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Method Development, Validation and Impurity Profiling of Ticagrelor by Acid Degradation Method

Rajendra B. Kakde[1]*, Dinesh D Satone[2], Geyata Dongare[1], Rahul Chilbule[1], Yogesh Malkhede[1]

[1]Department of Pharmaceutical Sciences Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-33, Maharashtra, India.
[2]Analytical Development Division, Orbicular Pharmaceutical Technologies Pvt Ltd, Hyderabad-500072, India

Abstract : A simple method is developed for impurity profiling of Ticagrelor by High Performance liquid Chromatography. Forced degradation under acidic condition were carried out. Only one degradant has been found. Cosmocil C_{18} (250 x 4.6 mm i.d., 5 µ) column with mobile phase composed of 0.1% Formic acid:ACN in the ratio of 55:45%v/v at flow rate 1.0 mL/min was shown sharp peak and good resolution between drug and its degradant. The detection wavelength was used at 254nm. Acid degradant was isolated by prep-HPLC by using Thermo (100 x 10mm i.d., 5µ) Hypersil Gold semi-preparative column at flow rate 4.0 mL/min. The complete spectral analysis IR, ^{1}H NMR, ^{13}C NMR and LC-MS confirmed the chemical structure of impurity.
Keywords : Ticagrelor, Method Development, Validation, Isolation and Characterization of Unknown Impurity.

1. Introduction

Ticagrelor (trade name Brilinta) is a platelet aggregation inhibitor produced by AstraZeneca. It is an antagonist of the PZY_{12} receptor. Chemically it is (1S,2S,3R,5S)-3-[7[[(1R,2S)-2-(3,4-difluorophenyl) cyclopropyl]amino]-5-propylsulfanyltriazolo-[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol.

The drug was approved for use in the European Union by the European Commission on December 3, 2010. The drug was approved by the US Food and Drug Administration on July 20, 2011. The Drug Controller General of India (DCGI), based on the New Drug Advisory Committee's (NDAC) recommendations granted its approval in May 2012 for marketing Brillinta (Ticagrelor) tablets in India.

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Ticagrelor is used for the prevention of thrombotic events (for example stroke or heart attack) in people with acute coronary syndrome or myocardial infarction with ST elevation. The FDA regulation for ticagrelor is reduction of the rate of cardiovascular death, myocardial infarction (MI), and stroke in people with acute coronary syndrome or history of myocardial infarction.

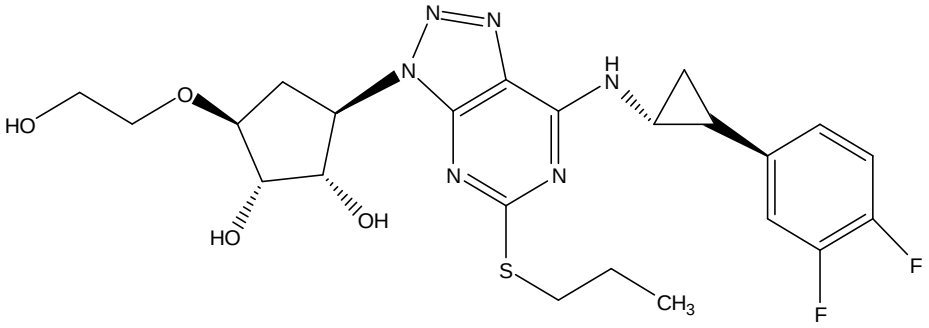


Fig. 1. Structure of Ticagrelor

Literature survey revealed that several HPLC, UPHC/MS-MS, and LC-MS/MS methods were reported for the estimation of Ticagrelor in bulk and also in human plasma. One UV method was also reported. Four process related impurities, majar degradation pathway and its identification were reported. An extensive literature survey disclosed that no one reported impurity profiling of standard drug by Acid degradation method and its identification.

2. Experimental

2.1.Material and Reagents:

Standard drug of % purity 99.06% was used. HPLC grade water (Merck) was used throughout the study as required. Acetonitrile (Merck), Methanol (Merck) and Formic acid (Merck) of HPLC grade were used. Hydrochloric acid and Sodium hydroxide of Analytical Reagent Grades were used for the study.

2.2. Instrumentation:

2.2.1. Analytical and Preparative HPLC:

Analytical High Performance Liquid Chromatograph (HPLC) equipped with Shimadzu LC-20AD LC solution software. The system consisted of Shimadzu Rheodyneinjector 7725 I as loop injector, SPDM-20A prominence Photo Diode Array (PDA) detector as a source of detection and DGU-$20A_5$ prominence degasser for removing the dissolved gases in the mobile phase.

Preparative HPLC separation and fraction collections were carried out using Shimadzu prominence LC-6AD equipped binary gradient module, SPD-M20A prominence PDA detector, FRC-10A Fraction collector and CBM-20A Communications Bus Module. The Lab Solution Software was used for the data process.

2.2.2. FTIR:

IR experiment was carried out on Shimadzu FT-IR (Shimadzu, Japan). Sample preparation was carried out with the help of KBr powder, mixed well and instrument was operated on principle of transmission technique. Spectra were acquired by accumulation of 45 scans form the range of 4000 cm^{-1} to 400 cm^{-1} with 4 cm^{-1} resolution. Instrument was controlled by IR solution software.

2.2.3. NMR: Nuclear Magnetic Resonance (NMR) spectra were obtanined using FT NMR Spectrometer model Avance-ll (Bruker). The instrument were equipped with a cryomagnet of field strength 9.4 T. ^{1}H and ^{13}C frequency was 400 MHz, 100 MHz. The Instrument were accompanied by TOPSPIN NMR data system.

2.3.Chromatographic Conditions:

Analytical HPLC: Cosmocil, C_{18} column (250 x 4.6 mm, 5 µ) was used for separation. 0.1% formic acid and ACN (55:45%v/v) employed as mobile phase. The flow rate and injection volume was 1.0mL/min and 20µL respectively. The detection wavelength was 254 nm.

Preparative HPLC: Thermo, (100 x 10mm, 5µ) Hypersil Gold semi-preparative column was used for isolation. Mobile phase used same as in analytical HPLC. The flow rate and injection volume was 4.0mL/min and 1mL respectively.

2.4.Preparation of solutions:

a)Working Standard solution:

Accurately weighed quantity of Ticagrelor10 mg was dissolved in 5 mL of methanol and sonicated for 15 min. The solution was cooled to room temperature and diluted up to the mark with methanol,volume was made to 10 mL (solution A: conc.1000 ppm).1.0 mL of solution A was diluted to 10 mL with methanol (conc. 100 ppm). To that 2.0 mL of solution was pipette out and diluted up to the mark with methanol, volume was made up to 10 mL (conc. 20 ppm) and 10 ppm solution (Solution A) was made for validation study.

b) Acid Degradation:

One mL of solution A was mixed with 0.1 N MethanolicHCl in 10mL volumetric flask. This solution refluxed for 2 hr at 70^0 C and cooled. Two mL of the cooled solution diluted with methanol upto 10 mL and neutralized with 0.1 N MethanolicNaOH. The neutralized solution injected into HPLC system.

c)Working SampleSolution

For sample preparation, methanol was used as diluent. Ten tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 25 mg of Ticagrelor (83.6 mg) was transferred into a 25 mL volumetric flask. About 15 mL of methanol was added and mixture was sonicated for 15 min. The solution was cooled to room temperature and diluted up to the mark with methanol (stock solution 1000 ppm). The resultant solution was filtered through Whatman Grade I filter paper. 1.0 mL of filtrate was transferred to a 10 mL volumetric flask and then volume was made up to the mark with methanol to obtain a concentration of 100 ppm. One millilitre of that solution was diluted to 10 mL with methanol to get working sample solution of concentration 10 ppm (Solution-B).

3.0Result and Discussion:

3.1. Development of HPLC Method for Impurity Profiling of Ticagrelor by Acid

Degradation

The main object of this work was to develop new impurity profiling method of Ticagrelor and isolation, characterization of isolated impurity. Forced degradation studies were carried out to identify the degradation product.

3.1.1.Optimized Degradation Condition on Analytical HPLC

Initially, different mobile phases were tried in isocratic mode to get an adequate retention of Ticagrelor. Mobile phase containing 10 mM Ammonium acetate (pH 3.0):methanol (50:50%v/v), 10 mM Ammonium acetate (pH 3.0):methanol (70:30%v/v), 10 mM Ammonium acetate(pH 3.0):ACN (70:30%v/v) etc. were tried, but retention time of Ticagrelor was above 20 min, did not give sharp peak and no adequate resolution between drug and its degradant.

To get separation of Ticagrelor from its degradation products chromatographic method development was carried using Cosmocil, C_{18} column (250 x 4.6 mm i.d., 5 µ), 0.1% formic acid and Acetonitrile

(55:45%v/v) as mobile phase seems to be good resolution and peak shape. Chromatogram of Standard Ticagrelor was shown in fig. 2.

3.1.2. Identification of Degradation Products of Ticagrelor

Only one degradation found in acid hydrolysis. The acid hydrolysis as a total of 27.015% degradation was found (0.1N HCl at 70˚C, upto 2 hr). The method was specific because degradation products were separated to base line from main component which confirms the method is selective and homogeneity of the drug product. Acid degradation chromatogram of Ticagrelor shown in fig. 3.

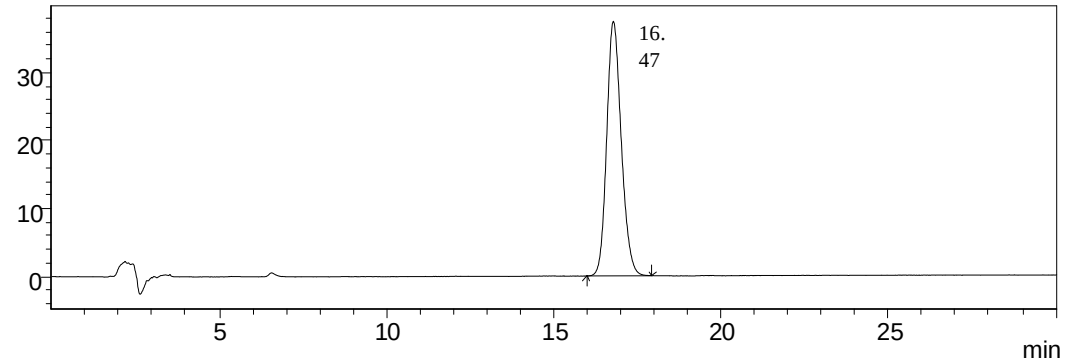


Fig. 2. Chromatogram of Standard Ticagrelor

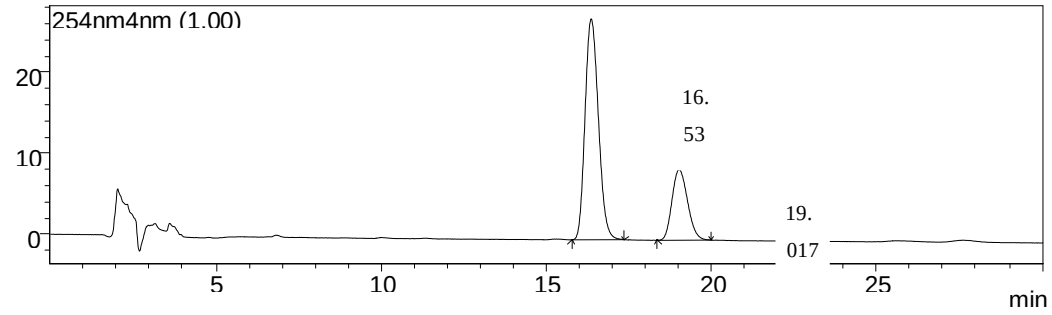


Fig. 3. Chromatogram of Ticagrelor and its acid degraded product.

3.1.3. Isolation of Degradation Product of Ticagrelor

Acid degradation product were isolated by Prep-HPLC. The fractions have been collected on the basis of mass based Purification. The collected fraction lyophilized to get free solids.

3.2.Structure Elucidation of Unknown Acid Degradation Product

The isolated degradation product weresubjected to complete identification and characterization. The retention time and UV spectrum obtained in the PDA detection during HPLC analysis was matched with degradation product intended to isolate for characterazation.

3.2.1.IR Spectroscopic Analysis of Ticagrelor and its Isolated Impurity

The IR spectra of the ticagrelor and isolated impurity are recorded, and the functional groups wavelengths are shown in following table 1. The functional group -N-H, –OH, and -N=N, present in ticagrelor as well as impurity. Only C-F functional group absent in isolated impurity as compaired to Ticagrelor.

Table: 1. IR data of Ticagrelor and it's isolated impurity

Product	**IR absorption band cm^{-1}**
Ticagrelor	3435.98 (O-H str), 3290.56 (N-H str), 1624.06 (C=C str), 1585.49 (C=N str), 1519.91 (N=N str), 1274.95 (C-F str).
Isolated impurity of Ticagrelor	3440.21 (O-H str), (3300.20 (N-H str), 1625.15 (C=C str), 1523.76 (N=N str).

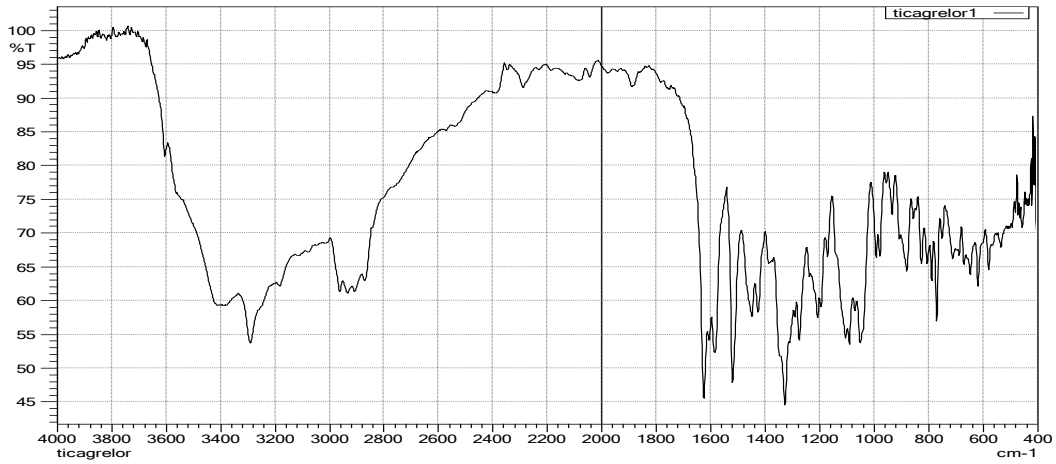


Fig. 4. IR Spectra of Ticagrelor

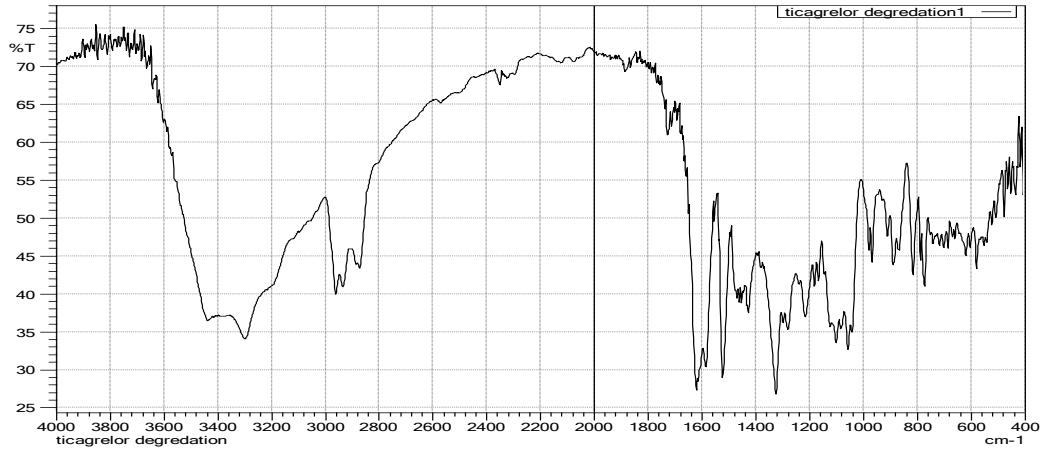


Fig. 5. IR Spectra of Isolated Impurity of Ticagrelor

3.2.2. LC-MS Study of Ticagrelor and its Isolated Impurity

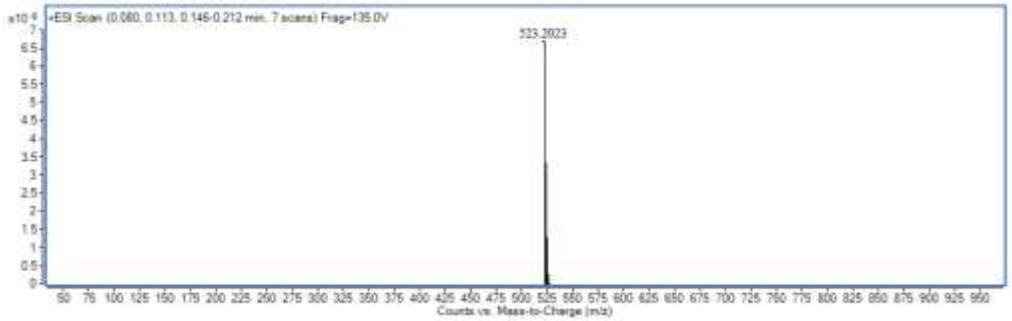


Fig. 6. LC-MS of Ticagrelor

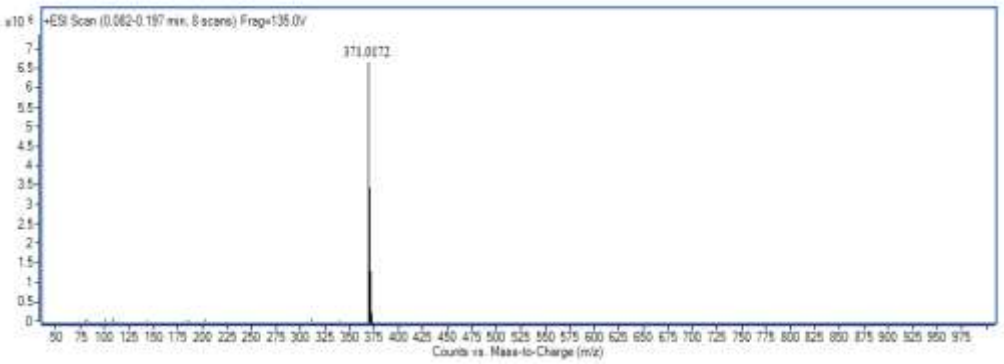


Fig. 7. LC-MS of Isolated Impurity of Ticagrelor

3.2.3. 1H and 13C NMR Spectra of Ticagrelor and its Isolated Impurity

The NMR spectra of Ticagrelor its Isolated Impurity are shown in fig. 8, 9 and 10, 11 respctively. The NMR results of Ticagrelor its Isolated Impurity are shown in table 2.

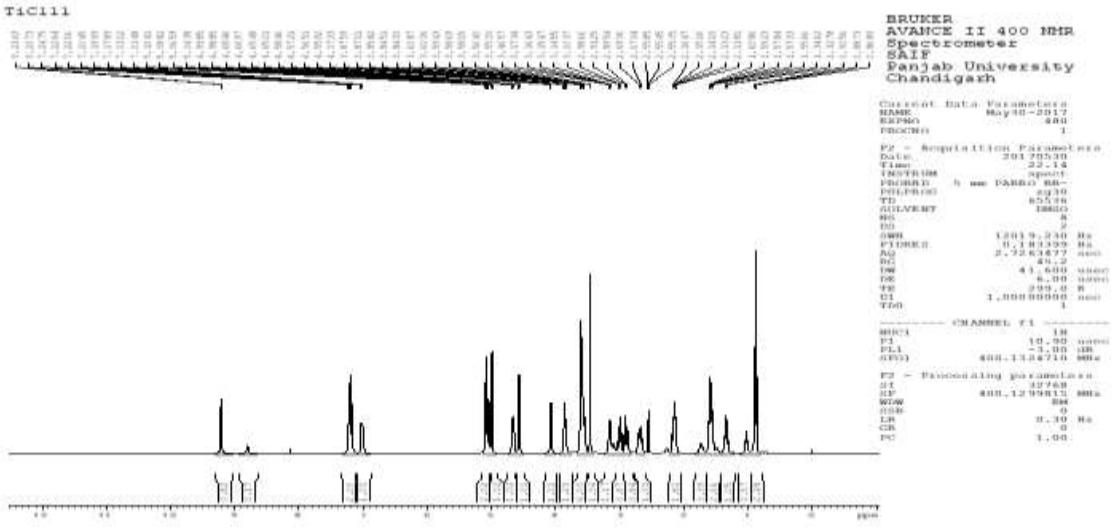


Fig. 8. 1H NMR spectra of Ticagrelor

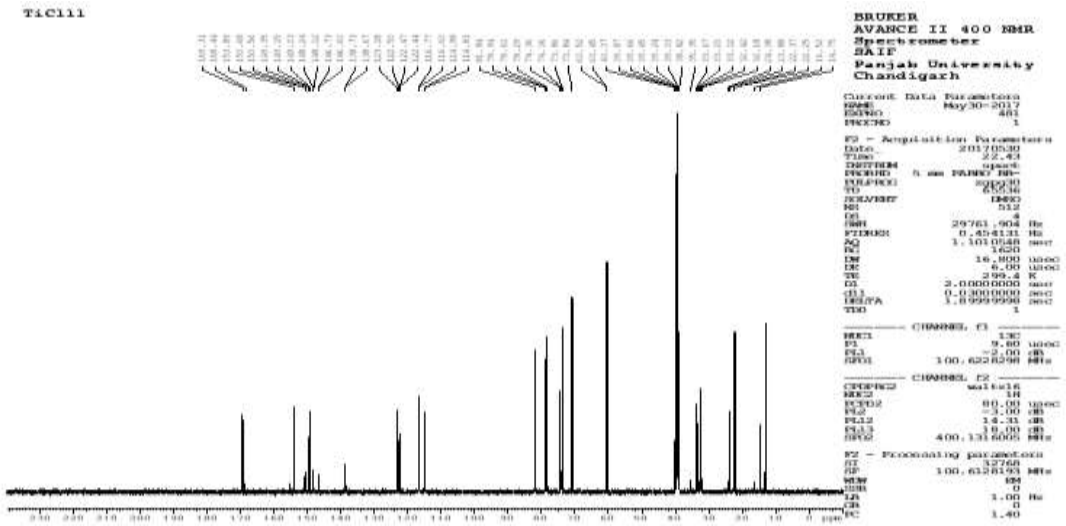


Fig. 9. 13C NMR spectra of Ticagrelor

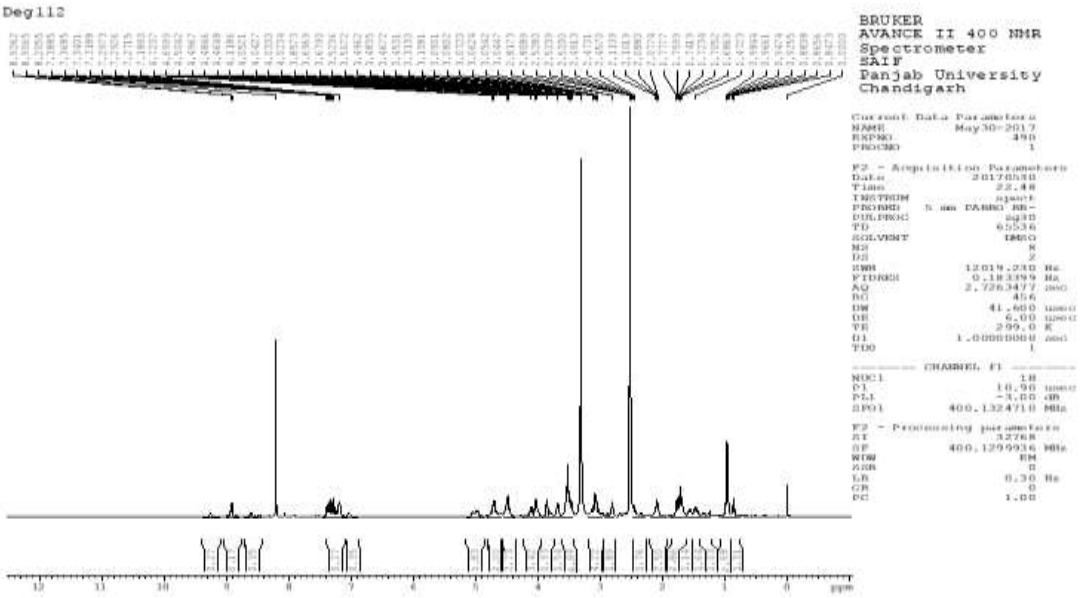


Fig. 10. 1H NMR spectra of isolated Impurity of Ticagrelor

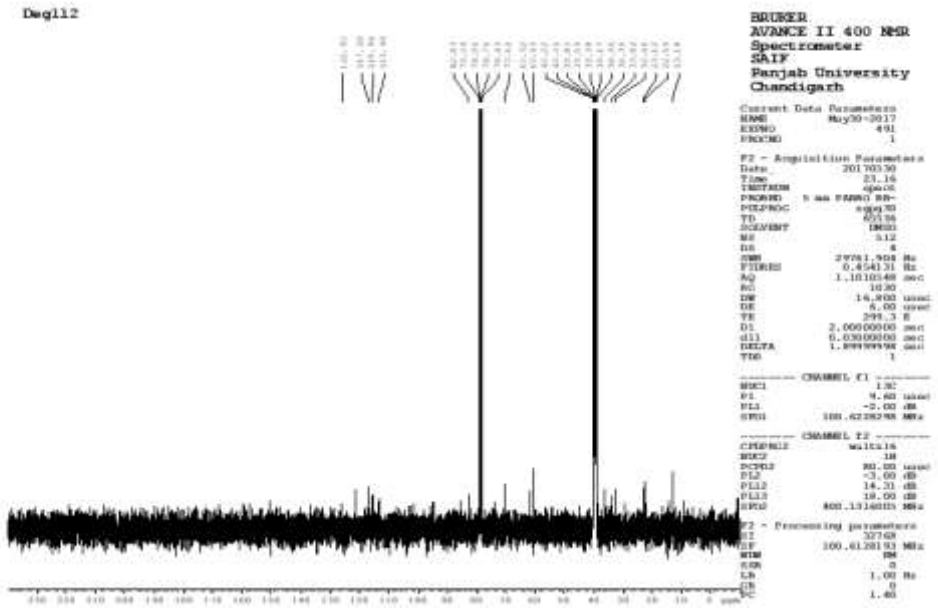


Fig. 11. 13C NMR of isolated impurity of Ticagrelor

Table 2. NMR results of Ticagrelor and its isolated Impurity

Position	Ticagrelor[a]		Isolated Impurity of Ticagrelor[b]	
	Chemical shift of 1H (ppm)	Chemical shift of 13C (ppm)	Chemical shift of 1H (ppm)	Chemical shift of 13C (ppm)
1	---	---	0.84 (s)	13.18
2	---	---	0.88 (s)	22.59
3	---	---	0.94 (s)	23.12
4	---	114.98	0.98 (s)	32.46
5	---	---	1.47 (d)	33.82
6	---	116.77	1.75 (d)	36.36
7	---	---	2.11 (s)	60.43
8	---	122.50	2.81 (d)	70.62
9	---	123.18	3.31 (s)	78.96

10	3.14 (s)	---	3.52 (s)	82.83
11	3.17 (m)	33.67	3.69 (s)	113.44
12	3.00 (m)	16.52	3.85 (s)	115.58
13	3.46 (s)	24.38	4.04 (s)	117.18
14	---	138.73	4.69 (s)	120.28
15	7.03 (d)	153.89	---	---
16	---	148.24	---	---
17	---	146.73	---	---
18	7.24 (m)	169.31	---	---
19	7.37 (m)	168.44	---	---
20	---	---	---	---
21	1.34 (m)	32.42	---	---
22	1.60 (m)	23.88	---	---
23	0.90 (t)	14.75	---	---
24	3.60 (m)	33.20	---	---
25	3.87 (t)	81.84	---	---
26	4.68 (m)	78.94	---	---
27	4.55 (s)	74.36	---	---
28	2.89 (m)	35.35	---	---
29	---	---	---	---
30	3.58 (s)	70.84	---	---
31	4.07 (s)	60.52	---	---
C_{25}	5.10 (d)	---	---	---
C_{26}	5.24 (d)	---	---	---
C_{31}	4.65 (s)	---	---	---

a- Position according to structure of Ticagrelor
b- Position according to starting to ending of NMR peak

Analysis of all spectral data leads to the conclusion of structure of isolated impurity of ticagrelor was identified as (IUPAC Name) (1S,2S,3S,5R)-3-(2-hydroxyethoxy)-5-(7-amino-5-(propylthio)-3H-[1,2,3]triazolo [4,5-d]pyrmidin-3-yl)cyclopentane-1,2- diol.

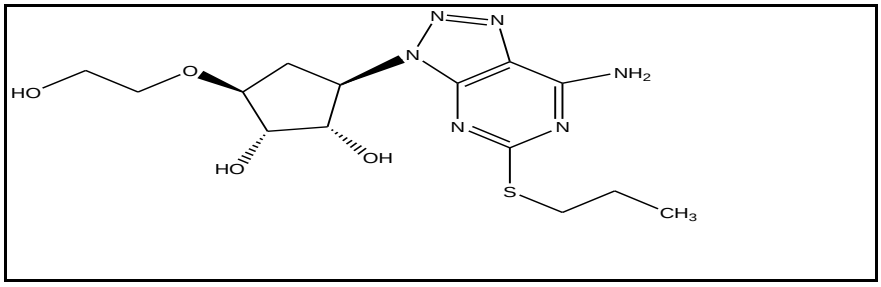


Fig. 12. Structure of isolated impurity of Ticagrelor

3.3. Pre-Validation Performance

3.3.1.System Suitability Parameters

Standard solution of Ticagrelor (10 ppm) injected into the developed HPLC method to know the system suitability results. The retention time of Ticagrelor is 16.479 min, USP tailing 1.21 and USP Plate count is 5633.852.

3.3.2. Robustness

Robustness was checked by analysis of sample solutions with standard on same column, after making

small changes in methodparameters. The results are shown in table 3.

Table 3. Robustness study of Ticagrelor byHPLC

Brillinta (Avg. Wt.34.50 mg for 10 mg of Ticagrelor)				
	Flow rate (1.0mL/min ±0.1)		**Change in wavelength (254nm±2nm)**	
%Estimation	**0.9 ml/min**	**1.1ml/min**	**252nm**	**256nm**
Mean*	101.12	98.471	97.83	99.82
±SD	1.2581	0.6392	0.2564	1.0125
%RSD	1.2441	0.6491	0.2620	1.014
RetentionTime(R_t)	18.320	15.31	---	---

*Mean of 3reading

3.3.3.Analytical Method Validation

3.3.3.1. Specificity

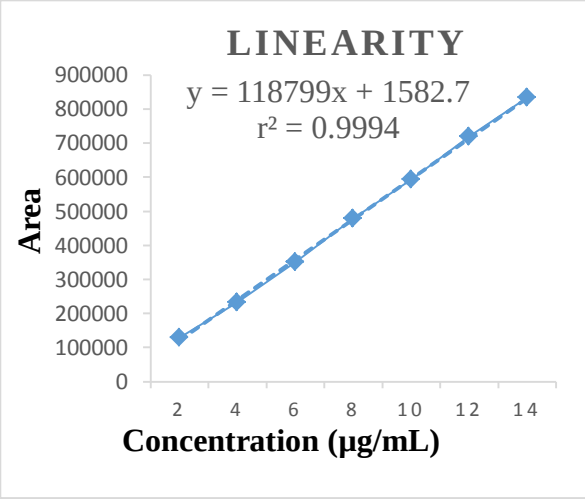
Forced degradation study of the drug substance can help to identify the stability of the molecule and possible degradation products and also validate the stability and specificity of the analytical procedures. Acid, base, thermal, oxidative and photolytic degradation sample solutions were injected into the developed method to know the specificity of the method. Degradation was observed in acidic, basic and oxidative conditions. Developed method was able to separate all degradation products from drug compound, so it can be employed as stability indicating method. Degradation was not observed in thermal, and photolytic conditions. Degradation results were shown in table 4.

Table 4. Specificity Studies of Ticagrelor

Conditions	Normal	Acid	Base	Oxidation	Thermal	Photolytic
%Assay	100.00	72.985	60.97	75.53	99.23	99.20

3.3.3.2. Linearity andrange

Linearity was determined for seven concentrations (2-14 µg/mL) of each three replicate injections. The linearity test results were shown in table 5. Linearity curve was shown in fig. 13.



Concentration	**Peak Area***
2 µg/mL	129919
4 µg/mL	232889.33
6 µg/mL	350196.33
8 µg/mL	477931
10 µg/mL	593896.33
12 µg/mL	719655.667
14 µg/mL	832966.667
*Mean of three readings	

Fig. 13. Calibration curve for linearity of Ticagrelor

Table 5. Analytical performance data of Ticagrelor

Parameters	**Ticagrelor**
Linear dynamic range (µg/mL)	2-14
Slope	118799
Correlation coefficient(r^2)	0.9994
LOD(µg/mL)	0.362
LOQ(µg/mL)	1.095

3.3.3.3.Accuracy

Accuracy method was conducted to determine % Recovery of the drug. The study was carried out at 80%, 100% and 120% for three replicate injections of each concentration of the analyte results were shown in table 6.

Table 6. Accuracy study of Ticagrelor byHPLC

Level	**Detector response* (Peak area)**		**%**
	Standard	**Sample**	**Recovery**
80%		495990	98
100%	632204	622202	98.4
120%		752593	99.16
***Mean of three observation**		**Mean**	**98.52**
		±SD	**0.5892**
		%RSD	**0.5980**

3.3.3.4. Precision

Intraday precision (Repeatability) was performed by analysis of three independent samples within a day. Three replicate injection of each of sample solutions were made separately and chromatograms were recorded. Similarly three replicate injections on another day of three independent samples for interday precision. Precision results are shown in table 7.

Table 7. Precision study of Ticagrelor byHPLC

Sr. No.	**Observation**	**%Labelled claim**		
		Intra-day	**Inter-day**	**Different Analyst**
1	I	99.05	98.64	100.86
2	II	100.396	98.91	99.84
3	III	102.09	98.398	102.32
Mean		**100.512**	**98.65**	**101.007**
SD		**1.5233**	**0.2561**	**1.2464**
%RSD		**1.5155**	**0.2596**	**1.2339**

3.3.3.5.Limit of Detection (LOD)and Limit of Quantification (LOQ)

LOD and LOQwere done with method based on based on standard deviation of the response and the slope of calibration curve.

$$\mathbf{LOD} = \frac{\mathbf{3.3\sigma}}{\mathbf{S}} \text{ and } \mathbf{LOQ} = \frac{\mathbf{10\sigma}}{\mathbf{S}}$$

Signal to noise ratio (k) = 3.3 and 10 for LOD and LOQ respectively.
σ =Standard deviation of the response
S = slope of the calibration curve.
The results of LOD and LOQ studies are shown in table 5.

3.3.3.6. Assay

Solution A and Solution B were injected into developed chromatographic conditions and % of assay was calculated and the % assay was found to be 98.88 %. The results of assay procedure shown in table 8.

Table 8.Analysis of marketed formulation of Ticagrelor byHPLC

Sr. No.	Wt. of sample (mg)	Area of sample	Area of standard	% Assay
1.	24.795	615849	632204	97.13
2.	23.775	621512		97.96
3.	24.668	643223		101.39
4.	24.09	619538		97.65
5.	25.015	632591		99.78
6.	24.35	630915		99.40
			Mean	98.88
			±SD	1.6001
			%RSD	1.6182

4. Conclusion

The simple, accurate, precise and economical method was developed for analysis of Ticagrelor. The developed method were validated according to ICH R2 guidelines. In Impurity Profiling Study, drug were forced fully degraded by 0.1 N HCl (Methanolic). In aciddegradation study one degradant was observed and that degradant was isolated and confirmed the structure by using spectroscopic techniques like NMR, LC-MS and FT-IR. The accurate mass of the isolated impurity of Ticagrelor were subjected to LC-MS. The elemental compositions of isolated impurity was $C_{14}H_{22}N_6O_4S$ calculated as 370.15. 1H NMR Spectra of isolated impurity of Ticagrelor shows singlets at 0.98 ppm, 2.11 ppm, 3.31-3.69 ppm, 3.85 ppm, 4.05-4.69 ppm indicate presesnce of $-CH_3$, $-CH_2$, -OCH, -NH and $–OCH_2$ group respectively. It shows doublet at 1.47 ppm, 1.75 ppm and 2.81 ppm due to presence of $-CH_2$ group. 13C NMR Spectra strongly support the above data, as peaks 13.18-36.36 ppm are due to aliphatic carbon atoms. It shows peaks at 113.44 ppm, 115.58 ppm, 117.18 ppm and 120.28 ppm of carbon atoms in Pyrimidine ring. The peaks shows at 60.43-82.83 ppm due to the presence of hydroxyl groups attached to carbon atoms.

In FT-IR study it was found that all are groups present in isolated impurity of Ticagrelor except C-F (str.) is absent as compaired to pure Ticagrelor.

All the above spectral study of isolated impurity of Ticagrelor was confirmed the structure as (1S,2S,3S,5R)-3-(2-hydroxyethoxy)-5-(7-amino-5-(propylthio)-3H[1,2,3]triazolo[4,5-d]pyrmidin-3-yl)cyclopentane-1,2-diol.

5. References

1. www.drugbank.ca/drugs/DB04902.
2. Assessment Report for Brilique, European Medicines Agency, January 2011. http://www.ema.europa.eu/ema/index.
3. European Public Assessment Report Possia, Human Medicines Development and Evalution, April 2013. http://www.ema.europa.ea.

4. "FDA approves blood-thinning drug Brilinta to treat acute coronary syndromes. FDA 20 July 2011.
5. "Impurities in New Drug substances" ICH Q3A (R2), 2006.
6. "Impurities in New Drug Products" ICH Q3B (R2), 2006.
7. "Impurities: Guideline for Residual Solvents", ICH, Q3C (R3), 2006.
8. "Stability Testing of New Drug Substances and Products", ICH, Q1A (R2), 2006.
9. A. Lakshamana Rao and L. Kalyani, *J. Pharm. Sci.*, **3**, 634 (2013).
10. N. Kumar, S.R. Devineni, P.R. Gajjala, D.K. Gupta, S. Bhat, R. Kumar, S.K. Dubey and P. Kumar, *J. Pharm. Biomed. Anal.*, **120**, 248 (2016); doi:10.1016/j.jpba.2015.12.037.
11. H. Sadou Yaye, P.-H. Secrétan, T. Henriet, M. Bernard, F. Amrani, W. Akrout, P. Tilleul, N. Yagoubi and B. Do, *J. Pharm. Biomed. Anal.*, **105**, 74 (2015); doi:10.1016/j.jpba.2014.11.046.
12. P.R. Kulkarni and G.K. Gajare, *Int. J. Res. Pharmacy Chem.*, **6**, 733 (2016).
13. M.A. Ambasana, N.P. Kapuriya, K.M. Mangtani and K.D. Ladva, *Int. J. Pharmaceut. Sci. Res.*, **7**, 2009 (2016).
14. L.M. Bueno, J.W. Manoel, C.F.A. Giordani, A.S.L. Mendez, N.M. Volpato, E.E.S. Schapoval, M. Steppe and C.V. Garcia, *Eur. J. Pharmaceut. Sci.: Official J. Eur. Federation Pharmaceut. Sci.*, **97**, 22 (2017); doi:10.1016/j.ejps.2016.11.004.

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