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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF PRASUGREL HYDROCHLORIDE IN BULK AND IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new, simple, specific, accurate, precise, and rapid reverse phase high performance liquid chromatographic method was developed and validated for the determination of prasugrel Hydrochloride in pure and tablet dosage forms. The HPLC separation was carried out by reverse phase chromatography on Kromasil C18 (100 x 4.6mm; 5µm) with a mobile phase consist of methanol buffer (680mg potassium dihydrogen phosphate in 500 ml water, pH-2.1 adjusted with ortho phosphoric acid) in the ratio of 70:30 v/v delivered in isocratic mode at a flow rate of 0.8 ml/min. The prasugel Hydrochloride was quantified at 220nm. The retention time of Prasugrel Hydrochloride was 1.9 min. The developed method was validated according to ICH guidelines. The interday and intraday precision were found to be within limits. The developed method has adequate sensitivity and specificity for the determination of prasugrel Hydrochloride in bulk and its tablet dosage forms. Accuracy (recoveries: 98.92-101.19%) and reproducibility were found to satisfactory. The developed method was found to be cost effective and was successfully employed for the determination of Prasugrel Hydrochloride in various pharmaceutical preparations.

KEYWORDS: Method development, Prasugrel, RP-HPLC, Validation

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INTRODUCTION

Prasugrel Hydrochloride chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno [3,2- c] pyridin-2-yl acetate hydrochloride [1] (Fig: 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 & HPTLC, HPLC for its analysis of pure drug [5,6,7,8,9,10]. 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].

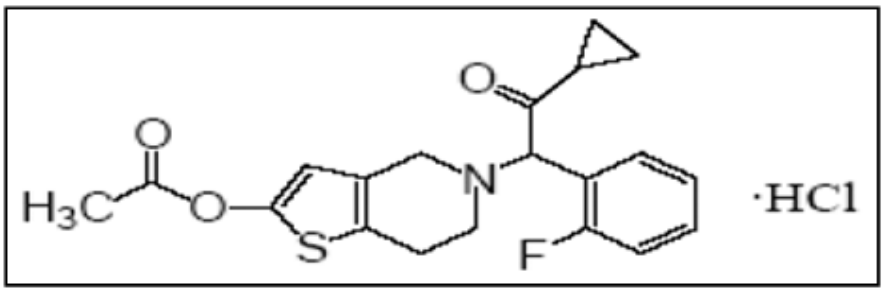

Figure 1
Chemical structure of Prasugrel Hydrochloride

MATERIALS AND METHODS

Apparatus

A HPLC (Perkin Elmer Binary LC Pump 200B/ 250) equipped with an inbuilt solvent degasser, Series 200 Pump, Series 200 UV/VIS detector and Kromasil (5µm; 100 x 4.6mm) column was used with Total Chrome Navigator Software.

Reagent and Materials

The branded formulation (Effient tablets containing 10mg of Prasugrel Hydrochloride) was procured from the local market. HPLC grade methanol (Merck Specialties Pvt. Ltd), AR grade O-Phosphoric acid as HPLC buffer (Spectrochem Pvt. Ltd), potassium dihydrogen phosphate (Mylochem) and distilled water filtered through a 0.45 µm filter (Millipore) were used.

Diluent: Methanol

Solvent system: The solvent system employed for chromatography consisted by methanol buffer (pH 2.1) (10: 90).

Chromatographic conditions

Kromasil C18 (100 x 4.6mm; 5µm) was the column used for separation. Mobile phase consisting of a mixture of methanol: buffer (680mg potassium dihydrogen phosphate in 500 ml water, pH-2.1 adjusted with O-phosphoric acid) in the ratio of 70:30 v/v delivered in isocratic mode at a flow rate of 0.8 ml/min quantified at 220 nm. The mobile phase was filtered through a 0.45 nylon filter and sonicated for 10 min.

Method development

Buffer (680mg Potassium Dihydrogen Phosphate in 500 ml water, pH-2.1 adjusted with O-phosphoric acid) and Methanol in different proportions were tried and finally buffer (680mg Potassium Dihydrogen Phosphate in 500 ml water, pH-2.1 adjusted with O-phosphoric acid) and methanol (70: 30 v/v) was selected as an appropriate mobile phase which gave good resolution.

Procedures

Sample preparation

Take 20 tablet of Prasugrel, weighed & calculate the net content. Crush them in mortal & pastel. Transfer an accurately weighed quantity of tablet powder equivalent to about 10mg of Prasugrel to a 10ml volumetric flask, add about 8ml of diluent and sonicate with intermittent shaking for about 10min , then make volume up to the mark with diluent and mix. Filter the solution through 0.45µ Millipore PVDF filter, collect the filtrate by discarding first few ml of the filtrate. Dilute 1ml of this solution to 10ml with mobile phase and mix.

Standard solution for Prasugrel

Transfer an accurately weighed quantity of about 10 mg of Prasugrel working standard to a 10 ml volumetric flask. Add about 8 ml of diluent and sonicated to dissolve. Make volume up to the mark with diluent and mix. Dilute 1.0 ml of this solution to 10 ml with mobile phase and mix.

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 1.113. 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 1.566. From the data obtained the developed RP-HPLC method was found to be precise.

Table 1
Precision data of the proposed method

INTERDAY PRECISION				INTRADAY PRECISION			
Conc. (ppm)	**Mean Peak area**	**SD**	**% RSD**	**Conc. (ppm)**	**Mean Peak area**	**SD**	**% RSD**
20	557592.49	6206.01	1.113	**20**	563271.3767	8822.42	1.566
30	826790.69	4504.31	0.545	**30**	822064.5267	5226.53	0.636
40	1018839.57	8115.74	0.797	**40**	1014666.25	14202.5171	1.399

Repeatability

Repeatability was obtained by injecting 6 injections of 100µg/ml and % RSD was found to be 0.83.

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 (80, 100 and 120%). The analyzed samples yielded high recovery values from the proposeded method. The % recovery results of the method are given in below Table.

Table 2
Recovery studies of Prasugrel HCl

Concentration % of spiked level	% Recovery	Statistical Analysis of % Recovery	
80% SAMPLE 1	100.58	**MEAN**	100.43
80% SAMPLE 1	99.53	**SD**	0.84
80% SAMPLE 1	101.19	**%RSD**	0.84
100% SAMPLE 1	100.76	**MEAN**	100.41
100% SAMPLE 1	99.40	**SD**	0.89
100% SAMPLE 1	101.06	**%RSD**	0.88
120% SAMPLE 1	101.02	**MEAN**	99.62
120% SAMPLE 1	98.93	**SD**	1.21
120% SAMPLE 1	98.92	**%RSD**	1.21

Linearity

The method was linear in the range of 10µg/ml to 100µg/ml for Prasugrel Hydrochloride. Linear regression data was given in Table 3.

Table 3
Linearity data of Prasugrel Hydrochloride

Linearity range (µg/ml)	Correlation coefficient	Slope	Regression Equation
10-50	0.9930	22837	y = 22837x + 76879

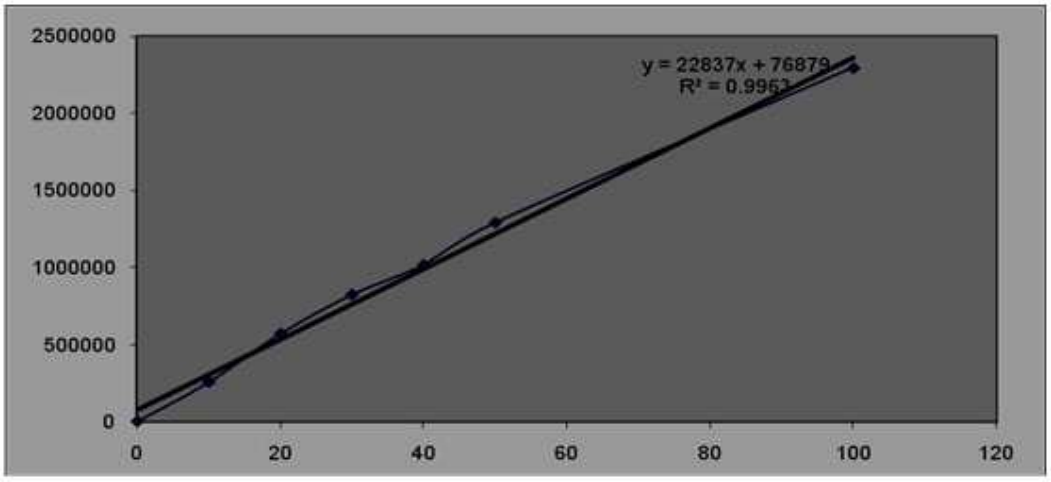

Figure 2
Linearity plot of Prasugrel Hydrochloride

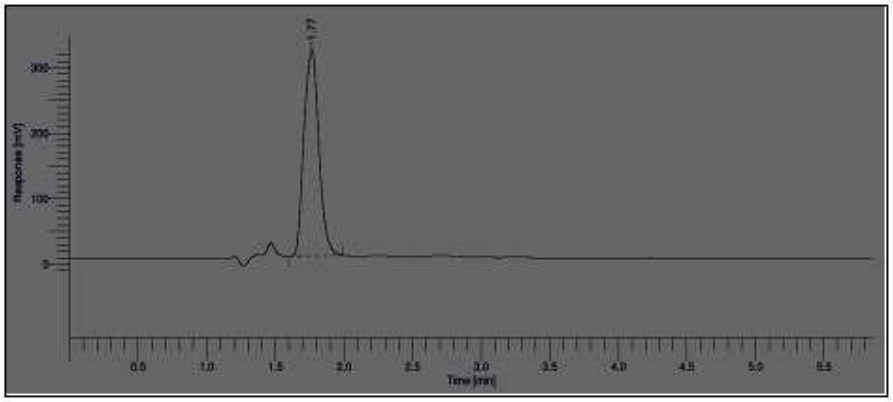

Figure 3
A Representative Chromatogram of Prasugrel Hydrochloride

Robustness

As seen in table 5 it can be said that in all varied chromatographic conditions, there was no significant change in chromatographic parameters.

Table 4
Robustness study (n=3)

Concentration	Conditions changed	% RSD	Mean RT
100 µg/ml	**Flow rate**		
	0.6 ml/min	0.87	2.36
	1.0 ml/min	0.63	1.48

Specificity

There should be no interference from the peaks due to diluents and placebo peaks at the retention time of Prasugrel. There was no interference from the blank, placebo with the main peak. The peak purity obtained was within the limit, **Hence the method can be termed as specific.**

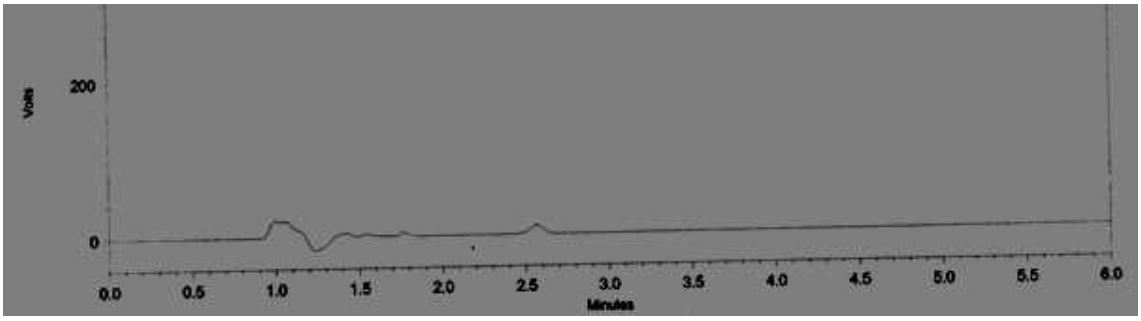

Figure 4
Chromatogram of Diluent

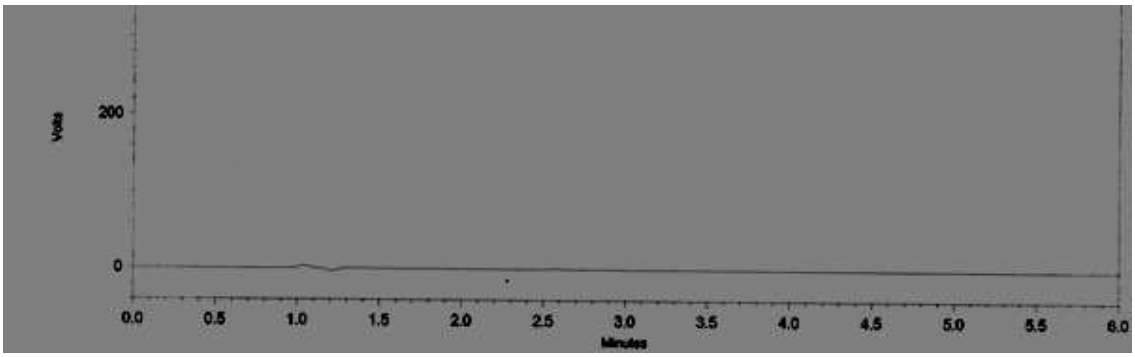

Figure 5
Chromatogram of Placebo

RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 10 to 100µg/ml for Prasugrel Hydrochloride. The method was specific because excipients in the formulation did not interfere in the analysis of Prasugrel Hydrochloride. Accuracy of the method was determineded by recovery values from 98.92 to 101.19% for Prasugrel Hydrochloride. Precision is indicated by % RSD values less than 1.5. These low values suggest sensitivity of the developed method.

CONCLUSION

The developed RP-HPLC method was simple, rapid, sensitive, precise, accurate and hence can be used for the determination of Prasugrel Hydrochloride in bulk as well as in pharmaceutical preparations.

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