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Identification and characterization of synthetic impurity in Ticagrelor drug substance

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Abstract

Three process impurities ranging from 0.43 to 1.42% in Ticagrelor were detected by a simple gradient reverse-phase high-performance liquid chromatography (HPLC). LC–MS was performed to identify the mass of the impurities. These impurities in the Ticagrelor crude sample have been isolated by using a column. Based on the spectral data (NMR, IR, and MS), the structures of these impurities were characterized as **TIC-1:** (1S,2S,3R,5S)-3-(7-(((1R,2S)-2-(3,4-difluorophenyl)cyclopropyl)amino)-5-(propylsulfonyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-5-(2-hydroxyethoxy)cyclopentane-1,2-diol,**TIC-2:**2-(((3aR,4S,6R,6aS)-6-((5-((5-amino-6-chloro-2-(propylthio)pyrimidin-4-yl)amino)-6-chloro-2-(propylthio)pyrimidin-4-yl)amino)-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)oxy)ethan-1-ol,**TIC-3:** (1S,2S,3R,5S)-3-((3-((1R,2S)-2-(3,4-difluorophenyl)cyclopropyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)amino)-5-(2-hydroxyethoxy)cyclopentane-1,2-diol. The structure was elucidated by various techniques MS, 1D NMR (1H, 13C, and DEPT), 2D NMR(HSQC, HMBC and ^{15}N HSQC, HMBC) and IR confirmed the proposed chemical structures of impurities.Identification, isolation, structural characterization, and prospects of the formation of these impurities were first reported in this paper.

Keywords; Ticagrelor, Characterization, Spectroscopy, Structure elucidation

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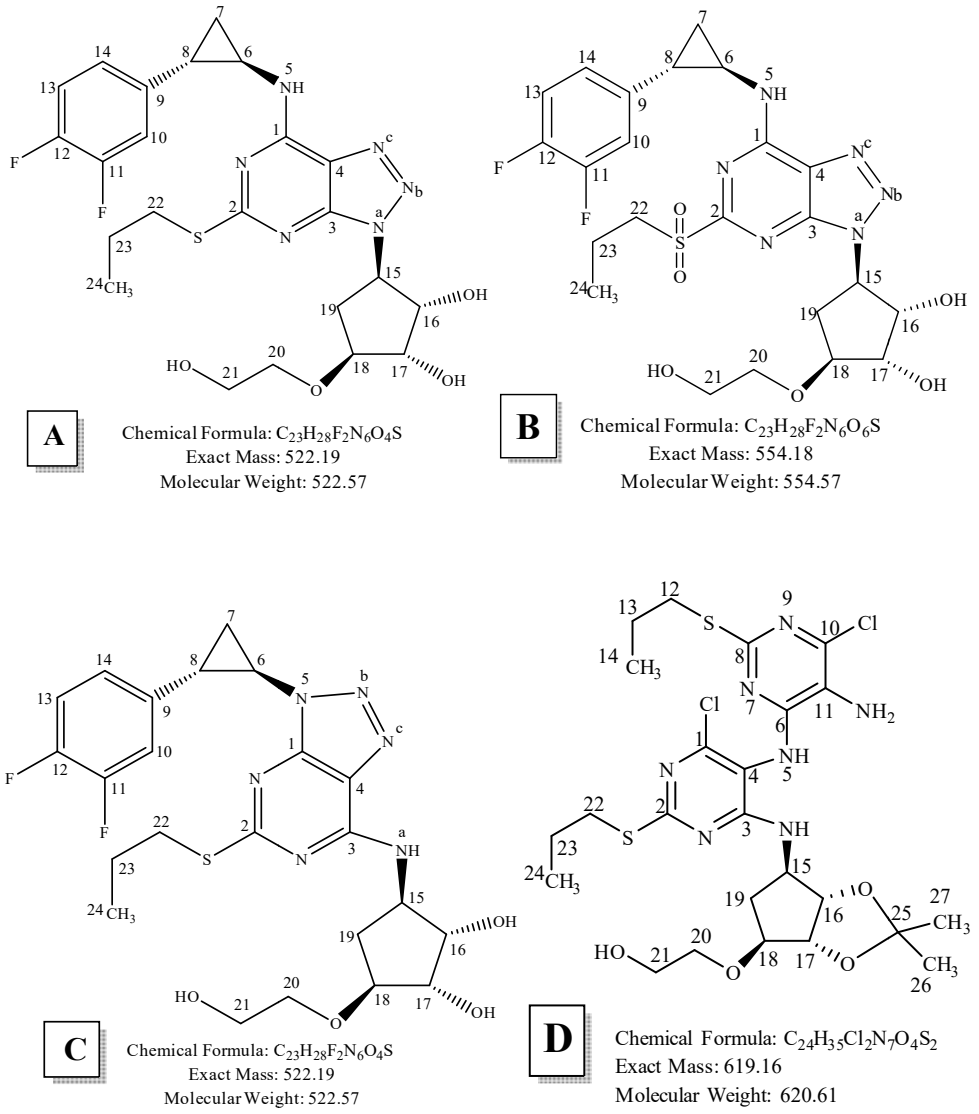

Fig-1:A) Structure of Ticagrelor drug substance, B) Structure of TIC-1 impurity, C) Structure of TIC-2 impurity and D) Structure of TIC-3 impurity.

1.0 Introduction: Ticagrelor is indicated for the prevention of thrombotic events. Ticagrelor is a direct-acting and reversible P2Y12-adenosine diphosphate (ADP) receptor blocker. Its higher potency led to improved prognosis in acute coronary syndrome (ACS) patients. Adenosine is released in the plasma by endothelial cells and myocytes during ischemia, hypoxia, or oxidative stress. Most of the plasma adenosine is quickly taken up by red blood cells (RBC) through a facilitated diffusion transport system or converted into inosine by adenosine deaminase activity (ADA). An increase in APC may, therefore,

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result from the inhibition of RBC uptake, reduced ADA, or both. Increased APC impacts the cardiovascular system by purinergic receptors, named A1, A2A, A2B, or A3 depending on their pharmacological properties.

During the analysis of laboratory batches of Ticagrelor drug substance three unknown impurities with area percentages of 0.1 to 0.4% were detected by a simple gradient HPLC method. To commercialize an active pharmaceutical ingredient (API), as per regulatory requirements, it is mandatory for the manufacturer to identify and characterize all the unknown impurities that are present in API at a level of even below 0.05%. in this context, a comprehensive study has been undertaken to identify and characterize this unknown impurity present in laboratory batches of Ticagrelor drug substance using spectroscopic and spectrometric techniques. During the literature survey of Ticagrelor, no reports were found regarding these unknown impurities' isolation and characterization. The study towards the identification and characterization of impurity in Ticagrelor was not reported in the literature to date, to the best of our knowledge, and this impurity profiling study will be of immense importance for process development chemists to understand the source of impurity during the synthesis of Ticagrelor.

Material and methods:

Materials and reagents: Ticagrelor API and impurity samples were obtained from the chemical research division, Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC-grade acetonitrile was purchased from Merck India Limited. De-ionized water was prepared using Millipore Milli-Q plus purification system. Analytical reagent grade ammonium acetate was purchased from Merck India Limited,and HPLC grade acetonitrilewas purchased from Rankem. Dimethyl sulfoxide-d_6 and deuterium oxide D_2O (for NMR) were from CIL. KBr was purchased from Merck.

Detection by chromatography: The HPLC studies were carried out on Agilent 1100 series quaternary pump with a degasser and as an autosampler. BetasilC18 column (250x4.6 mm. 5µm,) was used for chromatographic separation. The mobile phase consists of a mixture of buffer (0.01M;pH4.0 potassium dihydrogen phosphate) and Acetonitrile in the ratio of 70:30, and mobile phase B is a mixture

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of Acetonitrile and water in the ratio of 65:35(v/v) was used. The separation achieved with gradient program [T/A- 0.01/95, 3/95, 12/55, 30/55, 40/0.0, 75/0.0, 76/95, 85/95]. The flow rate was maintained at 0.8mL/min with UV detection at 210 nm. The column temperature was maintained at 30ºC.

Mass spectrometry: The LC-MS/MS studywas carried out on AB Sciex 4000-Q-trap spectrometer. The source voltage was kept at 4.0kV and the capillary temperature at 250°C. Nitrogen was used as both sheath and auxiliary gas. The mass range was kept at m/z 70-1200 and 3sec scan time under positive polarity with electrospray ionization. The LC part consisted of an Agilent 1100 series quaternary pump with a degasser and anautosampler. AnX-Bridge phenyl column (150x4.6 mm. 3.5µm,) was used for chromatographic separation. The mobile phase consisted of a mixture of buffer (0.02M;pH6.0 ammonium acetate) and Acetonitrile in the ratio 95:5, and mobile phase B is a mixture of buffer and Acetonitrile in the ratio of 40:60(v/v) was used. The separation achieved with gradient program [T/A- 0.01/85, 5/85, 25/50, 45/50, 55/5, 70/5, 71/85, 80/85]. The flow rate was maintained at 1.0mL/min with UV detection at 240 nm. The column temperature was maintained at 35ºC.

HRMS DIP analysis:

The High-resolution mass spectrum of Ticagrelor impurities was recorded on Waters Synapt G2si time of flight (TOF) LC-HRMS system. The sample was introduced into the system through U-HPLC by bypassing the column. The ESI +ve ionization mass spectrum of Ticagrelor impurities displayed the sodium and potassium adducts.The proposed molecular formulae by the software werewithin1ppm deviation.

NMR: The NMR spectra of Ticagrelor and isolated impurities and synthesized compounds were recorded on Bruker Avance III 400MHz instrument at 25°Cand operating at 400MHz for ^{1}H NMR and 100MHz for ^{13}C NMR using DMSO-d_6 as solvent. The ^{1}H and chemical shift values were reported on δ scale in ppm, relative to DMSO-d_6 (δ= 2.5ppm) and in the ^{13}C chemical shift values were reported relative to DMSO-d_6 (δ=39.5 ppm). DEPT, ^{15}N HSQC, HMBC,

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HSQC,and D_2O Exchange, experiments were also carried out at 25°C using the same instrument.

IR: The IR spectrum of Ticagrelor and isolated impurities were recorded on a PerkinElmer (spectrum-one) FT-IR spectrophotometer over the range 4000 to 400 cm-1 by pressing pallet method using KBr power dispersion.

Results and Discussion:

Detection of impurity by HPLC and LCMS

For identification of the impurities and their molecular weights LC-MS method was utilized described in section 2.3 and analyzed. The impurities and Ticagrelor are well separated in the chromatograph. The relative retention time (RRT) for impurities respective to Ticagrelor are 0.6, 1.1, and 0.4 for TIC-1, TIC-2, and TIC-3 impurities respectively. The protonated molecular mass obtained from mass spectrometer are m/z = 523 for Ticagrelor, m/z = 555 for TIC-1, m/z = 523 for TIC-2 and m/z = 621 for TIC-3 impurity. The impurities TIC-1, TIC-2, and TIC-3 do not match with any reported impurities, so these are inferred to be new and have been taken considerable attention for their structural characterization.

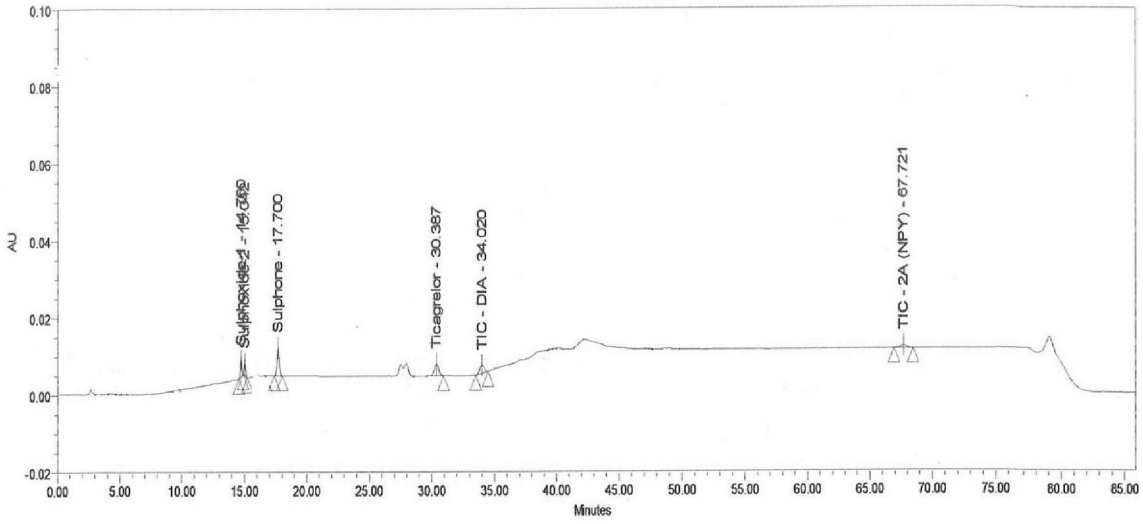

Fig-2:HPLC chromatogram for impurity standards spiked in diluent.

Elemental composition by HRMS:

The impurity samples were subjected tohigh-resolution mass spectrometry analysis to confirm the chemical formula. Based on the isotopic ratio system has

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predicted the elemental composition of these impurities. TIC-1 was observed as a sodium adduct of the molecule [M+Na] at m/z = 577.1655 and dimmer [2M+Na] at m/z = 1131.3469. The elemental composition for m/z = 577.1655 with -0.3 ppm error is $C_{23}H_{28}N_6O_6F_2NaS$. TIC-2 was observed as sodium and potassium adduct of the molecule [M+Na] at m/z = 545.1757 and [M+K] at m/z = 561.1504. The elemental composition for m/z = 545.1757 with -0.4 ppm error is $C_{23}H_{28}N_6O_4F_2NaS$. TIC-3 was observed as sodium and potassium adduct of the molecule [M+Na] at m/z = 642.1465 and [M+K] at m/z = 658.1262. The isotopic pattern presented in the mass spectrum revealsthe presence of two chlorine atoms in the molecule. The elemental composition for m/z = 642.1465 with -0.3 ppm error is $C_{24}H_{35}N_7O_4NaS_2Cl_2$.

Table-1: Mass, MS/MS and IR assignments for Ticagrelor and impurities

Products	RRT	M.W.	Mass daughter ions in (+) mode	IR absorption band (cm^{-1})
Ticagrelor API	1.00	522.57	495, 335, 293, 153	3392 (O-H str), 3293 (N-H str), 2964, 2932 (C-H str), 1625, 1588 (C=C str), 1275 (C-N str), 1196 (C-F str), 1050 (C-O- str)
TIC-1	0.58	554.60	511, 395, 289, 153	3392 (O-H str), 3293 (N-H str), 2964, 2932 (C-H str), 1625, 1588 (C=C str), 1275 (C-N str), 1196 (C-F str), 1050 (C-O- str)
TIC-2	1.11	522.57	495, 335, 293, 153	3392 (O-H str), 3293 (N-H str), 2964, 2932 (C-H str), 1625, 1588 (C=C str), 1275 (C-N str), 1196 (C-F str), 1050 (C-O- str)
TIC-3	2.22	620.60	562, 343, 299, 246	3392 (O-H str), 3293 (N-H str), 2964, 2932 (C-H str), 1625, 1588 (C=C str), 1275 (C-N str), 1109 (C-Cl str), 1050 (C-O- str)

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Structural elucidation of impurity

The isolated impurities of Ticagrelor and Ticagrelor API were subjected to spectroscopic analysis such as 1D NMR (^{1}H, ^{13}C, and DEPT), 2D NMR (HSQC, HMBC, COSY, and ^{15}N HSQC, ^{15}N HMBC), HRMS and IR (section 2.7). The numbering was given to all impurities as shown in Fig.1. The NMR (^{1}H, ^{13}C, and DEPT) data of Ticagrelor API and TIC imp-1, TIC imp-2, and TIC imp-3are presented in Tables 2 and 3 respectively. The HSQC and HMBC assignments of Ticagrelor API and TIC imp-1, TIC imp-2, and TIC imp-3are presented in Tables 4 and 5 respectively.

TIC-1 impurity has consisting comparable chemical shift values whencompared with Ticagrelor NMR spectral data (Tables 2 and 4). In the proton NMR spectrum of the TIC-1 impurity n-propylene chain, H-22, and H-23 are shifted downfield, 3.30 ppm and 1.65 ppm from 2.86 ppm, 2.95 ppm and 1.51 ppm (observed in Ticagrelor) respectively. In the carbon NMR spectrum of TIC-1 impurity C-22, the carbon attached to the 'S' atom was observed at 51.8 ppm in contrast to Ticagrelor (32.3 ppm). In the HMBC spectrum, the protons resonated at 3.3ppm are coupled with C-23 (2JCH) C-24 (3JCH), and C-2 (3JCH) carbons, the protons at 1.62ppm are coupled with C-22 (2JCH) and C-24 (2JCH) and the proton at 0.83 are coupled with C-22 (3JCH) and C23 (2JCH) carbons. We accomplished from observations that oxidation occurred at sulfur.

In comparison with the parent drug, TIC-2 impurity retains all proton and carbon signals. The chemical shifts for some protons at position number 1, 3, 6, 15, and N5 are different when compared with Ticagrelor. H-15 was shifted to up-field at 4.47ppm as pentate in TIC-2 impurity, whereas in the parent drug, it was observed at 4.96ppm as a quartet. This pentate changes to a quartet in the D_2O exchange experiment. This confirms the presence of an exchangeable proton in the vicinity of H-15. The H-6 proton chemical shift value was stimulated to downfield at 4.08ppm from 3.16ppm presented in Ticagrelor. In the COSY experiment (^{1}H-^{1}H correlation) it was revealed that the exchangeable proton at

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9.03ppm was coupled with the H-15 proton at 4.47, whereas in the parent drug, it (5-H, at 9.36ppm) was coupled with H-6 proton at 3.16ppm. In the ^{13}C NMR spectrum of TIC-2 impurity, the carbon C-15 and C-6 chemical shifts were observed at 54ppm and 36.9; these were at 60ppm and 34ppm in Ticagrelor. It reveals that C-15 was more shielded and C-6 was more de-shielded when compared with Ticagrelor. This supports the formation of the N5-Nb bond, as this explains the shielding and de-shielding of C-15 and C-6 carbon.

To confirm this compared the ^{15}N-HSQC and ^{15}N-HMBC data of TIC-2 impurity with that of Ticagrelor (refer to table no 6), it was observed that these compounds have only one exchangeable NH proton at 9.03ppm (position N-a) and 9.36ppm (position N-5) respectively in TIC-2 impurity and Ticagrelor. In the ^{15}N-HMBC data of TIC-2 impurity the Na (at 110ppm) and N5 (at 230ppm) shows coupling with H-19 at 1.4ppm (3JNH) and cyclo-propane ring protons (H-6, H-7, H-8) respectively. However, in Ticagrelor ^{15}N HMBC data it was observed that N-5 (at 110) couples with H-7 proton at 1.57ppm and N-a (at 230ppm) couples with H-19 at 2.03ppm and H-16at 4.56ppm protons.

TIC-3 impurity formed in the second stage of the synthetic process, in proton NMR spectrum of TIC-3 impurity chemical shifts due to difluoro benzene and cyclopropane are observed in trace level (indicates impure nature of TIC-3 impurity sample). The structures were elucidated considering all the data generated.

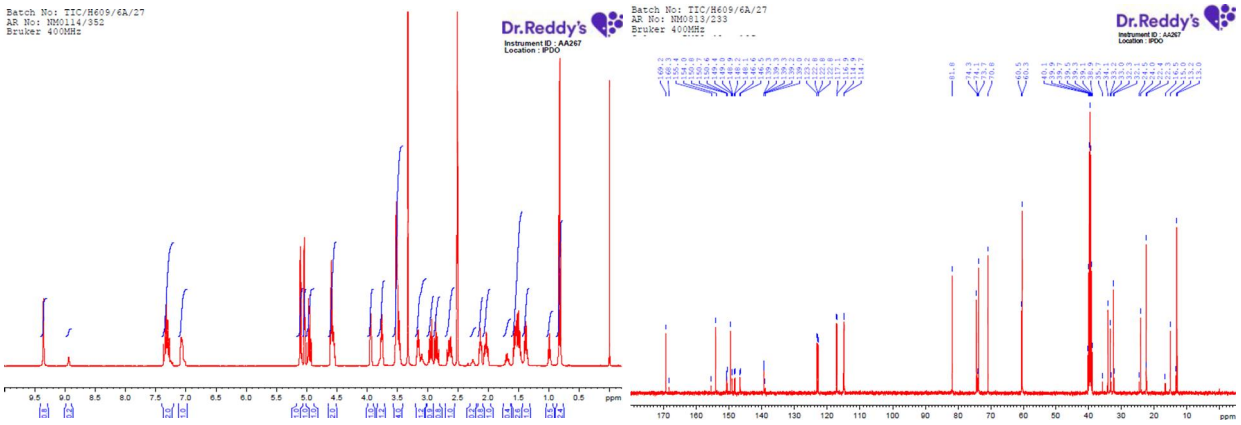

Fig-A1: ^{1}H and ^{13}C NMR NMR spectrum of Ticagrelor API in DMSO-d_6

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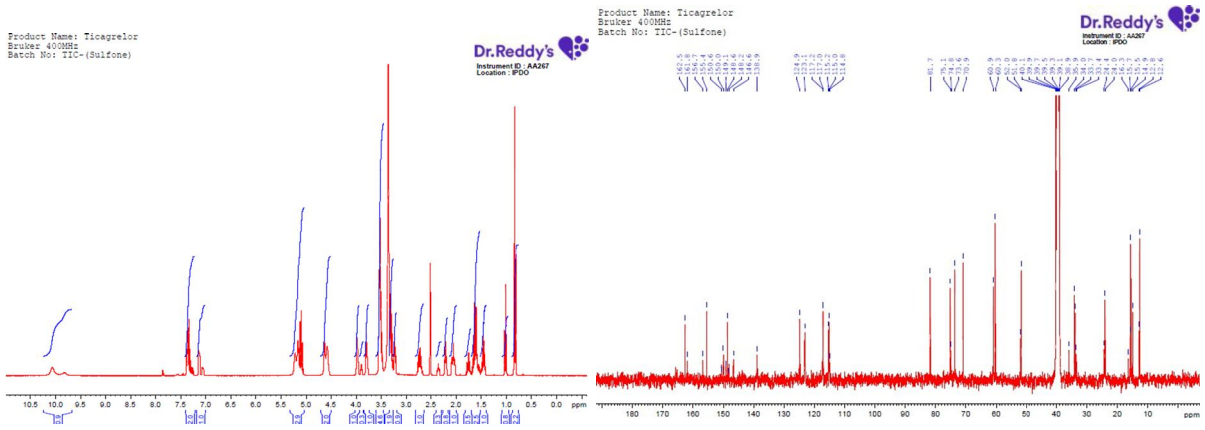

Fig-B1: ^{1}H and ^{13}C NMR spectrum of TIC-1 impurity in DMSO-d_6

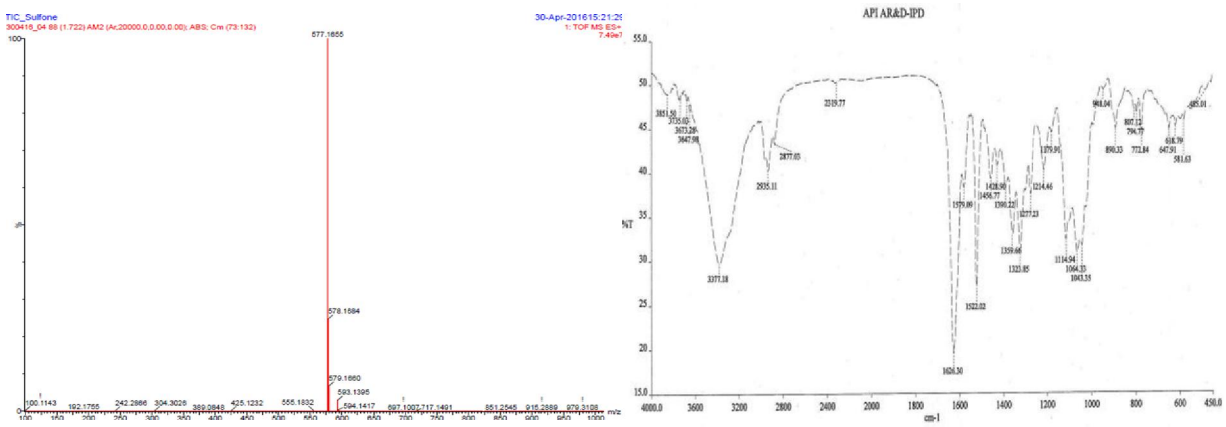

Fig-B2: HRMS +ve mass spectrum and FT-IR spectrum of TIC-1 impurity

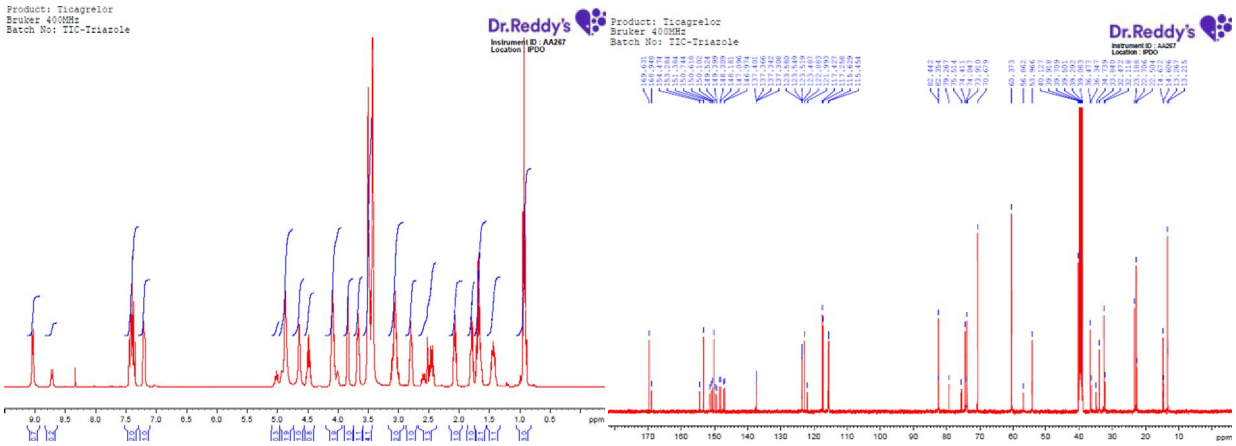

Fig-C1: ^{1}H and ^{13}C NMR spectrum of TIC-2 impurity in DMSO-d_6

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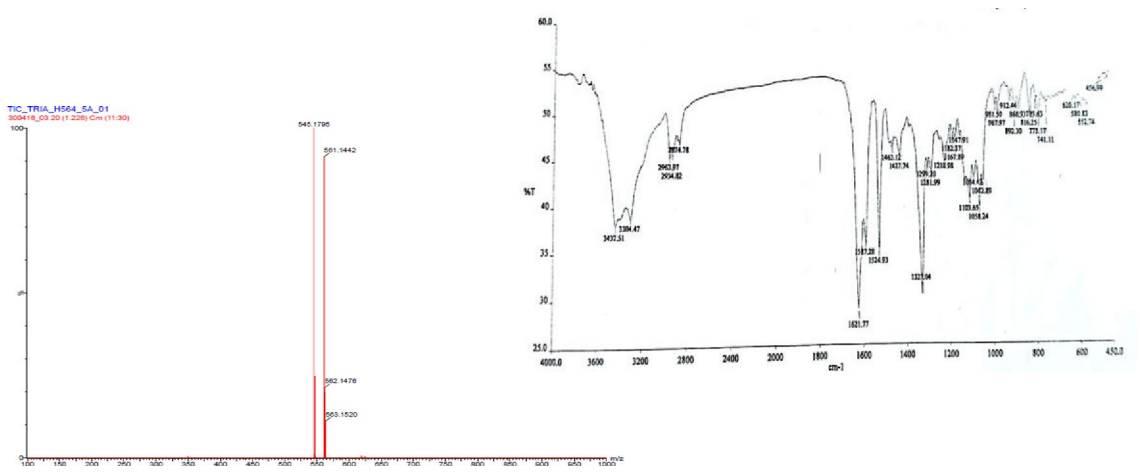

Fig-C2: HRMS +ve mass spectrum and FT-IR spectrumof TIC-2 impurity

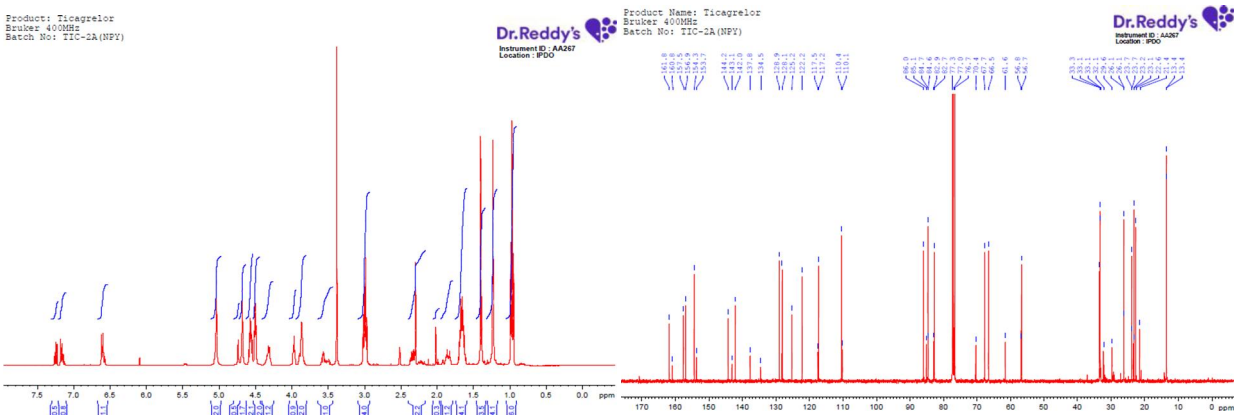

Fig-D1: ^{1}H and $^{13}CNMR$ spectrum of TIC-3 impurity in DMSO-d_6

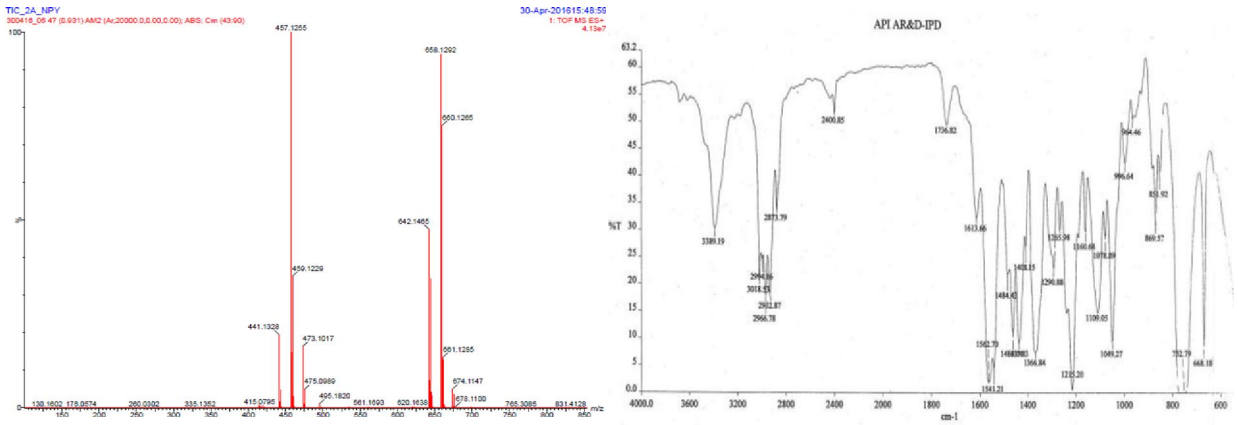

Fig-D7: HRMS +ve mass spectrum and FT-IR spectrum of TIC-3 impurity

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Table-2: Structural assignments for Ticagrelor and TIC-1 impurity

	Ticagrelor API			TIC-1 Sulfone imp		
Position[#]	δ (ppm)^{1}H	^{13}C	DEPT	δ (ppm)^{1}H	^{13}C	DEPT
1	-	154.0	-	-	155.4	-
2	-	169.2	-	-	162.5	-
3	-	149.4	-	-	149.4	-
4	-	123.2	-	-	124.9	-
5	9.36 (NH, d 4.06)	-	-	10.06 (NH, br)	-	-
6	3.16 (1H, m)	34.1	CH	3.23 (1H, m)	34.0	CH
7	1.37(Ha, dd 6.014.0)	15.0	CH_2	1.46 (Ha, m)	14.9	CH_2
	1.57 (Hb, m)	-	-	1.58 (Hb, m)	-	-
8	2.13 (1H, m)	24.0	CH	2.21 (1H, m)	24.0	CH
9	-	139.2	-	-	138.9	-
10	7.30 (1H, m)	114.8, d, 17.2*	CH	7.36 (1H, m)	115.1	CH
11	-	149.3 dd, 243.0, 12.5*	-	-	149.4	-
12	-	147.7 dd, 241.5,12.5*	-	-	147.8	-
13	7.35 (1H, m)	117.0, d, 16.6*	CH	7.36 (1H, m)	117.1	CH
14	7.08 (1H, m)	122.7, d, 5.8*	CH	7.14 (1H, m)	123.1	CH
15	4.96 (1H, q 9.2)	60.5	CH	5.11 (1H, q 9.2)	60.9	CH
16	4.56 (1H, dd 5.2 8.8)	74.3	CH	4.58 (1H, m)	75.1	CH
16OH	5.11 (OH, br)					
17	3.95 (1H, m)	73.7	CH	3.98 (1H, m)	73.6	CH
17OH	5.04 (OH, br)					
18	3.76 (1H, m)	81.8	CH	3.80 (1H, m)	81.7	CH
19	2.03 (Ha, m)	33.2	CH_2	2.06 (Ha, m)	33.7	CH_2
	2.64 (Hb, m)	-	-	2.72 (Hb, m)	-	-
20	3.50 (2H, m)	70.8	CH_2	3.52 (2H, m)	70.9	CH_2
21	3.53 (2H, m)	60.3	CH_2	3.52 (2H, m)	60.3	CH_2
21OH	4.56 (OH, br)					
22	2.86 (Ha, m)	32.3	CH_2	3.30 (2H, m)	51.8	CH_2
	2.95 (Hb, m)	-	-	-	-	-
23	1.51 (2H, m)	22.3	CH_2	1.62 (2H, m)	15.5	CH_2
24	0.82 (3H, t 7.2)	12.9	CH_3	0.83 (3H, t 7.2)	12.6	CH_3

[#]Refer to the structural formula in Fig-1 for numbering
p-pentate, m-multiplet, br-broad.
*^{19}F-^{13}C coupling

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Table-3: Structural assignments for TIC-2and TIC-3 impurity

	TIC-2 Triazole imp			TIC-3 NPY imp		
Position[#]	δ (ppm)^{1}H	^{13}C	DEPT	δ (ppm)^{1}H	^{13}C	DEPT
1	-	150.6	-	-	138.8	-
2	-	169.6	-	-	152.3	-
3	-	153.3	-	-	156.0	-
4	-	123.5	-	-	119.7	-
5	-	-	-	4.7 (NH, m)	-	-
6	4.08 (1H, m)	36.5	CH	-	157.5	-
7	1.80 (Ha, m)	14.6	CH_2	-	-	-
	2.06 (Hb, m)	-	-	-	-	-
8	2.81 (1H, m)	22.7	CH	-	153.4	-
9	-	137.4	-	-	-	-
10	7.40 (1H, m)	115.4, d, 20*	CH	-	139.3	-
11	-	149.4 dd, 245.0, 10.0*	-	-	123.6	-
11NH_2	-	-	-	5.0 (NH_2, br)	-	-
12	-	148.7 dd, 245.0,10.0*	-	3.00 (2H, m)	33.3	CH_2
13	7.40 (1H, m)	117.3, d, 20*	CH	1.67 (2H, m)	23.1	CH_2
14	7.2 (1H, m)	123.5	CH	0.96 (3H, m)	13.4	CH_3
15	4.47 (1H, p 9.2)	54.0	CH	4.32 (1H, m)	56.7	CH
15NH	9.03 (NH, d 4.0)	-	-	6.60 (NH,m)	-	-
16	4.08 (1H, m)	74.4	CH	4.57 (1H, m)	84.7	CH
17	3.95 (1H, m)	73.9	CH	4.57 (1H, m)	86.0	CH
18	3.76 (1H, m)	74.0	CH	3.97 (1H, m)	82.8	CH
19	1.40 (Ha, m)	34.7	CH_2	1.86 (Ha, m)	32.1	CH_2
	2.40 (Hb, m)	-	-	2.34 (Hb, m)	-	-
20	3.42 (2H, m)	70.7	CH_2	4.50 (2H, m)	66.8	CH_2
21	3.35 (2H, m)	60.4	CH_2	3.87 (2H, m)	67.7	CH_2
22	3.08 (2H, m)	32.5	CH_2	3.00 (2H, m)	33.1	CH_2
23	1.65 (2H, m)	22.7	CH_2	1.67 (2H, m)	22.6	CH_2
24	0.90 (3H, t 9.2)	13.3	CH_3	0.96 (3H, m)	13.4	CH_3
25	-	-	-	-	110.4	-
26	-	-	-	1.23 (s)	23.7	CH_3
27	-	-	-	1.4 (s)	26.1	CH_3

[#]Refer to the structural formula in Fig-1 for numbering
p-pentate, m-multiplet, br-broad.
*^{19}F-^{13}C coupling

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Table-4: HSQC and HMBC correlation data of Ticagrelor and TIC-1 Impurity

	Ticagrelor API		TIC-1 Sulfone imp	
Position[#]	HSQC(H-C)	HMBC (C-H)	HSQC(H-C)	HMBC (C-H)
1	-	154.0 - 9.36(5-NH)	-	155.4
2	-	169.2 - 2.86, 2.95 (22-H)	-	162.5
3	-	149.4 - 4.96 (15-H)	-	149.4 - 5.11 (15-H)
4	-	123.2	-	124.9
5	-	-	-	-
6	3.16 - 34.1	34.1 - 1.57 (7-Hb)	3.23 - 34.0	34.0 - 1.58 (7-Hb)
7	1.37 - 15.0 1.57 - 15.0	15.0 -	1.46 - 14.9 1.58 - 14.9	14.9 -
8	2.13 – 24.0	24.0 - 1.37 (7-Ha)	2.21 - 24.0	24.0 - 1.46 (7-Ha)
9	-	139.2 - 1.37, 1.57 (7-H), 7.35 (13-H)	-	138.9 - 1.46, 1.58 (7-H), 7.36 (13-H)
10	7.30 - 114.8	114.8 - 7.08 (14-H)	7.36 115.1	115.1 - 7.14 (14-H)
11	-	149.3 - 7.35 (13-H)	-	149.4 - 7.36 (13-H)
12	-	147.7-7.30 (10-H), 7.08 (14-H)	-	147.8 - 7.36 (10-H), 7.14 (14-H)
13	7.35 - 117.0	117.0	7.36 - 117.1	117.1
14	7.08 - 122.7	122.7 - 7.30 (10-H)	7.14 - 123.1	123.1 - 7.36 (10-H)
15	4.96 - 60.5	60.5 - 5.11 (16-OH)	5.11 - 60.9	60.9 - 2.06, 2.72 (19-H)
16	4.56 - 74.3	74.3 - 5.04 (17-OH), 2.64 (19-Hb)	4.58 - 75.1	75.1 5.15 (17-OH), 2.72 (19-Hb)
17	3.95 - 73.7	73.7 - 5.11 (16-OH), 4.96 (15-H)	3.98 - 73.6	73.6 - 5.21 (16-OH)
18	3.76 - 81.8	81.8 - 5.04 (17-OH)	3.80 - 81.7	81.7 - 2.06, 2.72 (19-H)
19	2.03 - 33.2 2.64 - 33.2	33.2 - 4.96 (15-H) -	2.06 - 33.7 2.72 - 33.7	33.7 - 5.11 (15-H) -
20	3.50 - 70.8	70.8 - 4.50 (21-OH), 3.53 (21-H)	3.52 - 70.9	70.9
21	3.53 - 60.3	60.3 - 4.50 (21-OH), 3.50 (20-H)	3.52 - 60.3	60.3 - 3.52 (20-H)
22	2.86 - 32.3 2.95 - 32.3	32.3 - 0.82 (24-H) -	3.30 - 51.8 -	51.8 - 0.83 (24-H), 1.62 (23-H)
23	1.51- 22.3	22.3 - 2.86, 2.95 (22-H), 0.82 (24-H)	1.62 - 15.5	15.5 - 0.83 (24-H), 3.30 (22-H)
24	0.82 - 12.9	12.9 - 2.86, 2.95 (22-H)	0.83 - 12.6	12.6 - 1.62 (23-H), 3.30 (22-H)

[#]Refer to the structural formula in Fig-1 for numbering

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Table-5:HSQC and HMBC correlation data of TIC-2 and TIC-3 Impurity

	TIC-2 Triazole imp		TIC-3 NPY imp	
Position[#]	HSQC (H-C)	HMBC (C-H)	HSQC (H-C)	HMBC (C-H)
1	-	150.6- 9.03 (15N-H)	-	138.8- 4.7 (5N-H)
2	-	169.6- 3.08 (22-H)	-	152.3- 3.0 (22-H)
3	-	153.3- 9.03 (15N-H)	-	156.0- 4.7 (5N-H), 6.6 (15N-H)
4	-	123.5	-	119.7- 6.6 (15N-H)
5	-	-	-	-
6	4.08 - 36.5	36.5- 1.8 (7-Ha), 2.06 (7-Hb), 2.81 (8-H)	-	157.5- 4.7 (5N-H), 5.0 (11N-H)
7	1.80 – 14.6	14.6	-	-
	2.06 – 14.6	-	-	-
8	2.81 – 22.7	22.7- 7.2 (14-H), 7.4 (10-H)	-	153.4- 3.0 (12-H)
9	-	137.4- 7.2 (14-H), 1.8 (7-Ha), 2.06 (7-Hb)	-	-
10	7.40 – 115.4	115.4- 2.81 (8-H)	-	139.3- 5.0 (11N-H)
11	-	149.4	-	123.6
12	-	148.7	3.00 – 33.1	33.3- 1.67 (13-H), 0.96 (14-H)
13	7.40 - 117.3	117.3	1.67 – 23.1	23.1- 3.0 (12-H), 0.96 (14-H)
14	7.20 - 123.5	123.5- 2.81 (8-H)	0.96 - 13.4	13.4- 3.0 (12-H), 1.67 (13-H)
15	4.47 - 54.0	54.0- 9.03 (15N-H), 4.08 (16-H), 1.40 (19-Ha), 2.40 (19-Hb)	4.32 - 56.7	56.7- 6.6 (15-NH), 4.57 (16 & 17-H), 3.97 (18-H), 1.86 (19-Ha), 2.34 (19-Hb)
16	4.08 - 74.4	74.4- 4.47 (15-H), 2.40 (19-Hb)	4.57 – 84.7	84.7- 4.23 (15-H), 3.97 (18-H), 1.86 (19-Ha), 2.34 (19-Hb)
17	3.95 - 73.9	73.9- 4.47 (15-H), 2.40 (19-Hb)	4.57 – 86.0	86.0- 4.23 (15-H), 3.97 (18-H), 1.86 (19-Ha), 2.34 (19-Hb)
18	3.76 - 74.0	74.0- 4.47 (15-H), 2.40 (19-Hb)	3.97 – 82.8	82.8- 4.23 (15-H), 1.86 (19-Ha), 2.34 (19-Hb), 4.50 (20-H)

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19	1.40 - 34.7 2.40 - 34.7	34.7- 4.47 (15-H) -	1.86 – 32.1 2.34 – 32.1	- -
20	3.42 - 70.7	70.7	4.50 - 66.8	66.8- 3.97 (18-H)
21	3.35 - 60.4	60.4- 3.42 (20-H)	3.87 – 67.7	67.7- 4.5 (20-H)
22	3.08 - 32.5	32.5- 1.65 (23-H), 0.90 (24-H)	3.00 – 33.1	33.1- 1.67 (23-H), 0.96 (24-H)
23	1.65 – 22.7	22.7- 3.08 (23-H)	1.67 - 22.6	22.6- 3.0 (22-H), 0.96 (24-H)
24	0.90 - 13.3	13.3- 3.08 (23-H), 1.65 (23-H)	0.96 - 13.4	13.4- 3.0 (22-H), 1.67 (23-H)
25	-	-	-	110.4- 1.23 (26-H), 1.4 (27-H)
26	-	-	1.23 – 23.7	23.7
27	-	-	1.4 - 26.1	26.1

[#]Refer to the structural formula in Fig-1 for numbering

Table-6: ^{15}N HSQC and ^{15}N HMBC correlation data of Ticagrelor API and TIC-2 Impurity

	TIC-3 NPY imp		TIC-2 Triazole imp	
Position[#]	HSQC (H-N)	HMBC (N-H)	HSQC (H-N)	HMBC (N-H)
N2’	-	220 - 9.36 (5N-H)	-	220 - 9.03 (Na-H)
N3’	-	-	-	-
Na	-	230 - 4.56 (16-H), 2.03 (19-Ha)	9.03-110	110 - 1.40 (19-Ha)
Nb	-	364.5 - 4.96 (15-H)	-	-
Nc	-	-	-	-
N5	9.36-110	110 - 1.57(7-Hb)	-	230 - 4.08 (6-H), 2.06 (7-Hb), 1.80 (7-Ha), 2.81 (8-H)

[#]Refer to the structural formula in Fig-1 for numbering

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Synthetic root for the formation of Impurities:

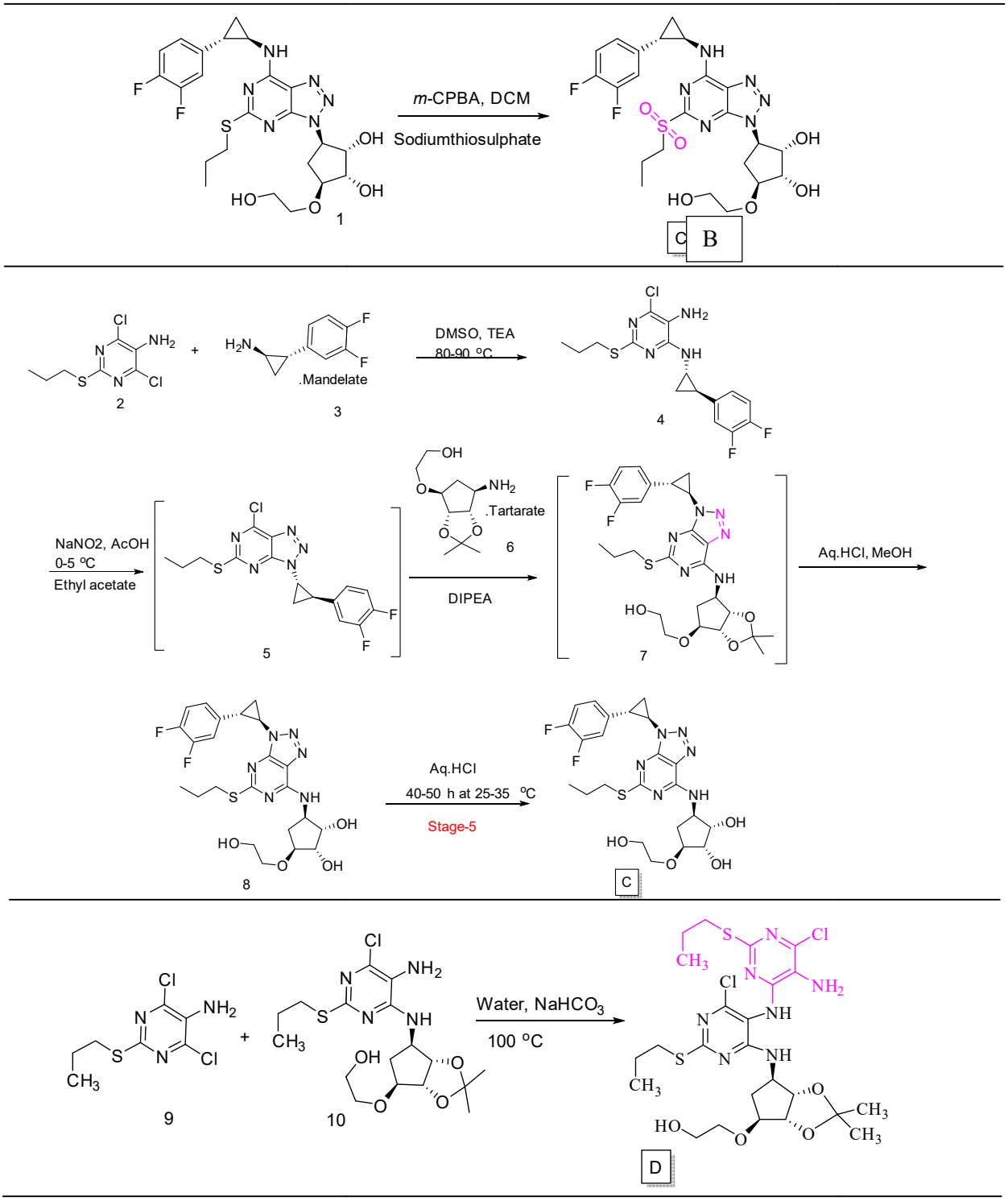

Fig-3: Synthetic routes to prepare **B**) Structure of TIC-1 impurity, **C**) Structure of TIC-2 impurity and **D**) structure of TIC-3 impurity

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Conclusion

Three new process-related impurities in the preparation of Ticagrelordrug substance were identifiedand the structures were elucidated by various techniques HRMS, MS, 1D NMR (^{1}H, ^{13}C, and DEPT), 2D NMR(HSQC, HMBC, and ^{15}N HSQC, ^{15}N HMBC) and IR.The proposed chemical structures of impurities were confirmed and identified the root of synthesis of these impurities. Based on this knowledge formation of impurities was controlled in the root of synthesis for the Ticagrelor drug substance and a pure compound was obtained.

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