Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (9):206-212
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of RP- HPLC method for determination of ticagrelor in pharmaceutical dosage formulation

Eena Joshy, Anu Babu, Delma D'cruz and Aneesh T. P.*

Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Sciences Campus, Kochi, Kerala, India

---

ABSTRACT

The quantitative determination of Ticagrelor in pharmaceutical dosage forms was developed by using a simple accurate precise and cost effective method. The separation was achieved by isocratic method using phenomenex C18 column on a Shimadzu binary gradient liquid chromatographic system equipped with LC-20AD solvent delivery system, SPD-20A photo diode array detector and rheodyne injector with 20 µl loop volume. The mobile phase developed for the estimation of the drug in dosage form and in human plasma contains a mixture of acetonitrile and methanol in the ratio of 70:30% v/v at a flow rate of 1 ml/min at a detection wavelength 254 nm. Retention time of Ticagrelor in pharmaceutical formulation was found to be 3.793 min with a run time of 7 min. The drug showed linearity in the range of 10-100 µg/ml with a correlation coefficient (r^2) 0.9967.The results were found to be within the acceptance of ICH, USP guidelines. The described method was validated with respect to the system suitability parameters, linearity, precision and accuracy. Each solution was injected in triplicate, and %RSD was measured. The validation results obtained in the study shows that the proposed method can be easily and conveniently adopted for routine analysis of ticagrelor in pharmaceutical formulation.

Key words: Ticgrelor, RP-HPLC, Method Development, Validation

---

INTRODUCTION

Analytical method development and validation used to distinguish the primary drug components from the impurities. Analytical methods are validated in order to demonstrate that method is suitable for intended purpose [1]. Analytical method will provide information on drug uniformity, drug release, evaluation of drug characteristics, potency, impurities etc. during the drug development and manufacturing. Analytical studies concentrate on spectrophotometric analysis and HPLC. Reverse chromatography is widely used in HPLC. Method development requires which instrument is used and why it's so. Choice of column, mobile phase, detectors, should be considered during the HPLC method development [2].The use of analytical methods during the drug development and manufacturing provide information on, Potency which can relate directly to the requirement of a known dose, Impurities which can relate to the safety profile of drug and evaluation of key drug characteristics such as drug release, drug uniformity, properties which can compromise bioavailability. Ticagrelor belongs to an oral antiplatelet agent, which inhibits the platelet activation and aggregation which is mediated by the P2Y12 ADP receptor. It blocks adenosine diphosphate (ADP) receptors of subtypeP2Y12 in contrast to other antiplatelet drugs, ticagrelor has a binding site different from ADP, making it an allosteric antagonist and the blockage is reversible[3]. Chemically it is 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 (fig. 1). Ticagrelor is used to prevent signal transduction and platelet activation which inhibits platelet aggregation and thrombus formation in atherosclerotic disease. Ticagrelor is a nucleoside analogue, the cyclopentane ring is similar to the sugar ribose, and the nitrogen ring aromatic system resembles the nucleobase purine giving the adenosine molecule [4-5]. The results describes the method is simple, precise and accurate for the estimation of ticagrelor in pharmaceutical dosage form. The method was validated as per ICH guidelines.

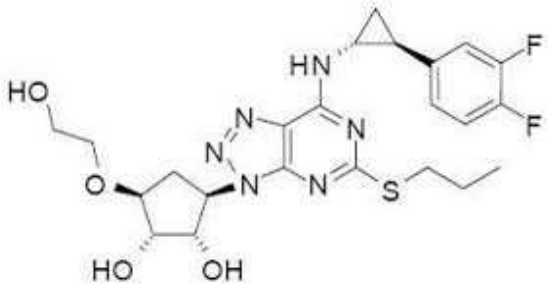

Figure .1: Structure of Ticagrelor

MATERIALS AND METHODS

Chemicals and reagents:
The Active pharmaceutical ingredient was obtained from Swapnaroop drugs and Pharmaceuticals, Maharashtra, India. The pharmaceutical formulation was purchased from local market which contains Ticagrelor 90 mg. All the chemicals and reagents used were of Analytical or HPLC grade.

Optimization of Chromatograhic conditions
The separation was performed by using Phenomenex C18 (250 × 4.6 mm, 5 µm) column on a Shimadzu binary gradient liquid chromatographic system equipped with LC-20AD solvent delivery system, SPD-20A photo diode array detector and rheodyne injector with 20 µl loop volume. Analysis was carried out by isocratic method with a mobile phase consist of a mixture of acetonitrile and methanol in the ratio of 70% v/v in pump A and 30% v/v in pump B. It was found to be the most suitable mobile phase for ideal chromatographic separation of Ticagrelor. Mobile phase was pumped through the column at a flow rate of 1.0 ml/min with a run time of 7 min at a detection wavelength of 254 nm. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution.

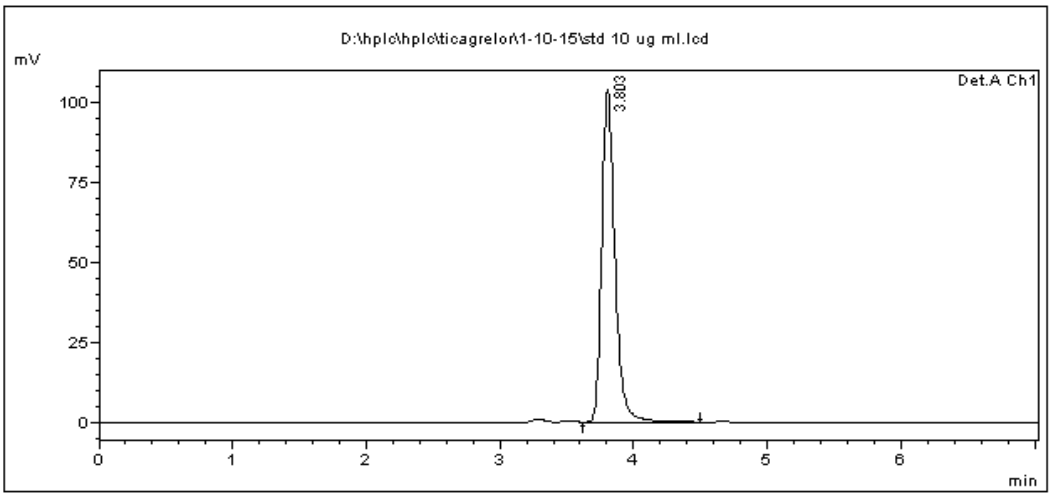

Figure.2: Chromatogram of Ticagrelor 10 µg/ml

Preparation of stock solution

10 mg of Ticagrelor was accurately weighed and 1000 µg/ml concentration of Ticagrelor was prepared as standard stock solution, using methanol as solvent. From the standard stock solution different concentrations were prepared ranging 10-100 µg/ml using mobile phase. 20 µl of each was injected in HPLC & chromatogram was taken. Chromatogram of standard is shown in (fig.2)

Calibration curve

Calibration curve was plotted against peak area and concentration which obeys Beer's law. Linearity was obtained by analysis of serially diluted sample in the range of 10-100 µg/ml. Calibration graph is shown in (fig.3)**.**

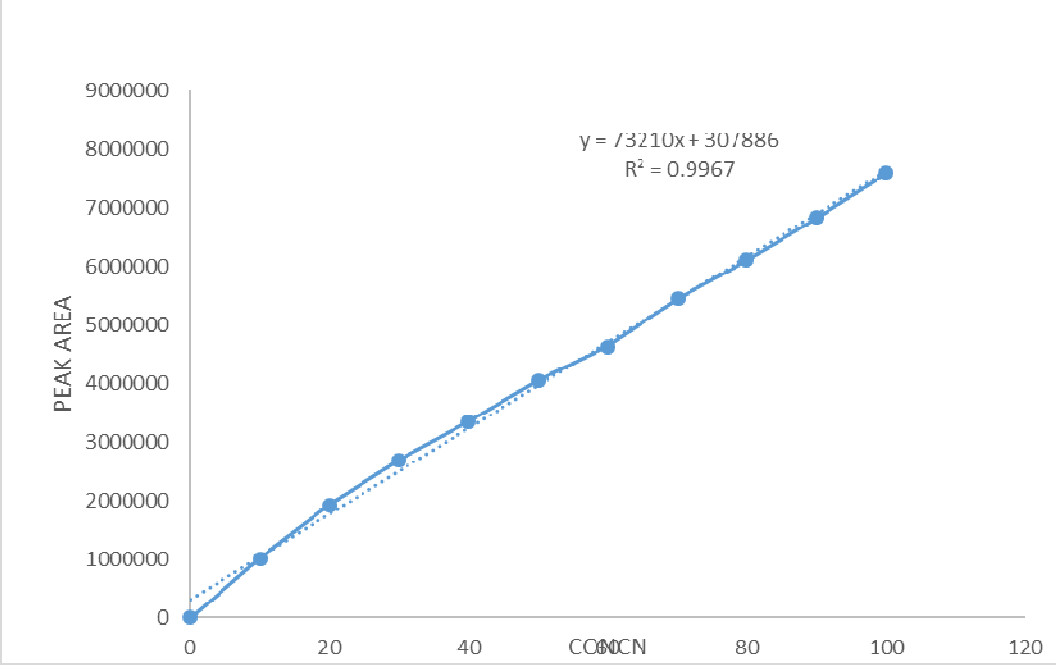

Figure.3. Calibration curve of Ticagrelor

Validation of the method

Validation parameters are done based on the ICH guidelines by evaluating linearity precision, accuracy etc [6].

Table.1: Linearity results of Ticagrelor

Concentration (µg/ml)	Area
10	1010823
20	1932626
30	2696877
40	3355496
50	4050929
60	4628695
70	5448596
80	6109169
90	6827269
100	7591809

Linearity

10-100µg/ml concentrations were prepared by serial dilution in different 10ml standard volumetric flask. Linearity of ticagrelor ranges from 10-100 µg/ml. Evaluation were done using U.V detector at 254 nm. The plotted calibration revealed good linear relation with concentration and area**.** The regression equation was found to be y = 73210x+307886 and correlation coefficient was found to be 0.9967. According to Beer's law the correlation

coefficient for linearity is 0.9999. Since the result is approximately close to the true value. The method is indicated as high significance [7-8]. The linearity results were shown in (Table.1).

Precision

Precision is related to repeatability and reproducibility. It is measurement of same results obtained from unchanged conditions with homogeneous sample. Repeatability was determined as intraday and reproducibility as interday precision, in accordance with ICH recommendations [9]. For intraday precision, evaluation was carried out by injecting standard solution at various time intervals and %RSD of ticagrelor was found to be 0.06% shown in **(**Table 2) where, inter-day precision was carried out in consecutive days with %RSD of 0.02% and 0.01% shown in **(**Table 3**)**. The %RSD can be reach upto 2%. Since the results, less than 1% values, were found to be satisfactory, which indicates method is precise.

Table 2: Intraday precision studies

Days	Standard Concentration	Peak Area n=3	
1^{ST} DAY	40	Morning	afternoon
		3355496	3355587
		3354251	3354362
		3351794	3351694
	MEAN	3353847	3353881
	STD DEV	28577.6	28517.4
	% RSD	0.06%	0.06%

Table 3: Interday precision studies

Standard Concentration	Peak Area n=3	
50	Day 1	Day 2
	4227541	4227274
	4228652	4228176
	4226663	4227285
MEAN	4227618.6	4227578.3
STD DEV	996.77	517.623
% RSD	0.02%	0.01%

Accuracy

In order to evaluate accuracy analytical recovery experiments are carried to study the interference of formulation additives, by standard addition of 50, 80 and 100% level in triplicates. Results were obtained by percentage recovery and relative standard deviation. The percentage recovery of Ticagrelor were found to be in the range of 99.6 - 99.75% and the mean recovery of ticagrelor was found to be 99.68%, shown in (Table 4**)** which shows very low interference from excipients, indicates the method is accurate.

Table 4: Recovery studies

Recovery Level	Concentration Added (µg/ml)	Concentration Found (µg/ml)	% Recovery	Mean Recovery
50	40	39.9	99.75%	99.68%
80	60	59.32	99.6%	
100	80	79.8	99.7%	

Limit of Detection

LOD was determined by standard deviation method and also from the slope of the calibration plot by using formula 3.3×σ/S. It was observed to be 0.971 µg/ml. Since the observed concentration was very low the method is sufficiently sensitive.

Limit of Quantification

LOD was determined by standard deviation method and also from the slope of the calibration plot by using formula 10×σ/S. It was observed to be 2.94 µg/ml. As the amount of analyte was found to be less, we can estimate the drug at very low concentration.

System suitability parameters

In HPLC method the system suitability parameters were determined and analyzed for retention time, Number of theoretical plates, Tailing factor, Asymmetric factor, Calibration range and compared with standard values which shown in (Table 5).The retention time of Ticagrelor was 3.793 min .So the method is cost effective as it utilizes very less quantity of mobile phase. The theoretical plates were 2556.31. Since higher number of theoretical plates improves the efficiency of column, elution will be faster and tailing factor was 1.1 which shows the column is highly efficient [10].

Table 5: System suitability parameters

Parameters	Results
Retention time	3.793
Tailing factor	1.2
Theoretical plates	2556.31
Calibration range	10 -100
Asymmetric factor	1.5

Robustness

Robustness shows that the results are unaffected by slight but deliberate variations in analytical method [11-12].It provides an indication about variability of the method during normal laboratory conditions like flow rate, mobile phase ratio, and wavelength shows in (Table 6). It was observed that an only slight variation in the chromatogram shows that the HPLC- method development is robust.

Table 6: Robustness

Parameters	Condition	Retention time	Tailing factor
Flow rate	0.8	3.808	1.5
	1	3.793	1.2
	1.2	3.768	1.5
	Mean(n=3)	3.789	1.4
Mobile Phase	70:30	3.793	1.2
	80:20	3.810	1.25
	90:10	3.820	1.5
	Mean(n=3)	3.807	1.31
Detection Wavelength	254	3.793	1.2
	230	3.748	1.25
	240	3.771	1.5
	Mean(n=3)	3.770	1.5

Quantification of Ticagrelor in dosage form

Accurately weighed 10 mg equivalent of Ticagrelor from the dosage form and made 1000 µg/ml of Ticagrelor as stock solution using methanol as solvent. From above solution 20, 30, 40, 50 µg/ml concentrations were prepared using mobile phase and 20 µl of each was injected in HPLC & chromatogram was taken for quantifying the drug in dosage form. Chromatogram of sample was shown in (fig 4). The quantification of the drug is done using linear regression equation method and direct comparison method.

Using linear regression method,

Linear equation is y = 73210x + 307886

By substituting peak area of sample in y can obtain concentration of the sample

The quantified drug concentration in 20, 30, 40, 50 µg/ml were found to be 19.8, 29.6, 39.8, and 48.4 µg/ml respectively which is shown in (Table 7).

Direct comparison method

Is done by using formula,

$$\frac{\text{Peak area of Sample}}{\text{peak area of Standard}} \times \text{Dilution factor} \times \frac{\text{Weight of the sample}}{\text{Weight to be taken}}$$

The quantified drug concentration in 20, 30, 40, 50 µg/ml were found to be 19.5, 28.6, 39.5, and 48.5 µg/ml respectively which is shown in (Table 8).

Table 7. Quantification of Ticagrelor in dosage form by linear regression method

Concentration (µg/ml)	Peak Area Sample	Peak Area of standard	Drug Concentration Using (µg/ml)	Assay (%)	Mean % Assay
20	1751444	1932626	19.8	99%	97.6%
30	2476877	2696877	29.6	98.6%	
40	3221794	3355496	39.8	96%	
50	3851426	4050929	48.4	96.8%	

Table 8. Quantification of Ticagrelor in dosage form by Direct comparison method

Concentration (µg/ml)	Peak Area of sample	Peak Area of standard	Drug Concentration Using (µg/ml)	Assay (%)	Mean % Assay
20	1751444	1932626	19.5	97.5%	97.1%
30	2476877	2696877	28.6	95.3%	
40	3221794	3355496	39.5	98.75%	
50	3851426	4050929	48.5	97%	

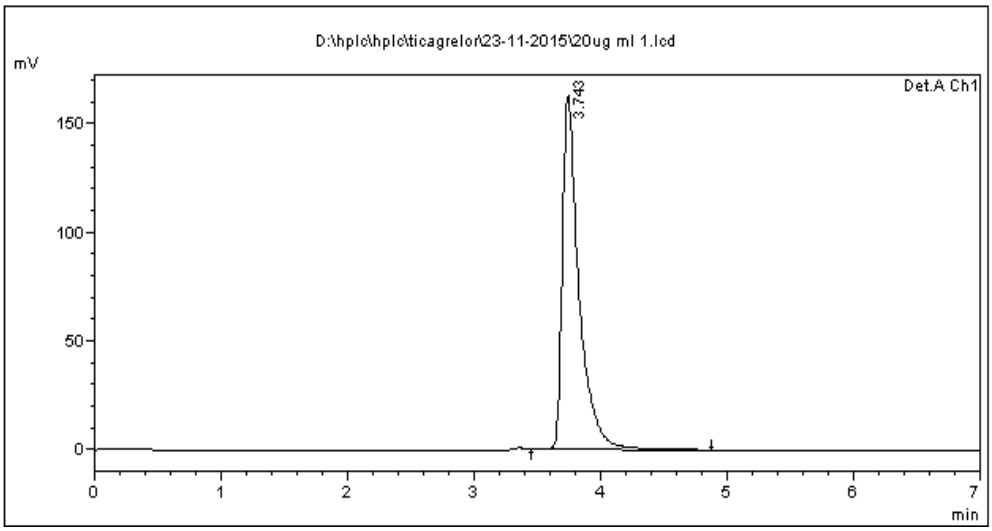

Figure.4: Chromatogram of sample in dosage form 20 µg/ml

CONCLUSION

The developed and validated RP-HPLC method was found to be simple, precise, accurate and sensitive for the estimation of Ticagrelor in Pharmaceutical formulation**.** Validation of results according to ICH and USP carried out high accuracy and good precision. The RSD for all the parameters are found to be less than one, which indicates the validity of method is suitably fine. The mobile phase used in dosage form contains a mixture of acetonitrile and methanol in the ratio of 70% v/v in pump A and 30% v/v in pump B at a flow rate of 1 ml/min. The detector wavelength was found to be 254 nm in U.V spectroscopy. Retention time of Ticagrelor in pharmaceutical dosage form was found to be 3.793 min with a run time of 7 min. The drug shows linearity in the range of 10-100 µg/ml with a correlation coefficient of 0.9967. Hence, this method can be easily and conveniently adopted for the quality control analysis of Ticagrelor in Pharmaceutical formulation.

REFERENCES

[1] R.Singh, *J. Pharm. Edu. Res*., **2013**, 4(1),26-33.
[2] B.Prathapa, Dey. Akalanka, G.H. Srinivasa, P.Johnson & P.Arthanariswaran, *I.J.N.T.P.S.*, **2013**, 3(1), 15-23.

[3] D. Goel, *Int. J. Appl. Basic. Med. Res.*, **2013**, 3(1), 19-21.
[4] RaeAnne and Benjamin Chavez, *Pharmacy and Therapeutics*., **2012**, 37(10), 562-68.
[5] R.Teng, S.Oliver, M.A.Hayes, K.Butler., *Drug Metab. Dispos*., **2010**, 38(9), 1514-21.
[6] ICH Harmonised Triparite Guidelines. Validation of Text and methodology Q2(R) 2005. In International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use., **1996**, 4.
[7] A. Mrunal Ambasana, P.Naval Kapuriya, J. Nilkanth Faldu and D. Karthik Ladva, *Der. Pharmacia. Lettr*., **2014**, 6(4), 237-40.
[8] Rao. Lakshmana and L. Kalyani, *Int. J. Pharm*., **2013**, 3(3), 634-42.
[9] S.Savita, R.Janhavi, *Asi. J. Pharm. Clin. Res*., **2014**, 7(1), 137-40.
[10] P.L. Pingale, P.V. Nandasana, *Int.J. Drug. Dev.Res*., **2012**, 4, 247-52.
[11]H.K. Pannu, M.P. Mahajan, S.D. Sawant, *Der. Pharma. Chemica*., **2012**, 4, 996-1002.
[12]B. Dimple, M.Vinodh, M.Vinayak, *Mal. J. Anal. Sci*., **2013**,17, 387-93.