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# Development and Validation of Stability Indicating RP- HPLC Method for the Estimation of Ticagrelor in Formulation

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***Abstract*** **- A simple, accurate, selective and stability-indicating RP-HPLC method was developed for the simultaneous estimation of ticagrelor in formulation. Effective chromatographic separations were achieved on a Shimadzu HPLC C18G (250× 4.6mm) with reverse phase elution. The mobile phase composed of methanol acid and water in the ratio of 20:80 v/v at a flow rate of 1.0 ml/min. The detection was carried out at 254 nm. The retention times were 5.786 min for ticagrelor. The linearity range for ticagrelor was 10 to 100 µg/ml, respectively with correlation coefficient 0.99. The developed method was validated statistically with respect to linearity, precision, accuracy, robustness, ruggedness, detection and quantification limits and also subjected to stress conditions like hydrolytic, oxidation, photolysis and thermal degradation. The method was accurate, precise, specific, rapid and found to be suitable for the analysis of commercial samples.**

***Keywords: Ticagrelor, HPLC, Degradation, Validation.***

## 1. INTRODUCTION

Ticagrelor (Fig. 1) is an orally active antiplatelet agent, inhibitor of platelet activation and aggregation mediated by the P2Y12 ADP-receptor1. Chemically it is (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-difluorophenyl) cyclopropyl ]amino} -5-(propylthio)-3 *H*-[1,2,3]-triazolo [4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxycyclo pentane-1,2-diol2. Ticagrelor is indicated to reduce the rate of thrombotic cardiovascular events in patients with acute coronary syndrome. Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation, which inhibits platelet aggregation and thrombus formation in atherosclerotic disease. Literature survey revealed that few LC-MS7-8 methods were reported for the estimation of Ticagrelor. No author reported the HPLC method for determination of Ticagrelor in pharmaceutical formulations. Hence an attempt has been made to develop and validate a novel, simple, rapid and sensitive RP-HPLC method in accordance with ICH guidelines for the estimation of Ticagrelor in its tablet formulation.

## 2. EXPERIMENTAL MATERIALS AND METHODS

The working standard of Ticagrelor was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation BRILINTA tablets (Ticagrelor 90 mg) were procured from local market. HPLC grade acetonitrile, methanol and water were purchased from E.Merck (India) Ltd, Mumbai, India.

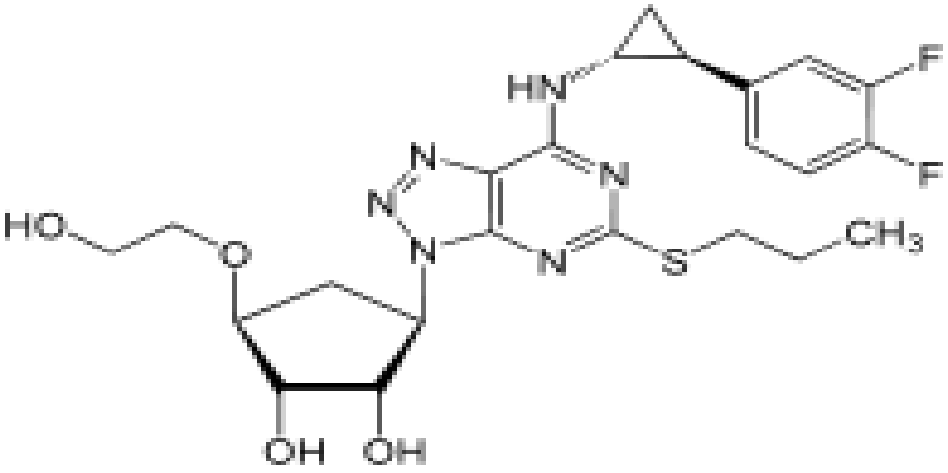


Fig.1 Chemical structure of Ticagrelor

*Chromatographic conditions:*
A mixture of methanol and water in the ratio of 20: 80 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Ticagrelor. The solvent mixture was filtered through 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 10 µL. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 254 nm. The run time was set at 10min.

*Preparation of mobile phase*
Methanol and HPLC water in the ratio of 20:80 v/v was filtered through 0.45 µ membrane filter and degassed. Methanol was used as diluent. The mobile phase was filtered and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 ml/min. The detection of the drug was carried out at 254nm.

*Preparation of standard solution*
From the pure sample, 100mg of Ticagrelor were accurately weighed and transferred into 100ml volumetric flask separately. They were dissolved in 100ml Methanol to obtain 1000µg/ml of stock solutions . From these stock solutions 1ml of Ticagrelor was taken into 10ml volumetric flasks separately and further diluted remaining with a methanol to get 100µg/ml concentration of Ticagrelor. The solutions were then filtered through 0.45µm Nylon filter.

*Sample preparation*
Tablets were taken and their average weight was determined, they were triturated to fine powder. Then powder equivalent to 35.55 mg of Ticagrelor was taken into 10ml volumetric flask and dissolve in Methanol with sonication for 5-10minutes. The supernatant liquid was transferred into 100ml volumetric flask through a whatman filter paper No.41. The residue was washed twice with solvent and combined filterate was made up to 100ml mark. After that 1ml of the above solution was taken in 10ml volumetric flask and was diluted remaining with Methanol with a mixture of to get 100µg/ml concentrations of Ticagrelor. The solution was then filtered through 0.45µm Nylon filter.

*Forced degradation and stability-indicating tests:*
Acid degradation studies: To 1 mL of sample solution of Ticagrelor, 1 mL of 2N hydrogen chloride was added and refluxed for 30 mins at 60$^0$C . The resultant solution was diluted to obtain 100 µg/mL solutions and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies: To 1 mL of sample solution of Ticagrelor, 1 mL of 2N sodium hydroxide was added and refluxed for 30 mins at 60$^0$C . The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies: To 1 mL of sample solution of Ticagrelor, 1 mL of 30% hydrogen peroxide ($H_2O_2$) was added . The solutions were kept for 30 min at 60$^0$C. The resultant solution was diluted to obtain 90 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies: The sample of ticagrelor tablets was placed in oven at 1050C for 1 hrs to study dry heat degradation. The resultant solution was diluted to obtain 90 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral degradation studies: Stress testing under neutral conditions was studied by refluxing the Ticagrelor in water for 1 hrs at a temperature of 60ºC. The resultant solution was diluted to obtain 90 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photostability studies*:* The photochemical stability of the Ticagrelor was studied by exposing the sample of ticagrelor tablets to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m$^2$ in photo stability chamber. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Sunlight Degradation: The sample of Ticagrelor tablets was placed in sunlight for one hr to study dry heat degradation. The resultant solution was diluted to obtain 100 µg/mL solution and 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

## RESULTS AND DISCUSSION

Method validation was performed following ICH guideline specifications. System suitability is anintegral part of the method validation and performed to evaluate the parameters like tailing factor, theoretical plates, resolution and %RSD for replicate injections.The results were within the limits and were presented in Table. 1 while in figure 2 displayed the system suitability chromatomagram . In the blank chromatogram, there were no peaks observed at the retention times of Ticagrelor and also the degradation studies showed that there was no interference with degradants. To determine the accuracy of the proposed method, recovery experiments were conducted; known concentration of pure drug was spiked at three different levels, 50, 100 and 150% and was calculated. Accuracy was calculated as the percentage of recovery and the results were tabulated in Table 2. The precision was evaluated at three levels, repeatability, reproducibility and intermediate precision each level.

Table 1: SYSTEM SUITABILITY RESULTS

| PARAMETER | RESULTS |
|---|---|
| | TICAGRELOR |
| % RSD of peak area | 0.61 |
| Tailing factor (T) | 1.60 |
| Theoretical plate (N) | 2421 |

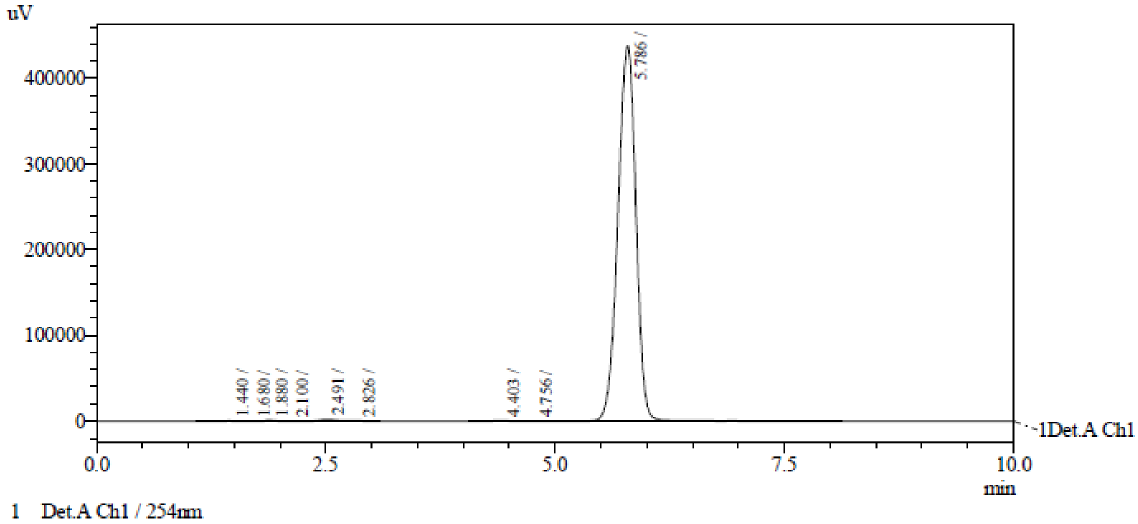


Figure 2 typical HPLC chromatogram of ticagrelor

TABLE 2: ACCURACY DATA

| Spiked level % | Amount taken (µg/ml) | Amount found (µg/ml) | Percent recovery (%w/w) | Average recovery % |
|---|---|---|---|---|
| 80 | 40 | 40.05 | 99.87 | 99.2 |
| 80 | 40 | 40.02 | 99.95 | |
| 80 | 40 | 40.02 | 99.95 | |
| 100 | 50 | 50.01 | 99.98 | 99.92 |
| 100 | 50 | 50.02 | 99.96 | |
| 100 | 50 | 50.09 | 99.82 | |
| 120 | 60 | 60.50 | 99.17 | 99.42 |
| 120 | 60 | 60.31 | 99.48 | |
| 120 | 60 | 60.23 | 99.61 | |

Precision was investigated by six replicate injections of concentrations 120, 100 and 80 µg/ml of Ticagrelor. The result of precision was expressed as percentage relative standard deviation (% RSD) and was tabulated in Table 3.The linearity of the measurement was evaluated by analyzing different concentrations 10-100 µg/ml of the standard solutions of Ticagrelor were used. Calibration curve was constructed by plotting concentration against mean peak area and the regression equation was computed. The summary of the parameters is shown in Table 4. LOD and LOQ determined were 0.2 µg/ml and 0.6 µg/ml. The robustness of the method was evaluated by subjecting the 100% test concentration to small but deliberate changes in the chromatographic conditions like, flow rate, mobile phase composition and wavelength and the results were shown in Table 5. The ruggedness of the proposed method was determined.

TABLE 3: PRECISION STUDIES

| Parameter | Ticagrelor |
|---|---|
| Repeatability RSD of Peak Area | 0.37 |
| Reproducibility RSD of Peak Area | 0.36 |
| Intermediate Precision RSD of Peak Area | 0.47 |

TABLE 4: REGRESSION EQUATION PARAMETERS

| Parameter | Ticagrelor |
|---|---|
| Linearity range(µg/ml) | 10-100 |
| Correlation co-efficient | 0.990 |
| Slope | 30580 |
| Y-intercept | -660.8 |

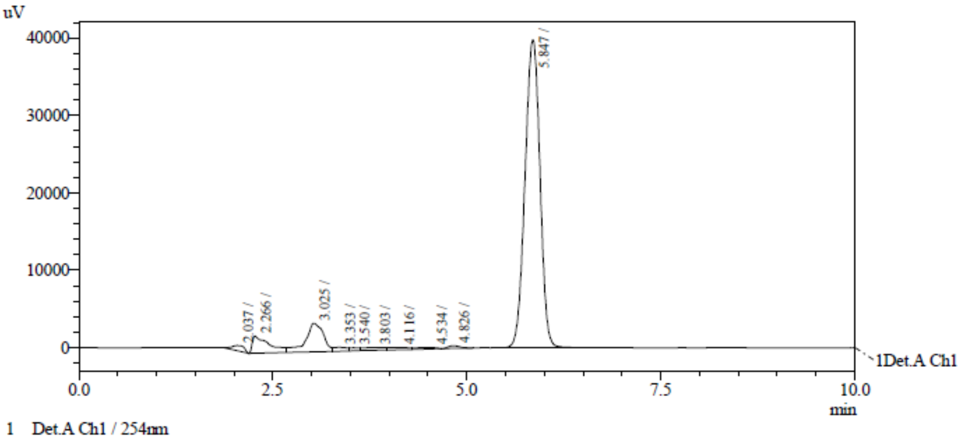
TABLE 5: ROBUSTNESS STUDY

| Parameter | variation | Ticagrelor |
|---|---|---|
| | | %RSD of Peak area |
| Flow change | 0.9 ml/min | 0.33 |
| | 1 ml/min | 0.45 |
| | 1.1 ml/min | 0.23 |
| Mobile phase | 20:80 | 0.44 |
| | 30:70 | 0.46 |
| | 70:30 | 0.54 |
| Wavelength | 254 | 0.12 |
| | 253 | 0.16 |
| | 255 | 0.52 |

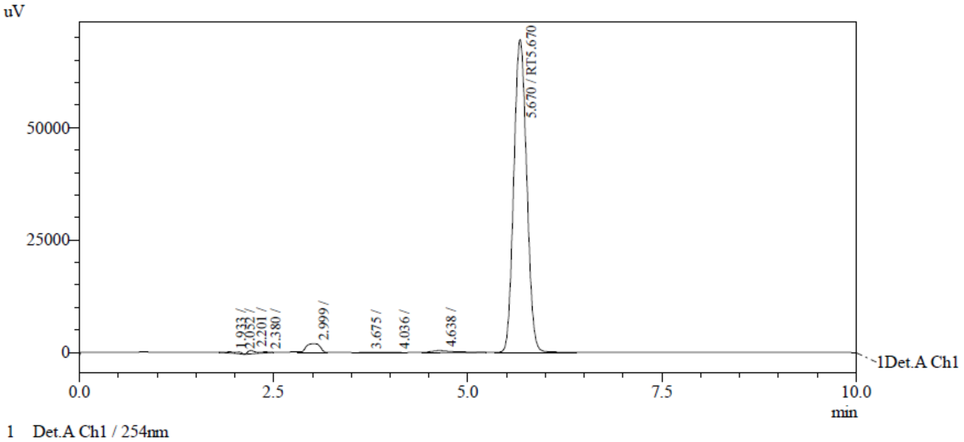
By analyzing the same sample using different columns, different analysts, and on different instruments. The stability studies of the standard solution were conducted at intervals of 24 h and 48 h at room temperature. There were no significant changes observed in system suitability parameters like theoretical plates, tailing factor, retention time and resolution. Hence the standard solution was found to be stable up to 48 h on the bench top. The stability study of the mobile phase was conducted at intervals of 24 h and 48 h at room temperature. There were no significant changes.

TABLE 6: DEGRADATION STUDIES

| Stress conditions | Peak Area | % Degradation | % of active drug present after degradation |
|---|---|---|---|
| Control sample | 3587709 | - | - |
| Acidic<br>(2N/60$^0$C/30 mins) | 2947045 | 17.8 % | 82% |
| Alkaline<br>(2N/60$^0$C/30mins) | 3179435 | 11.3% | 88.6% |
| Oxidative<br>(3%$H_2O_2$/60$^0$C/30mins) | 3509865 | 2.16% | 97.8% |
| Thermal<br>(60$^0$C/1 hr) | 3234216 | 9.85% | 90.1% |
| Neutral<br>(60$^0$C/1 hr) | 3370461 | 6.05% | 93.9% |
| Photostability<br>(7 days) | 3207415 | 10.5% | 89.4% |
| Sunlight<br>(1 hr) | 2924621 | 18.4% | 81.4% |


uV
40000
30000
20000
10000
0
2.037 /
2.266 /
3.025 /
3.353 /
3.540 /
3.803 /
4.116 /
4.534 /
4.826 /
5.847 /
1Det.A Ch1
0.0
2.5
5.0
7.5
10.0
min
1 Det.A Ch1 / 254nm

A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25000
0
1.933 /
2.052 /
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3.675 /
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4.638 /
5.670 / RT5.670
1Det.A Ch1
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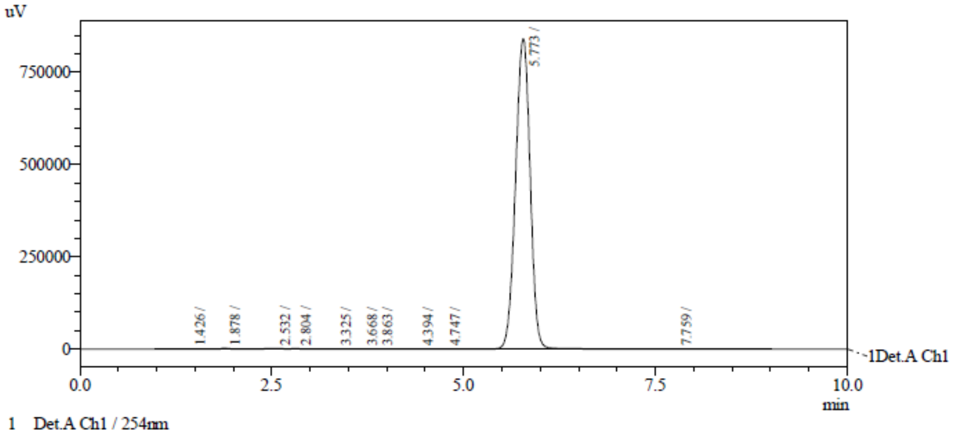
2.5
5.0
7.5
10.0
min
1 Det.A Ch1 / 254nm

B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1.878 /
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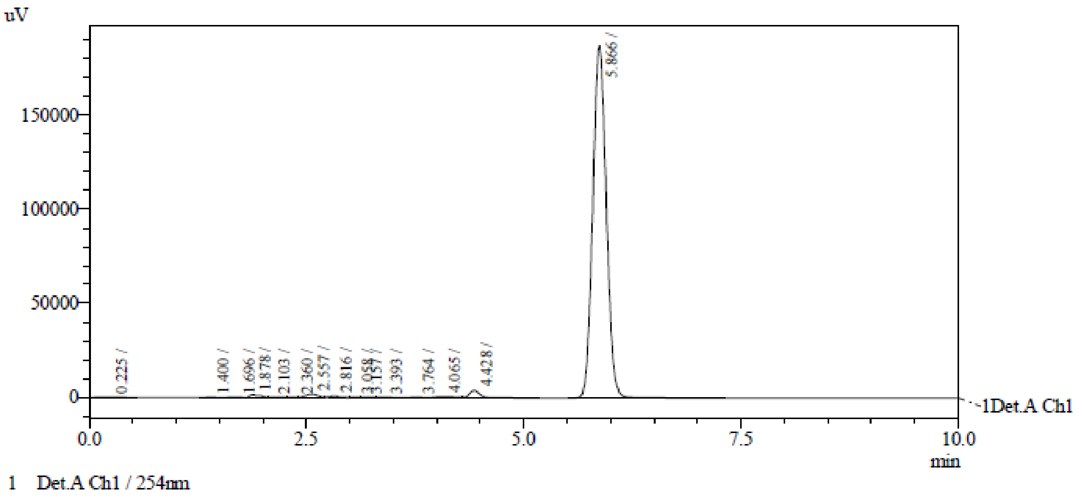
4.394 /
4.747 /
5.773 /
7.759 /
1Det.A Ch1
0.0
2.5
5.0
7.5
10.0
min
1 Det.A Ch1 / 254nm

C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3.058
3.157
3.393
3.764
4.065
4.428
5.866
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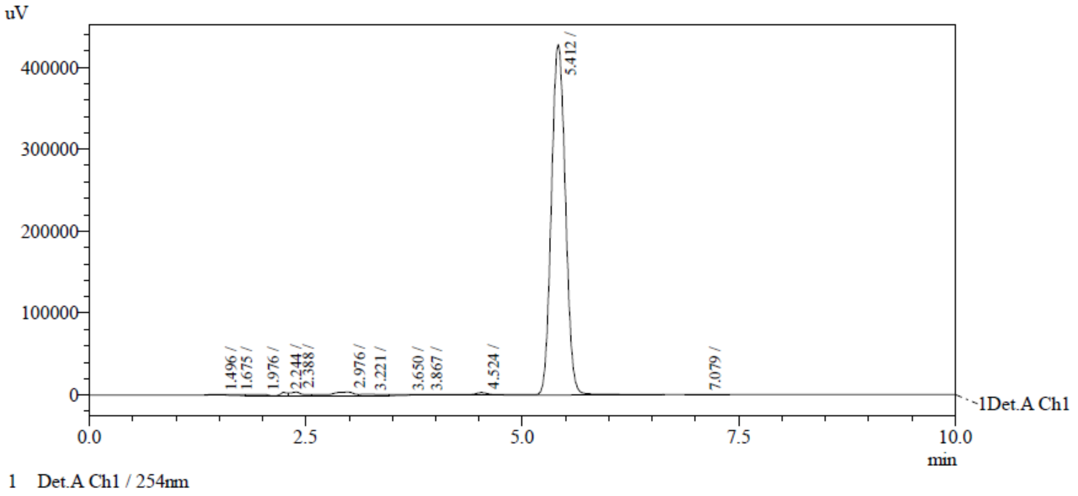
0.0
2.5
5.0
7.5
10.0
min
1 Det.A Ch1 / 254nm

D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2.244
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3.221
3.650
3.867
4.524
5.412
7.079
1Det.A Ch1
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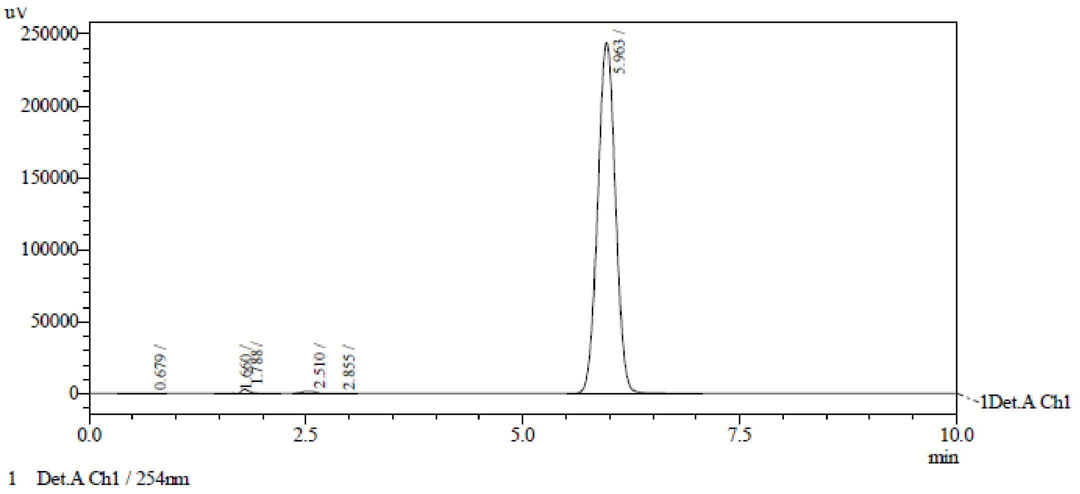
2.5
5.0
7.5
10.0
min
1 Det.A Ch1 / 254nm

E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100000
50000
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1.660
1.788
2.510
2.855
5.963
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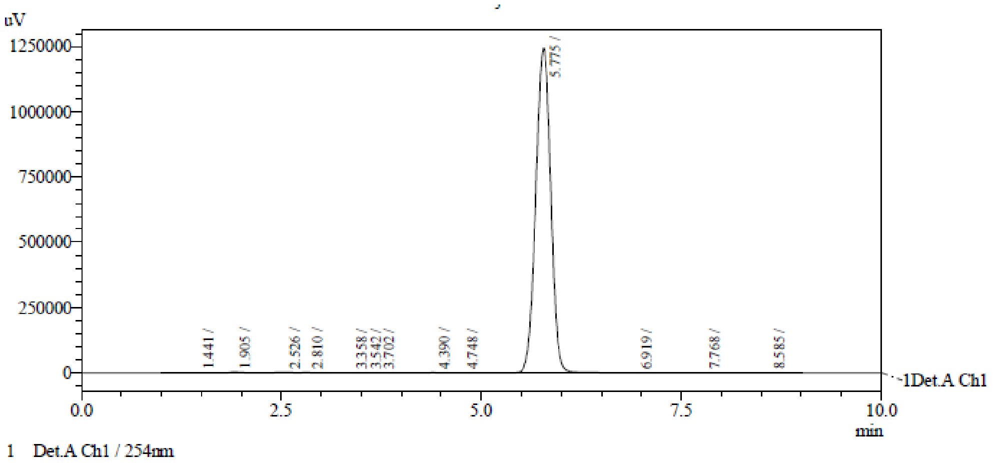
0.0
2.5
5.0
7.5
10.0
min
1 Det.A Ch1 / 254nm

F



G

Fig. 3: Chromatograms of test sample subjected to different forced degradation conditions.A. acid degradation; B. alkali degradation; C. oxidative degradation; D. thermal degradation E.Sunlight degradation.F.Photostability degradation.G.Neutral degradation

## CONCLUSION

Observed in system suitability parameters like peak area, theoretical plates, tailing factor and retention time .Hence the mobile phase is stable up to 48 h at room temperature. The proposed method was applied successfully for the analysis of Ticagrelor in tablet dosage forms, satisfactory results were obtained. .The results were summarized in Table 6. Fig. 3 displays chromatograms of samples subjected to different stress degradations. A simple, specific and reliable reverse phase HPLC method was developed for the estimation of Ticagrelor in their pharmaceutical formulation. The compounds were subjected to forced degradation applying several stress conditions. The proposed method successfully separated the compound with degradants, the active contents were estimated. The proposed method is specific and stability-
Indicating. Hence the developed method can be adapted to regular quality control analysis.

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