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Research Article

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Tranexamic Acid and Ethamsylate in Bulk and Formulation

Narasapalli Narasimha Reddy[1], J Sumalatha[2]*, P Salomi[1] and S Nelson Kumar[3]

[1]Department of Pharmaceutical Analysis, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, – 516003, India

[2]Department of Pharmaceutical Chemistry, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, – 516003, India

[3]Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, – 516003, India

***Corresponding Author:** J Sumalatha, Department of Pharmaceutical Chemistry, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, – 516003, India.

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Abstract

A simple, swift, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Tranexamic acid and Ethamsylate in pharmaceutical dosage form. Chromatographic separation of Tranexamic acid and Ethamsylate was achieved on Waters Alliance-e2695 by using Zorbax SB C18 (150x 4.6mm, 3.5µ) column and the mobile phase containing Ethanol: 0.1% TFA in the ratio of 5:95% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 250nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Tranexamic acid and Ethamsylate were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The analytical method developed in this study underwent rigorous validation adhering to the International Council for Harmonisation guidelines to ensure its suitability for intended pharmaceutical analysis applications. The method was found to be simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Tranexamic acid and Ethamsylate study of its stability.

Keywords: HPLC; Stability Indicating; Tranexamic Acid and Ethamsylatum

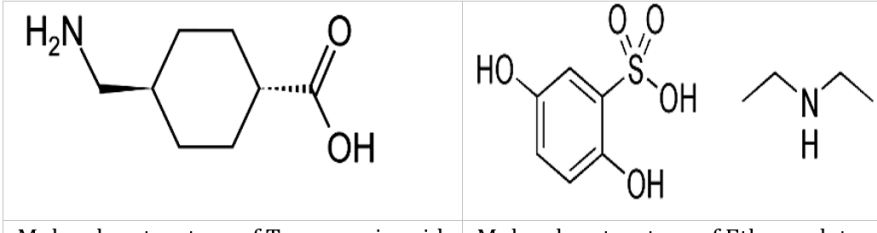
Introduction

Tranexamic acid (TXA) and ethamsylate (ETS) are commonly prescribed hemostatic agents employed to prevent and treat various forms of hemorrhage. Tranexamic acid is a synthetic derivative of the amino acid lysine that acts as an antifibrinolytic by reversibly blocking lysine binding sites on plasminogen molecules, thereby inhibiting fibrinolysis and stabilizing blood clots [1]. Ethamsylate, on the other hand, functions through a different mechanism by improving platelet adhesion and restoring capillary resistance, thus facilitating primary hemostasis without directly influencing the coagulation cascade [2].

The simultaneous quantification of tranexamic acid and ethamsylate is crucial in combined pharmaceutical formulations, where both agents may be used synergistically to achieve enhanced hemostatic control. Reliable analytical methods are necessary for ensuring the quality, efficacy, and safety of these formulations throughout their shelf life. High-performance liquid chromatography (HPLC) has been widely utilized for the separation and quantification of such agents due to its high sensitivity, specificity, and reproducibility [3].

Analytical method development involves the process of selecting conditions that will allow for the efficient, accurate, and reproducible quantification of the target analytes. Method validation, as per the International Council for Harmonisation (ICH) guidelines [4], is essential to confirm that the developed method meets the necessary requirements for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, and system suitability.

The review of the literature indicates that only a few analytical techniques [5-11] are available for the quantification of Tranexamic acid and ethamsylate. The developed method has been validated as per ICH guidelines [12,13]. The present work aims to develop and validate a robust, accurate, and reliable analytical method for the simultaneous estimation of tranexamic acid and ethamsylate in pharmaceutical dosage forms, providing a scientific foundation for routine quality control and regulatory submission.



Molecular structure of Tranexamic acid	Molecular structure of Ethamsylate

Figure 1

Materials and Methods

Chemicals and reagents

Tranexamic acid and Ethamsylate pure drugs were received from Pharma life research lab, Hyderabad. Tranexamic acid and Ethamsylate tablets were obtained from the local market. Acetonitrile, Ethanol HPLC grade was received from Rankem, Water (Milli Q) was produced in-house, and Ammonium Formate, orthophosphoric acid, Formic Acid and Tri fluoro acetic acid of HPLC grade were obtained from analytical reagents.

Preparation of standard solution

Precisely weigh and transfer 10mg of Tranexamic acid and 5mg of Ethamsylate working standard into a 10ml volumetric flask, add Diluent, and sonicate to fully dissolve it, then bring the volume up to the mark with the same solvent. (Stock solution).

Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (100ppm of Tranexamic acid, 50ppm of Ethamsylate).

Preparation of sample solution

Weigh and transfer 17mg of Tranexamic acid and Ethamsylate sample into a 10 mL clean dry volumetric flask, add diluent, and sonicate for up to 30 minutes to ensure complete dissolution, then centrifuge for 30 minutes. Filter the solution through 0.45 micron injection filter (Sample stock solution).

Further pipette 1 ml of the above stock solutions into 10 ml volumetric flask and dilute up to the mark with diluents. (100ppm of Tranexamic acid, 50ppm a of Ethamsylate).

Inject 10µL of the standard, sample into the chromatographic system and measure the areas for Tranexamic acid and Ethamsylate peaks and calculate the %Assay by using the formulae.

Preparation of Diluent (Mobile Phase): Mobile phase was prepared by mixing Ethanol and 0.1% TFA taken in the ratio 5:95. It was passed through a 0.45µ membrane filter to eliminate the impurities that could affect the final chromatogram.

Method validation

Specificity

The ability of an analytical method to measure the analyte of interest without interference from blank and known impurities is its specificity. For this purpose, chromatograms of the blank, standard, and sample were recorded. The chromatogram of the blank showed no response at the retention times of the drugs, confirming that the drug responses were specific".

Linearity

Preparation of stock solution

Accurately weigh 10 mg of Tranexamic Acid and 5 mg of Ethamsylate working standards, and transfer them into a clean, dry 10 mL volumetric flask. Add an appropriate volume of diluent and sonicate to ensure complete dissolution. Subsequently, dilute to volume with the same diluent.

From the above stock solution 25, 50, 75, 100, 125, 150 ppm of Tranexamic acid, 12.50, 25, 37.50, 50, 62.50, 75 ppm of Ethamsylate were prepared using the diluents.

Inject each concentration level into the chromatographic system and record the corresponding peak areas. Construct a graph plotting concentration on the X-axis and peak area on the Y-axis, then determine the correlation coefficient.

Range

According to ICH guidelines, the range of an analytical method is defined as the interval between the lowest and highest concentrations of the analyte (inclusive) that have been demonstrated to provide acceptable levels of precision, accuracy, and linearity.

Preparation accuracy sample solutions

For preparation of 50% solution (With respect to target Assay concentration)

Accurately weigh and transfer 8.5 mg of Tranexamic acid and Ethamsylate sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Transfer 1 mL of the prepared stock solution into a 10 mL volumetric flask and dilute to volume with the diluent. (50ppm of Tranexamic acid and 25ppm of Ethamsylate).

For preparation of 100% solution (With respect to target Assay concentration)

Accurately weigh and transfer 17mg of Tranexamic acid and Ethamsylate sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Transfer 1 mL of the prepared stock solution into a 10 mL volumetric flask and dilute to volume with the diluent. (100ppm of Tranexamic acid and 50ppm of Ethamsylate).

For preparation of 150% solution (With respect to target Assay concentration)

Accurately weigh and transfer 25.5mg of Tranexamic acid and Ethamsylate sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Transfer 1 mL of the prepared stock solution into a 10 mL volumetric flask and dilute to volume with the diluent. (150ppm of Tranexamic acid and 75ppm of Ethamsylate).

Procedure

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Precision

Precision refers to the reproducibility of an analytical method under routine operating conditions. System precision is evaluated using a standard chemical substance to verify that the analytical system is performing correctly.

In this peak area and %of drug of six determinations is measured and % RSD should be calculated.

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD.

The precision of the instrument was checked by repeatedly injecting(n=6) solutions of 100ppm of Tranexamic acid, 50ppm of Ethamsylate).

Robustness

As part of the robustness evaluation, deliberate variations were introduced in the flow rate, mobile phase composition, and temperature to assess their impact on the method.

The flow rate was varied at 0.9 ml/min to 1.1ml/min.

A standard solution of 100 ppm Tranexamic Acid and 50 ppm Ethamsylate was prepared and analyzed using both the method flow rate and varied flow rates. Upon evaluating the results, it can be concluded that changes in flow rate significantly impacted the method. However, the method proved to be robust, even with a ±10% variation in the flow rate.

The variation of Organic Phase ratio.

Standard solution of 100ppm of Tranexamic acid, 50ppm of Ethamsylate was prepared and analysed using the varied in mobile phase ratio.

Limit of detection (LOD) and limit of quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the drug were calculated using the following equations, in accordance with the guidelines set by the International Conference on Harmonisation (ICH)

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Results and Discussion

The chromatographic analysis was performed using a Zorbax SB C18 (150x4.6 mm, 3.5 µ) with a mobile phase consisting of Ethanol: 0.1% TFA (5:95). The flow rate was set at 1 ml/min, and the detector wavelength was 250 nm. The column temperature was maintained at 25°C, and an injection volume of 10 µL was used. The total run time for each analysis was 5.0 minutes. Both peaks have good resolution, tailing factor, Theoretical plate count, and resolution. The total runtime for each validation parameter was set to 5 minutes.

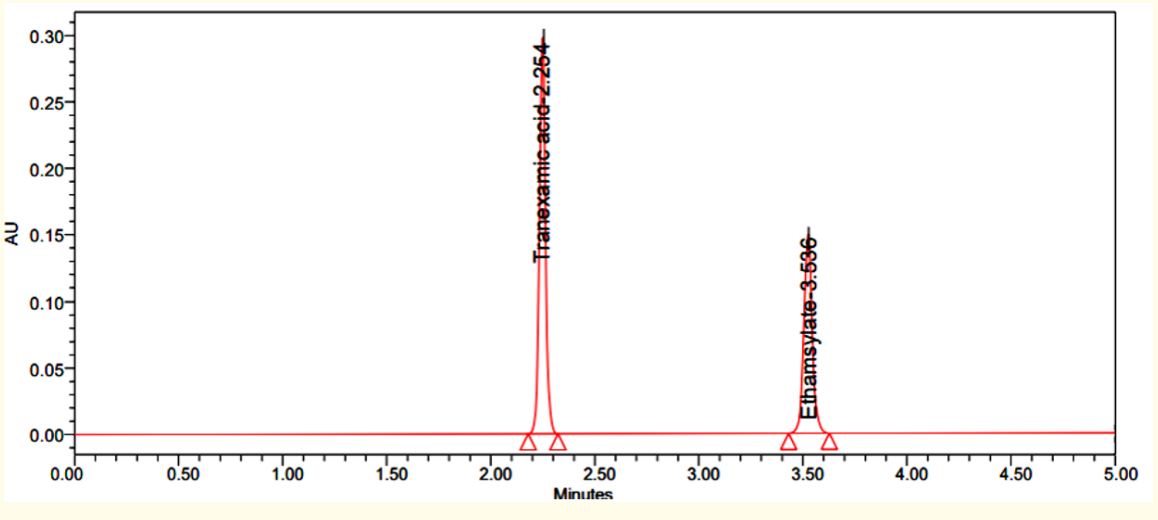


Figure 2: Optimized Chromatogram.

With the optimized chromatographic conditions, the Tranexamic acid peak was observed at 2.254 min with peak area 2857456, tailing factor 1.12 Ethamsylate peaks was observed at 3.536 min, with peak area 1538763, tailing factor 1.09 and resolution 10.48.

Method validation

The HPLC method for the determination of Tranexamic Acid and Ethamsylate was validated in accordance with the protocol to demonstrate its suitability for the intended purpose. All validation parameters were conducted following ICH guidelines.

Specificity

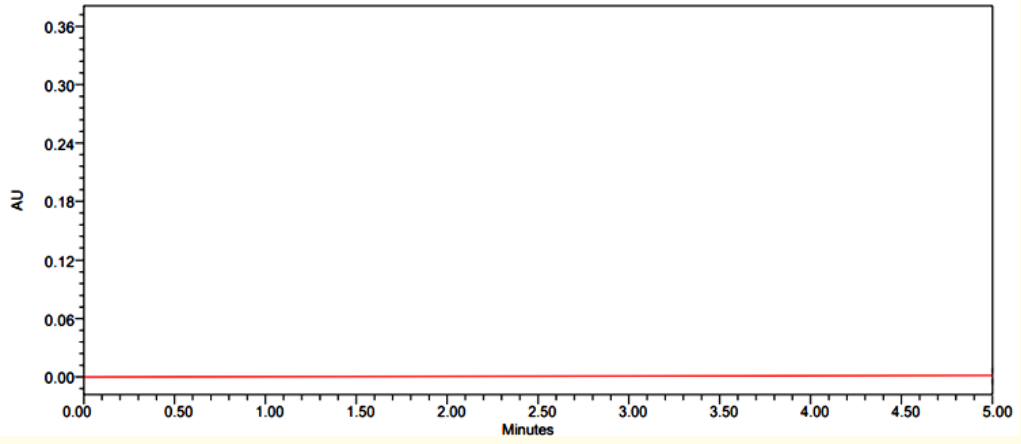


Figure 3: Chromatogram of blank.

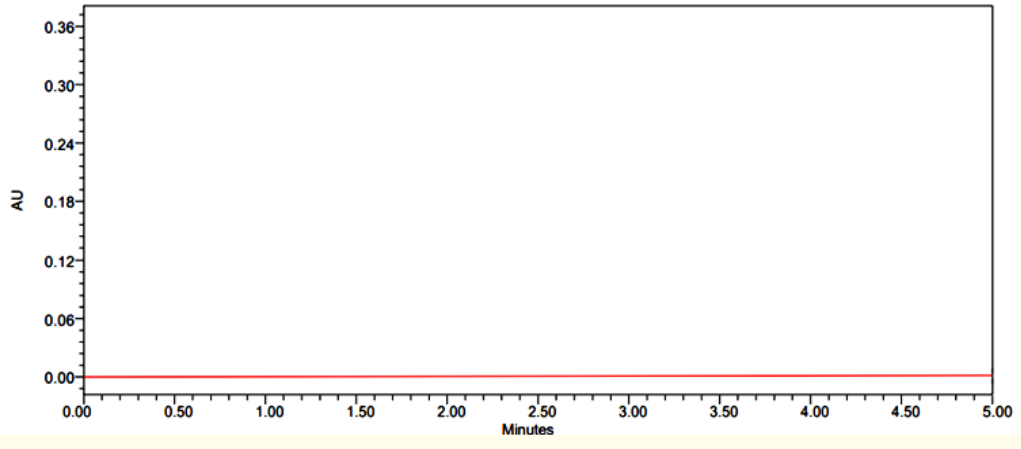


Figure 4: Chromatogram of placebo.

Linearity

Six linear concentrations of Tranexamic acid (25–150 µg/ml) and Ethamsylate (12.50-75.00 µg/ml) were injected and duplicated. Average areas were mentioned in table No. 1, and the linearity equations obtained for Tranexamic acid was y = 28592.23x +4916.32, and for Ethamsylate, was y =29567.73x +10663.04. The correlation coefficient obtained was 0.999 for the two drugs.

Precision

Method precision

The %RSD (Relative Standard Deviation) for Tranexamic acid and Ethamsylate was calculated using six replicate injections. The mean area for Tranexamic acid was 2837835 with a standard deviation (S.D) of 12487.94, resulting in a %RSD of 0.44%. Similarly, the mean area for Ethamsylate was 1525666, with a standard deviation of 7744.427, giving a %RSD of 0.51%. These low %RSD values indicate high precision and reproducibility of the method for both compounds.

S. No.	Tranexamic acid		Ethamsylate	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	25.00	705496	12.50	396854
2	50.00	1456303	25.00	738965
3	75.00	2147554	37.50	1102437
4	100.00	2847978	50.00	1528756
5	125.00	3630134	62.50	1854852
6	150.00	4257870	75.00	2214306
Regression equation	y = 28592.23x +4916.32		y =29567.73x +10663.04	
Slope	28592.23		29567.73	
Intercept	4916.32		10663.04	
R^2	0.99983		0.99966	

Table 1: Results of linearity for Tranexamic acid and Ethamsylate.

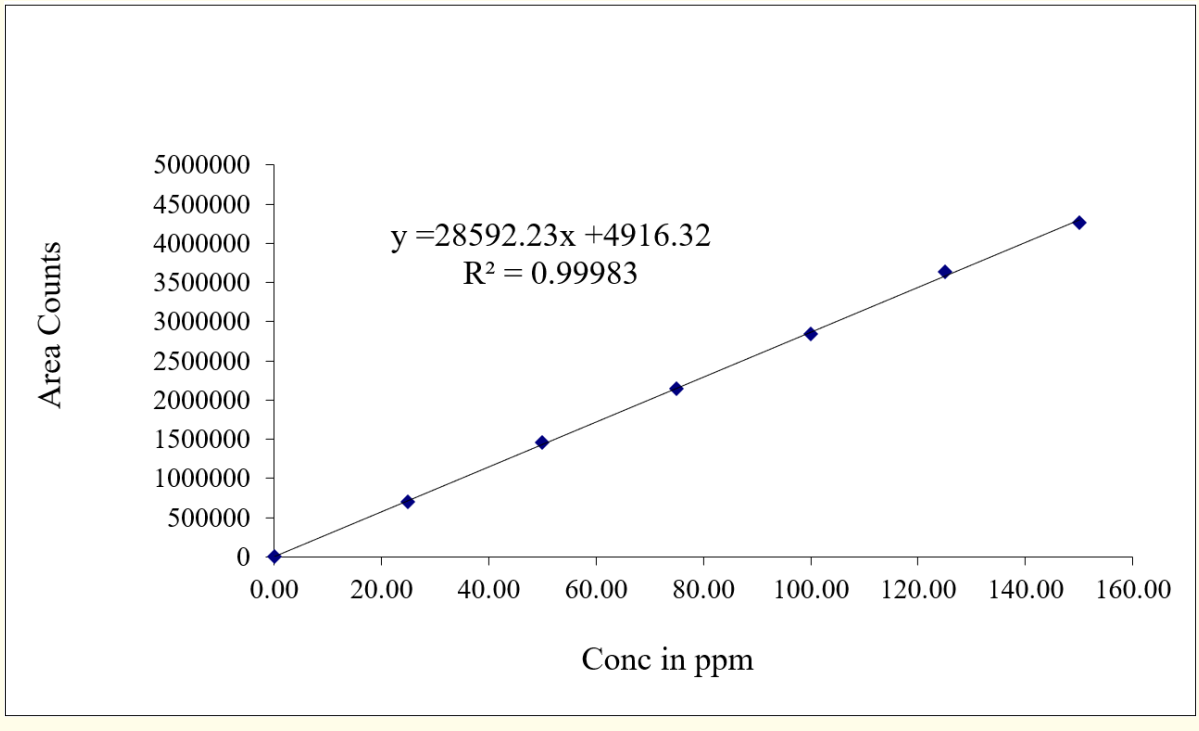


Figure 5: Calibration curve for Tranexamic acid 250 nm.

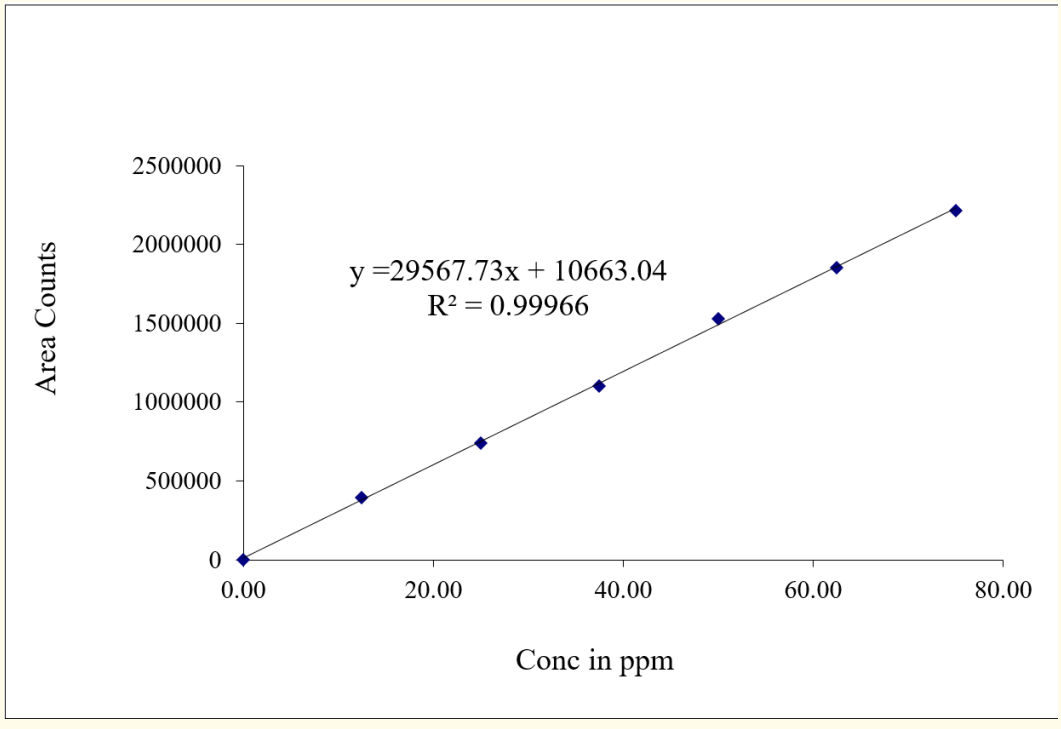


Figure 6: Calibration curve for Ethamsylate at 250 nm.

Intermediate precision (Day_ Day Precision)

Intermediate precision of Tranexamic acid and Ethamsylate based on six replicate injections. The mean area for Tranexamic acid on day-1 and day-2 was 2837747, 2836379 with a standard deviation (S.D) of 12453.74, 17404.10 resulting in a %RSD of 0.44%, 0.61% respectively. Ethamsylate mean area on day-1 and day-2 was 1522266, 1513972 with a standard deviation of 12706.86, 11029.49 yielding a %RSD of 0.83%, 0.73% respectively. These results indicate good intermediate precision for both analytes, demonstrating the method's consistency when tested under different conditions or over different days.

Accuracy

Three levels of accuracy samples were prepared using the standard addition method. Triplicate injections were performed for each accuracy level, and the mean % recovery was found to be 99.9% for Tranexamic Acid and 100.0% for Ethamsylate.

Sensitivity

The Limit of Detection (LOD) for Tranexamic acid was 0.6 µg/ml, and the Limit of Quantitation (LOQ) was 2 µg/ml. Ethamsylate LOD was 0.3 µg/ml, and the LOQ was 1 µg/ml. These values indicate the method's ability to detect and quantify deficient concentrations of both analytes with high sensitivity.

Assay

Lysteda®, bearing the label claim Tranexamic acid and Ethamsylate. An assay was performed with the above formulation. The average % Assay for Tranexamic acid and Ethamsylate obtained was 100.6% and 100.8%, respectively.

Robustness

The method's robustness for Tranexamic acid and Ethamsylate was evaluated under various conditions by altering the flow rate, mobile phase composition, and temperature. When the flow rate was decreased to 0.9 ml/min, the %RSD for Tranexamic acid was 0.21%, and for Ethamsylate, was 0.15%. When the flow rate was

increased to 1.1 ml/min, the %RSD values were 0.4% and 0.75%, respectively. For mobile phase composition changes, a ratio of 27A:73B resulted in %RSD values of 0.25% for Tranexamic acid and 0.21% for Ethamsylate, while a 33A:67B ratio yielded %RSDs of 0.35% and 0.63%. Lastly, temperature variations at 27°C showed %RSDs of 0.6% and 1.0%, indicating that the method is robust under these conditions.

Table 2: Robustness data for Tranexamic acid and Ethamsylate.

S. No.	Condition	%RSD of Tranexamic acid	%RSD of Ethamsylate
1	Flow rate (-) 0.9 ml/min	0.21	0.15
2	Flow rate (+) 1.1 ml/min	0.40	0.75
3	Mobile phase (-) 27A:73B	0.25	0.21
4	Mobile phase (+) 33A:67B	0.35	0.63

Degradation studies

The method's specificity was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, and thermal degradation. The sample was exposed to these conditions, and the main peak was studied for peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

Regulatory guidelines outlined in ICH Q2A, Q2B, Q3B, and FDA 21 CFR Section 211 necessitate the development and validation of stability-indicating potency assays.

Table 3: Forced Degradation results for Tranexamic acid and Ethamsylate.

Degradation	Area	% Assay	% Deg	Area	% Assay	% Deg
Control	3871216	100	0	99632	100	0
Acid	3454213	89.2	10.8	87596	87.9	12.1
Alkali	3352232	86.6	13.4	88696	89.0	11.0
Peroxide	3312784	85.6	14.4	86220	86.5	13.5
Reduction	3834218	99.0	1.0	96584	96.9	3.1
Thermal	3765041	97.2	2.8	95989	96.3	3.7
Photolytic	3698359	95.5	4.5	98795	99.1	0.9
Hydrolysis	3742106	96.6	3.4	98632	98.9	1.1

Conclusion

The mobile phase and solvents are easy to replace, economical, reliable, sensitive, and time-efficient. The developed HPLC method for the estimation of Tranexamic Acid and Ethamsylate is simple, rapid, accurate, precise, robust, and cost-effective. The mobile phase and solvents used are straightforward to prepare, economical, and reliable, offering sensitivity with reduced analysis time. The sample recoveries for both drugs were in excellent agreement with their respective label claims (99.9% for Tranexamic Acid and 100.0% for Ethamsylate), demonstrating no interference from formulation excipients. As such, this method is suitable for routine analytical applications in laboratories, providing a reliable approach for the determination of these drugs.

Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

Conflict of Interest

No conflict of interest exists.

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