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A Validated Rp-Hplc Method for the Estimation of Ticagrelor in Bulk and Pharmaceutical Formulation

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ABSTRACT:A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Ticagrelor in Bulk and Pharmaceutical Tablet Formulations. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, Shimadzu shim pack C18 (250mm x 4.5µm x 5µ) column. Shimadzu Prominence-i LC-2030C Plus equipped with Auto sampler as the instrument model. Mobile phase consists of mixture of Methanol: Acetonitrile: 0.1% Ortho phosphoric acid in Millipore water in the ratio (90:5:5v/v) at a flow rate of 1.0 mL /min with injection volume of 10 µg/ml UV detection was performed at 255 nm. The Linearity was established for Ticagrelor in the range of 0.5-16 µg/ml with correlation coefficient of 0.9997. LOD and LOQ were found to be 0.2040µg/ml and 0.6184 µg/ml respectively. Retention time of Ticagrelor were found to be 3.30 min. % Recovery was found to be 99.84 to 100.49 and %RSD was found with in 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Ticagrelor in bulk and pharmaceutical dosageform.

KEYWORDS: Ticagrelor, RP-HPLC,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].

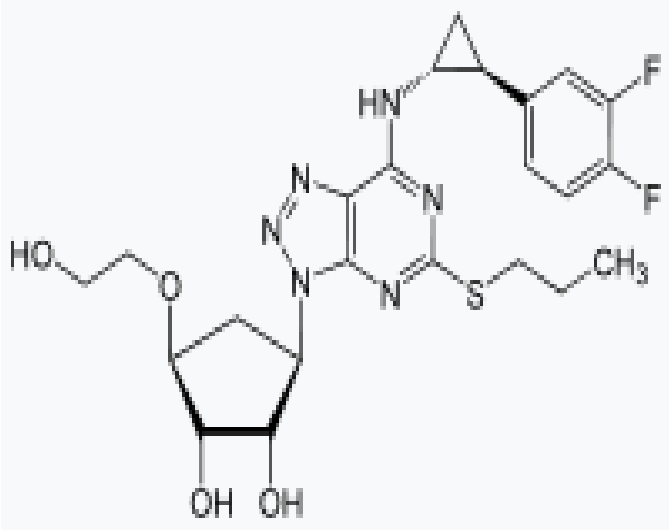

Fig.1: Chemical structure of Ticagrelor

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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 RP- HPLC[2-12]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:

Instrumentation:

Chromatographic separation was performed on a Shimadzu Prominence-i LC-2030C plus equipped with Auto sampler comprising a variable wavelength programmable UV detector. Shimadzu shim pack C18 (250mm x 4.5µm, 5µ) column is used.

Materials and Reagents:

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. Methanol, Acetonitrile and 0.1% Ortho Phosphoric Acid were obtained from Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. Distilled water was used throughout the experiment.

Chromatographic conditions:

Table 1: HPLC method development parameters.

HPLC method development parameters	
Column	C18, 250mm X 4.5 µ m X 5µ
Column Temperature	30°C
Wavelength	255 nm
Run time	10min
Injection Volume	10 µL
Flow rate	1.0 mL / min
Diluents	Mobile phase
Elution	Isocratic

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Preparation of solutions Mobile phase preparation:

The Mobile phase consisted of a mixture of Methanol (90%), Acetonitrile (5%) in the ratio of v/v, which was filtered through a membrane and degassed before use. pH Adjusted with 0.1% Ortho phosphoricacid in Millipore water.

Preparation of sample Standard Solution:

The formulation tablets of Ticagrelor (Brilinta- 90mg) were crushed to give finely powdered material. From the Powder prepared a 100mg of Ticagrelor was accurately weighed, transferred in a 100 ml volumetric flask, add 30 ml of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10 mL of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent, And an appropriate concentration of sample was prepared at the time of analysis. 10µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of Standard Solution:

10 mg of Ticagrelor was dissolved in 10ml of Methanol in 10 ml volumetric flask (1000µg/ml). Further dilution was made from above in such a way that the final concentrations consist of 0.5, 1, 2, 4, 8, 16 µg/ml.

System suitability requirements from stock and standard solutions:

Tailing factor:NMT 1.131

Theoretical Plates:NLT4678

III. RESULTS AND DISCUSSION:

Validation of the proposedmethod:

The proposed method was validated as per ICH guidelines.[14] The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity:

From the chromatograms of blank, standard (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good retention time. The specificity results are shown in Table2.

Table 2: Specificity of Ticagrelor:

Name of the solution	Retention time in min
Blank	0
Ticagrelor (Standard)	3.3

BLANK

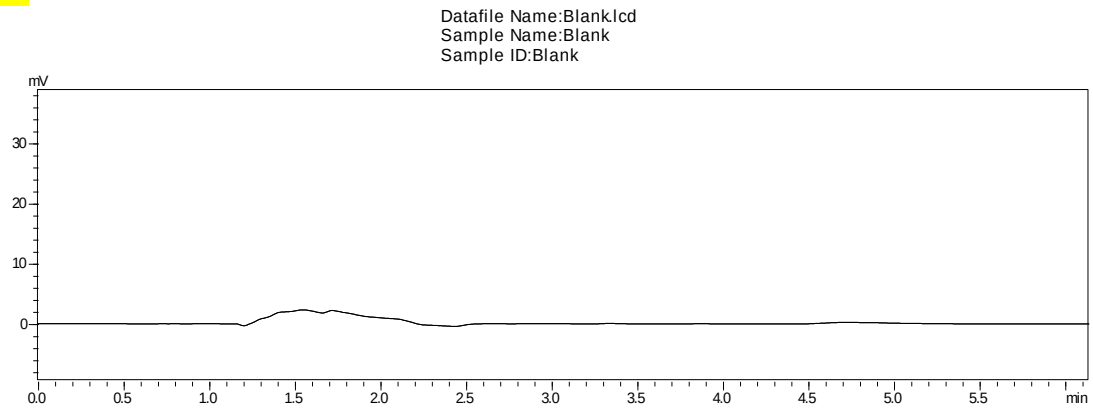

Figure 2: Chromatogram of Blank solution of Ticagrelor.

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TICAGRELOR STANDARD 100mcg/ml

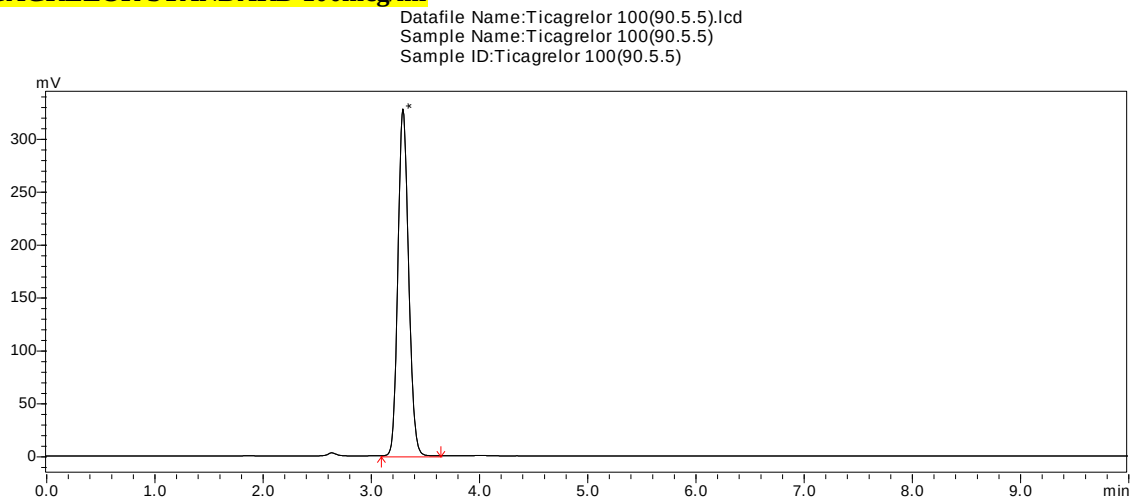

Figure 3: Chromatogram of standard solution of Ticagrelor.

Linearity:

The linearity of the response of the drug was verified at six concentration levels, ranging from 0.5-16mcg/ml of Ticagrelor in each linearity level were prepared. 10µl of each concentration was injectedinto the HPLC system. The response was read at 255 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table 3.

Table 3: Linearity of Ticagrelor.

Concentration (mcg/ml)	Peak area* (mv)
0.5	13707
1	25342
2	45340
4	90991
8	189715
16	369878

*Average of six determinations

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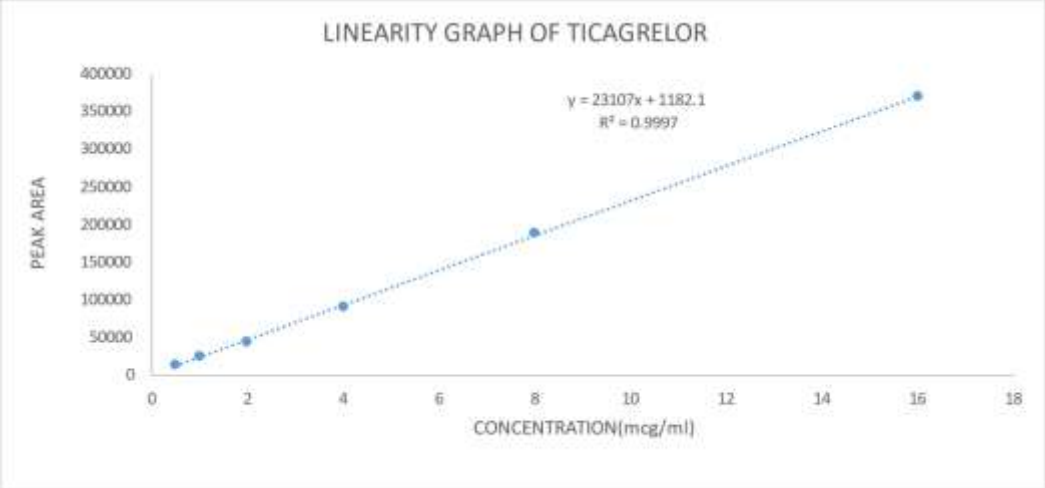

Figure 4: Linearity of Ticagrelor.

Precision:

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions of Ticagrelor were injected. % RSD was calculated and it was found to be 1.105 and interday precision done same as intraday, six replicate standard solutions of Ticagrelor were injected. % RSD was calculated and it was found to be 0.9622 which are well within the acceptance criteria of not more than 2.0. Results of system precision are shown in Table 4.

Table 4: Results of Precision of Ticagrelor.

SI. NO	Intra Day Name	Studies Peak area	Inter Day Name	Studies Peak area
1	Injection-1	25456	Injection-1	25325
2	Injection-2	25955	Injection-2	25397
3	Injection-3	25784	Injection-3	25743
4	Injection-4	25463	Injection-4	25811
5	Injection-5	25198	Injection-5	25229
6	Injection-6	25347	Injection-6	25695
	AVG	25533.83	**AVG**	25533.33
	STDEV	282.3936	**STDEV**	245.684
	%RSD	1.105	**%RSD**	0.9622

Accuracy:

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (90 mg of Ticagrelor). The recovery values for Ticagrelor ranged from 98.0 -102.0 %. The average recoveries of three levels of Ticagrelor were found to be 99.84 – 100.49 %. The results are shown in Table 5.

Table 5: Results of recovery of Ticagrelor.

Recovery level	Amount taken (mcg/ml)	Peak Area	Average	% Recovery ± SD*	% RSD
50%	2	91196 90542	90906.666	100.49	0.7317

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		90982		± 0.7354	
100%	4	181093 182258 182165	181838.666	99.84 ± 0.7115	0.7126
150%	6	262015 260129 257197	259780.333	100.27 ± 1.8717	1.866

*Average of three determinations

Limit of Detection and Limit of Quantification:

The limit of detection is an analytical method in which the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure in which the smallest amount of the analyte in sample which can be quantitatively determined LOD and LOQ were calculated using formula LOD = 3.3(SD)/S and LOQ = 10(SD)/S. Results were shown in Table 6.

Table 6: System suitability parameters.

Parameters	**Ticagrelor**
Linearity	0.5-16mcg/ml
Regression equation	y = 23107X+ 1182.1
Correlation coefficient	R^2 = 0.9997
Retention time	3.3 min
Run time	10 min
Limit of Detection (LOD)	0.2040 µg/ml
Limit of Quantitation(LOQ)	0.6184 µg/ml
Tailing factor	1.131
Theoretical Plate	4678

Ruggedness:

The ruggedness of test method by carrying out precision study in six preparations of sample on a single batch sample by different analysts andby different instrument, the results of the precision study are tabulated as below Table 7. The % RSD values are less than 2.

Table 7: Results of Ruggedness of Ticagrelor:

By changing the Analysts:

Concentration	**T1**	**T2**	**Mean**	**SD**	**%RSD**
0.5	13617	13854	13735.5	167.584	1.22008
1	25258	25114	25186	101.823	0.4042
2	45566	45329	45447.5	167.584	0.36874
4	91923	90874	91398.5	741.755	0.81156
8	189923	189597	189760	230.516	0.12147
16	3699964	368442	369203	1076.216	0.29149

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By changing the Instrument:

Concentration	T1	T2	Mean	SD	%RSD
0.5	14071	13753	13912	224.859	1.61630
1	25882	26105	25993.5	157.684	0.60663
2	45634	45297	45465.5	238.294	0.52412
4	91831	90958	91394.5	617.304	0.67542
8	189916	189587	189751.5	232.638	0.12260
16	369461	368854	369157.5	429.213	0.11626

Robustness:

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analyzing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±0.2 ml/min), column temperature (±5°C) and detection wavelength (±2 nm) shown in Table 8.

Acceptance criteria:System suitability should pass as per test method at variable conditions.

Table 8: Robustness results for Ticagrelor

Robustness	Condition	Tailing Factor	% RSD
Flow Rate	Decreased(-0.2ml/min)	1.12	0.63
	Increased (+0.2ml/min)	1.084	
Column Temperature	Decreased (-5^{0}C)	1.08	0.40
	Increased (+5^{0}C)	1.078	
Wavelength	Decreased (-2nm)	1.077	1.18
	Increased (+2nm)	1.08	

ASSAY:

Brand name	Available form	Label claim	Amount found	Assay
BRILINTA	Tablets	90mg	89.72mg	99.91

IV. CONCLUSION:

The present analytical method was validated as per ICH guidelines[13] and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Ticagrelor in bulk drug and pharmaceutical dosage forms.

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