GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Application of Stability-indicating RP-HPLC Method for quantification of Ticagrelor In Oral dispersible tablet dosage form

VDL Abhisri Kalla, Shanthi Kalavala, Padma Kandala, Kamuju Satya Priyanka Kamuju, Abhiram Kandula, Vijay Juttaka and Prasad Cheepurupalli *

Department of Pharmaceutical Analysis, Pydah College of Pharmacy, Kakinada, Andhra Pradesh- 533461, India.

GSC Biological and Pharmaceutical Sciences, 2024, 28(01), 054–070

Publication history: Received on 13 May 2024; revised on 30 June 2024; accepted on 03 July 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.1.0247

Abstract

A simple, rapid, accurate and precise stability-indicating HPLC method was developed and validated for the determination of Ticagrelore in oral dispersable tablets. Separation of the drug was achieved on Shimadzu HPLC comprising of LC- 20 AD binary gradient pump, a variable wavelength programmable SPD-20A detector and SCL system controller. C18G column (250 mm x 4.6 mm, 5 µ) as stationary phase with mobile phase consisting of methanol and water in the ratio of 25: 75 v/v. The method showed a good linear response in the concentration range of 10-90 µg/ml with correlation coefficient of 0.999. The flow rate was maintained at 1.0 ml/min and effluents were monitored at 254 nm. The retention time was 5.786 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity and forced degradation studies. Stress conditions including acid, alkali hydrolysis, water stress, oxidation, photolysis, and heat were applied. The degradation products did not interfere with the detection of Ticagrelor, thus the method can be considered as a stability indicating method. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the determination of Ticagrelore in oral dispersible tablets.

Keywords: Ticagrelore; HPLC; Stability-indicating; Oral Dispersable Tablets; ICH

1. Introduction

Ticagrelor (1S,2S,3R,5S) – 3 - (7-{[(1R,2S) – 2 -(3,4difluoro phenyl) cyclopropyl]amino}-5-(propylsulfanyl)-3H-[1,2,3] triazolo[4,5-d]pyrimidin-3-yl)-5-(2-hydroxyethoxy) cyclopentane-1,2-diol. (TCG) is a platelet aggregation inhibitor used for the prevention of thrombotic events (myocardial infarctions, strokes) in patients with acute coronary syndromes. It belongs to the class of triazolopyrimidines which are polycyclic aromatic compounds containing triazole ring fused to a pyrimidine ring. Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation. It is a white crystalline powder with an aqueous solubility of approximately 10µg/ml at room temperature. It has a log P of 2.30, pKa of strong acidic function 12.94 and strong basic function 2.90. It prevents platelet aggregation and thrombus formation in atherosclerotic disease which reduces chances of cardiac arrest due to blockage. The marketed formulation of ticagrelor replaced clopidogrelcontaining formulations due to higher efficacy and lower side effects. It was approved for use in the European Union by the European Commission on December 3, 2010, by the US Food and Drug Administration on July 20, 2011. It is marketed under the name Brilinta® in the USA and Brilique® in the EU.The Brilinta® is available as tablet dosage form and Brilique® is available in orodispersible tablet contains 90 mg ticagrelor. The orodispersible tablets may be used as an alternative to Brilique 90 mg film-coated tablets for patients who have difficulty swallowing the tablets whole or for whom there is a preference for orodispersible tablets. The tablet should be placed on the tongue, where it will rapidly disperse in saliva.

* Corresponding author: Cheepurupalli Prasad

Literature survey reveals various analytical methods reported for estimation of Ticagrelor in tablets dosage forms includes spectrophotometry[1-5] and HPLC[6-7] There are some chromatographic methods repored for estimation of ticagrelor in presence of its impurities. Very few stability indicating RP-HPLC methods[8-9] reported of estimation of Ticagrelor in tablets dosage form. Ticagrelor is also available in orodispersible tablets (Brilique®) but to date no stability indicating RP-HPLC methods reported of estimation of Ticagrelor in orodispersible tablets dosage form. There were accurate and sensitive analytical methods available in literature for other drugs.[10-70] According to Current Good Manufacturing Practices, all drugs must be tested with a stability-indicating assay method before release. It was felt necessary to develop a stability-indicating liquid chromatography (LC) method for the determination of Ticagrelor as bulk drug and as pharmaceutical dosage forms and to separate the drug from the degradation products under the International Conference on Harmonization (ICH) suggested conditions[71] (hydrolysis, oxidations, photolysis and thermal stress). Therefore, the principal objective of this study was to develop a new, simple, economical, precise, and reproducible stability indicating RP-HPLC method with a wide linear range and good sensitivity for assay of Ticagrelor in the bulk drug and in the pharmaceutical dosage forms (orodispersible tablets).

2. Materials and methods

2.1. Chemicals

Ticagrelor pure drug (purity >99.8%) was obtained as gifted sample from RA Chem Pharma Ltd(Hyderabad,India). HPLC grade water and acetonitrile was from MERCK India Ltd. HPLC grade methanol was from standard reagent Pvt Ltd Hyderabad. Analytical grade hydrochloric acid, sodium hydroxide and hydrogen peroxide was from SD Fine chemicals Mumbai, India. Nylon membrane filters 0.2 µm and 0.45 µm were from PALL life sciences Mumbai, India.

2.2. Equipment

The instruments used in the study were electronic balance (Apex,India), sonicator (LAB india Ltd Mumbai, 3.5 L), hot air oven (Accumaxindia), and digita pH meter (Elico LI 120). HPLC (Shimadzu, Kyoto, Japan) was monitored and integrated using LC solutions software. Additionally, syringe [Hamilton (Rheodyne-20 µL)] and syringe filter [Himedia Syringe-driven filters (0.22 µm)] were used.

2.3. Chromatographic conditions

The chromatographic system used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector 7725i fitted with a 20 µL loop was used and data were recorded and evaluated by use of LC solutions software version 5.0. Separation was performed on a Enable C18G (250 × 4.6 mm i.d.,5µ) at ambient temperature. A mixture of methanol and water in the ratio of 20: 80 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 before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at room 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 254 nm. The run time was set at 10 min

2.4. Preparation of mobile phase

Mobile phase was prepared by mixing 200 mL of HPLC grade methanol with 800 mL of HPLC grade water. The mobile phase was sonicated for 10 min and filtered through the 0.45 µm membrane filter.

2.5. Preparation of standard stock solutions

The standard stock solutions of 100 µg/mL of the drug were prepared by dissolving 50 mg of pure drug in the methanol in a 50 mL volumetric flask and the volume was made up to the mark Resulting solutions were further diluted with mobile phase to obtain a final concentration of 100 µg/mL and stored under refrigeration. Aliquots of standard stock solutions were put in a 10 mL volumetric flask and diluted up to the mark with mobile phase. In such a way, the final concentrations of the drug were in the range of 10–90 µg/mL.

2.6. Preperation of sample solution

Ten tablets were accurately weighed and tablet powder equivalent to 5 mg of Ticagrelor was taken and dissolved in 50 ml of methanol. The resulting solution (5 ml) was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase. The final solution was filtered through 0.45µ membrane filter using injection filter.A 20 µL of the

filtrate was injected into chromatographic system. The peak area of the Ticagrelor was determined and concentration was found using linear regression equation obtained from calibration curve.

2.7. Method validation

The developed method was validated by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficients of variation and relative errors of less than 2 % were considered acceptable, except for the quantification limit, for which these values were established at 2%, as recommended in the literature.

2.7.1. System Suitability Test

Before performing validation experiments, system suitability test (SST) has to be applied to indicate that HPLC system and method are capable of providing data with admissible quality. SST was performed by investigating capacity factor, tailing factor, theoretical plates number, resolution and also relative standard deviation (RSD) of the peak areas.

2.7.2. Stability

Stability was assessed by analyzing QC standard solutions after keeping them at room temperature for 48 h. Obtained results were investigated as recovery values and compared to the freshly prepared solutions.

2.7.3. Linearity

A stock solution of ticagrelor of 1000 µg/mL was prepared with methanol. From it, various working standard solutions were prepared in the range of 10 to 120 µg/ml using mobile phase as diluent and injected into HPLC. It was shown that the selected drug had linearity in the range of 10–90 µg/mL. The calibration plot (peak area of Ticagrelor versus Ticagrelor concentration) was generated by replicate analysis (n=9) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

2.7.4. Accuracy

The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value.

2.7.5. Precision

The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (50 µg/mL). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra- and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (RSD).

2.7.6. Robustness

Robustness of the proposed method for Ticagrelor was carried out by the slight variation in flow rate, pH and mobile phase ratio. The percentage recovery and RSD were noted for Ticagrelor.

2.7.7. Ruggedness

The test solutions were prepared as per test method and injected under variable conditions. Ruggedness of the method was studied by different analysts.

2.7.8. Detection limit and quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formulas:

$$LOD=3.3SD/slope$$

$$LOQ=10SD/slope$$

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y-intercept of regression line, and s is the slope of the calibration curve.

2.8. Forced degradation studies

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic, and ultra violet (UV) degradations. The sample was exposed to these conditions, and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

2.8.1. Hydrolytic degradation

Hydrolytic stress testing was performed to force the degradation of the drug substance to its primary degradation products by exposure to neutral, acidic and basic conditions over time. Functional groups likely to undergo hydrolysis are amides (lactams), esters (lactones), carbamates imides, imines, alcohols (epimerization for chiral center) and aryl amines.

To initiate hydrolytic studies, a preliminary solubility screen of the drug substance was performed. Solubility of at least 1 mg/mL in neutral, acidic and basic conditions was recommended for the neutral/ acid/base stress testing. However, concentration less than 1 mg/mL can be used, if solubility is an issue. In some cases, a co-solvent may be necessary to achieve the target concentration. Special attention should be given to the drug substance structure when an appropriate co-solvent was chosen.

2.8.2. Oxidative degradation

Oxidative degradation of drug substances in pharmaceutical formulation is well documented. Although exact mechanistic details about what promotes reaction between drug substance and molecular oxygen in pharmaceutical formulations are not fully understood, such reactions are generally thought to be in the category of auto-oxidation process.

There are three major pathways: (i) autoxidation or radical mediated oxidation, (ii) peroxide-mediated oxidation and (iii) photo chemically induced oxidation. Traditionally, dilute aqueous peroxide solutions have been used for oxidative stress testing of pharmaceuticals. In addition to the auto-oxidation processes, peroxide-mediated oxidative degradation can occur, which may not be observed using radical indicator. The process of oxidation depends on the amount of oxygen in the air and the nature of the material it touches. True oxidation happens on the molecular level. We can only see the large scale effects as the oxygen causes free radicals on the surface to break away.

2.8.3. Photolytic degradation

For photolytic degradation, the drug was exposed to the direct sunlight. Sufficient amount of the drug was taken in a closed petri-dish and exposed to sunlight. At different time intervals, the drug was taken out, diluted appropriately and injected into HPLC to determine the amount of degradation of the drug.

2.8.4. UV-degradation

The goal of UV-degradation studies is to force the degradation of drug substance via UV and fluorescent condition over time to determine the primary degradation products. A molecule absorbs light when an absorption band overlaps to some extent with the incident light energy and a valence electron in the relevant atmosphere rose to an excited state. A near UV fluorescent lamp has a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm, and the significant proportion of UV should be in both bands of 320–360 nm and 360–400 nm.

2.8.5. Thermal degradation

To evaluate thermolytic pathways, evaluated temperatures (e.g. 50 °C and −80 °C) in the solid state and/or in solution can be used. Many compounds began to degrade via different mechanisms above 80 °C, giving rise to degradation products. To solid-state stressing, the use of high and low humidity atmosphere of the evaluated temperature is appropriate. To evaluate stability under the temperatures above, stress conditions were selected based on a conservative estimate of the Arrhenius expression, a quantitative relationship of reaction rate and temperature using average activation energy.

$$K_{obs} = A\exp^{-Ea/RT}$$

3. Results and Discussion

3.1. Optimization of chromatographic conditions

Optimization studies were carried out to develop the HPLC method for estimation of Ticagrelor in the presence of its degradation products. In this case, various parameters were investigated including composition of mobile phase, sationerry phase, wavelength, flow rate and injection volume.The optimum parameters based on peak shapes and retention time values. Selection of wavelength depends upon UV spectra of Ticagrelor in which it shows maximum absorbance at 254 nm, so this wavelength was selected as analytical wavelength. Different mobile phases were tested in the development stage of the method. According to literature, ACN in combination with water, phosphate buffer and ammonium acetate buffer were reported for the determination of Ticagrelor. With methanol as organic phase no HPLC method is reported, so we made a trails with methanol as organic phase and water as aqueous phase. With the increase methanol the retention time decreased but increase in methanol causes less number of theoretical plates.To make the method economical we chosen less percentage of methanol in the composition. Mobile phase in the ratio of methanol : water (20:80,v/v) provided good peak shape and retention,so this composition is optimized. Injection volumes of 5, 10, 15 and 20 µL were investigated. Peak area values increased with the increasing injection volume and better peak shapes were obtained. Therefore, 20 µL was found to be the optimum injection volume. To determine the optimum flow rate, flow rates of 0.8, 1.0 and 1.2 mL min−1 were tested. There was no significant difference on the obtained peak area values. The highest peak area values were obtained with the flow rate of 0.8 mL min−1, however, the peak tailing was a little bit more compared to other flow rates. Due to the fact that increase on flow rate caused high column pressure, the lowest possible flow rate was selected. For this purpose, the flow rate of 1 mL min−1was chosen as the flow rate of the method. Figure 2 illustrates a typical chromatogram obtained at optimum conditions. The optimized chromatographic conditions are shown in Table 1.

Table 1 Optimized chromatographic conditions

Parameters	**Conditions**
Stationary Phase(Column)	C_{18} (250 × 4.6 mm i.d.,5µ)
Mobile Phase	Methanol: Water (20:80,v/v)
Flow rate(ml/min)	1.0 mL/min
Run time(min)	10 min
Column temperature (°C)	ambient
Volume of injection loop(µL)	20
Detection wavelength(nm))	254 nm
Retention time(min)	5.786

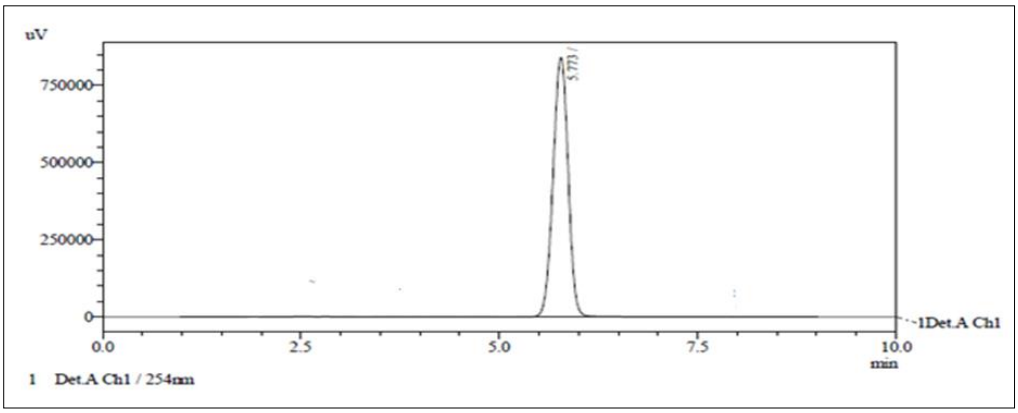

Figure 1 Chromaogram of Ticagrelor

3.2. Method validation

3.2.1. System Suitability Test

After setting the optimum conditions, system suitability parameters for the developed method were determined and compared with recommended limits. To determine the parameters, the study was performed with standard solution of 50 µg/ml concentration and the results were acquired from six injections. System suitability parameters of the method were demonstrated in Table 2. According to results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

Table 2 Results of system suitability test (n = 6)

Parameter	Criteria	Result
Capacity factor(k')	$k' > 2$	4.824
Tailing factor (T)	T < 2	1.211
Theoretical plates (N)	N> 2000	2421
% RSD (peak area)	% RSD ≤ 1	0.87

3.2.2. Stability

The sample solution stability was analyzed by injecting the same solution at 0, 12, 24, and 48 h. Identical change was not observed in the developed method. Also, results were found within acceptable limits (RSD <2), which are summarized in Table 3

Table 3 Stability data of Ticagrelorn(standard solutions)

Time (hr)	Assay(%)	% Difference
Initial	100.08 ---	----
After 12 hr	100.02	0.05
After 24 hr	99.87	0.21
After 36 hr	99.16	0.92
After 48 hr	98.32	1.76

3.2.3. Linearity and sensitivity

Linearity study was performed with calibration standards with 10, 20, 30, 40, 50,60,70,80 and 90 ppm concentrations. The standards were injected in triplicate. Calibration curves were obtained by plotting the peak areas against the given concentrations. The calibration curve was evaluated by the determination coefficient. The determination coefficient (R^2) of the calibration curves was 0.9999. Therefore, the calibration curve for Ticagrelor was found to be linear within the range of 10–90 µg/ml concentrations. The regression equations were calculated from the calibration graphs. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ are given in Table 4. The low values of LOD and LOQ indicates the sensitivity of method.

Table 4 Spectral and statistical data for determination of Ticagrelor by proposed RP-HPLC method.

Parameter	Result
Detection wavelength (nm)	254
Linearity range (µg/ml)	10-90
Coefficient of determination (r^2)	0.9988
Regression equation (Y^a)	Y= 29989x+34171
Slope (m)	29989

Intercept (c)	34171
Limit of detection, LOD (µg/ml)	0.05
Limit of quantitation, LOQ (µg/ml)	0.21

[a]*Y* = *mx* + *c*, where *x* is the concentration (µg/ml).

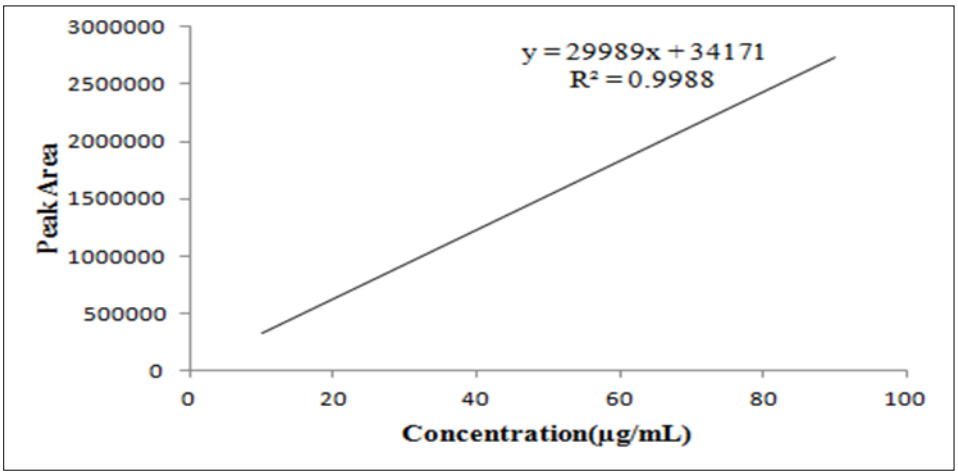

Figure 2 Calibration curve

3.2.4. Accuracy

To study the reliability, the suitability, and the accuracy of the method, recovery experiments were carried out. Known quantities of the pure drug were added to the placebo to make samples at the levels of 80 %, 100 %, and 120 %, and were assayed by the proposed method. Accuracy was calculated as the percentage of recovery and the results are shown in Table 5.

Table 5 Accuracy

% Level	Concentration(µg/mL)	Recovery(%)	Statistical Results
	Formulation Pure drug		Mean SD %RSD
80	50 40 98.4		99.1 1.47 1.48
80	50 40 98.1		
80	50 40 100.8		
100	50 50 100.5		101.3 0.72 0.71
100	50 50 101.9		
100	50 50 98.8		
120	50 60 99.4		99.8 0.51 0.52
120	50 60 99.7		
120	50 60 100.4		

3.2.5. Precision

The precision was demonstrated at three levels: repeatability, intermediate precision, and reproducibility (between laboratories' precision). Each level of precision was investigated by 3 sequential replicate of injections of three concentrations of 40, 50 and 60 µg/mL. The precision was expressed as relative standard deviation (RSD) or coefficient of variation (CV). The results of three levels of precision are shown in Table 6.

Table 6 Precision Data

Precision	**Results**
	Concentration(µg/mL) RSD of Peak area RSD of Retention Time
Repeatability	40 0.89 0.021
	50 1.21 0.088
	60 1.11 0.123
Intermediate precision	40 1.42 0.087
	50 0.75 0.066
	60 0.67 0.062
Reproducibility	40 1.64 0.111
	50 0.78 0.17
	60 0.85 0.094

3.2.6. Robustness and ruggedness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (0.5±0.1 mL/min) and different pH values. The results are given in Figure 3. Ruggedness of the method was carried out by different analysts. The results are displayed in Figure 4.

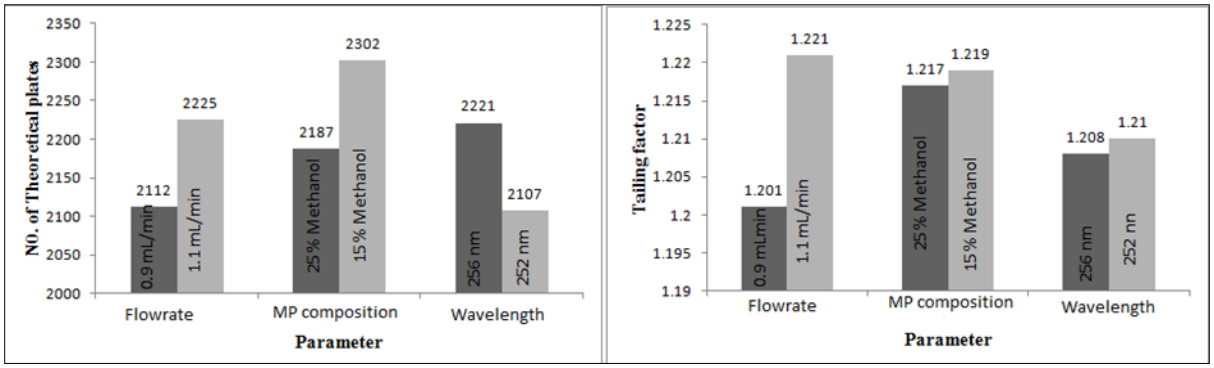

Figure 3 Robustness

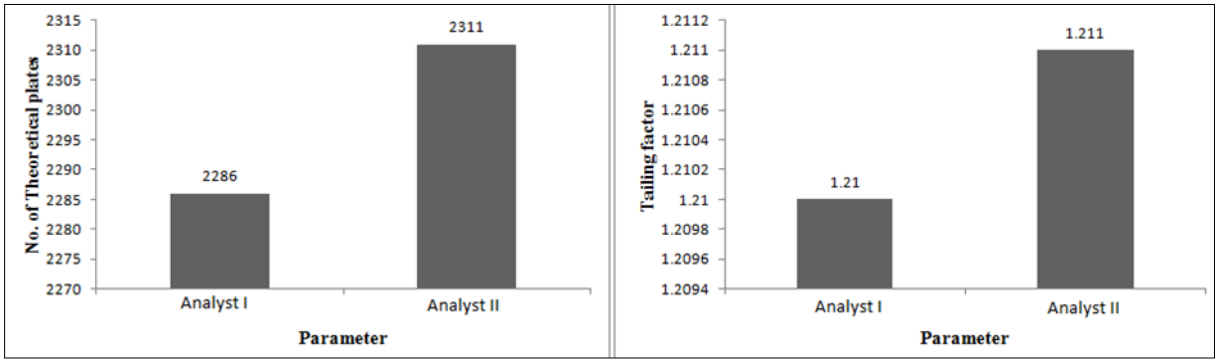

Figure 4 Ruggedness

3.2.7. Mobile phase stability

The stability of the mobile phase was evaluated, so the mobile phase was stored at 4–8 °C for 1 week. The aged mobile phase was compared using a freshly prepared one. The mobile phase was stable up to 1 week at 4–8 °C.

3.3. Forced degradation studies

3.3.1. Degradation in neutral condition

Neutral degradation of Ticagrelor was performed using distilled water. Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of distilled water. Then the volumetric flask was heated on a water bath at 80 °C. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to yield a concentration of 50 µg/mL in the mobile phase. It was then filtered through a 0.22 µm filter and 20 µL was injected into the HPLC for analysis. The obtained chromatogram was observed for any degradation occurred during the time. The results Fig. 5.

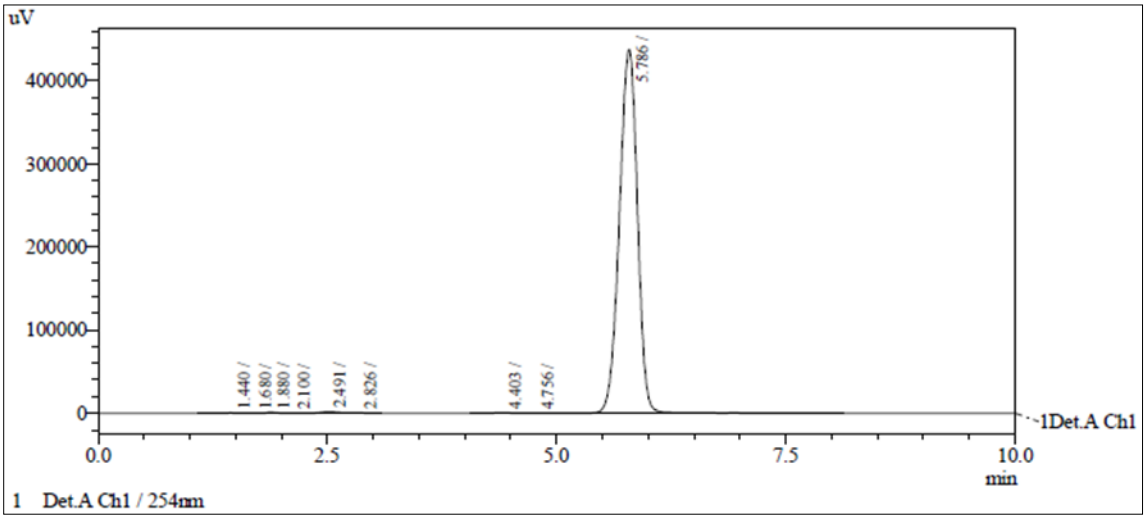

Figure 5 Neutral degradation

3.3.2. Degradation in acidic condition

Acid degradation of Ticagrelor was performed using 0.1 M HCl. Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of 0.1 M HCl and then was subjected to heat on a water bath at 80 °C. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to obtain a concentration of 50 µg/mL in the mobile phase. It was then filtered through a 0.22 µm filter and injected into the HPLC. The obtained chromatogram was analyzed for any degradation happened during the time. The results are given in Fig.6.

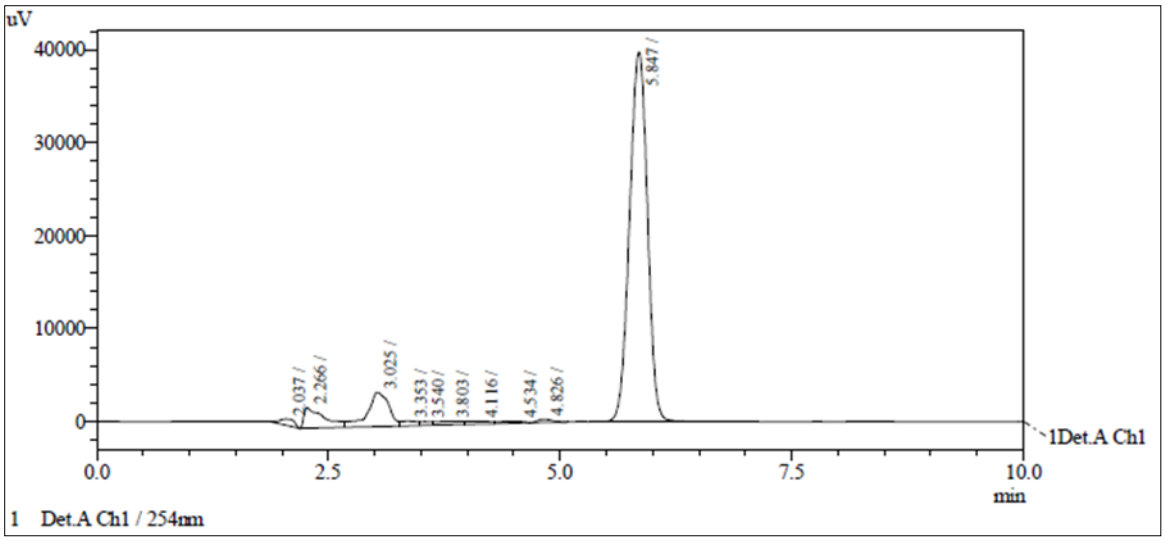

Figure 6 Acid degradation

3.3.3. Degradation in basic condition

Alkaline degradation of Ticagrelor was performed using 0.1 M NaOH. Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of 0.1 M NaOH, and then was heated on a water bath at 80 °C. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to obtain a concentration of 50 µg/mL in the mobile phase. It was then filtered through a 0.22 µm filter and injected into the HPLC. The obtained chromatogram was studied for any degradation happened during the time. The results are given in Fig. 7.

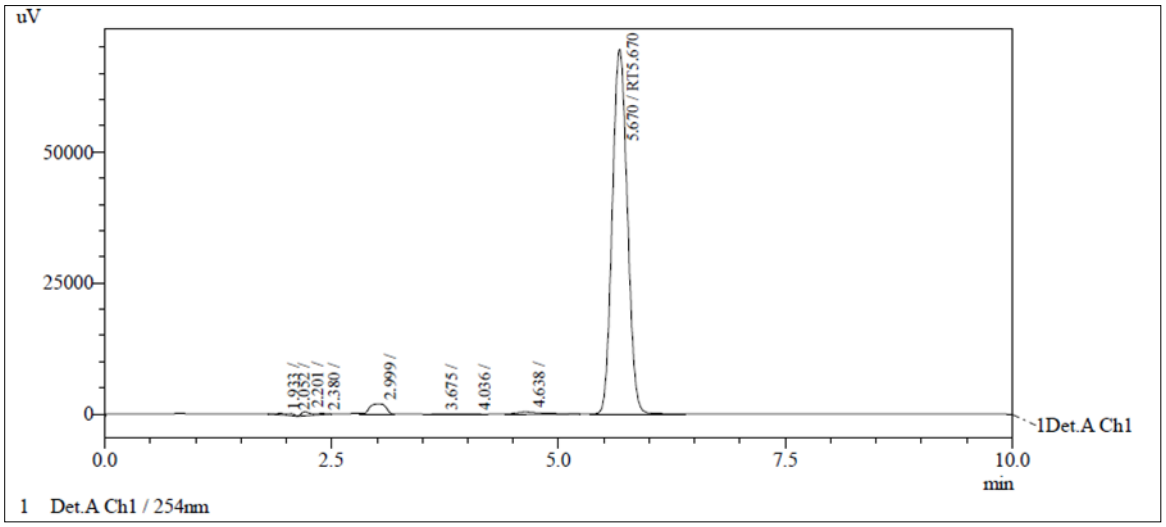

Figure 7 Base degradation

3.3.4. Oxidative degradation

For oxidation, the reagent chosen was hydrogen peroxide (3%). Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of H_2O_2. Then it was placed at room temperature for degradation. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to yield a concentration of 50 µg/mL in the mobile phase. It was then filtered through a 0.22 µm filter and injected into the HPLC.

The obtained chromatogram was studied for any degradation underwent during the time given. The results are shown in Fig. 8.

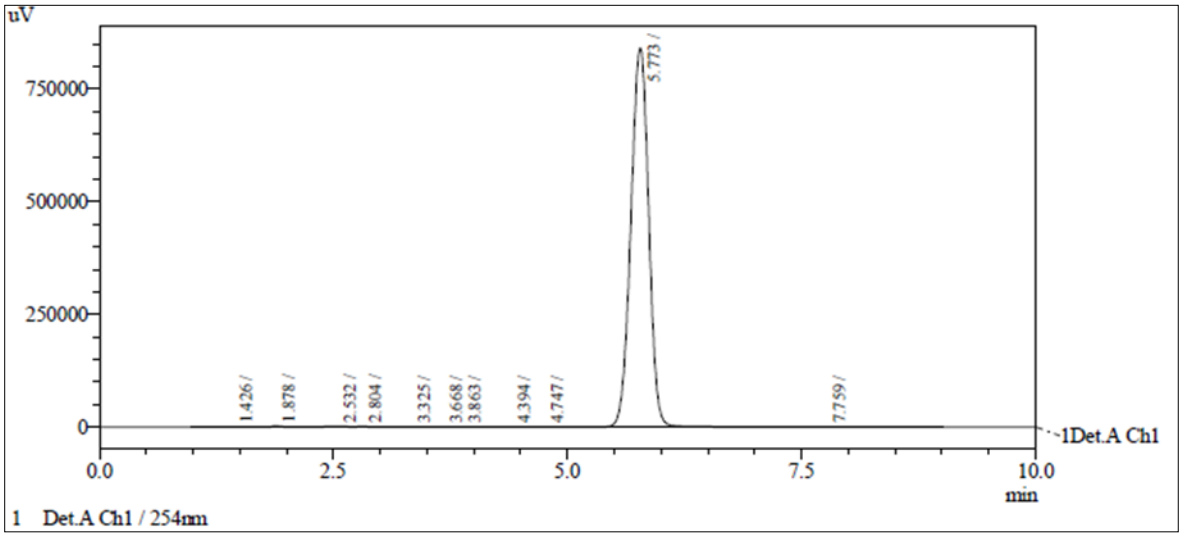

Figure 8 Oxidative degradation

3.3.5. Photolytic degradation

For photolysis, 100 mg of the Ticagrelor bulk was weighed accurately and transferred into a clean petridish. Then the closed petridish was placed under direct sunlight for degradation. At different time intervals, 10 mg of sample was taken out. From it, a stock solution of 1000 µg/mL was prepared. Then it was sonicated for 5 min and diluted to obtain a working solution of 50 µg/mL in the mobile phase. It was then filtered through a 0.22 µm filter and injected into the HPLC. The obtained chromatogram was observed for any degradation occurred during the time. The results are given in Fig. 9.

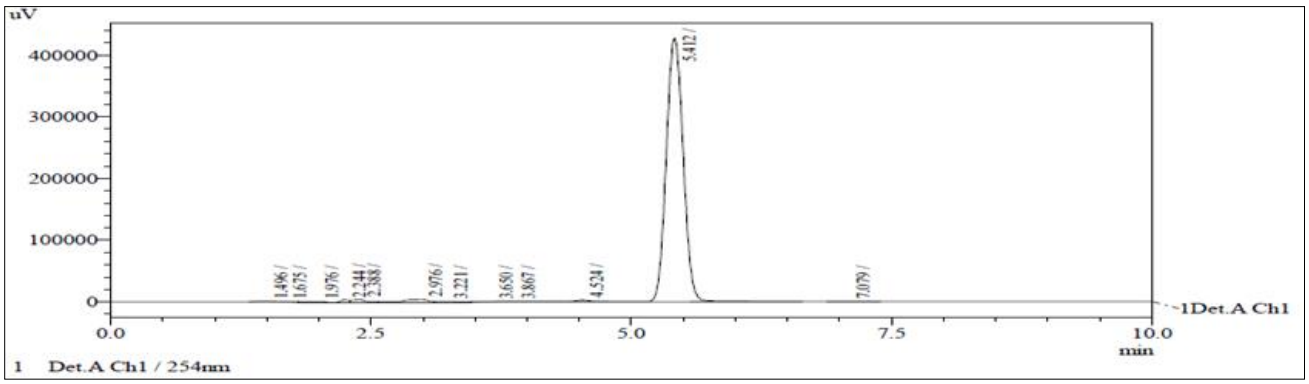

Figure 9 Oxidative dagradation

3.3.6. UV-degradation

For UV degradation, 100 mg of the Ticagrelor bulk was weighed accurately and transferred into a clean petridish. Then the petridish was placed under a UV chamber 30 cm at distance from the UV lamp. The cover of the petridish was removed for degradation. After 3 h, the UV lamp was switched off and 10 mg of sample was taken out. From it, a stock solution of 1000 µg/mL was prepared with the mobile phase, from which 50 µg/mL of working solution was prepared. It was sonicated and filtered through a 0.22 µm filter. Twenty microlitres of the sample was injected into the HPLC. The degradation of Ticagrelor under UV light is given in Fig. 10.

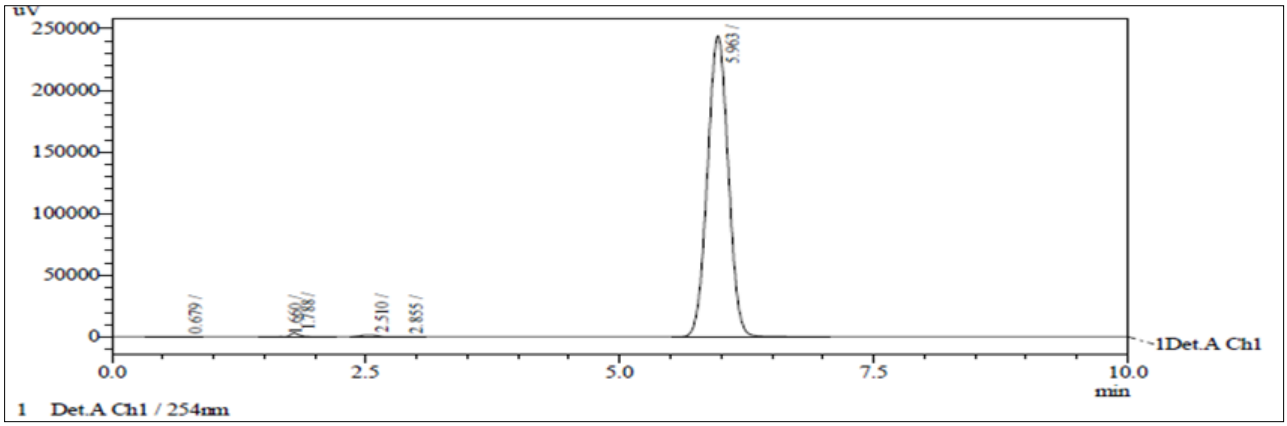

Figure 10 Photytic degradation

3.3.7. Thermal degradation

Thermal degradation was performed by placing the Ticagrelor bulk in the hot air oven at 40 °C. Samples were drawn at definite time intervals. The weighed amount of sample was added to 5 mL of HPLC grade methanol and sonicated for 5 min. Volume was made up to mark with methanol, which is the bulk standard stock solution of 1000 µg/mL. From the stock, a working standard solution of 50 µg/mL was prepared with the mobile phase. It was sonicated and filtered through a 0.22 µm filter, and 20 µL of the sample was injected into the HPLC. The obtained chromatogram was analyzed for any degradation underwent during the time, and the results are given in Fig. 11.

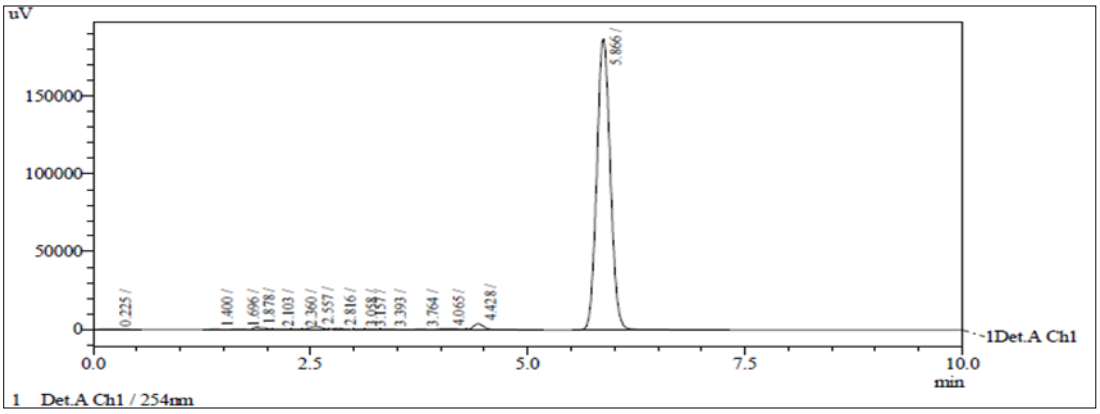

Figure 11 Thermal degradation

When stress conditions were applied to Ticagrelor, the HPLC results showed that there was no interference between the tested drug and the degradation products indicates the specificity and stability indicating nature of the method. It was observed that the drug showed extensive degradation in oxidative condition, alkali hydrolysis, and thermal. Figs. 5–111 show the chromatograms obtained after degradation under different stress conditions.

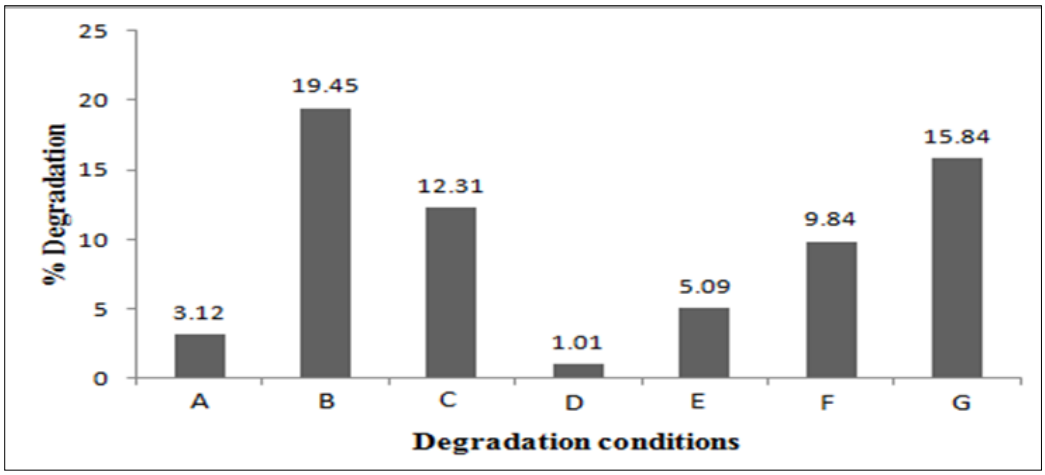

Figure 12 Percentage of degradation

3.4. Sample Analysis

The developed and validated method was applied for analysis of oral suspension contain Ticagrelor. The samplewas analyzed in triplicate. Analysis results were evaluated using a calibration curve. The amount of Ticagrelor in the samples was calculated from calibration curve equation and recovery and RSD values were determined. The results of analysis are given in Table 7. The recoveries were in good agreement with the label claims. It was concluded that the method can be applied successfully for theanalysis of Ticagrelor in suspension dosage form.

Table 7 Assay

Sample	Labelled amount(mg)	Amount obtained* (mg)	Percentage Recovery*±SD
Ticagrelor oral dispersible tablets(Brilique®)	5	4.92	98.4 ± 0.92

* Average of five determinations

Compliance with ethical standards

Acknowledgments

Authors are highly grateful to Sun Pharma Limited for providing us with the pure drug samples.The author thanks the Pydah College of Pharmacy for providing the instrumental and chemical facilities.

Disclosure of conflict of interest

The authors attest that they have no conflict of interest in this study.

References

[1] Anil Kumar N, Naga Swathi PR, Sharmila D, Sharmila SK, Pawar AK. A validated stability indicating method of UV-Spectrophotometry for the estimation of ticagrelor in bulk & marketed formulation. Der Pharmacia Lettre. 2016;8(19):309-15..

[2] Pandya D, Patel M, Ghediya R, Shah A, Khunt R. UV-Vis spectrophotometric assay determination of oral antiplatelet ticagrelor drug in pharmaceutical formulation: Application to content uniformity. Journal of Chemical and Pharmaceutical Research. 2016 ;8(1):316-21.

[3] Sales Oliveira S, da Silva Bitencourt A, Gobetti C, Sebastian Loureiro Mendez A, Virginia Garcia C. Assaying the antiplatelet ticagrelor by validated UV spectrophotometric method with performance equivalent to HPLC. Current Pharmaceutical Analysis. 2017 ;13(6):538-42.

[4] Souri E, Hamid KM, Tehrani MB, Jalalizadeh H. Spectrophotometric methods for determination of ticagrelor in dosage forms. Asian Journal of Pharmacy and Pharmacology. 2017;3(5):172-6.

[5] Bairam R, Tatapudi HK, Gajji N, Rasheed SH. UV spectrophotometric and HPLC method for quantification of ticagrelor in bulk and tablet dosage form. Asian Journal of Pharmaceutical Analysis. 2022;12(3):159-65.

[6] Joshy E, Babu A, D'cruz D, Aneesh TP. Development and validation of RP-HPLC method for determination of ticagrelor in pharmaceutical dosage formulation. Scholars Research Library, Der Pharmacia Lettre. 2016;8(9):206-12.

[7] Ambasana, MA, Kapuriya NP, Mangtani, KM, Ladva, KD.. An improved assay method for the estimation of ticagrelor hydrochloride by reverse phase liquid chromatography. International Journal of Pharmaceutical Sciences and Research.2016; 7: 2009–2014.

[8] Omaima J, Shyamala, JVC Sharma, Development and validation of stability indicating UPLC method for the estimation of ticagrelor in bulk and its tablet dosage form, Journal of Drug Delivery and Therapeutics. 2019; 9(1-s):201-205.

[9] L. Kalyani, a. Lakshmana rao, A validated stability-indicating hplc method for determination of ticagrelor in bulk and its formulation. International Journal of Pharmacy, 2013, 634-642, 2249-1848.

[10] Debnath R, Manjunath SY, Kumar TH. Stability Indicating UV Spectrophotometric method for the Determination of Voglibose in Pharmaceuticals Dosage Form. Research Journal of Pharmacy and Technology. 2022;15(6):2543-6.

[11] Debnath R, Manjunath SY, Kumar TH.Development and Validation of New RP-HPLC Method For The Estimation of Voglibose In Bulk And Pharmaceutical Dosage Form. International Journal of Biomedical Research.2020; 11: 1-10.

[12] Sankar DG, Kumar TH, Krishna MV. UV spectrophotometric determination of duloxetine hydrochloride. Asian Journal of chemistry. 2009 ;21(6):4951.

[13] Kumar TH, Reddy KM, Rishika D, Kumar RP. Estimation of Orlistat by UV Spectrophotometric method. International Journal of Pharmaceutical Sciences and Research. 2011 ;2(9):2469.

[14] Kumar HT, Sri SD, Rao VPK and Rao YS. Visible Spectrophotometric methods for estimation of Lansoprazole in pharmaceutical formulation. Indian Drugs. 2014; 51(12), 26-31.

[15] Rao SY, Gandi P, Rao VPK, Kumar HT**.** Validated Spectrophotometric Methods For Estimation Of Olmesartan Medoxomil In Bulk And Pharmaceutical Tablet Dosage Form. Indian drugs.2015;52(1),33-39.

[16] Gowthami G, Vara PRK, Hemant KT and Srinivasa RY. Spectrophotometric Methods For The Estimation Of Duloxetine Hydrochloride In Bulk And In Pharmaceutical Dosage Form. Indian Drugs. 2015; 5(52), 27-32.

[17] Gandi P, Rao YS, Rao KV, Kumar TH. RP-HPLC method for the determination of zaltoprofen in bulk and pharmaceutical dosage form. Der Pharmacia Lettre. 2015;7(7):6-10.

[18] Srinivasa RY, Gandi P, Rao KV, Kumar HT. Development and validation of visible spectrophotometric method for the estimation of zaltoprofen in tablet dosage form. Der. Pharmacia. Lett. 2015;7(1):196-201.

[19] Lakshmi GT, Rao YS, Kumar TH and Rao KVP**.** UV Spectrophotometric method for the estimation of candesartan cilexetil in bulk and pharmaceutical dosage form. Der Pharmacia Lettre. 2015; 7 (3):17-22.

[20] Sindhu SN, Rao YS, Kumar TH, Rao KV. Method development and validation of RP-HPLC method for estimation of imatinib mesylate in pure and pharmaceutical dosage form. Der Pharmacia Lettre. 2015;7(3):33-8..

[21] Gowthami G, K. Rao KVP, Kumar TH and Rao YS. RP-HPLC Determination Of Lafutidine In Bulk Drug And Pharmaceutical Dosage Form. International Journal of Pharmacy. 2015.

[22] Srinivasa RY, Gandi P, Rao KV, Kumar HT. Development and validation of RP-HPLC method for the determination of zaltoprofen in bulk and pharmaceutical tablet dosage form. Journal of Chemical and Pharmaceutical Research. 2014; 7(1): 5-9

[23] Rao KV, Tanuja M, Rao YS, Kumar TH. Development and validation of spectrophotometric methods for estimation of zileuton in bulk and its dosage form. International Journal Chemical Sciences. 2015;13(2):714-24.

[24] Rao KV, Tanuja M, Rao YS, Kumar TH. Development and Validation of RP-HPLC method for the estimation of Zileuton in bulk and its dosage form. Int. J. Drug Dev. & Res., 2015, 7(1).,101-106.

[25] Swathi S, Kumar HT, Rao PK. Validated RP-HPLC method for simultaneous determination of rosuvastatin calcium and ezetimibe in pharmaceutical dosage form. Int. J. Pharm. And Pharm. sci. 2015;7(4):209-13.

[26] Rao MT, Ratna VJ, Rao YS, Kumar TH. Development and validation of RP-HPLC method for the determination of itraconazole in bulk and capsule dosage form. International Journal of Pharmaceutical Science Review and Research. 2015;31(2):221-5.

[27] Rao KV, Tanuja M, Rao YS, Kumar TH. Visible spectrophotometric methods for determination of zileuton in bulk drug and pharmaceutical formulation. International Journal of Research in Pharmacy and Chemistry. 2015;5(2):251-7.

[28] Lakshmi TG, Rao SY, Rao VP, Kumar HT. Visible spectrophotometric methods for the estimation of orlistat in bulk and pharmaceutical dosage form. Journal of Pharmaceutical Sciences and Research. 2015 Mar 1;7(3):155.

[29] Kumar HT, Sri SD, Rao VP, Rao SY. Validated RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical formulation. International Journal of Pharmaceutical Sciences and Research. 2015 Jul 1;6(7):2913.

[30] Lakshmi GT, Rao YS, Rao K, Prasada V, Kumar TH. RP-HPLC method for estimation of atomoxetine hydrochloride in bulk and pharmaceutical dosage form. Research Journal Of Pharmaceutical Biological and Chemical Sciences. 2015 ; 6(2):1208-14.

[31] Kumar HT and Sankar DG. Simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in pharmaceutical formulation by RP-HPLC method with forced degradation studies. Der Pharmacia Lettre, 2016, 8 (6): 288-298.

[32] Kumar HT, Sankar DG**.** Stability indicating RP-HPLC method development and validation for simultaneous estimation of Metoprolol Succinate and Cilnidipine in bulk and pharmaceutical formulation. Indo American Journal of Pharmaceutical Research, 2016, 6(04):5338-5348.

[33] Kumar HT, Sankar DG**.** RP-HPLC method for simultaneous estimation of Metformin Hydrochloride and Canagliflozin in pharmaceutical formulation. Indian Drugs. 2021; 58(6) :49-59.

[34] Shaik S, Kumar TH**,** Rao YS, Rao KVP. New Visible Spectrophotometric Methods for Estimation of Rabeprazole in Bulk and Pharmaceutical Formulation. Der Pharma Chemica, 2018, 10(11): 34-39.

[35] Sailusha Ratnam K, Kumar H, Srinivasa Rao Y. Visible Spectrophotometric Method for Determination of Eletriptan Hydrobromide in Pharmaceutical Formulation Using Marquis Reagent. Indo American Journal of Pharmaceutical Research. 2018:8(12).

[36] Madhuri PL, Kumar TH, Rao YS, Rao K. UV spectrophotometric method for estimation of sacubitril in synthetic mixture. Asian Journal of Research in Chemistry. 2019;12(1):7-10.

[37] ChaitanyaPSV KH, Rao YS, Rao KV. Estimation of vortioxeine in bulk and pharmaceutical formulations by UV spectrophotometry. European Journal of Pharmaceutical and Medical Research. 2019;6(2):389-92.

[38] Yamini M, Kumar HT, Rao YS, Rao KV. Determination of Zonisamide in Bulk and Pharmaceutical Formulation by UV Spectrophotometric Method. International Journal of Pharmaceutical Sciences Review and Research. 2019;54(2):79-8.

[39] Kumar TH, Jhanavi D, Rao KVP and Rao YS: RP-HPLC method for quantification of orotic acid in capsule formulation. Int J Pharm Sci & Res 2019; 10(5): 2343-47.

[40] Kumar HT and Asha C. New Validated Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Amlodipine Besylate And Valsartan In Pharmaceutical Formulation. Int J Pharm Sci & Res. 2019; 10(5): 2633-43.

[41] Kumar HT, Asha CH, Sankar DG. Estimation of amlodipine besylate and irbesartan in pharmaceutical formulation by RP-HPLC with forced degradation studies. International Journal of Applied Pharmaceutics. 2019 May 7;11(33):159-67.

[42] Sailusha Ratnam K, Kumar H, Srinivasa Rao Y**.** Spectrophotometric Methods For The Estimation Of Nebivolol Hydrochloride In Pharmaceutical Formulation. International Journal of Chemical Studies 2019;11(4): 2362:2368.

[43] Kumar TH, Dakshayani P, Prajna CK, Rao KV. Stability Indicating HPLC Method Development and Validation for the Estimation of Zonisamide in Bulk and Pharmaceutical Dosage Form. Saudi J Med Pharm Sci. 2019;5:688-92.

[44] Banu T, Kumar HT, Rao VK, Rao SY. Application Of Simultaneous Equation Method For Estimation Of Sacubitril And Valsartan In Combined Dosage Form. Asian Journal of Research in Chemistry.2021;14(2): 111-114.

[45] Kumar HT and Sankar DG. Simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in pharmaceutical formulation by RP-HPLC method with forced degradation studies. Der Pharmacia Lettre, 2016, 8 (6): 288-298.

[46] Kumar HT and Sankar DG Stability indicating RP-HPLC method development and validation for simultaneous estimation of Metoprolol Succinate and Cilnidipine in bulk and pharmaceutical formulation. Indo American Journal of Pharmaceutical Research, 2016, 6(04):5338-5348.

[47] Kumar TH, Banu T, Ravindar B, Rasheed SH, Gajji N. Quantification of Sacubitril and Valsartan in tablet formulation By RP-HPLC Method. International Journal of Pharmaceutical Technology Letters. 2023 ;1(1):64-71.

[48] Hemant KT, Krishna PC, Varaprasad RK, Srinivasa RY. RP-HPLC method for estimation of tramadol hydrochloride and paracetamol in pharmaceutical formulation. GSC Biological and Pharmaceutical Sciences. 2019;8(1):89-97.

[49] Kumar T, Ravindar B, Rasheed SH, Gajji N**.** Stability-Indicating RP-HPLC Method for the Estimation of Metoprolol Succinate and Hydrochlorothiazide in Tablet Dosage Form. International Journal of Pharmaceutical Technology Letters.2021,1(1),13-21.

[50] Siridevi MP, Kumar HT, Rao SY, Rao VP. RP-HPLC method for quantification of Empagliflozin in pharmaceutical formulation. Asian Journal of Pharmacy and Technology. 2019;9(3):208-11.

[51] Bhavana V, Kumar HT, Rao SY, Rao VP. RP-HPLC method for estimation of Solifenacin Succinate in API and Tablet dosage form. Asian Journal of Pharmaceutical Analysis. 2019;9(3):118-22.

[52] Siridevi MP, Hemant KT, Srinivasa RY, Varaprasad RK. Simultaneous Spectrophotometric Estimation of amlodipine besylate and Perindopril Erbumine in tablet Formulation. Research Journal of Pharmacy and Technology. 2019;12(12):6101-6.

[53] Kumar TH, Samantaray S, Sankar DG. RP-HPLC Method for estimation of metoprolol succinate and Olmesartan medoxomil in pharmaceutical formulation with forced degradation studies. International Journal of Applied Pharmaceutical Sciences and Research. 2019 Jul 1;4(03):34-42

[54] Kumar TH, Begum A, Sankar DG. Determination of azilsartan medoximil and chlorthalidone in tablets exposed to forced degradation by using RP-HPLC. Biomedical Research. 2019;30(5):10-35841.

[55] Sony**,** Kumar HT**,** Manjunath SY. RP-HPLC Method For The Estimation Of Venlafaxine Hydrochloride In Capsule Dosage Form. Indo American Journal of Pharmaceutical Research.,2021,11(1):1517-1524.

[56] Mahivish R, Manjunath SY, Kumar H. UV spectrophotometric method for estimation of zileuton in pharmaceutical formulation. GSC Biological and Pharmaceutical Sciences. 2020;13(1):212-9.

[57] Soundarya K, Hemant Kumar T, Manjunath SY. New UV Spectrophotometric Method For The Estimation Of Atazanavir Sulfate In Bulk And Pharmaceutical Dosage Form. Scholars Academic Journal of Pharmacy,2020,9(10).

[58] Nagamani P, Manjunath SY, Hemant Kumar T. Development and validation of RP-HPLC method for estimation of amlodipine besylate and celecoxib in pharmaceutical formulation. Journal of Drug Delivery and Therapeutics.2020 ;10(6):31-36.

[59] Mahivish R, Manjunath SY, Kumar H.RP-HPLC Method Development And Validation For The Estimation Of Zileuton In Tablet Formulation. International Journal of Research GRANTHAALAYAH, 2021; 9(1): 141-149.

[60] Soundarya K, Kumar HT, Manjunath SY. New RP-HPLC method for the estimation of atazanavir sulphate in pharmaceutical dosage form. Research Journal of Pharmacy and Technology. 2022;15(7):2928-32.

[61] Afreen, Manjunath SY, Hemant Kumar T. UV Spectrophotometric Method for Estimation Of Famciclovir In Pharmaceutical Formulation. GSC Biological and Pharmaceutical Sciences. 2021; 16(3): 212-219.

[62] Bhavana V, T Hemant Kumar, Srinivasa Rao Y and Vara Prasada Rao K. UV Spectrophotometric Method For Simultaneous Estimation Of Solifenacin Succinate And Tamsulosin Hydrochloride In Combined Dosage Form By Derivative Spectroscopy. Indian Journal of Pharmaceutical Sciences.2021; 83(2): 322-326.

[63] Kumar HT and Sankar DG**.** Stability Indicating RP-HPLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Dosage Form. Indian Drugs.2021;58(12):75-78.

[64] Manjunath SY, Naresh P, Hemant Kumar T. Application of Vierdot's method for estimation of Tapentadol and Paracetamol in combined dosage form by UV spectrometry. Research Journal of Chemistry and Environment.2021; 25 (10): 1-7.

[65] Koduru S, Tatapudi HK, Kalepu S, Mitta C, Bairam R. Stability-indicating RP-HPLC method applied to the quantification of anti-histaminic drug ebastine in its oral suspension dosage form. Drug Analytical Research. 2022;6(2):8-17.

[66] Rao K, Prasad V, Vasudha D. Visible Spectrophotometry Methmdsfor The Estimationof Modafinil In Pharmaceutical Formulation. Indian Drugs. 2022 ;59(9).

[67] Kumar ST, Manjunath SY, Rasheed SH. Development and Validation of Kinetic Spectrophotometric Method for Determination of Venlafaxine Hydrochloride. Journal of Applied Spectroscopy. 2022 Nov;89(5):886-91.

[68] Koduru S, Kumar T H, Mitta C, Kalepu S, Bairam R. Utilization Of The Stability-Indicating RP-HPLC Method For Dietry Supplement Calcium Orotate Quantification In Capsule Dosage Form. Bulletin of Pharmaceutical Sciences. Assiut. 2023 ;46(1):141-56.

[69] Koppisetty BR, Prasad YR, Amgoth KM, Yarraguntla SR, Dadı V, Tatapudı HK. Utility Of Quality By Design Approach In Rp-Hplc Method Development For Quantification Of Lamivudine And Effavirenz In Combination Formulation. Journal of Faculty of Pharmacy of Ankara University. 2023 ;47(2):625-36.

[70] Koppisetty BR, Tatapudi HK, Dadi V, Gayathri PR, Komali P, Challa GN, Kollabathula VR, Yarraguntla SR. QbD based RP-HPLC method for simultaneous determination of a emtricitabine, tenofovir diproxil fumarate and efavirenz in tablet dosage form-an application to stability indicating assay. Analytical Chemistry Letters. 2023;13(3):267-88.

[71] ICH Proceedings of the International Conference on Harmonisation of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guidelines). Validation of Analytical Procedures: Methodology, Q2B.