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

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INTRODUCTION

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon,

nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies

The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays

The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and Applied research

The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

METHODOLOGY

Validation parameters

Validation

Blank

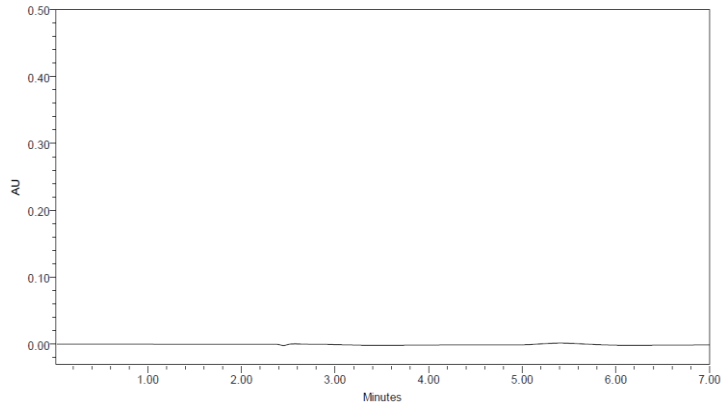

Fig: Chromatogram showing blank (Mobile phase preparation).

System suitability

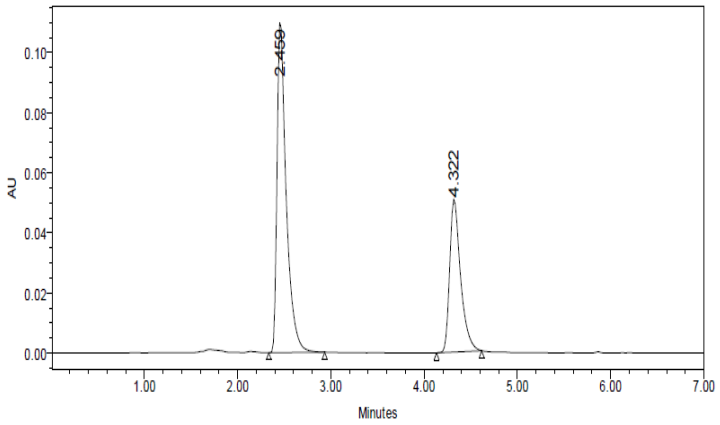

Fig: Chromatogram showing injection -1.

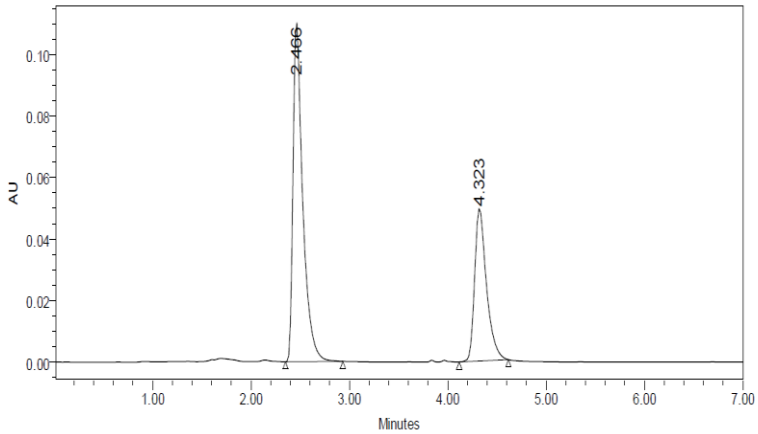

Fig: Chromatogram showing injection -2.

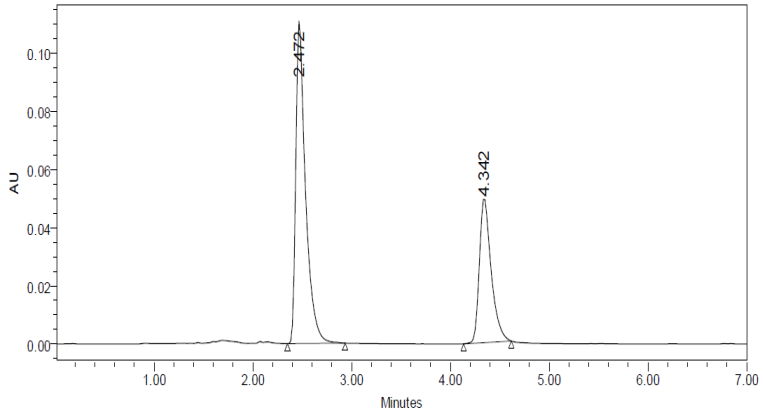

Fig: Chromatogram showing injection -3.

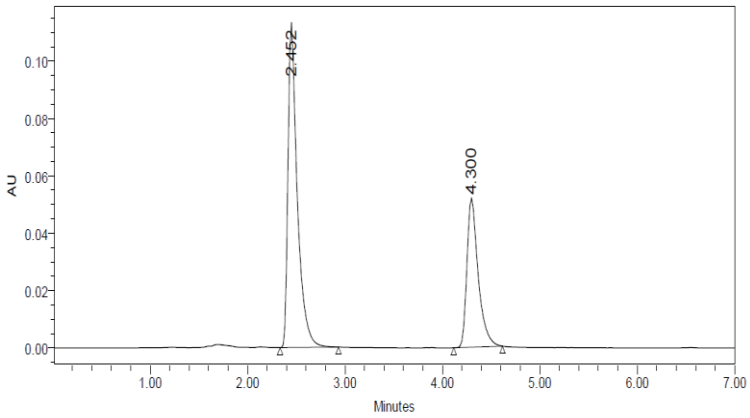

Fig: Chromatogram showing injection -4.

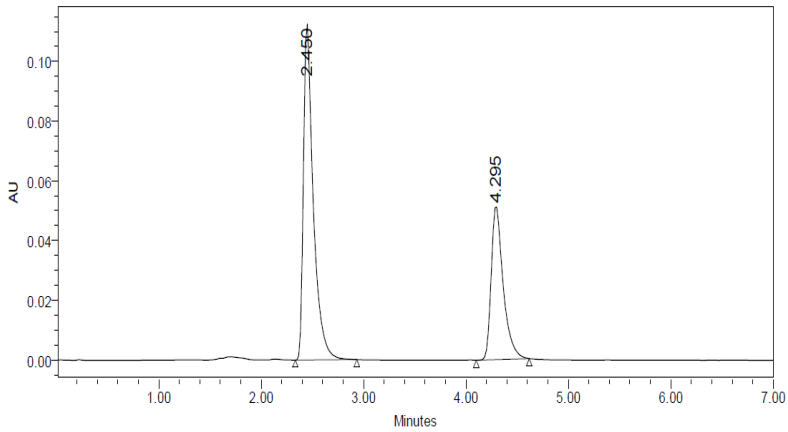

Fig: Chromatogram showing injection -5.

Table: Results of system suitability for Aspirin.

S. no.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.459	602561	111160	5123	1.4
2	Aspirin	2.466	600543	53992	5023.2	1.4
3	Aspirin	2.472	601288	55420	5061.3	1.3
4	Aspirin	2.452	600776	112478	5147.3	1.6

5	Aspirin	2.450	600758	111779	5101.8	1.7
Mean			601185.2			
Std. Dev			816.3576			
% RSD			0.13			

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Prasugrel.

S. no.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.322	422674	50988	5949	1.5	3.2
2	Prasugrel	4.323	424692	49813	5890.0	1.5	3.3
3	Prasugrel	4.342	421255	49826	5952.5	1.4	3.2
4	Prasugrel	4.300	415235	51804	5926.4	1.50	3.2
5	Prasugrel	4.295	416260	51274	5898.5	1.49	3.2
Mean			420023.2				
Std. Dev			724.7845				
% RSD			0.17				

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Aspirin and Prasugrel in drug product.

Assay (Standard)

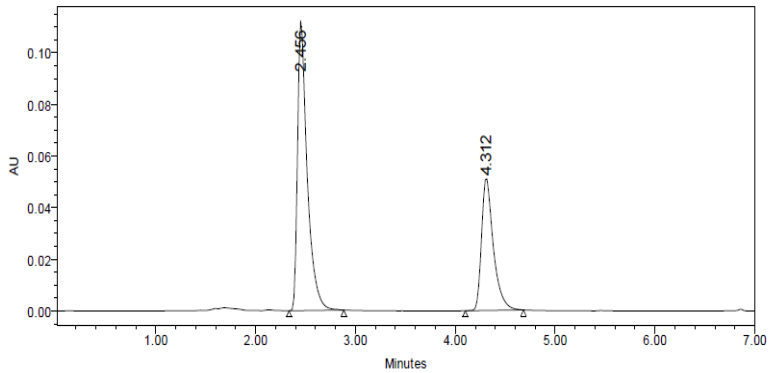

Fig: Chromatogram showing assay of standard injection -1.

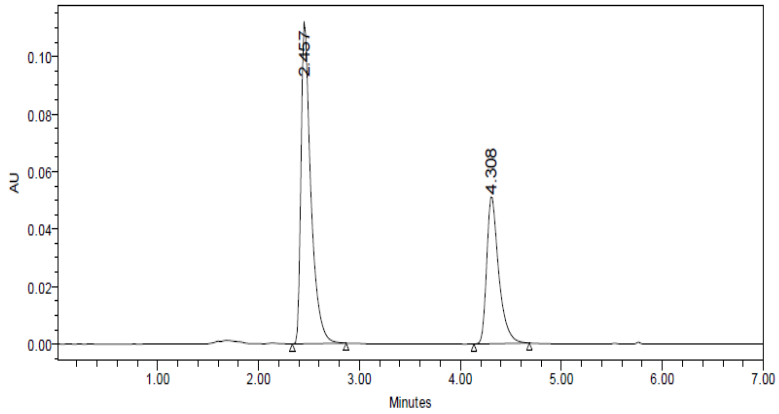

Fig. Chromatogram showing assay of standard injection -2.

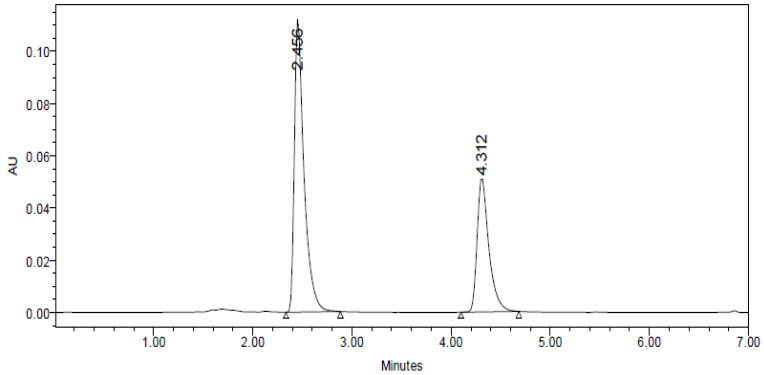

Fig: Chromatogram showing assay of standard injection -3.

Table: Peak results for assay standard.

S. no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Aspirin	2.456	600122	112157		1.5	5023	1
2	Prasugrel	4.312	420842	51068	3.3	1.4	5946	1
3	Aspirin	2.457	600205	112399		1.2	5149	2
4	Prasugrel	4.308	422034	51511	3.3	1.4	5848	2

5	Aspirin	2.456	600213	11201		1.5	5046	3
6	Prasugrel	4.312	420191	52014	3.2	1.5	5941	3

Assay (Sample)

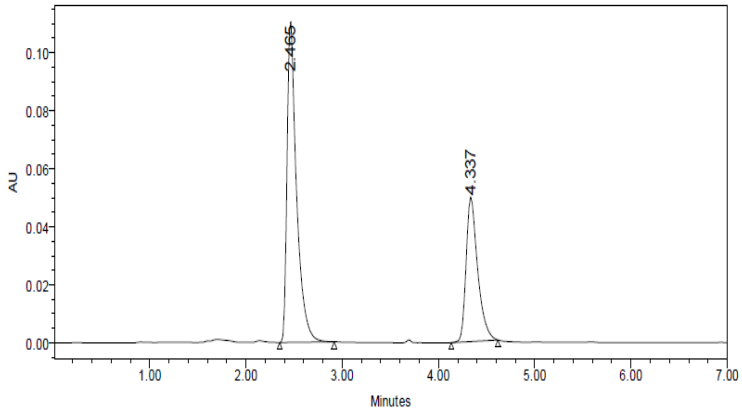

Fig: Chromatogram showing assay of sample injection-1.

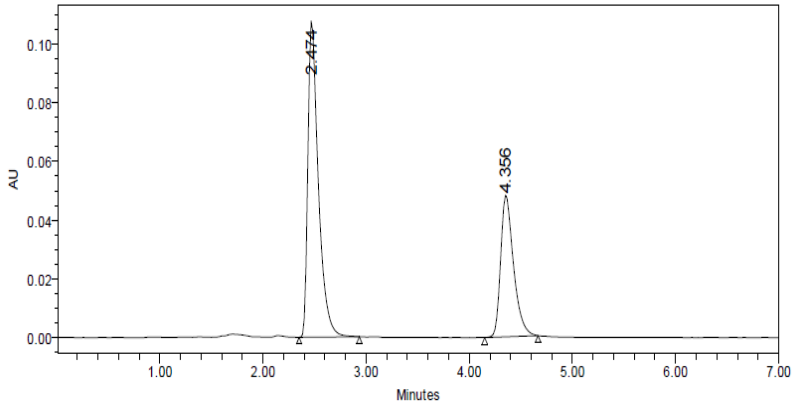

Fig. Chromatogram showing assay of sample injection-2.

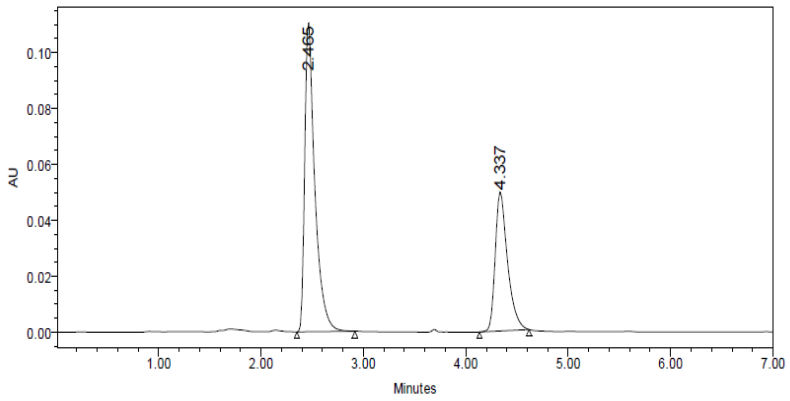

Fig: Chromatogram showing assay of sample injection-3.

Table: Peak results for assay sample.

S. no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Aspirin	2.465	601812	110102		1.6	5028	1
2	Prasugrel	4.337	414764	49842	3.2	1.5	5949	1
3	Aspirin	2.474	600435	108333		1.6	5189	2
4	Prasugrel	4.356	418130	48360	3.3	1.5	5818	2
5	Aspirin	2.465	600212	112453		1.6	5061	3
6	Prasugrel	4.337	413645	48641	3.2	1.5	5812	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 600819.7/600180×10/150×150/0.0265×99.7/100×0.2655/100×100

= 99.7%

The % purity of Aspirin and Prasugrel in pharmaceutical dosage form was found to be 99.7 %.

Linearity

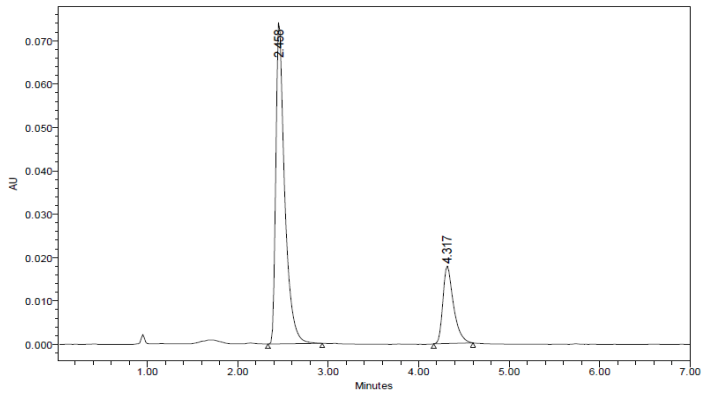

Figure: Chromatogram for linearity concentration-50µg/ml of Aspirin& 5 µg/ml of Prasugrel.

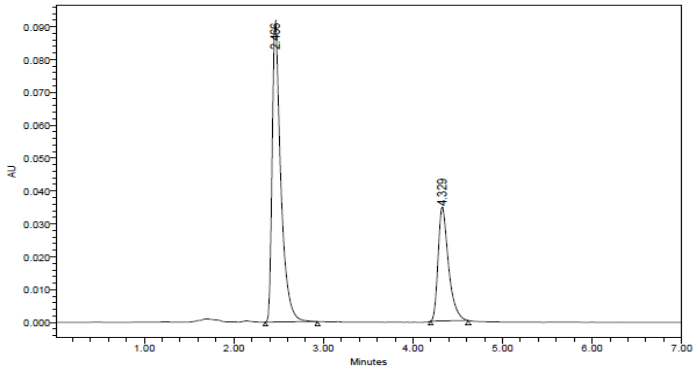

Figure: Chromatogram for linearity concentration-100 µg/ml of Aspirin & 10µg/ml of Prasugrel.

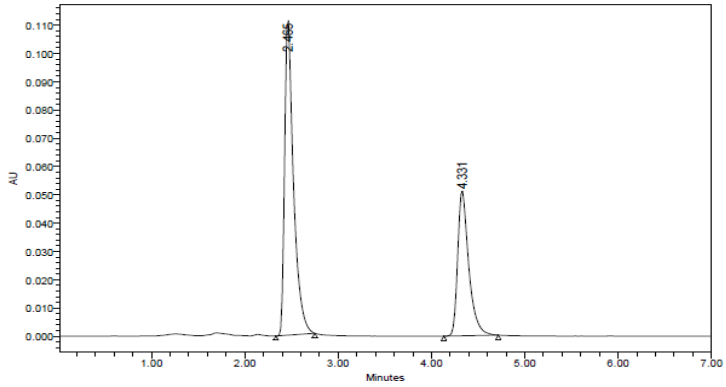

Figure: Chromatogram for linearity concentration-150 µg/ml of Aspirin & 15µg/ml of Prasugrel.

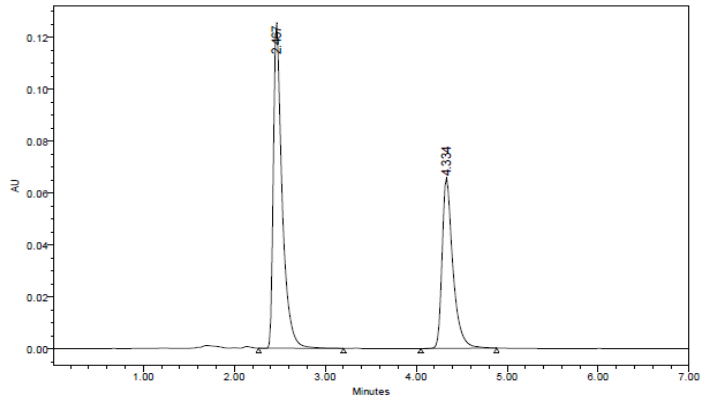

Figure: Chromatogram for linearity concentration-200 µg/ml of Aspirin & 20 µg/ml of Prasugrel.

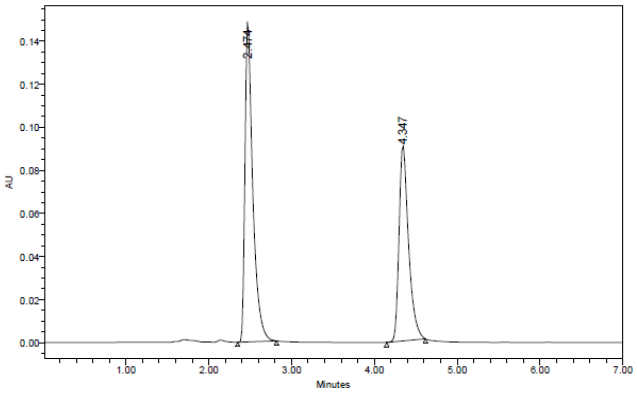

Figure: Chromatogram for linearity concentration-250 µg/ml of Aspirin & 25 µg/ml of Prasugrel.

Chromatographic data for linearity study

Aspirin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	50	215760
66.6	100	417001
100	150	600435
133.3	200	791969
166.6	250	974736

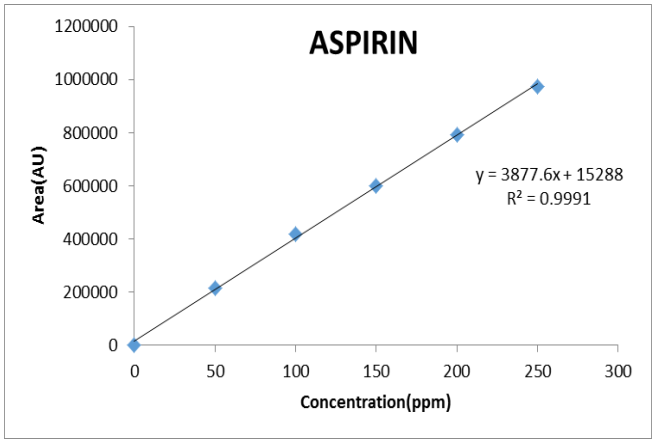

Figure 6.3.4 calibration graph for Aspirin.

Linearity plot

The plot of Concentration (x) versus the Average Peak Area (y) data of DRUG is a straight line.

Y = mx + c

Slope (m) = 3877

Intercept (c) = 15288

Correlation Coefficient (r) = 0.999

Validation criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 15288. These values meet the validation criteria.

Prasugrel

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	145474
66	10	279372
100	15	421045
133	20	562151
166	25	721671

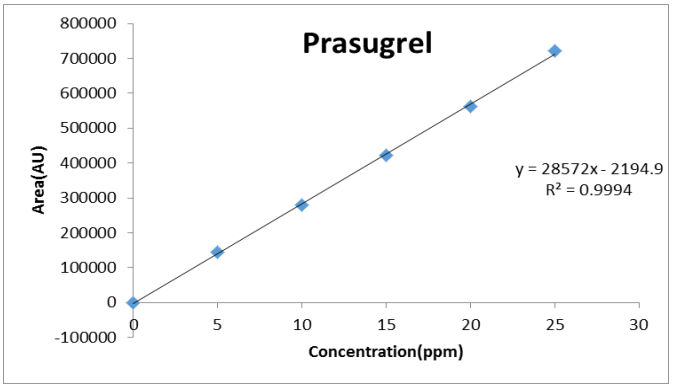

Figure 6.3.4 calibration graph for Prasugrel.

Linearity plot

The plot of Concentration (x) versus the Average Peak Area (y) data of DRUG is a straight line.

Y = mx + c

Slope (m) = 28572

Intercept (c) = 2194

Correlation Coefficient (r) = 0.999

Validation criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 2194. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

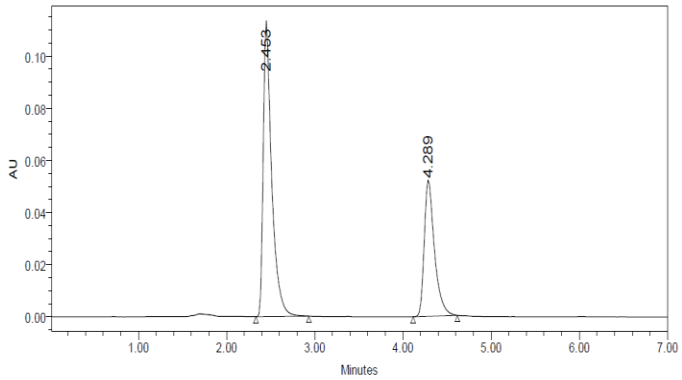

Fig. Chromatogram showing precision injection -1.

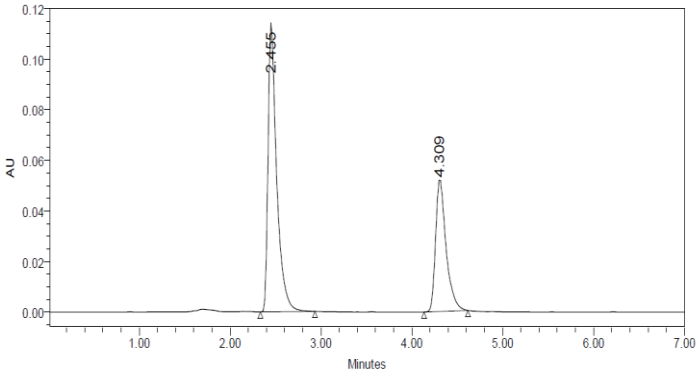

Fig. Chromatogram showing precision injection -2.

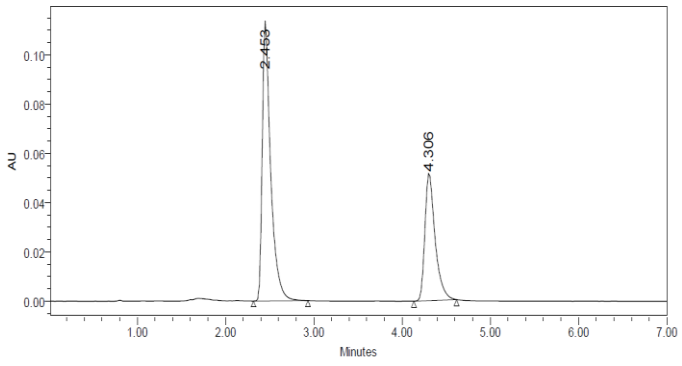

Fig. Chromatogram showing precision injection -3.

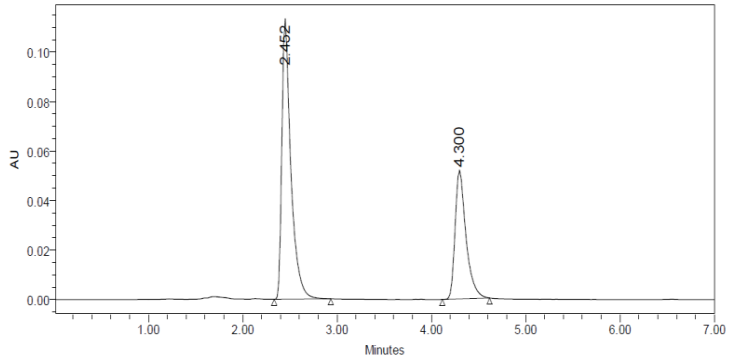

Fig. Chromatogram showing precision injection -4.

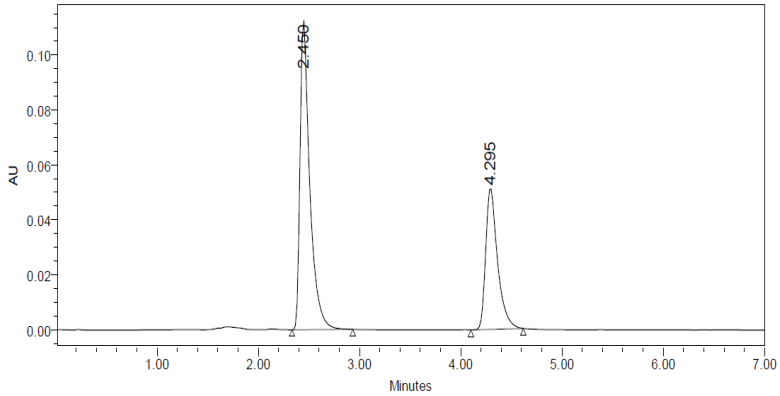

Fig. Chromatogram showing precision injection -5.

Table: Results of repeatability for Aspirin.

S. no.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.453	603403	112688	5881.3	1.4
2	Aspirin	2.455	608107	113637	5844.1	1.3
3	Aspirin	2.453	607266	112849	5918.1	1.3
4	Aspirin	2.452	608776	112478	5847.3	1.4
5	Aspirin	2.450	609758	111779	5801.8	1.5
Mean			607462			
Std. Dev			2445.82			
% RSD			0.40			

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of method precession for prasugrel.

S. No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.289	429183	52411	5050.9	1.49	3.2
2	Prasugrel	4.309	416643	52475	5084.8	1.5	3.2
3	Prasugrel	4.306	424052	51841	5000.1	1.4	3.2
4	Prasugrel	4.300	425235	51804	5026.4	1.51	3.2
5	Prasugrel	4.295	416260	51274	5098.5	1.51	3.2
Mean			422274.6				
Std. Dev			5646.668				
% RSD			1.3				

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day 1:

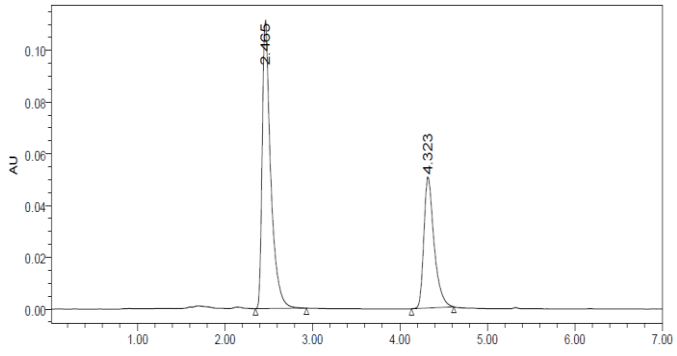

Fig: Chromatogram showing Day1 injection -1.

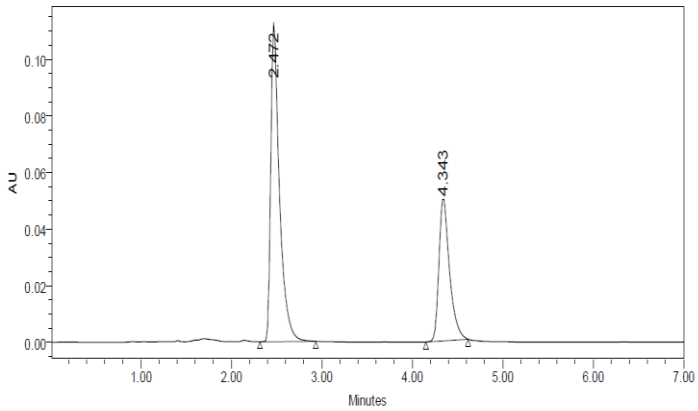

Fig: Chromatogram showing Day1 injection -2.

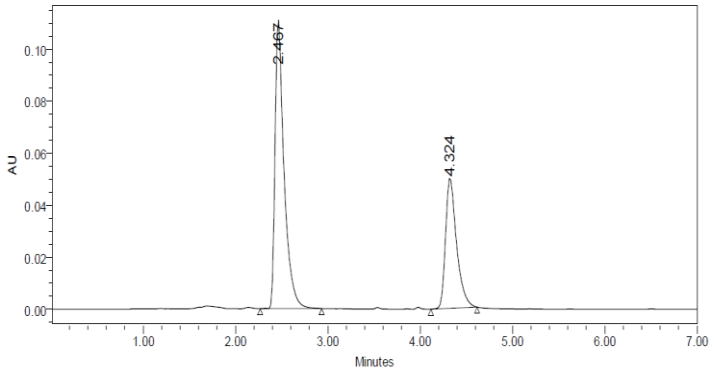

Fig: Chromatogram showing Day1 injection -3.

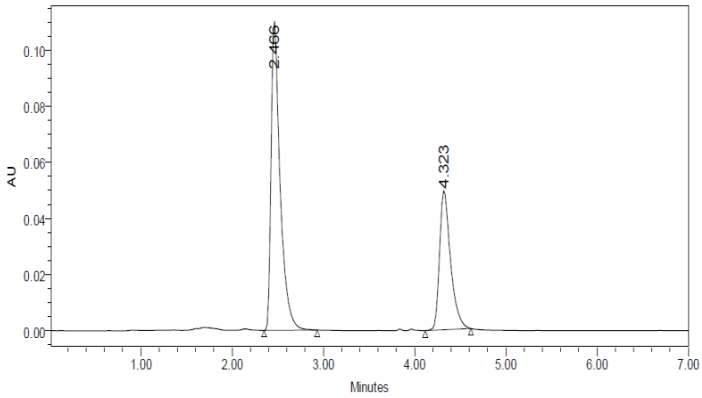

Fig: Chromatogram showing Day1 injection -4.

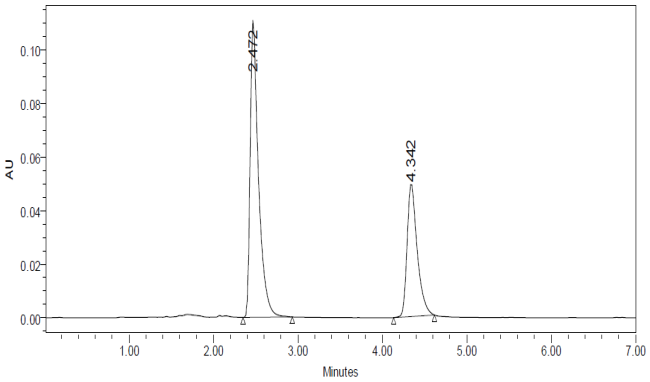

Fig: Chromatogram showing Day1 injection -5.

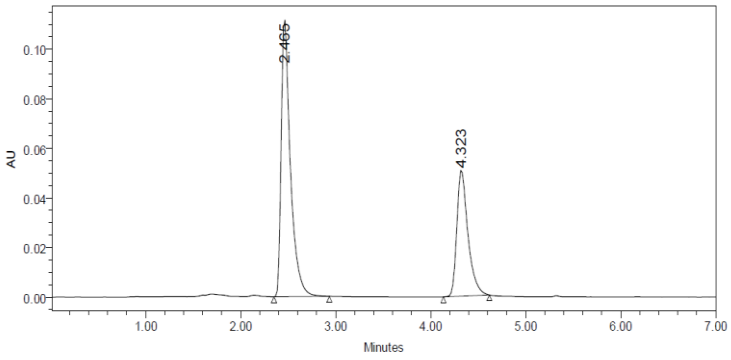

Fig: Chromatogram showing Day1 injection -6.

Table: Results of Intermediate precision for Aspirin.

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.465	602386	111226	5075.9	1.5
2	Aspirin	2.472	608118	112497	5043.2	1.3
3	Aspirin	2.467	605566	110347	5029.9	1.5
4	Aspirin	2.466	608543	53992	5023.2	1.4
5	Aspirin	2.472	609288	55420	5061.3	1.4
6	Aspirin	3.424	607315	54154	5078.4	1.3
Mean			606869.3			
Std. Dev			2538.025			
% RSD			0.41			

Acceptance criteria

- %RSD of five different sample solutions should not more than 2

Table: Results of Intermediate precision for Prasugrel.

S. no.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.323	422252	50991	5886.2	1.6	3.2
2	Prasugrel	4.343	418090	50664	5947.5	1.5	3.2
3	Prasugrel	4.324	424361	50295	5907.8	1.55	3.2
4	Prasugrel	4.323	424692	49813	5890.0	1.50	3.2
5	Prasugrel	4.342	411255	49826	5852.5	1.49	3.2
6	Prasugrel	4.323	422252	50991	5756.8	1.50	3.2
Mean			420483.7				
Std. Dev			5096.974				
% RSD			1.2				

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2

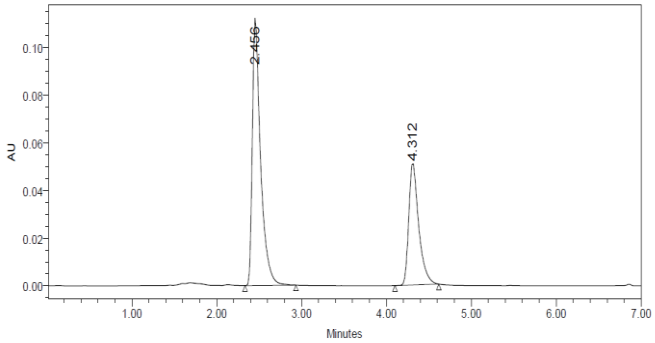

Fig: Chromatogram showing Day 2 injection -1.

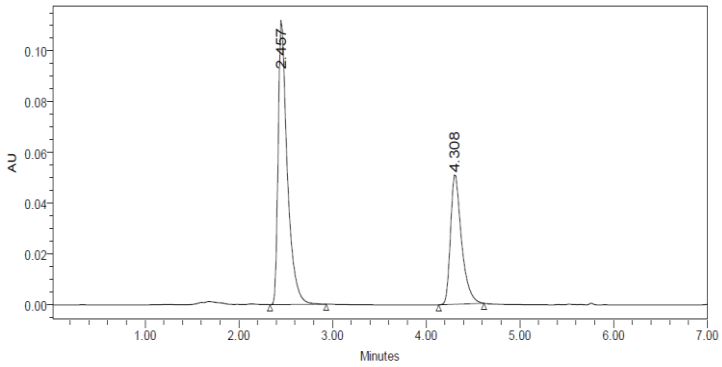

Fig: Chromatogram showing Day 2 injection -2.

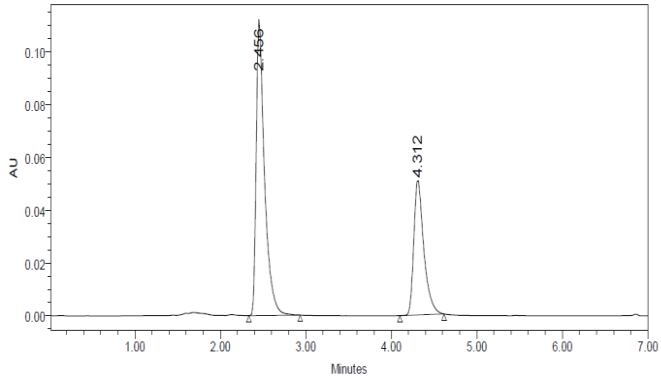

Fig: Chromatogram showing Day 2 injection -3.

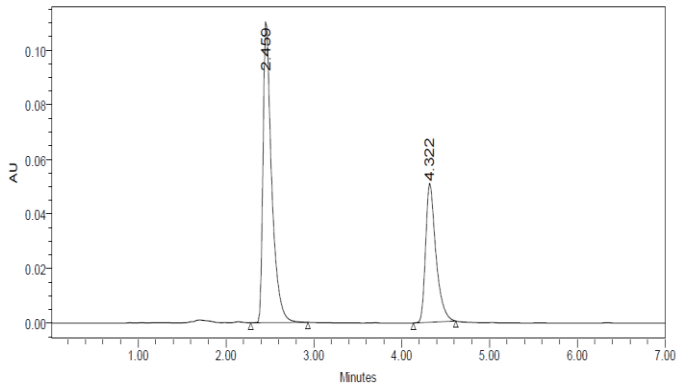

Fig: Chromatogram showing Day 2 injection -4.

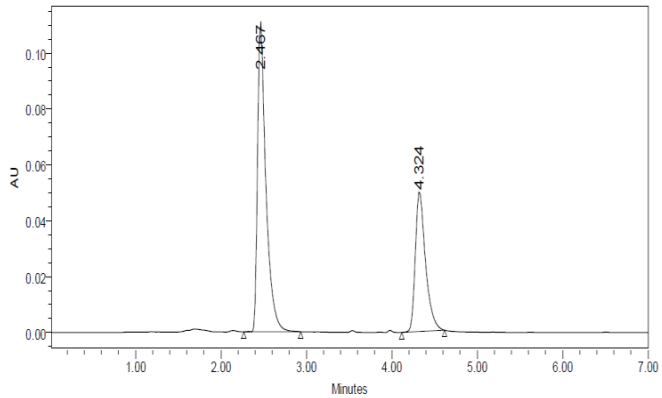

Fig: Chromatogram showing Day 2 injection -5.

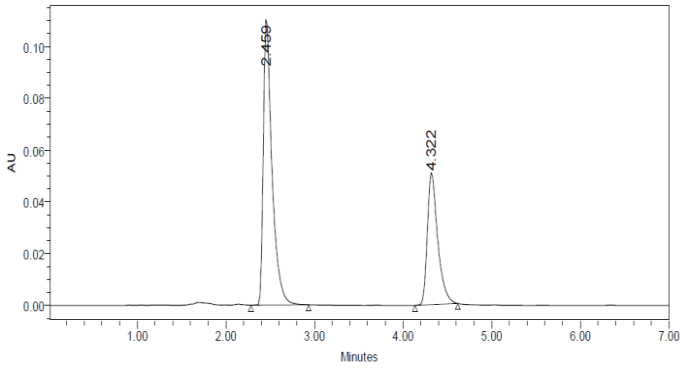

Fig: Chromatogram showing Day 2 injection -6.

Table: Results of Intermediate precision Day 2 for Aspirin.

S. no.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aspirin	2.456	602581	112175	5013	1.7
2	Aspirin	2.457	600985	112422	5007	1.7
3	Aspirin	2.456	600145	114513	5198	1.8
4	Aspirin	2.459	600332	111580	5246	1.7
5	Aspirin	2.467	600566	110347	5096	1.8
6	Aspirin	2.459	600332	111580	5178	1.8
Mean			600823.5			
Std. Dev			908.2622			
% RSD			0.15			

Acceptance criteria

- %RSD of five different sample solutions should not more than 2

Table: Results of Intermediate precision for Prasugrel

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Prasugrel	4.312	425263	50936	5981	1.5	3.2
2	Prasugrel	4.308	427069	51400	5887	1.49	3.2
3	Prasugrel	4.312	424231	51236	5928	1.5	3.2
4	Prasugrel	4.322	423569	51084	5898	1.50	3.2
5	Prasugrel	4.324	414361	50295	5887	1.5	3.2
6	Prasugrel	4.322	413569	51084	5940	1.5	3.2
Mean			421343.7				
Std. Dev			5841.789				
% RSD			1.38				

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 238nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry C_{18} because it was giving good peak.

40ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Acetonitrile: water (35:65% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 7min because analyze gave peak around 2.456, 4.312 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 50-250µg/ml of Aspirin and 5-25 µg/ml of Prasugrel of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Aspirin and Prasugrel in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Aspirin and Prasugrel was freely soluble in ethanol, methanol and sparingly soluble in water. Acetonitrile: water (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Aspirin and Prasugrel in bulk drug and in Pharmaceutical dosage forms.

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