ANALYTICAL METHOD VALIDATION OF TRANEXAMIC ACID INJECTION

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Abstract:
The purpose of this study is to verify the method developed for estimation of tranexamic acid in injection formulation, to verify any interference between placebo and Mobile phase, to check the stability test using HPLC. A stainless-steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS) was used for separation at UV 220 nm with flow rate 0.9 ml/min and injection volume 20 µl, and observed that there is no interference between active Tranexamic acid and mobile phase. The method was linear, accurate, precise and robust. The linearity range from 0.8 mg/ml to 1.2 mg/ml for Tranexamic acid with R^2 = 0.999. The recovery % of the Tranexamic acid injection (test) and standard lies between 99.67% to 100.28% and 99.35% to 100.68% respectively. The RSD of intermediate precision on Day 1, Day 2, and Day 3 was observed to be 0.239 %, 0.402 % and 0.137 % respectively. The RSD by changing Flow rate, wavelength injection volume and column length was observed to be 0.640%, 1.145 %, 0.924 % and 0.924 % respectively, and was found that the retention time and column length are directly proportional to each other. The stability test of Mobile phase, sample solution and standard solution was performed and found that Mobile phase was stable up to 48 hours and sample solution was found Stable at 2-8ºC for 24 hours, LOD and LOQ was observed to be 0.4062 and 1.2311 respectively.

Index Terms: Validation, Tranexamic Acid, HPLC

1. Introduction:

Blood can leak from organs or blood vessels inside the body, causing bleeding, or from a skin break or other natural opening on the outside of the body can cause bleeding (ear, mouth, nose, vagina, or rectum). Bleeding may be cause due to Hormone imbalance, Dysfunction of the ovaries, Pregnancy complication, cancer, Uterine fibroids and accidents or during surgery[1]. Excess blood loss of people lead to anemia and death. To overcome this problem antifibrinolytic agents such as aminocaproic acid, fibrinogen Amicar are used, among these tranexamin acid is one of them[2].

Tranexamic acid was first developed in the 1960s and used in clinical settings 40 years ago. A synthetic lysine derivative, Trans-4-(aminomethyl)cyclohexane-1-carboxylic acid, is Tranexamic acid. It has an antifibrinolytic effect and is a biologically active substance. It interferes with action of plasmin and prevents the dissolution of the fibrin clot by reversibly blocking the lysine binding sites on plasminogen through the formation of a reversible complex of the drug with plasminogen molecules[3]. It is hydrophilic in nature and has been used as an antifibrinolytic and hemostatic medication. It has been used to treat particularly heavy menstrual bleeding since it was first released on the market, in addition to reducing blood loss during all kinds of surgeries[4].

Many papers describe drug validation with various reagents, followed by their HPLC determination, according to a review of the literature[5]. The literature survey also revealed that many methods have been developed for estimating tranexamic acid, including HPLC, liquid chromatography fluorescence technique, HPLC linked electrospray ionisation mass spectrometry, gas liquid chromatography, potentiometric method, volumetric method, and UV-spectroscopic approach. [6]. The estimate and validation of Tranexamic acid in tablet dose form were also covered in certain publications. However, no report for the technique validation and Tranexamic acid injection estimation has been located in pharmaceutical formulation[7].

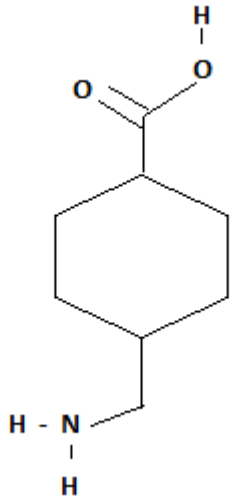

Figure no. 1.1 Tranexamic acid structure

2. Material and Method:

2.1 Material required:

Tranexamic acid injection 5 ml and HPLC grade water will be the gift sample given by National Healthcare Pvt. Ltd. Bara Nepal. Tranexamic acid pure form obtained from china Jiangsu Int. Eco. (China).

2.2 Instruments required:

The method is developed using a Shimadzu HPLC Prominence I LC-2030 equipped with SPD 20 A detector, isocratic pump system, auto injection, A stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS). Other instruments such as Electronic balance (Schimadzu AP 135 W), pH meter (Pico+ labindia) and vacuum pump (PCI Analytical) is used for analysis procedure.

2.3 Standard preparation:

0.2% w/v of tranexamic acid will be prepared by dissolving and diluting 100 microgram of tranexamic acid to 50 millileter with water. It will be then sonicated for 15 minutes.

2.4 Sample preparation: the sample solution will be prepared by diluting 2 ml of injection equivalent to 200 microgram of tranexamic acid to 100 millileter with water to produc a solution containing 0.2% w/v of tranexamic acid.

2.5 Chromatographic conditions: The mobile phase (Prepared by dissolving 11g of anhydrous sodium dihydrogen orthophosphate in 500 ml of water, add 5 ml of triethylamine and 1.4 g of sodium dodecyl sulfate, adjust the pH to 2.5 with 2 M orthophosphate acid and add sufficient water to procude 600 ml. Add 400 ml of methanol and mix.) will be pumped by at a flow rate of 0.9 ml per minute. A stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS) is used for separation. The elution was monitored at UV 220 nm and the sample injection volume was 20 µl.

2.6 Method validation:

2.6.1 Specificity:

The 3-spike sample was prepared as follow. One gram of placebo and 100 mg of Tranexamic acid WS was transferred in to 50 ml volumetric flask, and diluted up 25 ml with water. Sonicate for 10 minutes. Shake and make up the volume with water.

2.6.2 Linearity:

Three concentrations (Such as 80%, 90%, 100%, 110% and 120%) of sample solution and Standard solution was prepared and the calibration curve was constructed by plotting graph of peak area VS concentration.

2.6.3 Accuracy:

Three concentrations (Such as 0.8 mg/ml, 1 mg/ml and 1.2 mg/ml) of sample solution and Standard solution was prepared and the calibration curve was constructed by plotting graph of peak area VS concentration.

2.6.4 Precision:

Precision (repeatability) of the instruments was checked by repeatedly injecting (n=6) the mixture of Standard solution of Tranexamic acid in three different instrument in three different days by 3 different analyst.

2.6.5 Limit of quantification (LOQ) and Limit of detection (LOD):

Limit of detection and Limit of Quantification was determined using following equation.

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where, σ = Standard deviation of response and the y- intercept of the regression line
S = slope of regression line.

2.6.6 Robustness:

The Robustness was determined by changing column length (Such as 15 cm and 25 cm), Wavelength in the variation of ± 2 nm (Such as 220 nm, 218 nm and 222 nm), Injection volume in the variation of 100%, 90% and 80% (Such as 20 µl, 18 µl and 16µl) and Flow rate in the variation of ±10% (Such as 1.5 ml per minutes, 1.35 ml per minutes and 1.65 ml per minutes) of Sample solution.

2.6.7 **Solution stability:**

The Stability of Sample solution and Standard solution was determined after storing the Samples for 6 hours and 24 hours at real time ($25^{O}C$) and Storing for 24 hours at $2^{O}C$ - $8^{O}C$ in refrigerator. The Stability of Mobile phase was concluded by storing it for 6 hours, 24 hours and 48 hours at $25^{O}C$.

3. Result and discussion:

3.1 Specificity:

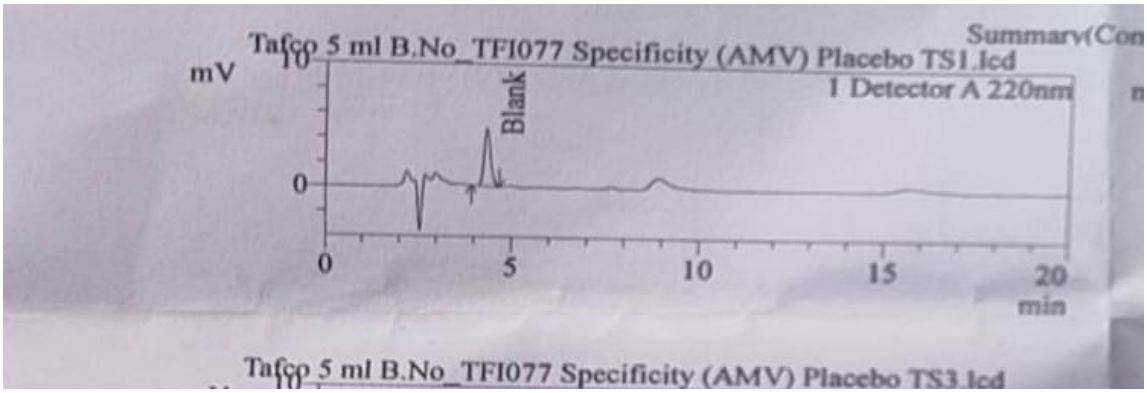

Figure no. 3.1: blank (mobile phase)

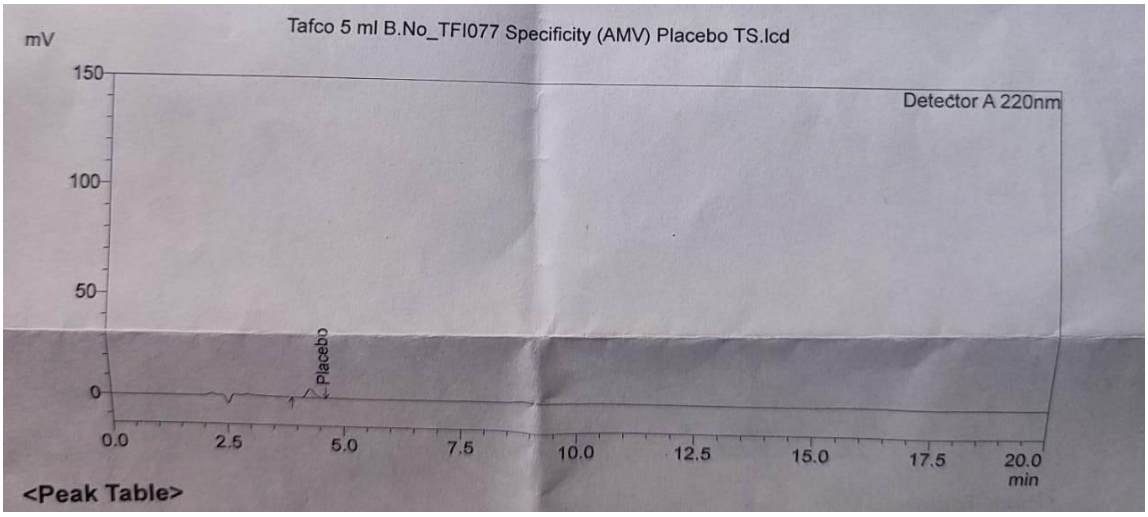

Figure no. 3.2: placebo

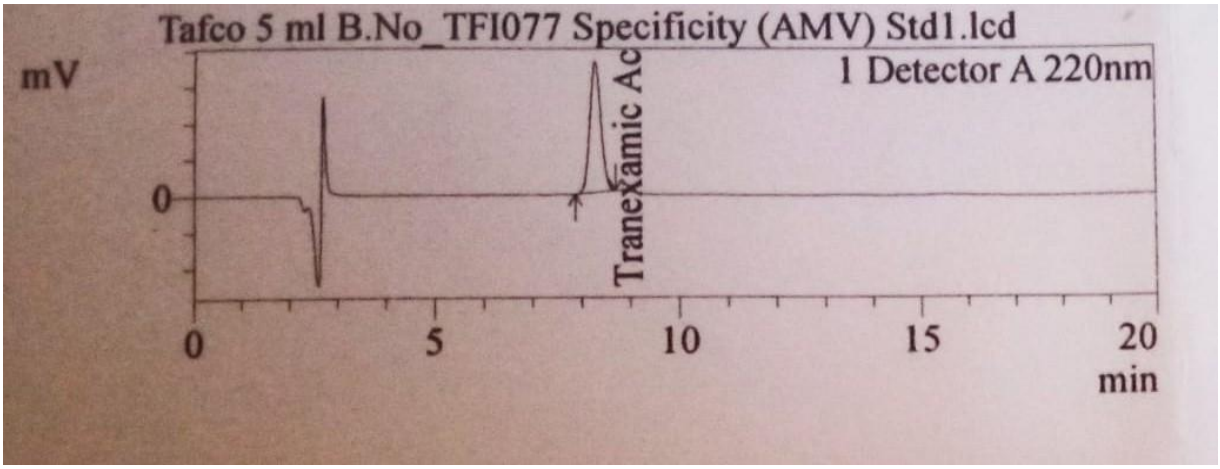

Figure no. 3.3: standard (tranexamic acid)

The chromatogram of Blank (Mobile phase), Placebo and Tranexamic acid Working Standard is shown in figure No. 3.1, 3.2 and 3.3 respectively. It is observed from the above chromatogram that there is no interference of blank and placebo of Working Standard

(Tranexamic acid). In placebo sample there is no peak detection at sample retention time so, the method was specific for Tranexamic acid.

3.2 Linearity:

The linearity test was performed on five different concentration of test and Standard solution. The five different concentrations (Such as 80%, 90%, 100%, 110% and 120%) of sample solution and Standard solution was prepared and the calibration curve was constructed by plotting graph of peak area VS concentration.

Table 3.1: Concentration and area for linearity of tranexamic acid (standard liniarity)

S. No.	Conc. %	Area					
		Std 1	Std 2	Std 3	Mean	SD	%RSD
1.	80	451285	451423	451316	451341	72	0.016
2.	90	507733	509788	509396	508972	1091	0.214
3.	100	571487	571634	570922	570932	565	0.099
4.	110	627777	621261	627776	625605	3762	0.601
5.	120	680131	680336	683664	681377	1983	0.291

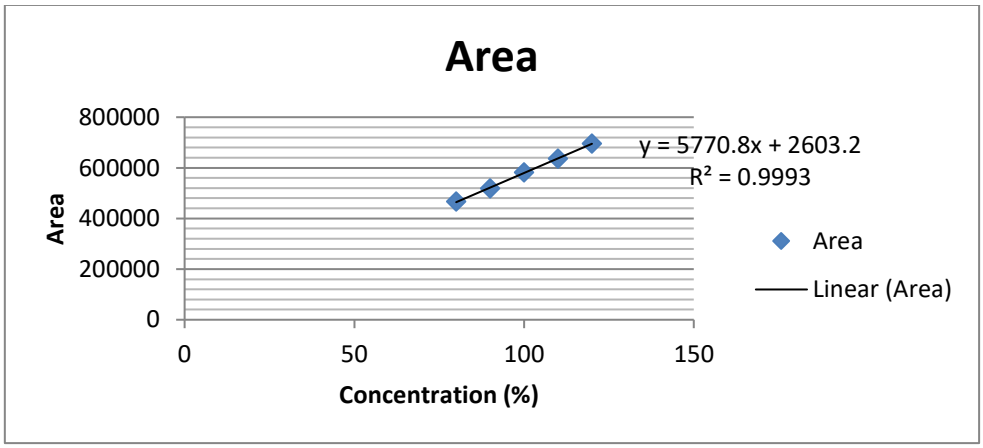

Figure no. 3.4: Calibration curve for area under curve versus concentration in mg/ml of tranexamic acid (standard solution)

Table 3.2: Concentration and area for linearity of Tranexamic acid (Test Linearity)

S.No.	Conc. %	Area					
		Std 1	Std 2	Std 3	Mean	SD	%RSD
1.	80	466076	466469	466092	466212	222	0.048
2.	90	517995	518466	518253	518238	236	0.046
3.	100	582850	581727	581528	581767	539	0.093
4.	110	636323	636833	636705	636620	265	0.042
5.	120	695778	695457	695443	695559	190	0.027

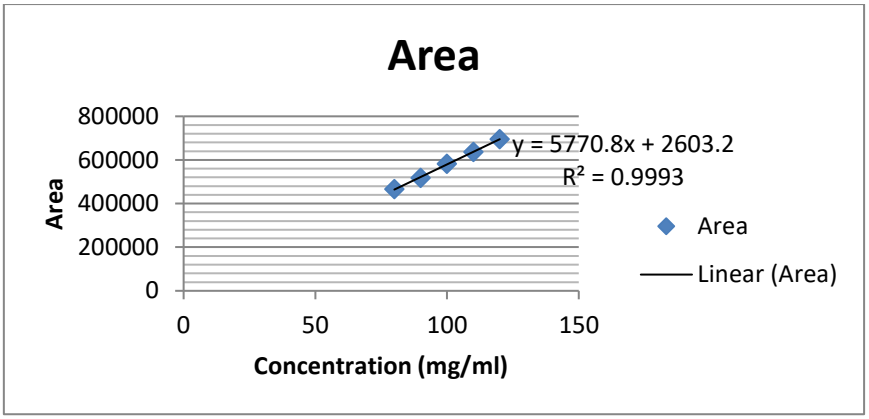

Figure no. 3.5: calibration curve for area under curve versus concentration in mg/ml of tranexamic acid (test solution)

In Figure 3.4 area against the concentration for Standard (Tranexamic acid) was plotted which gives a straight line with R^2 = 0.999 with regression equation y = 5770x + 2603. In Figure 3.5 the area against the concentration for test sample was plotted which gives a straight line with R^2 = 0.999 with regression equation y = 5770x + 2603. In Figure the straight line was obtained with R^2 = 0.999 with regression equation y = 5770x + 2603, with the slope of 5770. The linearity curve obtained by using lower concentration of Tranexamic acid (Standard solution) (i.e. 0.8 mg/ml to 1.2 mg/ml) by plotting area against concentration. This showed that the proposed method was linear for Tranexamic acid with slope of 5770.

3.3 Accuracy:

The accuracy was determined by recovery test. The accuracy results at three concentrations (Such as 0.8 mg/ml, 1 mg/ml and 1.2 mg/ml) for sample solution and Standard solution in all drug products showed good recovery and are summarized in table given below.

$$Standard\ preparation = \frac{100.2\ mg}{100\ ml} \qquad C_S = 0.998\ mg/ml$$

$$Test\ preparation\ (80\ \%) = \frac{0.8\ ml}{100\ ml} \qquad C_{U80} = 0.8\ mg/ml \quad [Note: 1\ ml\ injection = 100\ mg]$$

$$Test\ preparation\ (100\ \%) = \frac{1\ ml}{100\ ml} \qquad C_{U80} = 1\ mg/ml \quad [Note: 1\ ml\ injection = 100\ mg]$$

$$Test\ preparation\ (120\ \%) = \frac{1.2\ ml}{100\ ml} \qquad C_{U80} = 1.2\ mg/ml \quad [Note: 1\ ml\ injection = 100\ mg]$$

$$Assay\ (\%)\ (80\ \%) = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 80$$

$$Assay\ (\%)\ (100\ \%) = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100$$

$$Assay\ (\%)\ (120\ \%) = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 120$$

Where, r_U = Area of test solution

r_S = Area of Standard solution

Table 3.3: Area of standard solution

Area of Standard solution	
Std 1	571487
Std 2	571634
Std 3	570922
Std 4	570885
Std 5	570526
Std 6	570138
Mean	570932
Standard deviation	516.141
RSD %	0.090

Table 3.4: Recovery study of tranexamic acid

Area of Test			
	80%	**100%**	**120%**
TS1	466076	582850	695778
TS2	466469	581727	695457
TS3	466092	581528	695443
Assay %			
TS1	81.51 %	101.93 %	121.68 %
TS2	81.58 %	101.74 %	121.63 %
TS3	81.51 %	101.70 %	121.62 %
Recovery %			
TS1	101.89	101.93	101.40
TS2	101.97	101.74	101.36
TS3	101.89	101.70	101.35
Avg. (%)	**101.69**		
Recovery as per Average %			
TS1	100.19	100.24	99.71
TS2	100.28	100.04	99.67
TS3	100.20	100.01	99.67
		Average	**100.0011**
		Standard deviation	**0.253892**
		RSD in %	**0.253892**

Table 3.4 shows the obtained recovery % which lies between 99.67 % to 100.28 % with mean recovery of 101.69 %. The Standard deviation was observed to be 0.253892% and relative Standard deviation was observed to be 0.253892%, which is < 2.0%. Thus, the Accuracy meets the requirement of the analytical method validation specification (limit) and fount to be accurate for Tranexamic acid Injection.

3.4 Precision:

Precision (repeatability) of the instrument was determined by repeatedly injecting (n=6) the mixture of Standard solution of Tranexamic acid in Three different instrument in three different days by three different analyst and on 100% test concentration Standard concentrasion, which is shows below in table

Table 3.5: Shows results of test solution (concentration) precision done by first analyst on first day on instrument no 1. (day 1)

Injection	Area	RT of Tranexamic acid injection	Tailing Factor	Theoretical Plate
1	250390	8.766	1.165	6595
2	251681	8.764	1.199	6459
3	250403	8.755	1.168	6605
4	251009	8.749	1.169	6606
5	250221	8.738	1.185	6323
6	250116	8.663	1.184	6310
Mean	**250637**	**8.739**	**1.178**	**6483**
Standard deviation	**598.094**	**0.039**	**0.013**	**140**
% RSD	**0.239**	**0.444**	**1.123**	**2.167**

From Table 3.5, it was found that the % Relative Standard deviation of area of Test solution on Day 1 by First analyst was 0.239%, which was observed to be within the range that is < 2.0%. The TF was observed to be < 2. The Theoretical plate was found to within the range which is not < 2000.

Table 3.6: Shows results of standard solution (concentration) precision done by first analyst on first day on instrument no 1.

Injection	Area	RT of Tranexamic acid injection	Tailing Factor	Theoretical Plate
1	251237	8.766	1.186	6288
2	251061	8.764	1.189	6268
3	251531	8.755	1.189	6263
4	251612	8.749	1.189	6201
5	252770	8.738	1.191	6158
6	251650	8.663	1.193	5855
Mean	**251644**	**8.739**	**1.198**	**6172**
Standard deviation	**598**	**0.039**	**0.004**	**163**
% RSD	**0.237**	**0.444**	**0.337**	**2.636**

From Table 3.6, it was found that the % Relative Standard deviation of area of Standard solution on Day 1 by First analyst was 0.237%, which was observed to be within the range that is < 2.0%. The TF was observed to be < 2. The Theoretical plate was found to within the range which is not < 2000.

Table 4.7: Shows Results of Test solution (concentration) precision done by second analyst on second day on instrument No 2.

Injection	**Area**	**RT of Tranexamic acid injection**	**Tailing Factor**	**Theoretical Plate**
1	251009	7.358	1.245	5438
2	250307	7.358	1.249	5426
3	253059	7.357	1.235	5392
4	251784	7.358	1.237	5401
5	251103	7.358	1.239	5434
6	250486	7.358	1.241	5440
Mean	**251291**	**7.358**	**1.241**	**5422**
Standard deviation	**1009.953**	**0.000**	**0.005**	**20**
% RSD	**0.402**	**0.003**	**0.420**	**0.369**

From Table 3.7, it was found that the % Relative Standard deviation of area of Test solution on Day 2 by Second analyst was 0.402%, which was observed to be within the range that is < 2.0%. The TF was observed to be < 2. The Theoretical plate was found to within the range which is not < 2000.

Table 3.8 Showing results of standard solution (concentration) precision done by second analyst on second day on instrument no 2.

Injection	Area	Retention time of Tranexamic acid injection	Tailing Factor	Theoretical Plate
1	251619	7.357	1.235	5403
2	254341	7.355	1.230	5365
3	253265	7.354	1.246	5419
4	252259	7.354	1.245	5396
5	252187	7.361	1.260	5404
6	254625	7.359	1.239	5411
Mean	253050	7.357	1.242	5400
Standard deviation	1234	0.003	0.011	19
% RSD	0.488	0.041	0.853	0.352

From Table 3.8, it was found that the % Relative Standard deviation of area of Standard solution on Day 2 by Second analyst was 0.488%, which was observed to be within the range

that is < 2.0%. The TF was observed to be < 2. The Theoretical plate was found to within the range which is not < 2000.

Table 3.9: Showing Results of Test solution (concentration) precision done by third analyst on third day on instrument No 3. (Day 3)

Injection	Area	RT of Tranexamic acid injection	Tailing Factor	Theoretical Plate
1	243584	7.354	1.401	4295
2	243257	7.361	1.396	4296
3	243686	7.360	1.399	4298
4	243655	7.359	1.399	4295
5	242939	7.359	1.396	4306
6	243841	7.360	1.403	4290
Mean	**243494**	**7.359**	**1.399**	**4297**
Standard deviation	**333.313**	**0.003**	**0.003**	**5**
% RSD	**0.137**	**0.036**	**0.197**	**0.123**

From Table 3.9, it was found that the % Relative Standard deviation of area of Test solution on Day 3 by Third analyst was 0.137%, which was observed to be within the range that is < 2.0%. The TF was observed to be < 2. The Theoretical plate was found to within the range which is not < 2000.

Table 3.10: Showing results of standard solution (concentration) precision done by third analyst on third day on instrument no 3. (day 3)

Injection	Area	RT of Tranexamic acid injection	Tailing Factor	Theoretical Plate
1	250390	8.587	1.165	6595
2	251681	8.460	1.199	6459
3	250403	8.706	1.168	6605
4	251009	8.764	1.169	6606
5	250221	8.776	1.185	6323
6	250116	8.771	1.184	6310
Mean	**250637**	**8.678**	**1.178**	**6483**
Standard deviation	**598**	**0.128**	**0.013**	**141**
% RSD	**0.239**	**1.477**	**1.130**	**2.169**

From Table 3.10, it was found that the % Relative Standard deviation of area of Standard solution on Day 3 by Third analyst was 0.239%, which was observed to be within the range that is < 2.0%. The TF was observed to be < 2. The Theoretical plate was found to within the range which is not < 2000.

3.5 Limit of quantification (LOQ) and Limit of Detection (LOD):

The Limit of Detection and Limit of Quantification was obtained from th**e** regression analysis of Linearity curve plotted by lower dilute of Standard solution from the concentration of 80% to 120% (0.8 mg/ml to 1.2 mg/ml). The Detection limit (LOD) and Quantification limit (LOQ) may be indicated as:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where, σ = Standard deviation of response and the y- intercept of the regression line
S = slope of regression line.

In Figure No 3.4 the straight line was obtained with $R^2 = 0.999$ with regression equation y = 5770x + 2603, with the slope of 5770. The linearity curve obtained by using lower concentration of Tranexamic acid (Standard solution) (i.e. 0.8 mg/ml to 1.2 mg/ml) by plotting area against concentration. This showed that the proposed method was linear for Tranexamic acid with slope of 5770. Standard deviation of intercept was observed to be 710.3715. Thus, LOD and LOQ for Tranexamic acid was observed to be 0.4062 and 1.2311 respectively.

3.6 Robustness:

The Robustness was determined by changing column length (Such as 15 cm and 25 cm), Wavelength in the variation of ± 2 nm (Such as 220 nm, 218 nm and 222 nm), Injection volume in the variation of 100%, 90% and 80% (Such as 20 µl, 18 µl and 16µl) and Flow rate in the variation of ±10% (Such as 1.5 ml per minutes, 1.35 ml per minutes and 1.65 ml per minutes) of Sample solution.

Table 3.11: Showing Results of effects of changing column length (Test Solution)

S.No.	Parameter	Test	Assay %	RT of test	Area of test
1	Column Length-1 15 cm	TS1	103.88	7.358	263795
2		TS2	103.92	7.358	263903
3		TS3	102.92	7.358	261442
Average			**103.57**	**-**	**263046.7**
Standard deviation			**0.566157**	**-**	**1390.731**
RSD%			**0.546624**	**-**	**0.528701**
4	Column Length-2 25 cm	TS1	102.04	7.914	252327
5		TS2	101.57	7.903	251155
6		TS3	102.95	7.891	254557
Average			**102.1867**	**-**	**252679.7**
Standard deviation			**0.701593**	**-**	**1728.202**
RSD%			**0.68658**	**-**	**0.68395**

Table 3.12: Showing Results of effects of changing column length (Standard solution)

S.No.	Parameter	Standard	RT of Std.	Area of Std.
1	Column Length-1 15 cm	Std 1	7.357	251619
2		Std 2	7.355	254341
3		Std 3	7.354	253265
4		Std 4	7.354	252259
5		Std 5	7.361	252187
6		Std 6	7.359	254625
			Average	**253050**
			Standard deviation	**1234**
			RSD%	**0.488**
7	Column Length-2 25 cm	Std 1	7.993	245230
8		Std 2	7.980	248522
9		Std 3	7.962	245712
10		Std 4	7.944	246284
11		Std 5	7.927	248426
12		Std 6	7.980	244276
			Average	**246408**
			Standard deviation	**1731**
			RSD%	**0.702**

Table 3.11 shows the results of Tranexamic acid by changing its Column length i.e. 15 cm and 25 cm. The RSD of Test solution of tranexamic acid was observed to be 0.528701and 0.68395 for 15 cm column and 25 cm column respectively, which was < 2.0%.

Table 3.12 shows the results of Tranexamic acid by changing its Column length i.e. 15 cm and 25 cm. The RSD of Standard solution of tranexamic acid was observed to be 0.488 and 0.702 for 15 cm column and 25 cm column respectively, which was < 2.0%. From Table it is found that column length and retention time are directly proportional to each other.

Table 3.13: Showing Results of effects of changing wavelength

S.No.	Parameter	Test	Assay %
1	Standard Robustness (By BP) 220 nm	TS1	102.21
2		TS2	102.92
3		TS3	103.75
4	Variation in WL by (-2) WL= 218 nm	TS1	102.01
5		TS2	103.56
6		TS3	102.13
4	Variation in WL by (+2) WL= 222 nm	TS1	100.45
5		TS2	101.43
6		TS3	100.61

Average	**102.12**
Standard deviation	**1.169**
RSD%	**1.145**

Table 3.13 shows the results of Tranexamic acid by changing its Wavelength i.e. 220 nm, 218 nm and 222 nm. The Standard deviation and RSD was observed to be 1.169 and 1.145 respectively, which was < 2.0%.

Table 5.14: Showing Results of effects of changing Flow rate

S.No.	Parameter	Test	Assay %
1	Standard Robustness (By BP) FR= 1.5 ml per minutes	TS1	102.21
2		TS2	102.92
3		TS3	103.75
4	Variation in FR by (-10%) FR= 1.35 ml per minutes	TS1	102.54
5		TS2	101.73
6		TS3	102.01
4	Variation in FR by (+10%) FR= 1.65 ml per minutes	TS1	103.25
5		TS2	103.26
6		TS3	102.60
		Average	**102.70**
		Standard deviation	**0.658**
		RSD%	**0.640**

Table 3.14 shows the results of Tranexamic acid by changing its Flow rate by 1.5 ml per minutes, 1.35 ml per minutes and 1.65 ml per minutes. The Standard deviation and RSD was observed to be 0.658 and 0.640 respectively, which was < 2.0%.

Table 3.15: Showing Results of effects of changing Injection volume

S.No.	Parameter	Test	Assay %
1	Standard Robustness (By BP) IV = 20 µl	TS1	102.21
2		TS2	102.92
3		TS3	103.75
4	Variation in IV by (-2 µl) IV = 18 µl	TS1	101.59
5		TS2	101.39
6		TS3	101.05
4	Variation in IV by (-4 µl) IV = 16 µl	TS1	101.82
5		TS2	101.02
6		TS3	101.08
		Average	**101.87**
		Standard deviation	**0.941**
		RSD%	**0.924**

Table 3.15 shows the results of Tranexamic acid by changing its injection volume by 18 µl and 16 µl. The Standard deviation and RSD was observed to be 0.941 and 0.924 respectively, which was < 2.0%.

3.7 Stability:

The Stability of Sample solution and Standard solution was determined after storing the Samples for 6 hours and 24 hours at real time (25^{O}C) and Storing for 24 hours at 2^{O}C - 8^{O}C in refrigerator. The Stability of Mobile phase was determined by storing it for 6 hours, 24 hours and 48 hours at 25^{O}C.

By mixing freshly made Standard and sample solutions with original solutions that had been kept at room temperature in the auto sampler (for 6 hours, 24 hours and 48 hours at 2ºC - 8ºC), solution stability for the method was examined. Which original solution was prepared as a triplicate sample at 100% concentration. The mean assay percentages of freshly prepared solutions and those obtained from solutions kept in an auto sampler at room temperature for 6 hours, 24 hours and 48 hours were compared.

3.7.1 For Mobile phase:

$$Stadard\ preparation = \frac{100.6\ \ g}{100\ \ ml} \qquad C_S = 1.00248\ \ mg/ml$$

$$Test\ \ preparation = \frac{1\ \ ml}{100\ \ ml} \qquad C_U = 1\ \ mg/ml$$

Note: Each ml of injection contains 100 mg of tranexamic acid.

$$Assay\ in\ \ \% = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100$$

Where,

r_U = Area of test preparation

r_S = Area of Standard preparation

C_S = Concentration of Tranexamic acid in Standard preparation (mg/ml)

C_U = Concentration of Tranexamic acid in Test preparation (mg/ml)

Table 3.16: Showing the result of effect of stability of Mobile phase

S.No.	Parameter	Sample	Area	Assay %
1	Fresh Sample	Std.	573663	-
2		TS1	576205	100.69
3		TS2	574061	100.32
4		TS3	571616	99.89
Average				**100.30**
5	Store at Real time	Std.	587384	-

6	(6 hours)	TS1	584062	99.68
4		TS2	582940	99.49
5		TS3	583605	99.60
Average				**99.59**
6	Store at Real time	Std.	581764	-
7	(24 hours)	TS1	582281	100.34
8		TS2	582300	100.34
9		TS3	581762	100.25
Average				**100.31**
10	Store at Real time	Std.	587731	-
11	(48 hours)	TS1	583833	99.58
12		TS2	584886	99.76
13		TS3	585292	99.83
Average				**99.73**
RSD%				**0.377**

3.7.2 For Test and Standard solution:

$$Stadard\ preparation = \frac{1002\ \ g}{100\ \ ml} \qquad C_S = 0.9985\ \ mg/ml$$

$$Test\ \ preparation = \frac{1\ \ ml}{100\ \ ml} \qquad C_U = 1\ \ mg/ml$$

Note: Each ml of injection contains 100 mg of tranexamic acid.

$$Assay\ in\ \ \% = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100$$

Table 3.17: Showing the result of effect of stability of Test and Standard solution

S.No.	Parameter	Sample	Area	Assay %
1	Fresh Sample	Std.	278529	-
2		TS1	286660	102.76
3		TS2	284500	101.99
4		TS3	281533	100.93
Average				**101.89**
5	Store at Real time	Std.	279181	-
6	(6 hours)	TS1	284096	101.61
4		TS2	281798	100.79
5		TS3	281607	100.72
Average				**101.04**
Between 98.0% and 102.0% in comparison to the freshly prepared solution.				**99.16**

6	Store at Real time (24 hours)	Std.	574923	-
7		TS1	585039	101.61
8		TS2	584516	101.52
9		TS3	584565	101.52
Average				**101.55**
Between 98.0% and 102.0% in comparison to the fresh solution.				**99.66**
10	Store at 2 - 8ºC (24 hours)	Std.	571948	-
11		TS1	583346	101.84
12		TS2	583801	101.92
13		TS3	584108	101.97
Average				**101.91**
Between 98.0% and 102.0% in comparison to the freshly prepared solution.				**100.02**

The Table 3.16 shows the results observed from the stability test of Mobile phase at freshly prepared, after 6 hours store, after 24 hours store and after 48 hours store during real time i.e. 25ºC ± 2ºC, RH 60% ± 5% according to ICH guidelines. The Table 5.17 shows the results observed from the stability test of Solution (Test solution and Standard solution) at freshly prepared, after 6 hours of store, after 24 hours of store and after storing for 24 hours at 2ºC - 8ºC. The RSD of all the assay results upto 48 hours for Mobile phase was observed to be 0.377%, Which is < 2.0% and The assay % was observed to be 99.16%, 99.66% and 100.02% for sample solution stored at 6 hours, 24 hours and 24 hours (by storing at 2ºC - 8ºC) respectively, by comparing it with freshly prepared solution at room temperature (i.e. 25ºC ± 2ºC, RH 60% ± 5%). Thus; the obtained assay value lies inside the specified limit so the Solution Stability meets the requirement of the analytical method validation specification (limit).

4. Conclusion:

The aim of the study was to validate a method for the simultaneous estimation of Tranexamic acid injection. The interference of active Tranexamic acid with mobile phase was observed and found to be no interference between them, as active was not affected by it. The method was observed to be accurate, linear, robust and precise. The linearity range from 0.8 mg/ml to 1.2 mg/ml for Tranexamic acid with R^2 = 0.999. The recovery % of the Tranexamic acid injection lies between 99.67% to 100.28% and the recovery % of Standard Tranexamic acid was observed to be 99.35% to 100.68%. The intermediate precision and robustness was observed to be within the RSD < 2.0%. The RSD of intermediate precision on Day 1, Instrument 1 by 1st analyst, Day 2, Instrument 2 by 2nd analyst and Day 3, instrument 3 by third analyst was observed to be 0.239 %, 0.402 % and 0.137 % respectively. The TF was observed to be < 2.0% and theoretical plate was observed to be not < 2000. The RSD by changing Flow rate (i.e. 1.5 ml per minutes, 1.35 ml per minutes and 1.65 ml per minutes) was observed to be 0.640%. The RSD of robustness by changing wavelength (i.e. 220 nm, 218 nm and 222 nm) was observed to be 1.145 %. The RSD of robustness by changing injection volume (i.e. 20 µl, 18 µl and 16

µl) was observed to be 0.924 %. The RSD of robustness by changing column length (i.e. 15 cm and 25 cm) was observed to be 0.924 % and was found that the retention time and column length are directly proportional to each other. The peak was obtained earlier in small column i.e. 15 cm than 25 cm, thus beneficial for pharmaceutical industries. The stability test of Mobile phase, sample solution and Standard solution was performed and found that Mobile phase was stable up to 48 hours and sample solution was found Stable at 2-8ºC for 24 hours. The LOD and LOQ for Tranexamic acid was observed to be 0.4062 and 1.2311 respectively.

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