RESEARCH PAPER

QBD Assisted Estimation Of Ticagrelor By A Novel Rp-Hplc Method: Development, Validation And Forced Degradation

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ABSTRACT
A new, simple, accurate and selective method using reverse-phase high-performance liquid chromatography was developed for the assay of ticagrelor in bulk drug and dosage form. Mobile phase prepared with 0.1% orthophosphoric acid in water: acetonitrile (52.5: 47.5v/v) and C 18 column (150 mm, 4.6mm,3.5 m; column dimensions) used as stationary phase, which provided fine peak. The wavelength selected for analysis is 254 nm. The retention time is 4.6 minutes. Linearity obtained in concentration 10 to 50 g/mL. The recoveries at 80%,100%,120% level of concentrations were 99.43 %,99.85 %,100.09%. Percentage results were under in stated acceptable limits for precisions. Small changes in chromatographic condition did not appear any deviation; thus, confirming the reliability of method. The selectivity of method revealed no interference by impure substances or pharmaceutical ingredients. Forced degradation was carried out under different condition, (Acidic, basic, oxidative, and thermal). The developed method applied on routine analysis in pharmaceutical dosage form according to International Council for Harmonisation guidelines.

Keywords: Ticagrelor, method development, RP-HPLC, validation, forced degradation studies.
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INTRODUCTION

Ticagrelor an oral antiplatelet drug which reduces the risk of heart thrombotic events in patients with acute coronary syndrome. Ticagrelor is part of triazolopyrimidine class and IUPAC named 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-hydroxyethoxy) cyclopentane-1,2-diol". Its molecular formula is C23H28F2N6O4S, and its molecular weight is 522.57 g/mol.[1]

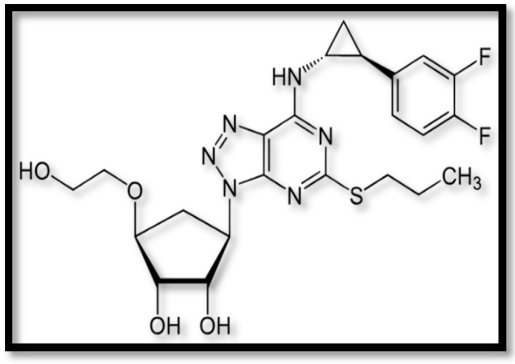

Figure 1. Chemical structure of Ticagrelor drug

Figure 1 depicts the chemical structure of ticagrelor, highlighting its characteristic heterocyclic rings, difluorophenyl group, and hydroxyl-containing side chain, which are important structural features of the drug.

MATERILS AND METHODS

Chemicals and Reagents

Ticagrelor API was purchased from Dhamatech Pharma, Navi Mumbai. Marketed Brilinta 90 mg (ticagrelor) tablets were purchased from a local pharmacy. Other reagents such as acetonitrile, HPLC-grade Orthophosphoric Acid and water were bought from Merck India Limited Mumbai. Hydrochloric acid and hydrogen peroxide of analytical grade and NaOH used in the study.

Method development

A Box–Behnken design (BBD), was employed for the systematic method optimization. The method was optimized by identifying the independent method variables that might affect the method performance. Hence the three major method variables which were mobile phase, flow rate and wavelength were chosen as independent variables. The dependent responses, tailing factor (TF) and retention time, were selected as critical analytical responses for evaluating chromatographic performance and method robustness. The experimental design comprised 17 runs, including five center points, to assess the main, interaction, and quadratic effects of the selected factors.

Instrumentation and Chromatographic Conditions

Chromatography was performed using a HPLC Agilent Technologies 1260 Infinity II model with a DAD detector and Open Lab software. All weights were measured using an electronic weighing

balance (Shimadzu Model), and an ultrasonicator (Life Care Equipment) was used in the study.

a. Column: C18 (150 x 4.6 mm, particle size 3.5µm)
b. Wavelength: 254 nm
c. Autosampler Injection Volume: 10 µl
d. Analysis Run time: 10 Minutes

Preparation of standard stock solution

10mg of the drug was taken and diluted in 10ml volumetric flask with the use of the 5ml of the solvent. To remove air from in between add solvent up to that point, sonicate for ten minutes then made up to 10ml which will give 1000µg/ml. Then filter the sample. Then 1ml from the above filtrate taken in 10ml volumetric flask and again diluted till 100µg/ml. Then further dilute stock solution till the final concentration will be 10µg/ml.

Preparation of sample solution

BRILINTA 90 mg ticagrelor tablet, ten tablets were accurately weighed and finely crushed. A quantity of the powder equivalent to 10 mg was added into a 100 mL volumetric flask. Add the 100mL of volumetric flask with approximately 75 mL of solvent, then sonicate for ten minutes to completely dissolve, then made up to 100mL to get a solution of concentration 1000 g/ mL. Diluted stock solutions to achieve 10µg/ ml.

Preparation of the mobile phase

0.1 % orthophosphoric acid in water: acetonitrile was used as the mobile phase in ratio of 47.5:52.5 (v/v). The prepared mixture was thoroughly mixed and degassed via sonication for 5 min prior to use.

Assay method

Standard and sample solutions were prepared and analysed, and the percentage assay was calculated.

Method validation

Accuracy:

Recovery studies were used to validate the proposed method in terms of accuracy. Samples were prepared by spiking known amounts at three levels (80%, 100% and 120%).

Precision:

Six replicate preparations of the standard solutions were analysed for interday and intraday precision, and %RSD was calculated. The % RSD should be less than 2.0.

Specificity:

The specificity was determined by blank and standard solutions.

Linearity:

The linearity range was 10-50 µg/ml. The relationship between the peak area and concentration of ticagrelor was linear over the examined range.

Robustness:

Three replicates of the standard solution were prepared by varying the flow rates, and the mobile phase compositions were checked for robustness.

System suitability:

A single blank injection and five replicates of the standard solutions containing 20 µg/ml of the drug were analysed to assess system suitability.

Forced Degradation Studies

Acid degradation:

The Five ml of stock solution was treated with five ml of 0.1N HCl, and refluxed for 4 hours. The reaction mixture was neutralized with 0.1 N NaOH solution.

Basic Degradation:

The five ml of the stock solution was treated with five ml of the 0.1N NaOH solution were refluxed for 4 hours. Then the 0.1 N HCl solutions was added to neutralize the mixture.

Oxidative Degradation:

Mixed 5ml of stock solution with 5ml of 3% hydrogen peroxide solution and. refluxed for 4 hours.

Thermal Degradation:

5 ml of stock solution was exposed to 60°C in a hot air oven for 6 hours. The sample was taken out of the oven and allowed to cool to room temperature.

All samples were diluted and analysed by the proposed HPLC method.

RESULTS AND DISCUSSIONS

The results obtained during the proposed method are presented and discussed in this section.

Method Optimization by AQBD approach:

The analytical method has been improved with QbD principle, where Box-Behnken design (BBD) approach was used to examine effect of the critical variables i.e. Mobile phase composition, flow rate and wavelength towards the chromatographic responses.

Table 1. Factors screening by Box – Behnken Design (BBD)

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	**Runs**	**A: MOBILE PH**	**B: FLOW RATE**	**C: WAVELENGTH**	**R1 AREA**	**R2 TP**	**R3 TF**

		ASE					
					AUC	TP	TF
3	1	40	0.8	252	2404.45	8270	0.97
4	2	50	0.8	252	2863.62	8306	0.98

9	3	45	0.7	254	2895.90	9072	0.96
11	4	45	0.7	254	2850.53	9111	0.95
6	5	52.0711	0.7	252	3353.22	9874	0.96
2	6	50	0.6	254	3733.17	11003	0.94
7	7	45	0.558579	256	3698.71	10279	0.93
1	8	40	0.6	256	3301.07	8872	0.93
10	9	45	0.7	254	3125.98	9169	0.96
8	10	45	0.841	254	2452.14	8037	0.98
			421				
5	11	37.9289	0.7	256	2761.24	8275	0.95
13	12	45	0.7	254	3057.15	8980	0.96
12	13	45	0.7	254	3057.44	8982	0.95
17	14	40	0.6	252	3473.15	8409	0.94
14	15	50	0.8	256	3733.17	8680	0.95
15	16	50	0.7	254	3624.28	8551	0.93
16	17	40	0.7	254	3365.29	8291	0.97

Table 1. indicates the inclusion of five centre-point runs following the Box–Behnken design trials.

Table 2. Confirmation location		
A: MOBILE	**B: FLOW RATE**	**C: WAVELENGTH**
PHASE (%ACN)		
47.5%	0.7 ml/min	254 nm

Table 2. confirms the selected levels of the mobile-phase composition (47.5%), flow rate (0.7 mL/min), and detection wavelength (254 nm) as the optimized conditions.

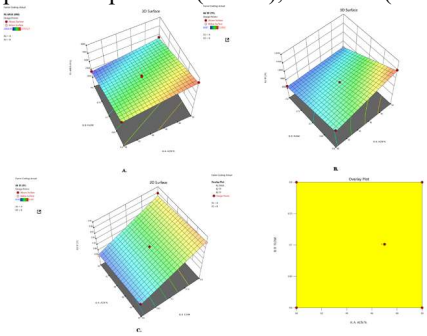

Figure 2. A) 3D Surface Plot for response 1(Area), 2.B) 3D Surface Plot for response 2(TP), 2. C) 3D Surface Plot for response 3(TF) and 2. D) Overall plot

Figure 2 demonstrates the 3D surface plots generated using the Box–Behnken design. Figure 2A, 2B, and 2C represent the effects on peak area, theoretical plates (TP), and tailing factor (TF), respectively. Figure 2D presents the overall plot, supporting the identification of the optimized analytical conditions.

Assay method:

Table 3: Assay of Ticagrelor			
Sr.no.	**Concentration (µg/ml)**	**Standard Area**	**Sample Area**

1.	10	3634131	3640462
2.	10	3596216	3535471
3.	10	3656529	3540472
4.	10	3605422	3635461
5.	10	3574814	3630452
6.	10	3625142	3545481
Average		3615376	3587966.5

Tablet Average Weight	280.61 mg
Standard Weight	10 mg
Sample Weight	31.14 mg
Labelled Amount	90 mg
Standard Purity (%)	99.06%
Assay (%)	98.45%

As seen from Table 3, the amount of ticagrelor present in tablet dosage form was 98.45% of labelled claim.

Method development:

Various experimental trials were conducted to determine the most suitable mobile phase composition for the RP-HPLC method.

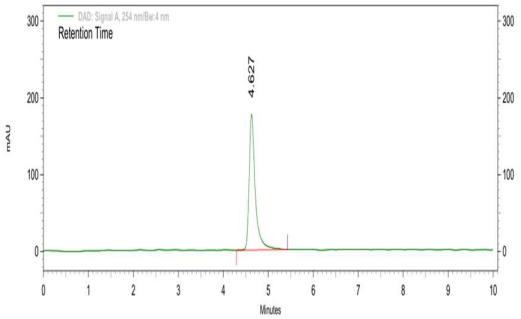

Figure 3. Optimised Chromatogram of Ticagrelor using 0.1 % OPA in water: acetonitrile (52.5: 47.5 v/v)

As shown in the optimized chromatogram (Figure 3), after systematically evaluating the chromatographic performance of different compositions. The optimum mobile phase composed of 0.1 % orthophosphoric acid in water and acetonitrile in the ratio of 52.5: 47.5 v/v was chosen.

Method validation

The method was validated by the described procedure following the guidelines of the ICH Q2.

Accuracy:

Table 4. Accuracy of Ticagrelor

Levels	Amount added (µg/ml)	Amount found (µg/ml)	Peak Area	% Recovery	Mean % Recovery	% RSD
80 %	72	71.55	3228792	99.37	99.43	1.42
		72.63	3277211	100.88		
		70.59	3185758	98.04		
100 %	90	90.13	4060334	100.15	99.85	0.57
		90.20	4063305	100.22		
		89.28	4022034	99.19		
120 %	108	108.30	4873555	100.28	100.09	0.55
		107.42	4833885	99.46		
		108.57	4885407	100.53		

Table 4. shows the percentage recovery at the 80%, 100%, and 120% concentration levels was found to be 99.43%, 99.85%, and 100.09%, respectively.

Precision:

Table 5. Precision data of Ticagrelor

Sr.no.	Concentration (µg/ml)	Intraday Precision	Interday Precision

		Area	
1.	**20**	3656942	3634131
2.	**20**	3642362	3596216
3.	**20**	3599026	3656529
4.	**20**	3624855	3605422
5.	**20**	3570918	3574812
6.	**20**	3708449	3708232
Mean		3633758.667	3629224
Standard Deviation (SD)		47801.07317	48225.79133
%RSD		1.31	1.32

Table 5. shows the %RSD values for intraday and interday precision were determined and found to be 1.31% and 1.32%, respectively.

Specificity:

Typical chromatograms of blank (A) and standard (B) are given in Figure 4. No interference of mobile phase, solvent, or impurities was found.

Figure 4. A) Untreated Chromatogram and B) Treated Chromatogram of Ticagrelor for specificity.

Linearity:

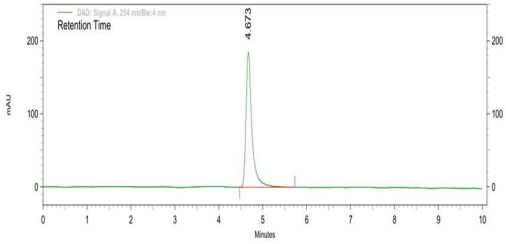

Figure 5. Linearity data of ticagrelo

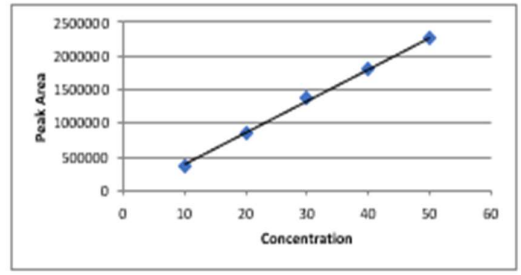

Figure 5 depicts the linearity of ticagrelor.

Table 6. Linearity of Ticagrelor		
Sr.no.	**Concentration (µg/ml)**	**Peak Area**
1.	10	866745
2.	20	1852276
3.	30	2763920
4.	40	3618558
5.	50	4458500
Correlation coefficient		0.9996

Table 6 presents the linearity data of ticagrelor over the given concentration range.

Robustness:

Table 7. Robustness study data				
Parameters	**Altered Conditions**		**Mean**	**% RSD**
Flow rate (ml/mins)	0.6	AREA	3875138	0.52
		RT	5.167	0.13
		NTP	7041	0.56
	0.8	AREA	3244953.3	0.76
		RT	4.205	0.096
		NTP	6555	0.43
Mobile phase (v/v)	53.5 : 47.5	AREA	3932019.3	0.59
		RT	4.855	0.35
		NTP	6652	0.77
	51.5 : 48.5	AREA	3901508.6	1.41
		RT	5.527	0.12
		NTP	6978.6	0.79

Table 7. shows that the robustness of the developed method was within the acceptance limits at different flow rates and mobile phase compositions.

System Suitability:

Table 8. Result of System Suitability Test		
Sr. No.	**Parameters**	**Results**
1.	Tailing Factor	1.63
2.	NTP	7010.83
3.	Retention time	4.6
4.	%RSD	0.96

Table 8 indicates that system parameters for suitability were acceptable to use.

Forced degradation results:

Table 9. Forced degradation results				
Stress conditions	**Peak areas**	**Degradation peaks**	**% Degradation**	**Actual % degradation**
Control	3535471	-	-	-
Acid Degradation	3535471	3466882	99.14	1.94
Basic Degradation	3535471	3312028	93.68	6.32
Oxidative Degradation	3535471	3457626	97.79	2.21
Thermal Degradation	3535471	3505065	99.14	0.86

Table 9. summarizes the results of the forced degradation.

CONCLUSION:

A simple and precise stability indicating method was developed for the ticagrelor estimation, using a quality by design approach. Method optimization was performed using a Box–Behnken design to ensure robust system parameters. For optimized method, C18 column and mobile phase of 0.1% orthophosphoric acid in water-acetonitrile were used along with 254 nm wavelength. Validation of the method demonstrated excellent linearity, precision, accuracy, specificity, system suitability, and robustness. The evaluation of analytes under various stressed situations confirmed the stability-indicating method. The optimized setting of the suggested HPLC technique resulted in a retention time of 4.6 minutes. For routine control of quality and assessment of stability of ticagrelor formulations, this method is greatly advisable.

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