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Application News

Liquid Chromatograph Nexera™ XS

Determination of Ticagrelor and its related impurities content from oral tablets as per proposed IP monograph UHPLC method

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User Benefits

- Shimadzu Nexera XS can be effectively used for assay and related substances test of Ticagrelor tablets as per the proposed IP monograph UHPLC method
- The Nexera XS easily meets with all the acceptance criteria as per the proposed IP monograph for Ticagrelor tablets

■ Introduction

Ticagrelor (see Fig. 1) is a pharmaceutical drug used for the prevention of stroke, heart attack and other events in people with acute coronary syndrome, meaning problems with blood supply in the coronary arteries. It acts as a platelet aggregation inhibitor by antagonising the $P2Y_{12}$ receptor.

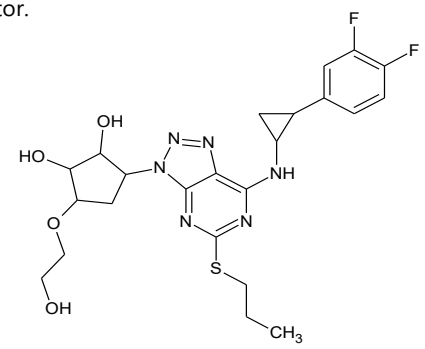

Fig. 1 Structure of Ticagrelor

The proposed Indian Pharmacopeia (IP) monograph defines an UHPLC (Ultra High-Performance Liquid Chromatography) method for high throughput fast analysis demanding a competent UHPLC system.

Here, we demonstrate the analysis of Ticagrelor tablets as per the proposed monograph in Indian Pharmacopeia using Shimadzu Nexera XS (see Fig. 2) fast LC system in compliance with system suitability requirements of the proposed monograph.

Nexera series

Key features- Analytical Intelligence

-Automated support functions utilizing digital technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.

-Allows a system to monitor and diagnose itself, handle any issues during data acquisition without user input, and automatically behave as if it were operated by an expert.

-Supports the acquisition of high quality, reproducible data regardless of an operator's skill level for both routine and demanding applications.

Fig. 2 Nexera™ XS system

■ Experimental

Chromatographic conditions, mobile phase preparations, standard and sample preparations were done in accordance with the proposed IP monograph for Ticagrelor tablets (see Table 1 for assay and Table 2 for related substances). System suitability parameters were also checked as per the requirements of IP monograph.

For Assay

Buffer solution preparation: A 0.2 percent w/v solution of monobasic ammonium phosphate. Adjusted to pH 2.5 with orthophosphoric acid (OPA).

Mobile phase preparation: A mixture of 70 volumes of methanol and 30 volumes of buffer solution.

Solvent mixture: 35 volumes of acetonitrile and 65 volumes of water.

Reference solution preparation: A 0.05 percent w/v solution of Ticagrelor RS in the solvent mixture (0.5 mg/mL).

Test solution preparation: Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Ticagrelor with 60 mL of the solvent mixture, with the aid of sonication and dilute to 100 mL with the solvent mixture (0.5 mg/mL).

Table 1 LC acquisition parameters for Assay

Column	: 150 mm × 4.6mm I.D., 1.8 µm USP packing L1
Oven temperature	: 55°C
Mobile Phase	: Methanol/Buffer = 7:3
Flow Rate	: 1.2 mL/min
Total Run Time	: 10.0 min
Injection Volume	: 5 µL
Autosampler Temperature	: 15°C
Detector wavelength	: 242 nm

For Related Substances

Buffer solution, Solvent mixture, and Test solution: Prepare as directed in the Assay.

Reference solution (a): A solution containing 0.05 percent w/v of Ticagrelor RS and 0.0001 percent w/v of Ticagrelor related compound B RS in the solvent mixture.

Reference solution (b): A 0.0001 percent w/v solution of Ticagrelor RS in the solvent mixture.

Reference solution (c): Dilute 5.0 mL of reference solution (b) to 10.0 mL with the solvent.

Table 2 LC acquisition parameters for Related Substances

Column	: 150m x 4.6 mm I.D., 1.8-µm USP packing L1
Oven temperature	: 55°C
Mobile Phase A	: Buffer/Water/Acetonitrile = 10:890:100
Mobile Phase B	: Buffer/Water/Acetonitrile = 10:290:700
Gradient program (B %)	: 0.0 min → 10.0 (%); 0.0-7.0 min → 10.0-65.0(%); 7.0-15.0 min → 65.0(%); 15.0-16.0 min → 65.0-100.0(%); 16.0-23.0 min → 100.0(%); 23.0-28.0 min → 100.0-10.0(%);
Flow Rate	: 1.0 mL/min
Total Run Time	: 35.0 min
Injection Volume	: 5 µL
Autosampler Temperature	: 15°C
Detector wavelength	: 242 nm

■ Results for Assay

The retention time of Ticagrelor in standard and sample solutions is found to be about 5.518 minutes (see Fig. 3). The tailing factor for peak due to Ticagrelor in the standard solution is well within the system suitability criteria of NMT 1.5 (see Table 3). The % relative standard deviation (RSD) for retention time and peak area for five replicates of standards solution complies with the acceptable criteria of RSD NMT 2.0% (see Table 3) Overlay of five replicates of standards is shown in Fig. 4.

Table 3 Ticagrelor Assay standard

Parameter	Observed	USP Criteria
%RSD Retention Time (n=5)	0.032	NMT 2.0
%RSD Area (n=5)	0.135	NMT 2.0
Tailing factor	1.048	NMT 1.5

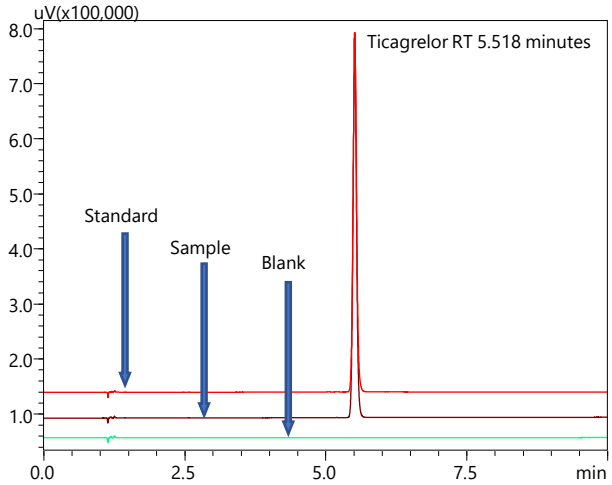

Fig. 3 Overlay of Assay Standard, sample and blank

Sample analysis for Assay

The results obtained for analysis of market sample (label claim 60 mg and 90 mg per tablet) using this method is found to be within the acceptance criteria of 90.0-110.0% (see Table 4).

Table 4 Content of Ticagrelor

Sample	Label claim (mg)	Content (%)	USP Criteria (%)
Preparation-1	60	98.5	90.0-110.0
Preparation-2		99.7	
Preparation-1	90	97.3	90.0-110.0
Preparation-2		96.9	

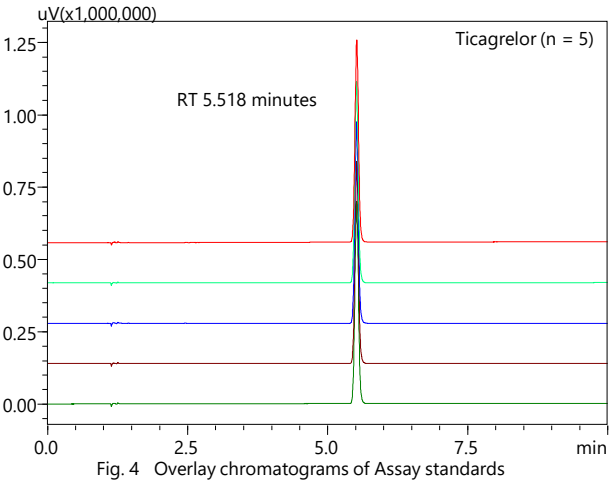

Fig. 4 Overlay chromatograms of Assay standards

■ Results for Related Substances

The retention time of Ticagrelor in standard and sample solutions is found to be about 12.9 minutes (see Fig. 5).

The observed relative retention time for Ticagrelor related compound B with regards to Ticagrelor is 1.074.

The resolution between the peaks due to Ticagrelor and Ticagrelor related compound B is found to be 6.336 which well within the acceptance criteria of NLT 1.5. in the chromatogram obtained with reference solution (a). (see Fig.6).

The observed %RSD for area and retention time for replicate injections of reference solution (b) is within the criteria of NMT 2.0 (see Table 5).

The signal-to-noise ratio for Ticagrelor in the chromatogram obtained with reference solution (c) is 14.79 which meets the criteria of NLT 10.

Table 5 Ticagrelor Related substances standard

Parameter	Observed	USP Criteria
%RSD Retention Time (n=5)	0.050	NMT 2.0
%RSD Area (n=5)	0.977	NMT 2.0

Sample analysis for Related substances

In the test chromatogram obtained with the test solution the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with the reference solution (b) i.e., NMT 0.2 percent and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) i,.e NMT 0.5 percent.(see Table 6 and Table 7). The typical chromatogram of sample showing overlay with blank, and placebo is shown in Fig. 7.

Table 6 Ticagrelor Related substance sample

Label claim	Main peak area %	Individual impurity area %	Sum of all Impurities area %
60 mg (n=2)	99.856	0.033	0.144
90 mg (n=2)	99.848	0.045	0.153
IP criteria	-	NMT 0.2	NMT 0.5

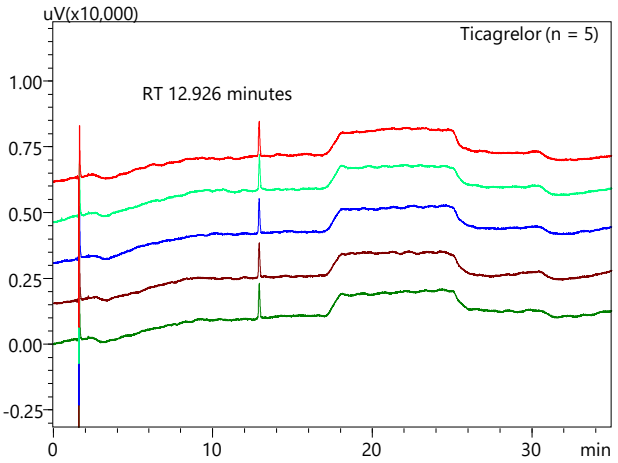

Fig. 5 Overlay chromatograms of Related substances standards

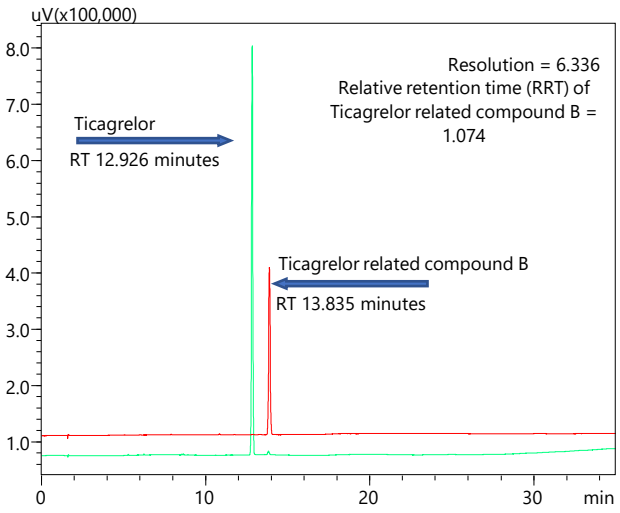

Fig. 6 Identification of Ticagrelor and Ticagrelor related compound B

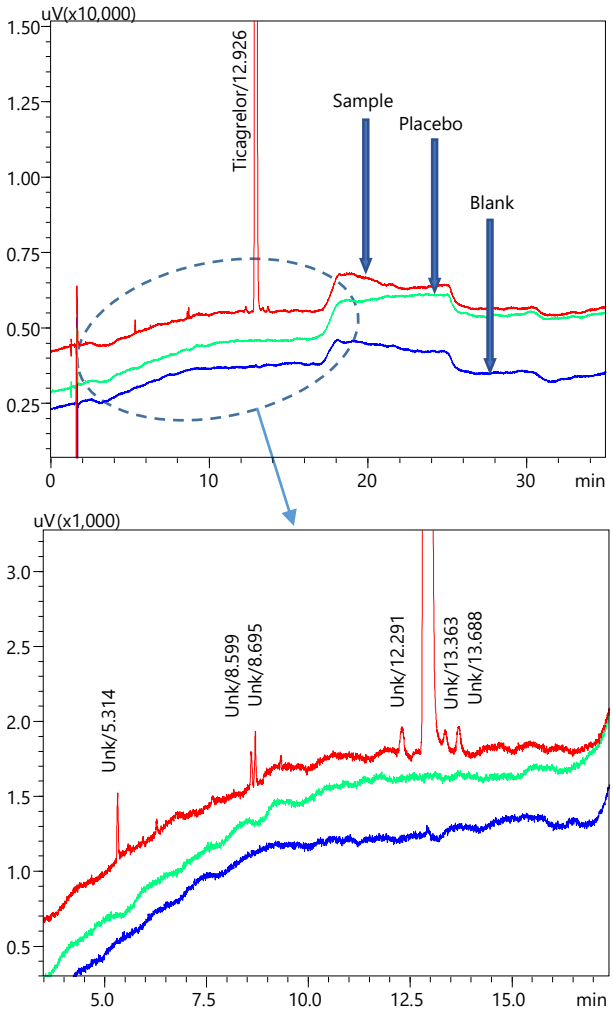

Fig. 7 Overlay of Related substances sample, placebo and blank

Table 7 Percent area for impurity peaks in samples

Peak#	Name	RRT	Sample 60mg-1	Sample 60mg-2	Sample 90mg-1	Sample 90mg-2
1	Unk	0.411	0.028	0.023	0.026	0.027
2	Unk	0.665	0.016	0.017	0.015	0.014
3	Unk	0.673	0.023	0.019	0.021	0.02
4	Unk	0.951	0.029	0.036	0.045	0.044
5	Ticagrelor	1.000	99.857	99.854	99.848	99.847
6	Unk	1.034	0.015	0.017	0.017	0.014
7	Unk	1.059	0.032	0.034	0.027	0.035

■ Conclusion

- This study successfully demonstrated the performance of Shimadzu Nexera series UHPLC system to determine the content of Ticagrelor and its related impurities from tablet formulation in conformity with the proposed IP monograph acceptance criteria and system suitability requirements.
- The reproducibility i.e., the relative standard deviation of retention time and area for standard are well within the acceptance criteria of NMT 2.0
- The tailing factor for peak due to Ticagrelor in both assay and related substances test standard solution is well within the system suitability criteria of NMT 1.5
- The resolution between Ticagrelor and Ticagrelor related compound B in the reference solution is found to be 6.336.
- The reproducibility i.e., the relative standard deviation of retention time and area for standard are well within the acceptance criteria of NMT 2.0
- The relative retention time (RRT) for Ticagrelor related compound B is found to be 1.070.
- The S/N ratio for Ticagrelor is also found to be within the acceptance criteria of NLT 10.
- The sample shows content of Ticagrelor and impurities within the permissible limits of IP.

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