Development and Validation of UV Spectroscopic Method for the Estimation of Ticagrelor in Bulk Andtablet Dosage Form.

Meena Iyer. *1, SowmyaH.G. *2, Jose Gnana Babu C.*3

*1(2nd yearM pharma, Student of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422)
*2(Assistant Professor of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422
*3(Professor and HOD of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422)

ABSTRACT: Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Ticagrelor in bulk and Pharmaceutical dosage form. The drug shows maximum absorption(λ_{max}) at 253nm in Acetonitrile solution andArea under Curve [AUC] in absorption spectra were measured between the wavelength range 248 to 258 nm which obeys Beer's law in the concentration range of 5-30 µg/ml. The linearity studycarried and regression coefficient was found to be 0.9997 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 99.42-99.86.TheLOD and LOQ were found to be 0.1380 and 0.4141µg/ml. The % relative standard deviation werefound less than 2.According to ICH guidelines the method has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for reliable quantification of Ticagrelorin bulk form and pharmaceutical dosage form.

KEYWORDS: Ticagrelor, Area under curve spectroscopy, validation, pharmaceutical formulations.

I. INTRODUCTION:

Ticagrelor is a platelet aggregation inhibitor reduces the rate of thrombotic cardiovascular events in patients with the acute coronary syndrome. Ticagrelor belongs to the category of triazolo pyrimidine 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 stop signal transduction and platelet activation[1].
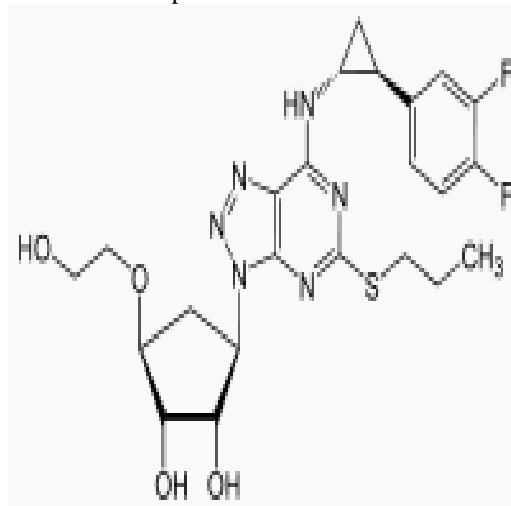

Fig.1: Chemical structure of Ticagrelor

Literature survey revealed that there were few analytical methods have been reported for the determination of Ticagrelor in pure drugand pharmaceutical dosage forms by using UV spectrophotometric[2-6],UPLC [7], HPLC [8-14]and HPTLC[15] so far.

The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area under curve Spectrophotometric method for estimation of Ticagrelor in bulk and tablet dosage form.

II. MATERIALS AND METHODS:
Instrument:
UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe

software. All weights were taken on analytical balance.

Chemicals:
Ticagrelor pure drug was obtained as a gift sample fromMicro Labs Ltd Bommasandra, Bengaluru and its pharmaceutical dosage form Ticagrelor 20 tablet labelled claim 90mg from local pharmacy manufactured by Astra Zeneca Pharma India Ltd.

Solvent:
Acetonitrile is used as a solvent.

Selection of analytical wavelength:
Appropriate dilutions of Ticagrelor were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm.Area under Curve [AUC] in absorption spectra were measured between the wavelength range 248 to 258 nmas the wavelength for detection (Fig-2).

Preparation of standard stock solution:
100mg of Ticagrelor was weighed accurately and transferred in to 100ml volumetric flask and dilute in Acetonitrile up to mark. From this, the solution was further diluted into 100µg/mland pipette out 0.5,1.0,1.5,2.0,2.5 and 3.0ml into 10ml individual volumetric flask and dilute in Acetonitrile up to mark, this gives 5, 10, 15, 20, 25 and 30µg/ml concentration.

Preparation of sample solution:
20 tablets of Ticagrelor marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 100mg of Ticagrelor was transferred into a 100ml of volumetric flask then it was diluted with Acetonitrile and made up to the mark.

METHOD AND VALIDATION:
The method was validated according to ICH guidelines.

III. RESULTS AND DISCUSSION:
Method: Area under curve spectroscopy.
Linearity:
The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 5-30µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 248 to 258 nm asabsorbance values are shown in table-1 (Fig-3). The calibration curve was prepared by plotting

graph against the concentration and absorbance and therefore the graph shown in (Fig-4). Statistical parameter like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (table-2).

Precision:
The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. (table-3).

Accuracy:
The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levelsas 50%, 100% and 150%. In which the formulation concentration kept constant and varied pure drug concentration.(table-4).

Ruggedness:
The ruggedness is defined as the reproducibility of results when the method is performed under the variation in conditions. This includes different analyst, laboratories, instruments, temperature etc.Ruggedness was determined between different analyst, thevalue of %RSD was found to be less than 2. (table-5).

LOD and LOQ:
Thelimit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. Thelimit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated using formula.
LOD = 3.3(SD)/S and LOQ = 3(LOD)
LOD and LOQ value of were found Ticagrelor be 0.1380 and 0.4141µg/ml.

IV. CONCLUSION:
As per ICH guidelines, the present analytical was carried and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for

routine analysis of Ticagrelor in bulk drug and in pharmaceutical dosage forms.

V. ACKNOWLEDGEMENT:

REFERANCE:

[1]. Swetha V, Prasad SV, Akhila Y. Analytical method development and validation of stability indicating assay method of Ticagrelor tablets by using RP-HPLC. World Journal of Pharmaceutical and Medical Research. 2017;3(10):235-41.

[2]. Souri E, Hamid KM, Tehrani MB, Jalali Zadeh H. Spectrophotometric methods for determination of Ticagrelor in dosage forms. Asian Journal of Pharmacy and Pharmacology. 2017;3(5):172-76.

[3]. 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.

[4]. Narware H, Malviya K, Sirohi B, Omray LO. UV spectrophotometric methods for estimation of Ticagrelor in Pharmaceutical formulations. Asian Journal of Pharmaceutical Education and Research. 2018;7(4):94-106.

[5]. Gupta A, Jadhav V, Jain A. Analytical method development and validation of Ticagrelor from bulk and formulation. Asian Journal of Pharmaceutical Research. 2019;9(3):141-46.

[6]. Kumar NA, 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.

[7]. Omaima J, Sharma JV. 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):201-05.

[8]. Swetha V, Prasad SV, Akhila Y. Analytical method development and validation of stability indicating assay method of Ticagrelor tablets by using RP-HPLC. World Journal of Pharmaceutical and Medical Research. 2017;3(10):235-41.

[9]. Tabassum K, Sarvesh R. Analytical method development and validation studies of Ticagrelor tablets by RP-HPLC. International Journal of Applied Pharmaceutics. 2017;9(4):10-21.

[10]. Kulkarni PR, Gajare GK. Development and validation of RP-HPLC method for estimation of Ticagrelor in bulk form. International Journal of Research in Pharmacy and Chemistry. 2016;6(4):733-37.

[11]. Cruz D, Babu A, Joshy E, Aneesh TP. Bioanalytical method development and validation Of Ticagrelor by RP-HPLC. International Journal of Applied Pharmaceutics, Innovare Academics Sciences Pvt. Ltd. 2017;9(3):51-54.

[12]. Shane NL, Chamle AH, VasanthaRaju SG, Pai A, Muddukrishna BS. Method development and validation for the estimation of Ticagrelor in bulk and comparison with other published methods. Journal of Global Pharma Technology. 2016;8(12):1-6.

[13]. Mehta AR, Maheshwari DG. Development and Validation of first UV Spectroscopic method and RP-HPLC method for Simultaneous estimation of Rivaroxaban and Ticagrelor in synthetic mixture. Journal of Global Trends in Pharmaceutical Science. 2018; 9(2):5275-97.

[14]. Bueno LM, Manoel JW, Giordani CF, Mendez AS, Volpato NM, Schapoval EE, Steppe M, Garcia CV. HPLC method for simultaneous analysis of Ticagrelor and its organic impurities and identification of two major photodegradation products. European Journal of Pharmaceutical Sciences. 2017 Jan 15; 97:22-9.

[15]. DA S, Shunmuganathan Elakkiya L, Mehta FA, Chhalotiya UK. Article Details Stability indicating HPTLC method for the estimation of Ticagrelor in bulk and in Pharmaceutical dosage form.

TABLES:
Table 1: Results of calibration curve at 248-258nm by Area under curvemethod

SL NO	Concentration in µg/ml	Absorbance ±Standard deviation*
1	0	0
2	5	0.130±0.00483
3	10	0.281±0.00466
4	15	0.431±0.00416
5	20	0.583±0.00516
6	25	0.730±0.00531
7	30	0.880± 0.00450

*Average of six determinations.

Table 2: Regression parameter for Ticagrelor at 248-258nm byArea under curvemethod.

Regression parameter	Results
Range(µg/ml)	5-30
λ_{max}(nm)	253
Regression Equation	Y= 0.0296x+0.0101
Slope(b)	0.0296
Intercept(a)	0.0101
Correlation coefficient(r^2)	0.9997
Sandell'sequation	0.03558
Limit of detection(µg/ml)	0.1380
Limit of quantitation(µg/ml)	0.4141

Table 3: Determination of precision results for Ticagrelor at 248-258nm by Area under curvemethod.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
5	0.130±0.00086	0.6636	0.130±0.00086	0.6636
10	0.280±0.00057	0.2054	0.280±0.00293	1.04
15	0.431±0.001155	0.2674	0.431±0.00293	0.6809
20	0.580±0.001500	0.2581	0.581±0.00477	0.8231
25	0.730±0.001041	0.1425	0.730±0.00278	0.381
30	0.879± 0.001528	0.1736	0.880± 0.0035	0.3981

*Average of six determinations, **percentage relative standard deviation.

Table 4: Determination of Accuracy results for Ticagrelor at 248-258nm by Area under curve method.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	10	5	14.91	99.42 ±0.361	0.363
100	10	10	19.90	99.52 ±0.270	0.271
150	10	15	24.63	99.86 ±0.213	0.214

*Average of six determinations, **percentage relative standard deviation.

Table 5: Determination of Ruggedness results for Ticagrelor at248-258nm by Area under curve method.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.431	0.432
±Standard deviation*	0.001	0.0015
%RSD	0.231	0.353

*Average of six determinations, **percentage relative standard deviation.
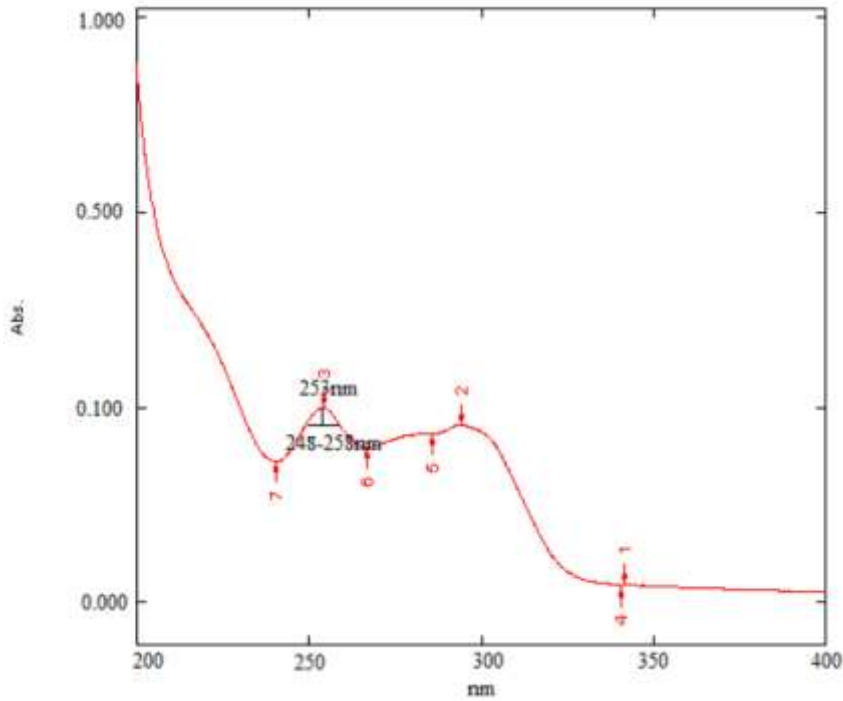
FIGURES:

Fig.2: Area under curve spectrum of Ticagrelor at 248-258nm.
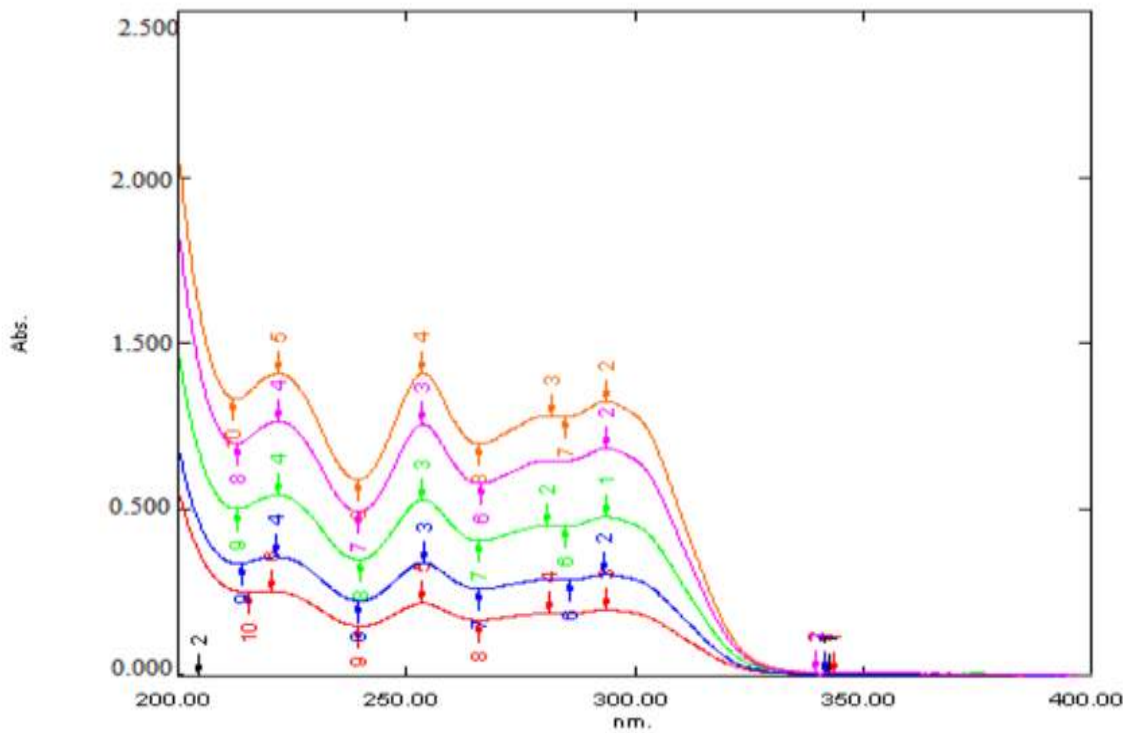

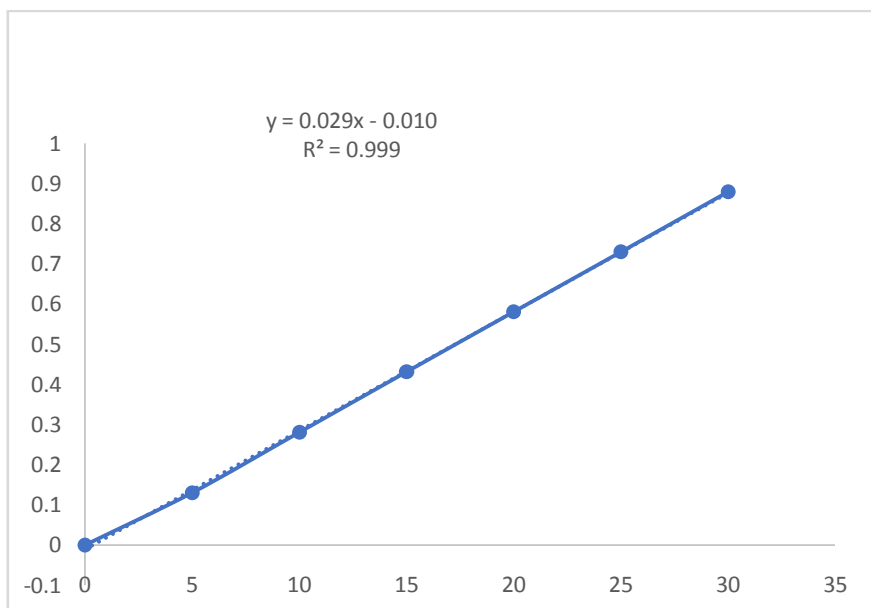**Fig.3: Area under curve overlain spectra of Ticagrelor showing absorbance at 248-258nm.**

Fig.4: Calibration curve of Ticagrelor at 248-258nm by Areaunder curve method.