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(RESEARCH ARTICLE)

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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 10-30µg/mL, 10-30µg/ml, with regression 0.999. The percentage recoveries for Tranexamic acid and Ethamsylate were found to be in the range of 100.41% and100.31 %, 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

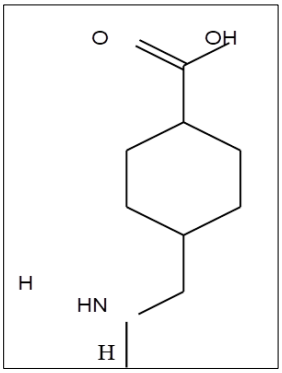

Figure 1 Chemical Structure of Tranexamic acid

IUPACNAME	4- (Amino ethyl) cyclo hexane carboxylic acid
Molecular Formula	C8H15NO2
Molecular Weight	157.21g/mol
Solubility	Freely Soluble in ethanol; water
Category	Antifibrinolytic
Storage conditions	Store at room temperature

1.2.2. ETHAMSYLATE

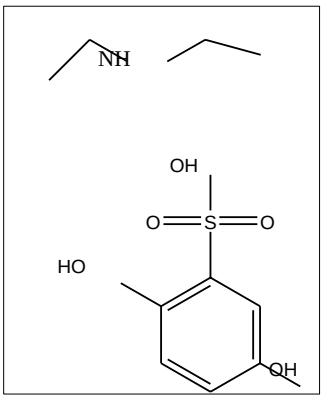

Figure 2 Chemical Structure of Ethamsylate

IUPACNAME	2;5-dihydroxybenzenesulfonic acid
Molecular Formula	C9H9NO5S
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
Tranexamicacid	Freely Soluble	Soluble	Poorly Soluble
Ethamsylate	soluble	Soluble	Soluble

2.1. Determination of wavelength by UV-Visible spectrophotometric method:Preparation of Standard stock solution (1000µg/ml)

Weighaccurately10mgofTranexamic acidand10mgofEthamsylateaccuratelyandtransferintoatwodifferent10mLcleandryvolumetricflaskwith7mL of diluent to dissolve and volume madeup to the mark with diluent (1000 µg/mL).

2.2. Preparation of sample solution

Weigh accurately 820 mg of tablet powder and transfer into 25ml volumetric flask. Then; 15 mLof 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 0.45Millipore Nylon filter. From the above solution pipette; out the 1mL and transferred into 10mL volumetric flask made up the volume with 10 ml diluent. Pipette 0.5mL of solution into a volumetric flask that holds10mL; 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 µg/mL of Tranexamic acid and 10 µg/mL of Ethamsylate solutions. By overlaying the individual spectra; the detection wavelength was found to be 280 nm. The HPLC system's PDAdetectorwassetat280nmforthe analysis

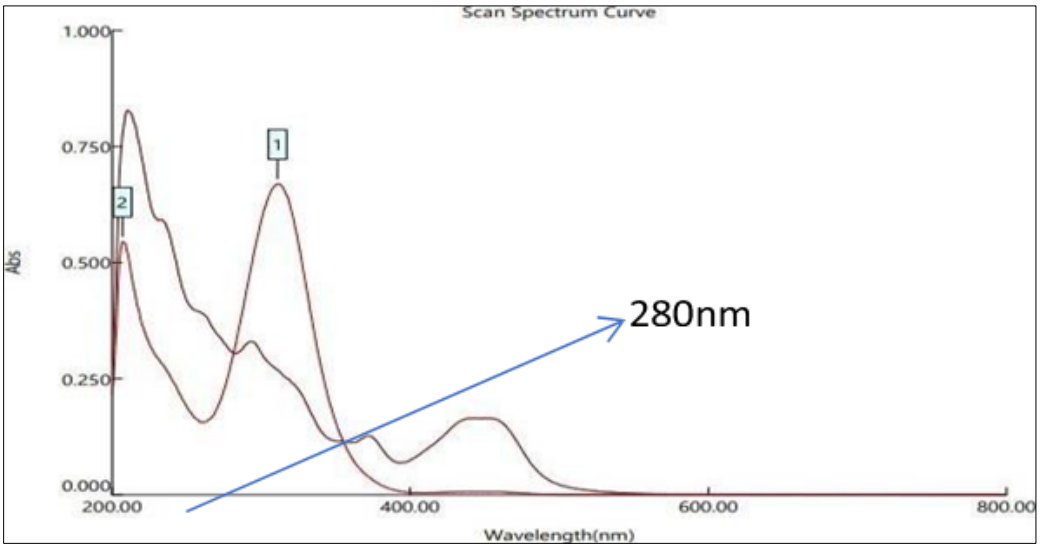

Figure 3 Combined spectrum of Tranexamic acid and Ethamsylate

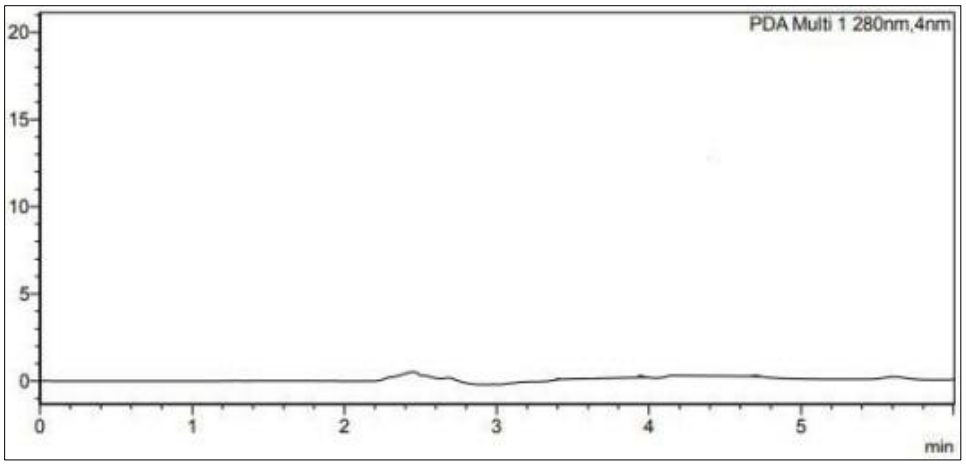

Figure 4 Blank chromatogram

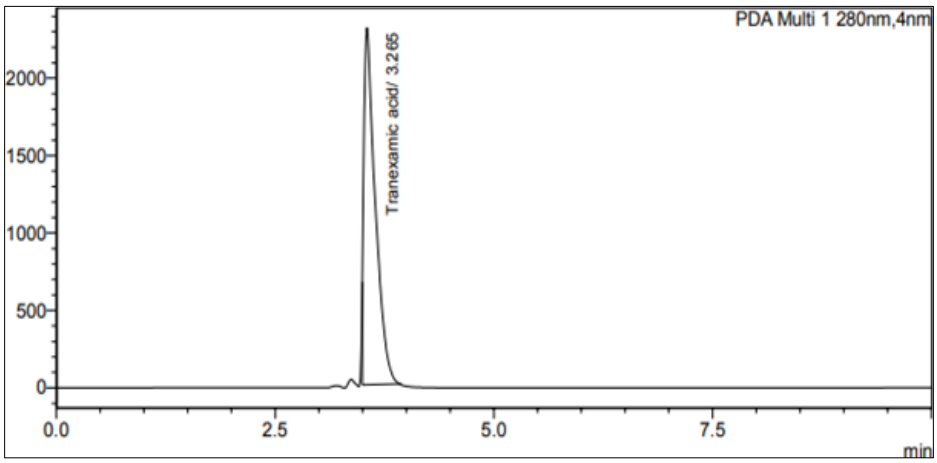

Figure 5 Tranexamic acid chromatogram

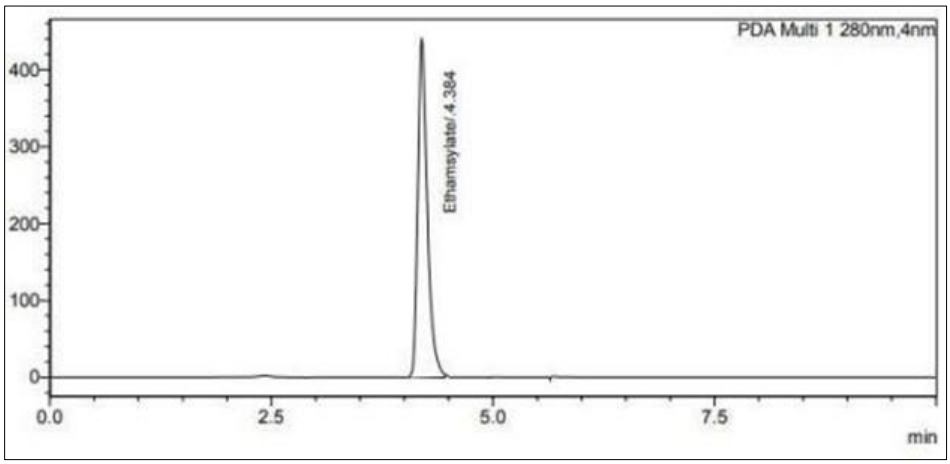

Figure 6 Ethamsylate chromatogram

2.4. Method development

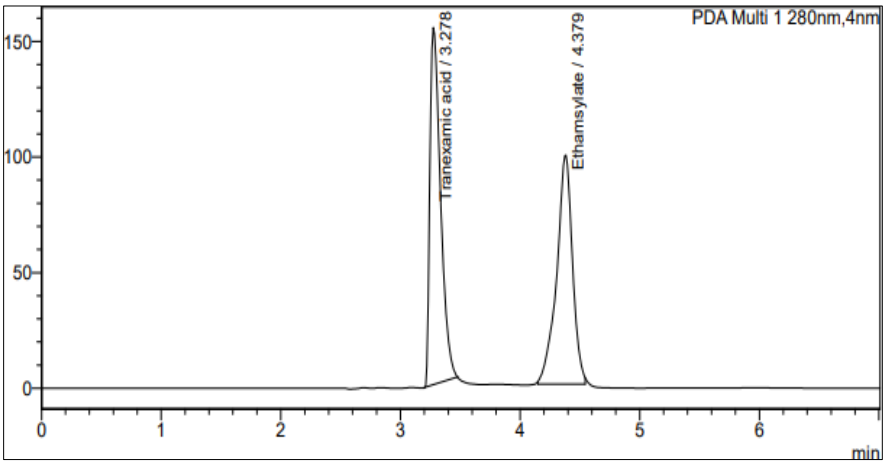

Figure 7 Standard chromatogram

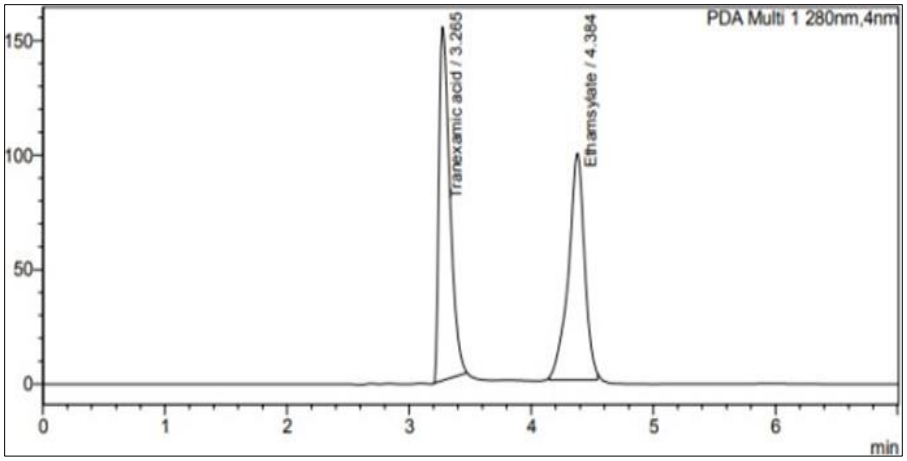

Figure 8 Sample chromatogram

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:

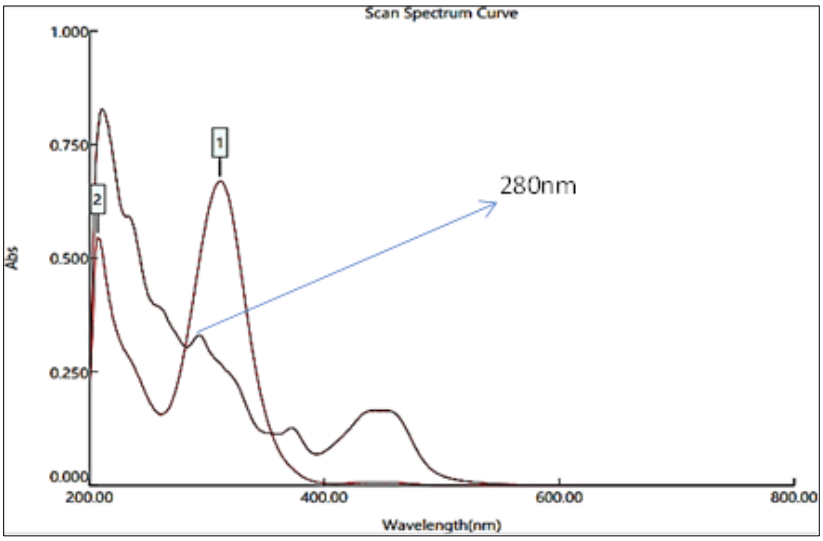

Figure 9 Combined Spectrum of Tranexamic acid and Ethamsylate

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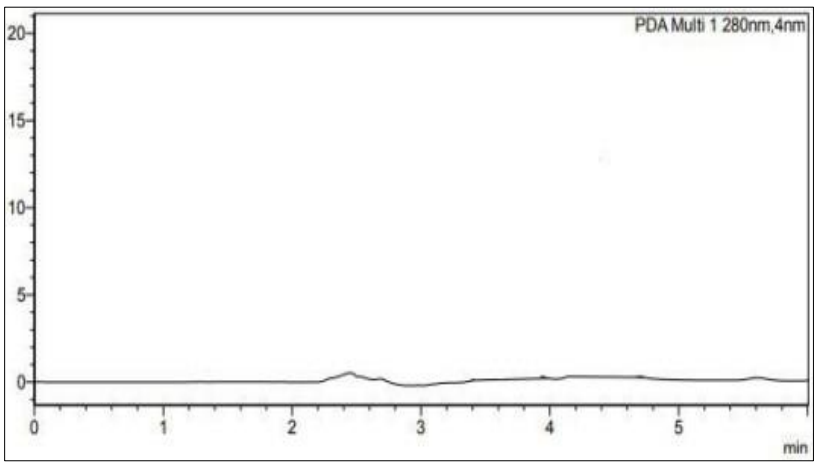

Figure 10 Blank chromatogram

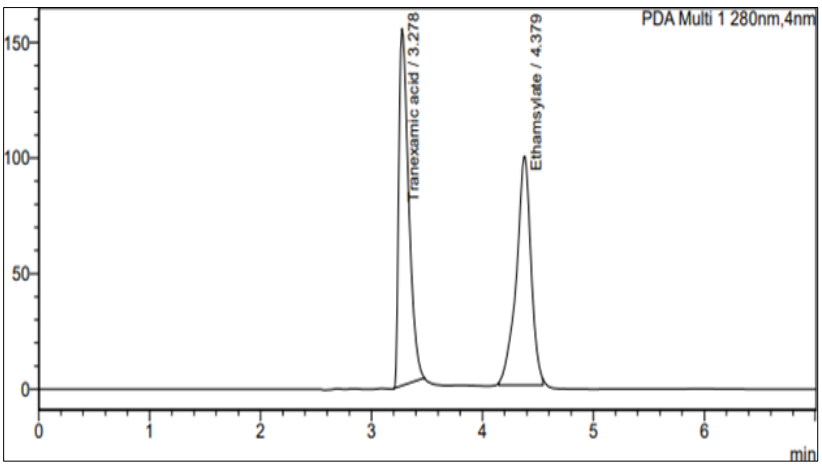

Figure 11 Chromatogram of standard

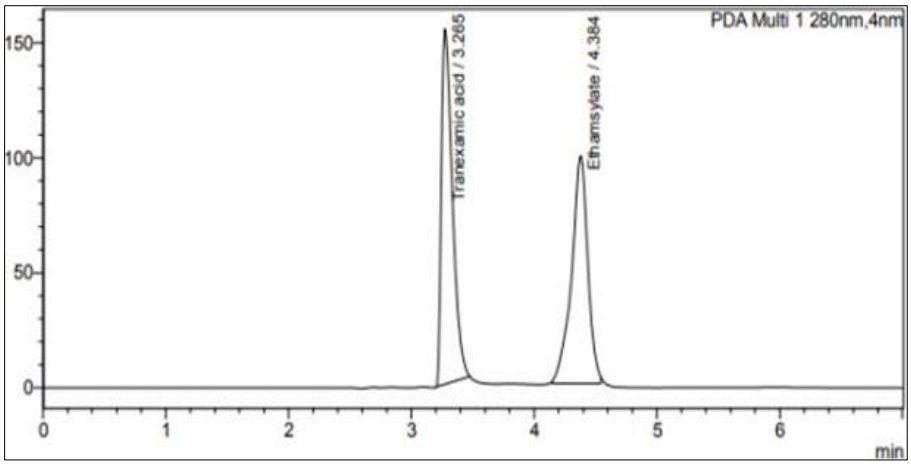

Figure 12 Chromatogram of sample

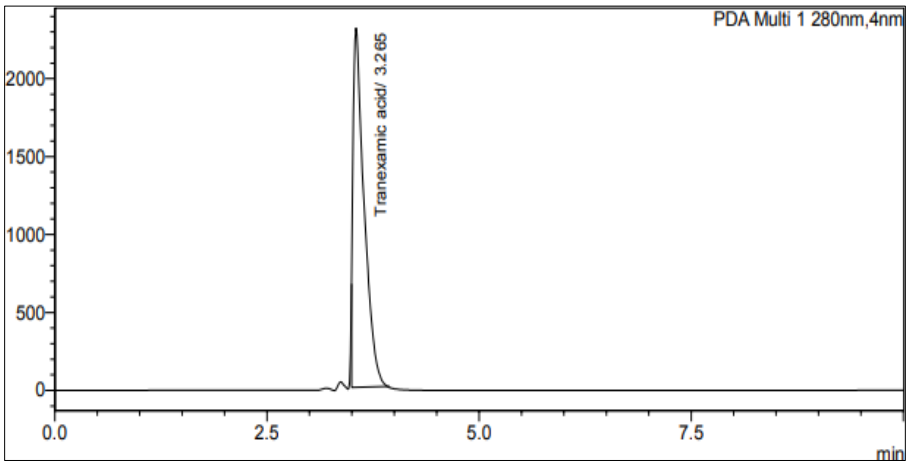

Figure 13 Chromatogram of Tranexamic acid

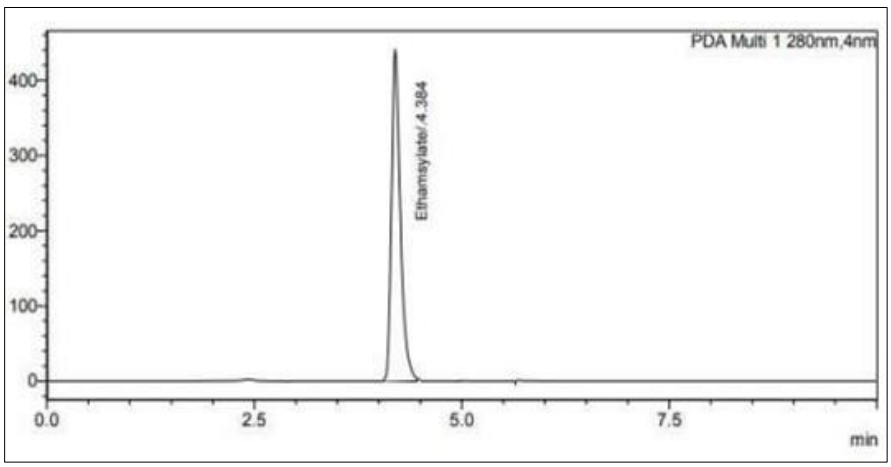

Figure 14 Chromatogram of Ethamsylate

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

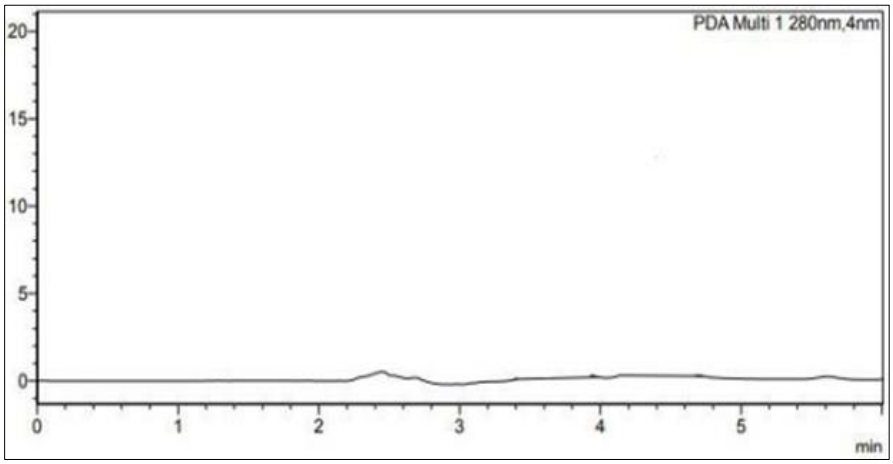

Figure 15 Blank chromatogram

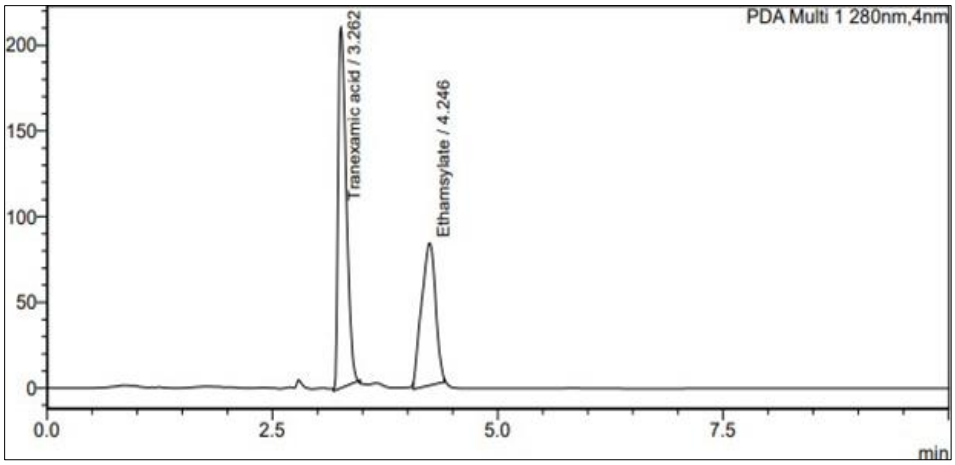

Figure 16 System Suitability Chromatogram1

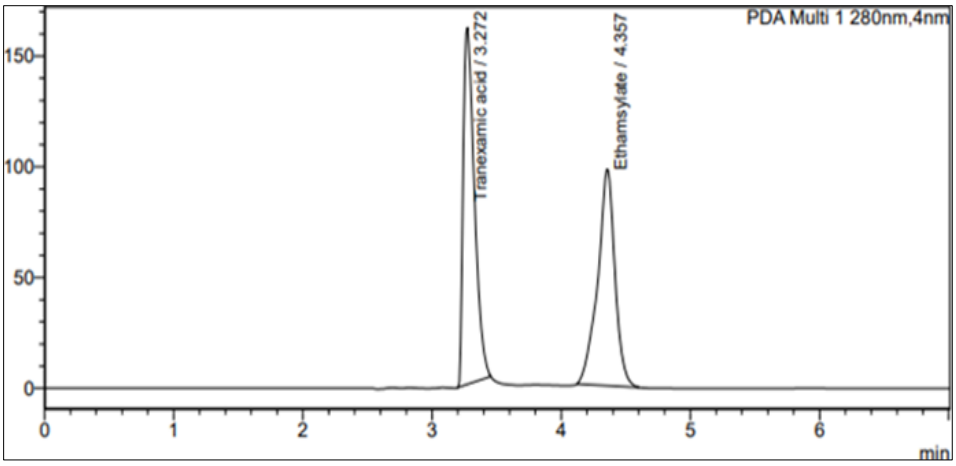

Figure 17 System Suitability Chromatogram2

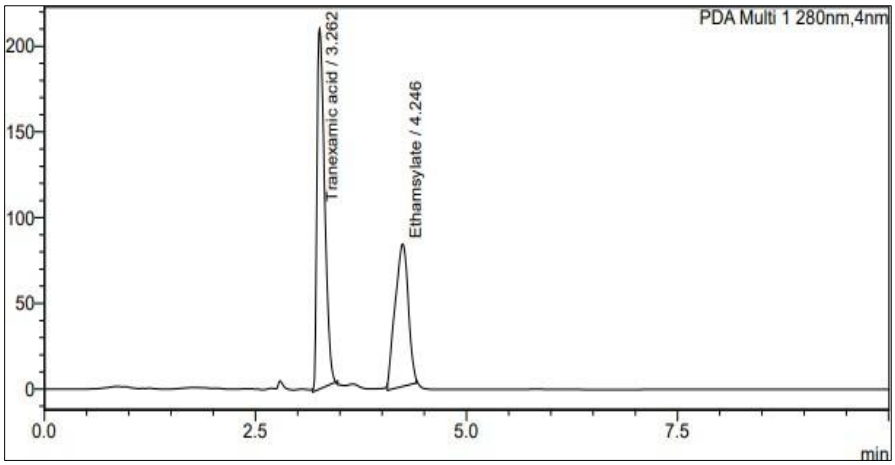

Figure 18 System Suitability Chromatogram 5

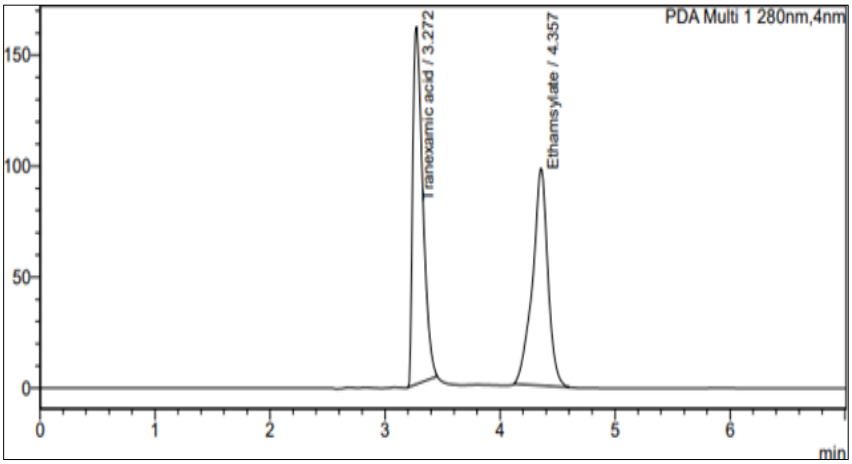

Figure 19 System Suitability Chromatogram 6

Table 4 System suitability parameter for Tranexamic acid and Ethamsylate

InjectionNo	Tranexamic acid		Ethamsylate	
	R_t(min)	Peak area	R_t(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 thatmay be expected to be present. Using blank; standard and sample solutions to a HPLC systemshowed that the method is specific.

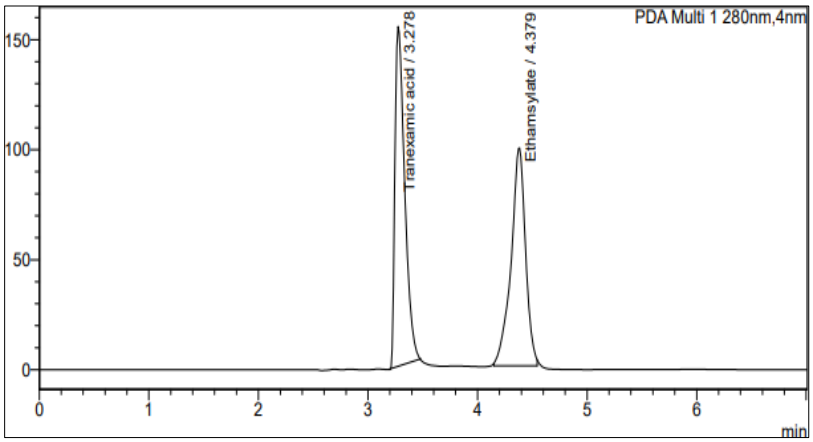

Figure 20 Standard chromatogram

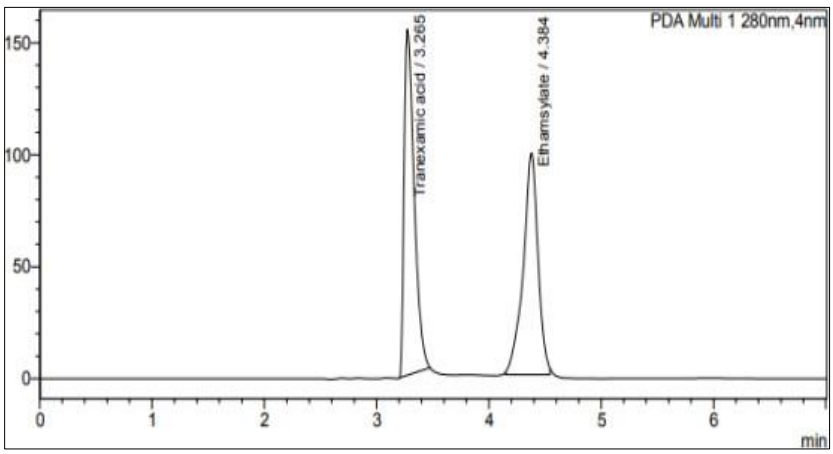

Figure 21 Sample chromatogram

3.3. Linearity

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

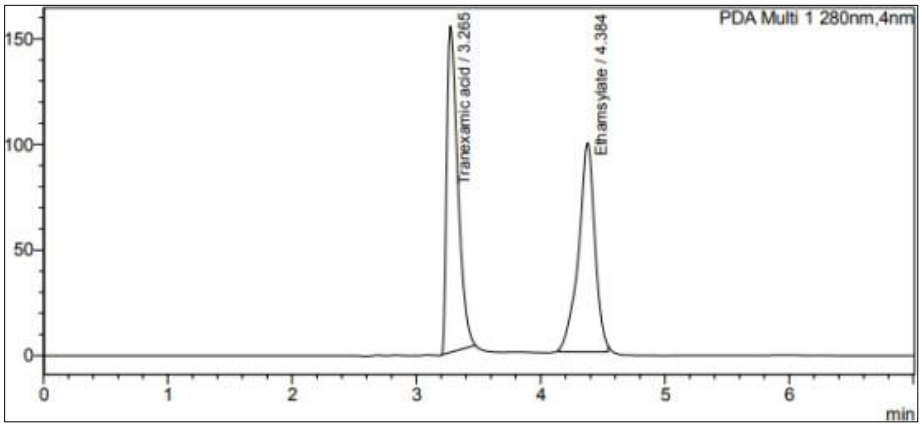

Figure 22 Linearitylevel-01

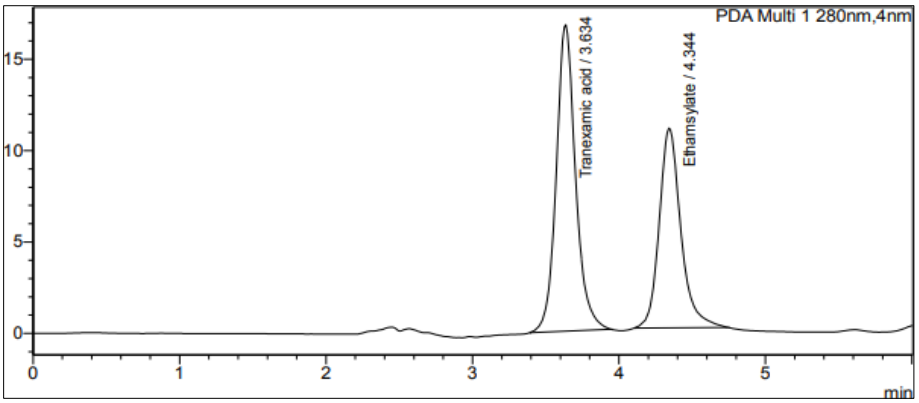

Figure 23 Linearitylevel-04

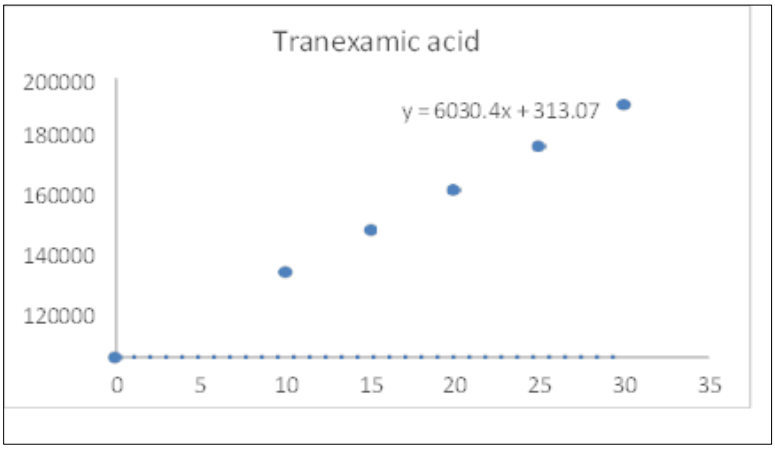

Figure 24 Tranexamic acid Linearity

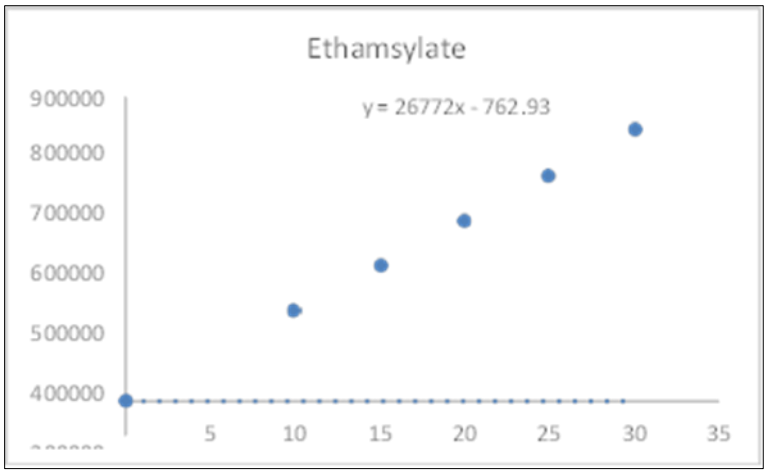

Figure 25 Ethamsylate Linearity

Table 5 Linearity data for Tranexamic acid and Ethamsylate

Tranexamic acid		**Ethamsylate**	
Concentration(µg/ml)	**Peak area**	**Concentration(µg/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 aconventional true value; or established reference value and found is very close to agreement.

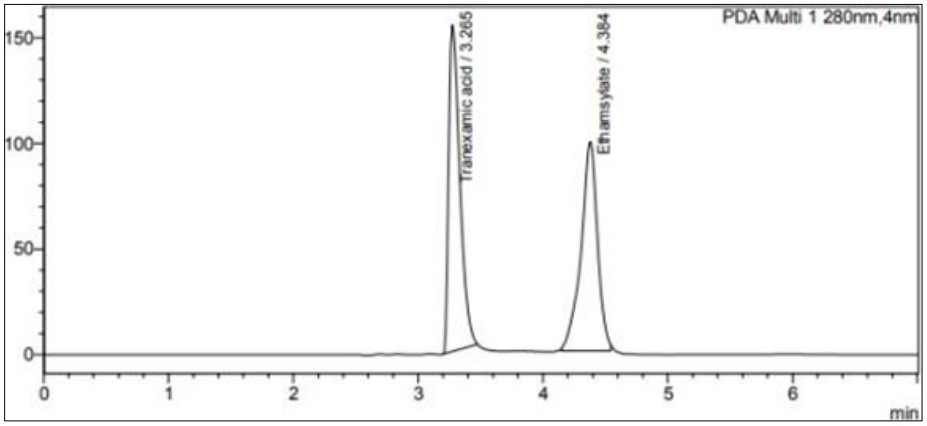

Figure 26 Accuracy chromatogramofsample-50%-1

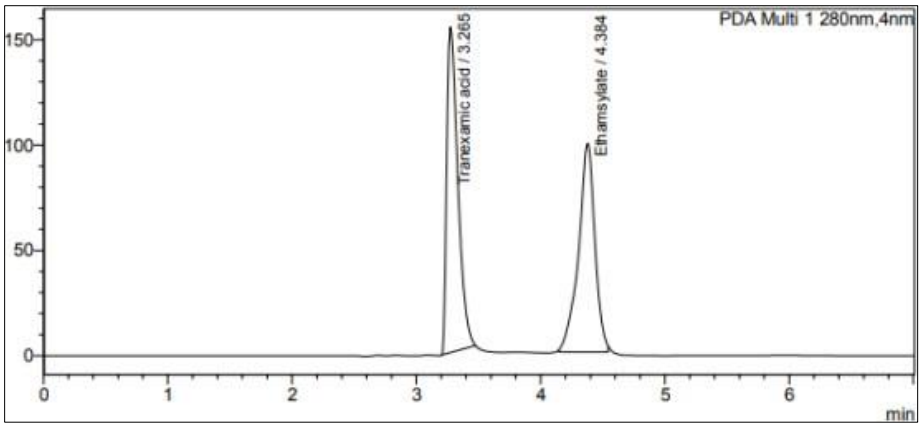

Figure 27 Accuracy chromatogram of sample-50%-2

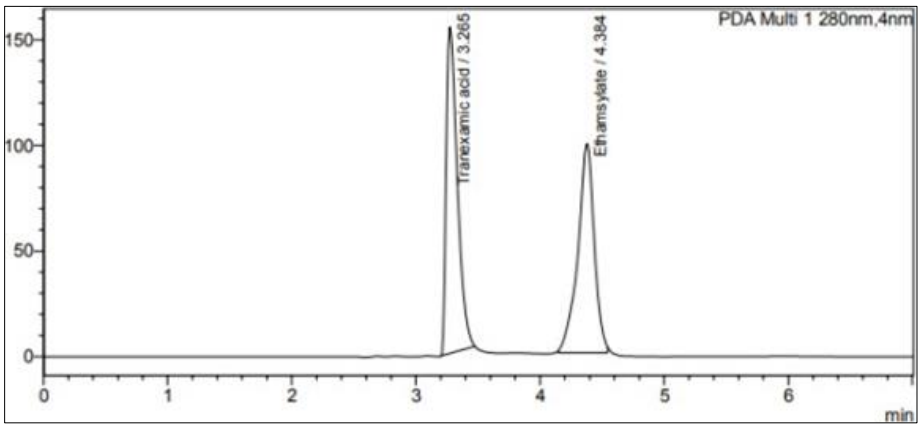

Figure 28 Accuracy chromatogram of sample-50-3

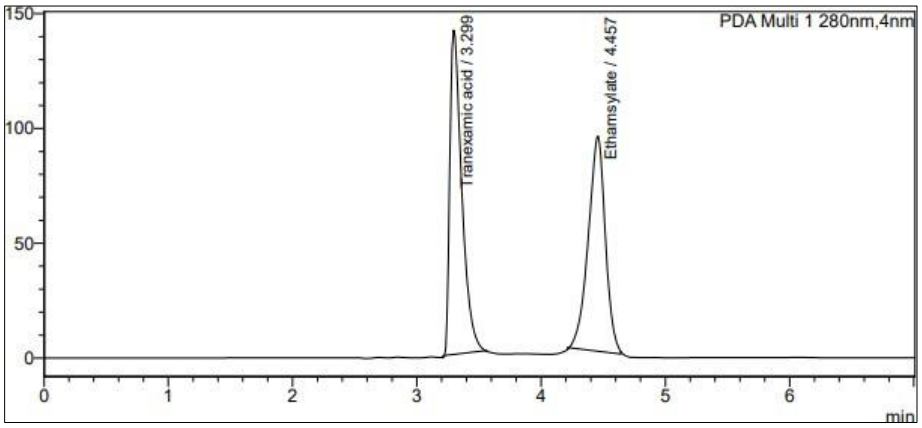

Figure 29 Accuracy chromatogram of sample-100%-1

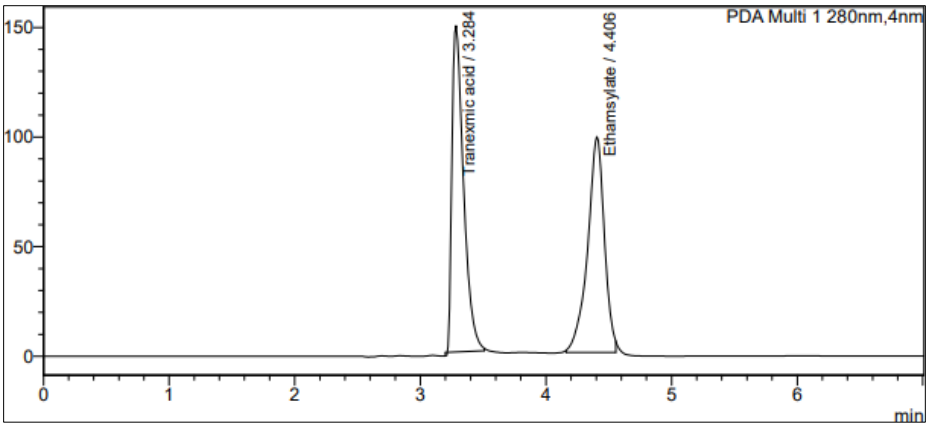

Figure 30 Accuracy chromatogram of sample-150%-1

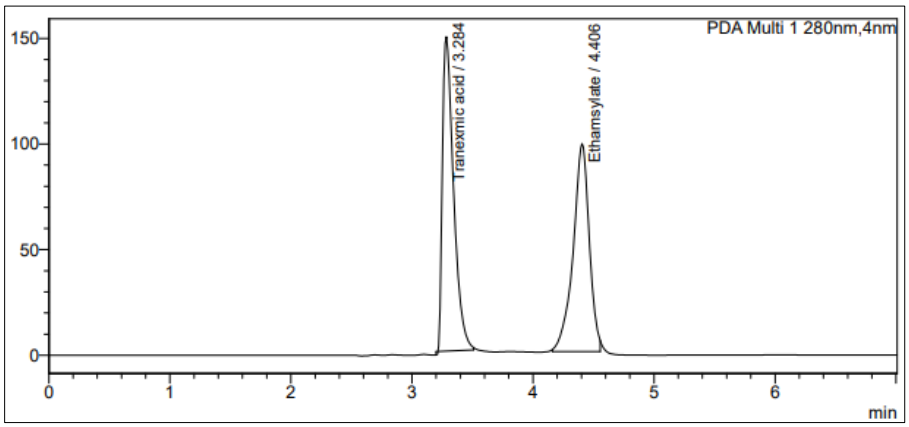

Figure 31 Accuracy chromatogram of sample-150%-3

Table 6 Accuracy data for Tranexamic acid

% Level	Standardpeak 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

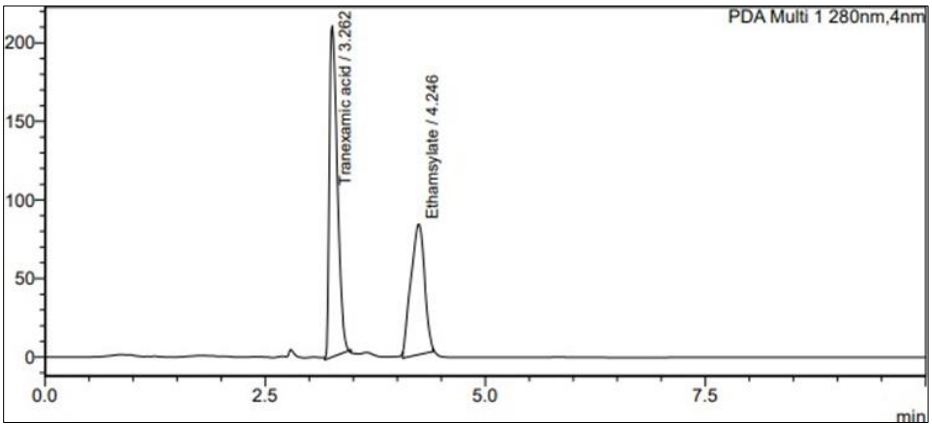

Figure 32 System precision Chromatogram-01

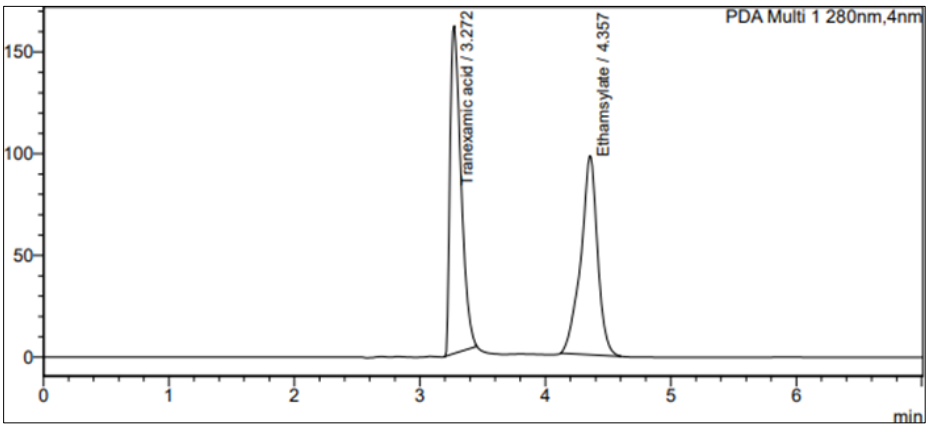

Figure 33 System precision Chromatogram-02

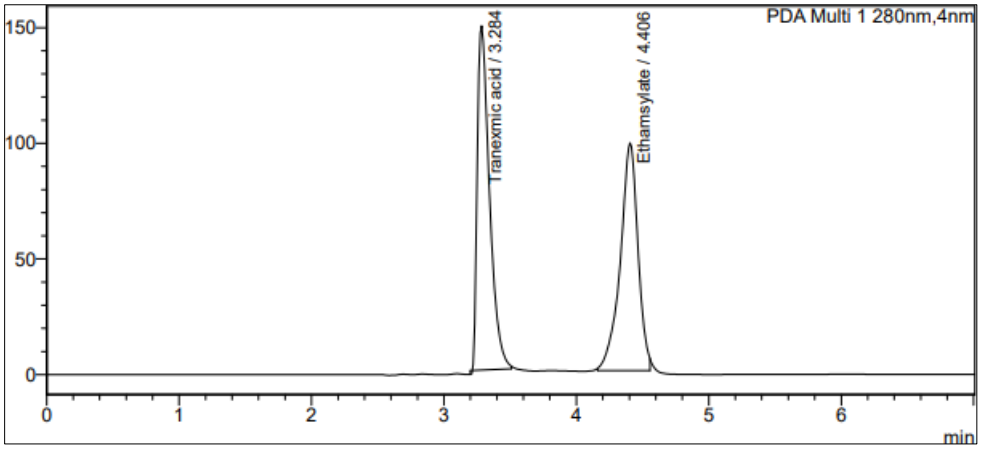

Figure 34 System precision Chromatogram-03

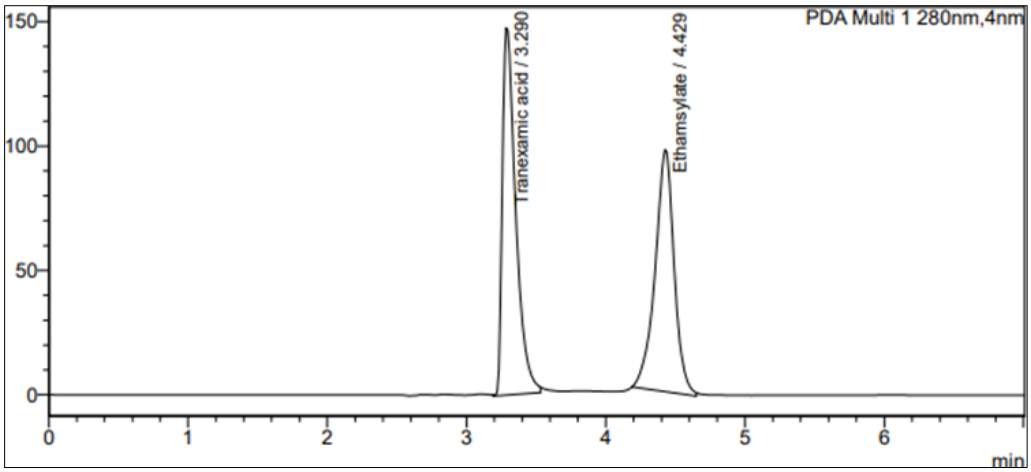

Figure 35 System precision Chromatogram-04

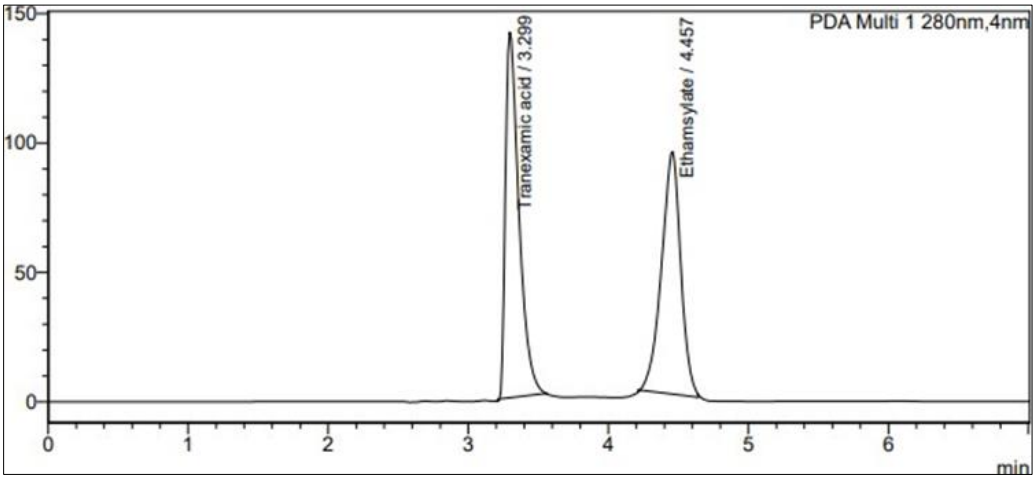

Figure 36 System precision Chromatogram-05

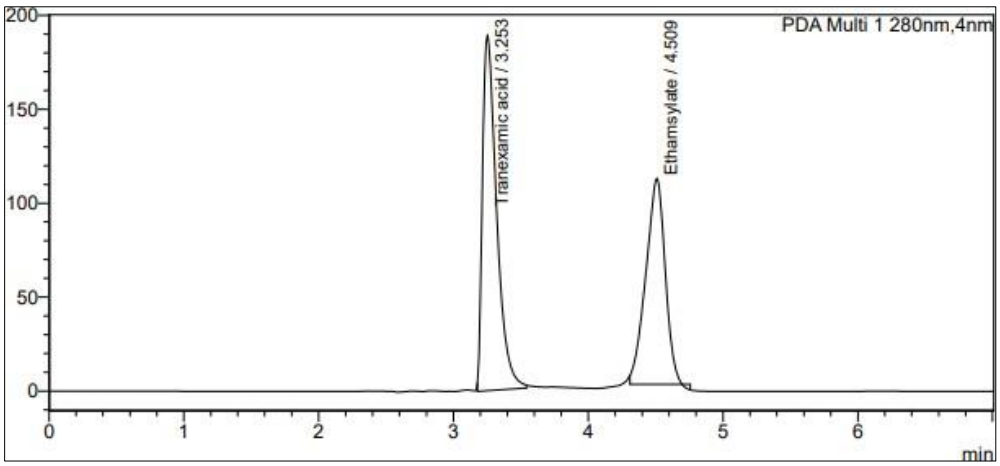

Figure 37 System precision Chromatogram-06

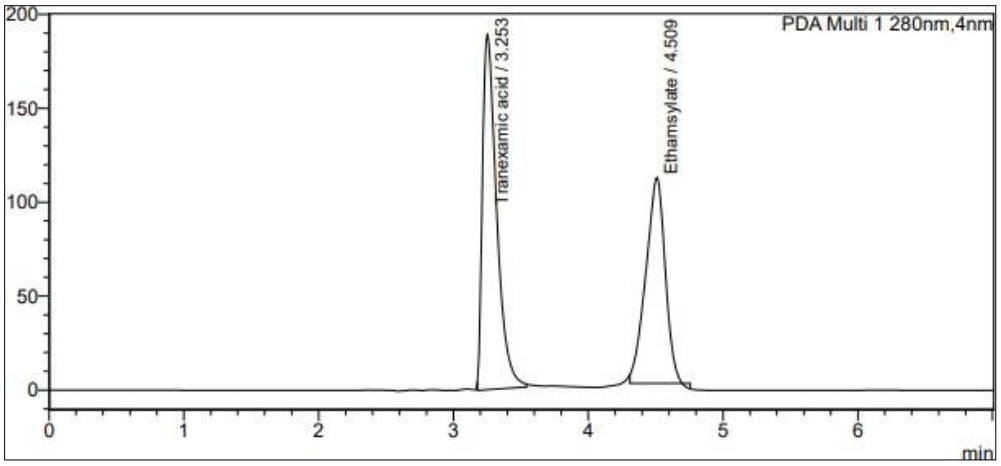

Figure 38 Method precision chromatogram-4

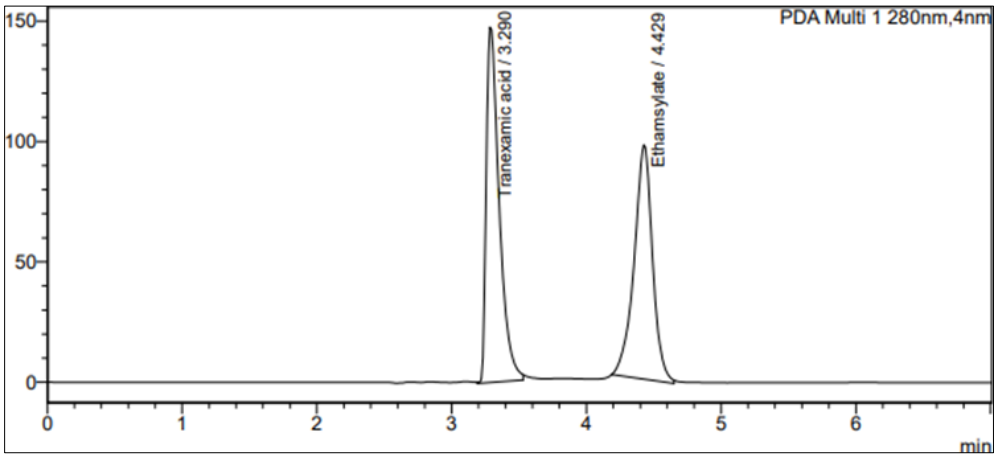

Figure 39 Method precision chromatogram-5

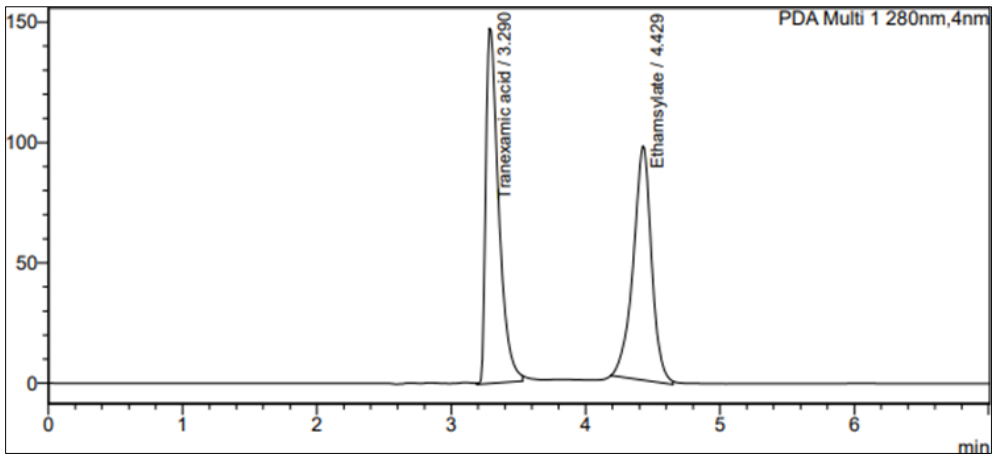

Figure 40 Method precision chromatogram-6

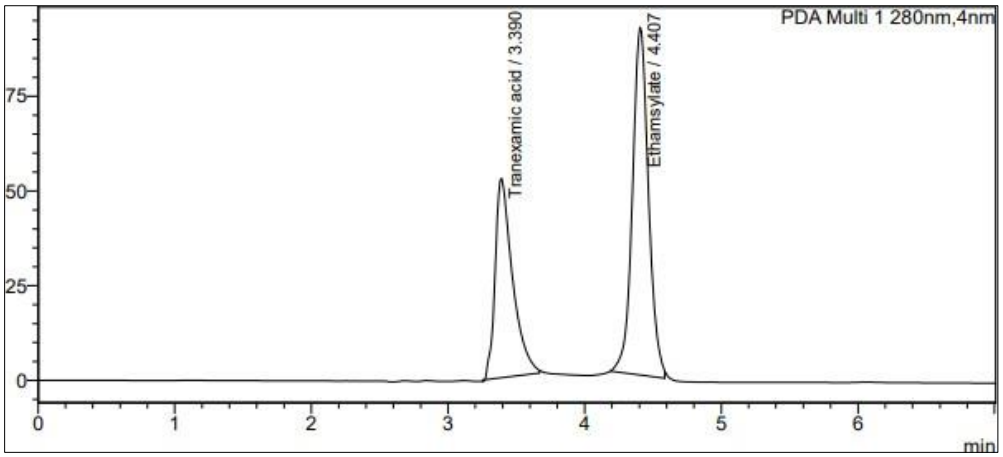

Figure 41 LOD Chromatogram

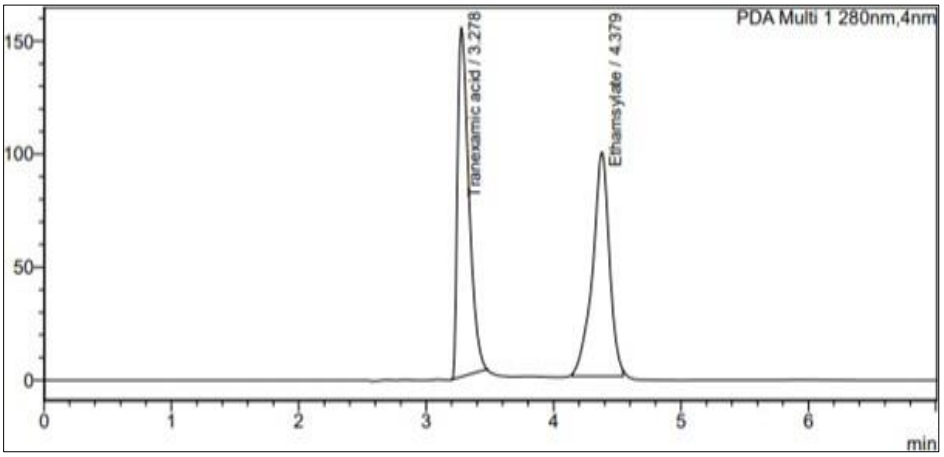

Figure 42 LOQ Chromatogram

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

Parameter	Tranexamicacid(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 ofthe 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 Flowrate0.8Ml /min	3.299	1303714	0.01	4.457	523585	0.01
	3.299	1303615		4.457	523612	
Change in Flowrate0.6ml/min	3.272	1303818	0.02	4.357	523814	0.01

	3.272	1303714		4.357	523915	
Changein Mobilephaseratio70:30v/v	3.253	1320951	0.02	3.509	534112	0.02
	3.253	1321534		3.509	534256	
Change in Mobilephaseratio60:40v/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

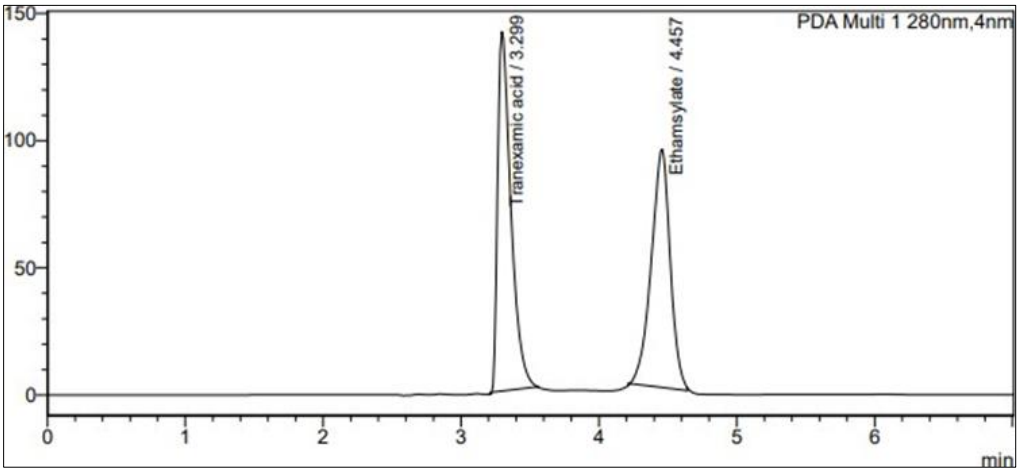

Figure 43 Chromatogram of change in flow rate 0.6ml/min (1)

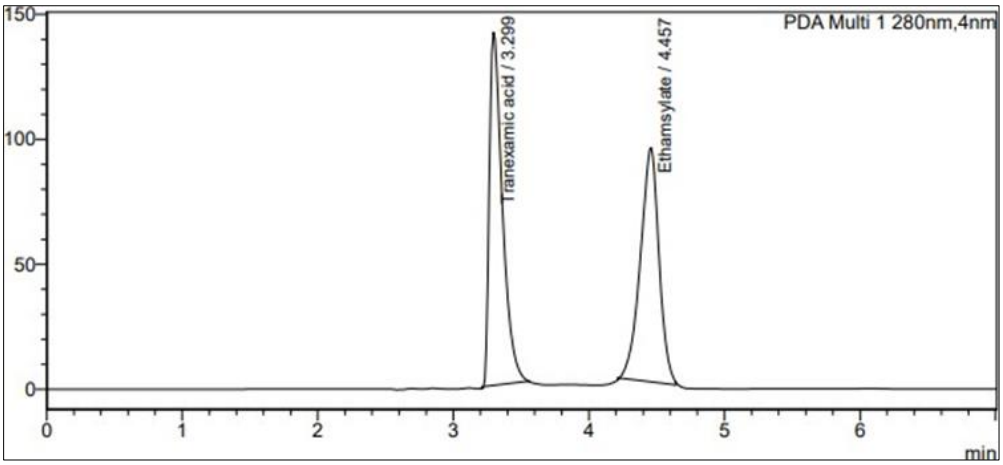

Figure 44 Chromatogram of Change in flow rate 0.6ml/min (2)

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%

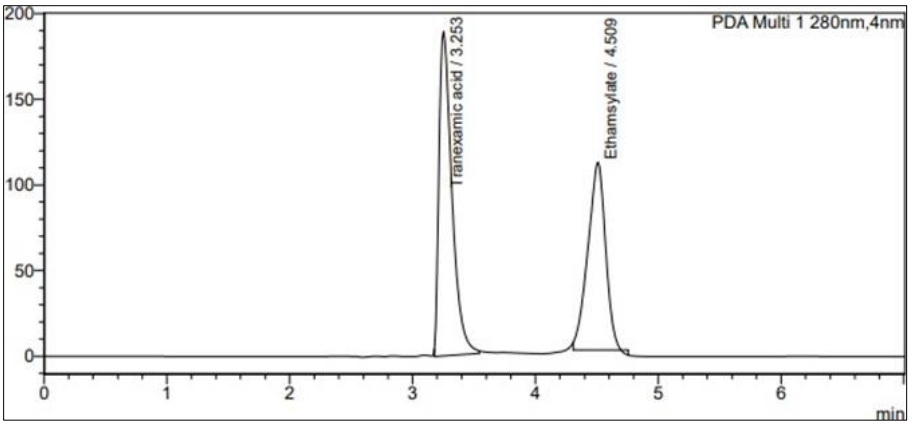

Figure 45 Change in increased Mobile phase ratios Buffer : organic phase (70:30v/v)2

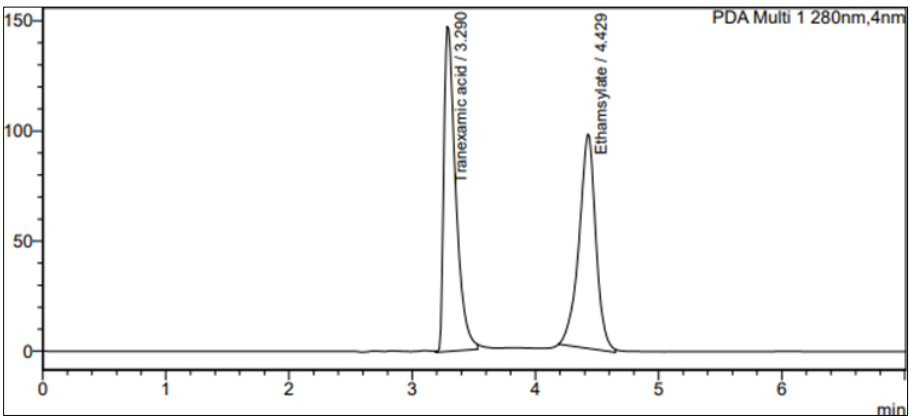

Figure 46 Change in decreased mobile phase ratios Buffer : organic phase in the ratiosof (60:40v/v)-1

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 size5m) 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. Thelinearityconcentrationrangewas10-30µg/mL for Tranexamic acid and 10-30µg/mL for Ethamsylate with a correlation coefficient (r^2) of 0.9999 and 0.9995respectively. 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

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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