

Design, development and validation of an RP-HPLC method for concurrent estimation of tranexamic acid and ethamsylate in bulk and pharmaceutical formulations

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Abstract

A reversed-phase high-performance liquid chromatography method is developed and validated for the determination of tranexamic acid Ethamsylate in bulk drug and marketed dosage forms. The chromatographic determination was performed on Shimadzu Lab solutions with a variable wavelength detector. The separation was conducted using thermoscientific Hypersil BDS (150 mm x 5 mm) with a mobile phase consisting of phosphate buffer: acetonitrile (80:20, %v/v) ratio. The mobile phase was delivered at a flow rate of 1.0 mL/min. The eluents were monitored at wavelength 280 nm and found sharp and symmetrical peaks with retention times of 3.27 and 4.27 min. The method was validated for linearity, accuracy, precision, and system suitability. The method was found to be linear over the concentration range 10-30 μ g/mL, 10-30 μ g/ml, with regression 0.999. The percentage recoveries for Tranexamic acid and Ethamsylate were found to be in the range of 100.41% and 100.31 %, respectively. The developed HPLC technique is precise, specific, accurate, and stable. Hence, this study proves that the method is reproducible, selective, and suitable to be applied for the analysis of tranexamic acid Ethamsylate in commercial pharmaceutical dosage form for quality control applications.

Keywords: Tranexamic acid; Ethamsylate RP-HPLC; Dosage form; Quality control

1. Introduction

1.1. Analytical techniques: [1;2]

Analytical methodology is a technique or procedure for determining either a physical or chemical change in a chemical substance; chemical element; or combination; or both. Analytical techniques used for analysis range from basic weighing in gravimetric analysis through titrimetric approaches to quite sophisticated techniques requiring highly specialized apparatus.

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1.2. Drug profile

1.2.1. Tranexamic acid

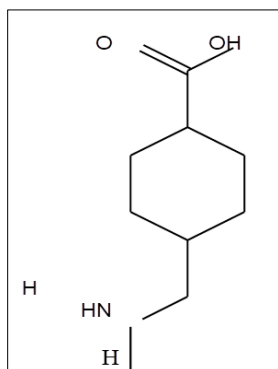


Figure 1 Chemical Structure of Tranexamic acid

IUPACNAME	4- (Amino ethyl) cyclo hexane carboxylic acid
Molecular Formula	C ₈ H ₁₅ N ₂ O ₂
Molecular Weight	157.21g/mol
Solubility	Freely Soluble in ethanol; water
Category	Antifibrinolytic
Storage conditions	Store at room temperature

1.2.2. ETHAMSYLATE

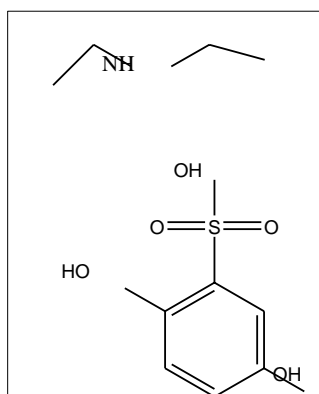


Figure 2 Chemical Structure of Ethamsylate

IUPACNAME	2;5-dihydroxybenzenesulfonic acid
Molecular Formula	C ₉ H ₉ N ₂ O ₅ S
Molecular Weight	243.24gms/mol
Solubility	Freely Soluble in ethanol; water; poorly soluble methanol; insoluble in glacial acetic acid.
Category	Haemostatic
Description	White or slightly yellowish crystalline powder

2. Method development

Table 1 Solubility studies

Solvent	Water	Ethanol	Methanol
Tranexamic acid	Freely Soluble	Soluble	Poorly Soluble
Ethamsylate	soluble	Soluble	Soluble

2.1. Determination of wavelength by UV-Visible spectrophotometric method: Preparation of Standard stock solution (1000 $\mu\text{g/ml}$)

Weigh accurately 10 mg of Tranexamic acid and 10 mg of Ethamsylate accurately and transfer into two different 10 mL clean dry volumetric flask with 7 mL of diluent to dissolve and volume made up to the mark with diluent (1000 $\mu\text{g/mL}$).

2.2. Preparation of sample solution

Weigh accurately 820 mg of tablet powder and transfer into 25 mL volumetric flask. Then; 15 mL of diluent was added and mixed with a cyclone mixer. The volume was then made up to the specified level using the diluent and filtered through a 0.45 Millipore Nylon filter. From the above solution pipette; out the 1 mL and transferred into 10 mL volumetric flask made up the volume with 10 mL diluent. Pipette 0.5 mL of solution into a volumetric flask that holds 10 mL; and then add diluent to fill the flask to the required level.

2.3. Selection of wave length (max)

The wavelengths of Tranexamic acid and Ethamsylate were determined separately by scanning the spectrum from 200 to 400 nm using the UV-Visible spectrophotometric technique. Scanning was done with 20 $\mu\text{g/mL}$ of Tranexamic acid and 10 $\mu\text{g/mL}$ of Ethamsylate solutions. By overlaying the individual spectra; the detection wavelength was found to be 280 nm. The HPLC system's PDA detector was set at 280 nm for the analysis.

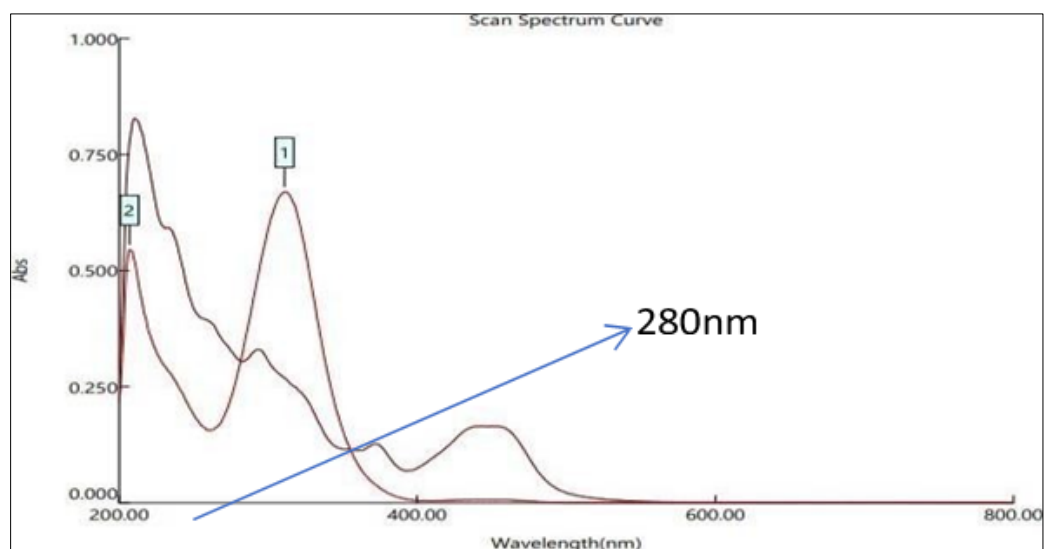


Figure 3 Combined spectrum of Tranexamic acid and Ethamsylate

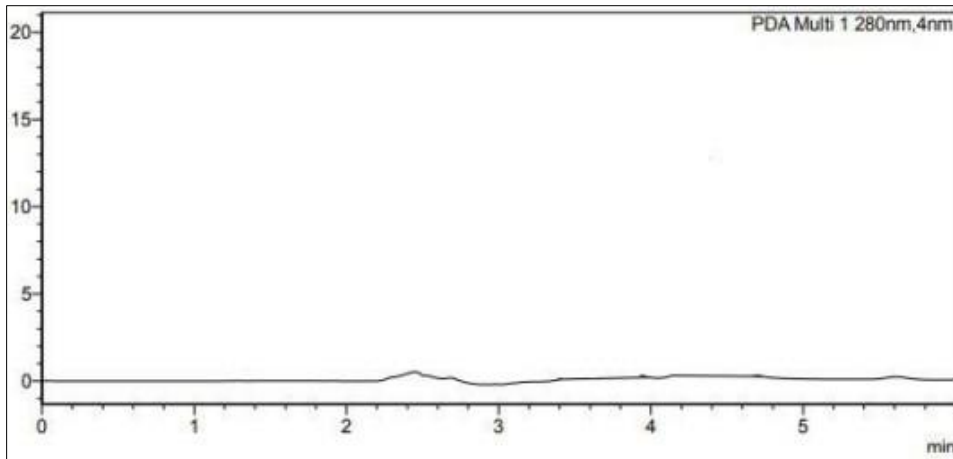


Figure 4 Blank chromatogram

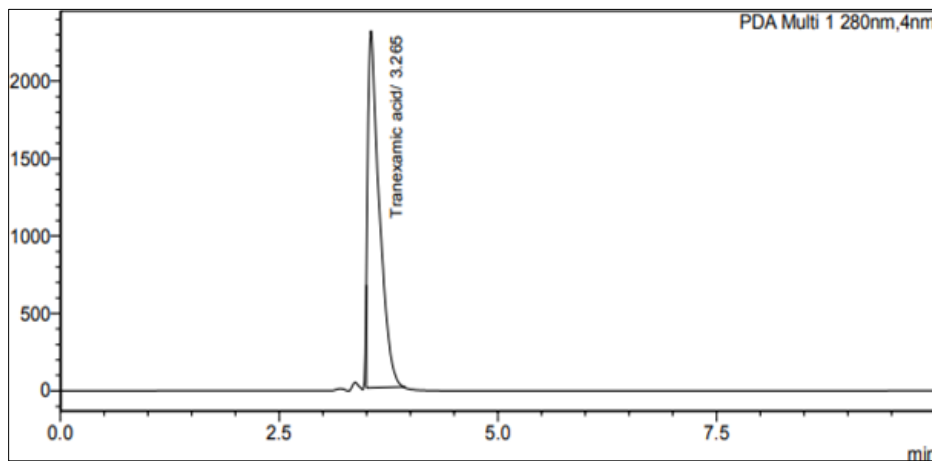


Figure 5 Tranexamic acid chromatogram

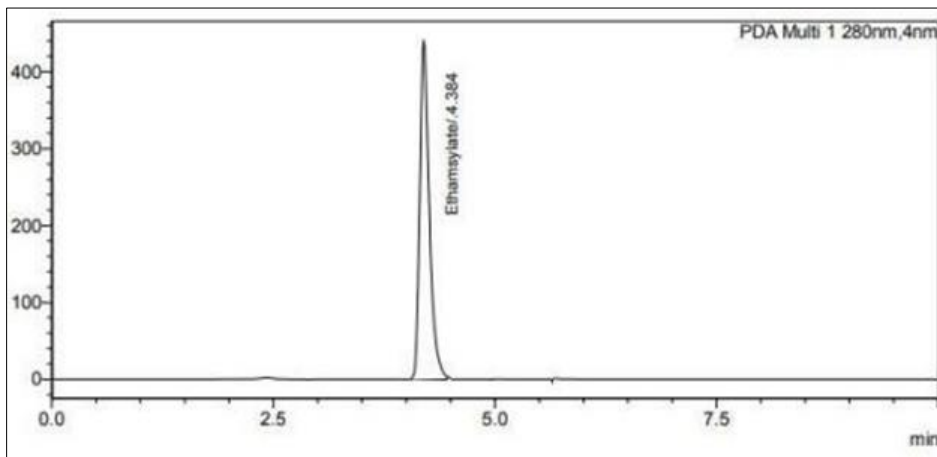


Figure 6 Ethamsylate chromatogram

2.4. Method development

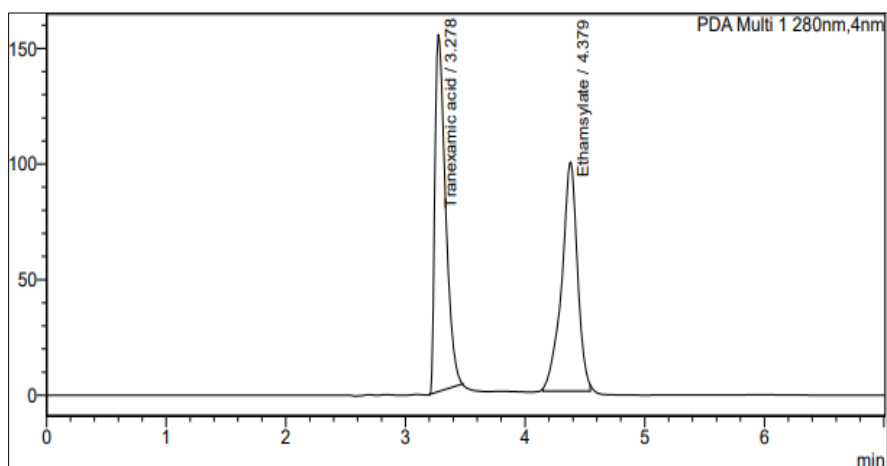


Figure 7 Standard chromatogram

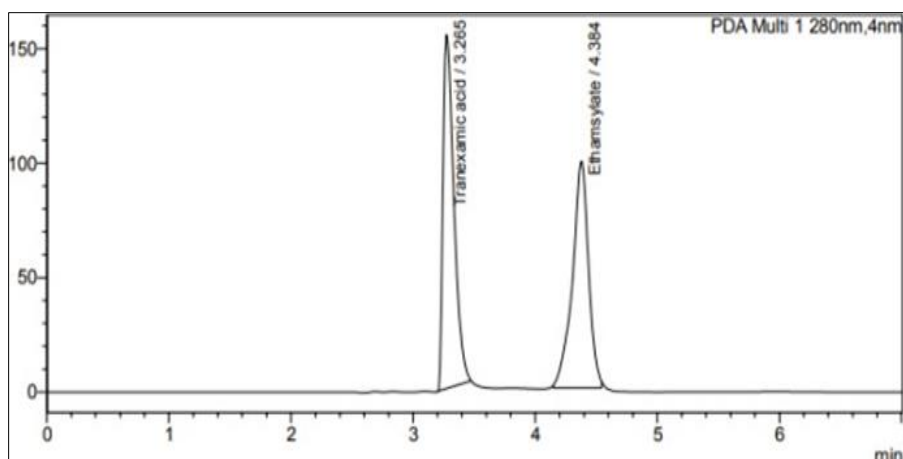


Figure 8 Sample chromatogram

3. Results and discussion

Table 2 Solubility studies

Solvent	Water	Ethanol	Methanol
Tranexamic acid	Freely Soluble	Freely Soluble	Soluble
Ethamsylate	Insoluble	Soluble	Low Soluble

3.1.1. Determination of wavelength by UV-Spectrum:

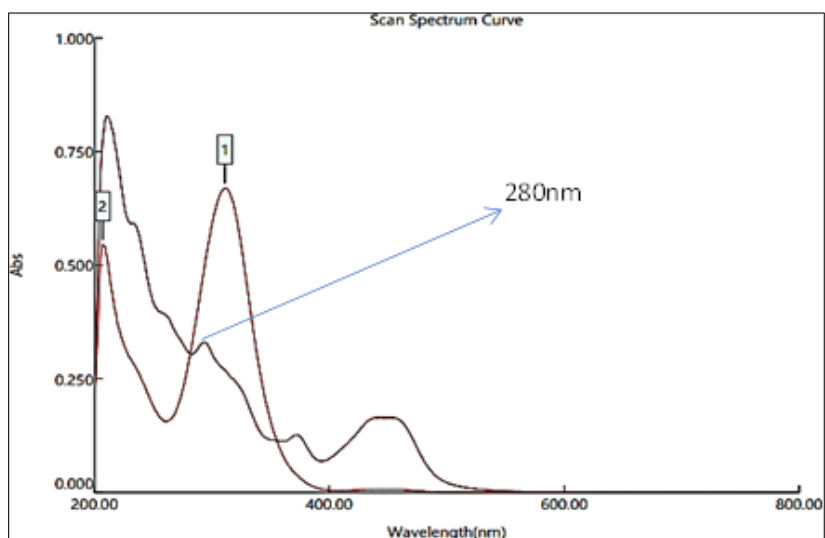


Figure 9 Combined Spectrum of Tranexamic acid and Ethamsylate

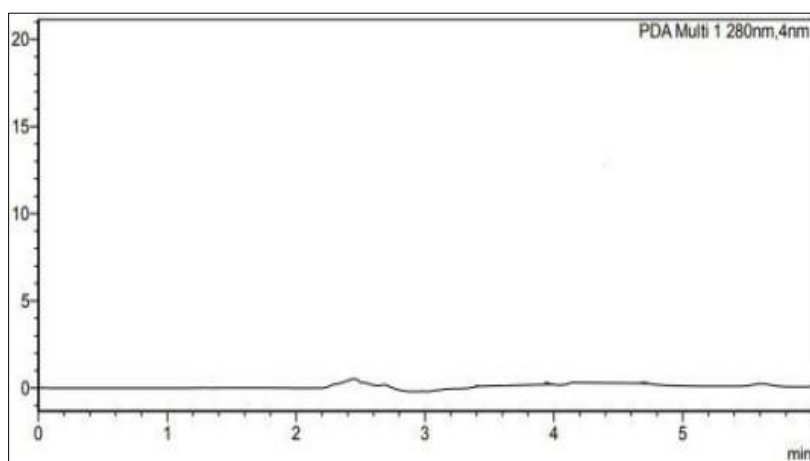


Figure 10 Blank chromatogram

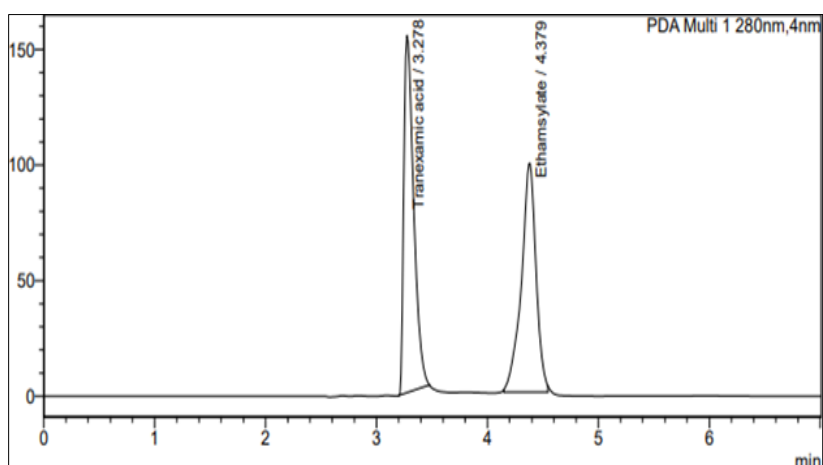


Figure 11 Chromatogram of standard

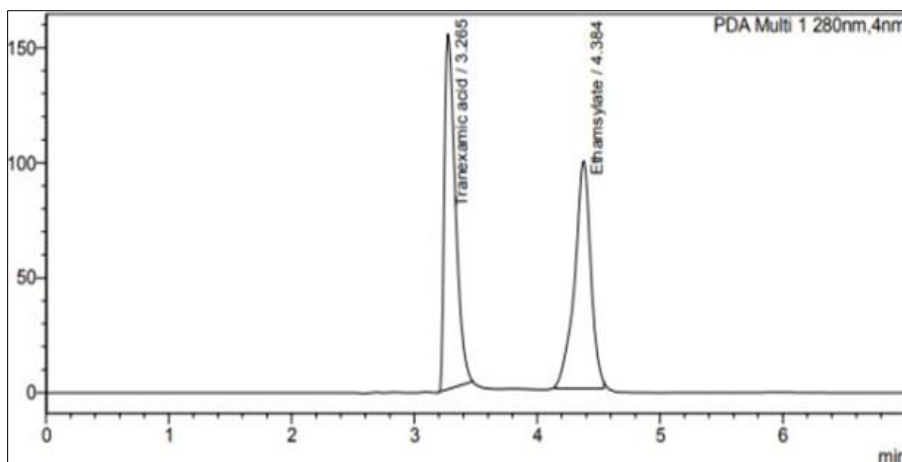


Figure 12 Chromatogram of sample

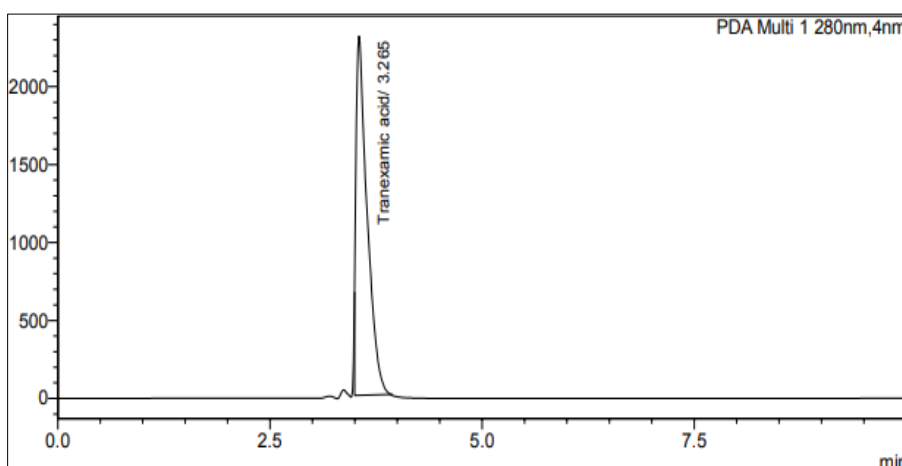


Figure 13 Chromatogram of Tranexamic acid

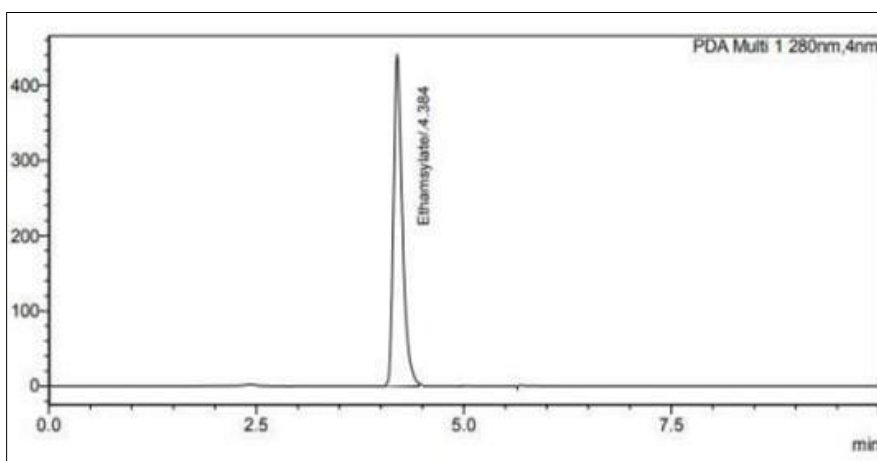


Figure 14 Chromatogram of Ethamsylate

Table 3 Parameters of Optimized Chromatogram

Parameters	Tranexamic acid		Ethamsylate	
	Standard	sample	Standard	Sample
Retention time	3.278	3.285	4.379	4.384
Tailing Factor	1.797	0.882	1.697	1.127
Theoretical plates	5552	4918	4152	4265
Resolution	-	-	5.176	5.054

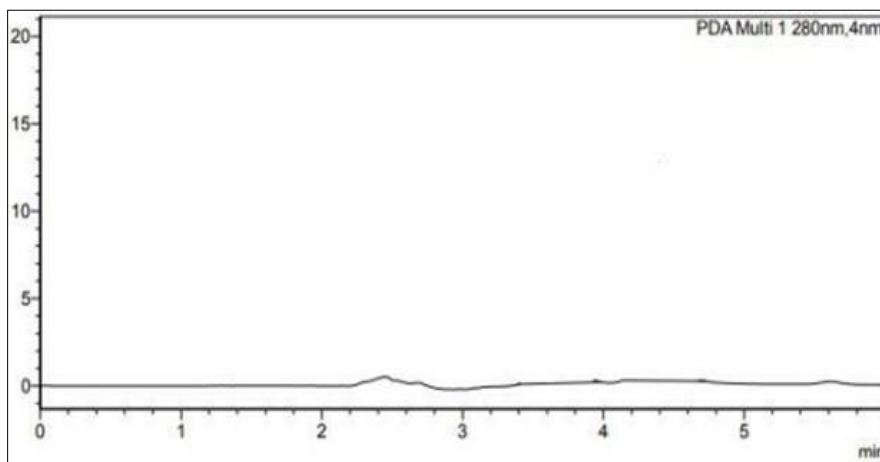


Figure 15 Blank chromatogram

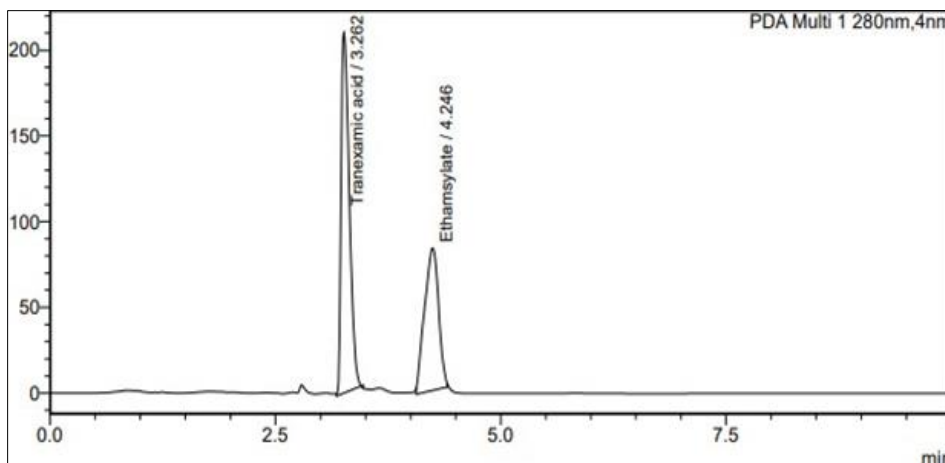


Figure 16 System Suitability Chromatogram1

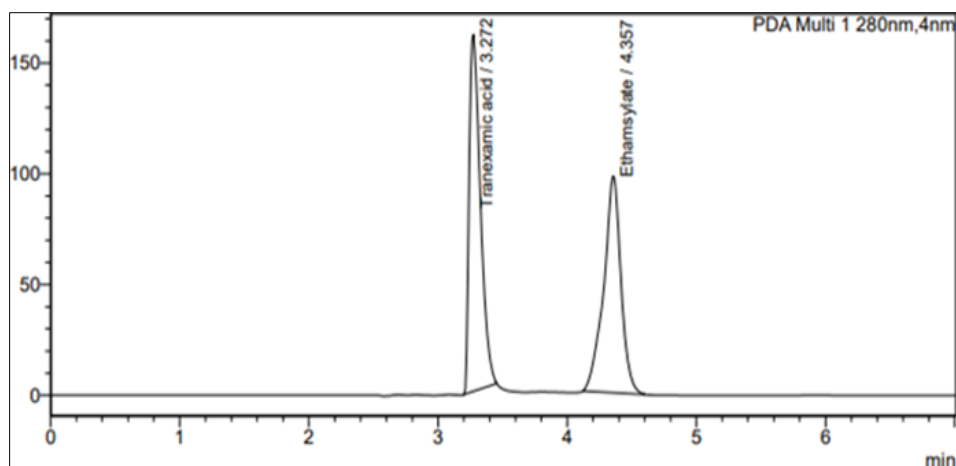


Figure 17 System Suitability Chromatogram2

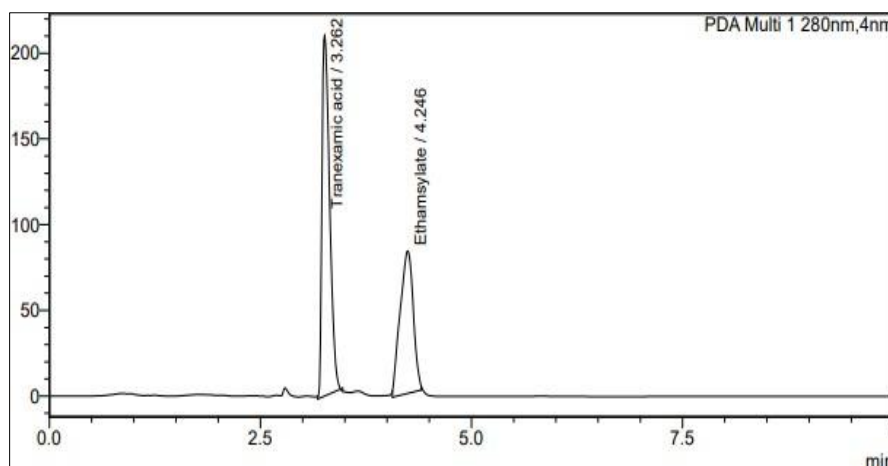


Figure 18 System Suitability Chromatogram 5

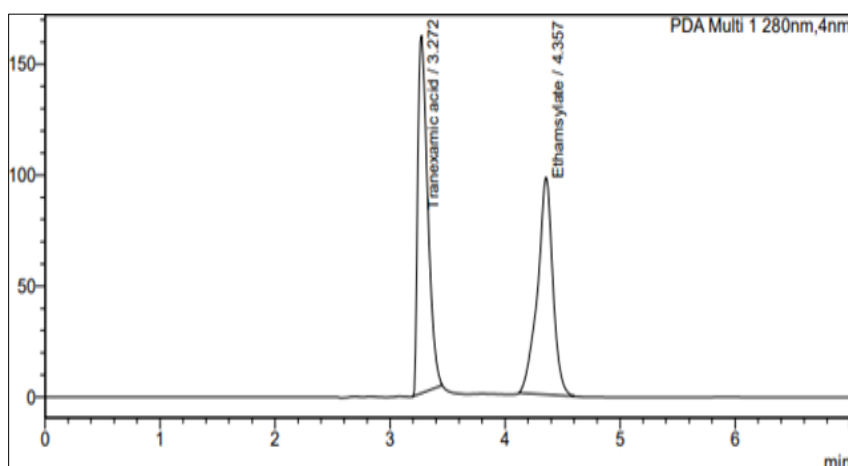


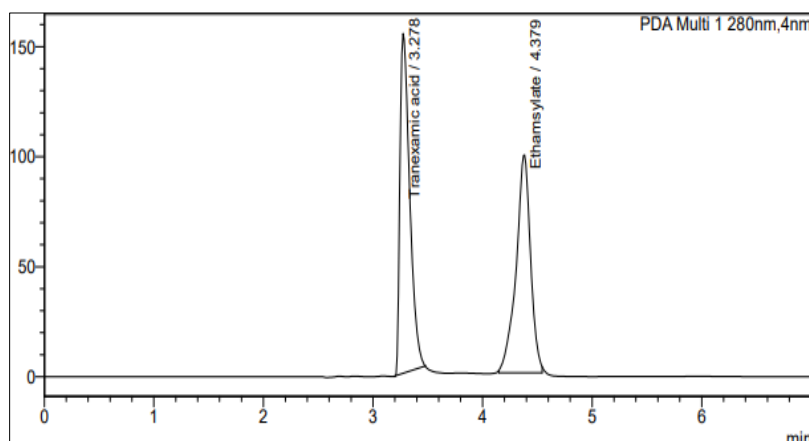
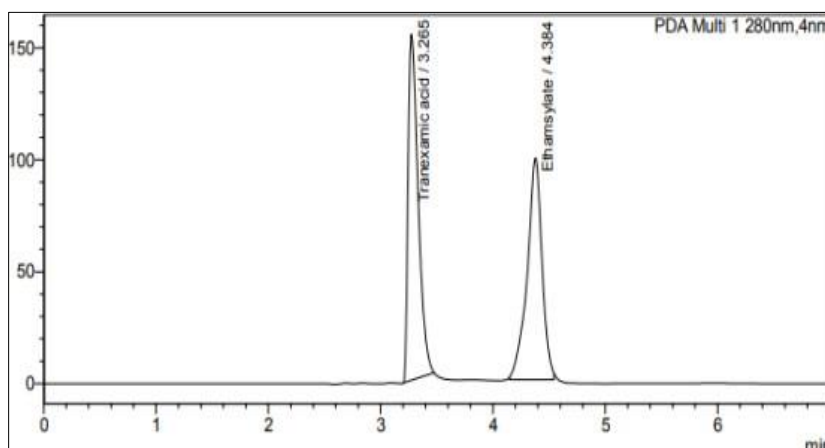
Figure 19 System Suitability Chromatogram 6

Table 4 System suitability parameter for Tranexamic acid and Ethamsylate

InjectionNo	Tranexamic acid		Ethamsylate	
	Rt(min)	Peak area	Rt(min)	Peak area
1	3.262	121456	4.246	533289
2	3.272	121125	4.357	533456
3	3.262	121380	4.246	534589
4	3.272	122505	4.357	535625
5	3.262	121568	4.246	533587
6	3.272	121459	4.357	533812
Mean		121582	Mean	534060
Standard Deviation		475.9795864	Standard Deviation	891.465685
%RSD		0.39	%RSD	0.17

3.2. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Using blank; standard and sample solutions to a HPLC system showed that the method is specific.

**Figure 20** Standard chromatogram**Figure 21** Sample chromatogram

3.3. Linearity

The linearity of an analysis shall be the possibility to obtain test results that are directly related to a sample's analyte concentration.

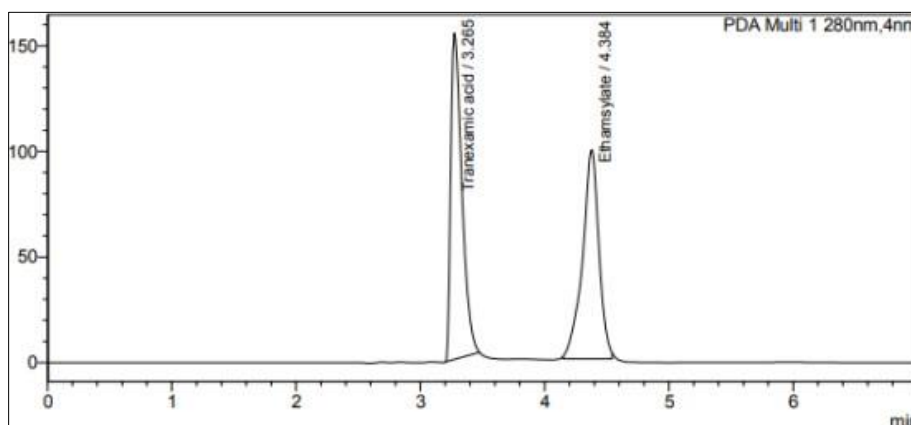


Figure 22 Linearitylevel-01

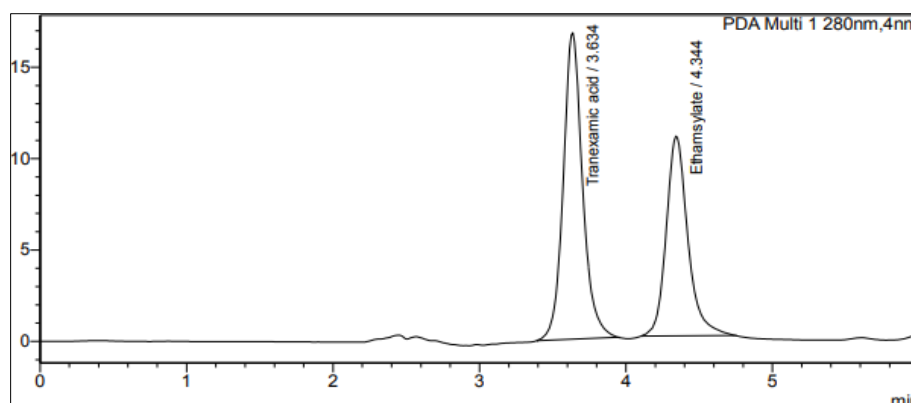


Figure 23 Linearitylevel-04

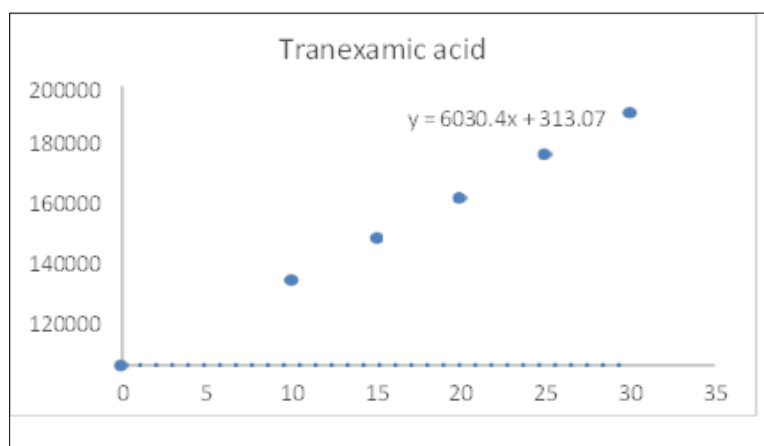


Figure 24 Tranexamic acid Linearity

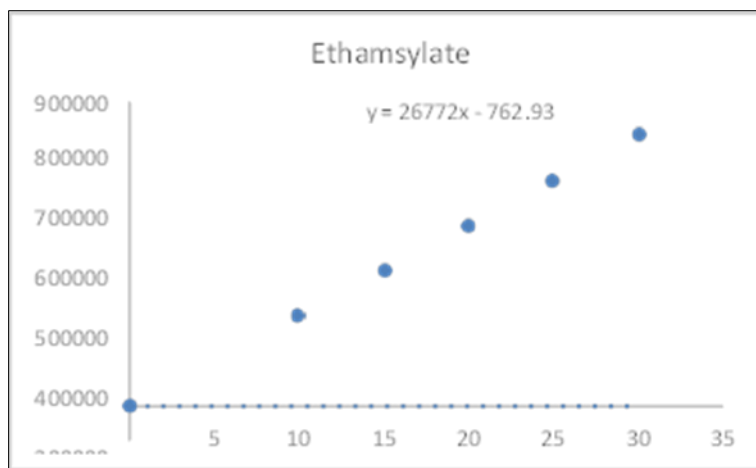


Figure 25 Ethamsylate Linearity

Table 5 Linearity data for Tranexamic acid and Ethamsylate

Tranexamic acid		Ethamsylate	
Concentration($\mu\text{g/ml}$)	Peak area	Concentration($\mu\text{g/ml}$)	Peak area
10	61078	10	266689
15	91242	15	401256
20	120136	20	533261
25	151254	25	666587
30	181205	30	804856
r^2	1	r^2	1

3.4. Accuracy

The accuracy of an analysis shows that the value which has been accepted either as a conventional true value; or established reference value and found is very close to agreement.

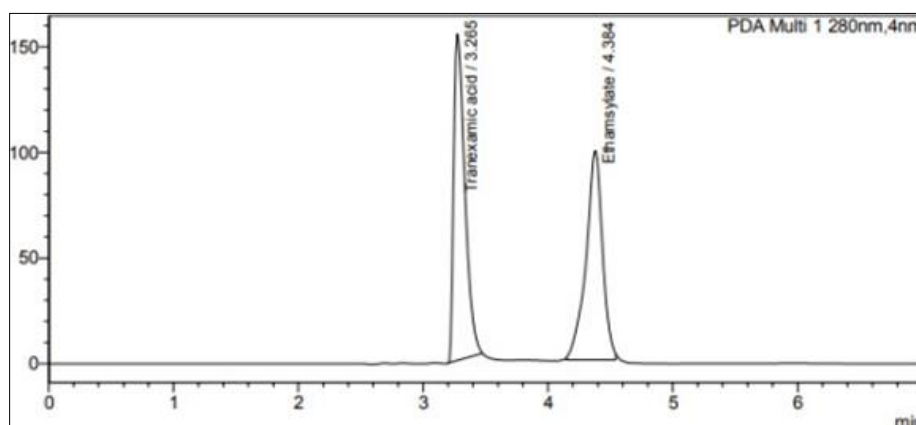


Figure 26 Accuracy chromatogram of sample-50%-1

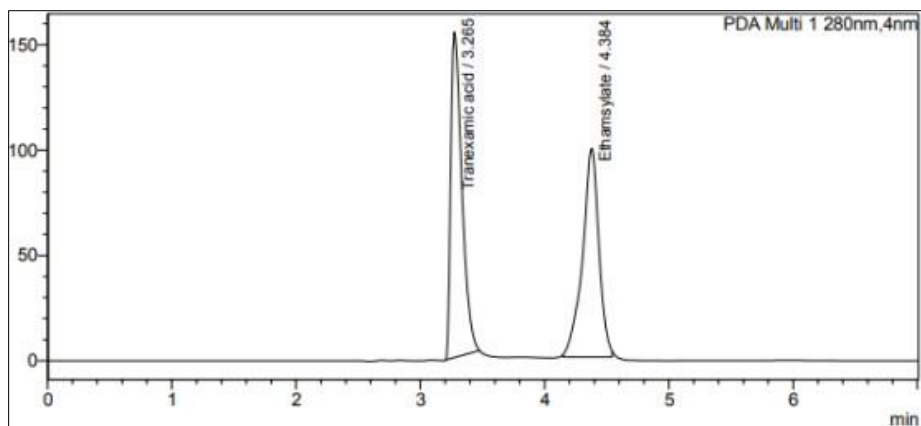


Figure 27 Accuracy chromatogram of sample-50%-2

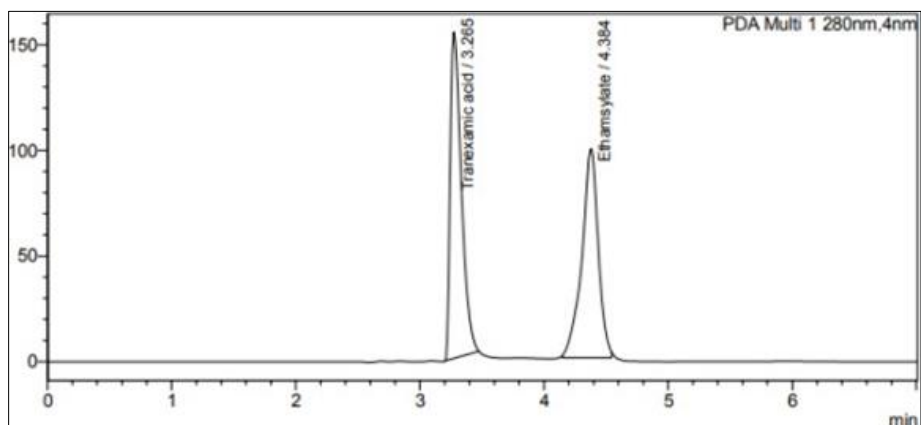


Figure 28 Accuracy chromatogram of sample-50%-3

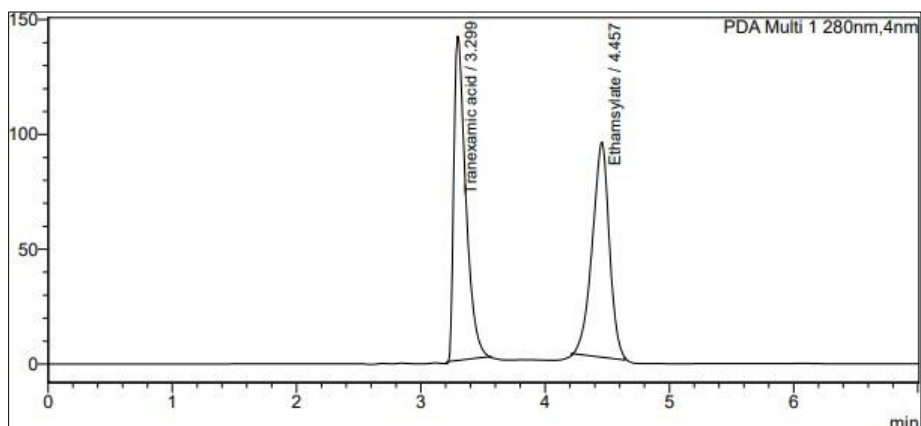


Figure 29 Accuracy chromatogram of sample-100%-1

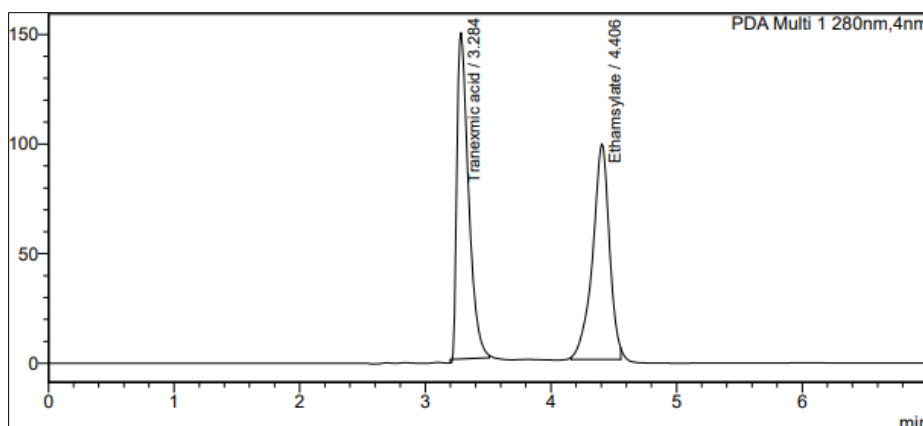


Figure 30 Accuracy chromatogram of sample-150%-1

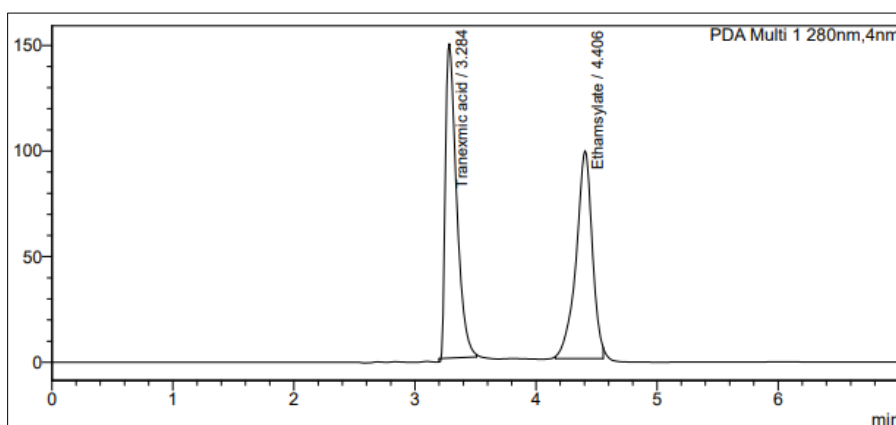


Figure 31 Accuracy chromatogram of sample-150%-3

Table 6 Accuracy data for Tranexamic acid

% Level	Standard peak area	Sample peak area	%recovery	Average % recovery	Mean % recovery
50	534060	269721	99.61	100.77	100.41
	534060	269895	100.38		
	534060	269756	100.41		
100	534060	533989	100.16	99.92	
	534060	534856	100.05		
	534060	535010	99.92		
150	534060	787854	100.78	100.55	
	534060	786897	100.77		
	534060	786125	100.72		

3.5. Precision

The method was determined by system precision and method precision using standard solution of Tranexamic acid and Ethamsylate given in 6 replicates to the chromatographic system.

3.5.1. System precision

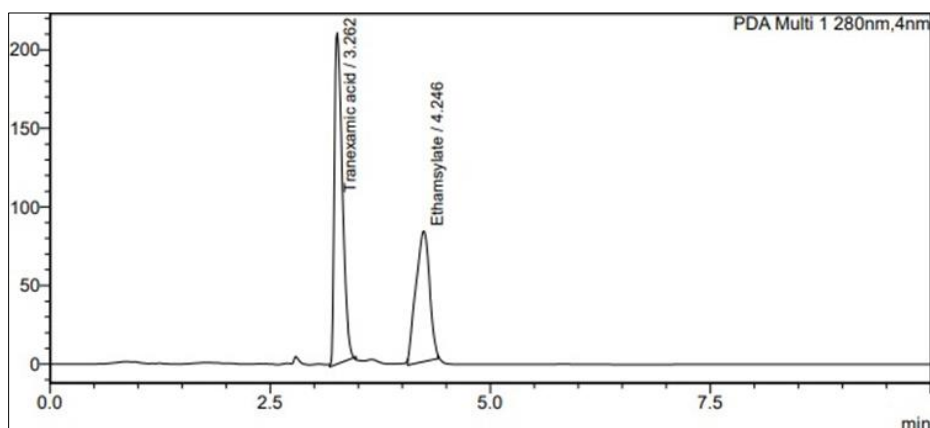


Figure 32 System precision Chromatogram-01

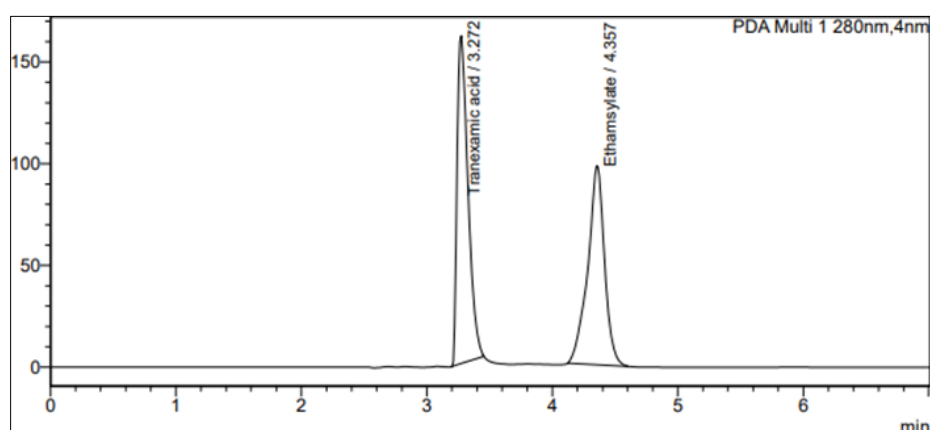


Figure 33 System precision Chromatogram-02

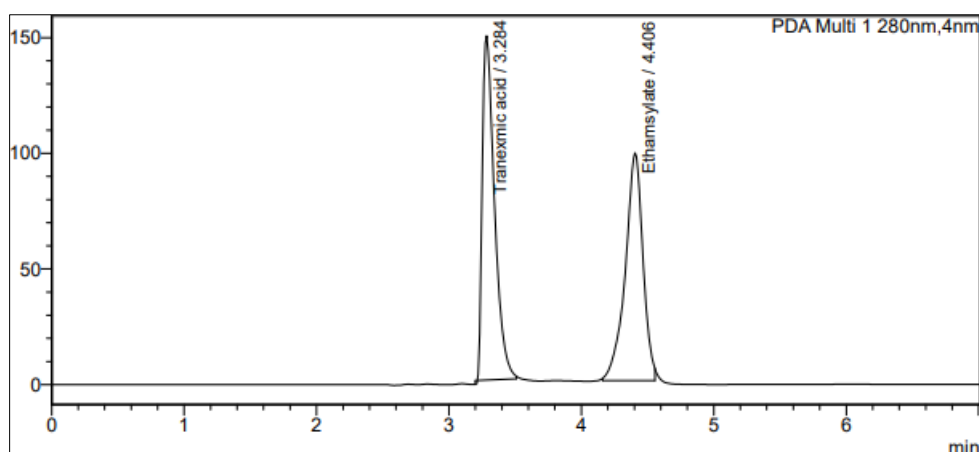


Figure 34 System precision Chromatogram-03

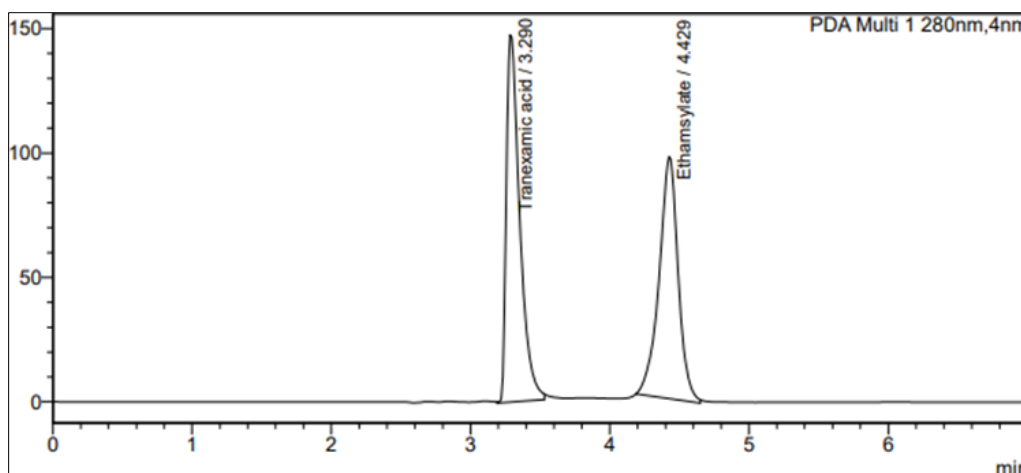


Figure 35 System precision Chromatogram-04

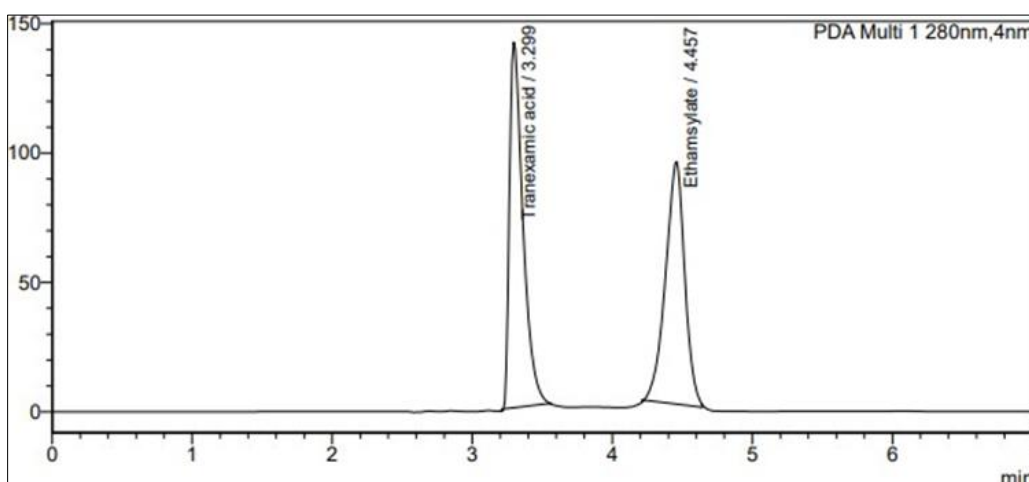


Figure 36 System precision Chromatogram-05

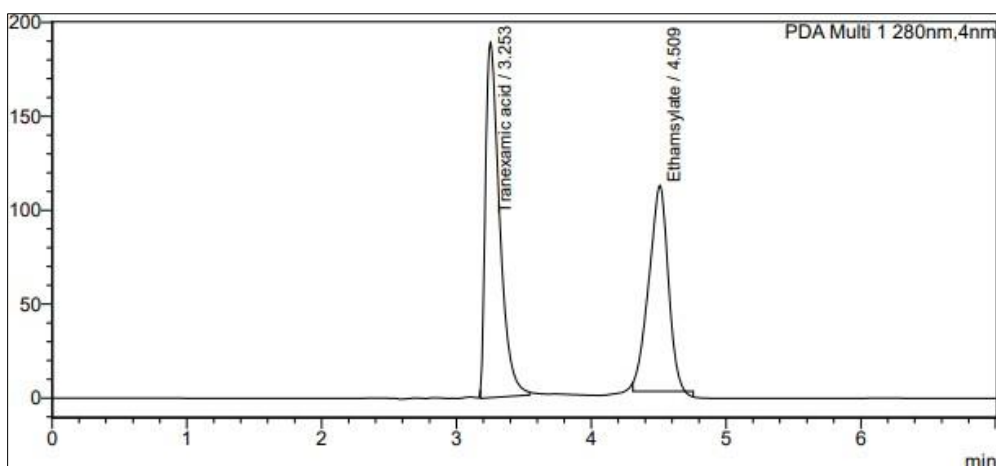


Figure 37 System precision Chromatogram-06

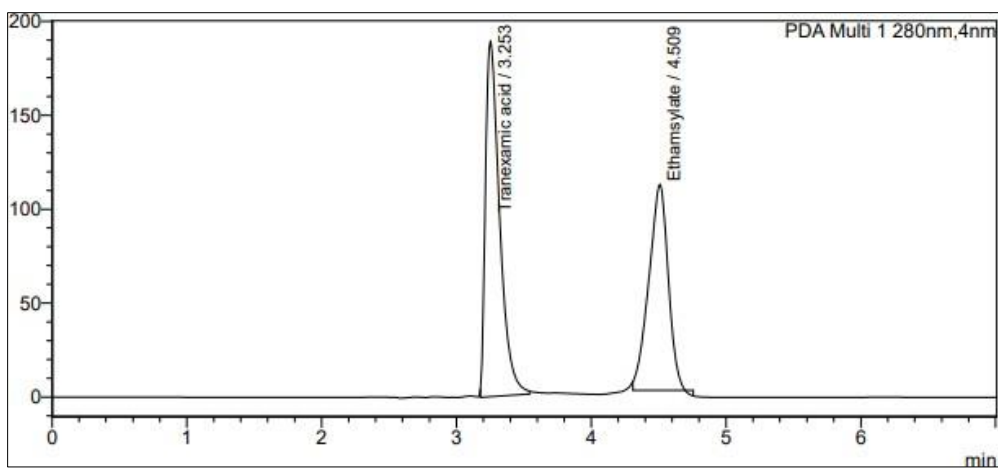


Figure 38 Method precision chromatogram-4

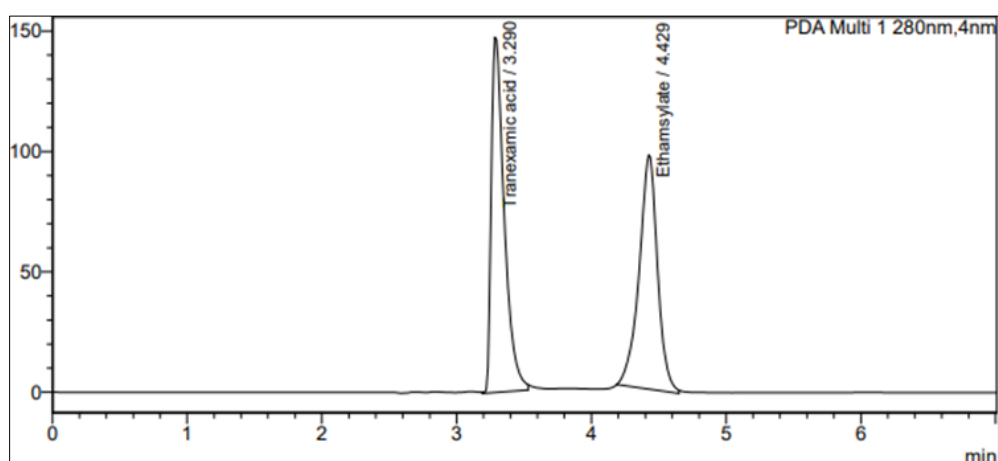


Figure 39 Method precision chromatogram-5

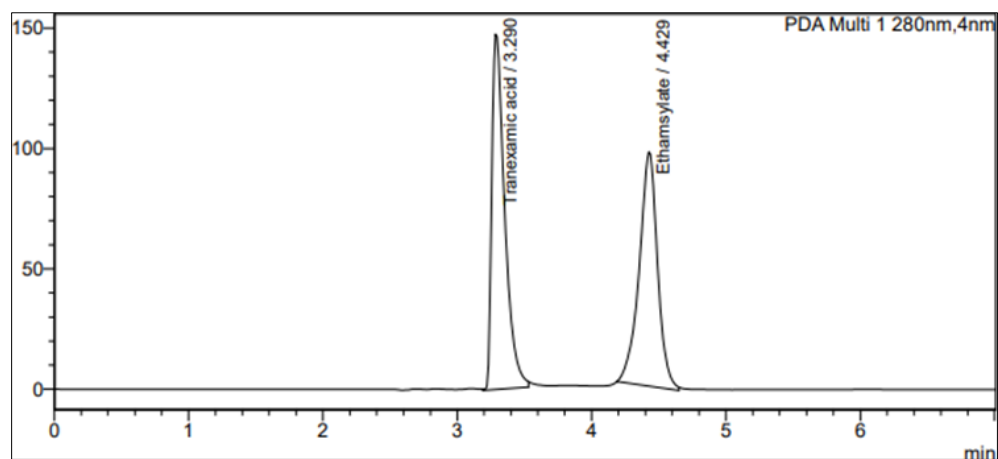


Figure 40 Method precision chromatogram-6

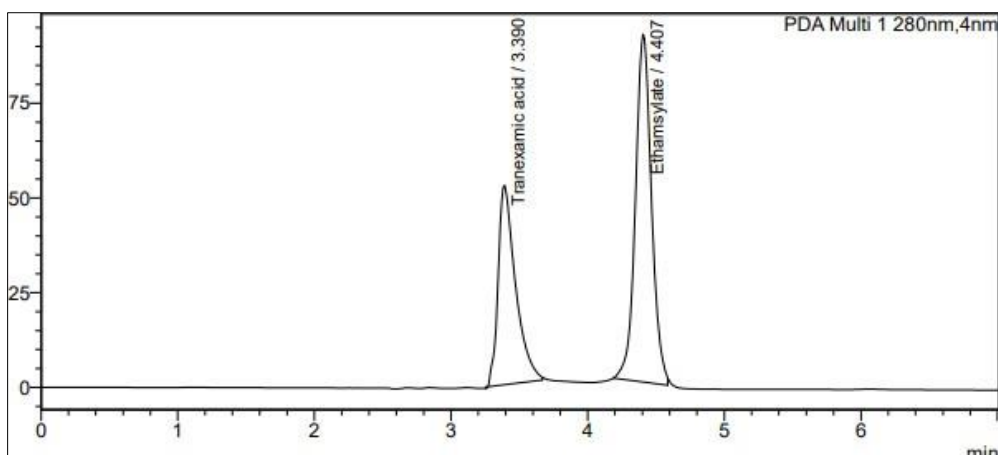


Figure 41 LOD Chromatogram

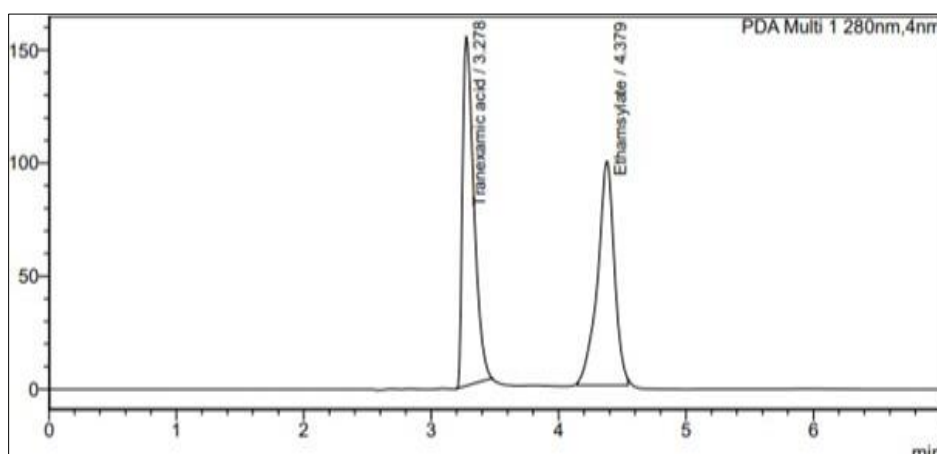


Figure 42 LOQ Chromatogram

Table 7 Limit of detection and limit of quantification of Tranexamic acid and Ethamsylate

Parameter	Tranexamic acid (µg/ml)	Ethamsylate (µg/ml)
LOD	1.0 µg/mL	3.0 µg/mL
LOQ	1.0 µg/mL	3.0 µg/mL

3.6. Robustness

The ICH defines the "ability of an analytical process to remain undisturbed by small but deliberate deviations in method parameters" as a measure of its robustness capacity to remain unaffected by minor changes in parameters such as temperature; the pH of the mobile phase; the percentage of organic solvent strength; the concentration of the buffer; etc.

Table 8 Robustness data for Tranexamic acid and Ethamsylate

PARAMETER	Tranexamic acid			Ethamsylate		
	R _t (min)	Peak area	%RSD	R _t (min)	Peak area	%RSD
Change in Flowrate 0.8 ml/min	3.299	1303714	0.01	4.457	523585	0.01
	3.299	1303615		4.457	523612	
Change in Flowrate 0.6 ml/min	3.272	1303818	0.02	4.357	523814	0.01

	3.272	1303714		4.357	523915	
Change in Mobile phase ratio 70:30v/v	3.253	1320951	0.02	3.509	534112	0.02
	3.253	1321534		3.509	534256	
Change in Mobile phase ratio 60:40v/v	3.290	1321043	0.01	4.429	534225	0.01
	3.290	1321654		4.429	534189	

3.7. Change in Mobile phase flow rate

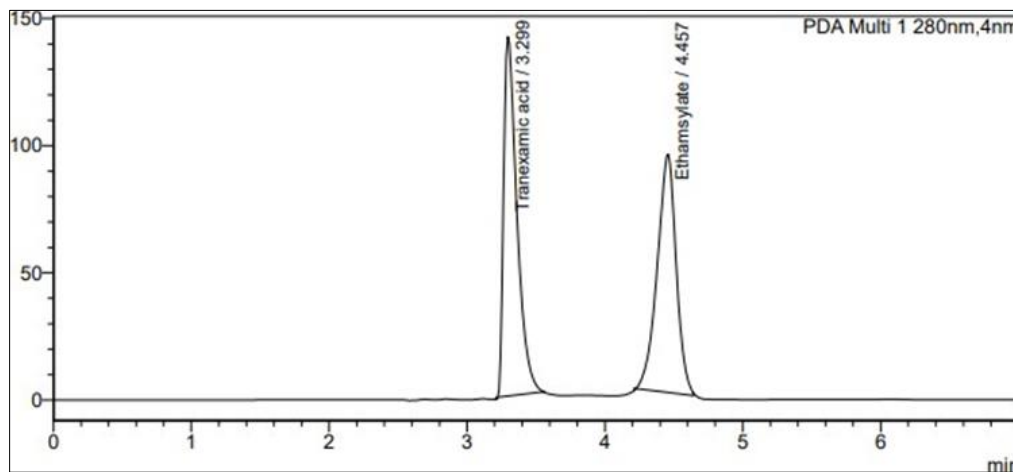


Figure 43 Chromatogram of change in flow rate 0.6ml/min (1)

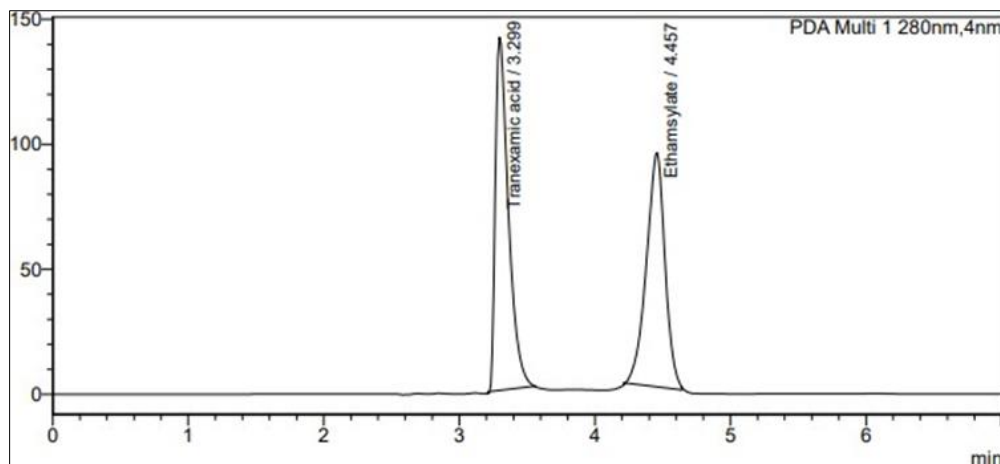


Figure 44 Chromatogram of Change in flow rate 0.6ml/min (2)

3.8. Change in mobile phase

Table 9 %Assay of TRAPIC-E® tablet formulation

DRUG	LABEL CLAIM (mg)	%ASSAY
Tranexamic acid	250	100.41%
Ethamsylate	250	100.31%

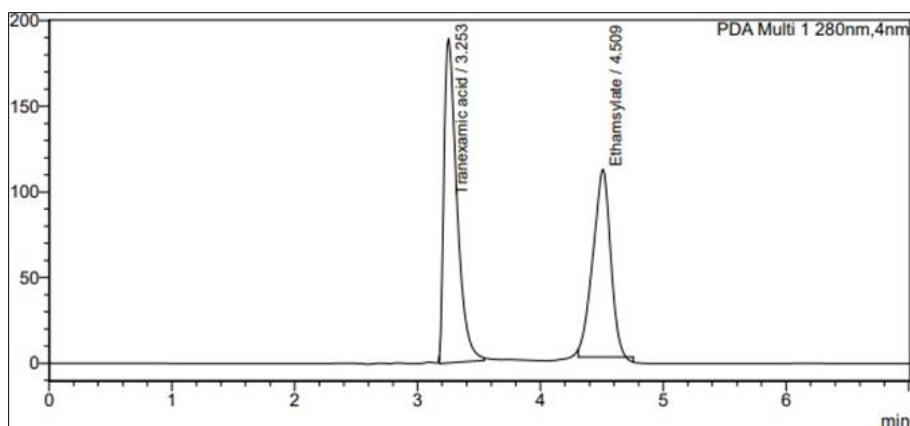


Figure 45 Change in increased Mobile phase ratios Buffer : organic phase (70:30v/v)2

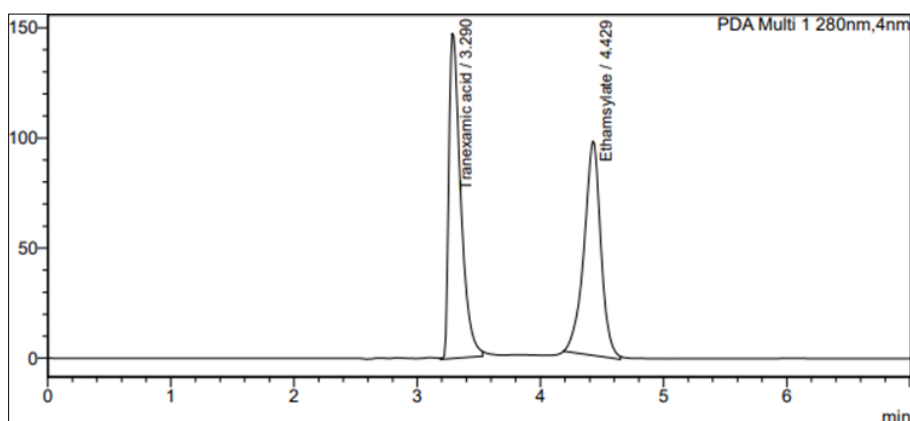


Figure 46 Change in decreased mobile phase ratios Buffer : organic phase in the ratios of (60:40v/v)-1

Table 10 Solubility studies

Solvent	Water	Ethanol	Methanol
Tranexamic acid	Freely Soluble	Freely soluble	Soluble
Ethamsylate	Insoluble	Freely soluble	Low Soluble

Table 11 Summary of the validation parameters

S. No	Parameters	Acceptance criteria	Name of the compound	Results		
				Theoretical plate count	Resolution	Tailing factor
1.	System suitability	Plate count should be more than 2000 and resolution must be more than 2	Tranexamic acid	>2000	-	<2.0
			Ethamsylate	>2000	>2.0	<2.0
2.	Linearity	R2≤0.999	Tranexamic acid	R2=0.9999		
			Ethamsylate	R2=0.9995		
3.	Accuracy	%Recovery should be between 98%to102%	Tranexamic acid	100.44		
			Ethamsylate	100.62		
4.	System precision	%RSD Not More Than 2%	Tranexamic acid	0.50		
			Ethamsylate	0.20		

5.	Method precision	%RSD not More than 2%	Tranexamic acid	0.30
			Ethamsylate	0.17
6.	LOD	-	Tranexamic acid	1.0µg/mL
			Ethamsylate	3.0µg/mL
7.	LOQ	-	Tranexamic acid	1.0µg/mL
			Ethamsylate	3.0µg/mL
8.	Robustness	Method should not be affected during change in the method parameters	Tranexamic acid	Method was not affected during changes done in the flow rate and wavelength
			Ethamsylate	
9.	Assay	-	Tranexamic acid	100.41%
			Ethamsylate	100.31%

4. Conclusion

I conclude that a simple; precise reverse phase high performance liquid chromatography (RP-HPLC) approach was developed for the estimation of Tranexamic acid and Ethamsylate in pharmaceutical dosage form. The separation was carried out by using a Hypersil BDS column (250x4.6; particle size 5µm) at room temperature. As the mobile phase; phosphate buffer and acetonitrile was used: At 1.0ml/min flow rate. At a detection wavelength of 280nm; an phosphate buffer :acetonitrile (80:20v/v) was injected onto the column. The linearity concentration range was 10-30µg/mL for Tranexamic acid and 10-30µg/mL for Ethamsylate with a correlation coefficient (r^2) of 0.9999 and 0.9995 respectively. The method found to be precise with % RSD values of NMT 2.0. According to ICH guidelines; the improved method was validated. Hence the developed method was improved; precise; and can be used in routine analysis.

Compliance with ethical standards

Acknowledgments

I am very grateful to SIMS College of Pharmacy for supporting me and providing everything without which it is not possible.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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