

Development and Validation for Quantitative Estimation of Ticagrelor by RP-HPLC with Cardiovascular Effects**Bhawna Lakhawat¹, Akash², Avinash Bairwa³**¹PhD Scholar, Department of Pharmacology, Index Medical College Hospital and Research Center, Indore, M.P., India²Associate Professor, Department of Pharmacology, Index Medical College Hospital and Research Center, Indore, M.P., India³PhD Scholar, Department of Biochemistry, Index Medical College Hospital and Research Center, Indore, M.P., India

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Corresponding author: Dr. Bhawna Lakhawat

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Abstract

An accurate, simple, highly sensitive, precise, rapid, and reproducible high-performance liquid chromatographic (HPLC) method has been developed for the determination of Ticagrelor (TCG) in bulk and tablet dosage forms. The detection was carried in the room temperature at 254 nm. The mobile phase consisting of potassium dihydrogen orthophosphate: ACN was selected for the study. The ratio of 40:60% v/v of selected mobile phase was found to produce asymmetric peaks with good resolution. The buffer pH 2.5 and ionic strength of 0.01M were utilized for the proposed method. The linearity was obtained with correlation factor (r²) 0.9962 in the concentration range of 0.5 µg/ml–3 µg/ml. The %RSD for all the parameters was found to be within the limits, that is, < 2% and % recovery was also found to be within the limit (97%-103%). The mean recovery results of assay were good and the label claim of rivaroxaban tablets was found to be satisfactory.

Keywords: RP-HPLC and Ticagrelor.**DOI:** 10.25258/ijcpr.18.9.141

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Introduction

The chemical name for Ticagrelor (TCG) is (1S,2S,3R,5S)-3-(7-([1R,2S]-2-(3,4-difluorophenyl)cyclopropyl)amino)-5-(propylsufanyl)-3H-1,2,3-pyrimidine-3-yl)-5-(2-hydroxyethoxy)cyclopentane-1,2-diol. Its triazole[4,5-d] molecular formula C₂₃H₂₈F₂N₆O₄S and a molecular weight of 522.6 g/mol. TCG is a CYP 3A4 substrate and weak CYP A4 inhibitor helps prevent problems such as heart attack, severe heart-related chest pain (unstable angina), stroke or formation of blood clots in stents in such people. This blood thinner prevents blood from clotting inside the veins and arteries. This helps the blood flow freely around the body thereby preventing a heart attack or stroke (or deep vein thrombosis or pulmonary embolism). [1-3] In general view, HPLC has been proven to be useful in diagnostic purposes and the pharmaceutical industry [4]. The aim and Objective of the present work was to develop and validate a precise, sensitive HPLC for TCG in its bulk and tablet dosage form. The analytical technique frequently employed for the estimation of drugs, and drug combinations is high-performance liquid chromatography (HPLC) [5,7]. The HPLC method offers a multitude of advantages

and it is suitable method for quantitative analysis [6]. The excellent sensitivity makes HPLC suitable for estimation of drugs in biological fluids [7].

Advantages of using HPLC include speed, automation and compatibility [8].

Materials and Method

Linearity: The linearity of the method was analyzed by recording absorbance of various concentrations of ticagrelor solutions by scanning against blank at λ_{max} of 254 nm. Calibration data and the optical characteristics were calculated from the calibration graph.

Specificity: The spectra obtained from the sample solution with excipients were compared with that of standard solutions for any change in the spectra due to the presence of excipients.

Accuracy: The standards were added at levels of 50, 100 and 150% and their recovery were estimated by recording the absorbance of the solutions by proposed method.

Precision: It was done by performing intraday and interday studies. The % RSD of the precision studies was determined.

LOD and LOQ: The LOD and LOQ values were calculated from standard equation using the slope of the calibration curve.

Stability of solution: The absorbance of solution stored under normal and refrigerated conditions subjected. The percentage decrease in concentration of the solutions was calculated.

Results

The selection of mobile phase was done by trials with different mobile phase compositions.

The mobile phase consisting of potassium dihydrogen orthophosphate: ACN was selected for the study. The ratio of 40:60% v/v of selected mobile phase was found to produce asymmetric peaks with good resolution. The buffer pH 2.5 and ionic strength of 0.01M were utilized for the proposed method. The standard chromatograph of ticagrelor recorded.

Linearity: Analyzing peak areas of various concentration of ticagrelor reveals linearity in the concentration range of 0.5-3.0 µg/ml. Calibration data of ticagrelor is given in table-1.

The recorded chromatograms of linear concentrations are shown in figure-1. Analysis of

calibration graph reveals correlation coefficient (r^2) of 0.9962 with a slope of 34993.8 and intercept of 40156.7.

Accuracy: The results of percentage recoveries of ticagrelor. The results reveal >98 % recoveries of ticagrelor by the proposed method. The good recovery reveals the method is accurate for the determination of ticagrelor.

Precision: Repeatability, intra-day and inter-day precision studies were performed. The calculated mean concentrations and % RSD are recorded in table-2. The relative errors of <2% reveals the proposed method is precise.

LOD and LOQ: the detection limit and quantitation limit calculated based on the standard deviation of the response and the slope. The calculated LOD of rivaroxaban was found to be 0.01986 µg/ml and LOQ was found to be 0.0685 µg/ml Table-3.

Robustness: In order to describe robustness, some chromatographic conditions were slightly altered. The changes include flow rate by ± 0.1 ml/min, volume of organic solvent in mobile phase by $\pm 0.5\%$ and variation in pH of buffer ± 0.5 . The peak shape and retention time were not altered due to the above changes. The results demonstrate the method is robust.

Table 1: Linearity data for ticagrelor

S. No	Concentration (µg/ml)	Peak area
1	0.5	19905
2	1	36892
3	1.5	54908
4	2	79212
5	2.5	97981
6	3	107129

Table 2: Precision data of ticagrelor

Conc. (µg/ml)	Intra-day		Inter-day	
	Mean Conc. $\pm$ SD*	% RSD*	Mean Conc. $\pm$ SD*	% RSD*
1.5	1.4824 $\pm$ 0.0137	0.9289	1.4935 $\pm$ 0.0089	0.6476
2	1.8923 $\pm$ 0.0256	1.2916	2.0219 $\pm$ 0.0159	0.8096
2.5	2.4987 $\pm$ 0.0269	1.0826	2.4022 $\pm$ 0.0409	1.2731

Table 3: LOD and LOQ ticagrelor

Limit of Detection concentration in µg/ml	Limit of Quantitation concentration in µg/ml
0.01986	0.0685

Discussion

The plot of peak areas against concentrations was evaluated by linear regression method and good linearity of ticagrelor was observed in concentration range of 0.05 to 3 µg/ml with correlation coefficient of 0.9962. The method precision was proved by the better results of intra-day and inter-day precision. Adequacy of the proposed method for determination of ticagrelor

was assured by ideal system suitability parameters and specificity. The recoveries and stability of the ticagrelor were found to be excellent; thus, the validated HPLC method was suitable for the analysis of formulation. The mean recovery results of assay were good and the label claim of ticagrelor tablets was found to be satisfactory.

The findings of the present study are comparable with earlier investigations on chromatographic

estimation of ticagrelor. Joshy et al. developed an RP-HPLC method using a C18 column and acetonitrile : methanol (70:30, v/v), with detection at 254 nm. Their method demonstrated linearity from 10–100 µg/mL and an r^2 of 0.9967, supporting the suitability of RP-HPLC for routine quantitative determination of ticagrelor [9].

The slightly broader concentration range in that study compared with the present work suggests that chromatographic methods can be adapted according to the intended analytical concentration and formulation requirements.

Similarly, Parida et al. reported a validated RP-HPLC method using methanol : phosphate buffer (75:25, v/v), with detection at 256 nm. Their method showed linearity over 5–50 µg/mL, an r^2 of 0.999, and recovery of 99.57–99.97% [10]. The present study also demonstrated good linearity and

recovery, although the chromatographic conditions and working concentration range differed. These differences illustrate that more than one chromatographic condition can provide reliable quantification of ticagrelor. The present results are also consistent with the findings of Gobetti et al., who developed a stability-indicating HPLC method for ticagrelor tablets. Their method demonstrated specificity against formulation excipients, linearity with $r=0.9990$, precision with RSD of 0.71%, and mean recovery of 99.61% [11]. The absence of significant interference from excipients in the present study similarly supports its applicability to tablet formulations. The current method additionally demonstrated robustness when flow rate, organic-solvent proportion, and buffer pH were slightly varied without significant alteration in peak shape or retention characteristics.

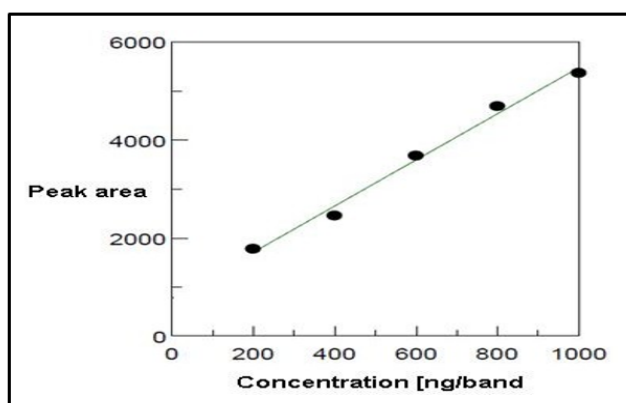


Figure 1: Linearity graph of ticagrelor

Tabassum and Sarvesh also reported a validated RP-HPLC method with a mean recovery of 99.93% and precision RSD of 0.069%; their forced-degradation work further demonstrated the ability of the modified gradient method to resolve related substances [12]. More advanced analytical approaches have subsequently applied quality-by-design principles to ticagrelor analysis and successfully separated multiple impurities and degradation products, emphasizing the continuing development of sensitive and selective analytical procedures [13].

In the present study, the LOD and LOQ were reported as 0.01986 and 0.0685 µg/mL, respectively, indicating good sensitivity. The combination of satisfactory linearity, accuracy, precision, specificity, robustness, and sensitivity suggests that the proposed RP-HPLC method can be applied for routine quality-control analysis of ticagrelor tablets.

Conclusion

According to the current study, stability RP-HPLC method is accurate, precise, specific, reproducible, and sensitive. The RP-HPLC method can also be

used to the Ticagrelor tablet's single dose formulation. No interference of additives, matrix etc. is encountered in these methods. Various validation parameters were carried out: specificity, linearity, precision, LOD, LOQ, accuracy, and forced degradation.

The linearity was obtained with correlation factor (r^2) 0.9962 in the concentration range of 0.5 µg/mL–3 µg/mL.

The %RSD for all the parameters was found to be within the limits, that is, < 2% and % recovery was also found to be within the limit (97%–103%). From the studies, it can be concluded that this RP-HPLC technique can be successfully used to estimate the Ticagrelor in tablet formulations.

Reference

1. Ambasana MA, Kapuriya N, Faldu NJ, Ladva K, Development and validation of a UV spectrophotometric method for the determination of ticagrelor in bulk form, *Der Pharmacia Lettre*, 2014; 6(4):237-240.
2. Ravisankar P, Sireesha M, Srinivasa Babu P, Purna Vyshnavi Ch, David Raj K,

- Development and Validation of UV Spectrophotometric Method for the Estimation of Ticagrelor (Oral Antiplatelet (OAP) in Pharmaceutical Dosage Form, *Int. J. Pharm. Sci. Rev. Res.*, 62(2), May - June 2020; Article No. 21, Pages: 135-140.
3. Anil Kumar N, Naga Swathi PR, Sharmila D, Sharmila SK, Pawar AKM, A validated stability indicating method of UV-Spectrophotometry for the estimation of ticagrelor in bulk & marketed formulation, *Der Pharmacia Lettre*, 2016, 8(19):309-315.
 4. Lavnya Chowdary G, Ravi Sankar P, Akhil Kumar G, Monika K, Srinivasa Babu P, Applications of HPLC in pharmaceutical analysis, *Int. J. Pharm. Sci. Rev. Res.*, 59(1), 2019, 117-124.
 5. Sethi PD. High-Performance Thin Layer Chromatography: Quantitative Analysis Pharmaceutical Formulations. 1st ed. CBS Publishers and Distributors, New Delhi. 1996; 1-4
 6. Sultana N, Arayne MS, Shafi N. In vitro interaction studies of Diltiazem with NSAID's using UV spectrophotometry and RP-HPLC techniques. *Pak J Pharm Sci.* 2007; 20(3): 202-13
 7. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Thomson Brooks. 2007; 816-820
 8. Kalyani L, Lakshmana Rao A, A validated stability-indicating HPLC method for determination of ticagrelor in bulk and its formulation, *International Journal of Pharmacy*, 3(3), 2013, 634-642.
 9. Joshy E, Babu A, D'Cruz D, Aneesh TP. Development and validation of RP-HPLC method for determination of ticagrelor in pharmaceutical dosage formulation. *Der Pharmacia Lettre*. 2016;8(9):206-212.
 10. Parida AK, Rao KS, Patnaik AK. A novel validated RP-HPLC method for the estimation of ticagrelor in bulk and pharmaceutical dosage forms. *Res J Pharm Technol.* 2018; 11(3): 867-872.
 11. Gobetti C, Pereira RL, Mendez ASL, Garcia CV. Determination of the new antiplatelet agent ticagrelor in tablets by stability-indicating HPLC method. *Curr Pharm Anal.* 2014;10(4).
 12. Tabassum K, Sarvesh R. Analytical method development and validation studies of ticagrelor tablets by RP-HPLC. *Int J Appl Pharm.* 2017;9(4).
 13. Bueno LM, et al. Application of quality by design to optimize a stability-indicating LC method for the determination of ticagrelor and its impurities. *J Pharm Biomed Anal.* 2018.