Forced degradation study indicating HPTLC method development and validation of Tranexamic Acid in bulk and tablet dosage form by using a quality by design approach

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Abstract—**A novel, stability-indicating High-Performance Thin-Layer Chromatography (HPTLC) method was developed and statistically optimized for the quantification of Tranexamic Acid in pharmaceutical formulations using a Box-Behnken Design under the Quality by Design framework. The study aimed to establish a robust and efficient chromatographic method capable of distinguishing Tranexamic Acid from its degradation products under various stress conditions. The critical method parameters, including mobile phase composition (Ethanol: Water: Glacial acetic acid), chamber saturation time, and development distance, were optimized using a three-factor, three-level Box Behnken Design to achieve maximum peak area and resolution. Chromatographic separation was performed on pre-coated silica gel 60 F_{254} TLC plates, and densitometric detection was conducted at 494 nm. Method was validated in accordance with** guidelines**, demonstrating specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantitation and assay. Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal and neutral conditions. The method effectively resolved the parent drug from its degradation products, confirming its stability-indicating nature. The use of Box-Behnken Design allowed for efficient optimization with minimal experimental trials, providing a statistically sound and time-efficient approach to method development. This validated HPTLC method is suitable for routine quality control and stability testing of Tranexamic Acid in bulk and dosage forms.**

Keywords—**Tranexamic Acid, High-Performance Thin Layer Chromatography, Stability-indicating method, Quality by Design, Box-Behnken Design, Validation, ICH guidelines.**

I. INTRODUCTION

Tranexamic acid is an antifibrinolytic drug commonly used to manage heavy menstrual bleeding. In addition, TA has a whitening effect against hyperpigmentation caused by melasma and ultraviolet (UV) radiation [1].3. The synthetic derivative of the amino acid lysine is known as tranexamic acid. It is a medication used to treat or prevent excessive blood loss from major trauma, surgery, tooth removal, nosebleeds, and heavy menstruation. It is also used for hereditary angioedema. It can be taken by mouth, injected into a vein, or applied topically[2]. Tranexamic acid, or trans-4-(aminomethyl) cyclohexane carboxylic acid, is an antifibrinolytic drug that works by inhibiting the activation of plasminogen into plasmin [2]. In the cosmetic industry, tranexamic acid is also used as an ingredient in whitening creams at a maximum concentration range of 1.5–2%. On the other hand, overuse of tranexamic acid on the skin can result in local toxicity, which includes allergies and irritation.Thus, it is important to take into account the presence of tranexamic acid in whitening cream formulations to ensure that the concentration stays within the allowed range.. Tranexamic acid (TA) is a hydrophilic drug used as an anti-fibrinolytic agent to reduce bleeding after cardiac surgery and arthro-plastic surgery. It also exhibits anti-inflammatory, whitening effects on skin [3].

Trans-4-aminomethyl-cyclohexanecarboxylic acid, Tranexamic Acid is a synthetic amino acid that is frequently used to control irregular bleeding in a variety of disorders [4].

Tranexamic acid (TA) is a synthetic lysine analogue that exerts its antifibrinolytic effect by reversibly blocking lysine-binding sites on plasminogen molecules, thereby inhibiting fibrinolysis and stabilizing blood clots [5]. It is extensively used in clinical settings for the prevention and treatment of bleeding associated with trauma, surgery, and

various hematological conditions [6]. Given its widespread therapeutic use, there is a growing need for reliable, sensitive, and cost-effective analytical methods for its quantification in pharmaceutical dosage forms.

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced form of TLC offering high resolution, reproducibility, and the capability of analyzing multiple samples simultaneously. Compared to HPLC, HPTLC is more economical and allows for higher sample throughput, making it suitable for routine quality control [7]. Despite the availability of various analytical methods for TA, such as HPLC and UV-spectrophotometry [8], HPTLC methods remain underutilized and offer significant potential.

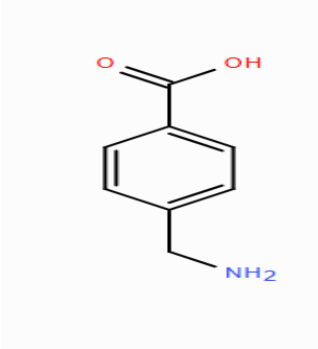

Fig.1 – Chemical Structure of Tranexamic Acid [9]

II. MATERIALS AND METHODS

Experimental

Materials: A sample of Tranexamic Acid API was purchased from Syngenesis ChemsciencePrivate Limited, Tathawade, Pune, India. Ethanol, Distilled Water and Glacial Acetic Acid were acquired from Loba Chemicals, Mumbai. Tranexamic Acid containing the marketed formulation (PAUSE 500) was purchased from the local market

Instrumentation: Samples were applied to plates in 6 mm-wide bands utilizing a 6 mm Linomat 5 sample applicator along with a Hamilton (100 µL) sample syringe. The analysis was conducted using aluminum silica gel 60 F254 HPTLC plates (Merck, Germany; dimensions of 20×20 cm and a thickness of 0.1 mm). A twin trough chamber (20×20 cm) facilitated the development and validation of the HPTLC method. For the observation of separated spots on the TLC plates, a UV-Visible chamber was employed. Densitometric scanning was performed using the TLC scanner 3 in reflectance/absorbance mode at a wavelength of 494 nm (CAMAG, Switzerland). Data processing and scanner control were efficiently managed by the Win CATS version 23.1.8 software.

Chromatographic Conditions: A suitable volume of both standard and sample solutions was applied to the HPTLC plate, forming bands of 6 mm in length. Before the chromatography process, the plates underwent a prewash with methanol and were activated at 100°C for 10 minutes. The mobile phase was composed of Ethanol: Water: Glacial Acetic Acid in a ratio of 7:3:0.1 (V/V/V). Ten minutes were given to the mobile phase to saturate. The mobile phase was permitted to saturate for 10 minutes. Following the development, the plates were allowed to air dry. For the scanning process, TLC Scanner 3 was utilized, operating at a wavelength of 494 nm, with a deuterium lamp serving as the light source. The scanning data was collected using Win CATS software.

Preparation of standard solutions: 5 mg of Tranexamic Acid dissolved in a 10 ml methanol to get concentration of 500 µg/mL.

Preparation of sample: 20 tablets of Tranexamic Acid (Pause 500) were precisely weighed and finely ground into a powder. The average weight of the tablets was determined, and a quantity of powder corresponding to 5 mg of Tranexamic Acid (7.91 mg) was placed into a 10 mL volumetric flask, followed by the addition of 10 mL of water. The resulting solution had a concentration of 791µg/mL. Subsequently, the solution was filtered using a 0.45 µm Whatman filter paper. This filtered sample solution was utilized for furtheranalysis.

Identification of QTMP, CMA, and Risk Assessment:

In the current investigation, the Quality Target Method Profile (QTMP), Critical Method Attributes (CMA), and Risk Assessment were identified and applied to establish a robust HPTLC method for Tranexamic acid. The QTMP was formulated by outlining essential analytical criteria such as specificity, accuracy, precision, robustness, and linearity, thereby confirming the method's appropriateness for routine quality control applications. The optimization of Critical Method Attributes (CMA), which encompassed mobile phase composition, saturation duration, and detection wavelength, band length and solvent front was carried out utilizing a Box-Behnken Design

(BBD) within the Quality by Design (QbD) paradigm. A comprehensive Risk Assessment was performed employing to pinpoint potential factors that could affect method performance. The primary risk factors mobile phase ratio, chamber saturation duration, and sample application volume were assessed, and their effects were mitigated through systematic optimization. The resulting method, developed through this structured approach, demonstrated exceptional separation efficiency, reproducibility, and robustness, in alignment with ICH Q2 (R1) guidelines. [10-11].

Optimization of method using design of experiments (DoE): Box–Behnken design:The Design of Experiment (DOE), specifically the Box-Behnken Design (BBD), serves as an effective approach for optimizing High-Performance Thin-Layer Chromatography (HPTLC) conditions. This study aims to enhance resolution, sensitivity, and reproducibility through a systematic optimization of key chromatographic parameters. The DOE, particularly BBD, allows for the simultaneous evaluation of multiple factors, thereby reducing the number of experiments needed while maximizing the information gathered. Typically, optimization studies assess components at three levels (-1, 0, +1) or fewer [12-14]. A design comprising sixteen experimental runs was developed. Utilizing this design, the HPTLC method was optimized, enabling the evaluation of both quadratic and interactive effects of each parameter on Rf values, with the assistance of State Ease Design Expert (Version 1.4.3.6336, Trial Version).

In the preliminary trials aimed at establishing the Thin-Layer Chromatography (TLC) method, several factors were examined. The selected wavelength for quantifying Tranexamic Acid was 494 nm. The volume of ethanol was determined to range from 6 to 8 mL. Furthermore, the volume of Water was set between 2 and 4 mL, while glacial acetic acid was adjusted to a range of 0.01 to 1 mL. A total of 17 unique experiments were performed, and the responses for all experimental runs are documented in Table 2.

Table.1- Chromatographic factors for Box-Behnken experimental design

Sr.No	Factors	Lower level (-(1)	Intermediate level (0)	Higher level (+1)
1	Saturation Time (min)	5	10	15
2	Band Length (mm)	4	6	8
3	Solvent Front (mm)	70	80	90

Establishment of method operable design region (MODR): After conducting the experimental runs guided by the Box Behnken Design, the gathered data was analyzed to develop regression models and understand the relationships between the variables and responses. The aim of this study was to identify the method operable design region (MODR). Subsequently, optimal chromatographic conditions were determined from the MODR using overlay plots, tailored to the specific objectives for each critical method parameter (CMP). Within the design space, all specifications outlined in the analytical target profile (ATP) were met at a defined level of risk. The criteria for the selected CMPs were utilized to identify the most favorable experimental runs. [15].

VALIDATION OF DOE- BASED HPTLC METHOD:

The method was validated as per ICH Q2 (R1) guidelines [16].

1)Linearity

The method's linearity was assessed by preparing six concentrations through dilution of a standard stock solution (500 µg/mL) to cover the range from 200 to 1200 ng/band of Tranexamic acid. A calibration curve was constructed using these six concentrations, ranging from 200-1200 ng/band. The solutions were injected into the chromatographic system, and a graph of concentration versus area was plotted to calculate the correlation coefficient.

2)Precision

Precision was evaluated by examining repeatability, intra-day, and inter-day variations toensure the method's accuracy.

- Repeatability: The percentage relative standard deviation (RSD) was determined by analyzing three replicates of three concentrations (400, 600, 800 ng/band).
- Intermediate Precision (Intraday and Interday): Intra-day variation was assessed by analyzing three concentrations (400, 600, and 800 ng/band) on the same day. Interday variation was determined by analyzing the same concentrations on three separate days, and the percentage RSD was calculated for both.

3)Accuracy
To validate the method's accuracy, recovery studies were conducted by spiking the standard medication into tablet solutions at concentrations of 80%, 100%, and 120% of the target concentration (800 ng/band). The percentage relative standard deviation (%RSD) was calculated to evaluate the accuracy of the results within the predefined limits.

4)LOD&LOQ
The Limit of Detection (LOD) and Limit of Quantitation (LOQ) are defined by analyte concentrations that correspond to signal-to-noise ratios of 3 for LOD and 10 for LOQ. For Tranexamic acid, the LOD and LOQ were determined by spotting a series of solutions until the signal-to-noise ratios reached 3 and 10, respectively. The amplitude of the analytical background was determined using a blank. The formulas are:
$LOD=3.3\times\sigma/S$
$LOQ=10\times\sigma/S$

5)Specificity
Specificity was assessed by applying an 800 ng/band spot of various components: solvent (Methanol), mobile phase, pure API, and the tablet sample. The specificity was determined by evaluating the Rf value and the corresponding spectrum of the analytes. The wavelength of 494 nm was used to ensure peak purity by comparing the spectra at three points: the beginning (S), the apex (M), and the end (E) of the peak.

6)Robustness
Robustness was tested by making slight modifications in the wavelength and mobile phase composition. Wavelength adjustments were made from 494 nm to 492 nm and 496 nm, and variations in the mobile phase were also tested. The impact of these changes on the retention factor and peak area was analyzed by calculating the %RSD. The acceptable threshold for the %RSD of the peak area was established as below 2.

7) Method Precision
The analysis of Pause 500 tablets was conducted following the sample solution preparation instructions. The process was repeated six times, and the peak area was recorded after applying the sample solution. The sample, with a basic concentration of 800 ng/band from the tablet solution, was used to calculate the percentage recovery. [17-21]

FORCE DEGRADATION STUDY [21-29]

1. Acid Degradation:
5 mg of the drug was placed in a 10 mL volumetric flask, and 3 mL of 0.1 N HCl was added. This solution stored at room temperature for 1 hour. After the time elapsed, the volume was adjusted to 10 mL with methanol. A 0.8 µL sample of the resulting solution was injected into the HPTLC system, and the chromatogram was recorded.

2. Alkali Degradation:
5 mg of the drug was placed in a 10 mL volumetric flask, and 3 mL of 0.1 N NaOH was added. This solution stored at room temperature for 1 hour. Afterward, the volume was made up to 10 mL with methanol. A 0.8 µL aliquot of the resulting solution was injected into the HPTLC system, and the chromatogram was captured.

Oxidative Degradation:
A quantity of 5 mg of the drug was transferred into a 10 mL volumetric flask, followed by the addition of 3 mL of a 3% H_2O_2 solution. The flask was maintained at room temperature for one hour. Subsequently, the volume was brought up to 10 mL using methanol. A 0.8 µL sample of the prepared solution was then injected into the HPTLC system, and the chromatogram was obtained.

3. Neutral Degradation:
5 mg of the drug was placed in a 10 mL volumetric flask, and 3 mL of distilled water was added. This solution stored at room temperature for 1 hour. After 1 hour, the volume was made up to 10 mL with methanol. A 0.8 µL portion of the resulting solution was injected into the HPTLC system, and the chromatogram was recorded.

4. Thermal Degradation:
Thermal degradation of Tranexamic Acid was studied by placing 5 mg of the standard drug powder in a hot air oven at 40°C for 30 minutes. Afterward, the volume was adjusted with methanol, and 0.8 µL of the resulting solution was injected into the HPTLC system, and the chromatogram was recorded.

III. RESULTS AND DISCUSSION

Chromatographic development: To identify the suitable mobile phase for the efficient separation of

Tranexamic Acid, multiple trials were conducted utilizing mobile phases with solvents of differing polarities and concentrations. Of the various combinations tested, the mobile phase comprising Ethanol, Glacial Acetic Acid, and Water in a ratio of 7:3:0.1 V/V/V provided the optimal resolution, yielding sharp, well-defined peaks with Rf values of 0.50 ± 0.02, as illustrated in Figure 2.

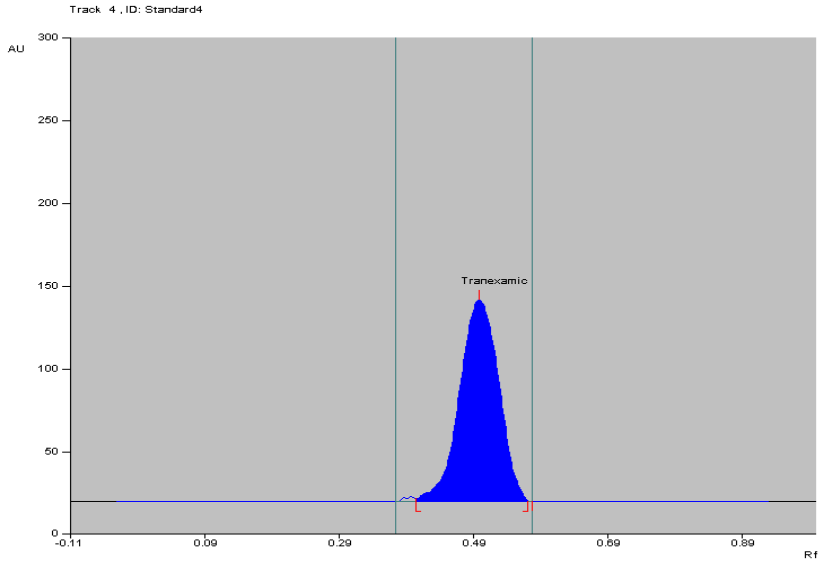

Fig.2 - Densitogram of a standard solution of Tranexamic Acid (800 ng/band)

Selection of the Wavelength: The efficacy of HPTLC densitometry is contingent upon the particular wavelength employed for the detection of analytes. In this research, the HPTLC plate was analyzed within the visible wavelength range of 400-800 nm utilizing a TLC scanner III (CAMAG). The most suitable wavelength for estimating Tranexamic Acid was determined to be 494 nm, as illustrated in Fig 3.

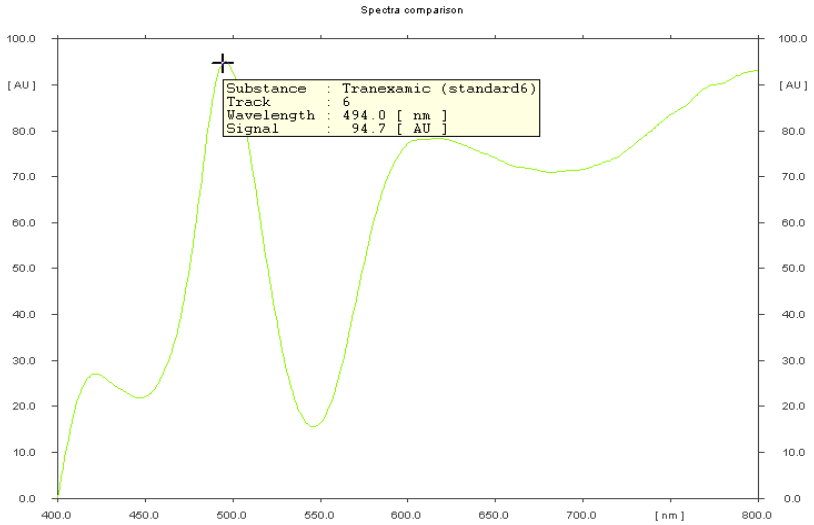

Fig.3 – VISIBLE Spectra of Standard (Tranexamic Acid)

Optimization of method using design of experiments (DoE): Box–Behnken design: The standard and sample solutions containing Tranexamic Acid were applied onto HPTLC plates and developed using linear ascending method in twin trough glass chamber. 17 runs were conducted for chromatographic conditions as per DoE (Table 2). After complete drying, the developed HPTLC plates were scanned at 494 nm. The final optimized chromatographic conditions included a mobile phase ratio Ethanol: Water: Glacial Acetic acid (7:3:0.1 V/V/V), saturation time 10 min, band length 6 mm and solvent front 80 mm (Table 3). These conditions produced Rf value of 0.50 with sharp peak and

highest resolution (Fig 2). The system suitability parameters for Tranexamic Acid are presented in (Table 4).17 experimental runs were conducted in a random manner to reduce bias in design. A typical second order regression equation obtained from State Ease design expert is

$Y=2.344-0.254*A-0.430*B+0.0047*C+0.0039*A^2+0.0256*B^2-0.0002*C^2-0.0008*AB+0.0024*AC+0.0017*BC$. Where Y is Rf value, A is Saturation time, B is band length and C is Solvent front. As per above equation, Saturation time (Factor A) shows the strongest positive influence on Rf, Band length (Factor B) and solventfront (Factor C) have minimal and statistically insignificant effects in this model.

The model is statistically significant ($p = 0.0017 < 0.05$) and explains most of the variation (SS Model = 0.1714 out of 0.1823). Individual linear effects of Saturation time ($p = 0.4471$) and Band length ($p = 0.1023$) are not significant, while Solvent front is marginally significant ($p = 0.0688$). All quadratic terms (A^2, B^2, C^2) are highly significant ($p < 0.05$), indicating strong non-linear effects. No interaction terms are significant, and the Lack of Fit is not significant, confirming a good model fit.

The 3D response surface plot shows a non-linear, bowl-shaped relationship between Rf value, band length, and saturation time. The Rf value is minimized when the band length is around 8–9 mm and saturation time is 9–10minutes. Rf decreases with increasing band length and saturation time up to a point, then begins to rise again, indicating optimal conditions lie near the center of the tested range. The contour plot verifies this minimum through the presence of concentric ellipses. This helps guide experimental settings to minimize Rf, likely linked to reducing impurities.

The perturbation plot shows that the R-value increases as any of the factors (A, B, or C) deviate from the reference point, with the lowest R-value occurring at the center (coded value = 0). Among the three, factor C has the steepest slope, indicating it has the strongest influence on the R-value. Factors A and B show lower sensitivity. This suggests that to minimize the R-value, all factors should be kept close to the reference point, especially factor C, which has the greatest impact on response variation.

The contour plot shows how Rf value varies with saturation time and band length. The highest Rf values occur in the dark blue central region, around 10–12 minutes of saturation time and 6–8 mm band length. Moving away from this optimal zone reduces the Rf value, indicating both factors significantly influence the response. The elliptical contours suggest a strong interaction between the two variables, meaning their combined effect is more impactful than individual changes. To maximize Rf, both variables must be carefully optimized together.

The three graphs show U-shaped trends between Rf value and the process parameters: pulsation delay (~10 ms), ventlength (~4 mm), and chamber flow (~15 l/min). The minimum Rf value is observed at these optimal points, with deviations in either direction leading to increased Rf. Chamber flow shows the steepest curve, indicating the Rf value is most sensitive to it. These findings suggest fine-tuning these parameters is essential for minimizing Rf.

Table.2 - Box Behnken design and their responses

Std	Run	Factor A: Saturation Time (min)	Factor B: Band Length (mm)	Factor C: Solvent front(mm)	Response (Rf)
7	1	5	6	90	0.57
16	2	10	6	80	0.5
6	3	15	6	70	0.74
3	4	10	6	80	0.5
8	5	15	6	90	0.73
2	6	15	4	80	0.57
14	7	10	6	80	0.5
5	8	5	6	70	0.72
9	9	10	4	70	0.74
17	10	10	6	80	0.5
10	11	10	8	70	0.77
1	12	5	4	80	0.69

15	13	10	6	80	0.5
3	14	5	8	80	0.69
4	15	15	8	80 80	0.72
12	16	108	8	90	0.73
11	17	10	4	90	0.7

Table. 3- Optimized Conditions for Tranexamic Acid

Sr.No	Factor	Optimized condition
1	Mobile Phase	Ethanol:Water:Glacial Acetic acid
2	Composition of mobile phase	7:3:0.1 V/V/V
3	Saturation Time	10 min
4	Band Length	6 mm
5	Solvent Front	80 mm
6	Response (Rf) for optimized condition	0.50

Table 4- System suitability parameters for Tranexamic Acid

Drug Name	Concentration (Working Standard)	Rf Value	Area
Tranexamic Acid	800 ng/band	0.50	5956

Table 5- Response of ANOVA by Box-Behnken design for TA

Source	Sum of squares	Df	M Mean square	F-value	P-value	
Model	0.1714	9	0.0190	12.20	0.0017	Significant
A- ASaturation time	0.0010	1	0.0010	0.6487	0.4471	
B B-Band length	0.0055	1	0.0055	3.53	0.1024	
C-Solvent front	0.0072	1	0.0072	4.61	0.0688	
Residual	0.0109	7	0.0016			
Lack of fit	0.0109	3	0.0036			
Pure Error	0.0000	4	0.0000			
Cor Total	0.1823	16				

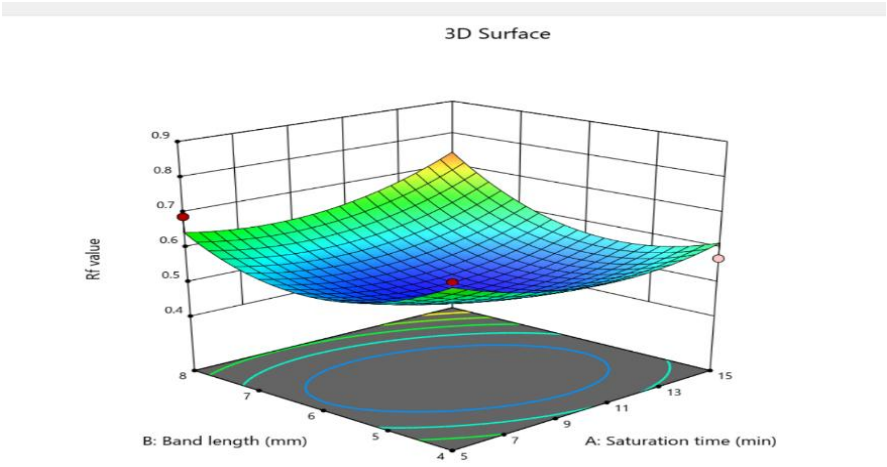

Fig.4 – 3D surface plots showing interaction between saturation time (A) and Band length (B)

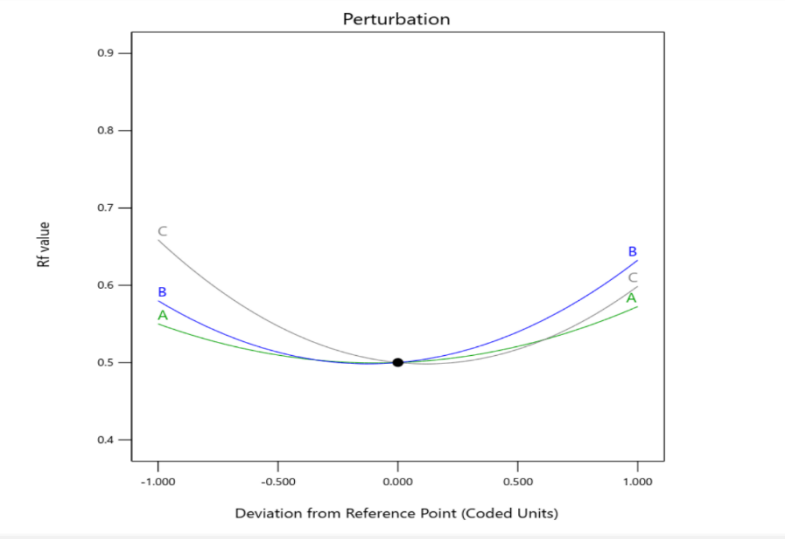

Fig.5 - Perturbation Plot for Factor A, Factor B and Factor C

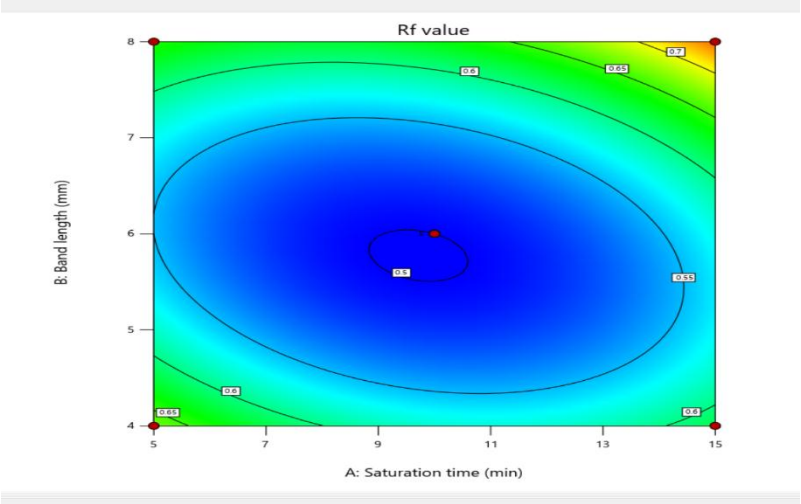

Fig.6- Method operable design region (MODR) for Factor A (Saturation time) and Factor (Band length)

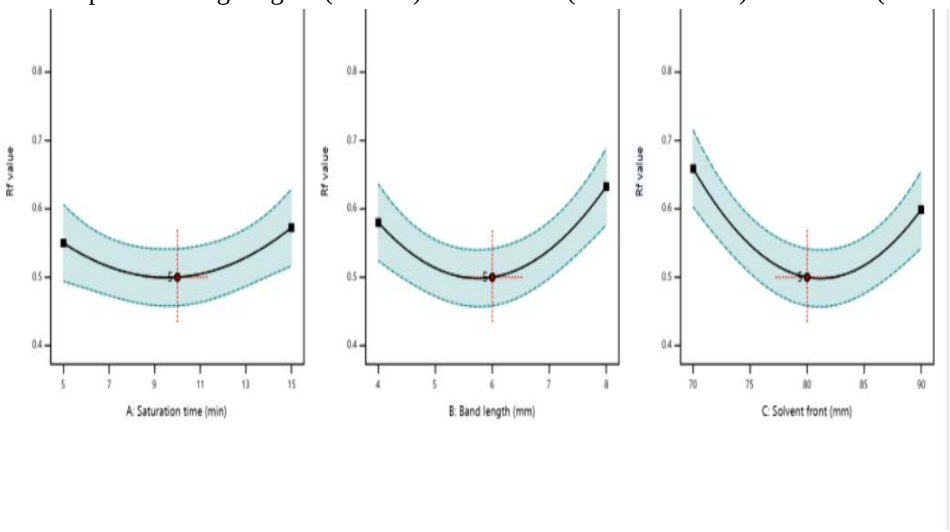

Fig.7- Relationship between saturation time (A), band length (B) and solvent front (C) and their response (Rf)

VALIDATION OF DOE -BASED HPTLC METHOD

1) Linearity

A linear relationship was established between peak area and concentration of Tranexamic acid within the range of 200-1200 ng/band. The linearity of

calibration curve was confirmed through the value known as regressioncoefficient (R^2)and the Y-intercept were found to be 0.993 and Y=3.7298x + 2805.4 respectively as shown in (Fig.8.).

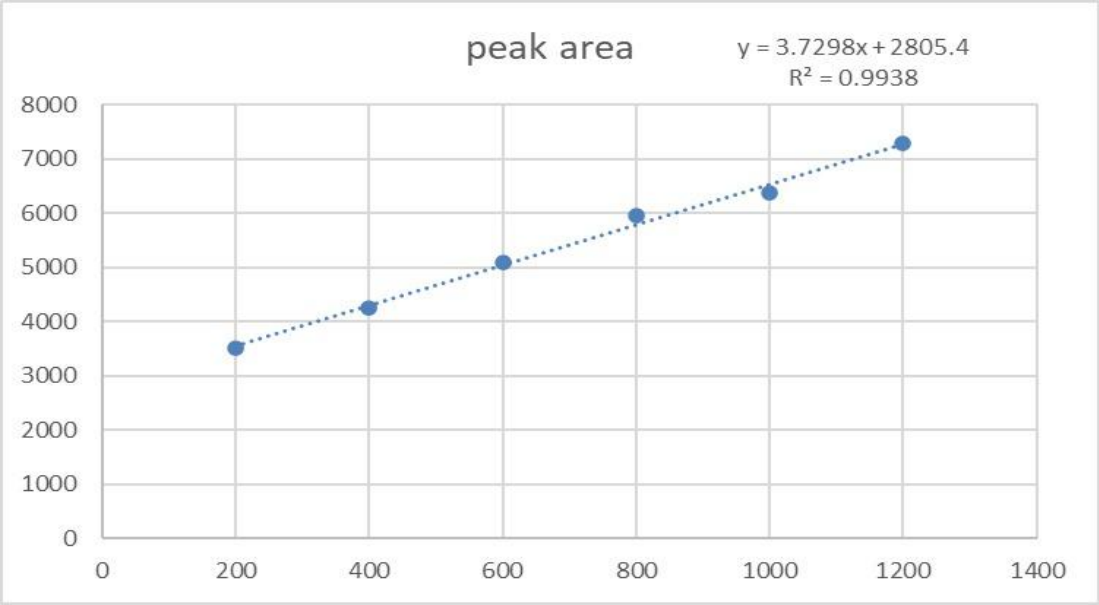

Fig.8 -Calibration curve for Tranexamic Acid (200-1200 ng/band)

2) Precision

The % RSD for intraday is found to be 0.39 to 0.61 % and for inter day % RSD was found and 0.085 to 0.20%, which denote the precision of method as shown in table 6. The measured %RSD for concentrations were below 2%, signifies the better precision of the developed method.

3) Accuracy

The percentage recovery was found to be 98.10 to 102.9% which signify the accuracy of the method as shown in table 8.

4) LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were found to be 4.12 ng/band and 12.4 ng/band for Tranexamic acid respectively, indicating the sensitivity of the developed method.

5) Specificity

There is no interference of solvent, and mobile phase. The developed method was found to be very specific for Tranexamic acid.

6)Robustness

For robustness of method, it was observed that the minor changes in the mobile phase volume and wavelength did not have significant impact on the developed method. The % RSD was found to be below 2, hence the method was found to be robust.

7) Method Precision (Assay)

Through experiments, it was determined that the amount of Tranexamic acid in the tablets, expressed as a percentage of the label claim, was in good agreement with the label claims. This suggests that none of the excipients that are generally present in tablets had interfered. It was discovered that the drug content was 98.24%± 1.38. Table No.6 provides a summary of the findings.

Table 6- Summary of pharmaceutical validation parameters

Sr. No.	Validation parameters	Results
1.	Linearity equation R^2	Y=3.7298x+ 2805.4 R^2= 0.9938
2.	Range	200-1200 ng/band
3.	Precision	(% RSD)
	Intra-day	0.39 to 0.61 %
	Inter-day	0.085 to 0.20%,
4.	% Assay (Mean ± %RSD)	98.24% ± 1.38 %
5.	Accuracy	Mean ± %RSD

	80 %	98.10% ± 1.63 %
	100 %	99.81%± 0.84
	120 %	102.9 %± 1.17%
6.	Limit of detection	4.12 ng/band
7.	Limit of quantitation	12.4 ng/band
8.	Specificity	Specific
9.	Robustness	Robust

FORCED DEGRADATION STUDY

Acid (0.1 N HCl) degradation: Acid (0.1 N HCl) degradation: Thedensitogram of the acid degradation sample displayed three additional peaks with Rf values of 0.11 and 0.52, along with the peak of Tranexamic acid. On acid degradation, 21.87% of degradation was observed in the Tranexamic acid as shown in fig no.9.

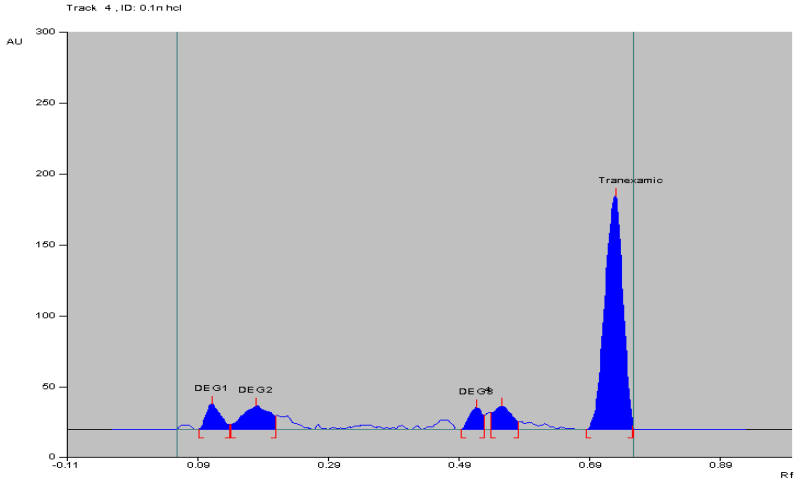

Fig. 9-Densitogram of Tranexamic Acid (800 ng/band) in acid exposure (0.1 N HCl) for 1 h

Alkali (0.1 N NaOH) degradation: The densitogram of the alkali degradation sample displayed two additional peaks with Rf values of 0.09 and 0.65, along with the peak of Tranexamic acid. On alkaline degradation, 14.68 % of degradation was observed in the Tranexamic acid as shown in fig no.10.

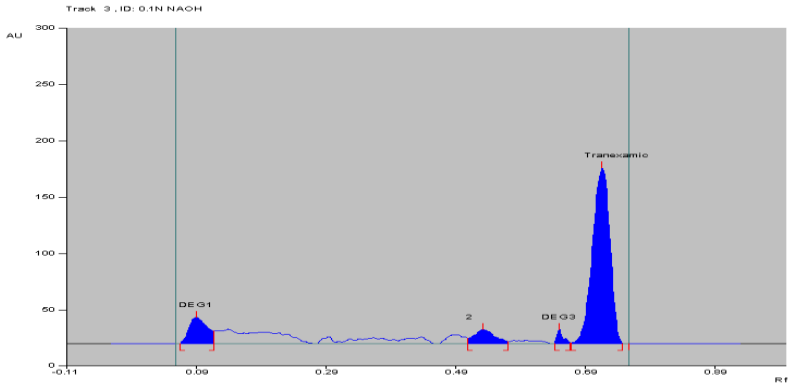

Fig. 10-Densitogramof Tranexamic Acid (800 ng/band) in alkaline exposure (0.1 N NaOH) for 1 h

Oxidative (3% H2O2) degradation: Thedensitogram of the alkali degradation sample displayed two additional peaks with Rf values of 0.54 and 0.65, along with the peak of Tranexamic acid . On oxidative degradation, 30.01 % of degradation was observed in the Tranexamic acid as shown in fig no.11.

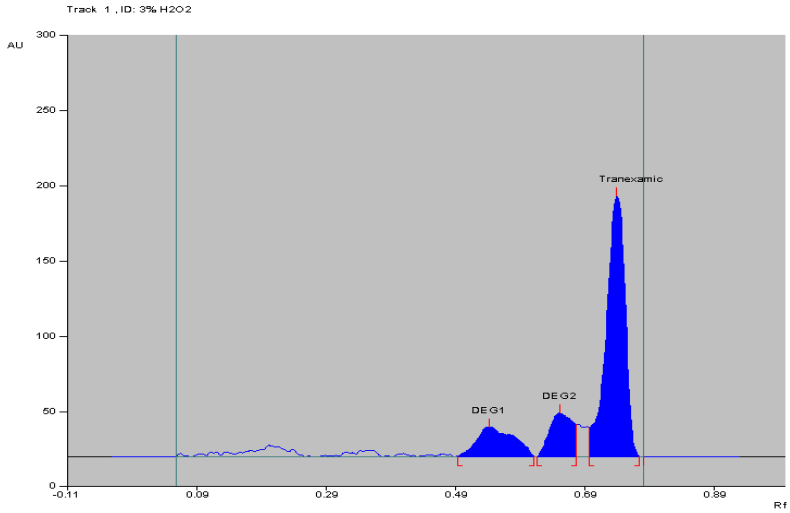

Fig. 11-Densitogram of Tranexamic Acid (800 ng/band) in oxidative exposure (3% H_2O_2) for 1 h

Neutral (Distilled water) degradation: Thedensitogram of the alkali degradation sample displayed one additional peak with Rf values of 0.57, along with the peak of Tranexamic acid. In neutral degradation, 19.78 % of degradation was observed in the Tranexamic acid as shown in fig no.12.

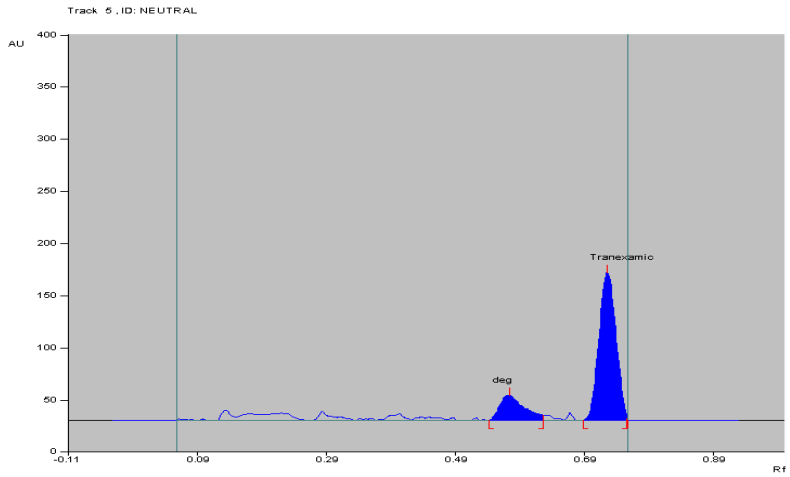

Fig.12-Densitogram of Tranexamic Acid (800 ng/band) in Neutral exposure (Distilled water) for 1 h

Thermal degradation: Thedensitogram of the thermal degradation sample displayed three additional peaks with Rf values of 0.52, 0.57 and 0.67, along with the peak of Tranexamic acid. On thermal degradation, 18.78 % of degradation was observed in the Tranexamic acid as shown in fig no.13.

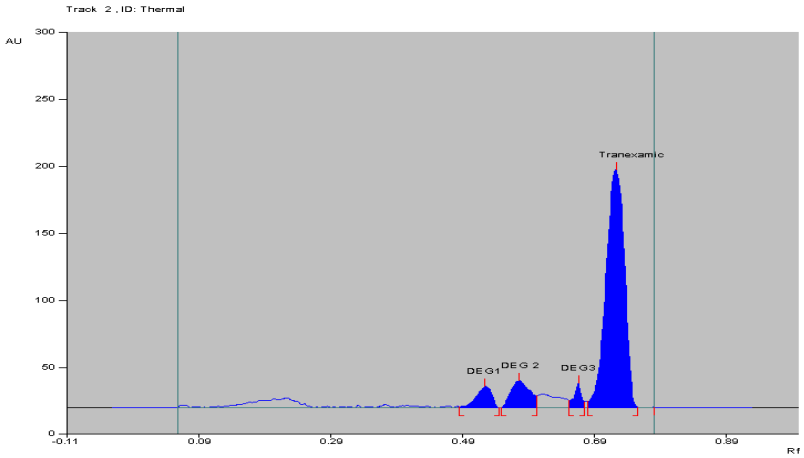

Fig. 13-Densitogram of Tranexamic Acid (800 ng/band) in Thermal exposure (40^0C) for 30 minutes.

Table 7 - Result of force degradation studies by proposed HPTLC method

Stress Conditions	Number of degradation peaks and Rf	Temperature and time	% Degradation of Tranexamic Acid
Acid (0.1N HCL)	3 (0.11, 0.18,0.52)	At room temp. for 1 h	21.87%
Base (0.1N NaOH)	2 (0.09, 0.65)	At room temp. for 1 h	14.68%
Oxidative (3% H_2O_2)	2 (0.54,0.65)	At room temp. for 1 h	30.01%
Neutral (DistilledWater)	1 (0.57)	At room temp. for 1 h	19.78%
Thermal	3(0.52, 0.57,0.67)	40^0C for 30 min	18.78%

IV. CONCLUSION

A robust, accurate, and precise stability-indicating HPTLC method for the estimation of Tranexamic Acid was successfully developed and optimized using the Box-Behnken design. This statistical design enabled the systematic evaluation of critical method parameters, such as saturation time, band length, and solvent front, and their interactive effects on the Rf value. The method demonstrated excellent linearity (R^2 = 0.9938), sensitivity (LOD: 4.12 ng/band, LOQ: 12.4 ng/band), and precision (intra- and inter-day %RSD < 2%).

The method effectively separated Tranexamic Acid from its degradation products under various stress conditions, confirming its stability-indicating capability. The optimization through Box-Behnken design provided a scientifically justified approach to achieve optimal conditions with minimal experimental trials. The method also proved to be accurate (recovery 98.10%–102.9%), specific, and robust, making it suitable for routine quality control and stability studies of Tranexamic Acid in pharmaceutical formulations.

Conflict of interest: It is hereby declared that there is no conflict of interest among authors.

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