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Development and Validation of a Novel HPTLC Method for the Simultaneous Estimation of Ticagrelor and Aspirin in Bulk and Pharmaceutical Tablet Formulations

Dr. Sachin Bhusari, Miss. Priyanka Paulzagade, Mr. Rohan Mane, Dr. Pravin Wakte

***Assistant Professor, Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar- 431001, Maharashtra, India.*
**University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra, India.*

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ABSTRACT: Accurate and simple HPTLC method was established for the simultaneous evaluation of two DAPT (Dual Antiplatelet Therapy) drugs (Ticagrelor and Aspirin). The method was optimized to analyze both drugs in their commercial dosage form (tablets). Chromatographic separation was achieved on precoated HPTLC plates (60 F 254S, 20 cm × 10 cm, thickness 200 mm, Merck, Darmstadt, Germany) with Cyclohexane, Ethyl acetate, and Glacial acetic acid as the mobile phase. Detection and estimation were done at 244 nm by spectrodensitometric analysis. The analytical performance of the proposed HPTLC method was confirmed based on the ICH guidelines for specificity, linearity, accuracy, precision, detection and quantification limits and robustness The calibration plots showed linearity in the ranges of 360–1080 and 300–900 ng/spot for Ticagrelor and Aspirin, respectively, with correlation coefficients (r^2) greater than 0.9994. The detection limits were 101.3 ng for Ticagrelor and 93.62 ng/spot for Aspirin, respectively. The HPTLC method was successfully validated and applied for simultaneous analysis of Ticagrelor and Aspirin in the bulk and marketed drug preparation.

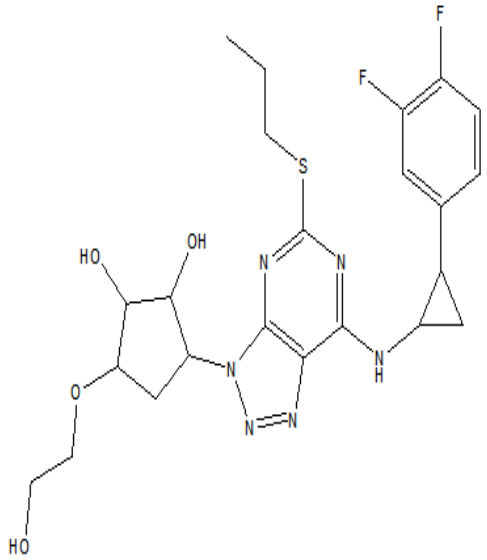
KEYWORDS: Ticagrelor, Aspirin, HPTLC, Method validation.

I. INTRODUCTION

High-Performance Thin-Layer Chromatography (HPTLC) is a widely used analytical tool for separating, identifying, and quantifying active pharmaceutical ingredients. HPTLC is easy to operate, inexpensive and capable of screening large numbers of samples simultaneously with limited solvent. weights. though not as broadly applicable due to low plate performance and development height, HPTLC remains a relevant technique for pharmaceutical analysis particularly where fast and accurate quantitation of samples is desired. [1,2]

This study aims to develop a new HPTLC method for the simultaneous quantitative estimation of ticagrelor and aspirin accurately, precisely, and reproducibly as per International Conference on Harmonization (ICH Q2) guidelines. The purpose of this study was to create a simple, dependable HPTLC method for the pharmacopeial evaluation and consistent analysis of aspirin and ticagrelor in both bulk and final pharmaceutical products.[6]

II. EXPERIMENTAL



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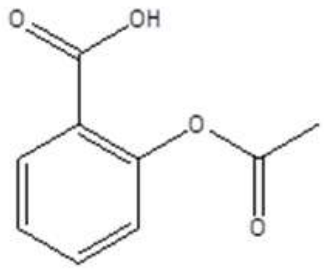

Chemicals and reagents:

Standard Ticagrelor and Aspirin were purchased from Tokyo Chemical Industry Co., Ltd. (TCI) India. The pharmaceutical drug formulation (MSN Laboratories, Pvt. Ltd. India) used in this investigation was obtained from the local market. All other chemicals and reagents were analytical grade.

Chromatographic conditions

The samples were applied as bands (6 mm wide) with a Hamilton microliter syringe (100 µl) under a controlled nitrogen flow utilizing a CAMAG Linomat V sample applicator (Switzerland). The slit size was maintained at 6 mm × 0.45 mm with a scanning speed of 20 mm/s. Precoated TLC silica gel aluminum plates 60 F254 (20 cm × 10 cm, 250 µm thickness, Merck, Darmstadt, Germany) were used. Precoated TLC plates were washed with methanol and activated at 105 ◦C for 5 minutes before applying the sample. A CAMAG twin trough glass chamber (20 cm x 20 cm) saturated with the mobile phase was used to develop the method. Cyclohexane, ethyl acetate, and glacial acetic acid (5.0: 5.0: 0.5 v/v/v) comprised the 10 mL mobile phase.

Before the experiment, the chamber was filled with the mobile phase for 20 minutes at room temperature (25 ± 5 ◦C) with a relative humidity of 60 ± 5%. Densitometric scanning was performed using a CAMAG TLC scanner III, which was operated with CATS software (V CATS 3.2SP2 CAMAG). The radiation source utilized was a UV spectrophotometer. The acquired bands were scanned at a wavelength of 244 nm.

Preparation of solutions

a. Standard stock solutions

Ticagrelor:

Stock solution (900 µg/mL) prepared by dissolving 90 mg of Ticagrelor in 50 mL ethanol in a 100 mL volumetric flask, followed by vortexing and sonication for 10 min. Volume adjusted to 100 mL with ethanol and filtered using a 0.22 µm syringe filter. From this, 1 mL diluted to 10 mL with ethanol to obtain a working solution of 90 µg/mL.

Aspirin:

Stock solution (750 µg/mL) prepared by dissolving 75 mg of Aspirin in 50 mL ethanol in a 100 mL volumetric flask, followed by vortexing and sonication for 10 min. Volume made up to 100 mL with ethanol and filtered using a 0.22 µm syringe filter. From this, 1 mL diluted to 10 mL with ethanol to obtain a working solution of 75 µg/mL.

b. Standard Mixture Preparation:

1 mL each of Ticagrelor (90 µg/mL) and Aspirin (75 µg/mL) working solutions combined in a 10 mL volumetric flask and volume adjusted with ethanol. Final mixture concentration: Ticagrelor 90 µg/mL, Aspirin 75 µg/mL

c. Sample stock solution

Sample Preparation (Label claim – Ticagrelor 90mg & Aspirin 75mg) One tablet each of Ticagrelor and Aspirin was dissolved in 50 mL ethanol in a 100 mL volumetric flask, vortexed, and sonicated for 10 min. The volume was adjusted to 100 mL with ethanol to obtain a stock solution (Ticagrelor 900 ppm; Aspirin 750 ppm). The solution was filtered through a 0.22 µm syringe filter. A 1 mL aliquot was further diluted to 10 mL with ethanol to yield a final concentration of Ticagrelor 90 ppm and Aspirin 75 ppm.

III. VALIDATION

a. Specificity

To verify the method specificity, Ticagrelor and Aspirin standard and sample solutions were applied to an HPTLC plate, and the plate was developed and scanned as described above. The peak purity of Ticagrelor and Aspirin was measured by evaluating the spectra of marker compounds at the peak apex, peak start and peak end positions of the spot.

b. Linearity

Prepared standard stock solution was diluted to obtain linearity standard solutions containing Ticagrelor and Aspirin in the concentration range of 360–1080 and 300–900 ng/band, respectively. Three sets of such solutions were evaluated. Every set was analyzed to obtain a calibration curve. The standard deviation (SD), coefficient of determination (r^2), slope and intercept of the calibration curves were estimated to determine the method's linearity.

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c. Accuracy

Accuracy was estimated through the % recoveries of known quantities of the combination of Ticagrelor and Aspirin added to solutions with marketed preparation. The marketed preparation was spiked with a known quantity of standard markers, and the % ratios between the recovered and expected concentrations were calculated. The analyzed samples were spiked with 0, 10, 20 and 30% of both Ticagrelor and Aspirin. The accuracy was calculated from the following equation:

$$\frac{\text{Spiked Concentration} - \text{Mean Concentration}}{\text{Spiked Concentration}} \times 100$$

d. Precision:

The proposed method was validated for both intraday and interday precision by evaluating three concentrations in triplicate on a single day and on different days for Ticagrelor (360, 720 and 1080 ng/band) and Aspirin (300, 600 and 900 ng/band). The interday precision of the method was verified by performing a similar method protocol on different days under the same set of experimental situations. The repeatability of the sample application and calculation of the peak area for the analyte were articulated in terms of the % RSD.

e. Limit of detection and limit of quantitation

As per ICH guidelines, the limit of detection (LOD) is the lowest analyte concentration distinguishable from background noise, while the limit of quantification (LOQ) is the lowest level quantifiable with acceptable precision and accuracy. Both are commonly determined using the signal-to-noise ratio method.

f. Robustness:

To determine the robustness of the present method, small changes in the chromatographic conditions were intentionally made, which may have affected the performance of the method, such as mobile phase composition ± 10% (v/v/v)and chamber saturation time by ±5 min.

g. Analysis of the marketed product:

To determine the contents of Ticagrelor and Aspirin in the tablet to an accurately weighed quantity of formulation equivalents of 90 mg of Ticagrelor and 75 mg of Aspirin, 50 mL of ethanol was added, followed by vortexing and sonication for 10 min. filter the solution by using a 0.22µm syringe filter. The volume of the resulting solution was 10 mL with ethanol. The % assay was determined. The possibility of the chemical constituent's interference with analysis was examined.

IV. RESULTS AND DISCUSSIONS

Chromatographic separation was performed on standard solutions of Ticagrelor and Aspirin. Briefly, the standard samples were spotted onto TLC plates. the TLC plates were developed by linear ascendingdevelopment using various solvents, such as acetone, benzene, chloroform, ethyl acetate, methanol and toluene. Based on the observations from preliminary trials, various solvent mixtures were evaluated to identify the optimal resolution between Ticagrelor and Aspirin within the solvent systems under investigation. initially, Chloroform and ethyl acetate were evaluated at several ratios, but inadequate separation between Ticagrelor and Aspirin occurred, even with the addition of a small quantity of acetic acid to the mobile phase. The optimized mobile phase consisting of Cyclohexane: Ethyl acetate: Glacial acetic acid (5.0: 5.0: 0.5 v/v/v) showed satisfactory resolution. Densitometric measurements were achieved with CAMAG TLC scanner III that was operated by win CATS software (V CATS 3.2SP2 CAMAG). At a wavelength of 244 nm, the optimized mobile phase showed the highest sensitivity for quantification of both drugs.

Other chromatographic parameters, such as the sample application rate and volume, sample application positions, chamber saturation time, total run length, and track-to-track separation, were also optimized to obtain precise, accurate, and reproducible Rf values, a symmetrical peak shape, and enhanced resolution for both compounds. The best chamber saturation time for the given mobile phase was found to be 20 minutes under room temperature (25 + 5 °C) with a relative humidity of 60 + 5%. The total length of the run of the chromatogram was approximately 80 mm. The distance between the two tracks was 10 mm. The peak shape grew more symmetrical and spots appeared more concentrated as the TLC plates were pre-treated with methanol and activated at 105 °C for 5 minutes. After the run, the TLC plates were dried in the air for 5 minutes. The slit width and the scanning speed were 6 mm × 0.45 mm and 20 mm/s, respectively. The optimized conditions for chromatography are listed in Table 1.

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Validation of the proposed method:

Validation of the proposed methods was performed by the International Conference on Harmonization (ICH Q2) guidelines (2005).

a. Specificity:

The peak purity of Ticagrelor and Aspirin was assessed by comparing their respective spectra at the peak apex, peak start and end positions of the peak. A good correlation (r^2 value more than 0.999) was obtained for both drugs. good correlation values and satisfactory peak purity suggest that there is no interference in the quantification of Ticagrelor and Aspirin in sample solutions. This verifies that the method is specific.

b. Linearity:

The proposed HPTLC method required evaluation of linearity through testing different concentrations of each analyte. The optimized conditions from earlier produced peak areas at 244 nm that directly correlated with Ticagrelor and Aspirin concentrations. The statistical parameters for the method include linearity ranges and correlation coefficients as well as slopes and intercepts, and % RSD of the slopes which are presented in Table 1. The linear graphs for Ticagrelor and Aspirin appear in Figs. 4 and 5.

Fig. 3: 3D Image of Linearity.

Table 1: Linear regression data for calibration plots of the analyzed drugs using the proposed HPTLC methods

Sr. No.	Parameters	Results	
		Ticagrelor	**Aspirin**
1.	Wavelength (nm)	244 nm	244 nm
2.	Retention factor (Rf) ± SD	0.180 ± 0.003	0.588 ± 0.004
3.	Linearity range (ng/band)	360–1080	300–900
4.	Correlation coefficient (r^2)	0.9996	0.9994
5.	Slope	0.000004	0.000005
6.	Intercept	0.0005	0.0004
7.	LOD (ng/band)	101.3	93.62
8.	LOQ (ng/band)	215.2	198.9

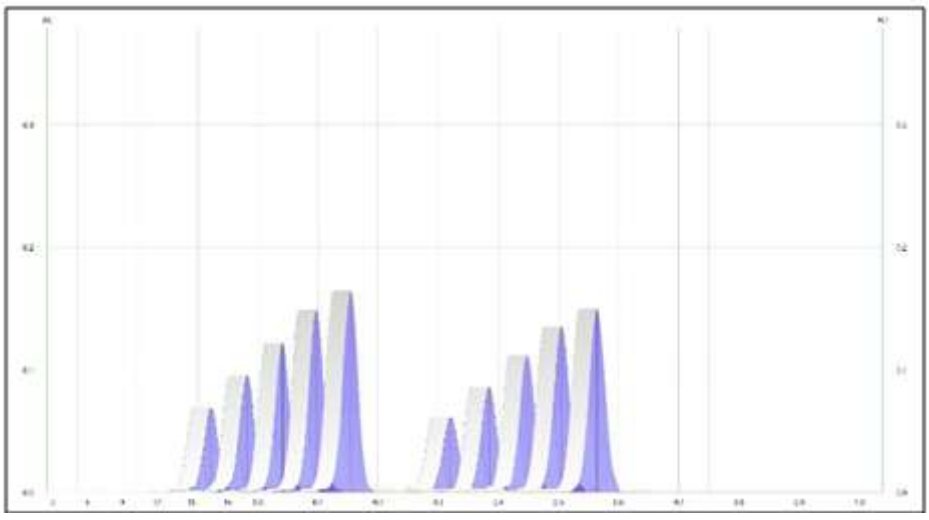

Table 2: Accuracy studies of Ticagrelor and Aspirin at 0%, 10%, 20% and 30% addition by the proposed TLC densitometric method.

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Analyte	Amount in sample (mg)	Amount added (mg)	Measured conc. ± SD	% Recovery	% RSD
Ticagrelor	90	0	0.0	98.92	0.54
	90	9	9.0	100.47	0.65
	90	18	18.1	100.40	0.67
	90	27	26.7	98.90	0.21
Aspirin	75	0	0.0	99.74	0.66
	75	7.5	7.5	99.68	0.73
	75	15	14.8	98.55	0.34
	75	22.5	22.4	99.67	0.42

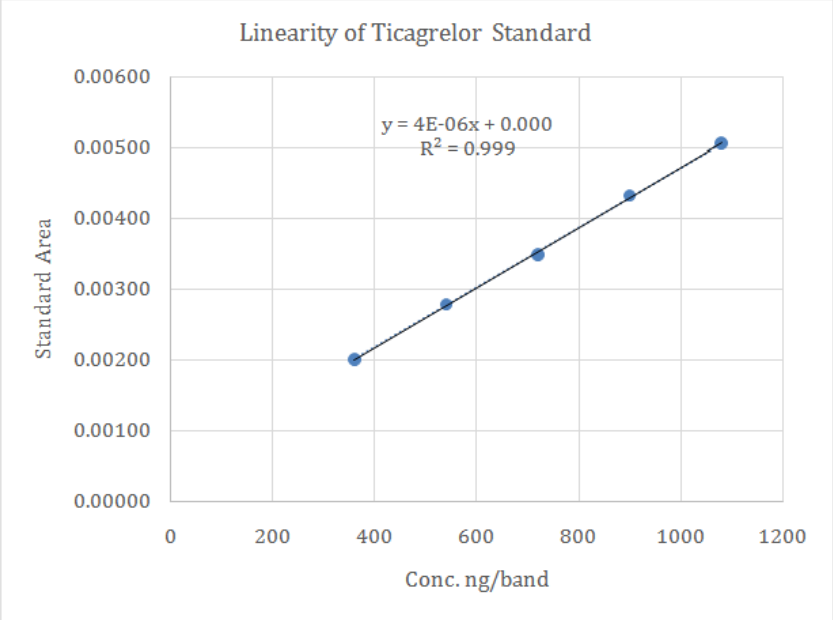

Fig. 4: Calibration curve for standard Ticagrelor.

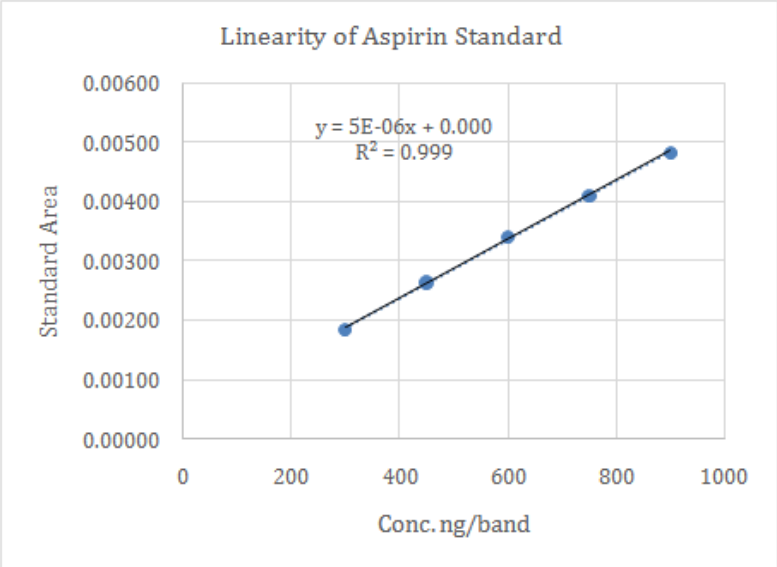

Fig. 5: Calibration curve for standard Aspirin.

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Table 3: Intra-day and inter-day precision of Ticagrelor and Aspirin.

Analyte	Concentration (ng/band)	RSD (%)
Intra-day precision		
Ticagrelor	360	1.33 %
	720	1.67 %
	1080	1.73 %
Aspirin	300	0.93 %
	600	1.50 %
	900	1.29 %
Inter-day precision		
Ticagrelor	360	1.46 %
	720	1.53 %
	1080	0.91 %
Aspirin	300	1.21 %
	600	0.79 %
	900	1.89 %

Where,
RSD % is relative standard deviation.

c. Accuracy:

Results from accuracy studies, provided in Table 2, were in an acceptable range (98.55–100.47%), indicating that the recovery of the proposed method was good. Additionally, a low %RSD value demonstrated the ruggedness of the proposed method.

d. Precision:

The developed method's intraday and interday precision were evaluated for the two drugs. As presented in Table 3, the results showed that both %RSD values for Ticagrelor and Aspirin under various precision conditions remained under two percent, thereby demonstrating excellent precision.

g. Analysis of marketed product:

The validated HPTLC method was used to analyse a marketed formulation, Tiare Kit. Both resulted Ticagrelor and Aspirin were adequately resolved at their Rf values along with number interference from excipients. This demonstrates that the analysis is both accurate and precise for quantifying the two drugs in a fixed-dose combination. (FDC) tablet (Figure 7).

e. Limit of detection and limit of quantitation:

The sensitivity of the method was adequate with respect to the sensitivity parameters tested, limit of detection (LOD) was 101.3 ng for Ticagrelor, and 93.62 ng for Aspirin while the limit of quantification (LOQ) for both was 215.2 ng and 198.9 ng respectively (Table 1).

f. Robustness:

The parameters of the optimized methods were intentionally varied. Each examined factor was charged at three levels (−1, 0 and 1). Each time a factor was subjected to change, the changes in the responses for both drugs were noted. The summary of the robustness study is given in Table 4. The values of the % RSD of the peak areas for both Ticagrelor and Aspirin in the analysis did not surpass 2.0%. The satisfactory low values of the % RSD of peak areas and unaffected Rf values suggested the robustness of the proposed method.

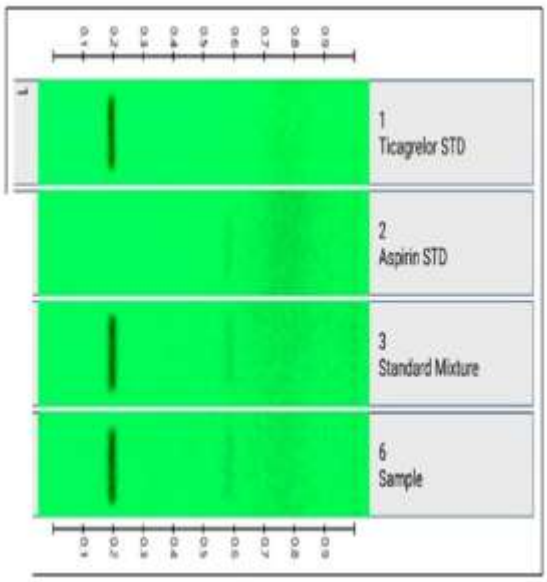

Fig. 6: Plate Image Including STD and Sample.

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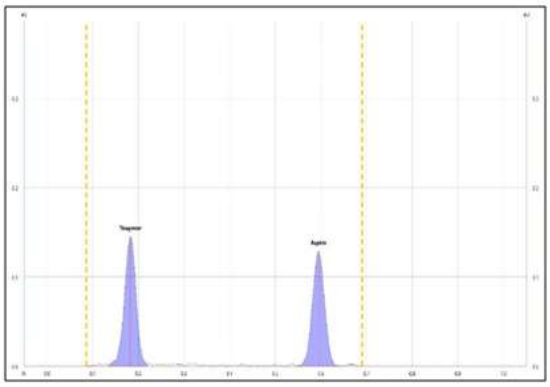

Fig. 7: Densitogram of Ticagrelor and Aspirin.

V. CONCLUSION

The present study illustrates a simple, accurate and precise HPTLC method for the simultaneous estimation of Ticagrelor and Aspirin. Additionally, the proposed method does not necessitate a complex treatment or sophisticated analytical units, which are usually associated with HPLC and LC–MS/MS analyses. Reliability was ascertained by testing a range of validation parameters of the proposed method. Finally, the proposed method was sensitive and specific; hence, it can be suggested for use for the routine analysis of the cited phytochemicals, either in bulk form or in their combined dosage forms.

Table 4: Robustness evaluation of the method for Ticagrelor and Aspirin.

Factor	Level	Ticagrelor		Aspirin	
		% RSD of peak area	Rf ± SD	% RSD of peak area	Rf ± SD
A: Mobile phase composition (v/v/v)					
+10	+1	1.94	0.1764 ± 0.0018	1.69	0.5824 ± 0.0048
0	0	1.56	0.173 ± 0.000	0.85	0.5643 ± 0.0012
-10	-1	1.35	0.1794 ± 0.0009	1.67	0.5832 ± 0.0016
B: Chamber saturation time					
25	+1	1.98	0.1766 ± 0.0018	0.83	0.5824 ± 0.0048
20	0	1.13	0.171 ± 0.002	7.56	0.562 ± 0.0017
15	-1	0.95	0.1794 ± 0.0009	1.36	0.5830 ± 0.0014

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