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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND PRASUGREL IN TABLET DOSAGE FORM

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Keywords:

Aspirin, Prasugrel, RP- HPLC, Validation

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ABSTRACT: Aim of the present work is to develop a rapid, simple, precise, accurate and reproducible reverse phase high performance liquid chromatographic method for simultaneous determination of Aspirin and Prasugrel in tablet dosage form. The estimation was carried out on a HIBAR (Lichrospher C-18) column with the dimensions of 250mm x 4.6mm, 5µm. Combination of Acetonitrile and 0.5% Potassium dihydrogen phosphate buffer (adjusted to pH-3 using orthophosphoric acid) in the ratio of 60: 40 was used as mobile phase. The flow rate is set at 1.0ml/min and eluents were monitored at 220 nm. Both drugs were properly resolved having run time of 3.3 min and 4.8 min for Aspirin and Prasugrel, respectively. The method was validated as a final verification of method development with respect to Precision, Linearity, Accuracy, Ruggedness and Robustness. Linearity for Aspirin and Prasugrel was in the range of 10-450µg/ml and 10-500µg/ml respectively. The mean recoveries obtained for Aspirin and Prasugrel were within the range of 98-102%.

INTRODUCTION: Aspirin (ASP), 2-acetoxy benzoic acid is cyclooxygenase inhibitor. The molecular formula is C9H8O4 and the molecular weight is 180.16 gm/mole. It is a Non-steroidal Anti-inflammatory drug and inhibits Platelet aggregation. Prasugrel chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4, 5, 6, 7-tetrahydrothienol [3, 2- c] pyridin-2-ylacetate. The molecular formula is C20H20FNO3Sand the molecular weight is 373.442gm/mole.

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It inhibits adenosine diphosphate induced platelet aggregation and used in the treatment of coronary artery disease. Structures for aspirin and Prasugrel were given in **Figures 1 and 2**.

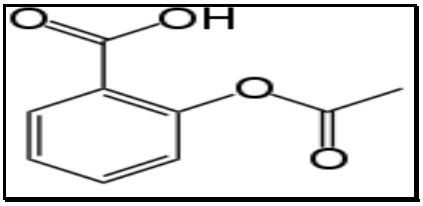

FIGURE 1.STRUCTURE OF ASPIRIN

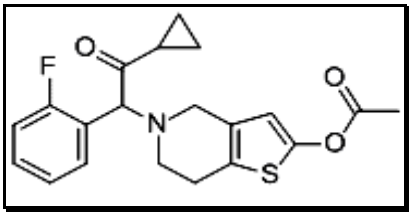

FIGURE 2.STRUCTURE OF PRASUGREL

Literature survey revealed that only few analytical methods (3- 18) such as HPLC, LC-MS, UV, and HPTLC have been reported for the determination of PRASU and ASP individually and in combination with other drugs. Only two methods (1-2) were reported till date using RP-HPLC which suffers with some drawbacks like high retention time, asymmetric peak shape and low range of linearity. The present study focuses on development of simple, rapid, sensitive, precise, accurate and cost effective analytical method for estimation of Aspirin and Prasugrel in Tablet dosage form.

MATERIALS AND METHODS:

Reagents and chemicals:

The bulk drug of Prasugrel was obtained as a gift sample from MSN Laboratories, Hyderabad. HPLC grade Acetonitrile, Orthophosphoric acid and Potassium dihydrogen phosphate, sodium hydroxide were obtained from Merck and Milli-Q water of HPLC grade was used for the experiment.

Stock solution and standards:

25mg of Aspirin and Prasugrel were accurately weighed and transferred in to a 25ml volumetric flask and required quantity of mobile phase was added to dissolve the drugs. Then volume was made up to the mark with mobile phase. This gives the standard stock solution of Aspirin and Prasugrel having concentration of 1000µg/ml. working standard solutions were prepared by transferring suitable aliquots of standard solution in to 10ml volumetric flask and made up to mark with mobile phase.

Apparatus and Chromatographic conditions:

HPLC analysis was performed on SHIMADZU 20-AD HPLC outfitted with dual head reciprocating pump with a manually operating Rheodyne injector of 20µl sample loop and a SPD-20A UV-Vis detector. The software equipped was LC solution software. The Chromatographic column, Lichrospher C-18 (250mm x4.6mm, 5µm) was used as a stationary phase. Acetonitrile and 0.5% potassium dihydrogen phosphate (pH-3) in the ratio of 60:40 was used as mobile phase. The pump flow rate was set at 1.0ml/min. The eluent was detected at 220nm and run time was 7min.

Preparation of sample solution:

Twenty tablets were weighed accurately and crushed to form a fine powder. Accurately weighed quantity of powder equivalent to 75 mg of Aspirin, and 10mg of Prasugrel were transferred in to a 100 ml of volumetric flask, 50ml of mobile phase was added.

The volume was made up to mark with mobile phase and then sonicated for 5min. This solution was then filtered through whatmann filter paper. Suitable aliquot of the filtrate was pipetted in to 10ml volumetric flask and volume made up with mobile phase to obtain concentration in the range of linearity previously determined. This solution was filtered through a 0.45µ membrane filter and sonicated for 2 min. This was marked as Test solution.

TABLE.1. SYSTEM SUITABILITY DATA FOR ASPIRIN AND PRASUGREL

System suitability Parameters	Aspirin	Prasugrel
Retention time (Rt)	3.364	4.832
Peak area	2239644	1823730
USP plate count (N)	6978.447	7719.533
USP tailing factor (T)	1.2545	1.321833
Resolution factor (Rs)	8.404	7.670
Relative retention time (RRT)	0	1.468
% RSD of(n= 6)		

Assay of Marketed Formulation:

Prepared sample solution was injected under identical chromatographic conditions as mentioned earlier and chromatograms were recorded. This was done in triplicate. The amount of Aspirin and Prasugrel is calculated from the calibration curve. The results were given in the **Table 2**. Representative chromatograms for blank, standard and sample were given in the **Figures 5, 6 &7.**

TABLE.2. ASSAY DATA FOR ASPIRIN AND PRASUGREL TABLET FORMULATION

Parameter	Aspirin	Prasugrel
Peak areas	18728452	2044350
	18725699	2053678
	18789989	2043645
Mean	18728937	2043671
% Assay	99.8%	100.07%

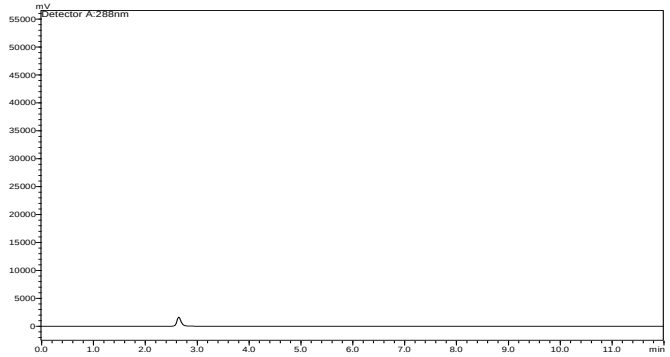

FIGURE 5. CHROMATOGRAM OF BLANK

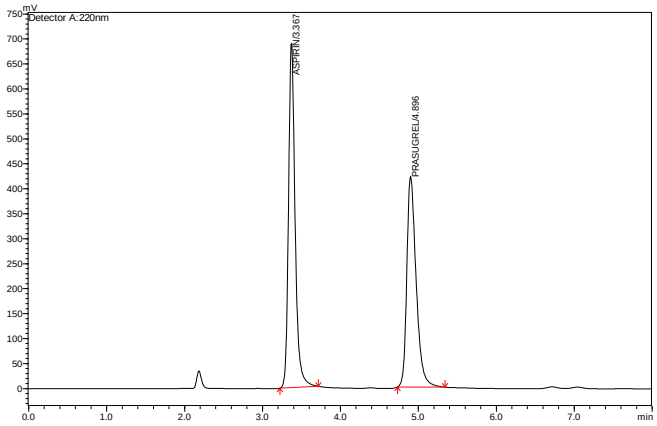

FIGURE 6. CHROMATOGRAM OF STANDARD MIXTURE

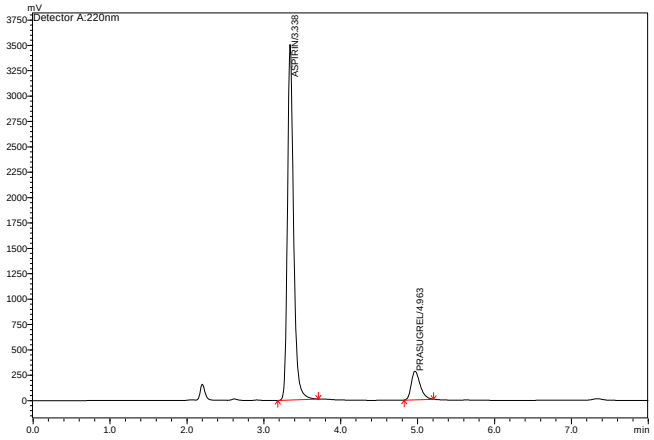

FIGURE 7. CHROMATOGRAM OF TEST FORMULATION

Validation of Assay Method:

Linearity:

Linearity of the method was demonstrated over wide concentration ranges of Aspirin and Prasugrel. Each concentration was determined in duplicate. 20µl of each of standard solutions were injected at the optimized chromatographic conditions and the chromatograms were recorded. The average peak areas were noted. Calibration curve for ASP and PRASU were constructed by plotting concentration on X-axis against mean peak area on Y-axis and regression equation was calculated by the method of least squares. The correlation coefficient, y-intercept, slope of the regression line were noted.

Recovery and Accuracy:

Accuracy of the method was established by performing recovery studies.It was ascertained on the basis of recovery studies by standard addition method. Recovery studies were carried out at five different levels (25%, 50%, 75%, 100%, and 125%) by the addition of standard drug to pre-analyzed sample solution having the concentration of 75µg/ml of Aspirin and 10µg/ml of Prasugrel. Triplicate determinations were carried out at each level. Mean percentage recovery values at five different levels of the two drugs were calculated.

Precision:

Precision was carried out at two levels i.e. repeatability of injections and intermediate precision.

Repeatability also called intra assay precision assessed by using a minimum of 9 determinations (3 concentrations/ 3 replicates). It was carried out at three different levels i.e. 50%, 100% and 150% under specified chromatographic conditions. 20µl of each level was injected in triplicate.

Intermediate precision expresses the precision within laboratory variations. It includes full analysis on different days, instruments or analysts. It was performed using standard concentration of 100µg/ml.

Robustness:

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized chromatographic conditions were done i.e. variation in flow rates (± 0.1ml/min), concentration of organic phase (±.2%) and detection wavelengths (± 3nm). It was performed using 50µg/ml.

RESULTS:

Method development and optimization:

To develop a suitable HPLC method for the determination of Aspirin and Prasugrel, trials were performed with different mobile phases, using

water and acetonitrile at different pH with different compositions of mobile phases like 50:50 (water: Acetonitrile pH-3), 50:50 (0.5% potassium dihydrogen phosphate buffer: Acetonitrile pH-3), 60:40 (Acetonitrile: 0.5% potassium dihydrogen phosphate pH-3 and also by changing different columns like YMC ODS column and HIBAR (Lichrospher) column. The method was optimized finally using combination of Acetonitrile and 0.5% potassium dihydrogen phosphate buffer pH-3 in the ratio of 60/40 v/v with a flow rate of 1.0 ml/min. The drugs were eluted at retention times of 3.3 minutes for Aspirin and 4.8 minutes for Prasugrel at a detection wavelength of 220nm.The run time was set for 7 minutes.

Validation:

Linearity:

Calibration curves were constructed by plotting concentration on X-axis against average peak area on Y-axis and regression equations were computed. Linearity was established over the concentration range of 10-450µg/ml for Aspirin and 10-500µg/ml for Prasugrel. Correlation coefficient for Aspirin and Prasugrel was found to be 0.999 and 0.9991 respectively. The results were given in the **Table 3**. Plot was given in **Figures 3& 4**

TABLE.3. LINEARITY DATA FOR ASPIRIN AND PRASUGREL

S. no	Concentration (µg/ml)	Mean peak area	
		Aspirin	Prasugrel
1	10	540423	44806
2	50	2237906	1943761
3	100	4513697	3802566
4	150	6561985	5600155
5	200	8983334	7290167
6	250	11201618	9079435
7	300	13874744	10968055
8	350	16086881	13281506
9	400	18428937	15258892
10	450	21266874	16651972
11	500	-	18529591

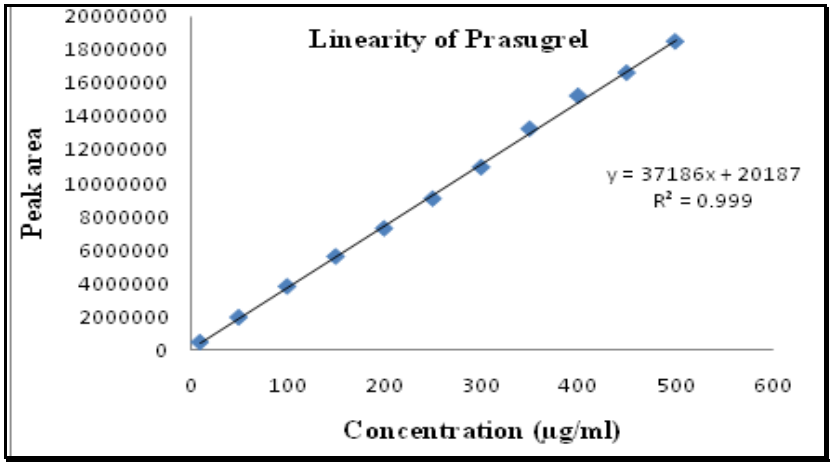

FIGURE 3. CALIBRATION CURVE FOR ASPIRIN

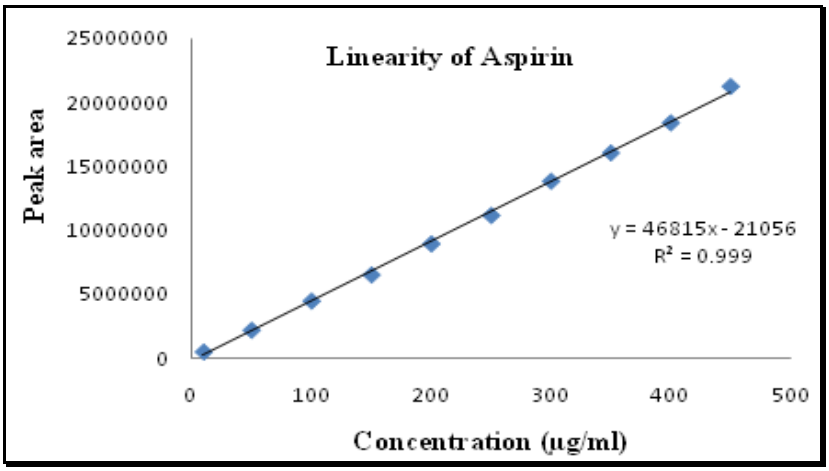

FIGURE 4. CALIBRATION CURVE FOR PRASUGREL

Recovery and Accuracy:

The % recoveries of Aspirin and Prasugrel at each level was within the limits of 98% and 102% given in the Table.7.0 which indicates that the method was accurate and also reveals that the excepients present in the pharmaceutical formulation has no interference with the analytes. The results were given in the **Table.4.**

TABLE.4. ACCURACY STUDY DATA FOR ASPIRIN AND PRASUGREL

% Level	Aspirin			Prasugrel		
	Amount of drug added (µg/ml)	Amount of drug recovered (µg/ml)	Average % Recovery	Amount of drug added (µg/ml)	Amount of drug recovered (µg/ml)	Average % Recovery
50%	175	173.04	98.8	110	109.6	99.6
75%	225	225.5	100.2	160	162	101.8
100%	275	274.3	99.7	210	206.11	98
125%	325	327.9	100.8	260	260.5	100.1
150%	375	377.4	100.64	310	311.2	100.3

Precision:

From the precision studies, it is evident that %RSD of the peak areas and %assay of both the drugs were below 2.0%. Thus, Precision was established. Hence, the developed method was precise. The results were given in the **Table. 5, 6 and 7**.

TABLE.5. INTRA-ASSAY PRECISION DATA FOR ASPIRIN AND PRASUGREL

Level	Aspirin		Prasugrel	
	Peak area	Mean% Recovery	Peak area	Mean % Recovery
50%	6463961 6775570 6482397	100.2	5658792 5668986 5656453	100.77
100%	13874744 13684218 13998756	100.2	10968055 10905785 10998172	99.21
150%	21465872 21266874 21384968	100.06	16249690 16651972 16622401	100.2
	%RSD	0.22	%RSD	0.78

TABLE.6.INTERMEDIATE PRECISION DATA FOR ASPIRIN AND PRASUGREL (100µg/ml)

Injections	Analyst-1				Analyst-2			
	Aspirin		prasugrel		Aspirin		Prasugrel	
	Peak area	% Assay	Peak area	% Assay	Peak area	% Assay	Peak area	% Assay
1	9817463	100.4	8293594	100.01	9776097	100.3	8220780	99.6
2	9688320	99.1	8278922	99.29	9706201	100.3	8294586	100.5
3	9773676	99.98	8318031	99.8	9762541	100.8	8256147	100.1
4	9733951	99.5	8232632	99.3	9715049	99.8	8234500	99.8
5	9848547	100.7	8203431	99.0	9685431	99.4	8245341	99.9
6	9821360	100.4	8284986	99.4	9724048	100.4	8232186	99.8
%RSD		0.61	%RSD	0.37	%RSD	0.49	%RSD	0.31

Robustness*:*

Even though by inducing variations in mobile phase composition, detection wavelength and flow rate, %RSD of the peak areas of Aspirin and Prasugrel was not more than 2.0% Hence, the analytical method is robust and is unaffected by small deliberate variations in the method parameters. The results were given in the **Table.8**.

TABLE.7. INTERMEDIATE PRECISION DATA FOR ASPIRIN AND PRASUGREL (100µg/ml)

Injections	Day-1				Day -2			
	Aspirin Peak area	% Assay	prasugrel Peak area	% Assay	Aspirin Peak area	% Assay	Prasugrel Peak area	% Assay
1	4503473	100.6	3726300	99.64	4455543	99.6	3708188	99.1
2	4473439	100.05	3709073	99.20	4426736	99.0	3756872	100.4
3	4458529	99.73	3749114	100.2	4455642	99.6	3728482	99.7
4	4464748	99.86	3758249	100.5	4446495	99.4	3760791	100.5
5	4485174	100.3	3715630	99.3	4482657	100.2	3726732	99.6
6	4496958	100.5	3733257	99.8	4477316	100.1	3748975	100.2
%RSD		0.35	%RSD	0.5	%RSD	0.44	%RSD	0.54

TABLE.8. ROBUSTNESS DATA FOR ASPIRIN AND PRASUGREL

Parameter	variation	Aspirin			Prasugrel		
		Mean Rt	Mean Peak Area	%RSD of Peak Areas	Mean Rt	Mean Peak Area	%RSD of Peak Areas
Flow rate (ml/min)	0.9	3.741	2443361	1.84	5.065	1868508	1.02
	1.0	3.386	2439364	0.93	4.835	1851181	0.71
	1.1	3.078	2466633	0.76	4.164	1863090	1.31
Wavelength	217nm	3.366	1938318	1.63	4.861	1668374	1.27
	220nm	3.384	2420403	1.20	4.848	1851181	0.71
	223nm	3.375	2036054	1.37	4.870	1443889	1.58
Mobile Phase (ACN: Buffer)	58:42	3.374	2477220	0.92	5.065	1857684	1.32
	60:40	3.386	2439364	0.93	4.835	1851181	0.71
	62:38	3.386	2460382	1.13	4.689	1877220	1.21

Stability of drug solutions:

Solution stability was estimated with standard concentration of 100µg/ml. The standard solution was injected and peak area values were recorded. This solution was then kept for 12hrs and injected in to HPLC and the peak areas were recorded. The same procedure was repeated at an interval of 12hrs until there was a significant change in the peak area value due to degradation (4%). The % degradation was estimated by comparing with peak areas with the areas of freshly prepared solutions. The drug solutions were found to be stable for about 24hrs from the time of preparation. At the 36^{th} hour significant amount was degraded. Hence, both the drug solutions prepared in the mobile phase were stable up to 36^{th} hourfrom the time of their preparation. The results were given below in the **Table.9**.

TABLE.9. SOLUTION STABILITY DATA FOR ASPIRIN AND PRASUGREL

Time of Data acquisition(hrs)	Aspirin		Prasugrel	
	Peak area	% Recovery	Peak area	% Recovery
0	4503473	100.6	3749114	100.2
12	4464748	99.8	3733257	99.8
24	4449862	99.54	3697907	98.9
36	4221362	96	3419177	96

CONCLUSION: The proposed method was found to be simple, rapid, precise, robust and accurate for the determination of Aspirin and Prasugrel in Tablet formulation. The sample recoveries from the formulation were in good agreement with the label claim, which suggests non-interference of formulation excipients in the estimation. The method was successfully validated in terms of

linearity, precision, accuracy and robustness as per ICH guidelines. The method provides a linear response across a wide range of concentrations. Moreover, the method is fast with respect to analysis time, covering wide concentration ranges when compared to reported chromatographic techniques.

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