2113493	0.54	0.62
4-Fluoro Imp	24.31	2157956	0.55	0.78
Prasugrel	31.21	380552323	97.18	1.00
Desacetyl Imp	39.51	2901751	0.74	1.27
Totals		391601766	100.00	

Fig. 3: Chromatogram of Prasugrel with impurities

Table 4: Linearity results of Prasugrel and its impurities

Name	Correlation coefficient	Slope	Y-Intercept	Residual sum square	RSD
Prasugrel	0.9998	428574.47	31233.6	4.426292×10^{9}	33265
Desfluoro Imp	1.0000	339138.91	2281.4	3.776929×10^{7}	3073
Acetyl impurity	1.0000	244038.23	-241.12	2.776117×10^{8}	8331
Desoxo impurity	1.0000	422952.61	1039.1	1.209725×10^{8}	5499
4-Fluoro impurity	0.9997	383520.16	34425.95	6.154546×10^{9}	39225
Desacetyl impurity	1.0000	360063.78	-14381.56	1.451443×10^{8}	6024

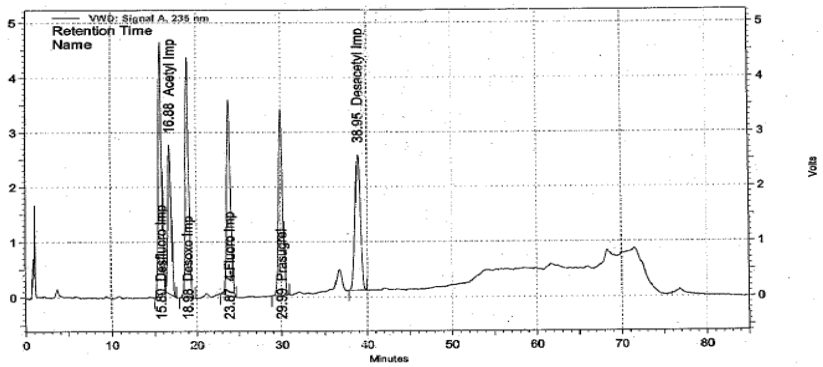


Fig. 4: Linearity Chromatogram of Prasugrel and its impurities

Linearity

To demonstrate the linearity with Prasugrel standard, impurities in the range of LOQ to 200% of specification limit**.** Correlation coefficients of Prasugrel and its related compounds were more than 0.999. Plotted a graph of Prasugrel standard and Impurities concentration (ppm) on X-axis and Area responses on Y-axis**.** Linearity data of Prasugrel and its relative impurities given in the below table- 4. The linearity chromatogram of Prasugrel and its related impurities shown in the figure-4

Accuracy

Accuracy study found that the mean % of recovery was more than 90% and less than 110% at each level LOQ to 200% of concentration levels, hence method is accurate. The accuracy results are given table-5, 6,7,8,9.

Table 5: Recovery of Desfluoro Impurity:

S. No.	Level in %	% Mean Recovery	% RSD
	LOQ	93.25	2.4
2.	50%	95.63	1.8
3.	100%	95.54	2.0
4.	150%	97.98	0.6
5.	200%	101.24	1.6

Table: 6 Recovery of Acetyl Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	105.83	0.7
2.	50%	96.56	0.7
3.	100%	98.71	0.7
4.	150%	99.75	0.6
5.	200%	103.34	1.1

Table: 7 Recovery of Desoxo Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	103.64	3.2
2.	50%	101.36	0.9
3.	100%	101.37	1.5
4.	150%	101.34	0.4
5.	200%	102.55	1.0

Table: 8 Recovery of 4-Fluoro Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	107.19	0.76
2.	50%	92.52	2.04
3.	100%	93.08	1.38
4.	150%	94.96	0.29
5.	200%	97.85	1.3

Table 9: Recovery of Desacetyl Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	98.17	2.1
2.	50%	103.24	1.7
3.	100%	102.48	1.6
4.	150%	103.33	1.0
5.	200%	104.54	1.0

Precision

The % RSD of the Retention time for peaks obtained from 6 injections of standard preparation was less than 10.0%.The RSD of % impurities from six spiked sample preparations should not be more than 10% by different instruments, analysts, columns and days. The RSD of % impurities from twelve spiked sample preparations should not be more than 15.0% by different instruments, analysts, columns and days. The precision data for different strengths given in the table- 10, 11, 12

Table 10: Precision results of 5 mg Prasugrel

S. No.	Compound Name	%RSD
1.	Desfluoro impurity	3.0
2	Acetyl impurity	1.3
3.	Desoxo impurity	0.8
4.	4-Fluoro impurity	1.6
5.	Desacetyl impurity	1.5

Table 11: Precision results of 10 mg Prasugrel

S. No.	Compound Name	%RSD
1.	Desfluoro impurity	3.0
2	Acetyl impurity	1.2
3.	Desoxo impurity	0.8
4.	4-Fluoro impurity	1.6
5.	Desacetyl impurity	1.5

Table 12: Intermediate Precision results of 10 mg Prasugrel

S. No.	Compound Name	%RSD
1.	Desfluoro impurity	2.3
2	Acetyl impurity	1.3
3.	Desoxo impurity	2.0
4.	4-Fluoro impurity	1.7
5.	Desacetyl impurity	2.9

LOD and LOQ

The distinct visible peak observed at LOD level concentration. The LOD and LOQ were established as per signal to noise ratio method data given table-13, 14. The LOQ chromatogram of Prasugrel and its related impurities shown in the figure-5

Robustness

The method robustness was studied by injecting the system suitability solution at change in the percentage of organic modifier, flow rate, and column temperature. The results were obtained as shown in the below table-15

Table13: LOQ establishment data

S. No.	Compound Name	S/N ratio	% level of comp. w.r.t to sample conc.
1.	Prasugrel	10.42	0.0230
2.	Desfluoro impurity	10.08	0.0243
3.	Acetyl impurity	10.05	0.0385
4.	Desoxo impurity	10.00	0.0225
5.	4-Fluoro impurity	10.14	0.0308
6.	Desacetyl impurity	9.75	0.0398

Table14: LOD establishment data

S. No.	Compound Name	S/N ratio	% level of comp. w.r.t to sample conc.
1.	Prasugrel	2.37	0.0230
2.	Desfluoro impurity	2.57	0.0243
3.	Acetyl impurity	2.79	0.0385
4.	Desoxo impurity	2.55	0.0225
5.	4-Fluoro impurity	2.68	0.0308
6.	Desacetyl impurity	2.35	0.0398

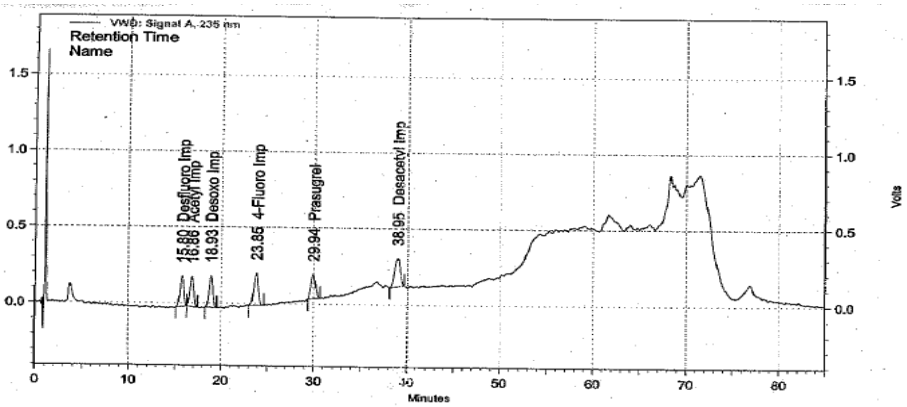


Fig: 5 LOQ Chromatogram of Prasugrel and its impurities

Table: 15 Robustness Results

Condition	Theoritical plates	Asymmetry	% RSD for STD preparation
Limits	**NLT 3000**	**NMT 2.0**	**NMT 5.0**
Normal Condition	17641	0.96	0.3
Flow rate 1.1ml/min	16416	0.95	0.5
Flow rate 0.9/min	18200	0.95	0.6
Column Temperature 30°C	18153	0.96	0.6
Column Temperature 20°C	16144	0.97	0.5
Buffer pH changed to 4.0	21062	0.96	0.8
Buffer pH changed to 3.6	11471	0.96	0.5
Change in minor component + 5%	19449	0.97	0.3
Change in minor component - 5%	16900	0.96	0.5

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CONCLUSIONS

A simple gradient RP-LC method has been developed and validated for the determination of related substances of Prasugrel drug substance. The developed method has been found to selective, sensitive, precise and robust. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination of related substances of Prasugrel table formulation.

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