

## BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TICAGRELOR BY RP-HPLC

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### ABSTRACT

**Objective:** The main purpose of this study was to develop a simple, precise, rapid and accurate RP-HPLC method for the quantitative determination of ticagrelor in human plasma.

**Methods:** The separation was accomplished by the isocratic method by utilizing phenomenex C18 column on a Shimadzu binary gradient liquid chromatography system furnished with LC-20AD solvent delivery system, SPD-20-A photo-diode array detector and 20 µl loop volume in a rheodyne injector. The analyte was extracted by protein precipitation in the involvement of diethyl ether as a protein precipitator. The mobile phase was developed for the estimation of the drug in human plasma consists of acetonitrile and methanol in the ratio of 60:40% v/v. Separation was done with a flow rate of 1 ml/min at a detection wavelength of 254 nm.

**Results:** Retention time was found to be 4.503 min with a run time 10 min. Linearity shows in a range of 20-100 µg/ml, with a correlation coefficient of 0.9992 respectively. Stability studies of ticagrelor in plasma were carried out by, short term stability, long term stability and bench top stability studies. Short term stability, long term stability and bench top stability of ticagrelor was carried out from 20 and 100 µg/ml concentration and %RSD was ascertained 0.12% and 0.08%, 0.18% and 0.15%, 1.19% and 1.30% respectively.

**Conclusion:** The outcomes were observed to be inside the knowledge of ICH guidelines. The prepared solution was injected in triplicate, and % RSD was measured. Acquired results demonstrate that proposed strategy can be effortlessly and advantageously applied for routine examination of ticagrelor in human plasma.

**Keywords:** Bioanalytical method, Reverse phase HPLC, Ticagrelor, ICH

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### INTRODUCTION

Bioanalysis is the strategy used to determine the concentration of drug and metabolites in the biological matrices like plasma, serum, cerebrospinal fluid, urine, saliva etc. Bioanalytical method and validation are utilized to build up, that a quantitative analytical method can be connected for the biochemical process. Validation involves documentation of laboratory investigations that the method is suitable and reliable for the intended applications [1]. It is utilized for the assessment of bioavailability and bioequivalence studies, quantitative evaluation of drug and metabolites, new drug development, clinical pharmacokinetics, research process and therapeutic drug monitoring. Bioanalytical techniques are constantly undergoing changes and improvements that they are the cutting edge of technology [2].

Ticagrelor is an anti-platelet drug which is chemically (1S,2S,3R,5S)-3-[7-[(1R,2S)-2-(3,4-Difluorophenyl)cyclopropylamino]-5-(propyl thiol)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-hydroxyethoxy)cyclopentane-1,2-diol. It blocks adenosine diphosphate (ADP) receptors of subtype P2Y12 in contrast to other antiplatelet drugs, ticagrelor has a binding site making it an allosteric antagonist and the blockage is reversible [3]. It inhibits platelet aggregation and thrombus formation in atherosclerotic disease by preventing the activation of platelet [4].

The present study portrays the improvement and acceptance of a delicate, particular, fast, straight forward and cost effective HPLC bioanalytical strategy for ticagrelor in human plasma as indicated by ICH Q2 (R1) and EMA guidelines [5, 6]. The objective of validation of bioanalytical procedure is to demonstrate that it is suitable for the intended purpose and it will be beneficial for the researchers.

### MATERIALS AND METHODS

#### Chemicals and reagents

The active pharmaceutical ingredients were benevolently acquired from swapnaroop drugs and pharmaceuticals, Maharashtra, India. The pharmaceutical formulations were purchased from the local

market which contains ticagrelor 90 mg. required chemicals, reagents, acetonitrile, methanol used are of HPLC grade.

#### Optimization of chromatographic conditions

Separation was done by using phenomenex C18 (250 × 4.6 mm, 5 µm) column on a shimadzu liquid chromatographic system with LC-20AD solvent delivery system, SPD-20A photodiode array detector and rheodyne injector with 20 µl loop volume. Mobile phase consists of a mixture of acetonitrile and methanol in the ratio of 60% v/v in pump A and 40% v/v in pump B was found to be the most suitable mobile phase for ideal chromatographic separation of ticagrelor. It was pumped with a flow rate of 1.0 ml/min through the column. The column was equilibrated by pumping the mobile phase for 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 254 nm. The run time was set at 10 min.

#### Preparation of stock solution

Blood samples were collected and centrifuged at 10,000 rpm for 2 min. The plasma thus obtained were separated and stored. 1 mg of standard ticagrelor was weighed accurately and spiked into 1 ml plasma. After centrifugation, subsequent aliquots of 20-100 µg/ml concentrations were prepared by diluting with 100 µl of diethyl ether and the mobile phase and clear supernatant liquid of each concentration were injected for analysis and chromatogram were taken [7]. The chromatogram is shown in fig. 1.

#### Calibration curve

The calibration plots were constructed by plotting peak area against respective concentrations. Linearity was obtained by analysis of serially diluted sample in the range of 20, 40, 60, 80, 100 µg/ml. Calibration graph is shown in fig. 2.

#### Validation of the method

The developed method was validated by evaluating linearity, precision, accuracy and specificity which were done in acceptance of ICH [8] and EMA guidelines.

### Linearity

Linearity was studied by standard solutions at different concentration levels. The linearity range for ticagrelor was found to be 20-100 µg/ml.

The regression equation was found to be  $y = 35798x - 9772.3$  with a coefficient of correlation ( $r^2$ ) 0.9992. The correlation coefficient for linearity is 0.9999. Since the result is approximately close to the true value, the method is indicated as highly significant.

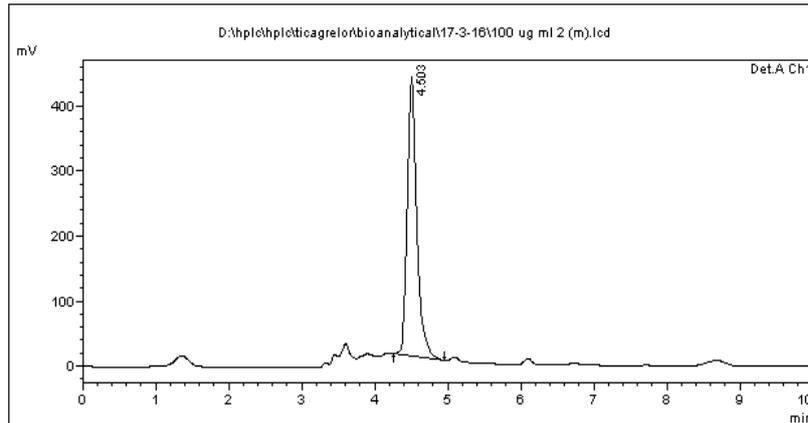


Fig. 1: Chromatogram of ticagrelor in human plasma 100 µg/ml

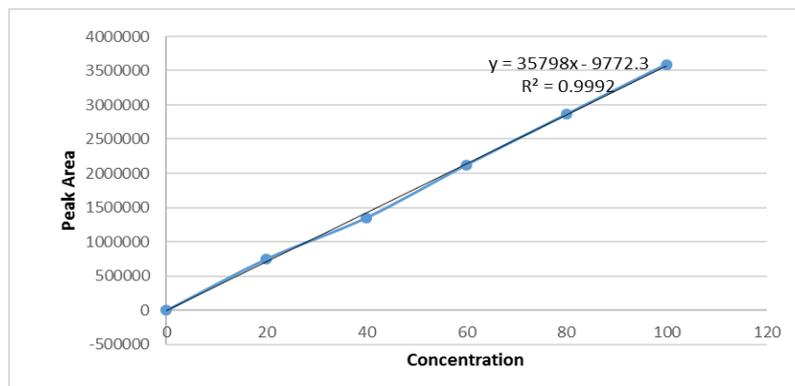


Fig. 2: Calibration curve of ticagrelor in human plasma

Table 1: Linearity of ticagrelor in human plasma

Concentration (µg/ml)	Area
20	750690
40	1352882
60	2124666
80	2861693
100	3590697

### Precision

Precision was determined by carrying three replicates of concentration 40 µg/ml and performed intraday (within a day) and interday (day to day) studies. The percentage relative standard deviation (%RSD) was found to be less than 1%. For Intraday precision study, evaluation was carried out by injecting a standard

solution at various time intervals and %RSD of Ticagrelor was found to be 0.56% and 0.51% shown in table 2 where inter-day precision was carried out in consecutive days with %RSD of 0.76% and 0.81% shown in (table 3). The %RSD can be reached up to 2%.

Since the outcome is less than 1% it was found to be satisfactory, which indicates method is precise [9].

Table 2: Intraday study of ticagrelor in human plasma

Days	Standard concentration	Peak area	
1 <sup>st</sup> day	40	Morning	Afternoon
		320815	3202864
		3212878	3218728
		3238005	3236005
	MEAN	3217899.33	3219199
	STD DEV	18124.41	16575.51
	% RSD	0.56%	0.51%

Table 3: Interday study of ticagrelor in human plasma

Standard concentration	Peak area	
	Day 1	Day 2
40	1730997	1730789
	1742154	1742415
	1757249	1758924
MEAN	1743466.66	1744042.66
STD DEV	13175.13	14137.94
% RSD	0.76%	0.81%

**Limit of detection (LOD)**

LOD was determined by standard deviation method and slope of the calibration plot by using the formula  $3.3\sigma/S$ . It was observed to be 0.382 $\mu$ g/ml. since the observed concentration is low, the method is sufficiently sensitive.

**Limit of quantification (LOQ)**

LOD was determined by standard deviation method and slope of the calibration plot by using the formula  $10\sigma/S$ . It was observed to be 1.158 $\mu$ g/ml. As the amount of analyte was found to be less, we can estimate the drug at very low concentration.

**Stability of ticagrelor in human plasma****Short term stability**

Short term stability was determined by storing concentration of 20 and 100  $\mu$ g/ml over a period of 6 h at room temperature. Stability was determined by performing three replicates and calculated %

RSD. It was found to be 0.12% and 0.08% respectively, shown in table 4 as it is less than 1% the method is said to be stable.

**Long term stability**

Long-term stability was determined by storing concentration of 20 and 100  $\mu$ g/ml over a period of 10 d at room temperature. Stability was determined by calculating the % RSD. It was found to be 0.18% and 0.15% respectively, as it is less than 1% the method can said to be stable.

**Bench top stability**

Three replicates of the lowest and higher concentration were determined. The samples were assessed after keeping at room temperature (bench top) against freshly prepared concentrations and %RSD was calculated [10]. It was found to be 1.19% and 1.30% respectively. The %RSD can be reached up to 2%. Since the result is less than 2% values were found to be satisfactory, which indicates method is stable.

Table 4: Short-term stability of ticagrelor

Concentration	Peak area	Mean	Standard deviation	%RSD
20	1929188	1927512	2385.189	0.12%
	1922566			
	1924781			
100	5491030	5495356	4249.185	0.08%
	5495513			
	5499524			

Table 5: Long-term stability of ticagrelor

Concentration	Peak area	Mean	Standard deviation	%RSD
20	1152882	1153686	2065.306	0.18%
	1156032			
	1152143			
100	4891697	489020	7398.169	0.15%
	4882466			
	4897096			

Table 6: Bench top stability of ticagrelor

Concentration	Old	Fresh	% RSD
20	1077812	1096036	1.19%
100	5342790	5442090	1.30%

**CONCLUSION**

The developed RP-HPLC method was found to be simple, precise, accurate and sensitive for the estimation of Ticagrelor in human plasma. Validation of results according to ICH and EMA carried out revealed high accuracy and good precision. The RSD for every one of the parameters are observed to be short of what one, which shows the legitimacy of the technique is reasonably fine. A mixture of acetonitrile and methanol mobile phase ratio of 60% v/v in pump A and 40% v/v in pump B at a flow rate of 1 ml/min. The wavelength was found to be 254 nm in U. V spectroscopy. Retention time was found to be 4.503 min with a run time 10 min. 20-100  $\mu$ g/ml concentration of ticagrelor shows linearity with a correlation coefficient of 0.9992. Thus we can conclude that this method can be easily and conveniently adopted for the quality control analysis of ticagrelor in human plasma.

**CONFLICTS OF INTERESTS**

Declare none

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