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Method development and validation for estimation of ticagrelor in pharmaceutical dosage form by using RP-HPLC

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Abstract
The present study was conducted to develop a simple and precise analytical method for the estimation of ticagrelor in tablet formulation. Reverse Phase HPLC was used for method development and validation studies of ticagrelor. The optimum chromatographic conditions comprised of C18 column (Enable; 4.6 x 250mm, 5µm)) as the stationary phase and acetonitrile and 0.01M phosphate buffer in the ratio of 60:40 v/v as the mobile phase. The flow rate was 1 ml/min with detection at 230 nm and a run time of 10 min. The retention time of ticagrelor was 5.45 min. The linearity studies indicated that the range of the developed method was 50-150 µg/ml with a correlation coefficient of 0.999. The method was specific with a percent mean recovery was found to be 100.6 %. The % RSD in the precision studies was 0.069. The validated method was applied to conduct the assay of ticagrelor in tablets with a percent mean recovery of 98.98 %.Conclusion: The developed and validated RP-HPLC isocratic method was simple, accurate and precise as per the ICH guidelines. It was suitable for the analysis of ticagrelor in bulk and tablet formulation.

Keywords: ticagrelor, HPLC, anti-platelet

Introduction
Ticagrelor is a tablet dosage form which acts an antiplatelet [1] medicine, makes your blood flow through your veins more easily. It can help prevent blood clots and sold under the brand name Brilinta, is a medication used for the prevention of stroke, heart attack and others with acute coronary syndrome and coronary arteries. It is a CYP3A4 substrate [2] and weak CYP3A4 inhibitor. The drug was approved for use in the European Union by the European Commission on December 3, 2010. The drug was approved [3] by the US Food and Drug Administration on July 20, 2011. Chemically it is (1S,2S,3R,5S)-3-(7-{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl] amino}-5-(propylsulfanyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-5-(2-hydroxyethoxy) cyclopentane-1,2-diol and molecular weight is 522.56. The main metabolite [4] of this drug is AR-C124910XX is formed by CYP3A4 by dehydroxyethylation at position 5 of the cyclopentane ring in figure 1.

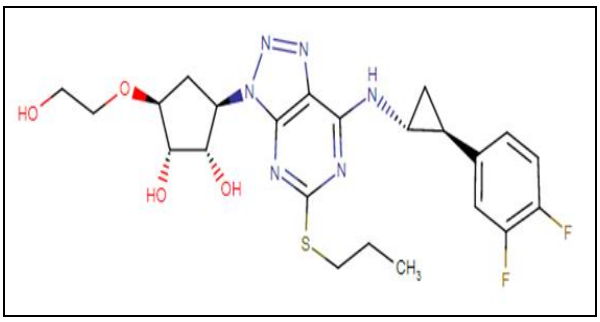

Fig 1: Chemical structure of Ticagrelor

Materials and Methods
Quantitative estimation of Ticagrelor was done by using an isocratic Shimadzu HPLC instrument on Enable C18 column (250 mm x 4.6 mm, 5µ). The Instrument is equipped with binary pump and variable wavelength PDA detector. A 20µL Hamilton syringe was used for injecting the samples. Data was analysed by using LC Solutions software. Shimadzu UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials. Ticagrelor pure drug was provided by Aurobindo Pharma Hyderabad., Optimised conditions: the mobile phase with Acetonitrile: 0.01M phosphate buffer (60:40 %v/v) was employed in isocratic mode at a flow rate of 1.0 mL/min. The run time was 8 min and 20µL of the sample was injected for every run into the column at wavelength of 230nm.
Chromatographic conditions: A mixture of acetonitrile and 0.01M phosphate buffer in the ratio of 60:40 %v/v was employed to be the most suitable mobile phase for identical chromatographic separation of Ticagrelor. The solvent mixture was filtered through 0.45 µ membrane filter and sonicated to dissolve it completely. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20µL and the column was maintained at ambient temperature and run time was set to be 10 min and detected of the drug was monitored at wavelength of 230nm and retention time was to be 5.45 min.

Preparation of standard stock solution
Accurately Weighed and transferred 90mg of ticagrelor working Standard into a 100ml clean dry volumetric flask, add 3/4th volume of HPLC graded mobile phase sonicated for 5 minutes and make up to the final volume with diluents to get concentrations like 0.9µg/ml of Ticagrelor.

Preparation of sample solution
Twenty tablets were accurately weighed, their mean weight was determined and they were mixed and finally powdered. Transfer the sample equivalent to 90mg of ticagrelor into a

100mL volumetric flask. Add about 85mL of diluents and sonicated to dissolve it completely and make volume up to the mark with diluents to concentration range 50-150 µg/mL. Mix well and filter through 0.45µ filter.

Linearity

The calibration graphs shows that the linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrate that the methods have adequate sensitivity to the concentrations of the analyte. Several aliquots of standard solution of Ticagrelor was taken in different 10 mL volumetric flasks and diluted up to the mark with diluents such that the final concentrations of Ticagrelor were in the range 50 to 150 µg/mL. Evaluation of the drug was performed with UV detector at 230 nm; peak area was recorded for all the peaks. The correlation coefficient value of Ticagrelor was 0.9976. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The data is tabulated in table 1& figure 2.

Table 1: Linearity values of Ticagrelor

Ticagrelor	
Concentration (µg/ml)	**Peak Area**
50	27287
70	46598
80	56931
100	76093
120	96186
130	106266
150	126359

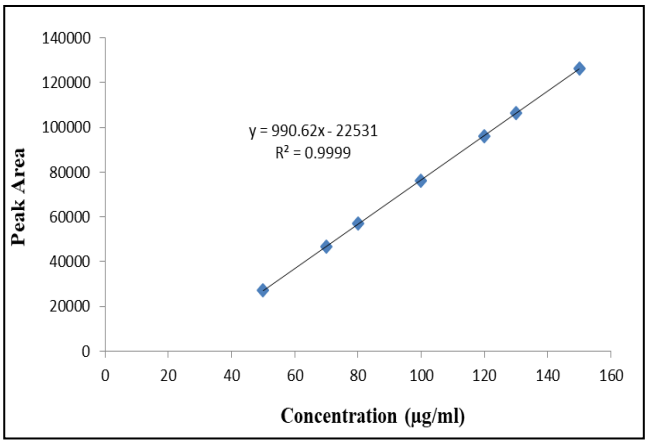

Fig 2: Calibration curve of Ticagrelor

System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (80, 100 and 120 µg/ml) and the percentage recoveries were calculated. The data is tabulated in table 2& figure 3, 4 & 5.

Table 2: Accuracy data of Ticagrelor

% level	Amount added (µg/ml)	Amount recovered (µg/ml)	Percentage of recovery	Percentage of Mean recovery
80	80	80.0	100.0	100.09
	80	80.12	100.15	
	80	80.1	100.13	
100	100	100.12	100.12	100.17
	100	100.22	100.22	
	100	100.17	100.17	
120	120	121.06	100.88	101.56
	120	122.12	101.76	
	120	122.43	102.03	

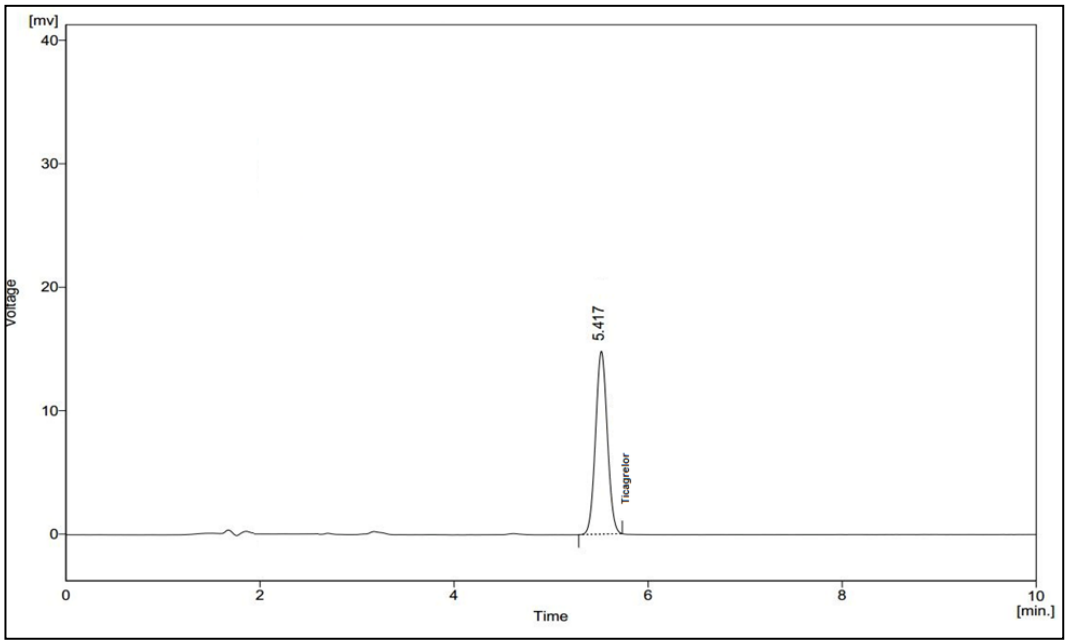

Fig 3: Accuracy 50% chromatogram of Ticagrelor

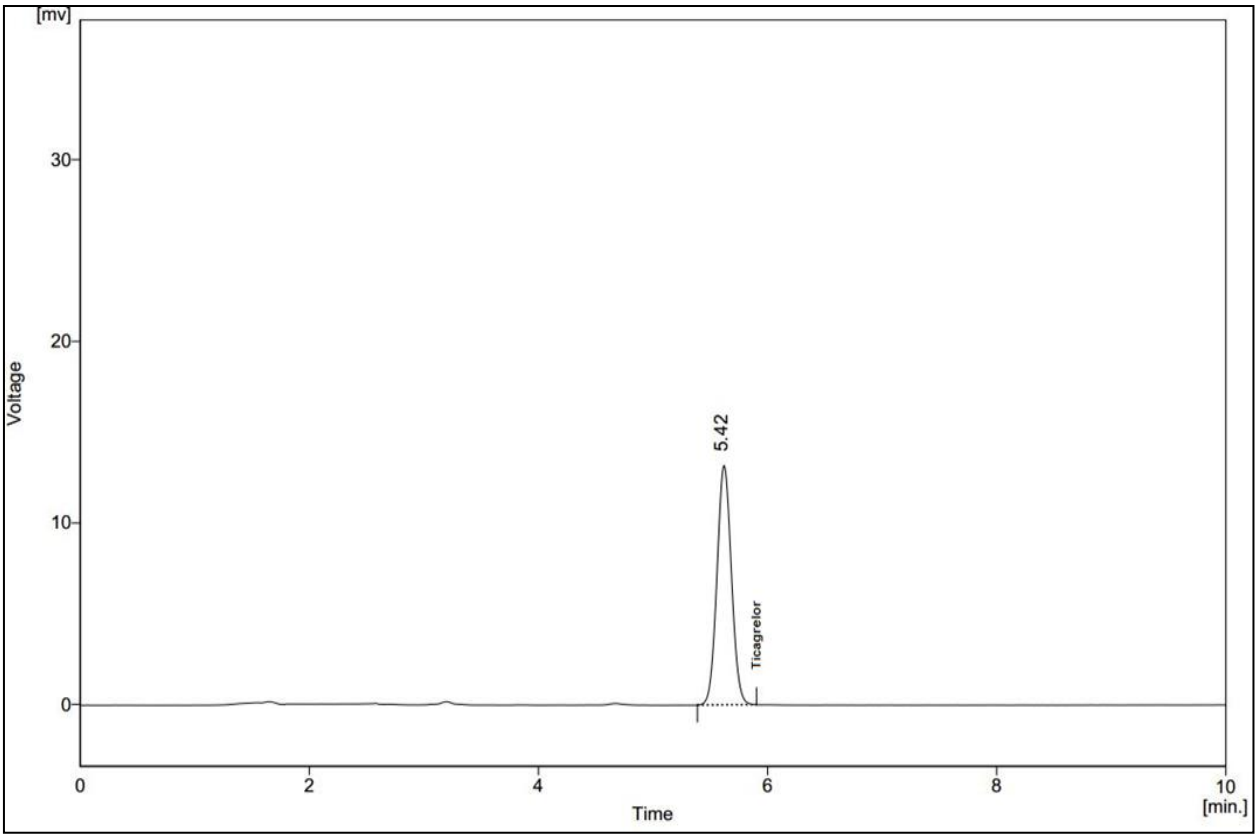

Fig 4: Accuracy 100% chromatogram of Ticagrelor

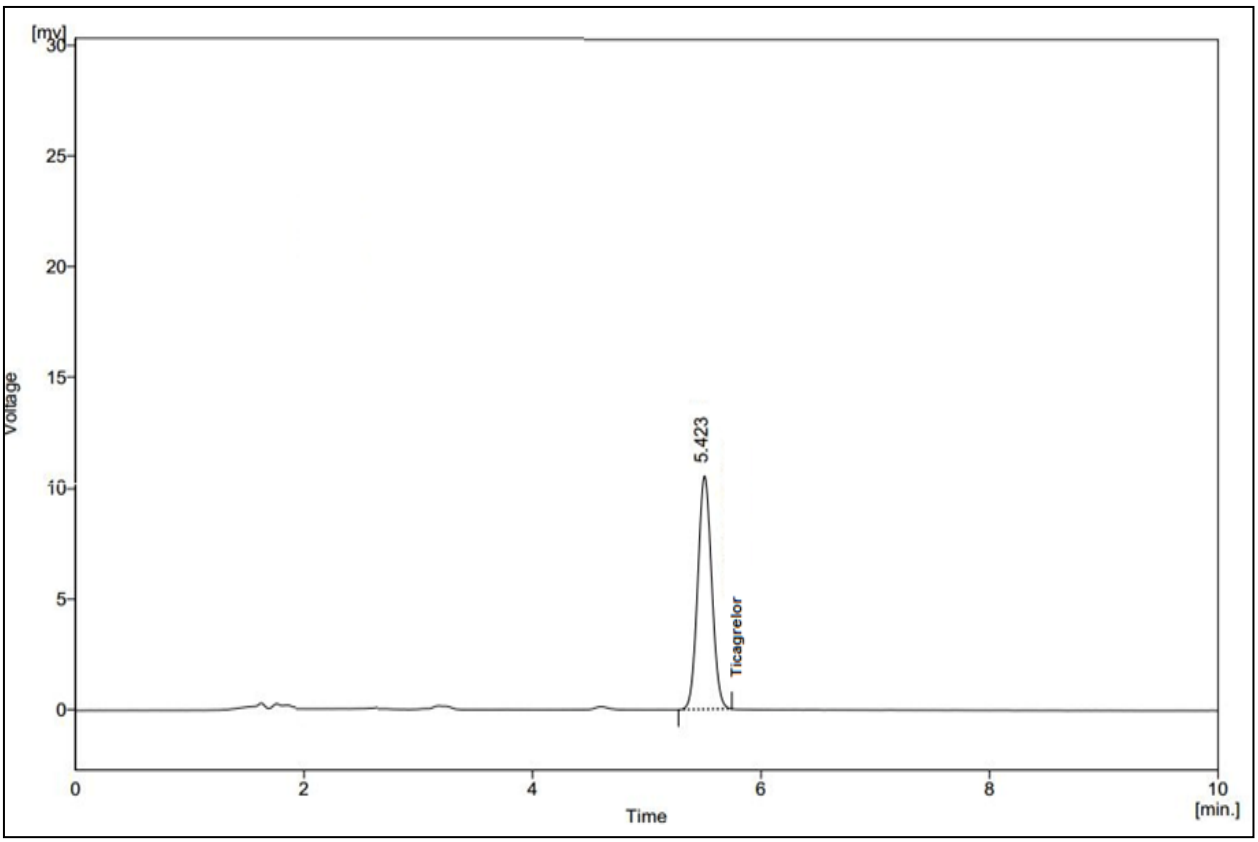

Fig 5: Accuracy 150% chromatogram of Ticagrelor

Precision

The precision of the method was determined by intra and inter precision studies. This was evaluated by injecting three independent sample preparations of ticagrelor from a single Formulation at three different concentration levels on the same day (Intraday) and on three different days (Inter day). The % RSD was then calculated. The data is represented in table 3 & 4 and figure 6 & 7.

Table 3: Precision (Intraday) values of Ticagrelor

Inj. Number	Area of Ticagrelor
1	75985
2	75036
3	75498
4	75268
5	75154
6	75167
Mean	75388
SD	347.08
%RSD	0.26

Table 4: Intermediate precision of Ticagrelor

Inj. Number	Area of Ticagrelor
1	75985
2	75036
3	75958
4	75268
5	75154
6	75280
Mean	75447
SD	415.99
%RSD	0.058

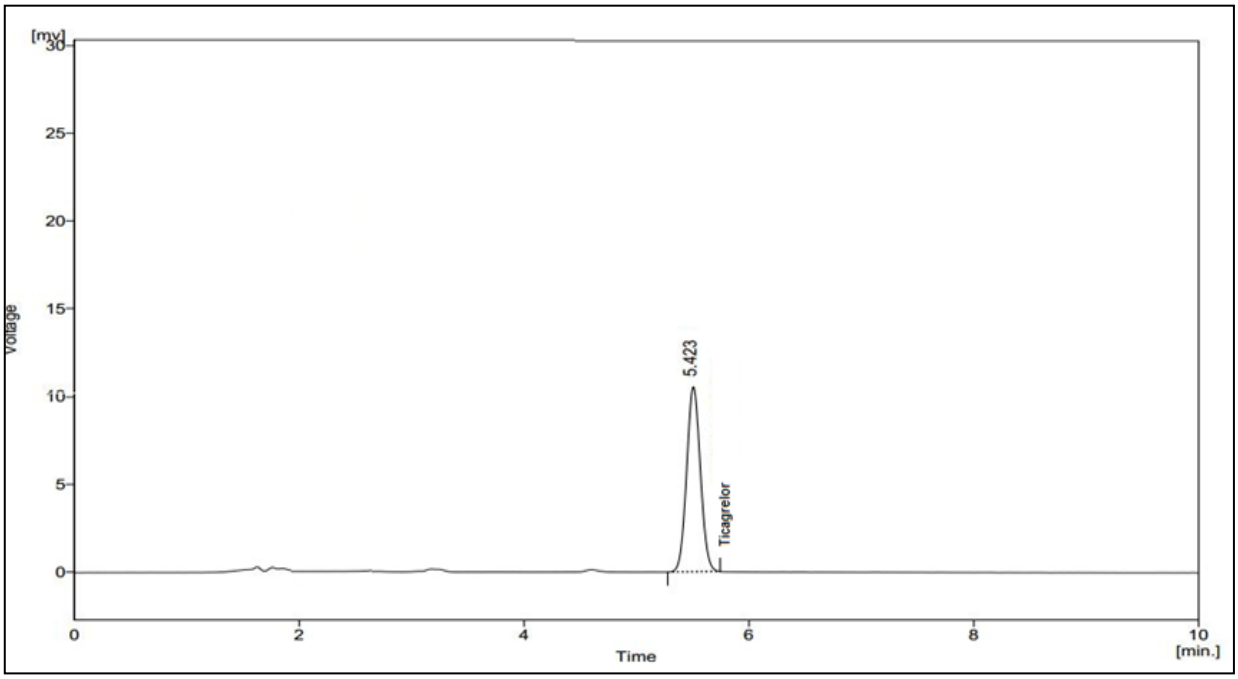

Fig 6: Repeatability of Ticagrelor

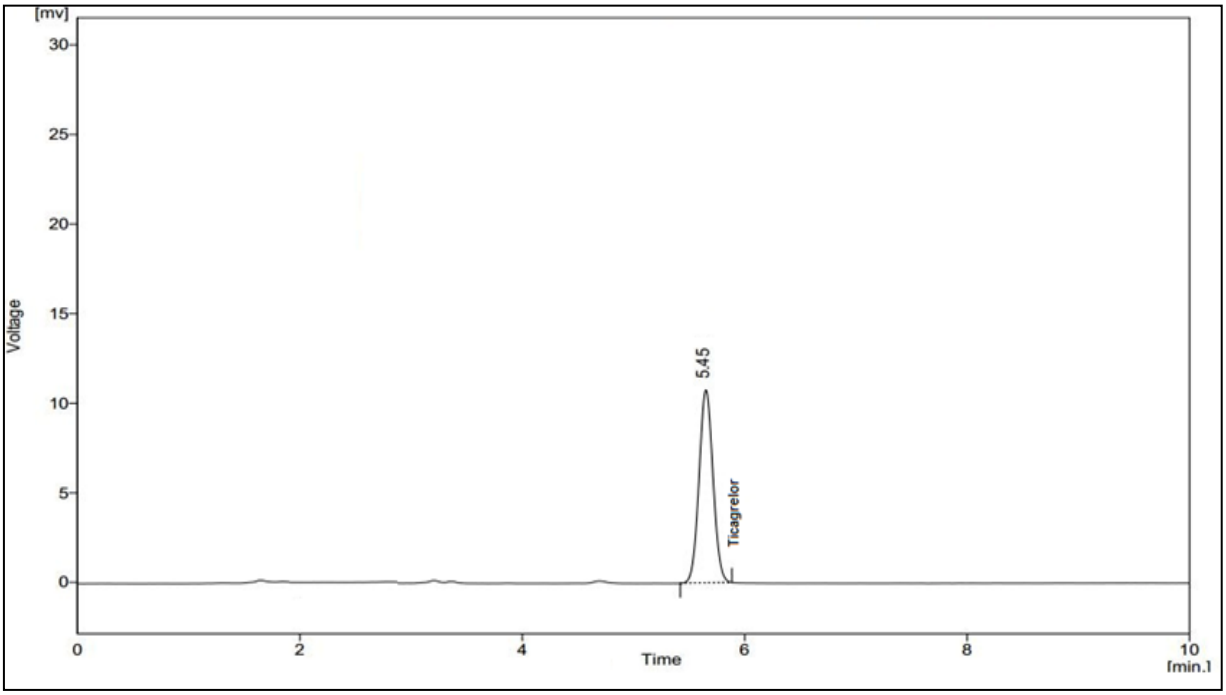

Fig 7: Intermediate precision of Ticagrelor

Limit of detection and limit of quantification

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values. Data is represented in table 5.

Table 5: LOD & LOQ value of Ticagrelor

Drug name	LOD	LOQ
Ticagrelor	0.17	0.52

Robustness

Robustness was established by introducing small changes in the HPLC optimised conditions which include mobile phase, flow rate and pH. Data is represented in table 6.

Table 6: Robustness of Ticagrelor

S.N	Condition	Changed condition	Ticagrelor
1	Flow rate: 1ml/min	0.9ml/min-1.1ml/min	0.0-1.7
2	Mobile phase composition: 60:40	58:42-62:38	1.2-0.4
3	pH: 3	pH-2-p.H-4	0.1-0.0

Results and Discussion

The proposed method was found to be simple. Linearity was observed in the concentration range of 50-150 µg/mL with the regression equation y= 990.62x - 22531 and the correlation coefficient of 0.999. System suitability parameters indicates high column efficiency with large number of theoretical plates (>2000).The tailing factor was found to be 1.08 which does not exceed the critical value (2).The average retention time was found to be 5.45. No interference was seen from any of the components of the pharmaceutical dosage form indicating the specificity of the method. The % RSD was found to be 0.26 for intraday and 0.058 for inter day precision studies. Thus the method was found to be accurate and precise as the %RSD was not more than 2%. The limit of detection and limit of quantification for Ticagrelor were found to be 0.17 and 0.52 µg/mL respectively. The RSD for the % assay of sample was calculated for each parameter in robustness and was found to be less than 2% confirming the robustness of the method.

Conclusion

A validated RP-HPLC method was developed for the determination of Ticagrelor in tablet dosage form and bulk forms. As the proposed method is simple, rapid, accurate, precise and specific it can be employed for the routine analysis of Ticagrelor in pharmaceutical dosage forms.

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