Development and Validation of RP-HPLC Method for the simultaneous determination of Aspirin and Prasugrel Hydrochloride

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Abstract: A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Aspirin and Prasugrel HCl in pharmaceutical dosage form. Chromatographic separation of Aspirin and Prasugrel HCl was achieved on Waters Alliance-e2695 by using Symmetry Shield RP-18 150x4.6 mm, 3.5µ column and the mobile phase containing **0.1% TFA & ACN** in the ratio of **50:50% v/v**. The flow rate was 1.0 ml/min; detection was carried out by absorption at **226nm** using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Aspirin and Prasugrel HCl was NLT 2000 and should not more than **2** respectively. % Relative standard deviation of peak areas of all measurements always less than **2.0.** The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Aspirin and Prasugrel HCl.

Key words: HPLC Aspirin and Prasugrel HCl Development, Validation, ICH Guidelines.

Introduction:

Aspirin (ASP), 2-acetoxyBenzoic acid is cyclooxygenase inhibitor. The Molecular formula is $C_9H_8O_4$ and the molecular weight is 180.16 gm/mole. It is a Non-steroidal anti-inflammatory drug and inhibits platelet aggregation. Acetylsalicylic acid binds to and acetylates serine residues in cyclooxygenases, resulting in decreased synthesis of prostaglandin, platelet aggregation, and inflammation. This agent exhibits analgesic, antipyretic, and anticoagulant properties. It is used for heart diseases.

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. 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.

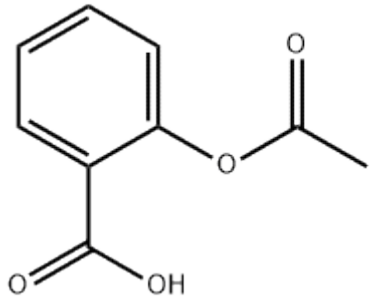

Figure 1 - Structure of Aspirin

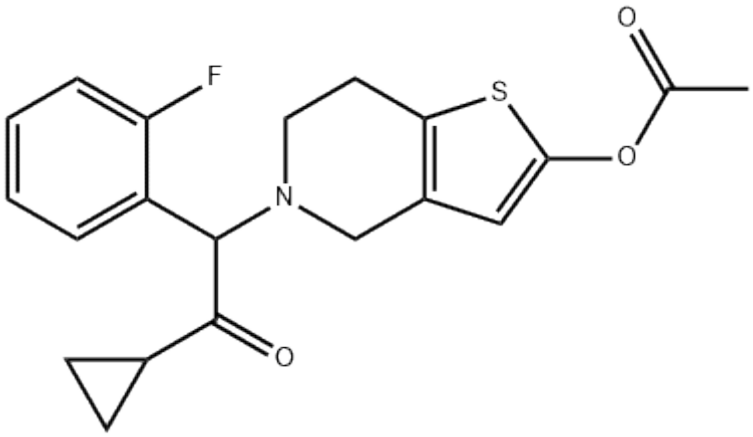

Figure 2 – Structure of Prasugrel

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.

Experimental study:

Materials and methods:

Reagents and chemicals: The reagents are HPLC grade Acetonitrile as diluent and 0.1% tri fluoro acetic acid(TFA) as buffer.

Preparation of standard solution

Accurately weigh and transfer 75mg of Aspirin, 10mg of Prasugrel HCl working standard into a 10 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (750ppm of Aspirin, 100ppm of Prasugrel HCl)

Sample Solution Preparation:

Accurately weighed and transfer 342mg of sample into a 10mL clean dry volumetric flask add diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter. (Stock solution). Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (750 ppm of Aspirin, 100ppm of Prasugrel HCl)

The Aspirin peak was observed at 2.336 min with peak area 2537472, tailing factor 1.12, Prasugrel HCl peak was observed at 4.873 min, with peak area 362893, tailing factor 1.04 and resolution 10.21. This trial was optimized.

General preparations

Preparation of Mobile Phase:

Mobile phase was prepared by mixing 0.1%TFA and ACN taken in the ratio 50:50. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of Diluent:

Mobile Phase used as a diluent.

System suitability:

Tailing factor for the peaks due to Aspirin and Prasugrel HCl in Standard solution should not be more than 2.0. Theoretical plates for the Aspirin and Prasugrel HCl peaks in Standard solution should not be less than 2000. Resolution for the Aspirin and Prasugrel HCl peaks in standard solution should not be less than 2.

Validation Process:

System Precision

The system's performance has been validated through assessment of device suitability parameters. Limits were found to be met for a variety of parameters, including plate count, tailing, and RSD percentage.

Specificity

Being able to identify and test a given analyte in the presence of other elements required to be combined in the Standard and the standard solution is known as specificity. Blank Standards and those with Aspirin and Prasugrel HCl will be tested using chromatograms.

Accuracy

Being close to the real meaning of the technique is what defines accuracy. Three concentrations will be used to test the recovery trials. The drug's quantity, percentage of recovery, and standard deviations were calculated after every injection at each level.

Precision

It is the level of agreement between the various test results that determines the precision of the analytical methodology. Researchers examined the effects of sampling a homogeneous population more than once. The current process was evaluated in terms of its ability to provide repeatable, intraday, and inter-day results. It was examined by sampling the materials on the same day and over the course of different days.

Linearity

Linearity is the feature of analytical process which allows for a direct proportion of analytical results in response to a certain concentration of the analyte in the Standard. A total of seven series of standard solutions were selected for the assessment of the linearity spectrum. The calibration curve was drawn by comparing regular solution concentration

with peak area. Using the least square method, the slope, intercept, and coefficient of correlation were calculated.

Robustness

Robustness refers to a procedure's resistance to small process parameter changes, as well as its reliability in normal operation. An organic solution was introduced into the HPLC system for a robustness analysis, and the chromatographic settings (such as flow rate and mobile-phase organic content) were modified. The separation factor, retention time, and peak asymmetry were determined by evaluating the effects of altered parameters.

Results and Discussion:

The aim of this study is to validate a stability indicating HPLC method for simultaneous estimation of Aspirin and Prasugrel HCl in bulk and multi branded formulations. According to the UV spectra of these compounds, an appropriate wavelength for simultaneous estimation of two drugs was chosen.

Determination of Working Wavelength (λ_{max}):

In simultaneous estimation of two drugs isobestic wavelength was used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are inter convertible. So this wavelength was used in simultaneous estimation to estimate two drugs accurately.

The wavelength of maximum absorption of the solution of the drugs in mixture of Acetonitrile and 0.1% TFA (50:50) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% TFA (50:50) as blank. The absorption curve shows isobestic point at 226nm. Thus 226 nm was selected as detector wavelength for the HPLC chromatographic method.

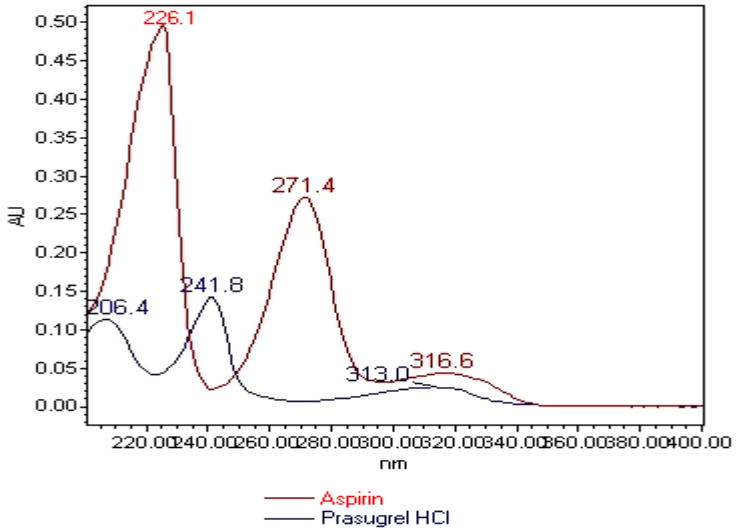

Fig No.:3 PDA - Spectrum of Aspirin & Prasugrel HCl

Chromatographic conditions:

During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method. Conditions for optimized chromatography are provided in table 1.

Table 1: Optimized chromatographic conditions

Parameters	Observation
Instrument used	Waters HPLC with auto sampler and PDA detector.
Injection volume	10µl
Mobile Phase	Acetonitrile and 0.1% TFA (50:50)
Column	Symmetry Shield RP-18 250x4.6 mm, 3.5µ
Detection Wave Length	226nm
Flow Rate	1 mL/min
Runtime	6min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

System suitability:

To attain results, the following device suitability parameters were established after six consecutive injections of normal solution: theoretical plate number, time, peak area, tailing factor, and resolution. All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The chromatogram in Figure 4 was the representative of the suitability results.

Table: 2 System suitability parameters for Aspirin & Prasugrel HCl

S.No	Parameter	Aspirin	Prasugrel HCl
1	Retention time	2.332	4.879
2	Plate count	5438	6628
3	Tailing factor	1.25	1.09
4	Resolution	----	10.24
5	%RSD	0.61	0.37

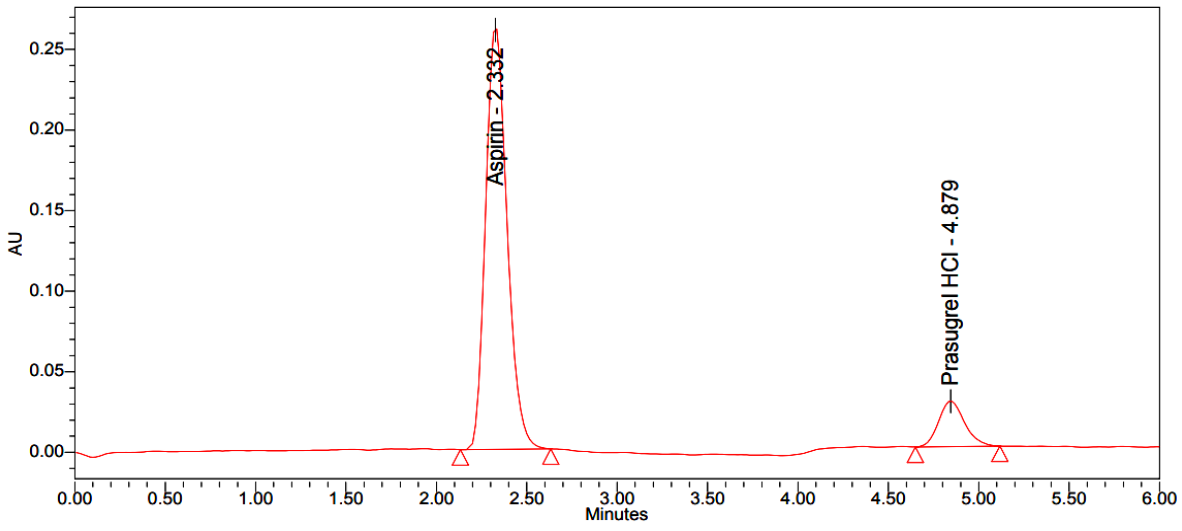

Fig No.4: Chromatogram of standard

Analytical method validation (HPLC)

The method was validated for its linearity range, accuracy, precision, and specificity. Method validation was carried out as per ICH guidelines.

Specificity:

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drugs was specific**.**

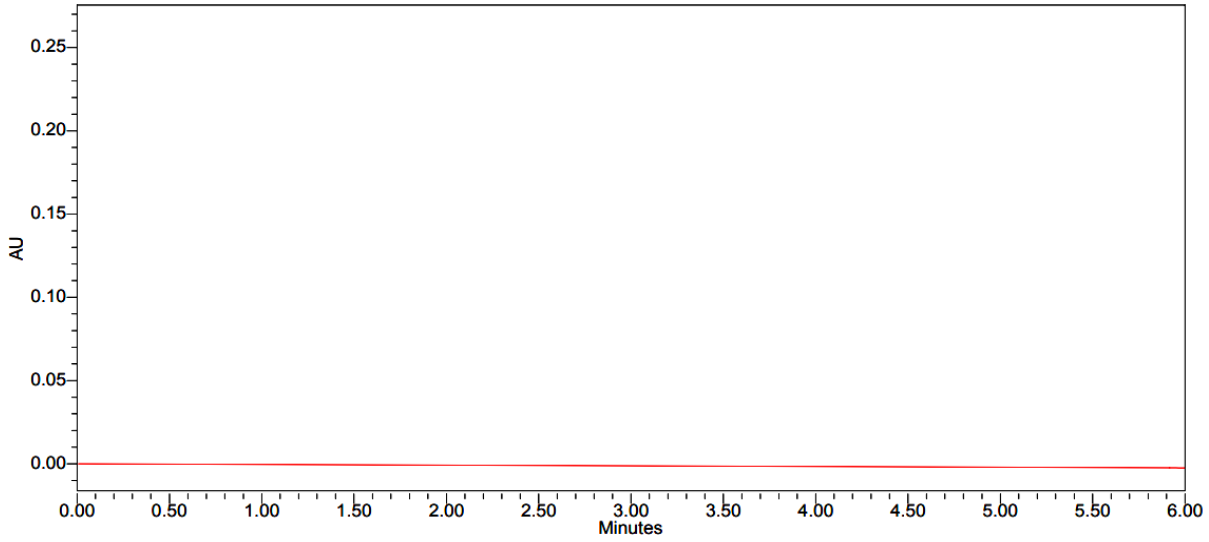

Fig No.5: Chromatogram of blank

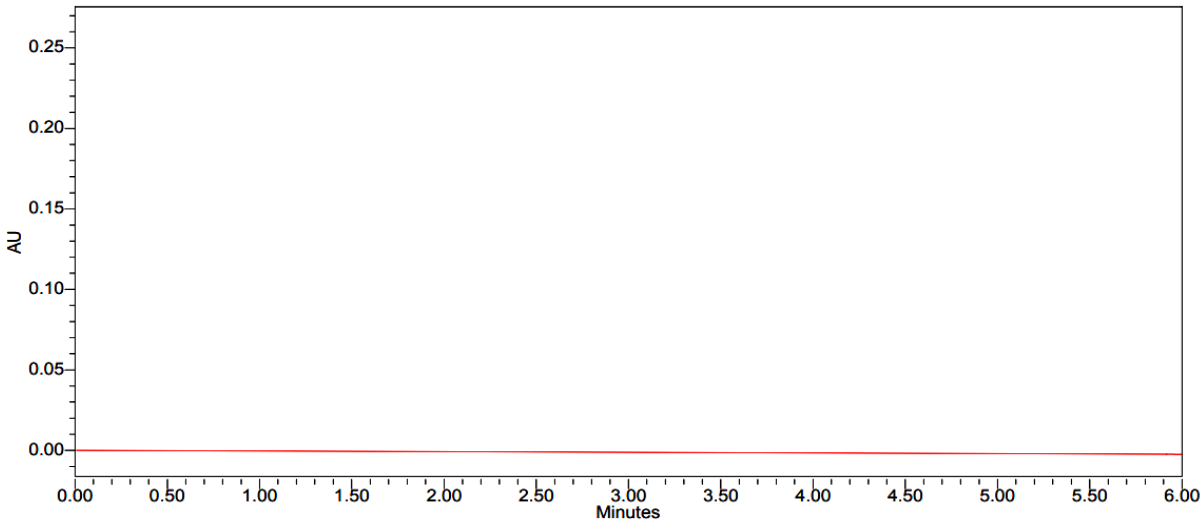

Fig No.6: Chromatogram of placebo

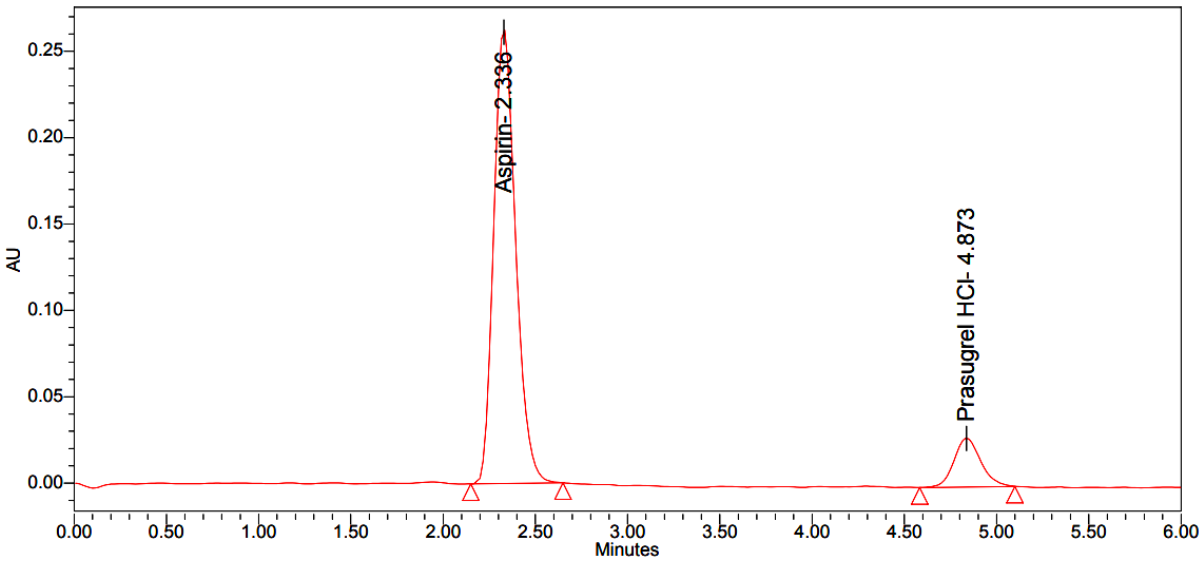

Fig. No. 7: Optimized chromatogram

Discussion:

Retention times of Aspirin and Prasugrel HCl were 2.336 min and 4.873min respectively as in Fig No 7. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this methodI.e., Fig No 5&6. So this method was said to be specific.

Linearity:

Accurately weigh and transfer 75mg of Aspirin, 10mg of Prasugrel HCl working standard into a 10 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. Linearity results were demonstrated in table 3.

Table No.3: Results of linearity for Aspirin & Prasugrel HCl

S.NO	Aspirin		Prasugrel HCl	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	187.50	639653	25.00	93123
2	375.00	1295299	50.00	189437
3	562.50	1963147	75.00	274437
4	750.00	2541563	100.00	363524
5	937.50	3188539	125.00	451032
6	1125.00	3770639	150.00	549932

Regression equation	y = 3363.40x +22410.75	y =3628.14x + 2387.04
Slope	3363.04	3628.14
Intercept	22410.75	2387.04
R^2	0.9998	0.9998

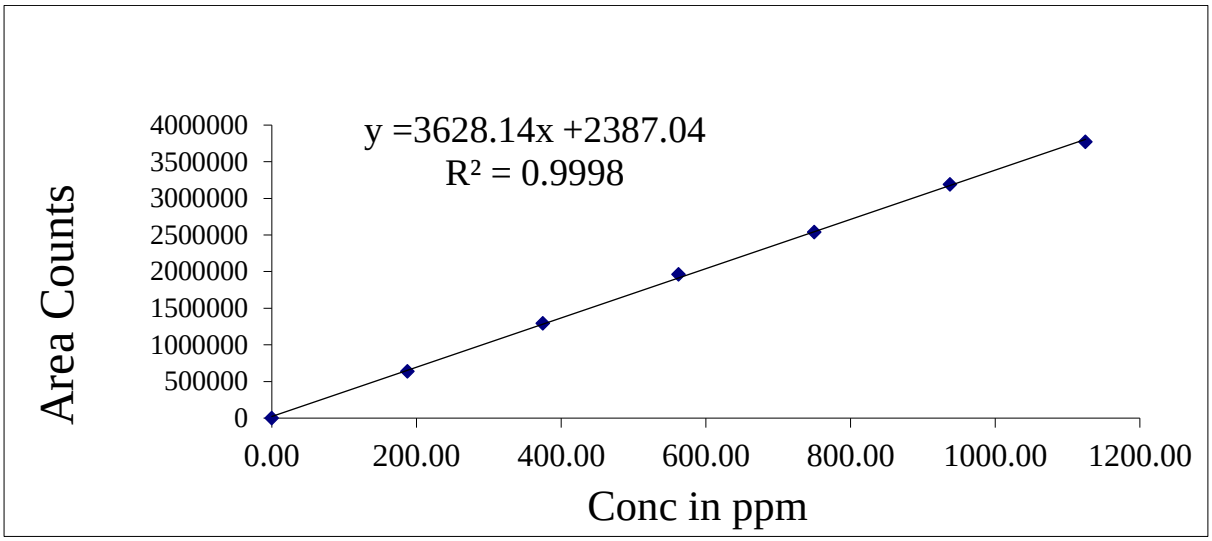

Fig No.8: Calibration curve for Prasugrel HCl at 226nm

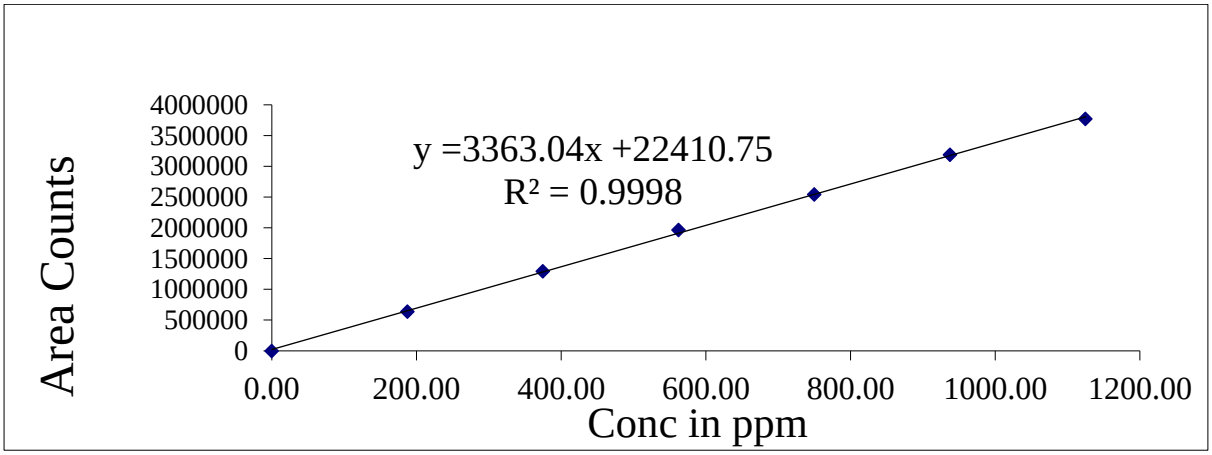

Fig No.9: Calibration curve for Aspirin at 226 nm

Precision

Precision is the degree of repeatability of an analytical method under normal operation conditions.In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD. The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 750ppm of Aspirin, 100pm of Prasugrel HCl). The % RSD for the absorbance of six replicate injections results should not be more than 2%. Table 4 shows the method precision results.

Table 4: System precision table of Aspirin & Prasugrel HCl

S. No	Concentration Aspirin (µg/ml)	Area of Aspirin	Concentration of Prasugrel HCl (µg/ml)	Area of Prasugrel HCl
1.	750	2537472	100	362893
2.	750	2545965	100	364560
3.	750	2512625	100	361950
4.	750	2530410	100	363591
5.	750	2505364	100	365786
6.	750	2520205	100	364268
Mean	2525340		363841	
S.D	15397.15		1343.09	
%RSD	0.61		0.37	

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.61% and 0.37% respectively for Aspirin and Prasugrel HCl. As the limit of Precision was less than “2” the system precision was passed in this method.

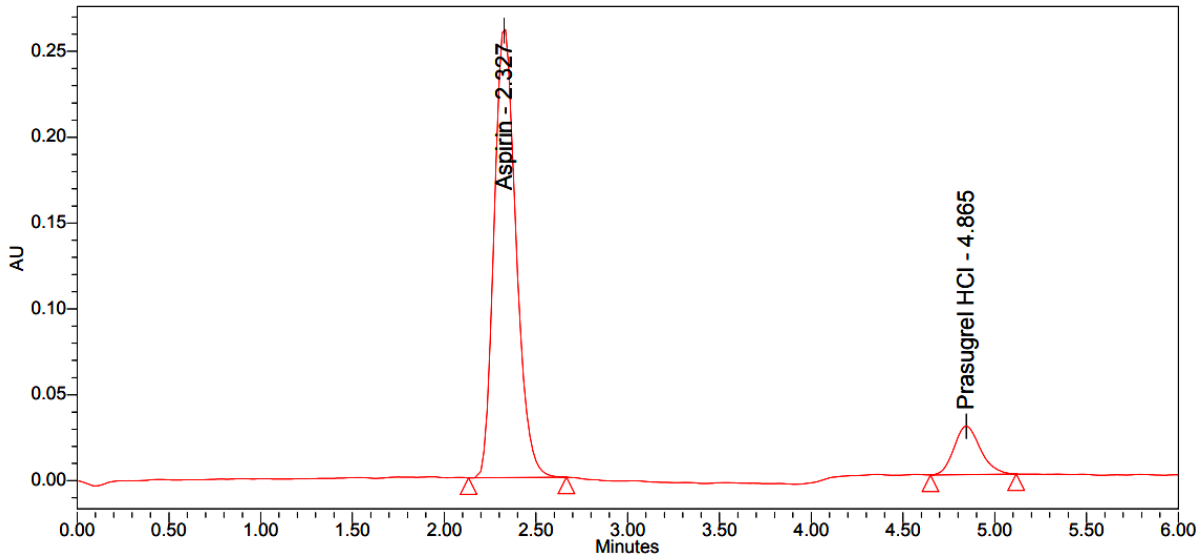

Figure 10. Chromatogram of method precision

Intermediate Precision (Ruggedness)

The % RSD for the area of six standard injections results should not be more than 2%. Intermediate precision results were shown in table 5.

Table No 5: Intermediate Precision (Analyst variation) for Aspirin and Prasugrel HCl by RP-HPLC method

S. No.	Area of Aspirin	Area of Prasugrel HCl
1	2524786	363430
2	2532367	362320
3	2571542	360461
4	2515143	362312
5	2556357	365761
6	2542687	364524
Average	2540480	363135
Standard Deviation	20860.891	1865.693
%RSD	0.82	0.51

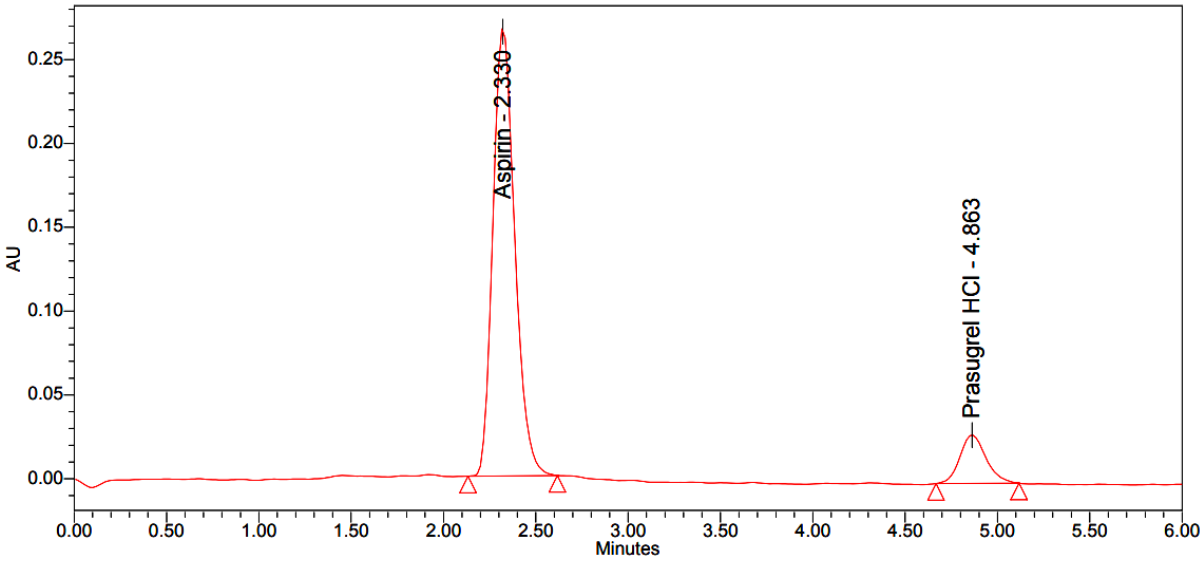

Fig. 11 : Inter day precision chromatogram

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 750ppm of Aspirin, 100ppm of Prasugrel HCl was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The variation of Organic Phase ratio 45:55, 50:50 & 55:45:

Standard solution of 750ppm of Aspirin, 100ppm of Prasugrel HCl was prepared and analysed using the varied in mobile phase ratio. Table 6 & 7 presents the robustness results of Aspirin and Prasugrel HCl by RP-HPLC.

Table No.6 : Robustness results of Aspirin by RP-HPLC

Parameter	Aspirin					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow (0.9ml)	2.545	2741736		1.16	5546
	Actual (1ml)	2.336	2537472		1.12	5482
	More flow (1.1ml)	2.088	2242709		1.07	5374
Organic Phase	Less Org	2.832	2836811		1.18	5571

change	(45:55)					
	Actual (50:50)	2.332	2545965		1.20	5438
	More Org (55:45)	1.926	2091340		1.11	5359

Table No.7: Robustness results of Prasugrel HCl by RP-HPLC

Parameter	Prasugrel HCl					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change (mL/min)	Less flow (0.9ml)	5.056	384210	9.89	1.10	6704
	Actual (1ml)	4.873	362893	10.21	1.04	6615
	More flow (1.1ml)	4.605	321542	10.05	1.00	6548
Organic Phase change	Less Org (45:55)	5.336	404417	9.92	1.13	6718
	Actual (50:50)	4.879	364560	10.23	1.09	6628
	More Org (55:45)	4.324	306259	9.86	1.02	6523

Conclusion:

In this proposed HPLC method for the selected drugs showed good linearity. Results for the recoveries of selected drugs were found to be within limits (98 – 102 %). These indicate that the proposed method was accurate for the analysis.

The developed HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

The sample recoveries were in good agreement with their respective label claims and they suggested non interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.

Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Aspirin and Prasugrel HCl.

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