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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF TICAGRELOR IN BULK FORM

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ABSTRACT

An accurate, Precise, Simple and Economical High Performance Liquid Chromatographic method for the estimation of Ticagrelor in bulk form has been developed. The method so developed is Reverse Phase High Performance Liquid Chromatographic method using Primesil C18 column (Length: 250nm, Diameter:4.6nm, Particle size:5µ) with a simple methanol and Water (0.05% OPA) mixed in a proportion of 95:05 v/v as mobile phase. The retention time for Ticagrelor was found to be 4.5 min. The linearity for the method was observed in a concentration range of 5-25µg/mL with the correlation coefficient of 0.997. The method so developed was validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool which comprises with the analytical method validation parameters like Linearity, Accuracy, Method precision, Specificity, System suitability, Robustness. The results obtained were well within the acceptance criteria.

Keywords: Ticagrelor, RP-HPLC, Method development, Validation.

INTRODUCTION

Ticagrelor [(1S,2S,3R,5S)-3-[7-[(1R,2S)-2-(3,4-Diflurophenyl)cyclopropylamino]-5-(propylthio)-3H-[1,2,3]Triazolo[4,5-d]pyrimidin-3-yl]-5-hydroxyethoxy)cyclopentane-1,2-diol] is a platelet aggregation inhibitor used for reduction of clinical thrombotic events in patients with acute coronary syndromes. Like the thienopyridines prasugrel, clopidogrel and ticlopidine, ticagrelor blocks adenosine diphosphate (ADP) receptors of subtype P2Y12. In contrast to the other antiplatelet drugs, Ticagrelor has a binding site different from ADP, making it an allosteric antagonist, and the blockage is reversible. Moreover, the drug does not need hepatic activation, which might work better for patients with genetic variants regarding the enzyme CYP2C19 (although it is not certain whether clopidogrel is significantly influenced by such variants). Ticagrelor is absorbed quickly from the gut, the bioavailability being 36%, and reaches its peak concentration after about 1.5 hours. The main metabolite, AR-C124910XX, is formed quickly via CYP3A4 by dehydroxyethylation at position 5 of the cyclopentane ring. It peaks after about 2.5 hours. Both Ticagrelor and AR-C124910XX are bound to plasma protein (>99.7%) and both are pharmacologically active. Blood plasma concentrations are linearly dependent on the dose up to 1260 mg. (the sevenfold daily dose). The metabolite reaches 30-40% of Ticagrelor's plasma concentration. Drug and metabolite are mainly excreted via bile and feces. Plasma concentrations of Ticagrelor are slightly increased (12-23%) in elderly patients, women, patients of Asian ethnicity, and patients with mild hepatic impairment. They are decreased in patients that described themselves as 'coloured' and those with severe renal impairment. These differences are considered clinically irrelevant.

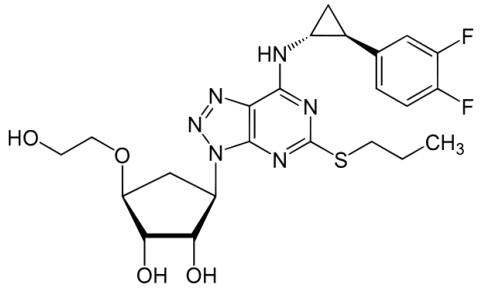

Fig. 1: Chemical Structure of Ticagrelor

MATERIALS AND METHOD

Instrumentation

Chromatographic separation was achieved using a C-18 column (250mm x 4.6mm i.d., 5µm particle size) of Younglin (S.K) Gradient system that is equipped with UV Detector

Materials Required

Ticagrelor pure standard was purchased from Swapnaroop Drug Agency, Aurangabad (MH), India. Methanol and Water of HPLC grade were purchased from Merck (India) and Qualigens (India) respectively. Ticagrelor tablets available under the brand name Brilinta (90mg, Astra Zeneca Ltd.) were purchased and used. Optimized Conditions The mobile phase with methanol and Water in the ratio of 95:05 %v/v was employed in isocratic mode at a flow rate of 1.0 ml/min .The run time was 10 mins and 20µL of the sample was injected for every run into the column. The wavelength of the UV detector was set at 252nm.

Chromatographic conditions

A mixture of methanol and water in the ratio of 95:05 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Ticagrelor. The solvent mixture was filtered through 0.45 µ membrane filter and sonicated for 20 mins before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 252 nm. The run time was set at 10min.

Preparation of Standard Stock Solution

Accurately about 10mg of Ticagrelor was weighed and transferred to a 10mL volumetric flask. 5mL of mobile phase was added to the flask and sonicated to dissolve it. The volume was then made up to the mark with mobile phase to get a standard solution of Ticagrelor at a concentration of 1000µg/mL

Preparation of sample Solutions

Working solutions for HPLC injections were prepared on daily basis. Aliquots of the standard stock solution were taken and diluted with the mobile phase to get solutions in a concentration range of 5-25 µg/mL.

Linearity

Several aliquots of standard solution of Ticagrelor was taken in different 10 mL volumetric flasks and diluted up to the mark with diluents such that the final concentrations of Ticagrelor were in the range of 5 to 25 µg/mL. Evaluation of the drug was performed with UV detector at 252 nm, peak area was recorded for all the peaks. The correlation coefficient value of Ticagrelor was 0.9976. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The data is tabulated in table 1.

System Suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (2.5, 5 and 7.5%) and the percentage recoveries were calculated. The data is tabulated in table 2.

Precision

The precision of the method was determined by intra and inter day precision studies. This was evaluated by injecting three independent sample preparations of Ticagrelor from a single formulation at three different concentration levels on the same day (Intra day) and on three different days (Inter day).The %RSD was then calculated. The data is represented in table 3.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values. Data is represented in Table no. 4.

Robustness

Robustness was established by introducing small changes in the HPLC optimized conditions which include mobile phase ratio (±1),flow rate ratio (±0.1) and wavelength(±1). This was studied using two replicates at a concentration level of 10µg/mL of Ticagrelor. Data is represented in Tables 5, 6 and 7.

Table 1: Data for Linearity

Concentration (µg/ml)	Area
5	567.98
10	1034.08
15	1583.81
20	2164.20
25	2771.20

Table 2: Recovery Studies for Ticagrelor

Recovery level (%)	Conc. taken(µg/ml)	Amount spiked(µg/ml)	Total amount	Amount found(µg/ml)	% recovery	Limit (98-102%)
50	5	2.5	7.5	7.56	102.17	Passed
100	5	5	10	9.91	99.10	Passed
150	5	7.5	12.5	12.58	100.64	Passed

Table 3: Data for Precision

Conc.(µg/ml)	Inter day		Intra day	
	Mean Area*±SD	%RSD	Mean Area*±SD	%RSD
10	1042.85±5.39	0.75	544.03±6.59	1.211
15	1589.11±7.39	0.50	1545.30±14.26	0.922
20	2141.30±21.22	0.99	2556.72±5.09	0.199

***Mean area of two injections.**

Table 4: LOD and LOQ

Standard Solution	LOD µg/ml	LOQ µg/ml
Ticagrelor	0.2125	0.6440

Table 5: Change in Flow Rate

Conc.µg/ml	Flow rate	RT	Mean area	SD	% RSD
10	0.9	4.1	1039.41	0.98	0.09
10	1.1	4.9	1016.86	14.36	1.41

Table 6: Change in Mobile Phase Composition

Conc.µg/ml	Mobile Phase	RT	Mean area	SD	% RSD
10	96:04	4.4	1003.23	15.78	1.5
10	94:6	4.6	1124.53	8.80	0.78

Table 7: Change in Wavelength

Conc.µg/ml	Wavelength (nm)	RT	Mean area	SD	% RSD
10	252	4.5	972.06	3.76	0.38
10	254	4.7	1683.67	8.80	0.52

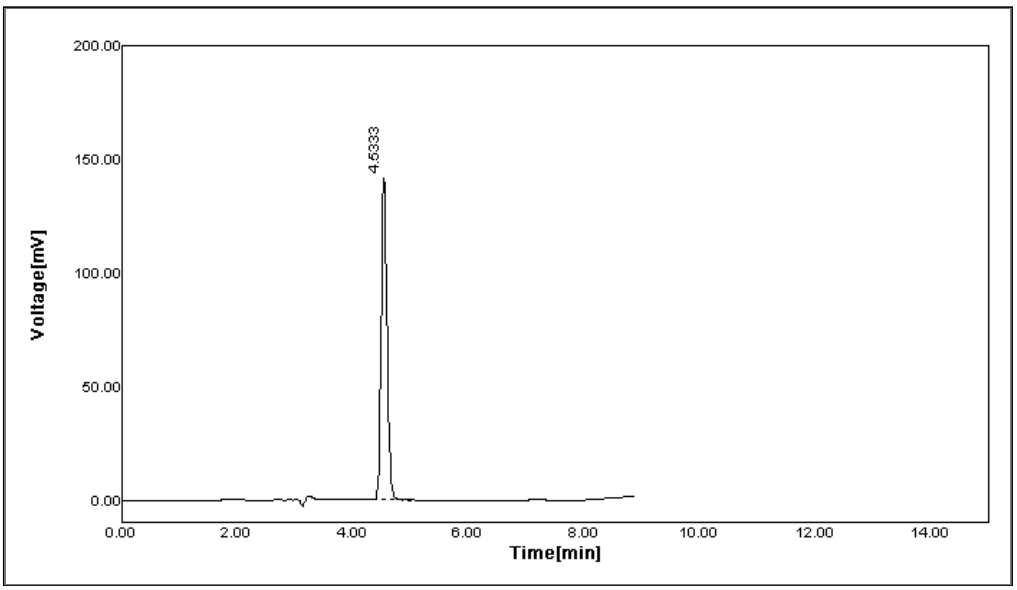

Fig. 2: Chromatogram of Ticagrelor

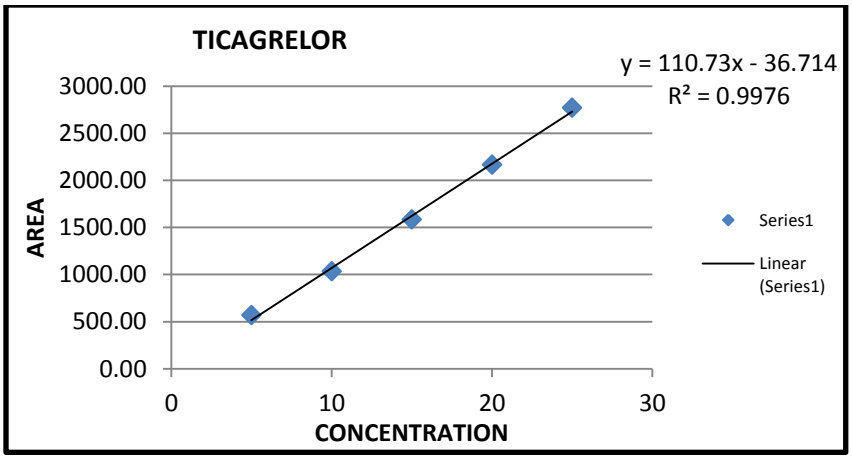

Fig. 3: Calibration Curve of Ticagrelor

RESULTS AND DISCUSSION

The proposed method was found to be simple. Linearity was observed in the concentration range of 5-25µg/mL with the regression equation y=110.73x-36.714 and the correlation coefficient of 0.9976. System suitability parameters indicates high column efficiency with large number of theoretical plates (>2000).The tailing factor was found to be 1.2699 which does not exceed the critical value (2).The average retention time was found to be 4.5. No interference was seen from any of the components of the pharmaceutical dosage form indicating the specificity of the method. The % RSD was found to be 0.75-0.99 for intraday and 1.211-0.199 for inter day precision studies. Thus the method was found to be accurate and precise as the %RSD was not more than 2%. The limit of detection and limit of quantification for Ticagrelor were found to be 0.2125µg/mL and 0.6440µg/mL respectively. The RSD for the % assay of sample was calculated for each parameter in robustness and was found to be less than 2% confirming the robustness of the method.

CONCLUSION

A validated RP-HPLC method was developed for the determination of Ticagrelor in tablet dosage form and bulk forms. As the proposed method is simple, rapid, accurate, precise and specific it can be employed for the routine analysis of Ticagrelor in pharmaceutical dosage forms.

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