DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF TICAGRELOR IN API FORM AND MARKETED TABLET DOSAGE FORM

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ABSTRACT: ***A novel, economical, rapid, precise, robust, rugged and accurate RP-HPLC method for estimation of Ticagrelor in bulk form and marketed Tablet Dosage form. The Chromatographic Separation was achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol: Phosphate buffer (0.02M and pH was adjusted with orthophosphoric acid) in the ratio of 45:55% v/v at a flow rate of 1.0mL/min and the detection was carried out at 255nm. The proposed method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Ticagrelor. The correlation coefficient was found to be 0.9995 for Ticagrelor. The LOD and LOQ for Ticagrelor were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Ticagrelor, which indicates that the proposed method is highly accurate. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.***

Keywords: Ticagrelor, RP-HPLC, Accuracy, Precision, ICH Guidelines.

I. INTRODUCTION

Ticagrelor is an oral antiplatelet drug that is used with low dose aspirin to decrease the risk of myocardial infarction and stroke in patients with acute coronary syndromes. Ticagrelor[1] has been linked to rare instances of hypersensitivity reactions accompanied by mild liver injury. Ticagrelor[2] is a triazolopyrimidine that is an adenosine isostere; the cyclopentane ring is similar to ribose and the nitrogen-rich [1, 2, 3] triazole [4, 5-d] pyrimidine moiety resembles the nucleobase adenine. A platelet aggregation inhibitor which is used for prevention of thromboembolic events in patients with acute coronary syndrome. It has a role as a platelet aggregation inhibitor and a P2Y12 receptor antagonist. It is a member of triazolopyrimidines, an organofluorine compound, an aryl sulfide, a secondary amino compound and a hydroxyether. Ticagrelor, or AZD6140, was first described in the literature in 2003. Ticagrelor[3] is an ADP derivative developed for its P2Y12 receptor antagonism. Unlike [Clopidogrel], Ticagrelor is not a prodrug. It is marketed by Astra Zeneca as Brilinta in the US and Brilique or Possia in the EU. Ticagrelor was granted EMA approval on 3 December 2010. Ticagrelor was granted FDA approval on 20 July 2011. Ticagrelor is a P2Y12 platelet inhibitor used in patients with a history of myocardial infarction or with acute coronary syndrome (ACS) to prevent future myocardial infarction, stroke and cardiovascular death. The IUPAC Name of Ticagrelor is (1S, 2S, 3R, 5S)-3-[7-[[(1R, 2S)-2-(3, 4-difluoro phenyl) cyclo propyl] amino]-5-propyl sulfanyl triazole [4, 5-d] pyrimidin-3-yl]-5-(2-hydroxy ethoxy) cyclopentane-1, 2-diol. The Chemical Structure of Ticagrelor is in fig-1.

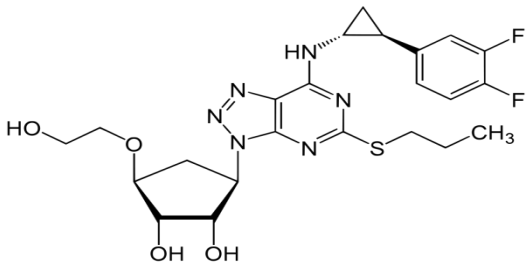

Fig.1. Chemical Structure of Ticagrelor

Therefore, it was thought of interest to develop simple, accurate, fast and cost effective method for the analysis of Ticagrelor in its tablet formulation. This paper describes development and validation[4] of simple, specific, sensitive, accurate and precise Chromatographic method[5] for the estimation of Ticagrelor in bulk and its formulation.

II. EXPERIMENTAL

Table-1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**T60-LAB INDIA** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Table-2: List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development

Wavelength Detection (Or) Selection of Wavelength:

The detection wavelength[6] was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range[7] from 200-400nm.

Preparation of Standard Solution:

10 mg of Ticagrelor working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Ticagrelor working standard[8] solution. The solution was mixed well and filtered through 0.45µm filter.

Preparation of Sample Solution:

Twenty tablets were taken and the average weight[9] was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Ticagrelor equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas[10].

Preparation of 0.02M Potassium dihydrogen orthophosphate Solution:

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted[11] to 1000ml with HPLC Grade water. The pH was adjusted to 3.60 with diluted orthophosphoric acid.

Preparation of Mobile Phase:

550ml of Phosphate buffer (0.02M) pH 3.60 and 450ml of HPLC Grade Methanol were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration[12].

Method Validation

Accuracy

The accuracy of the method was determined by calculating % recovery. A known amount of Ticagrelor was added to a placebo and the amounts were estimated by measuring the peak area. These studies were carried out in triplicate over the specified concentration range and the amount of Ticagrelor was estimated by measuring the peak area ratios. The percentage recovery[13] and standard deviation[14] of percentage recovery were calculated.

Precision

The precision[15] of the method was determined in terms of Intra-day and inter-day precision. For intra-day precision studies, a standard solution of 10 ppm was injected at various time intervals and percent related standard deviation (%RSD) was estimated. The inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the % RSD of the signal was calculated. The repeatability, intermediate precision[16] and reproducibility of the developed method were determined.

Specificity

Specificity[17] is the ability to assess unequivocally the analyte in the presence of components etc. The blank (diluent), placebo, standard (10 ppm), sample (10 ppm) were prepared and injected to prove that the method developed was specific to Ticagrelor.

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 12-28 ppm of Ticagrelor. A regression line was plotted of peak area v/s concentration. The correlation coefficient and equation of the regression line were calculated. The interval of lowest assessed concentration to the highest is the linearity[18] range[19] of the procedure.

LOD and LOQ

The detection limit[20] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit[21] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy[22].

$$LOD = 3.3 \times \sigma/S \text{ and } LOD = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response,

Slope = slope of the calibration curve[23].

Robustness

Robustness[24] of the developed method was studied by changing the flow rate and column temperature. The effect of flow rate was studied by keeping all chromatographic conditions[25,26] same except the flow rate, i.e. 0.9ml/min and in the next run 1.1ml/min respectively.

System Suitability

The system suitability[27,28] parameters like retention time, the number of USP theoretical plates, USP tailing, and peak area and peak height were evaluated.

III. RESULTS AND DISCUSSION

Method Development

Selection of Wavelength:

The UV spectrum[29] of Ticagrelor was obtained and the Ticagrelor showed absorbance's maxima at 255nm. The UV spectrum of drug is in fig-2.

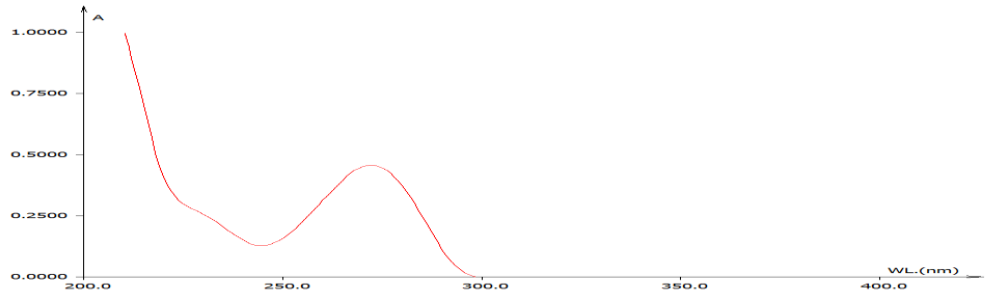

Fig.2. UV Spectrum of Ticagrelor

Observation: While scanning the Ticagrelor solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Method Optimization

Table-3: Optimized Chromatographic Method

Mobile phase	Methanol : Phosphate buffer (0.02M, pH-3.6) = 45:55
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic

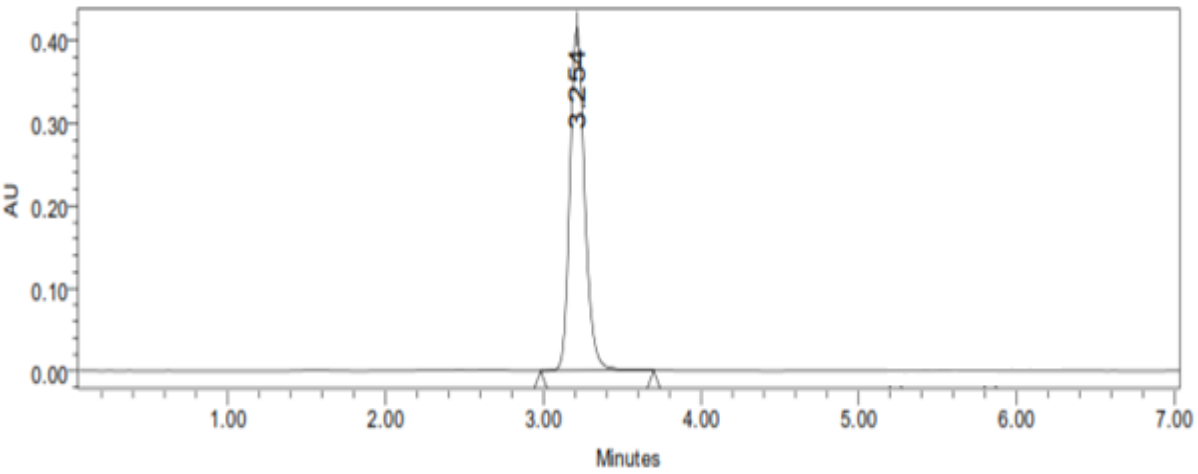

Fig.3. Chromatogram of Ticagrelor in Optimized Chromatographic Condition

Method Validation

System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system[30] that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-4.

Table-4: Data of System Suitability Test

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	3.253	284568	7368	1.26
2	Injection 2	3.254	285684	7295	1.25
3	Injection 3	3.215	283659	7346	1.27
4	Injection 4	3.297	284754	7394	1.29
5	Injection 5	3.253	283695	7425	1.25
6	Injection 6	3.213	284578	7385	1.27
Mean			**284489.7**	**7368.833**	**1.265**
S.D			**752.5617**		
%RSD			**0.26453**		

Table-5: System suitability results for Ticagrelor (Flow rate)

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Ticagrelor = 0.12
2	Theoretical plate	N > 2000	Ticagrelor = 7258
3	Tailing Factor	(Tf) < 2	Ticagrelor = 1.25

Linearity: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Ticagrelor. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve[31] was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Plotting of Calibration Graphs: The resultant areas of linearity peaks are plotted against Concentration.

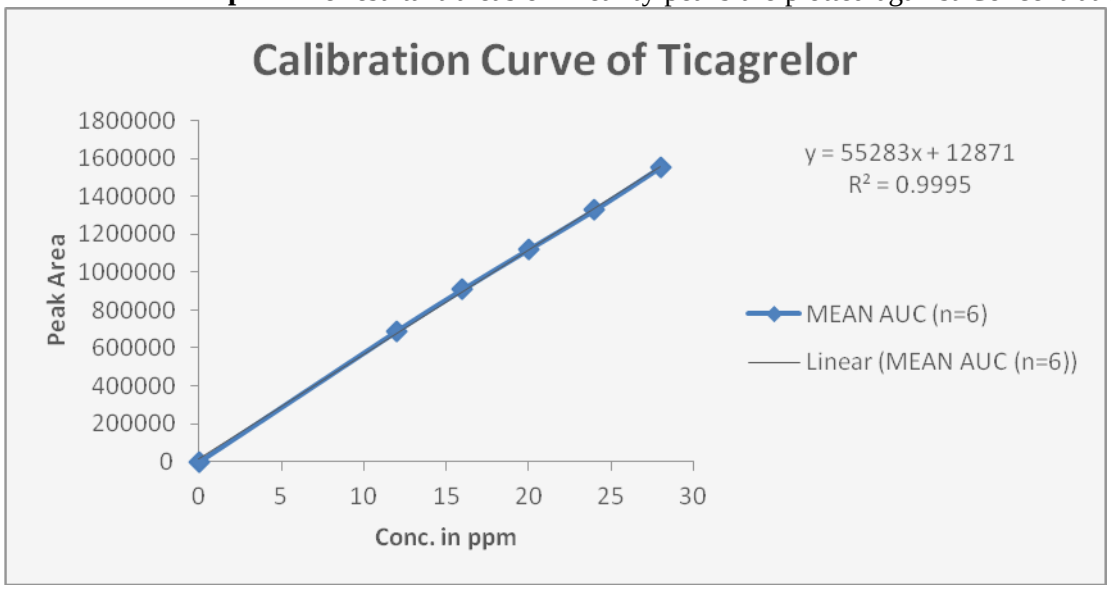

Fig.4. Standard curve for Ticagrelor

Observation: Linearity range was found to be 0-28µg/ml for Ticagrelor. The correlation coefficient[32] was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Ticagrelor.

Table-6: Linearity Readings for Ticagrelor

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Accuracy:

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Ticagrelor were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation y = 55283x + 12871. The results were shown in table-7.

Table-7: Accuracy results of Ticagrelor

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113% S.D. = 0.473694346 % R.S.D.= 0.471753
S_2 : 80 %	8	7.843532	446485	100.637	
S_3 : 80 %	8	8.19449	465887	100.73	
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S_5 : 100 %	10	9.978655	564521	100.868	
S_6 : 100 %	10	10.19623	576549	101.716	
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S_8 : 120 %	12	12.16785	685546	100.69	
S_9 : 120 %	12	12.18644	686574	100.823	

Observation: The mean recoveries were found to be 100.411, 100.664 and 100.463% for Ticagrelor. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision: The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Ticagrelor. The percent relative standard deviations were calculated for Ticagrelor are presented in the Table-8.

Table-8: Repeatability Results of Ticagrelor

HPLC Injection Replicates	AUC for Ticagrelor
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**

Standard Deviation	1318.666
% RSD	0.462558

Observation: The repeatability study which was conducted on the solution having the concentration of about 20µg/ml for Ticagrelor (n =6) showed a RSD of 0.462558% for Ticagrelor. It was concluded that the analytical technique showed good repeatability.

ii) Intermediate Precision / Ruggedness

Table-9: Ruggedness Results for Ticagrelor

Conc. of Ticagrelor (API) (µg/ml)	Observed Conc. of Ticagrelor (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.21	0.76	8.23	0.46
10	10.37	0.33	10.36	0.57
12	12.56	0.23	12.56	0.75

Observation: Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

Robustness: Robustness is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Table-10: Result of Method Robustness Test for Ticagrelor

Change in parameter	% RSD
Flow (0.8 ml/min)	0.554
Flow (1.2 ml/min)	0.867
More Organic	0.886
Less Organic	0.817
Wavelength of Detection (257 nm)	0.813
Wavelength of detection (253 nm)	0.794

Observation: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & organic phase (±5%) studied to determine the robustness of the method are also in favour of (Table-10, % RSD < 2%) the developed RP-HPLC method for the analysis of Ticagrelor (API).

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Observation: The LOD was found to be 5.004µg/ml for Ticagrelor.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Observation: The LOQ was found to be 15.164µg/ml for Ticagrelor.

Assay: – Assay refers to chromatography based purity assay where a compound of unknown activity or purity is compared to a reference standard with precisely determined bioactivity or purity.

AT WS DT P

$$\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

The assay was performed as explained in the previous chapter. The results which are obtained are following Table-11:

Table-11: Recovery Data for estimation Ticagrelor in Brilinta Tablet

Brand name of Ticagrelor	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Brilinta Tablet (AstraZeneca Pharmaceuticals)	90mg	89.893 (± 0.368)	99.698 (± 0.476)

Result & Discussion: The amount of drug in Brilinta Tablet was found to be 89.893 (±0.368) mg/tab for Ticagrelor & % Purity was 99.698 (± 0.476) %.

IV. SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Ticagrelor, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

Ticagrelor was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), sparingly soluble in aqueous buffers, Soluble in ethyl acetate, soluble in methanol, ethanol, n-propanol, isopropanol, 1-butanol, isobutanol, n-octanol, acetonitrile and ethyl acetate.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Ticagrelor it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently.

Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Ticagrelor in different formulations.

REFERENCES

[1] https://go.drugbank.com/drugs/DB08816
[2] https://pubchem.ncbi.nlm.nih.gov/compound/Ticagrelor
[3] https://en.wikipedia.org/wiki/Ticagrelor
[4] United States Pharmacopoeia 29 NF 24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, page no. 587.
[5] Skoog, West, Holler, Crouch, “Fundamentals of analytical chemistry”, eighth edition, 2009 (Indian edition), Cengage learning India Pvt ltd, New Delhi, Page no. 271-280.
[6] A.V Kasture, K.R Mahadik, S.G Wadodkar, H.N. More, “A textbook of pharmaceutical analysis, Instrumental methods”, Nirali Prakashan, vol.2, 9th edition, page no. 5-7, 28-30.
[7] Settle FA, In: Handbook of Instrumental Techniques for Analytical Chemistry. 1st ed, Singapore, Pearson Education Inc.2004.

[8] Willard HH and Dean AJ. Instrumental Methods of Analysis. CBS Publishers and distributors, 7th ed, 1986, 513-515.
[9] Connors AK. In: A Text Book of Pharmaceutical Analysis. A Wiley Interscience Publication, 3rd ed, 2005, 373-400.
[10] "Instrumental Method of Chemical Analysis" by Chatwal Anand, Himalaya Publishing House, p.no.615-623.
[11] Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), P1-7.
[12] "Practical Pharmaceutical Chemistry", 4th edition, Part 2, by Beckett and Stenlake, CBS Publishers and Distributors, P.No.157-174.
[13] Govt. of India, Ministry of Health and Family Welfare. Vol. 2. Delhi: Publication by Controller of Publication; 2007. Indian Pharmacopoeia; pp. 484–554.
[14] "Principles of Instrumental Analysis", 5th edition, Harcourt Publishes Int Company, Skoog, Holler and Nieman, Chapter 28, p.726-766.
[15] "HPLC Columns" Theory, Technology and Practice. Uwe D. Neue, Wiley-VC
[16] Handbook of HPLC, Vol.78, by Elena Katz et al. Marcel Dekker Inc.
[17] "Instrumental Methods of Chemical Analysis", 5th Edition, Galen W. Ewing, McGraw Hill Book Company 1988.
[18] "HPLC in Pharmaceutical Industry", Fong and Long, Marcel Dekker Series
[19] British Pharmacopoeia. (International ed.) 1993; Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act 1968, 1993.
[20] Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications. 2006.
[21] Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography. 6-8.
[22] Synder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development, 2nd ed, John Wiley and Sons Inc. Canada. 1997.
[23] Mohammad T et al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutica Sciencia. 2012, 2(3), 14.
[24] Snyder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development. 2nd ed, 2001.
[25] Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
[26] Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
[27] Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
[28] Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
[29] A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
[30] Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
[31] Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytica Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
[32] ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
[33] PR. Kulkarni1 * and GK.Gajare2, Development and Validation of RP-HPLC Method for Estimation of Ticagrelor in Bulk Form, International Journal of Research in Pharmacy and Chemistry, IJRPC 2016, 6(4), 733-737.
[34] Eena Joshy, Anu Babu, Delma D'cruz and Aneesh T. P.*, Development and validation of RP- HPLC method for determination of Ticagrelor in pharmaceutical dosage formulation, Scholars Research Library Der Pharmacia Lettre, 2016, 8 (9):206-212.
[35] K. Tabassum1*, R. Sarvesh2, Analytical Method Development and Validation Studies of Ticagrelor Tablets by RP-HPLC, International Journal of Applied Pharmaceutics, Vol 9, Issue 4, 2017, 10-21.
[36] Aswini Kumar Parida, K. Srinivasa Rao, Ajay Kumar Patnaik, A Novel Validated RP-HPLC Method for the estimation of Ticagrelor in Bulk and Pharmaceutical Dosage Forms. Research J. Pharm. and Tech. 2018; 11(3): 867-872. Doi: 10.5958/0974-360X.2018.00161.0.
[37] V. Kapil, K. Rambabu, K. Prasad, D. Nagaraju, N. Rajashekar, Dr. B. Tirumaleswara Rao and Dr. Challa Sudheer*, Analytical Method Development and Validation for the Estimation of Ticagrelor in Drug Substance by RP-HPLC Method, European Journal of Biomedical and Pharmaceutical Sciences, ejbps, 2017, Volume 4, Issue 5, 268-272.
[38] P. Shyamalambica , M. Vijaya Lakshmi, Development and Validation of Stability Indicating RP- HPLC Method for the Estimation of Ticagrelor in Formulation, International Journal Of Engineering Research & Technology (IJERT), Volume 07, Issue 10 (October – 2018).