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DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE DETERMINATION OF TICAGRELOR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

An anti-platelet drug decreases platelet aggregation and inhibit thrombus formation. The objective of the present study is to develop and validate the new analytical methods for the estimation of ticagrelor in bulk drug and pharmaceutical formulations. The new RP-HPLC method for the estimation of Ticagrelor was found out by using different chromatographic parameters. Chromatography was performed by gradient reverse phase separation using a Water's XBridge C18 column of particle size 5µ 250×4.6mm. The separations were achieved at the UV detection at 254nm using the mobile phase of Acetonitrile : Triethylamine Formic Acid in the ratio of 60:40. Flow rate was 1ml/min and the injection volume was set at 20 µl with 10 mins of runtime. The retention time was observed at 4.763. The method was validated by using various validation parameters like accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ). These results showed the method could find practical application as a quality control tool for analysis of the drug in its pharmaceutical dosage forms in quality control laboratories. The standard curve was linear over a working range of 10- 60 µg/ml and gave an average correlation factor of 0.9998 for ticagrelor. The limit of detection and the limit of quantitation were found to be 0.462µg/ml and 1.540µg/ml respectively. The method showed good recoveries and relative standard deviations of intra and inter day assay less than 2. This method can be easily and conveniently used for routine analysis of ticagrelor in bulk and tablet dosage forms.

KEYWORDS: Ticagrelor, RP-HPLC, Accuracy, Precision, Linearity, LOD, LOQ.

I. INTRODUCTION

Ticagrelor is a platelet aggregation inhibitor reduces the rate of thrombotic cardiovascular events in patients with the acute coronary syndrome. Ticagrelor belongs to the class of triazolo pyrimidines which are polycyclic aromatic compounds containing triazole ring fused to a pyrimidine ring. Ticagrelor is used to prevent a serious or life-threatening heart attack or stroke, or death in people who have had a heart attack or who have acute coronary syndrome (ACS; blockage of blood flow to the heart). It is also used to prevent blood clots from forming in people who have received coronary stents (metal tubes surgically placed in clogged blood vessels to improve blood flow) to treat ACS. Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation.[1-3] Ticagrelor is chemically known as (1S,2S,3R,5S)- 3-[7- [(1R,2S)-2-(3,4-Difluorophenyl) cyclopropylamino]-5-(propylthio)-3H-[1,2,3] triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxy ethoxy)cyclopentane-1,2-diol with a molecular formula of C23H28F2N6O4S and a molecular weight of 522.567 g/mol . It is freely soluble in Soluble in ethanol, dimethyl formamide sparingly soluble in water. Literature survey revealed that few HPLC, LC-MS and UV spectrophotometric methods have been developed and reported for the estimation of Ticagrelor.[4-8] Brilinta is the most available brand name in the market.[9] Ticagrelor is used to decrease the risk of a first-time heart attack or stroke in people at risk with coronary artery disease (CAD; reduced blood flow to the heart). It is also used to decrease the risk of another more serious stroke in people who are having a mild to moderate stroke or a transient ischemic attack (TIA; ministroke). Ticagrelor is in a class of medications called antiplatelet medications. It works by preventing platelets (a type of blood cell) from collecting and forming clots that may cause a heart attack or stroke. Ticagrelor was granted European Medicines Agency (EMA) approval on 3 December 2010.[10] Ticagrelor was granted FDA approval on 20 July 2011.[11]

Drug profile

IUPAC Name: (1S,2S,3R,5S)-3-[7-[(1R,2S)-2-(3,4-Difluorophenyl) cyclopropylamino]-5-(propylthio)- 3H- [1,2,3] triazolo[4,5-d] pyrimidin-3-yl]-5-(2- hydroxyethoxy) cyclopentane-1,2-diol.

Molecular formula: $C_{23}H_{28}F_2N_6O_4S$

Molecular weight: 522.57 g·mol-1.

Solubility: Water solubility 0.063 mg/ml. Ticagrelor is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF).

Pka value: Strongest Acidic 12.94 and strongest basic 1.11

Melting point: 140°C–142°C

Drug category: Antiplatelet

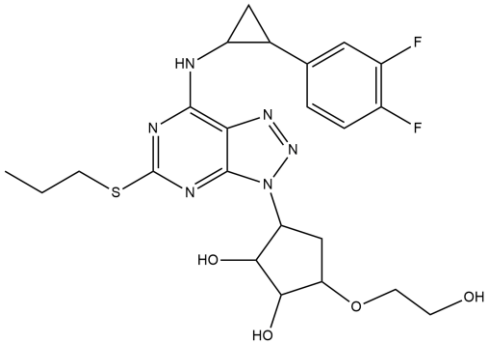

Figure 1: Structure of ticagrelor.

II. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Ticagrelor API procured from Manus Aktteva Biopharma LLP, Law Garden, Ellisbridge, Ahmedabad, Gujarat of these working standard drugs. HPLC grade Acetonitrile (Merck), Analytical grade Triethylamine and Formic acid was used as the solvents throughout the experiment. Pharmaceutical formulation tablet vasoglor (label claim containining 90mg) was used in HPLC analysis. HPLC grade water obtained in-house by using Direct-Q water purification system (Millipore, Milford,USA) was used in HPLC study.

2.2. Apparatus and Software

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on a waters X- Bridge C18 column 5µm 4.6x250mm with UV detection at 254nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON-230VAC, 50Hz), vaccum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. Double beam UV Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software- single channel was used for acquisition, evaluation and storage of chromatographic data.

2.3. Method development

After several trials with the different combination and ratio of solvents, the mobile phase Triethylamine and Formic acid (buffer): acetonitrile (40:60 v/v) was selected, because it was found that it ideals with retention time (Rt) 4.763 min. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component show reasonably good response at 255nm.

2.4. Selection of detection wavelength

To estimate the maximum λmax, ticagrelor 10 µg/ml of working standard solution was prepared and scanned in a UV wavelength range of 200 - 400 nm utilizing as water a blank. It was observed that the drug showed maximum absorbance at 255 nm which was hence chosen as the detection wavelength for the determination of ticagrelor. The overlay spectrum of Ticagrelor is shown in Fig. 2.

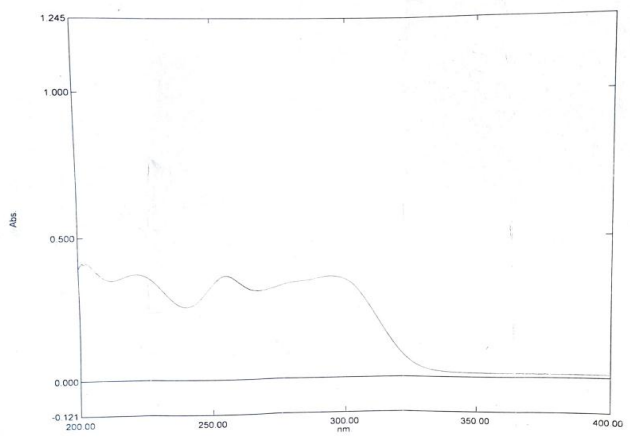

Figure 2: Absorption maxima for ticagrelor.

2.5. Preparation of the mobile phase

Triethylamine and Formic acid buffer solution was prepared by dissolving 0.5ml of each in 500 ml of HPLC grade water. HPLC experiments were carried out using binary pump A containing Acetonitrile and pump B containing Triethylamine and Formic acid.

2.6. Preparation of standard stock solution

Accurately weighed 50 mg of Ticagrelor is transferred into 50 ml of volumetric flask and was dissolved in acetonitrile and the volume were made up to the mark with the same solvent. This gave the concentration of 1000 µg ml-1 of Ticagrelor. From the above 5ml of Ticagrelor solution was pipetted out into a separate 50ml volumetric flask and the volume was made

upto the mark with acetonitrile. This gave the concentration of 100 µg ml-1 of Ticagrelor. From the above, six dilutions in between 10-60µg ml-1 of working concentration made up by using mobile phase as a solvent.

2.7. Preparation of sample solution

Ten tablets of Ticagrelor containing 90mg of Ticagrelor were weighed and powdered for further study. The powder equivalent to 10mg of Ticagrelor was accurately weighed and transferred to 10 ml volumetric flask. After that drug mixture is dissolved in acetonitrile. The volume is maintained with acetonitrile and is sonicated for 10 min. The above solution was carefully filtered through nylon membrane filter paper (0.45µ).

From this solution, required dilutions for HPLC method were prepared by using acetonitrile as a solvent.

2.8. Method development optimization

The optimized HPLC conditions of several mobile phases with different compositions have been tested to develop an optimized chromatographic conditions like tailing factor, peak shape, and the number of theoretical plates. For the selection of the mobile phase, primarily ethanol: water, acetonitrile: water has been tested for different compositions. Eventually, the gradient mode and mobile phase containing a mixture Acetonitrile: Triethylamine Formic Acid (50:50% v/v) at a flow rate of 1 ml/minute was found to be satisfactory and proper system suitability parameters obtained.

III. METHOD VALIDATION

Validation is the process of establishing a documented evidence, which provides a high degree of assurance, that a specific activity will consistently produce desired results or products, meeting its pre-determined specifications and quality characteristics. The method was validated as per ICH guidelines.[12-17]

3.1. System suitability

System suitability parameters are defined as the tests to ensure that a method can generate results of acceptable accuracy and precision. The requirements for system suitability are generally developed after method development and validation have been completed. The system suitability parameters like theoretical plates, retention time, tailing factor were studied for ticagrelor.

3.2. Specificity

Specificity is a procedure to detect quantitatively the analyte in presence ofthe components that may be expected to be present in the sample matrix. While selectivity is a procedure to detect the analyte qualitatively in presence of components that may be expected to be presented in the sample matrix. The excipients in tablet formulation were spiked in pre weighted quantity of drugs and then absorbance was measured and calculations were done to determine the quantity of the drugs.

3.3. Linearity

By using the working standard, aliquots of 10,20,30,40,50,60 µg/ml were prepared with acetonitrile. Six dilutions of each of the above mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration were injected into the HPLC system. Then their chromatogram was recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted. The correlation coefficient value of ticagrelor was found to be 0.9998.

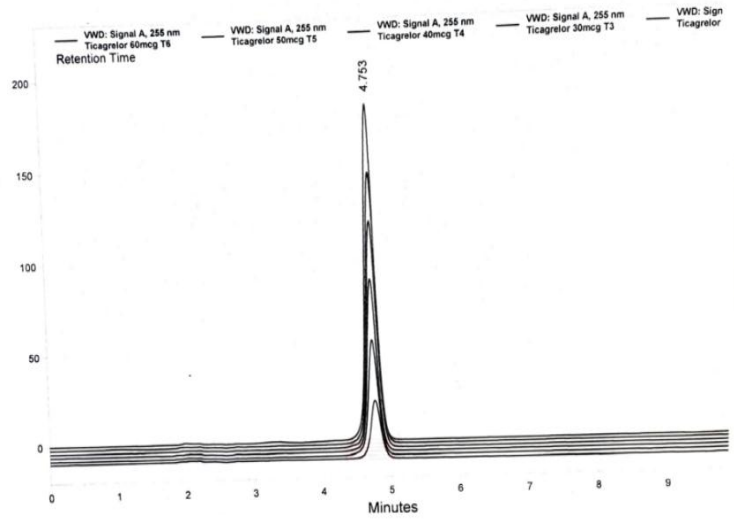

Fig. 1: Chromatogram of ticagrelor at concentration 10-60µg/ml.

3.4. Accuracy

The accuracy of the method was determined by calculating % recovery. A known quantity of Ticagrelor was spiked with placebo at three different levels such as 80%,100%,120% in triplicate over the specified concentration range and the amount of Ticagrelor estimated by measuring the peak area ratios. The percentage recovery and standard deviation of percentage recovery were calculated as per the proposed method. The mean % recovery at each level should be 98-102 %.

Table 1: Accuracy for ticagrelor.

Accuracy	Peak Area	Average	Standard Deviation	RSD	Recovery	Average
80%	13448151	134921 99.33	86015.11	0.64	99.67	100.00
	13437130				99.59	
	13591317				100.73	
100%	16443786	16406838	126467.87	0.77	100.23	100.00
	16510717				100.63	
	16266011				99.14	
120%	19137722	190150 21.67	115407.21	0.61	100.65	100.00
	18998697				99.91	
	18908646				99.44	

3.5. Precision

The precision of the method was determined in terms of Intra-day and inter-day precision. For intra-day precision studies, a standard solution of 30 µg/ml was injected at various time intervals and percent related standard deviation was estimated. The inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the percent related standard deviation of the signal was calculated. The repeatability, intermediate precision and reproducibility of the developed method were determined.

Table 2: Intra-day and inter-day precision

Morning				
S.N (Injection 30µg/ml)	**Areas**	**Average**	**SD**	**%RSD**
1	16207319	16245815.17	141979.40	0.87
2	16328891			
3	16416361			
4	16002691			
5	16216111			
6	16303518			
Afternoon				
S.N (Injection 30µg/ml)	**Areas**	**Average**	**SD**	**%RSD**
1	16242170	16381863.33	160867.40	0.98
2	16187296			
3	16394871			
4	16390147			
5	16645138			
6	16431558			

Table 3: Day-1 and day-2 precision.

Day -1				
S.N (Injection 30µg/ml)	**Areas**	**Average**	**SD**	**%RSD**
1	16373147	16422445.67	91212.82	0.56
2	16395075			

3	16305266			
4	16399452			
5	16512850			
6	16548884			
Day -2				
S.N (Injection 30µg/ml)	**Areas**	**Average**	**SD**	**%RSD**
1	16162867	16261647.17	144276.26	0.89
2	16037199			
3	16403849			
4	16354307			
5	16380771			
6	16230890			

Table 4: The summary output of ANOVA study of ticagrelor.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999898132
R Square	0.999796274
Adjusted R Squar	0.999755529
Standard Error	178782.3605
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7.84305E+14	7.843E+14	24537.814	2.01152E-10
Residual	5	1.59816E+11	3.196E+10		
Total	6	7.84465E+14			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	42734.42857	121819.6444	0.3508008	0.740034296	-270412.9365	355881.8	-270412.937	355881.794
X Variable 1	529253.3143	3378.669033	156.6455	2.01152E-10	520568.169	537938.5	520568.169	537938.46

3.6. Robustness

The robustness of an analytical method was studied by deliberately changing the variations in method parameters like flow rate, mobile phase compostion, detection wavelength.

3.7. Linearity and Range

A graph of peak area v/s concentration (µg/mL) were plotted for ticagrelor at concentration range between 10-60 µg/ml. The linear regression equation and correlation coefficient (R 2) were Y= 529253x – 42734 and 0.9998 respectively.

Table 5: Linearity data of ticagrelor.

Sl/No	Concentration	Peak Area at 255nm
1.	10µg/ml	5326582
2.	20µg/ml	10739718
3.	30µg/ml	15789135
4.	40µg/ml	21460093

5.	50µg/ml	26256710
6.	60µg/ml	31870099

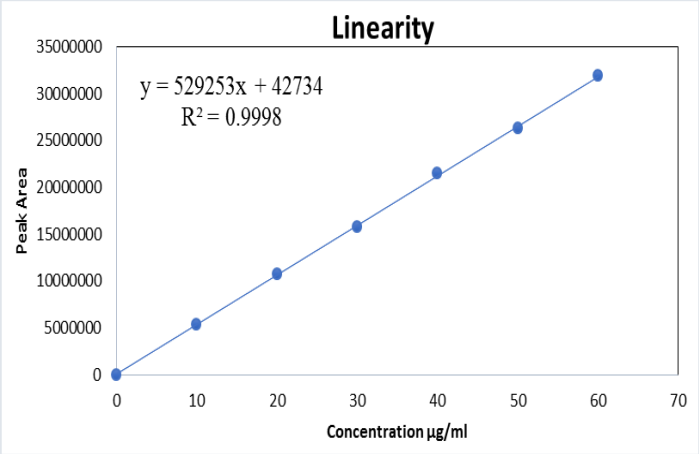

Fig. 2: Calibration graph of ticagrelor.

3.8. Stability of analytical solution

Regarding the stability of both the standard and sample solutions were analyzed over a period of 48 hours at 10°C. The results show that for both soultions, peak area and retention time almost unchanged and no significant degradation with in the 48 hours.Which indicates that both soultions were stable for atleast 2 days which was sufficient to complet hole analyctical process.

3.9. LOD and LOQ

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision. The LOD and LOQ were determined by injecting six replicates of the analyte at the progressively low concentrations of the standard solution using the developed HPLC method.

3.10. Analysis of ticagrelor in tablet formulation

Ticagrelor standard solution and ticagrelor sample solution were prepared as described in 2.6 and 2.7. The samples were analysed using the developed chromatographic method and the % content of the ticagrelor in the tablets was estimated.

IV. RESULTS AND DISCUSSION

Linearity

Between 10 and 60 µg/ml, the linearity was plotted, and the R2 value was found to be 0.9998. Tables 5 shows the final results. Figures 2 show the linearity graphs.

Precision

The 30 µg/ml concentration was used to determine intra-day and inter-day precision. In both cases, the percent RSD values were less than 2 percent. Tables 2 and 3 show the results.

Accuracy

It was found that the mean recovery was 100 percent for concentrations of 80%, 100%, and 120%. Table 1 show the results.

LOD and LOQ

LOD is the lowest amount of analyte in the sample that can be detected. LOQ is the lowest amount of the analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOD and LOQ were determined by the following equation. LOD = 3.3σ/S, LOQ = 10 σ/S. where σ is the standard deviation of the Y-intercept of the calibration curve and S is the slope of the regression equation. The LOD and LOQ values were found to be 0.462 µg/ml, 1.540 µg/ml respectively.

CONCLUSION

In addition to positive requirements for analytical methods, the striking advantage of all the developed method is that they are economical, cheap, precise. The proposed RP-HPLC method were suitable technique for the determination of Ticagrelor. All the parameters analysingTicagrelor met the criteria of ICH guidelines for Method Validation.

In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Ticagrelor in bulk and pharmaceutical formulations. The recoveries achieved were found good by the method. The HPLC method is more sensitive, precise and accurate compared to the spectrophotometric methods. The HPLC method developed may be recommended for the routine determination of Ticagrelor in bulk drug and pharmaceutical formulations.

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