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Der Pharmacia Lettre, 2016, 8 (19):309-315
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ISSN 0975-5071
USA CODEN: DPLEB4

A validated stability indicating method of UV-Spectrophotometry for the estimation of ticagrelor in bulk & marketed formulation

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ABSTRACT

To develop and validate a simple, rapid, accurate and economical UV Spectrophotometric method for the determination of Ticagrelor in bulk and its marketed product. UV- Spectrophotometric method was performed by using UV/Vis double beam spectrophotometer with spectral band width of 1 nm and 1.0 cm matched quartz cells and glass cells were used for UV regions respectively. Methanol and O-phosphoric acid was used as solvents in the ratio of 20:80 respectively and the maximum absorbance of Ticagrelor was observed in methanol & O-phosphoric acid at 237nm in UV region. The linear calibration range was found to be 2µg/mL to 10µg/mL in UV region. The correlation coefficient (R2) is 0.9855 and the regression equation is y=0.0808x-0.0022 in UV region. The method was validated in terms of Linearity, Precision, Accuracy, Robustness, LOD and LOQ as per ICH guidelines.

Keywords: UV-Visible Spectrophotometer, Ticagrelor, ICH guidelines.

INTRODUCTION

Ticagrelor (Fig. 1) is an orally active antiplatelet agent, inhibitor of platelet activation and aggregation mediated by the P2Y12 ADP-receptor. Chemically, it is (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl] amino}-5-(propylthio)-3*H*-[1,2,3]-triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxycyclo pentane-1,2-diol. A recent study indicated that Ticagrelor reduce the rate of thrombotic cardiovascular events in patients with acute coronary syndrome . Some detailed mechanistic studies pointed out that Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation, which inhibits platelet aggregation and thrombus formation in atherosclerotic disease. Literature survey revealed that few HPLC, LC-MS and very few UV spectrophotometric methods have been developed and reported for the estimation of Ticagrelor. However no efforts have been reported for the UV spectrometric method of Ticagrelor in bulk form by using methanol and O-Phosphoric acid as a solvent, which could be very economic and easily applicable as well. Hence an attempt has been made to develop and establish a novel, simple, rapid and sensitive UV spectrometric method in accordance with ICH guidelines for the estimation of Ticagrelor in bulk formulation.

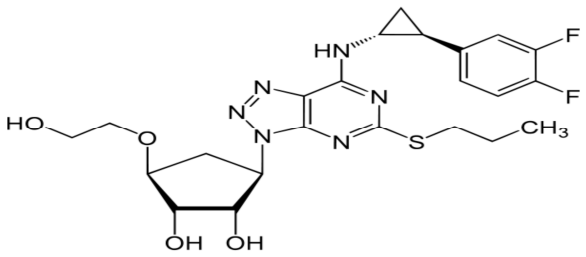

Figure 1: Structure of Ticagrelor [8]

MATERIALS AND METHODS

Instrumentation:
Lab India – T60, UV/Vis double beam spectrophotometer with spectral band width of 1 nm, wavelength accuracy of ± 0.3 nm and 1.0 cm matched quartz cells were used for UV determinations.

Chemicals and Solvents:
Ticagrelor was obtained as a gift sample from Raks pharma , Visakhapatnam , India and was used without further purification. All chemicals and reagents used were of analytical grade in UV method. AR grade Methanol is procured from Merck Pharmaceuticals Private Ltd., Mumbai, India.

Selection of Solvent and Detection wavelength:
Drug solution of 10µg/ml was scanned over the range of 200-400 nm in UV region using different solvents like water, hexane, ethanol, cyclohexane, methanol, Ortho phosphoric acid and Ammonium acetate buffer solutions. It was observed that the drug showed maximum absorbance in methanol and O-phosphoric acid in the ratio of 20:80 at 237 nm, hence methanol & O-phosphoric acid was used as solvent and 237 nm was used as detection wavelength for Ticagrelor for further study.

Preparation of standard stock solution:
Accurately weighed 100mg of Ticagrelor was dissolved in 20 ml of methanol and 80 ml of O- phosphoric acid to get 1000µg/ml stock solution. Working standard solutions were further diluted to get concentration range of 2-10µg/ml.

Method Validation [2-5]:
The proposed methods were validated for following parameters: System suitability, Linearity, Accuracy, Precision, Robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Linearity:
The calibration curve was obtained with five concentrations of the standard solution (2–10 µg/ml for UV method). The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Accuracy:
The accuracy of the method was evaluated by recovery study of Ticagrelor at three concentration levels (50%, 100% and 150 %). A study was carried out in triplicate at 6, 8 and 10 µg/ml in UV. A fixed amount of pre-analyzed sample and standard drug was added and recovery was studied for the quantification of Ticagelor. The percentage recovery and mean % recovery were calculated.

Precision:
The precision was determined for Ticagrelor in terms of intraday and interday. For intraday precision evaluation, standard solution (6 µg/ml for UV) was prepared from stock solution and calculate the absorbance's six times (n=6) at two different times in a day. The interday precision was studied by injecting the same concentration of standard solutions into the system six times on consecutive days. The standard deviation and the relative standard deviation were reported for precision. RSD for peak areas should be NMT 2% which indicate the precision of the developed methods.

Robustness:
Robustness of the method was determined by altering slight changes in detection of wavelength. It was observed that there were no marked changes in spectrum obtained, which demonstrated that the developed UV methods were robust.

Limit of Detection and Limit of Quantitation:
The LOD and LOQ were determined on the basis of response and slope of the regression equation.

Forced Degradation Studies:
Acidic Hydrolysis:
To 10 ml of stock solutions of Ticagrelor formulation and 10 ml of 1 M HCl, were refluxed separately for 1 hour at 80ºC on oil bath. The forced degradation in acid media was performed in the dark in order to exclude possible photo-degradation. The degradation samples were then cooled to room temperature. Suitable aliquots of resultant degradation samples were taken and neutralized for assay after suitable dilutions with solvent.

Alkaline Hydrolysis:
To 10 ml of stock solutions of Ticagrelor pharmaceutical formulation and 10 ml of 1 M NaOH, was added separately. These mixtures were refluxed separately for 1 hour at 80ºC on oil bath. The forced degradation in alkaline media was performed in the dark in order to exclude possible photo-degradation. The degradation samples were then cooled to room temperature. Suitable aliquots of resultant degradation samples were taken, neutralized and subjected to analysis after suitable dilutions with solvent.

Oxidative Hydrolysis:
To 10 ml of above stock solutions of Ticagrelor pharmaceutical formulation and, 10 ml of 3 %v/v H2O2 was added separately. These mixtures were refluxed separately for 1 hour at 80ºC on oil bath. The forced degradation in oxidative media was performed in the dark in order to exclude possible photo-degradation. The degradation samples were then cooled to room temperature. Suitable aliquots of resultant degradation samples were taken and subjected to analysis after suitable dilutions with methanol.

Dry Heat Degradation:
For dry heat degradation, Ticagrelor pharmaceutical formulation were placed in oven at 80ºC for 24 hours under dry heat condition in the dark and then cooled to room temperature. Degradation samples were subjected to analysis after suitable dilutions with methanol.

Photochemical Degradation:
For carrying out photolysis studies the drug was treated with UV light for 6 hours at 237 nm and also in sunlight. Then test solutions were prepared and the absorbance of the solutions (20 µg/ml) were measured for six times at 237 nm against blank

RESULTS AND DISCUSSION

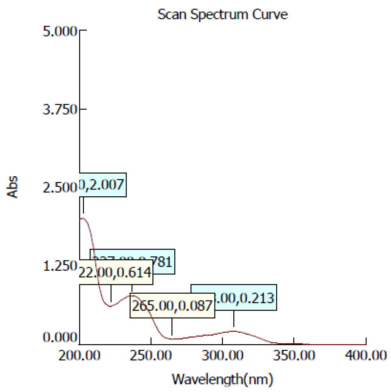

Fig 1: UV Spectrum of Ticagrelor

Linearity:
The standard calibration curve was constructed between concentration and absorbance and the linearity was found in the range of 2µg/mL to 10µg/mL in UV region. The regression equation and correlation coefficient were calculated and found to be within the required limits as per ICH guidelines.

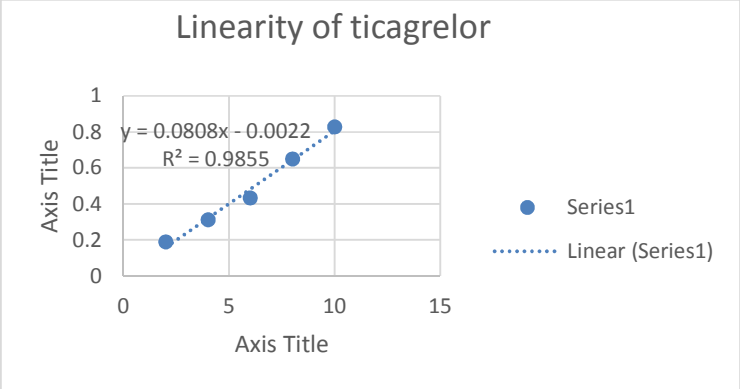

Fig: 2 Linearity Graph of Ticagrelor

Table 1: Linearity results for UV

Concentration (µg/mL)	Absorbance
2	0.189
4	0.312
6	0.434
8	0.650
10	0.828
Correlation Co-efficient(R^2)	0.9855
Slope(m)	0.0808
Intercept(c)	0.0022

Table 2: Results for regression analysis of Ticagrelor

S. NO	Parameters	Results
1	Regression equation (Y)	Y = 0.0808x-0.0022
2	Correlation coefficient (R^2)	0.9855
3	Slope (m)	0.0808
4	Y – intercept (c)	0.0022
5	Range	2-10 µg/mL
6	Limit of detection (LOD)	0.199 µg/mL
7	Limit of quantitation (LOQ)	0.66 µg/mL

Accuracy: The results represent the high percent recovery values indicating that the proposed method is accurate.

Table: 3 Results for Accuracy

S.No	Concentration Level (%)	Amount added(µg/ml)		Amount found(µg/ml)	%Recovery	Statistical parameters
		Std drug	Sample			
1	50	2	4	5.98	99.66	Mean=100. 21
2		2	4	6.01	100.16	SD=0.587
3		2	4	6.05	100.83	%RSD=0.59
4	100	4	4	8.01	100.12	Mean=100.24
5		4	4	7.99	99.87	SD=0.453
6		4	4	8.06	100.75	%RSD=0.45
7	150	6	4	12.01	100.08	Mean=99.97
8		6	4	12.01	100.08	SD=0.19
9		6	4	11.97	99.75	%RSD=0.19

Precision:
The precision of method was performed by intraday and interday variations study. The solutions of 6µg/mL were prepared and their absorbances are noted for the study. The %RSD for intraday and interday precision are found to be 0.07% & 0.08% respectively

Table 4: Results for Intraday precision

S.No	Absorbance	%Assay	Statistical parameter
1	0.430	100.02	Mean=99.92 SD=0.077 %RSD=0.08
2	0.437	99.93	
3	0.439	99.89	
4	0.432	99.85	
5	0.435	100.01	
6	0.435	99.84	

Table 5: Results for Inter day Precision

S.No	Absorbance	%Assay	Statistical parameter
1	0.431	99.81	Mean=99.78 SD=0.069 %RSD=0.07
2	0.433	99.85	
3	0.432	99.79	
4	0.438	99.80	
5	0.436	99.65	
6	0.435	99.81	

Robustness: All the experimental values for robustness obtained fall into the acceptance criteria.

Table 6: Results for Robustness

Concentration (µg/mL)	S.No	236nm	237 nm	238 nm
6.0	1	0.432	0.434	0.426
	2	0.426	0.428	0.424
	3	0.428	0.428	0.425
	4	0.421	0.425	0.427
	5	0.424	0.428	0.427
	6	0.424	0.431	0.426
	Mean	0.425	0.428	0.425
	SD	0.002	0.0008	0.0011
	%RSD	0.64	0.19	0.27

Table 7: Results for Marketed formulation of Ticagrelor

Formulation	Label claim (mg)	Amount of Drug Found In Tablet (mg)	Drug content (%±S.D)
BRILLIANTA	90mg	87.86	99.46±0.069

Stability Studies Spectrum:

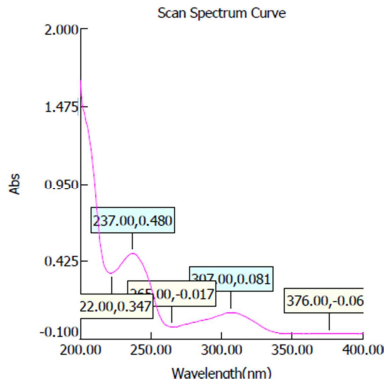

Fig:3 Spectrum of Acid Degradation

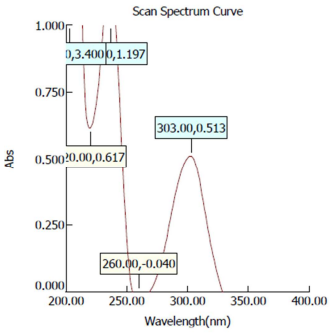

Fig:4 Spectrum of Basic Degradation

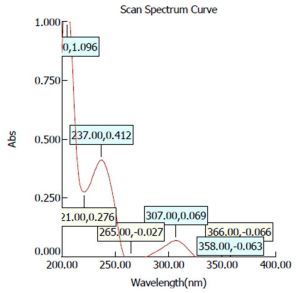

Fig:5 Spectrum of Oxidative Degradation

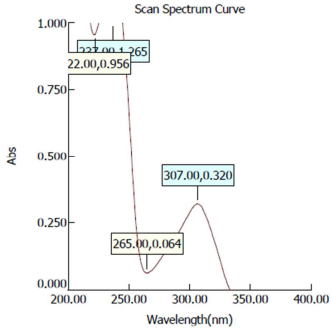

Fig:6 Spectrum of Thermal Degradation

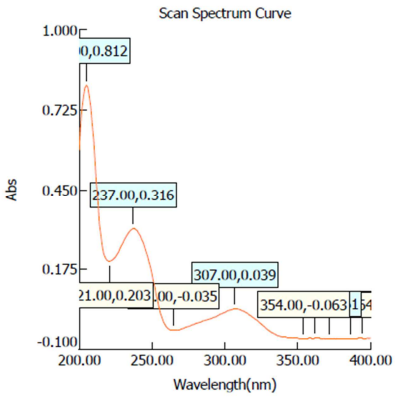

Fig:7 Spectrum of Photo Degradation

Type of stress conditions	Amount of drug to be taken(mg)	Amount of Solution taken(ug/ml)	Absorbance	% Degradable
Acidic Conditions	90mg	10ug/ml	0.480	57.97
Basic Conditions	90mg	10ug/ml	1.197	144.56
Oxidation	90mg	10ug/ml	0.412	49.75
Thermal	90mg	10ug/ml	1.265	152.7
Photo degradation	90mg	10ug/ml	0.316	38.16

CONCLUSION

A simple, sensitive and appreciable stability indicating UV spectrophotometric method has been developed for quantitative determination of Ticagrelor in bulk and marketed dosage form (tablet). The UV spectrum was scanned between 200 to 400 nm and 237 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 2 – 10 µg/ml. Accuracy (99.66 – 100.83) and the method were successfully applied to the pharmaceutical dosage form containing the Ticagrelor drug without any interference by the excipients. The method was fast and economical and it was also selective and sensitive for the desirable range. Results of the analysis were validated as per ICH guidelines and by recovery studies. Stability testing study includes the effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH

Acknowledgement
We are thankful to RAKS Pharma, Visakhapatnam, India for providing sample of Ticagrelor, A.U College of Pharmaceutical Sciences, Visakhapatnam for providing necessary facilities to carry out the research work.

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