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ESTIMATION OF TICAGRELOR IN COMMERCIAL DOSAGE FORM USING A SENSITIVE VALIDATED UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT

The aim was to develop and validate a simple, sensitive, cost effective and specific method for estimation of Ticagrelor HCl in bulk and formulation using ethanol as solvent. At λmax 255 nm the method obeyed Beer's law in the range of 5-30µg/ml with correlation coefficient (R^2=0.9982) and regression equation was found to be y=0.032x+0.0251. The % RSD was found to be 0.5303% and 0.5760% for inter-day and intra-day precision. The percentage recovery values ranged from 99.35%-100.65%. As per ICH guidelines method was validated for linearity, precision, LOD, LOQ, robustness, ruggedness and accuracy. Based on the obtained results the method was found to be new, reliable and sensitive and can be applied for estimation of Ticagrelor HCl in bulk and commercial dosage form.

INTRODUCTION

Ticagrelor (Fig.1) is chemically (1*S*,2*S*)-3-[7-[[(1*R*,2*S*)-2-(3,4-difluorophenyl) cyclopropyl]amino]-5-propyl sulfanyl triazolo [4,5-d]pyrimidine-3-yl]-5-(2-hydroxyethoxy) cyclopentane-1,2 diolhydrochloride[1].It is an oral antiplatelet[2-3]drug that inhibits platelet aggregation, myocardial infarction and thrombus formation in atherosclerotic disease. Literature review[4-8]for Ticagrelor revealed very few methods based on varies techniques like UV-Spectroscopic, LC-MS and HPLC for the estimation of Ticagrelor either in dosage form or in biological fluid. However only a few UV spectrophotometric methods are available for estimation of Ticagrelor and in this work the author developed a new UV method for its estimation.

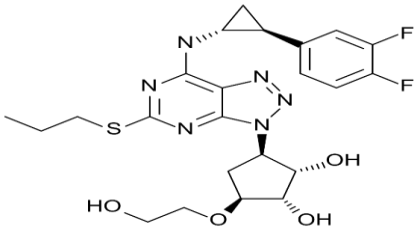

Fig 1:Structure of ticagrelor

MATERIALS AND METHODS

A gift sample of Ticagrelor HCL with an assay value of 99.2% w/w was obtained as gift sample. LABINDIA (T60) double beam UV / Visible Spectrophotometer and ELITE analytical balance were the instruments used.

Chemical used is Ethanol which is of 99.9% pure acquired from Fine chemicals. Ticagrelor tablets of 90 mg with a brand name **Brilinta**® were purchased from local market.

Preparation of standard stock solution: A standard drug solution of Ticagrelor HCl was prepared by adding 100 mg of drug into a 100 ml volumetric flask and made up to mark with ethanol to get a concentration of 1000 µg/ml.

Preparation of working standard solution: From the above standard stock solution 10ml of sample was transferred to100ml volumetric flask and made up to mark with ethanol to get a concentration of 100µg/ml.

Determination of lambda max (λmax) of Ticagrelor: Wavelength of maximum absorbance (λmax) was determined by scanning working standard solution using UV Spectrophotometer in the range of 200-400 nm using ethanol as reagent blank. The absorbance was found to be maximum at 255 nm against reagent blank (Fig.2)

Construction of calibration curve: Aliquots ranging from 0.5-3.0ml of working standard solutions were transferred to10 ml of volumetric flasks and made up to mark with ethanol to get solutions of 5-30 µg/ml concentration. The samples were then analysed at λ_{max} of 255 nm to get respective absorbances. The values [Table1] are then plotted to get a calibration curve (Fig. 3).

Preparation of test solution: 10 Tablets were weighed and powdered. The amount of powder equivalent to100mg was weighed and transferred to a volumetric flask and dissolved in100ml ethanol to get a concentration of 1000µg/ml. From the above solution 0.2ml was transferred to a 10ml volumetric flask and made up to get 20 µg/ml solution and this solution was analysed using ethanol as blank and assay was performed.

Method validation: Validation is a process of establishing documented evidence stating that a procedure, process, or activity carried out produces results within predetermined specifications[9].Validation parameters were validated as per ICHguidelines[10-12].

Range: The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Linearity: Ability of an analytical method to produce test results that are directly proportional to concentration of analyte. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. For estimation of linearity at least 5 concentrations are required.

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy is assessed by using 9 determinations covering a minimum of 3 concentrations. It is expressed in terms of % recovery which should be in between98-102%.

Precision: The closeness of agreement between the obtained values by analysing the sample for multiple times under prescribed conditions[13]. There are 3 levels repeatability, intermediate precision, and reproducibility.

Repeatability is a measure of the exactness under the same working conditions more than a short interim of time, that is, under ordinary working states of the scientific technique with the same hardware[14.] It is also known as intraday precision[15-16].

Reproducibility also is known as inter-day precision. Precision is expressed in terms of % Relative Standard Deviation, which should be less than 2%.

$$\%RSD = \frac{Standard\ deviation}{Mean} X100$$

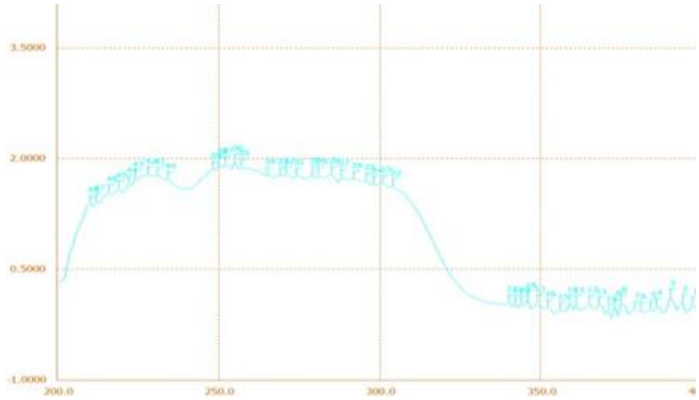

Fig 2: Determination of Lambda max

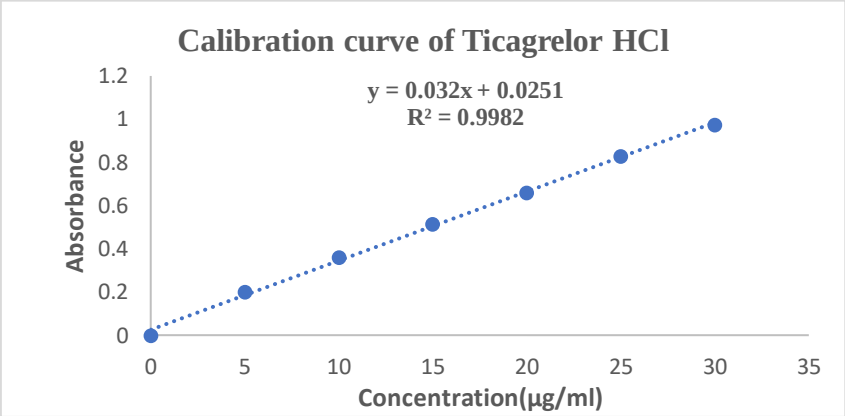

Fig: 3 Standard calibration curve of Ticagrelor HCl

Table 1: Linearity of working solutions

S. No	Concentration	Absorbance	Statistical analysis
1.	5	0.1998	Mean: 0.5890
2.	10	0.3591	Y= 0.032x + 0.0251
3.	15	0.5145	R^2= 0.998
4.	20	0.6589	
5.	25	0.8284	
6.	30	0.9736	

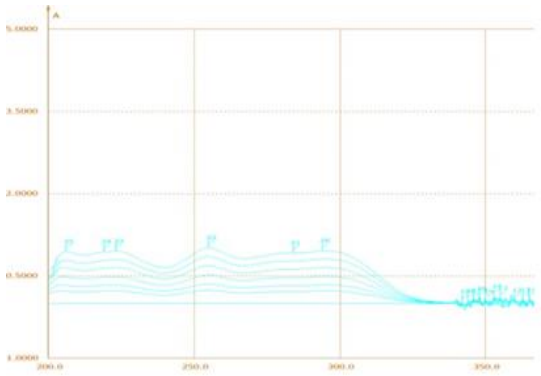

Fig 4: Overlay spectra

Table 2: Accuracy data

Ingredient	Level of addition (%)	Tablet amount (µg/ml)	Amount added (µg/ml)	Drug found (µg/ml)	% Recovery	Avg recovery ± (SD) %
Ticagrelor HCl	80	20	16	15.85	99.35	99.8% ± 0.311
	100	20	20	18.5	99.25	
	120	20	24	23.2	100.8	

Table 3: Repeatability data

Concentration	Absorbance	Statistical analysis
20	0.6509	Mean: 0.6578 % RSD: 0.717
20	0.6592	
20	0.6588	
20	0.6592	
20	0.6598	
20	0.6592	

Table 4: Intra assay study

Concentration (µg/ml)	% RSD			Average % RSD
	1	2	3	
20	0.593	0.576	0.547	0.572

Table 5: Inter assay study

Concentration (µg/ml)	% RSD					Average % RSD
	1	2	3	4	5	
20	0.5303	0.3142	0.410	0.4302	0.3927	0.415

Table 6: Ruggedness

Concentration (µg/ml)	Analyst 1		Analyst 2	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis
20	0.6592	Mean: 0.6580 % RSD: 0.5303	0.6601	Mean: 0.6612 % RSD: 0.576
20	0.6598		0.6593	
20	0.6592		0.6598	
20	0.6509		0.6598	
20	0.6592		0.6591	
20	0.6598		0.6592	

Table 7: Robustness data

Concentration (µg/ml)	λ1(254nm)		λ2 (255nm)		λ3(256nm)	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis	Absorbance	Statistical analysis
20	0.6471	Mean: 0.6569 % RSD: 0.153	0.6592	Mean: 0.6578 % RSD: 0.576	0.6402	Mean: 0.6578 % RSD: 0.153
20	0.6583		0.6588		0.6411	
20	0.6593		0.6592		0.6415	
20	0.6590		0.6509		0.6412	
20	0.6583		0.6592		0.6424	
20	0.6597		0.6598		0.6429	

Table 8: LOD& LOQ

Limit of Detection	Limit of Quantification
0.0204µg/ml	0.0621 µg/ml

Table 9: Validation parameters

Parameters	Results
Absorption maxima (nm)	255 nm
Linearity range	5-30 µg/ml
Regression equation	y= 0.032x+0.0251
Correlation coefficient(R^2)	0.9982
Molar Extinction coefficient	18159
LOD(µg/ml)	0.0241
LOQ(µg/ml)	0.0621
Accuracy (% Recovery± SD)	99.8%± 0.311
Inter day Precision (% RSD)	0.4560
Intraday Precision (% RSD)	0.5720
Sandell's sensitivity (µg/cm^2/0.001 absorbance units)	0.02287

Ruggedness: Ruggedness of an analytical procedure is the degree of reproducibility of results by analysing same sample under variety of conditions like laboratories, instruments, analysts, reagents etc.

Robustness: Robustness of an analytical procedure is capacity to remain unchanged by small but deliberate changes in parameters like wavelength, temperature, flowrates etc.

Sensitivity: (LOD) and (LOQ) of the drug were calculated by using equations according to ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

$$LOD = (3.3\times\sigma)/S$$

Limit of Quantification: It is an amount of analyte that can be quantitated with specified limit of accuracy and precision

$$LOQ = (10\times\sigma)/S$$

Linearity: To test linearity series of solutions ranging from 5-30 µg/ml were prepared from standard stock solution and analysed. Linearity was then evaluated by linear regression analysis. The linearity was shown in [Table 1].

Accuracy: The accuracy of method was determined by preparing solutions of concentrations 80%, 100%, 120%in which the amount of formulation **Brilinta®** to be added is kept constant 20 µg/ml and the amount of pure drug to be added varied from 16 µg/ml, 20 µg/ml, 24 µg/ml for 80,100, 120% respectively. The solutions for prepared in triplicate and accuracy was indicated by %RSD [Table 2].

Precision: For precision inter-day and intraday different solutions of same concentration (20µg/ml) were prepared and analysed 6 each in the morning, afternoon, and evening and absorbances were recorded [Table 4]. For inter-day study solutions of same concentration (20µg/ml) were prepared and analysed 5times each day for 5 consecutive days and the absorbances were noted [Table 5]. The results were indicated by % RSD

Ruggedness: The ruggedness of the method was carried out by analysing the sample using two different analysts and respective absorbances were recorded. The results are indicated in [Table6].

Robustness: The robustness of the method was carried out by analysing the sample using two different wavelengths (±1oflambda max) that were and respective absorbances were recorded. The results are indicated in [Table7].

Sensitivity: They are calculated by checking absorbance's using solvent and calculated using formulae and the results are shown in [Table8].

RESULTS AND DISCUSSION

Proposed UV spectrophotometric method was established for determination of Ticagrelor in bulk form as well as dosage form, which was developed and completely validated as per ICH guidelines. To decide the detection wavelength scanning of the standard solution was performed over a range of 200-400 nm. Detection wavelength of 255 nm was selected as λmax [Fig 2] as it showed maximum absorbance at this wavelength and also shown good results with good linearity. **Ticagrelor HCl** in ethanol. The method was found to be linear in the range of 5-30 µg/ml [Fig 3]. The regression equation was y=0.032x+0.0251, with 0.032 as slope and 0.0251 as intercept. Correlation coefficient(R^2) was found to be 0.9982.The method developed was found to be precise as %RSD was found to be less than 2%. Inter-day precision was carried out by analysing the same sample for 5times each in the morning, afternoon, evening and noted the absorbance values which stated that the method is precise indicated by %RSD which is less than 2%. And the Inter-day was carried out by analysing the same sample 6 times each day for 5 consecutive days. The method was found to be accurate, indicated by %recoveries ranging from 99.25%-100.8%. LOD&LOQ were found to be 0.0241 and 0.0621 respectively indicating that the method is sensitive. Robustness was carried out by analysing the sample at three different wavelengths and ruggedness by two different analysts and was found to be robust and rugged as the % RSD were found to be less than 2% for both robustness and ruggedness. All the parameters validated were shown in [Table 9].

CONCLUSION:

All the above parameters conclude that the proposed method is linear, accurate, precise, robust, and rugged and can be successfully applied for estimation of Ticagrelor HCl in bulk and formulation.

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