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Development of Some Analytical Methods for the Estimation of Aspirin and Ticagrelor Combined Formulation

Prince Yadav[1], Dr Vikas Kumar Mishra[2], Ravish Sahu[3], Ajay Singh Thakur[3], Yogesh Sharma[3], Ramdarshan Parashar[3], Yogesh Tiwari[4]

[1,3]*Assistant Professor, Vedic Institute of Pharmaceutical Education & Research, Sagar (MP)-470001*
[2]*Associate Professor, Ojaswini College of Pharmacy, Sagar (MP)-470001*
[4]*Assistant Professor, Shri Rawatpura Sarkar College, Sagar (MP)-470001*
Corresponding author: Yogesh Tiwari

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ABSTRACT
The quantitative analysis of the active constituents is an essential part for developing a pharmaceutical dosage form. Any changes in the quality or purity of the drug adversely affect the therapeutic value. Therefore, there is a need to develop better and reliable methods for the estimation of drug in pharmaceutical dosage form.
Present work is to develop simple, accurate, rapid & validated stability indicating RP-HPLC method for the simultaneous estimation of Aspirin and Ticagrelor in marketed formulation as per ICH guidelines.
It was also planned to validate the developed methods. In proposed project work we shall attempt to develop simple, accurate and precise UV spectroscopic and High Performance (HPLC) method for analysis of these drugs in the bulk and marketed formulations. Finally the developed methods will be validated for its reproducibility and robustness as well as for statistic.
The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can besuccessfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work
Keywords: RP-HPLC, Robustness, Reproducibility, Validation, accurate

I. INTRODUCTION

Analytical Chemistry is a vital part of pharmaceutical chemistry. It involves separating, identifying and determining the relative amounts of the component in the sample. Analytical method validation is the next important step in justification and acceptability of an analytical method, after method development. It enables scientists to communicate scientifically and effectively on technical matters. Set standards of evaluation procedures for checking compliance and taking remedial measures

High performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC separations involve both the mobile phase and the stationary phase. The amount of water in an HPLC mobile phase will determine how strongly a hydrophobic analyte is repelled into the stationary phase and how well retained it is.

Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase. RP-HPLC is a very powerful technique for the analysis of peptides and proteins.

Ticagrelor (TICA) in combination with Aspirin (ASP) represents a valuable, safe, and effective therapeutic regimen for the treatment of acute coronary syndrome (MI or unstable angina). Ticagrelor, a potent P2Y12 inhibitor together with Aspirin provides a standard therapy for non-ST-segment elevation acute coronary syndrome (NSTE-ACS) patients undergoing percutaneous coronary intervention (PCI). For the treatment of ACS, the recommended initial loading dose of Aspirin is 325 mg, which is to be followed by the maintenance dose of 75-100 mg/day. It is recommended not to exceed the Aspirin dose above 100 mg per day as it causes a reduction in the effectiveness of Ticagrelor.

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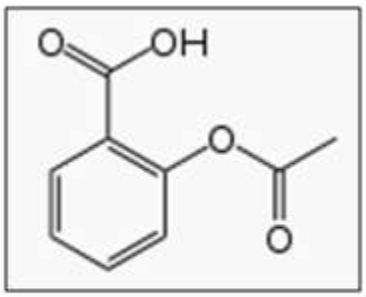
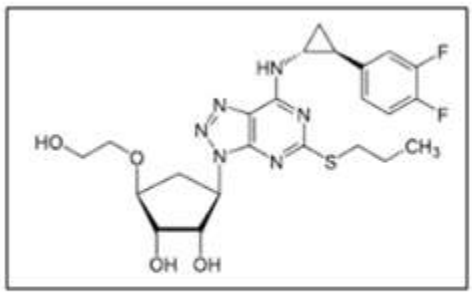


Aspirin



Ticagrelor

II. MATERIALS AND METHODS

Instrumentation

Alliance Waters e2695 Separations Module system was used for liquid chromatography method development and validation, equipped with a pump (Waters 515 Binary pump), and a Thermo C-18 column (4.6 x 250mm, 5µ particle size), and the detector consisted of UV/VIS operated at 260 nm and 282nm. Empower Pro was used for data processing and evaluation.

Chemicals and Reagents

ASP and TICA was a generous gift from industry. Commercial tablets of ASP, TICA BRILINTA Tablet were procured from the local drug market. Label claim of ASP and TICA in tablet is 81 and 90 mg respectively. HPLC grade and Acetonitrile were purchased from Merck (Merck Serono Amman, Jordan). The double distilled water was obtained from a local pharmaceutical company.

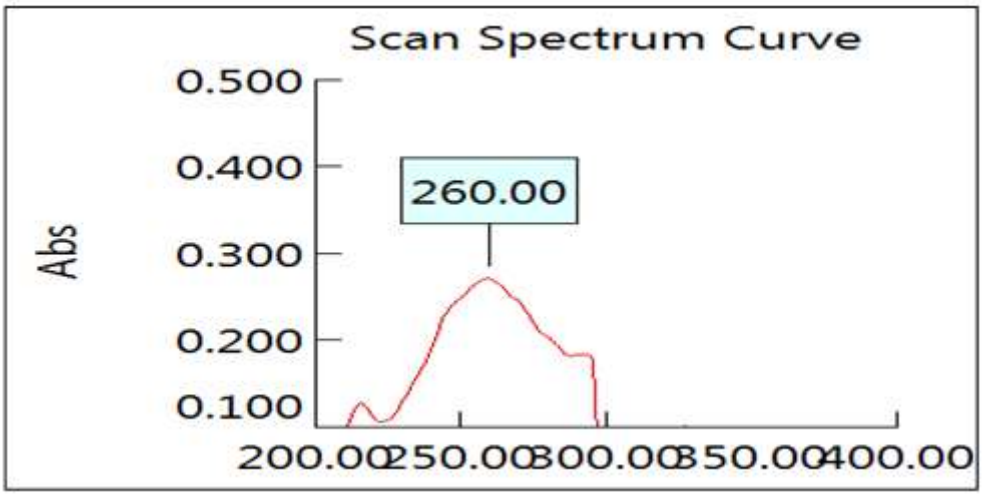
Chromatographic Conditions

The mobile phase was prepared by dissolving 1.0 gm ammonium carbonate in 1000 ml water. From the previous solution, 450 ml was mixed with 550 ml of acetonitrile. Prior to use the mobile phase was filtered through 0.45 µm membrane filters and degassed by sonication for 10 min.

Preparation of standard stock solution

Accurately weighed 10 mg of aspirin and ticagrelor was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of acetonitrile, and then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, Concentration of aspirin and ticagrelor was 200µg/ml. (Stock- A)

5 ml of solution was taken from stock-A of aspirin transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 µg/ml (Stock-B)



λ max of Aspirin

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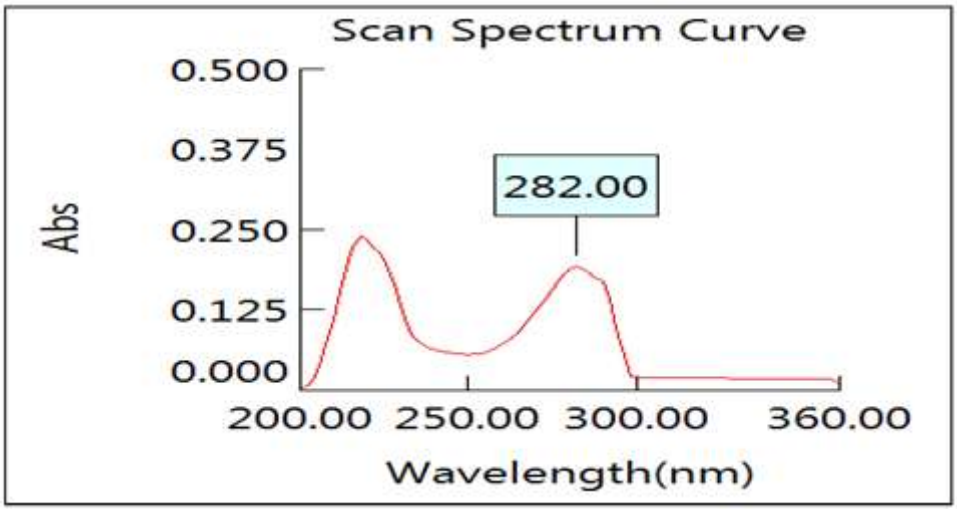

λmax of Ticagrelor

Method Validation

The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD), and robustness.

A. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration (Table given below).

B. Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

C. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

D. Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM Phosphate Buffer: acetonitrile (80:20 % v/v), to (85:15 % v/v).Results of robustness are reported in table.

III. RESULT AND DISCUSSION

A. Result of Method Developed By UV

The developed methods were found to be linear The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method as shown in Table:

Table: 1 Results of Linearity of ASP and TICA

PARAMETER	Simultaneous Equation Method	
	ASP	TICA
Working λ	260 nm	282 nm

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Beer's law limit (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.998	0.999

*Average of five determination

PARAMETER		Simultaneous Equation Method	
		ASP	TICA
Precision (%R.S.D.)*	Repeatability	0.033	0.043
	Day to Day	0.050	0.038
	Analyst to Analyst	0.033	0.150
	Reproducibility	0.071	0.039

Table: 2 Results of validation (%R.S.D.)

B. Method Development By RP-HPLC

The RP-HPLC method was developed for estimation of aspirin and ticagrelor in bulk and tablet dosage form by isocratically using 20 mM KH_2PO_4: acetonitrile (pH 3.0) in the ratio of 20:80 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 275 nm. Then developed method was validated by using various parameters.

1. System suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of referencestandard, 10 □ g/ml of aspirin and ticagrelor were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in table.

Table: 3 Results of system suitability parameters

Parameters	Aspirin	Ticagrelor
No. of Theoretical Plates	3253.333	3232.833
Tailing Factor	1.12	1.163
Retention time	4.252±0.001	8.104±0.001344

2. Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table

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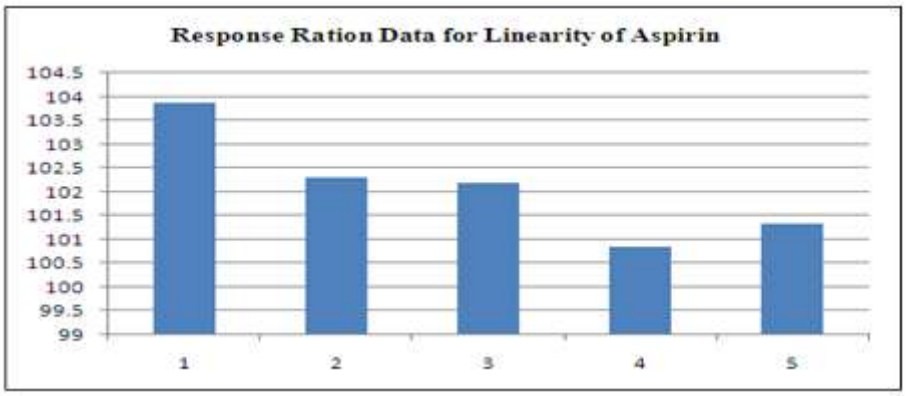
Table: 4 Response ratio data for linearity of Aspirin

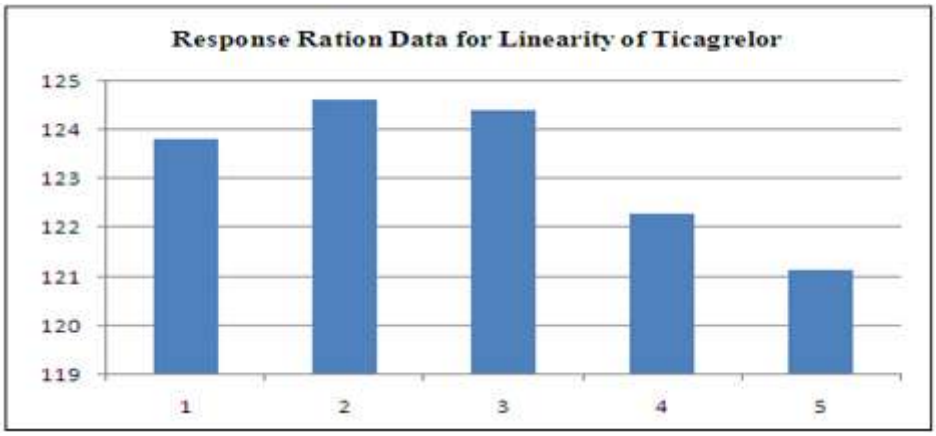
Replicates	Concentration(□g/ml)	Mean AUC	Response Ratio
Rep-1	5	519.427	103.8854
Rep-2	10	1023.192	102.3192
Rep-3	15	1532.948	102.1965
Rep-4	20	2017.082	100.8541
Rep-5	25	2533.370	101.3348
SD 1.037			
%RSD 1.015			



Response Ratio Curve of Aspirin

Table: 5 Response ration data for linearity of Ticagrelor

Replicates	Concentration (□g/ml)	Mean AUC	Response Ratio
Rep-1	5	619.035	123.807
Rep-2	10	1246.274	124.6274
Rep-3	15	1865.908	124.3939
Rep-4	20	2445.883	122.2942
Rep-5	25	3028.579	121.1432
SD 1.331			
%RSD 1.080			



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Response Ratio Curve of ticagrelor

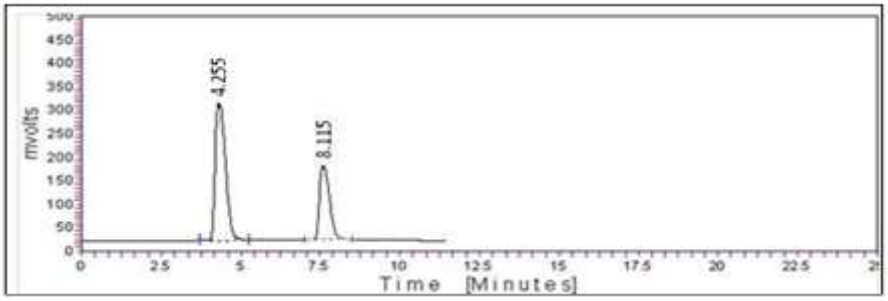
Table: 6 Results of linearity of aspirin and ticagrelor

Parameter	Aspirin	Ticagrelor
Conentration(µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.998
Slope (m)*	100.95	121.333
Intercept (c)*	8.888	16.913

***value of five replicate**

3. Specificity

Specificity of the method was determined and the peaks of diluent, mobile phase and excipients of tablets did not interfere with standard peaks aspirin and ticagrelor.



Chromatogram of both the drugs

4. Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less then 2 indicate the accuracy of method. Result of recovery study shown in table.

Table: 7 Results of recovery study

% LEVEL	% MEAN±SD*	
	Aspirin	Ticagrelor
80%	99.10±0.318	99.48±0.215
100%	99.72±0.235	99.14±0.440
120%	99.56±0.254	98.54±0.553

***** Value of three replicate and three concentrations.

5. Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. Result of precision shown in table.

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Table: 8 Results of Precision

Parameter	% MEAN±SD*	
	Aspirin	Ticagrelor
Repeatability	99.447±0.036	98.790±0.162
Intermediate precision		
Day to day precision	99.276 ±0.041	99.179±0.085
Analyst to Analyst	98.833±1.242	99.125±0.215
Reproducibility	99.172±0.026	

*** Value of five replicate and five concentrations**

6. Robustness

The robustness of developed method was checked by changing in the deliberate variationin solvent. Result of robustness shown in table.

Table: 9 Results of robustness

PARAMETER	% MEAN±SD*	
	Aspirin	Ticagrelor
Robustness	99.458±0.065	99.415±0.065

***** Value of five replicate and five concentrations

LOD AND LOQ

Detection limit and quantitation limit of described method were observed as 0.570 □ g/ml, 0.350 □ g/ml and 0.50 □ g/ml respectively and quantitation limit 1.54 □ g/ml, 0.95□ g/ml and 1.58□ g/ml respectively based on the SD of response and slope, which meet the requirement of new method.

IV. CONCLUSION:

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation. Proposed method was found to be linear in the range of 5-25 µg/ml aspirin and ticagrelor with the correlation coefficient near to one respectively. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (80%, 100% 120%) was ranging from 99.102 to 99.56%, 99.48 to 98.54% and 99.14 to 98.78% for aspirin and ticagrelor respectively.

The proposed methods were found to be linear in the range of 5-25 µg/ml with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent.

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can besuccessfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form.

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